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CLAY MINERALS IDENTIFIED IN THE RED QUATERNARY DEPOSITS FROM CETATE AREA (DOLJ COUNTY, ROMANIA), USING XRD METHOD

NEGREANU Ştefan, SOARE Barbara

Abstract. The area from which samples were collected is close to the limit between the counties of Dolj and Mehedinţi, about10 km north of the Danube. The Quaternary loess like red deposits from Oltenia are described in the specialized literature as "red clays" or "red loams". This paper renders data on the type and proportion of the clay minerals identified by X-ray diffraction of the clay fraction separated from both red loess like deposits and older deposits either ruditic with red matrix, mainly silty-sandy or sandy red levels. For a precise understanding of the conditions of the analysis, there are presented the methods used for this purpose in detail.

Keywords: Quaternary, loess like deposits, clay minerals, X-ray diffraction.

Rezumat. Minerale argiloase identificate prin difracţie de raze X, din depozite roşii cuaternare, zona Cetate (judeţul Dolj, România). Zona din care au fost culese probele pentru analize se găseşte în apropierea limitei dintre judeţele Dolj şi Mehedinţi la aproximativ 10 km nord de Dunăre. Depozitele loessoide cuaternare de culoare roşie din Oltenia sunt descrise în literatură ca „argile roşii" sau „luturi roşii". Lucrarea de faţă redă date referitoare la tipul şi proporţia mineralelor argiloase identificate prin difracţia de raze X din fracţia argiloasă, separată, atât din depozitele loessoide roşii, cât şi din depozite mai vechi, fie ruditice cu matrice roşie, preponderent silto-nisipoasă, fie nivele nisipoase roşii. Pentru o înţelegere exactă a condiţiilor de analiză sunt prezentate detaliat metodele utilizate în acest scop.

Cuvinte cheie: Cuaternar, depozite loessoide, minerale argiloase, difracţie de raze X.

INTRODUCTION

On the right side of the road from Cetate to Hinova, at about 8 km northwest of Cetate (Fig.1), there are several 400-500 m long open pits for the exploitation of sands and gravels. Vertically, the walls of the deepest open pit we took samples from are between 15 and 20 meters. The coordinates of this open pit are 44°9'10"N and 22°59 7"E.

At the top, there can be observed the soil horizons, developed on the first 1.5-2 m. Underneath, there appear dusty-sandy reddish deposits based on which soils developed. Their thickness varies between 1 and 2.5 m, sometimes even 3 m. The cause that induced these thickness variations is the erosion surface, which pushed down the soil horizons to the detriment of the silty deposit. The next level consists mainly of very fine and coarse gravels, the last ones having the lowest rate. The matrix of these gravels consists of sands and silts with a very low percentage of clay. Below the gravels with red matrix, on a thickness of 1.5-2 m, there appear thin alternations of sand and coarse gravel, with discontinuous red bands. From this site, there were taken samples from the loess like deposit located immediately below the soil profile as well as from the red gravel matrix. From the neighbouring open pits, there were collected samples only from the loess like deposit and the gravel matrix. Overall, there were analysed 8 samples. On the hydrogeological map 1:100 000, sheet 40d Cujmir (VLAD & ROBU, 1981), this type of deposit is considered to have been formed in the upper part the middle Pleistocene.

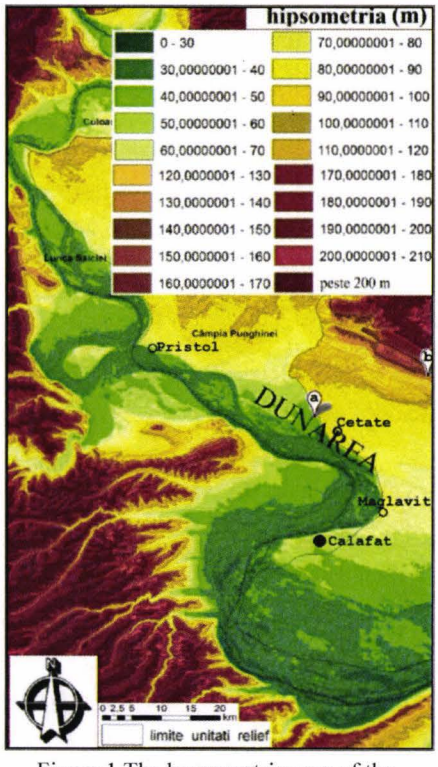

Figure 1.The hypsometric map of the area surrounding the sampling site; a-sampling site. (Original).

MATERIALS AND METHODS

Before starting the granulometric separation by sieving, the samples of gravel, sand and red clay were left in a thin layer to get dry. After drying, the agglomerations, reaching sometimes several centimetres in thickness, of sand, clay and fine and very fine gravel, could be easily disaggregated in particles under 2 mm, due to the partial loss of water absorbed by the clasts, especially by the clay. The next step was sieving using a sieve 2 mm mesh. After this stage, the pelitic fraction was obtained by centrifugation.

Through a series of chemical and physical processes, it is aimed at removingthe minerals, which do not belong to phyllosilicates, as much as possible from the lutitic fraction. This removal allows a clear formation, at maximum intensities, of the characteristic peaks of different phyllosilicates. In the detritic deposits containing clay or lutitic fraction, among the minerals that should be removed, we mention quartz, carbonates, feldspars, iron oxides and hydroxides.

The flocculation of clay minerals that can cause the formation of agglomerates larger than1mm in the natural environment (HILL, 1998) leads to the increase of the background of the diffractograms and produces the reflexes of diffuse diffraction. The phenomenon of aggregation of the fine particles in suspension is strongly influenced by the existence of organic matter.

The removal of the organic matterwas carried out using the method proposed by KUNZE & DIXON (1986), which requires the treatment with 10% hydrogen peroxide by successive additions up to its total removal, followed by 2-3 washings with distilled water.

The removal of the carbonates was made using diluted weak acids, attentively surveying the reaction, in order to avoid, the exposure of the phyllosilicates to an acid environment, together with the removal of carbonates, as this may affect their structure (OSTROM, 1961). In order to remove the carbonates from the analysed samples, we used the method presented by RABENHORST & WILDING (1984).

The extraction of the fraction < 2µ. After eliminating the causes that would prevent the dispersal of the fraction < 2µm, it follows the deflocculation process. This is done by adding a dispersant, a very commonly used one being sodium hexametaphosphate known under the commercial name of Calgon (JIPA, 1987).

For centrifugation, samples have to fill between a quarter and half of the glass tube; then, distilled water is added, leaving a free space at its top to avoid spilling during centrifugation. The sample is centrifuged for 20 seconds and, then, the solution is transferred into clean tubes. The new tubes are centrifuged for 10 minutes. After centrifugation, a part of the solution is removed, so that to keep about 1 cm sample (solid + liquid) in the tube, and then the remaining sample is homogenized. Of the < 2 µm fraction, 5 ml were used for the saturation with MgCl2 and 5 ml for the saturation with KCl.

Cation saturation. There were used potassium chloride (KCl) and magnesium chloride (MgCl2), the samples being covered by the solution of a chloride and then centrifuged 3 times. After the treatment, washing with distilled water was performed 3 times to remove all the salts that cause the flocculation of the clay minerals.

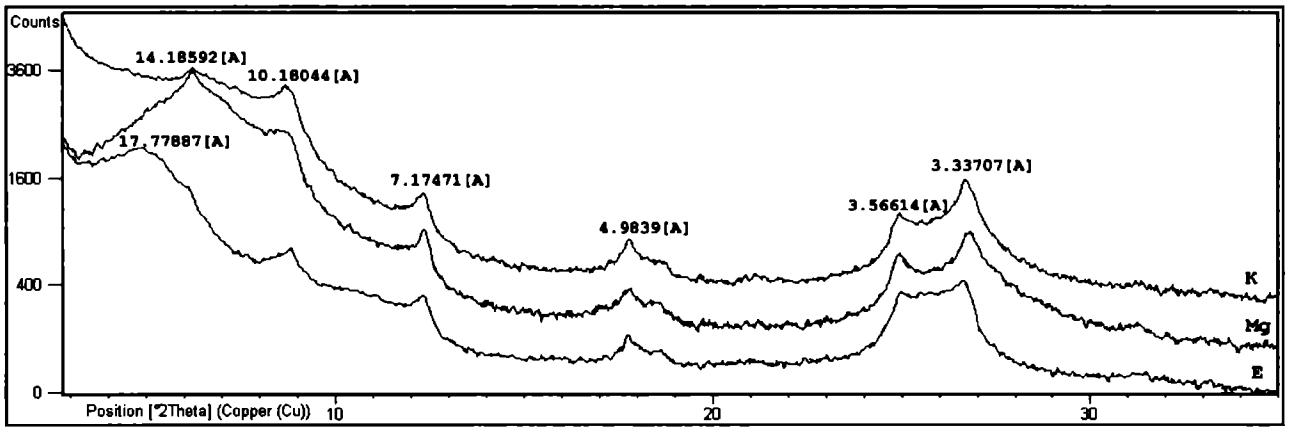

Figure 2. Diffractograms of the samples saturated with K^{+}(K), Mg^{2+}(Mg) and glycol ethylene (E) from the gravel matrix.

For **ethylene glycol treatment,** it was used a desiccator. There are added 100-200 ml of ethylene glycol in the desiccator and the sample slides are placed on a ceramic plate. They are left in an atmosphere with ethylene glycol vapours overnight or the desiccator is put in the oven at 60°C for at least 8 hours.

The heat treatment. The slides with samples were placed in an oven and heated at 330°C and 500°C for one hour. Immediately after removing them from the oven, they were analysed to prevent the rehydration of the sample.

A set of the samples saturated with Mg^{2+} was left to get dry at 20°C, while the other set was treated with ethylene glycol. Of the samples saturated with K^{+} there were made 3 sets of slides, 2 of them for the thermal treatment. Diffractograms were obtained from a range of 2θ, 2-40° using a Philips XPert MPD diffractometer, with Cu (40 mA, 40 kV) anticathode, Ni filter, with Kα radiation with a wavelength of 1.5406 Å, scanning step 0.01, step time 1s/step, scan range 2 ÷ 80° 2θ. Diffraction data were analysed and processed using X'PertQuantify and X'Pert High Scoreprograms. To obtain information about the amount of clay minerals identified based on X-ray diffractograms, it was used the calculation method of MOORE & REYNOLDS (1997).

RESULTS AND DISSCUSIONS

On the diffractograms of the samples saturated with K^{+}, rendered in Fig. 2, we can observe the presence of certain peaks corresponding to the interplanar distances with values of 14.18Å, 10.18Å, 7.17Å, 4.98Å, 3.56Å and 3.33Å.

The diffractogram of the sample saturated with Mg^{2+} presents the same intensity on most of the aforementioned peaks. The exception is represented by the 14.18 Å peak, where we can observe an increased intensity of the response compared to the sample saturated with K^{+}.

By applying the treatment with the ethylene glycol, there is noticed the increase of the interplanar distance from 14.18 Å to 17.77 Å, without any significant changes in the other peaks.

After heating the sample to 330°C, there is only a reduction of the intensity corresponding to the distance from 14.18 Å (Fig. 3). After heating the sampleto 500°C, this reflex disappears and the same thing happens with the 7.17 Å and 3.56 Å.

Based on the information, the clay minerals identified in both the gravel matrix and in the loess like deposits are kaolinite, smectite and illite.

Illite was identified according to the characteristic reflexes for the interplanar distances of 10.1 Å, 4.98 - 5.01 Å, 3.33 Å and 2.89-2.92 Å. Other characteristics of illite are lack of expansion and retention of the reflex plane (001) after the treatment with ethylene glycol and heating to 500°C .

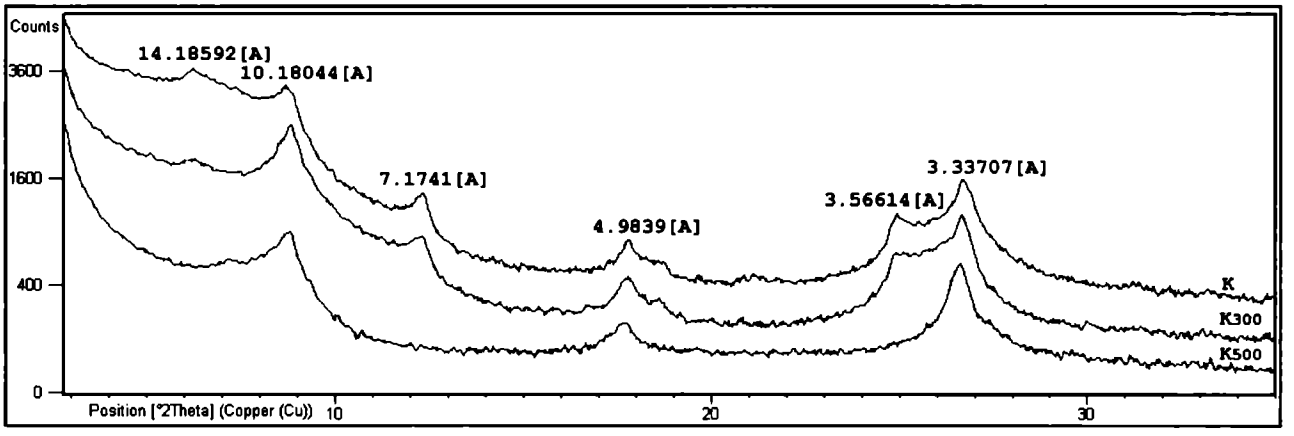

Figure 3. Diffractograms of the clayish fraction from the gravel matrix, saturated with K^{+}, (K), heated at 330°C (K300) and 500°C (K500).

The reflexes according to which kaolinite can be identified are those corresponding to the basal distance d (001) ranging from 7.15 to 7.20 Å and, when they are well crystallized, based on the doublets from 4.17-4.12 Å and 3.144-3097 Å (MATEI, 1986). Other features of the kaolinite are the lack of swelling, as well as in case of illite, when it is treated with ethylene glycol, structural collapse by heating to 500°C, the consequence of which is the total disappearance of kaolinite reflexes.

The term of smectite used in this paper refers to a clay phase with a structure of the type 2:1, dioctaedric and expandable. This was identified by taking into account that the basal distance of smectite is 14-15 Å. Another feature used for the identification was swelling induced by the treatment with organic molecules, resulting in the corresponding reflexes d (001) with values of 17-17.7 Å.

The value of 14 Å can also indicate the presence of chlorite or vermiculite minerals, but in their case, the swelling is not observed after the treatment with ethylene glycol. In addition, chlorite keeps the same basal distance after the 300 and 500°C heat treatments.

In case of typical smectites, after heating at 500°C, the basal distance decreases to 9.4-10 Å (the same as in case of illite), depending on the nature of the interfoliar cations.

In Fig. 3, it can be seen how the reflex from 14 Å of the saturated sample decreases in intensity after heating to 330°C, while at 500°C, it does not reach the value of 10 Å, but remains at the value of 12.29 Å.

The presence of a broad peak in the range 14 to 15 Å, which collapsed partly as a result of the heat treatment, it can be interpreted according to BARNHISEL & BERTSCH (1989) as coming from a hydroxy-interlayered smectite. Such interstratification, according to the same authors, is a solid solution with smectite and chlorite as extreme terms. Their formation is primarily due to the penetration of the expandable clay minerals, of the polymers of aluminium hydroxides in the interlamellar space (OLSON et al., 2000).

The percentages of the clay minerals in the pelitic fraction separated from the red loess like deposits from Cetateare 4% kaolinite, 16% smectite, 80% illite. In the matrix of the red gravels, there are present the same minerals with as lightly different quantitative share: 5% kaolinite, 22% smectite, 73% illite. The other samples show percentages close to the afore mentioned values, the average being 76% illite, 19% smectite, 5% kaolinite.

CONCLUSIONS

The clay minerals identified in the study area are illite, smectite and kaolinite. According to the behaviour observed after the treatment with ethylene glycol and heat, smectite seems to be partly chloritized.

The participation of the three minerals in the clay sized fraction is approximately uniform, illite having a share of over 70%, smectite of 15-25% and kaolinite of 4 or 5%.

At Cetate, it is observed the decrease in the smectite content of the fraction < 2μm in higher, loess like deposits compared with the lower ruditic deposits. The illite content varies inversely as compared with that of smectite, increasing in the upper horizons. This quantitative difference is not very large (a few percents), but it was found in all the analysed cases. The abundance of smectite in the ruditic layers can be explained by the petrographic diversity of the clasts, which can provide the cations necessary to the formation of the network of these minerals, or by the size of the smectite crystals, which are smaller than those of illite, and, thus can be easily moved to lower levels.

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THE USE OF IR SPECTROSCOPY FOR THE STUDY OF RED QUATERNARY DEPOSITS FROM VALEA LUNGULUI AREA (DOLJ COUNTY, ROMANIA)

NEGREANU Ştefan

Abstract. The investigated samples were taken from the left bank of the Raznic river, about 4 km upstream of its confluence with the Jiu river, from the red loess deposits and from the ruditic matrix of certain deposits of the same colour. Comparatively with the X ray diffraction, IR spectroscopy presents lower possibilities of identification of the minerals in the pelitic fraction, but, it can be successfully used for coarser fraction and easier identification of the quartz or carbonates. Regarding the clay minerals, this method enabled the identification of kaolinite, while the presence of the radiation absorption characterized by frequencies corresponding to Al-Al-OH bonds suggests the presence of dioctaedric minerals such as illite and/or smectite. For a precise understanding of the conditions of analysis, there are presented the methods used for this purpose.

Keywords: clay minerals, loesslike deposits, IR spectroscopy.

Rezumat. Folosirea spectroscopiei IR pentru analiza depozitelor roşii cuaternare, zona Valea Lungului (judeţul Dolj, România). Probele investigate au fost recoltate de pe malul stâng al râului Raznic, la aproximativ 4 km amonte de confluenţa acestuia cu Jiul, din depozite loessoide de culoare roşcată şi din matricea unor depozite ruditice de aceeaşi culoare. Comparativ cu difracţia de raze X spectroscopia IR are posibilităţi mai reduse de identificare a mineralelor din fracţia pelitică, putând fi utilizată cu succes în cazul fracţiei mai grosiere unde se identifică cu uşurinţă cuarţul sau carbonaţii. În ceea ce priveşte mineralele argiloase, prin utilizarea acestei metode a fost identificat caolinitul, iar prezenţa absorbţiei radiaţiei cu frecvenţe corespunzătoare legăturilor Al-Al-OH sugerează prezenţa unor minerale dioctaedrice de tipul illitului şi/sau smectitului. Pentru o înţelegere exactă a condiţiilor de analiză sunt prezentate metodele utilizate în acest scop.

Cuvinte cheie: minerale argiloase, depozite loessoide, spectroscopie IR.

INTRODUCTION

10 km W-NW of Craiova, on the left side of the road linking Craiova to Valea Lungului settlement, there are two outcrops with thicknesses of up to 11m. The coordinates of the sampling site are 44°20'58" N and 23°40'15" E (Fig. 1). At the bottom of these outcrops, on a thickness of 2-2.5 m, there can be found yellow-reddish gravels mixed with sands, while towards the top, this layer gets gray. Gravels are generally very fine to medium in the area, the clasts of coarse gravel or small cobbles representing 20%. There can be also found small lenses or layers of gray deposits that appear to be silts or clayish silts. Gravels and sands are heavily pigmented by black compounds present in the outcrop on a thickness of 50 – 75 cm and a length of 20 m, which are disposed in oblique bands. There follows a level composed of coarse and medium gravels dispersed in a mainly reddish-yellowish, medium - coarse sand mass. Above this level, on a thickness of 1.5 m, there appears a loess-like deposit, which has approximately the same colour as the previous one, located just below the soil profile.

The samples were taken from the two outcrops, namely the basis of the last level and the matrix of the reddish yellowish gravel that forms the lowest observed level. There were analysed four samples.

On the geological map 1:200 000, sheet 41 Craiova (MIHĂILĂ et al.), these deposits are indicated as being of lower Pleistocene age.

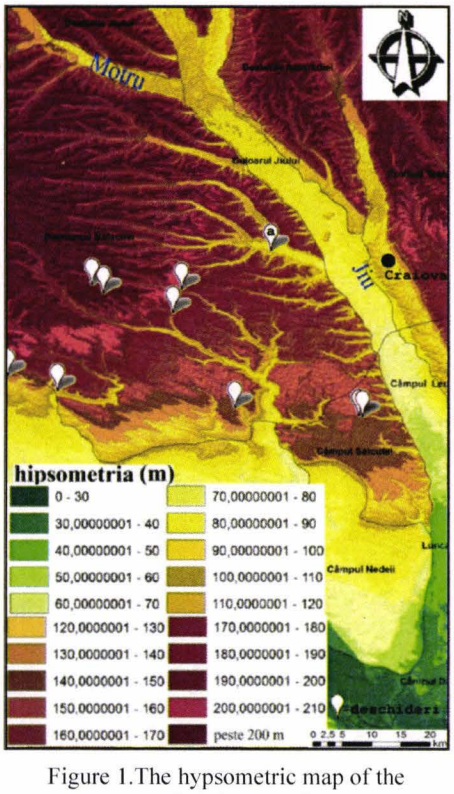

Figure 1.The hypsometric map of the area surrounding the sampling site; a-sampling site. (Original).

MATERIALS AND METHODS

The samples of gravels, sands and red clays were left in a thin layer to get dry before starting the granulometric separation by sieving. After this stage, the pelitic fraction was obtained by applying the pipette method. The sample dispersal for taking samples based on the pipette method was made according to the recommendations made by Krumbein in 1933 and presented by JIPA (1988); thus, samples are heated in distilled water up to the boiling point, without allowing the solution to boil. The heated solution was obtained by mixing about 500-750 grams of fraction smaller than2 mm,

obtained by sieving, with about 8-10 litters of distilled water. After heating, from the sedimentation cylinders, it was collected the solution by pipetting, using the data rendered by JIPA (1988) and JACKSON & BARAK (2005).

The layer of about 2-3 cm of suspension obtained in this way was left in an oven at a temperature of 50°C, in covered glasses, until water evaporated. The remaining material was milled in an agate mortar and then analysed as powder.

The samples were analysed in the form of disks obtained by pressing a mixture of KBr and clay fraction. The mixture used to obtain the disks contained 2 mg sample and 200 mg KBr (MADEJOVA, 2001). For a clearer observation, at a second analysis of the absorption spectra in the range of 3000-3800 cm^{-1}, the samples would have to be dried at 150°C over night. As this stage in sample preparation missed, there appeared the spectrum of adsorbed water in the aforementioned region of the spectrum, covering part of the absorption lines useful in the identification of clay minerals. The infrared spectra were obtained with BrukerOptics FTIR spectrophotometer, which, for the range 374-4000cm^{-1}, obtained 2,531 frequency-intensity of absorption couples.

RESULTS AND DISSCUSIONS

The IR spectra performed on the samples taken from the two outcrops indicated the presence of the same absorption bands, minor differences being identified only in terms of their intensity.

The frequencies corresponding to the Si-O-Si bond, 430cm^{-1}, with high and medium absorption intensity, were identified in all the analysed samples. This frequency is absorbed by both montmorillonite and illite, but the intensity is significant only in case of kaolinite. Consequently, this band was attributed to the presence of kaolinite.

The absorption around 470 cm^{-1}, also generated by Si-O-Si bonds, can not provide clear information on the mineral responsible for its appearance, because both kaolinite and montmorillonite show strong absorption rates in this area. However, the values, which always maintained above 470 cm^{-1}, may also indicate kaolinite, as montmorillonite displays slightly lower values.

The vibrations corresponding to the frequencies of 535 cm^{-1}originate from the bonds of the type Al-O-Si (STUBICAN & ROY, 1961) from the network of several clay minerals and, in this case, they cannot provide clear information on a specific mineral.

At 696 cm^{-1}, it is indicated the presence of the bond Si-O-Si, a bond which is also non-specific.

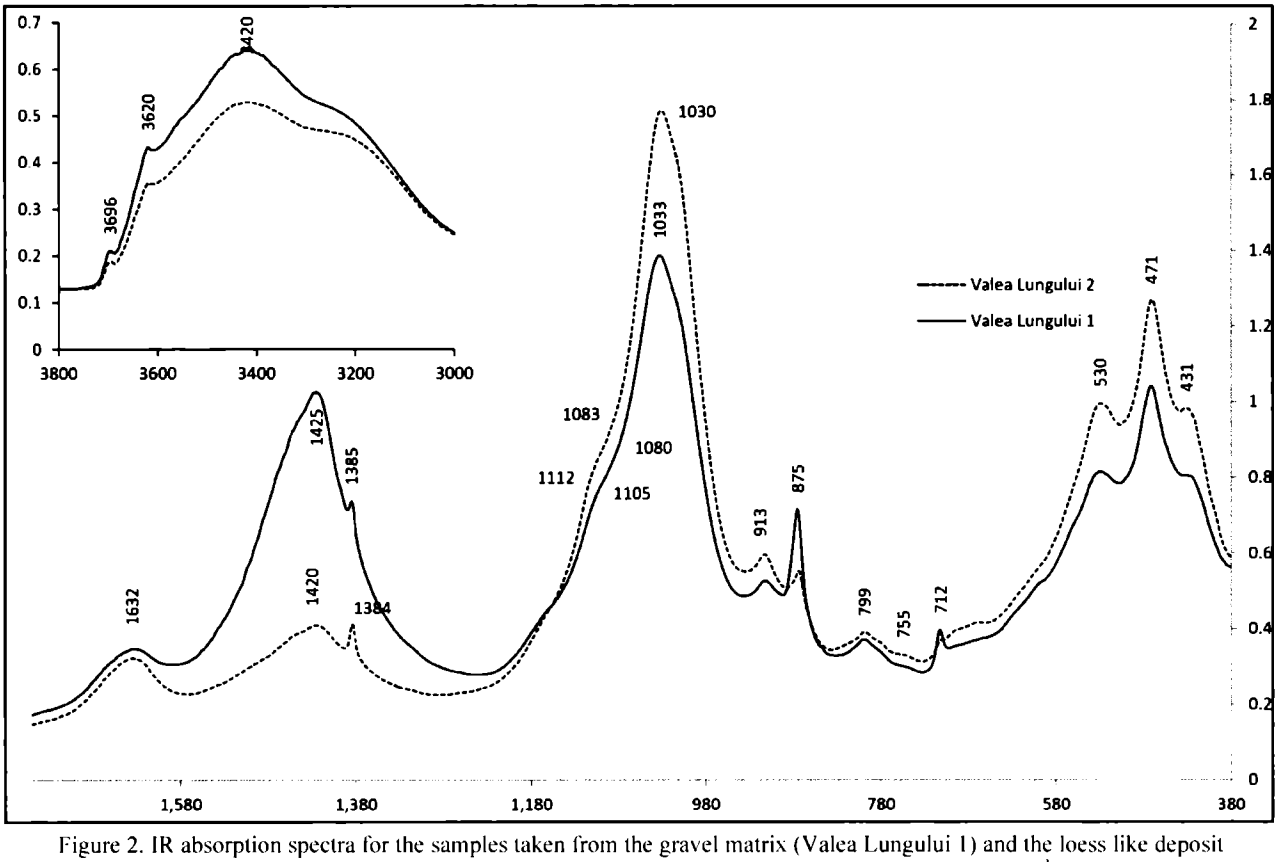

Figure 2. IR absorption spectra for the samples taken from the gravel matrix (Valea Lungului 1) and the loess like deposit (Valea Lungului2): on the horizontal axis, there are rendered the wavenumbers in cm^{-1}.

In Fig. 2, it is rendered the absorption spectrum for the sample taken from the matrix of lower yellow reddish gravel (Valea Lungului 1); the absorption band with an intensity reduced from 712 cm^{-1} is characteristic for the C = O bond from carbonates.

The absorption doublet at 779 cm^{-1} and 798 cm^{-1} is diagnostic for quartz. In the samples taken from Valea Lungului, the absorption intensity is weak and there appear only wide inflections. This indicates the extremely reduced presence of quartz.

The weak and very weak absorption at 755 cm^{-1} was present in all the samples, but it cannot be used to identify the minerals that generated it.

The vibrations with the frequency of 875 cm^{-1} are deformation vibrations, out of the plane, of $(CO_3)^{2-}$ (MADEJOVA & KOMADEL, 2001). For the absorption in the range 1382-1440 cm^{-1}, the specialize literature also indicates carbon-oxygen bonds: 1430 cm^{-1} stretching vibration $(CO3)^{2-}$ of calcite, MADEJOVA & KOMADEL (2001); 1410-1490 cm^{-1} frequencies that indicate $(CO3)^{2-}$COATES (2000); 6.90 to 6.97 μm (1449-1435cm^{-1}), bands considered to belong to the group $(CO3)^{2-}$, HUANG & KERR (1960); 1384 cm^{-1} stretching vibration of the bond C = O of the inorganic carbonyl group, JACKSON (1998); 1365 cm^{-1}, the stretching vibration in the bicarbonate ion COO^{-}, YRUELA et al. (1998).

The frequencies of 875 cm^{-1} and the 1382-1440 cm^{-1} range suggest the presence of carbonates, most probably of calcite. The frequencies of 913-915 $cm^{-1}$are absorbed byAl-Al-OH vibrations characteristic to the networks of montmorillonite, kaolinite and illite, showing strong absorption (40-80%) in kaolinite and average absorption (20-40%) in montmorillonite and illite, MATEI (1988). In all the analysed samples, the intensity was average, which would suggest the presence of montmorillonite and illite. The absence of a strong intensity at this frequency does not exclude the presence of kaolinite, which was indicated by the absorptions in the range of 430-538 cm^{-1}, but it is present in smaller amounts compared to illite and/or montmorillonite.

The inflection around the value of 1010 cm^{-1}, which follows, occurs in case of illite and montmorillonite. The maximum intensity of absorption registered at 1030-1034 cm^{-1} was attributed to illite, based on the conclusions drawn by HUNT et al. (1950), who distinguishes it from montmorillonite, indicated by the vibration absorption of 1041.66 cm^{-1} according to the same author.

The range present in all samples in the interval 1080-1106 cm^{-1} may indicate montmorillonite and/or illite, MATEI (1988). At values above 3000 cm^{-1}, only two frequencies can be used, one at 3620 cm^{-1}, absorbed by all the three aforementioned clay minerals and 3696-3699 cm^{-1}, which is a diagnostic band for the mineral kaolinite (FARMER, 1964). The 1640 cm^{-1} and 3620 cm^{-1} bands are assigned to the deformation vibrations, respectively the stretching vibrations of OH groups, belonging to the molecules of water (FARMER,1974).

CONCLUSIONS

Mineralogically, based on IR spectra, we can say that the analysed samples contain very small amounts of quartz and carbonates, probably calcite. The significant absorption in the range 1382-1440 cm^{-1} in the analysed samples from Valea Lungului is due to the frequent presence of rolled calcareous concretions,very poorly cohesive, in these deposits. These concretions led to the increase of the carbonates present in the concretions from which the aforementioned samples were separated. Kaolinite is present, without exception, in all the analysed samples, but it does not display the maximum intensity of the characteristic doublet 1012 cm^{-1} and 1032 cm^{-1}, which indicates the fact that the kaolinite amount is lower than the amount of the other two clay minerals.

Illite seems to be the dominant mineral, while montmorillonite, which shows similar bands of absorption, was not clearly indicated.

The presence of the bands characteristic to the vibration of the bond Al-Al-OH shows that the identified clay minerals are thus dioctaedric, such positions being occupied mostly by Al^{3+}.

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FUŞTEICA CAVE: A GEOLOGICAL APPROACH

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Abstract. Among the caves in the northwestern part of Oltenia, Fuşteica Cave is peculiar by the linear aspect of the ENE-WSW trended gallery and by the presence of a large volume of gravels with sandy-argillaceous matrix draping its entire length. Structural data on the fault plane along the gallery and the associated fissures in the host carbonate massive, related with the cave morphology and the underground waters drainage, support the tectonic control of the karstification processes. Petrographic data on pebbles from gravels and XRD data on argillaceous fraction from matrix show that the source area of the sedimentary fill is the Tismana granite.

Keywords: Fuşteica Cave, tectonic control, source area.

Rezumat. Peştera Fuşteica: o abordare geologică. Între peşterile din nord-vestul Olteniei, Peştera Fuşteica se individualizează prin aspectul liniar al galeriei, cu direcţie ENE-WSW, şi prin prezenţa unui volum mare de pietrişuri cu matrice nisipos-argiloasă pe toată lungimea sa. Date structurale privind planul de falie din lungul galeriei şi fisurile asociate din masivul carbonatic gazdă, corelate cu morfologia peşterii şi drenajul apelor subterane, susţin controlul tectonic al proceselor de carstificare. Date petrografice privind galeţii din pietrişuri şi date de difracţie RX obţinute pe fracţia argiloasă din matrice arată că aria sursă a umpluturii sedimentare este granitul de Tismana.

Cuvinte cheie: peştera Fuşteica, control tectonic, arie sursă.

INTRODUCTION

Fuşteica Cave is located in the carbonate massive in the southwestern extremity of the Vâlcan Mountains, Gorj County, Isvarna village. It is an easily accessible cave, with a width of 3 m and 5 m high entrance, located at 216 m altitude. The gallery has 1270 m in length, with +10 m difference in level. Characteristic for this cave is the linear development on a single gallery with ENE-VSV general direction (Fig. 1b), the height of which varies from 2.5 m to 7 m. Over its entire length, there are thick deposits of red terrigenous sediments, well preserved near the walls. The present paper tries to demonstrate the important role of tectonics in the cave formation and to contour the source area of its sedimentary fill.

GEOLOGIC AND TECTONIC SETTING

Fuşteica Cave is developed in Barremian-Aptian limestones representing the upper term of the carbonate Mesozoic cover lying on the metamorphic-magmatic basement of the Vâlcan Mountains. The basement rocks mainly consist in amphibolite gneisses of the Drăgşan Series, mica gneisses and micashists of the Lainici-Păiuş Series, and granitic rocks of the Tismana batholith. The sedimentary cover in Fuşteica Cave region is directly lying on granites.

The Tismana batholith consists in porphyritic granites with K-feldspar (microcline) phenocrysts, diorites, granodiorites and aplitic granites (BERZA, 1978). The granitic rocks in Fuşteica Cave area are covered by Lower Jurassic clastic formations represented by conglomerates and quartzo-feldspathic sandstones with interbedded silty clays, Middle Jurassic-Aptian carbonate formations, and Cenomanian-Middle Turonian formations, represented by marls, marly limestones and silty clays (POP et al., 1975). The lower part of the carbonate formations (Middle Jurassic-Neocomian) mainly consists in bedded limestones and dolomites while the upper one (Barremian-Aptian) is bearing massive Urgonian limestones (POP, 1973).

In the Alpine nappe system of the South Carpathians, result of the Middle Cretaceous (Austrian) and Late Cretaceous ("Laramian") tectogeneses, the Vâlcan Mountains belong to the Lower Danubian Units (IANCU et al., 2005). In Tismana - Baia de Aramă region, the Vâlcan Mountains join the bending area of the South Carpathians due to the Moesian microplate translation towards WNW, accommodated by Pre-"Laramian" compressions and Post-"Laramian" lateral faults (AIRINEI, 1983). Such lateral faults are E-W to ENE-WSW trended in the northern part of the Mehedinţi Plateau, and ENE-WSW to NE-SW trended in the southwestern part of the Vâlcan Mountains, some of these being seismically active (ATANASIU, 1961). For our discussion, the most important faults in this region are the Izverna fault, the Balta-Baia de Aramă tectonic trench, and the Motru fault (Fig. 1a).

The movement on the Izverna fault is a dextral strike-slip, with E-W direction on Izverna-Ponoarele sector and NE-SW direction on Ponoarele-Apa Neagră sector, where the fault joins Balta-Baia de Aramă tectonic trench, with the same direction and sense of movement (IANCU et al., 1986). The ENE-WSW to NE-SW trended faults on the southwestern border of the Vâlcan Mountains must represent the eastward prolongation of both Izverna and Balta-Baia de Aramă faults.

The two faults are intersected by the Motru fault on the Motru Valley, near Apa Neagră village, where the carbonate rocks show an extreme cataclastic deformation, visible in the quarry on the right slope of the valley. The movement on the Motru fault is a normal dip-slip, with the eastern block of the Vâlcan Mountains up-lifted towards the western block of the Mehedinţi Mountains and Plateau. A lot of secondary parallel faults occur in the up-lifted tectonic block affecting both the Tismana batholith and its sedimentary cover.

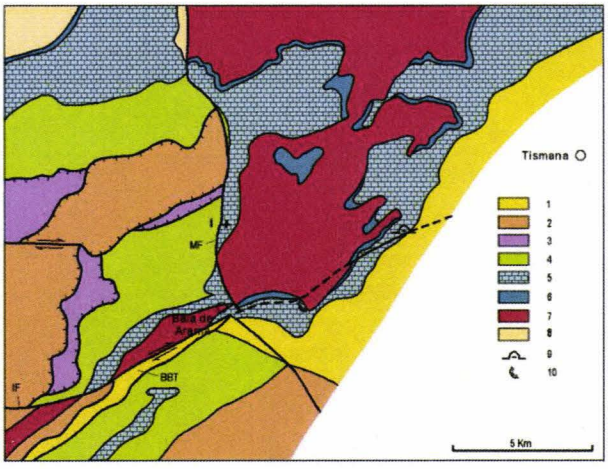

Figure 1a. Simplified geological map of Tismana-Baia de Aramă region. After NĂSTĂSEANU et al. (1968) and POP et al. (1975). 1- Tertiary sedimentary deposits; 2 - Getic Nappe; 3 - Severin Nappe. Danubian Mesozoic cover: 4 - Cenomanian-Middle Turonian non-carbonate cover; 5 - Middle Jurassic-Aptian carbonate cover; 6 - Lower Jurassic sandstones and conglomerates. Danubian basement: 7 - Tismana granite; 8 - Lainici-Păiuş Series. 9 - Fuşteica Cave; 10 - Apa Neagră Quarry. Abbreviation: BBT - Balta - Baia de Aramă tectonic trench; IF - Izverna fault; MF - Motru fault.

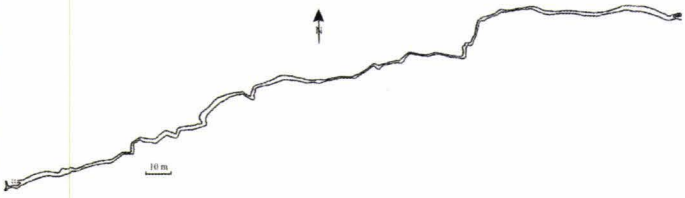

Figure 1b. Fuşteica Cave. After BURGHELE-BĂLĂCESCU & AVRAM (1966).

TECTONIC CONTROL OF THE KARSTIFICATION

The ENE-WSW general direction of Fuşteica Cave is coincident with the direction of a fault plan measured at the gallery entrance. The linear geometry of the gallery and its angular transverse cross section, of a tight and tall corridor with ogival ceiling (Fig. 2a), suggest the tectonic control of the karstification processes leading to the cave formation. The few issued data on this topic suggest the cave was formed along stratification and related fissures (BURGHELE-BĂLĂCESCU & AVRAM, 1966).

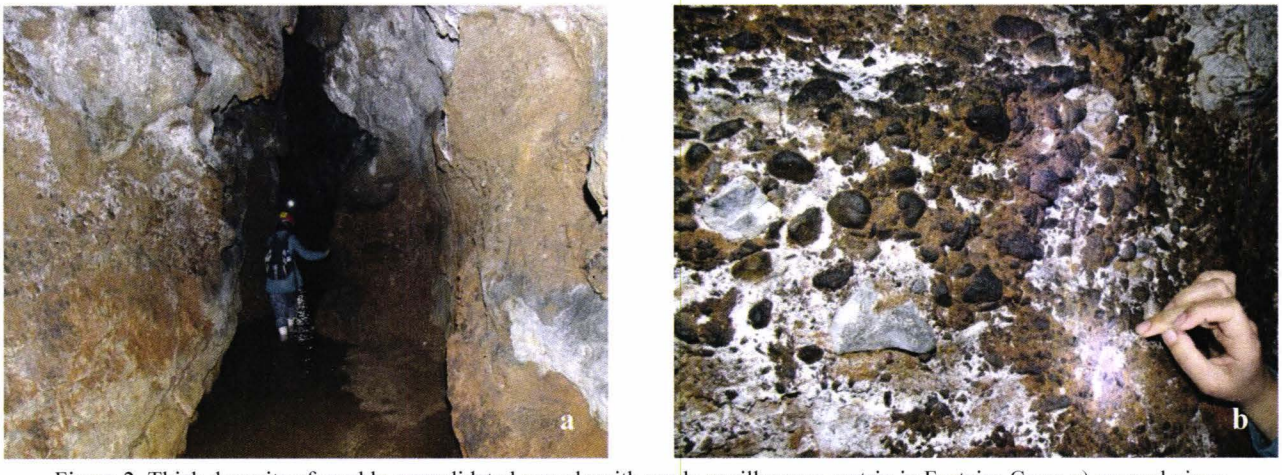

Figure 2. Thick deposits of weakly consolidated gravels with sandy-argillaceous matrix in Fuşteica Cave: a) general view; b) detail with calcareous (angular, gray) and silicates (rounded, dark) pebbles (photo: C. Munteanu).

Without denying the role of bedding in the water infiltrations inside the carbonate rocks, I consider that the faults are more important structural discontinuities for karstification. A strong reason for the tectonic control of karstification is the NE-SW underground drainage of karstic waters on the southwestern border of the Vâlcan Mountains, across the natural stream of surface waters (RĂDULESCU et al., 1987) but coincident with the main tectonic lines in this area.

Preliminary structural measurements in the carbonate massive hosting Fuşteica Cave show that the directions of the planar discontinuity correspond to the directions of the gallery sectors diverting from the general direction ENE-WSW. The structural discontinuities at the entrance of the gallery and Apa Neagră quarry, represent:

- a fault plane along the cave, with NE/ENE-SW/WSW direction (340°/80°);
- low-angle shear fissures, with NE/ENE-SW/WSW direction (170°/60°);

- tension fissures, with NW-SE direction (50°-60°/40°-55°);
- bedding surfaces, with NNE-SSW direction and ESE dip (110°/20°-40°);
- fissures parallel to the Motru fault, with N-S direction (270°/70°).

The low-angle shear fissures and the tension fissures are genetically related to the fault along the cave. Their orientation towards the fault plane shows a dextral strike-slip movement in transpressive tectonic regime (e.g. SANDERSON & MARCHINI, 1984). Quite informative, these data point out the role of tectonic discontinuities in the cave formation, more important than the role of stratification.

SOURCE AREA OF SEDIMENTARY FILL

The sedimentary fill of Fuşteica Cave consists in thick deposits of gravels well preserved near the walls (Fig. 1a), with quartzo-feldspathic sandstones, microconglomerates, limestones and granites pebbles into a red sandy-argillaceous matrix (Fig. 2b). The sandstones and the microconglomerates contain many clasts of quartz and microcline and lithoclasts of granites. The microscope examination of the granitic pebbles shows the biotite chloritisation and the advanced sericitization of plagioclase, contrasting with the fresh microcline (Fig. 3). The selective alteration of feldspars is a characteristic process for the Tismana granite. It is clear the granitic pebbles were provided by the Tismana batholith, directly or reworked from detrital sedimentary formations lying near the cave, Early Jurassic ("Lias") in age or younger (Miocene and Pleistocene).

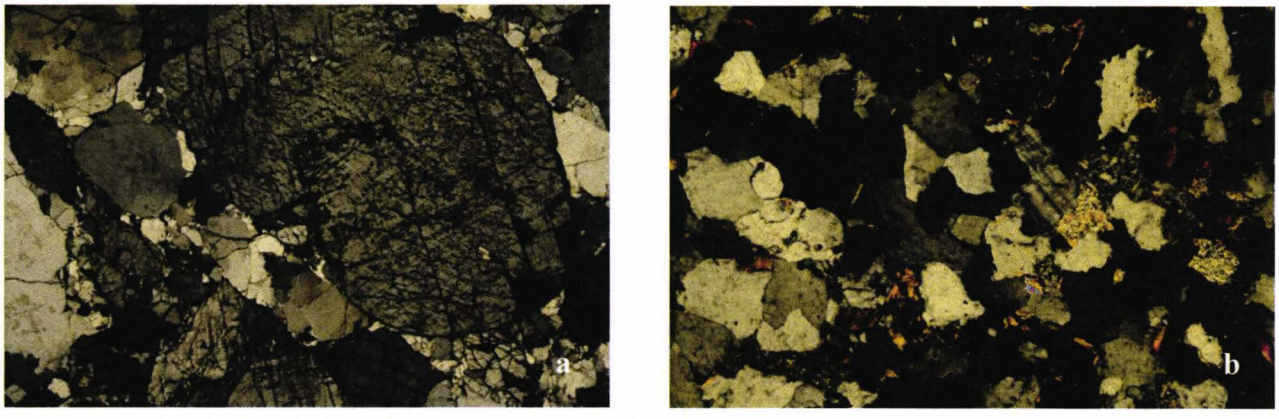

Figure 3. Microphotographs (N+, 30x) in pebbles from gravels: a) microconglomerates with rounded clasts of K-feldspar; b) aplitic granite (original).

The XRD analysis of the matrix pointed out the presence of K-feldspar, quartz and clay minerals. In the argillaceous fraction separated from matrix there were identified illite (67.5%), kaolinite (18%), vermiculite (8.3%), smectite (5.5%) and chlorite (0.7%). Quantitative information exclusively refers to proportions among clay minerals.

The high illite proportion in the argillaceous fraction is the result of the advanced sericitization of plagioclase in the Tismana granite (Fig. 4a). The fact that the K-feldspar is fresh (Fig. 4b), especially the microcline, explains why this is the most non-argillaceous mineral in the composition of analysed samples, beside quartz.

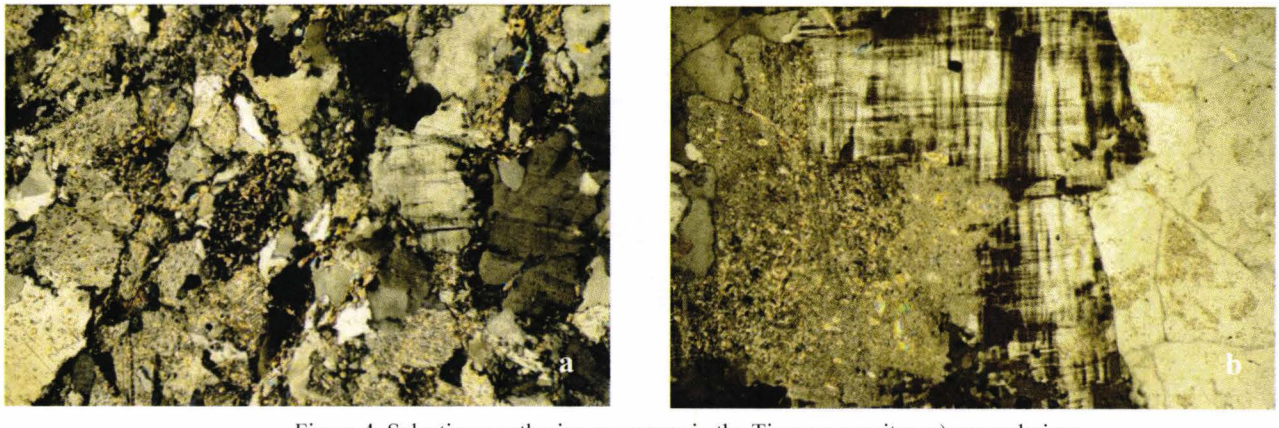

Figure 4. Selective weathering processes in the Tismana granites: a) general view; b) detail with sericitised plagioclase and fresh microcline. Microphotographs (N +, 30x) (original).

CONCLUSIONS

The karstification processes that led to Fuşteica Cave formation were tectonically controlled by a fault plane trended ENE-WSW on Apa Neagră sector, representing the Izverna fault prolongation eastward from the Motru Valley. The fault plane is accompanied by fissures of tectonic origin along which different oriented sectors of gallery formed. A small part of these short sectors formed on stratification. Other arguments for tectonic control are the galley morphology and the ENE-WSW direction of the underground waters drainage on the southern slope of the Vâlcan Mountains, coincident with the gallery direction.

In an advanced stage of its development, Fuşteica Cave was completely or almost filled with sediments. This event probably took place during the Pleistocene, when the host carbonate massive was covered by fluviatile gravels, now preserved as remnants of the Gornoviţa surface erosion (POP et al., 1975), at 400 m altitude, more than 100 m higher than their outcropping level in the Motru Valley. For the transportation and the deposition of such a large volume of sediments to the entire length of the gallery, an important stream water was required, also involving a western entrance, now buried by the Pleistocene gravels in the Motru Valley.

The petrographic and mineralogical study of the sediments from Fuşteica Cave shows that the main source area is the Tismana batholith. The cataclastic deformation related to fractures favoured the weathering and erosion processes in order to supply the detrital material.

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NEW CONTRIBUTIONS TO THE CIOCADIA MIDDLE MIOCENE FLORA (PART THREE)

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Abstract. Deciduous broad-leaved woody plants such as *Quercus drymeja* Unger, *Quercus gigas* Goeppert emend. Walther & Zastawniak, '*Castanea*' *kubinyii* Kováts ex Ettingshausen sensu Knobloch & Z. Kvaček, and *Fagus silesiaca* Walther & Zastawniak (Fagaceae) are established for leaves imprints from the middle Miocene Ciocadia Valley deposits of Southern Carpathian Foredeep, Romania. Well preserved plant fossil assemblages are to be found in rhythmically banded marlstones of marine shallow-water origin. The fossil plant record provides further evidence for the existence of rich warm temperate forests (comparable to the present Mixed Mesophytic Forest biome) with numerous exotic or endemic taxa. Carefully descriptions and documented revisions are made for the Fagaceae family representatives in the Ciocadia Valley paleoflora and various taphonomic, palaeoecological and palaeobiological aspects are discussed.

Keywords: leaf architecture, insect-plant interactions, damage types (DTs), Fagaceae.

Rezumat. Noi contribuţii la flora miocen medie de la Ciocadia (partea a treia). Plante fosile arborescente, cu frunze late, aparţinând taxonilor *Quercus drymeja* Unger, *Quercus gigas* Goeppert emend. Walther & Zastawniak, „*Castanea*" *kubinyii* Kováts ex Ettingshausen sensu Knobloch & Z. Kvaček, şi *Fagus silesiaca* Walther & Zastawniak (Fagaceae) sunt descrise pe baza amprentelor foliare descoperite în depozitele Miocenului mediu ce aflorează pe Valea Ciocadia, localizată în sudul Avanfosei Carpaţilor Meridionali, România. Aceste asociaţii de plante fosile, excelent conservate, au fost găsite în roci carbonatice laminate, de tipul marnelor cenuşii, acumulate în zona litorală a paleo-oceanului denumit Paratethysul Central. Punerea în evidenţă a acestor resturi de plante fosile oferă dovezi suplimentare referitoare la prezenţa în trecut pe teritoriul României a unor păduri temperate şi calde (comparabile cu aşa-numitul biom actual al pădurilor mixte mezofitice), cu numeroşi taxoni exotici şi/sau endemici. În acest articol au fost efectuate diagnoze precise şi revizuiri documentate doar asupra reprezentanţilor familiei Fagaceae prezenţi în paleoflora Văii Ciocadia şi au fost discutate diverse aspecte legate de tafonomia, paleoecologia şi paleobiologia acestora.

Cuvinte cheie: arhitectură foliară, interacţiuni mutuale plante-insecte, tipuri de distrugeri ale frunzelor, Fagaceae.

INTRODUCTION

This investigation was based on specimens from three genera, namely *Quercus*, '*Castanea*' and *Fagus* which co-occur in the Middle Miocene flora of Ciocadia. Now, new analyses of the gross morphological features of the plant remains show that the material previously described (by PARASCHIV & SEBE 2011) as *Quercus kubinyii* Kováts ex Ettingshausen must be re-assign to '*Castanea*' *kubinyii* Kováts ex Ettingshausen sensu Knobloch & Z. Kvaček. In the matter of *Fagus* remains there is no indication of more than one species of this genus.

MATERIAL AND METHODS

Besides their remarkable preservation as cleavage impression-compressions, the number of plant specimens from Ciocadia site is quite high and strongly suggestive of a very short transport (arguably of order of kilometres), although it can also be imagined that such a concentration has resulted of some eddy (turbionar) process. Leaves are preserved either parallel to the stratification or they lie at some angle to it (reworked hydrodynamically). Fine morphological features are often visible; cuticles are not preserved. For analytical observations and studies of the fossil plant remains, a Carl Zeiss - Jena Technoval Stereo Microscope (Binocular) was used, followed by examination of the literature. The repository place for the studied specimens is the National Museum of Geology of the Geological Institute of Romania, Bucharest. Systematic organization and taxonomic terminology in this article are based on the works of KUBITZKI (1993) and TAKHTAJAN (2009). Leaf remains were analysed morphologically according to the terminology proposed by HICKEY (1979) and WING et al., 1999.

RESULTS IN SYSTEMATIC PALEOBOTANY

Subinfradivision Angiospermae Brown & Doell ex Doell 1857 (=Magnoliophytina Cronquist, Takhtajan & Zimmermann 1966)
Class Dicotyledoneae de Candolle 1819 (=Magnoliopsida Brongniart 1843)
Subclass Hamamelididae Takhtajan 1967
Order Fagales Engler 1892
Family Fagaceae Dumortier 1829
Genus *Quercus* Linnaeus 1753

Quercus drymeja Unger
Text-fig. 1d

1847 *Quercus drymeja* Unger, Chloris protogea, p. 113-114, Pl. 32, Figs. 1-4.
2011 *Quercus drymeja* Unger; Z. Kvaček, Teodoridis & Roiron, p. 30-31, Pl. 6, Figs. 1-6; Pl. 11, Figs. 11-13; Pl. 17, Figs. 4-5.

Material. Complete slender lamina: BCI.0134.

Description. Leaf simple, ovate lanceolate (spear-shaped), ?6.8 cm long and 1.8 cm wide (microphyll 2 to microphyll 3), with incomplete base and no petiole preserved, slightly asymmetrical; base angle acute, apex prolonged, acute to acuminate, leaf margin toothed, simply serrate, with regular or irregular acute, blunt to spiny teeth, distinct, rarely disposed, with zigzag feature, deep sinuses with shape rounded (Urticoid-Hamamelid Tooth Type, Z. KVAČEK et al., 2011); venation simple craspedodromuos, with primary vein distinct, sinuate, strongly arcuated in the base (proximally) but also in the distal part, where the lamina become progressively narrowed; secondary veins thin, pinnate, alternate or opposite (in the apical part), arciform, 10-11 pairs, inserted at angles (from the base to the apex) of 50°-40°- 30° towards the median vein, the secondary veins run out till the simple teeth of the margin; tertiary veins, when are visible, alternate percurrent, venation of the higher orders not obvious.

Remarks and discussions. In the upper third, the lamina it is slightly folded under itself (laminar contortion) due to the arrangement or reworking of leaves during or after emplacement at the sediment-water interface. The leaf described was infected by ovoid dark blister-like areas, < 3 mm maximum diameter, thoroughly carbonized and randomly disposed all over the lamina. This could represent the major foliar diseases of oak trees when they are attacked by fungal spores. Our specimen has several points of similarity to *Q. drymeja* diagnosed by UNGER (1847), namely the narrowly lanceolate lamina, the acuminate apex, the asymmetrical shape, the spiny teeth, the number and type of secondaries or the angle between these and midrib, which makes specific assignment to the discussed morphospecies more precise. *Quercus drymeja* is one of the most common Miocene sclerophyllous oaks of the Central Europe and Mediterranean area (BOZUKOV et al., 2011; KOVAR-EDER et al., 2004).

Thermophilous palaeotropical element which displays xeromorphic features, such as the serratures or teeth terminated by spines, *Q. drymeja* was adapted to warm and dry climate from coastal and low mountainous areas. The relatively dry habitats of the mountain slopes, with low species diversity, were occupied by xeromesophytic elements (or hemixerophytic, cf. NAKHUTSRISHVILI 2013), where *Q. drymeja* may flourish sufficiently distinct to rank as representative species.

The nearest living relative of *Q. drymeja* can be traced (GRANGEON, 1958; Z. KVAČEK et al., 2002) in the group of the extant *Quercus sartorii* Liebmann (from Mexico, native in tropical mesic forests of mountain slope, at 1000-2000 m), or *Quercus serrata* Murray, from deciduous forests situated below 2000 m in China, Japan and Korea. According to UNGER (1847) and PALAMAREV & TSENOV (2004), *Q. drymeja* has also affinities with *Q. lancifolia* Schlechtendal & Chamisso (endemic to the upper elevations, of 700-2400 m, in tropical forests located in the central and southern Mexico (Veracruz, Chiapas), Guatemala, Costa Rica, Panama, Honduras, and Nicaragua), *Q. xalapensis* Humboldt & Bonpland (from Mexico, native in tropical mesic forests at elevation of 1500 m above sea level), or *Q. libani* G. Olivier ('Black forests' of mountain areas from Lebanon and Syria, Asia Minor (eastern Turkey, and northern Iraq and Iran), a semi-shade deciduous or semi-evergreen shrub or tree).

Occurrence of *Q. drymeja* leaves in the fossil floras of Romania: Miocene-**Slătioara**, Vâlcea County (wrongly designated *Quercus kubinyii* (Kováts ex Ettingshausen) Czeczott, PARASCHIV 2006); Pannonian B-C **Valea Neagră de Criș** (Valea Crișului I & II), Bihor County (GIVULESCU 1962); Pannonian E-**Delureni** (Beznea), Bihor County (GIVULESCU 1983); Early Pontian-**Cornițel**, Bihor County (GIVULESCU 1957); Early Pontian-**Gheghie**, Bihor County (GIVULESCU 1960); Late Pontian-Late Dacian (Early Pliocene) **Cărbunești**, Prahova County (denominated as *Quercus* aff. *Q. drymeja* Unger, GIVULESCU 2001).

Quercus gigas Goeppert emend. Walther & Zastawniak
Text-fig. 1a-c

1991 *Quercus gigas* Goeppert emend. Walther & Zastawniak, p. 160-169, Text-figs. 3-7; Pl. 4, Figs. 1-3; Pl. 5, 6, 7, Figs. 1-3; Pl. 8-10.

Material. Laminae incomplete: BCI.0127, BCI.0330, BCI.0250.

Description. Incomplete leaves and fragments, simple, slightly asymmetrical, oblong to obovate, ?10 (?11; ?5.5) cm long and ?2.7 (?4.7; ?4.2) cm wide (microphyll 2 to microphyll 3); without base preserved, and acute apex; leaf margin toothed, simple serrate, with mostly large and variable (coarse, hooked) wavy teeth, sometimes triangular, up to 4 mm high and 13 mm wide at the base, upcurved-flexuous (apical sides of teeth concave and basal sides convex), acute or acuminate-spinose (abruptly pointed to spine-tipped), deep sinuses with shape rounded (Urticoid-Hamamelid Tooth Type), apical angle of teeth acute to obtuse, regularly or irregularly spaced; venation simple craspedodromous, with primary vein straight, strong, up to 3 mm thick, tapering upward; secondary veins obvious, strongly pinnate (the secondary veins branch off at orderly intervals from the main central vein), at least 10 pairs, regularly-spaced, branching from midrib at intervals of 5-10 mm and at an divergence angle of 60° (median region), they are mostly opposite, passing through subopposite to alternate in the apical zone, running straight, slightly flexuous upwardly, but

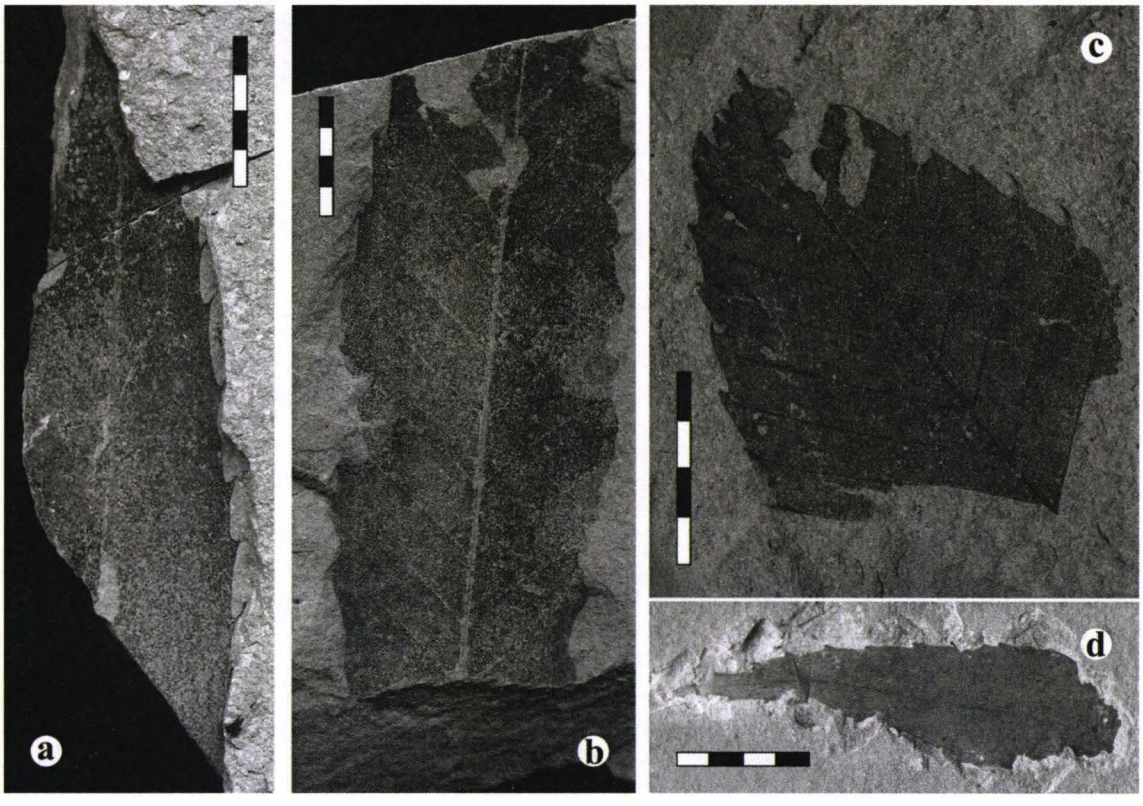
reversing its arching distally and crossing into long bristle-pointed teeth; tertiary veins when are visible, percurrent, more or less perpendicular to the secondaries.

Remarks and discussions. Although *Quercus gigas* presents an expanded diagnosis, WALTHER & ZASTAWNIAK (1991) strengthen the belief in the validity of this fossil species. The fossil oak leaves outlined great morphological variation, and in the *Q. gigas* type is now included *Q. kubinyii* Kovats pro parte, *Q. pontica miocenica* Kubát, *Q. czeczottiae* Hummel, and *Castanea atavia* Unger (WALTHER & ZASTAWNIAK, 1991). According to the same authors, *Q. gigas* may be related with the today living species of the monophyletic Group (Section or Subgenus) *Cerris* (Spach) Oersted, common in significant areas of Europe, Asia, and North Africa. *Q. gigas* is considered an important arctotertiary element of the Middle-Late Miocene floras in Europe (SONG et al., 2000; Z. KVAČEK et al., 2011). It was a representative broad-leaved deciduous element of warm-temperate mixed mesophytic forests (part of the zonal vegetation or climax vegetation of *Quercus* zone) from drier areas, in the middle altitude mountains to foothills. As noted by Z. KVAČEK et al. (2006), *Q. gigas* may occur partly on intrazonal (azonal) formations, probably riparian mixed forests, with *Ulmus* spp., *Acer* spp., *Cedrelospermum* spp., *Juglans* sp. and *Fraxinus macroptera*. This paleocommunity of sub-mountain to mountain forested wetlands was able to support dynamic groundwater near to surface, or short-term flooded sites, but never waterlogged.

The earliest verified macrofossils of *Quercus* are discovered in the Warman clay pit (Middle Eocene Claiborne Group), from western Tennessee, U.S.A., as staminate inflorescences (catkins) of *Q. oligocenensis* Daghlian & Crepet (WANG et al., 2013). Confirmed presence of fossil staminate flowers and pollen, belonging to either *Quercus* Group *Quercus* or *Lobatae*, is documented by CREPET (1989) from the Upper Eocene Baltic Amber (Prussian Formation). DENK et al. (2012) describes dispersed pollen belonging to *Quercus* Group *Ilex* from the Lower Oligocene (Rupelian) of Cospuden (Saxony, Germany) that may be the earliest unequivocal record of *Quercus* Group *Ilex* in Europe.

Nowadays the genus *Quercus* is widely distributed across the Northern Hemisphere with about 500 species in Europe, North Africa, Asia and North America (NIXON, 1989).

Occurrence of *Q. gigas* leaves in the fossil floras of Romania: Early Sarmatian (Volhynian-Early Basarabian) **Daia** (Thalheim), Sibiu County (wrongly assumed as *Castanea kubinyii* Kováts ex Ettingshausen, GIVULESCU 1975); Early Sarmatian-**Tâmpa**, Hunedoara County (described as *Castanea palaeopumilla* Andrae, BARBU 1932); Early Sarmatian-**Borod** (Valea Mâşca, drilling cores), Bihor County (wrongly assumed as *Castanea gigas* (Goeppert) Ilinskaya, GIVULESCU 1991); Pannonian-**Şoimi**, Bihor County (unfigured specimen, incorrectly attributed to *Quercus pontica miocenica* Kubát, GIVULESCU 1969); Pontian-**Borsec**, Harghita County (wrongly designated as *Quercus etymodrys* Unger, BARBU 1932); Late Pontian (= Pannonian s.l. G/H) **Chiuzbaia**, Maramureş County (wrongly attributed to *Castanea* sp., GIVULESCU 1984).



Text-fig. 1a-c - *Quercus gigas* Goeppert emend. Walther & Zastawniak. Scale bars, 20 mm.
Text-fig. 1d - *Quercus drymeja* Unger. Scale bar, 20 mm.

Genus (?) *Castanea* Miller 1759

'*Castanea*' *kubinyii* Kováts ex Ettingshausen sensu Knobloch & Z. Kvaček
Text-fig. 2a-d

1976 *Castanea kubinyii* Kováts ex Ettingshausen; Knobloch & Z. Kvaček, p. 35-38, Text-figs. 13, 14; Pl. 16, Figs. 7-9; Pl. 18, Figs. 1, 4-10; Pl. 23, Figs. 4, 6, 8-11; Pl. 31, Fig. 7.

Material. Laminae incomplete but also entire leaves: BCI.0301, BCI.0301a (counterpart), BCI.0305, BCI.0343.

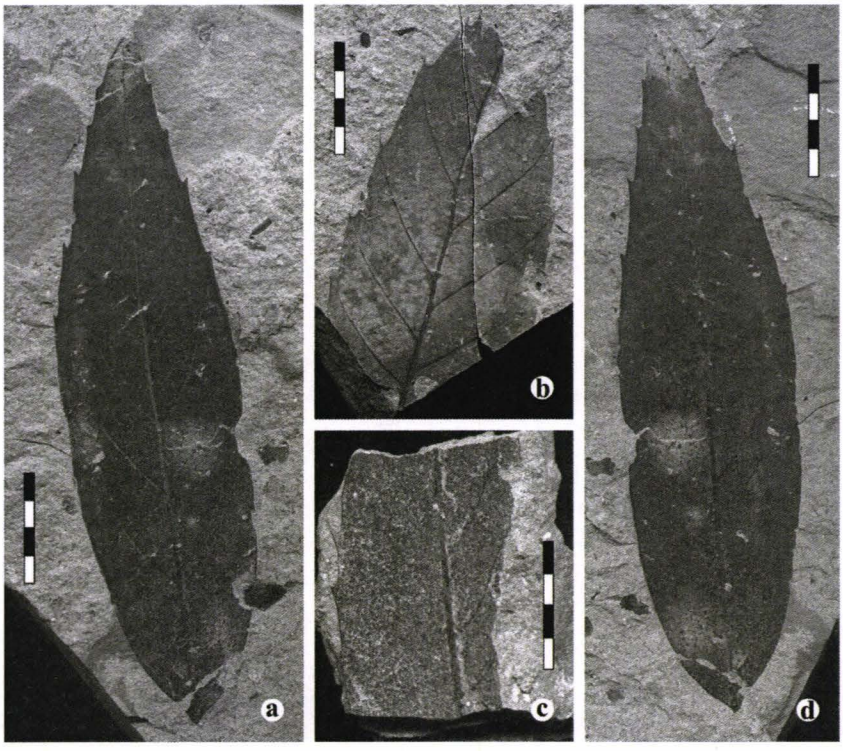
Description. Leaves entire or fragments, simple, symmetrical, with ovate-elliptic shape, 12.5 (?7.3; ?4.3) cm long and 3.4 (3.7; ?2.7) cm wide (microphyll 2 to microphyll 3); no petiole attached; base and apex angle acute, leaf base slightly asymmetrical, nearly rounded, partially decurrent; leaf margins toothed (dentate), simply serrated with each serration bearing a bristle tip (reaching up to 0.3 cm long), teeth moderate size or large (Text-fig. 2c), rarely spaced, narrow and point forward, apical sides of teeth deeply concave, basal sides concave to convex or acuminate (Text-fig. 2b), sinuses rounded (Urticoid-Hamamelid Tooth Type), tooth apex simple to spinose; in the first half (basal) the lamina margin is entire, more or less sinuous; venation simple craspedodromous, with the amendment that at the leaf base first pairs of secondary veins (suprabasal lateral veins) are interconnected into loops, primary vein strong (of moderate thickness or thick), straight, and obvious sinuate to the apex where it tapers gradually, secondary veins, 10-11 pairs, relatively thick, arise pinnate from the main vein at an angle of about 30°-40°, mainly alternate, few subopposite, slightly recurved, their course is arched and terminate upward in tooth apex passing into a bristle; tertiary veins ramified, reticulate or percurrent, convex or sinuous, forming an angle of 110-140° (commonly 120°) with primary vein, higher-orders of venation, when is observable, orthogonal reticulate, areoles well developed.

Remarks and discussions. The displayed leaf margins and the venation patterns are diagnostic to several fagacean fossil species such as *Castanea atavia* Unger, *C. gigas* (Goeppert) Ilinskaya, *C. kubinyii* Kováts ex Ettingshausen, *Quercus kubinyii* (Kováts ex Ettingshausen) Czeczott, and *Q. gigas* Goeppert emend. Walther & Zastawniak. Because such specimens are very similar in size, shape, and in general appearance, with both *Quercus* and *Castanea* (see WOROBIEC, 2003; Z. KVAČEK et al., 2011), it is still unclear whether the leaves of '*Castanea*' *kubinyii* belong to oak or chestnut. Although '*Castanea*' *kubinyii* was a conspicuous thermophilous element in many Middle-Late Neogene floras in Europe (Paratethys area), the details of the epidermal structure are not distinct enough (see Z. KVAČEK et al., 2002). Only a fortunate co-occurrence of fruits (nuts or/and cupules) together with this kind of foliage may help to clarify this problem. We assume, with some reserves, the denomination '*Castanea*' *kubinyii* for our specimens, giving credit to GIVULESCU (1990) who established that the comparison with *Quercus* is not morphologically or environmentally satisfactory. Moreover, the arctotertiary genus *Castanea* is unambiguously present in the Middle Miocene deposits of Romania, based on the rich fossil pollen record (*Tricolporopollenites cingulum* (Potonié) Thomson & Pflug, cf. PETRESCU, 2003).

Fossils of *Castanea* have been found throughout the Tertiary in the Northern Hemisphere beginning with the Palaeocene and until Late Pliocene (LANG et al., 2007). *Castanea* was a co-dominant element in the *Quercus-Carpinus-Castanea* association (MAI, 1995) of the upland warm-temperate mesophytic forests which correspond to deciduous broad-leaved forests (KOVAR-EDER et al., 2008).

GIVULESCU (1990) compared this fossil species to the extant species *Castanea sativa* Miller (*C. vesca* Gaertner), usually referred to as the European, Spanish or Sweet Chestnut, which is a large, deciduous tree, resistant to a very short dry season (limited by climate conditions) and distributed across the Mediterranean region, from the Caspian Sea to the Atlantic Ocean. The genus *Castanea* comprises three sections (*Castanea*-with three nuts per cupule, *Balanocastanon*-with one nut per cupule, and *Hypocastanon*-with a single nut per cupule) and seven species: *C. mollissima* Blume, *C. seguinii* Dode, *C. crenata* Siebold & Zuccharini, *C. dentata* (Marshall) Borkhausen, *C. sativa* Miller, *C. pumila* Miller, and *C. henryi* (Skan) Rehder & Wilson (DANE et al., 2003).

Occurrence of '*Castanea*' *kubinyii* leaves in the fossil floras of Romania: Miocene-**Slătioara**, Vâlcea County (wrongly assumed as *Castanea palaeovesca* Paolucci, BARBU 1942); Early Sarmatian (Volhynian-Early Bessarabian) **Cavnic**, Maramureș County (unfigured specimens, wrongly attributed to *Castanea atavia* Unger, GIVULESCU 1971b); Early Sarmatian-**Borod**, Bihor County (GIVULESCU 1944); Early Sarmatian-**Luncșoara**, Bihor County (GIVULESCU 1951); Sarmatian-**Porceni**, Gorj County (wrongly assumed as *Castanea palaeovesca* Paolucci, BARBU 1954); Sarmatian (Basarabian-Chersonian)-**Râmești** (Tănăseşti-Râmeşti), Vâlcea County (wrongly assumed as *Castanea palaeovesca* Paolucci, BARBU 1954); Pannonian B-C **Valea Neagră de Criș** (Valea Crișului I & II), Bihor County (GIVULESCU 1956); Pannonian E-**Delureni** (Beznea), Bihor County (GIVULESCU 1983); Early Pontian-**Cornițel**, Bihor County (GIVULESCU 1957); Early Pontian-**Gheghie**, Bihor County (wrongly assumed as *Quercus kubinyii* (Kováts) Czeczott, GIVULESCU 1960); Pontian-**Borsec**, Harghita County (reported as *Castanea palaeovesca* Paolucci, BARBU 1932); Late Pontian (= Pannonian s.l. G/H) **Chiuzbaia**, Maramureș County (wrongly attributed to *Castanea* cf. *sativa* Miller, GIVULESCU 1964); Late Pontian-Late Dacian (Early Pliocene) **Cărbunești**, Prahova County (denominated as *Castanea* sp., GIVULESCU 2001).



Text-fig. 2a-d - '*Castanea*' *kubinyii* Kováts ex Ettingshausen sensu Knobloch & Z. Kvaček. Scale bars, 20 mm.

Genus *Fagus* Linnaeus 1753

Fagus silesiaca Walther & Zastawniak
Text-fig. 3a-c

1991 *Fagus silesiaca* Walther & Zastawniak, p. 156-160, Text-fig. 1; Pl. 1, Figs. 1-6; Pl. 2, Fig. 1.

Material. Laminae incomplete but also entire leaves: BCI.0547, BCI.0545, BCI.0548, BCI.0566 (counterpart).

Description. Leaves entire or fragments, simple, slightly asymmetrical, elliptic to oblong-ovate, 8.4 (?5) cm long and 3 (2.5; 3.4) cm wide (microphyll 2 to microphyll 3); relatively short (2 mm) or absent petiole, stout, slightly bent sideways; apex acute or attenuate, triangular, base cuneate to rounded, partially decurrent (with the laminar tissue from half of the base extending proximally along the petiole at a gradually decreasing angle), slightly undulate; leaf margin toothed, serrate, with small teeth, regularly or irregularly spaced, shape variable, often concave-flexuous, simple to acuminate, sometimes spinose, occasionally blunt, apically oriented, with sinus shape rounded, region of the base entire; venation pinnate, simple craspedodromous, rarely semicraspedodromous, with primary vein straight, strong, gradually narrowed, slightly sinuous in the upper part of the lamina (adaxial), 8-13 pair of secondary veins, straight or subparallel, near leaf margin slightly upwardly curved, repeatedly curved abaxial and concave downwards, distributed at intervals of 0.8-1.2 cm, moderately thick, alternately disposed on the midrib, with the two lowermost pairs opposite; the angles with the primary vein varies between 40° and 60° (increasing in the median part of the lamina, very rare up to 70°), each secondary vein enters basally in a single, sharp tooth; intersecondary veins not clear; the tertiary venation when is preserved, very thin, percurrent, mostly opposite, rarely alternate, simple or forked, forming an orthogonal reticulum, they are consistently obtuse to the midrib, with a proximally increasing vein angle; higher order of venation, when is visible, forming a network with well-developed quadrangular areoles.

Some highly distinctive insect damage types (DTs) are to be found on the fossil leaves of *Fagus* from Ciocadia. The appearance can be attributed to the good preservation of impression-type of plant material. First, (see Text-fig.3c) there are irregular to circular perforations, 1 to 3 mm in diameter, undiagnostic, which are classified as hole feeding (DT02, LABANDEIRA et al., 2007), which is the standard bite marks, occurring on primary and secondary veins; second, an incipient excision that is incised on the interveinal tissue, toward the primary vein (see Text-fig.3b), of margin feeding type (DT15, LABANDEIRA et al., 2007), the sides of the leaf are eaten, common in orthopterans and weevils; and third, piercing and sucking punctures (see Text-fig.3c), undiagnostic, circular, < 2 mm in diameter (DT47, LABANDEIRA et al., 2007), with central dome distinct, thick, dense, and thoroughly carbonized, surrounded by a brim of reaction tissue, caused by sucking insects which pierce the leaf blade to suck out the nutrients, and the pierce marks are left behind.

Remarks and discussions. After the process of examination of previously described foliage of fossil *Fagus* from Romania and Europe it became apparent that the taxonomy of middle Tertiary species of this genus is still unclear or in open nomenclature. Apparently, many authors have erected new species on characteristics of little systematic value (e.g. the morphology of shade leaves vs. sun leaves, when polymorphism is common not only in the fossil record, but also in the today living species of *Fagus*); some ignored the initial descriptions of the name bearing specimens, or have assigned material of widely differing morphology to the same species. Revision of some historical types usually lowers diversity of morphospecies once synonyms (or erroneously assigned fossils) have been recognized.

The fossil beech leaves described above differ from the leaves of the Lower Pliocene *Fagus kraeuselii* Z. Kvaček & Walther (= *F. haidingeri* Kováts sensu DENK 2004 pro parte) in having a greater number of secondary veins (8-13 pairs), from the Middle Oligocene-Early Miocene *Fagus saxonica* Z. Kvaček & Walther (WALTHER 1994), in having fewer secondary veins (*F. saxonica* has 12-16), and from the Middle Miocene *Fagus friedrichii* Grímsson & Denk, because they display less densely spaced secondary and tertiary veins, and from the Late Miocene *Fagus gussonii* Massalongo emend. Knobloch & Velitzelos in generally smaller leaves (GRÍMSSON & DENK, 2005). On the other hand, the above-noted venation features of *Fagus* do not always provide a sufficient tool for distinction of infrageneric species. *F. silesiaca* is very similar with *Fagus menzelii* Z. Kvaček & Walther (possible junior synonym from the Middle Miocene, see Z. KVAČEK & WALTHER, 1991), and may be the same taxon, based on the morphological resemblance and epidermal structure (WOROBIEC, 2003; WOROBIEC et al., 2012).

No cupules or bud scales of *Fagus silesiaca* were described until now from the Ciocadia site and the only form-species *Fagus deucalionis* Unger emend. Denk & Meller is described based on the cupule-nut complex from the Late Oligocene to Early Miocene of Central Europe (DENK & MELLER, 2001).

F. silesiaca occurs frequently in fossil floras from the Late Miocene to the Early Pliocene of Central Europe (WALTHER, 1994). KOVAR-EDER et al., 2004 consider that the beech maximum of Europe is starting with the latest Early Miocene. Now we extend its early colonisation to the Middle Miocene (Late Badenian-Early Sarmatian). Anyway, such a short range during the Neogene turns our *Fagus* morphospecies to be of great value in biostratigraphy and in establishing correlations. Because such leaf remains have traditionally been assigned in Romania to various species of *Fagus*, yet a reliable assignment can be made to *F. silesiaca*, grace to its distinct characters.

The main synonyms of *F. silesiaca* are *Fagus attenuata* Goeppert, *Fagus haidingeri* Kováts sensu Knobloch (WOROBIEC, 2003), *Fagus pliocaenica* Saporta, *Fagus pristina* Saporta (BARRÓN & DIÉGUEZ, 1994).

Fagus silesiaca certainly inhabited wider areas and most probably belonged to zonal vegetation, of mesophytic deciduous or mixed broad-leaved evergreen forest formation (Z. KVAČEK et al., 2006). This tree was one of the arctotertiary dominant elements from the Ciocadia paleoflora. It populated moist, rich soils of uplands, or well-drained lowlands, and it sometimes formed either dense patches or larger and maybe pure stands (after the great number of leaf remains) due to vegetative propagation from stumps and trunks of young trees. By the presence of subtropical elements, like *Tetraclinis salicornioides*, *Cunninghamia*, *Glyptostrobus europaeus*, *Laurophyllum* etc. (PARASCHIV & SEBE, 2011), these forests certainly developed in a warmer climate than the present-day beech forests.

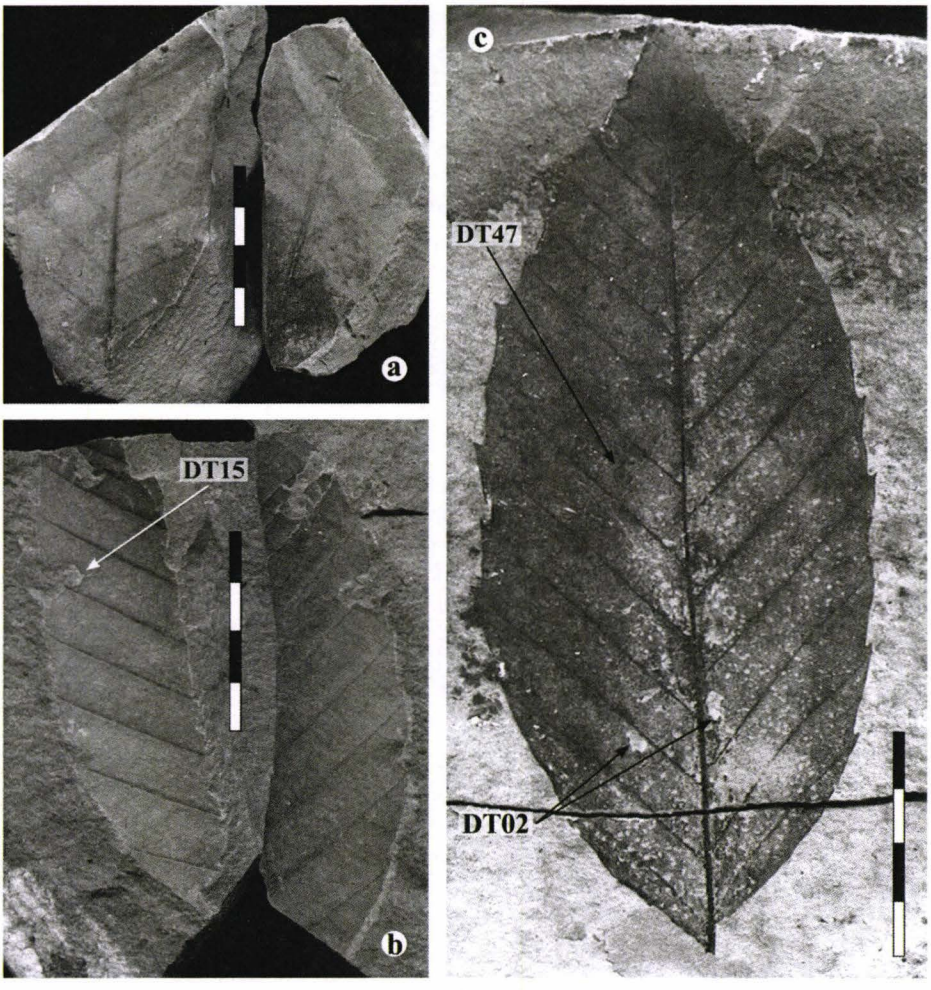
F. silesiaca is compared with the modern species *F. longipetiolata* Seemen (PALAMAREV & IVANOV, 2003), native to China and Vietnam, where it grows on broad-leaved evergreen and mixed mesophytic forests on mountain slopes, *F. hayatae* Palibin ex Hayata (WOROBIEC, 2003), which flourish in the mixed mesophytic forests of Central China, and *F. grandifolia* Ehrhart (PALAMAREV & MAI, 1998), whose area covers almost the entire eastern half of the U.S.A. from Labrador Peninsula to the north of Florida Peninsula, where it lives as a dominant species in mixed deciduous forests (Northern and Central Hardwood, and Southeastern Forest Region).

Fagus is a small genus (10 or 13 species) of broad-leaved deciduous trees (disjunctly distributed in temperate areas of the Northern Hemisphere-Holarctic ecozone) with two accepted subgenera, based on phylogenetic studies, *Engleriana* Shen with the species *F. engleriana* Seemen in Diels (incl. *F. multinervis* Nakai) of China and South Korea, *F. japonica* Maximovich from Japan and *F. okamotoi* Shen (Japan), and *Fagus* Shen, with the species *F. longipetiolata* Seemen (China), *F. brevipetiolata* Hu (China), *F. bijiensis* C. F. Wei & Y. T. Chang (limited to Western China), *F. tientaiensis* T. N. Liou (limited to Eastern China), *F. lucida* Rehder & E. H. Wilson in Sargent (China), *F. chienii* Cheng (limited to Western China), *F. hayatae* Palibin in Hayata (incl. *F. pashanica* C. C. Yang) from China mainland and Taiwan, *F. crenata* Blume (Japan), *F. sylvatica* Linnaeus (incl. *F. orientalis* Lipsky, and *F. moesiaca* (K. Malý) Czeczott, from Europe and southwestern Asia), and *F. grandifolia* Ehrhart (incl. *F. mexicana* Martinez or *F. ferruginea* Aiton) of Eastern North America and Mexico (cf. DENK et al., 2005). *Fagus* grows abundantly in undisturbed forest habitats of *Fagus* Zone (phytogeographical unit disposed on different altitudinal gradients from 1200-1300 meters up to 1500-1700 meters).

The oldest fossil occurrence of this genus based on both fruits and leaves, is provided by *Fagus langevinii* Manchester & Dillhoff from the Middle Eocene of McAbee, British Columbia, and Republic, Washington (MANCHESTER & DILLHOFF, 2004), which outperform the previous record of *Fagus pacifica* Chaney from the Early Oligocene Bridge Creek flora of Oregon (MEYER & MANCHESTER, 1997).

Occurrence of *F. silesiaca* leaves in the fossil floras of Romania: Miocene-**Slătioara**, Vâlcea County (wrongly assumed as *Fagus* aff. *ferruginea* Aiton, BARBU 1942); Sarmatian-**Porceni**, Gorj County (wrongly assumed as *Fagus* aff. *ferruginea* Aiton, BARBU 1954); Early Sarmatian-**Morilor Valley**, Mehedinți County (PARASCHIV 2004, conferred); Late Miocene (Maeotian)-**Negoești**, Dolj County (wrongly presumed as *Fagus sylvatica* Linnaeus

fossilis Laurent & Marty, BARBU 1954); Pannonian B-C **Valea Neagră de Criş** (Valea Crişului I & II), Bihor County (incorrectly assumed as *Fagus attenuata* Goeppert or *Fagus sylvatica* Linnaeus *fossilis* Laurent & Marty, GIVULESCU 1956); Pannonian E-**Delureni** (Beznea), Bihor County (wrongly ascribed to *Fagus attenuata* Goeppert, GIVULESCU 1983); Pannonian-**Guşteriţa**, Sibiu County (unfigured specimen, incorrectly attributed to *Fagus attenuata* Goeppert, GIVULESCU 1969); Early Pontian-**Corniţel**, Bihor County (incorrectly determined as *Fagus attenuata* Goeppert or *Fagus feroniae* Unger, GIVULESCU 1957); Early Pontian (Odessian) **Batoţi**, Mehedinţi County (PETRESCU et al., 2002, not figured or described; ŢICLEANU et al., 2002, incorrectly categorized, in both works, as *Fagus pliocaenica* Saporta); Pontian-**Borsec**, Harghita County (reported as *Fagus* aff. *ferruginea* Aiton, BARBU 1932); Pontian-**Crăguieşti**, Mehedinţi County (DIACONU 2007, not figured or described); Late Pontian (= Pannonian s.l. G/H) **Chiuzbaia**, Maramureş County (wrongly ascribed to *Fagus attenuata* Goeppert, GIVULESCU 1964); Late Dacian (Early Pliocene) **Dedoviţa**, Mehedinţi County (GIVULESCU 2001); Late Pliocene-**Biborţeni**, Covasna County, (incorrectly determined as *Fagus* cf. *grandifolia* Ehrhart or *Fagus* sp. aff. *sylvatica* Linnaeus, GIVULESCU 1971a).



Text-fig. 3a-c - *Fagus silesiaca* Walther & Zastawniak. Scale bars, 20 mm.

CONCLUSIONS

As a generality, a high floral diversity (as in the Middle Miocene floras of Romania) associated with high insect-feeding activity, indicate a 'healthy' plant-insect system (WAPPLER et al., 2009). The local palaeogeography and equable climate (oceanic influenced) enabled different fossil taxa to extend far outside their 'normal' ranges of distribution as we know today from the nearest living species. This can be sustained by the fact that deciduous fossil species (of *Fagus*, '*Castanea*', *Quercus*, *Carpinus*, *Betula*, *Ulmus*) grow together alongside evergreen taxa (*Laurophyllum*, *Daphnogene*, *Engelhardia*, *Ziziphus*) or taxonomically isolated and seemingly relict forms (as *Berberis* or *Cedrelospermum*). Their abundance in the fossil record and their representation by heavy organs such as foliage is interpreted as indicating that they lived on adjacent slopes near sites of deposition. An altitudinal differentiation of the vegetation in the Ciocadia area already existed in the Middle Miocene as suggested by the presence of *Tetraclinis salicornioides*, *Glyptostrobus europaeus*, *Ulmus pyramidalis*, *Carpinus* spp., *Quercus gigas*, *Fagus silesiaca*, *Platanus* sp., *Pinus* spp., *Picea* sp., *Cunninghamia* sp., etc. (see ŢICLEANU, 1984; PARASCHIV & SEBE, 2011; PARASCHIV, 2013).

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MEDICINAL AND AROMATIC PLANT VARIETIES DEVELOPED IN THE REPUBLIC OF MOLDOVA

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Abstract. The studies carried out in the area of genetics and breeding of aromatic and medicinal plants in the Republic of Moldova have resulted in the development of new high-efficient cultivars. The new cultivars of *Salvia sclarea* that are early-, medium- and late-ripening allow the production of 18-24.8 t/ha of inflorescences and 67-79 kg/ha of essential oil depending on the variety. New *Lavandula angustifolia* clone cultivars, named Moldoveanca-4, Alba-7, and Vis Magic-10 with a different harvesting time have been developed. Their producing capacity is 125-245 kg/ha of essential oil and varies with the variety. The works carried out on the breeding of *Anethum graveolens* have afforded a cultivar named Ambassador with a producing capacity of 10.5 t/ha of raw material and 88.9 kg/ha of essential oil with a carvone content of 39.8%. A new cultivar named Miracol has been developed in *Salvia officinalis*, which contributes to a producing capacity of 900 kg/ha of dry leaves or 18 kg/ha of essential oil for a single harvesting. The research conducted on *Silybum marianum* has provided a new cultivar named Argintiu, which is early-ripening with a fruit producing capacity of approximately 890-1.000 kg/ha. Hybridizations and individual selections have produced two new cultivars of *Calendula officinalis* – Nataly and Diana with a production making more than 1,000 kg/ha of dry inflorescences and a content of flavones and polyphenols of 0.624-0.873% and 0.988-1.038% respectively, depending on the variety.

Keywords: Medicinal and aromatic plants, variety, essential oil, yield, polyphenols, flavones.

Rezumat. Soiuri de plante medicinale şi aromatice elaborate în Republica Moldova. Cercetările în domeniul geneticii şi ameliorării plantelor medicinale şi aromatice în Republica Moldova au condus la obţinerea unor soiuri noi cu productivitate înaltă. Soiurile noi de *Salvia sclarea* având perioade de recoltare timpurii, medii şi tardive, asigură obţinerea unei producţii de 18.0-24.8 t/ha de inflorescenţe şi 67-79 kg/ha de ulei esenţial în funcţie de soi. La specia *Lavandula angustifolia* au fost create soiuri-clone Moldoveanca-4, Alba-7 and Vis Magic-10 cu perioada de recoltare diferită. Potenţialul de producţie a acestora este de 125-245 kg/ha de ulei esenţial în dependenţă de soi. Lucrările de ameliorare la *Anethum graveolens* s-au soldat cu crearea soiului Ambasador la care potenţialul de producţie este de 10.5 t/ha materie primă şi 88.9 kg/ha ulei esenţial cu conţinut de 39.8 % de carvonă. A fost creat şi un soi nou de *Salvia officinalis*, numit Miracol. Soiul poate asigura obţinerea unei producţii de 900 kg/ha frunze uscate sau 18kg/ha ulei esenţial la o singură recoltare. Cercetările de ameliorare efectuate la specia *Glaucium flavum* au rezultat elaborarea soiului Agat, cu randamentul de 24.0 t/ha de herba proaspătă în trei ani de exploatare a plantaţiei. La specia *Silybum marianum* cercetările s-au încununat cu crearea soiului timpuriu Argintiu, care are capacitatea de producţie de cca 890-1000 kg/ha. Prin hibridări şi selectări individuale au fost elaborate soiurile Nataly şi Diana de *Calendula officinalis*. Acestea formează inflorescenţe involte care asigură o producţie de peste 1000 kg/ha de materie primă uscată cu conţinut de flavone de 0.624-0.873% şi polifenoli de 0.988-1.038% în funcţie de soi.

Cuvinte cheie: plante medicinale şi aromatice, soi, ulei esenţial, productivitate, polifenoli, flavone.

INTRODUCTION

The importance of medicinal and aromatic plants is indisputable in view of the revival of phytopharmacy and herbal treatments. Thus, the share of drug products from medicinal and aromatic plants and their derivatives has been constantly growing. More than 50% of the prescribed drugs are chemical derivatives identified for the first time in medicinal plants. Estimated 50 -70 thousand of plant species are used in medicine throughout the world (ROSE, 1981). On the other hand, further harvesting of these plant species from wild flora is currently uncontrolled in many countries, thus damaging irreparably wild flora. Many of the medicinal plant species from the flora of the Republic of Moldova (NEGRU, 2007), as well as of other countries (MURPHY, 2008) have become rare endangered species. Preservation of vegetal biodiversity, including the biodiversity of medicinal plants may be accomplished only through cultivation of these species. Taking this into consideration and because the quality of raw vegetal material does not meet the standards of the European Pharmacopoeia in many cases, it is imperative to cultivate these species, while the cultivation requires development of varieties with specific characters and properties and a certain content of chemical components in a particular ratio for the specific pedoclimatic conditions of cultivation.

Given the fact that about 3,500 tons of essential oil, 10,000 tons of food additives, 13,000 tons of perfumery additives are produced in the world, development and cultivation of medicinal and aromatic plant varieties are strictly necessary, thus ensuring steadfast production of high quality pharmaceutical, perfumery, and food raw material with a much more enhanced content of active principles in comparison with the types collected in wild flora. Moreover, they provide an important source of income for population in rural areas.

The producing potential of the Republic of Moldova in the matter of pharmaceutical raw material, essential oil and concrete (200-250 tons), food, cosmetic, perfumery additives is relatively modest. However, the quality of the products derived from the medicinal and aromatic plants cultivated in the Republic of Moldova is very high due to the elevated concentration of active principles supported by the varieties developed here, that are distinguished by a unique ratio of the major components, as well as the pedoclimatic conditions specific to this area – abundant insolation, rich

soils, reduced atmospheric depositions. For example, the potential of our *Salvia sclarea* L. varieties for the concentration of sclareol in the concrete is 65-70% or 10-15% higher than the requirements of the European standard in this sphere. All these make this branch very attractive.

This work describes the most valuable varieties of medicinal and aromatic plants developed in Moldova.

MATERIAL AND METHODS

The biological material is represented by varieties, hybrid genotypes of medicinal and aromatic plants belonging to the species *Salvia sclarea* L. (Clary Sage), *Lavandula angustifolia* Mill. (Lavender); *Salvia officinalis* L. (Common Sage); *Anethum graveolens* L. (Dill); *Glaucium flavum* Cr. (Yellow poppy), *Coriandrum sativum* L. (Coriander), *Carum carvi* L. (Caraway), *Pimpinella anisum* L. (Anise), and *Calendula officinalis* L. (Common marigold).

S. sclarea varieties have been created through hybridization between depression non-affected inbred and male sterility lines (type 1). Simple, double, triple, backcross, and stepwise hybrids produced in the previous study were also used as parental forms. The general and specific combining capacity of the parental forms was assessed in top cross using two testers. The developed cultivars represent hybrids of different complexity. So, the varieties Dacia-50, Dacia-99, and Victor are simple hybrids; Nataly-Clay is a triple hybrid; Ambra Plus is a backcross hybrid; Balsam is a stepwise hybrid.

Iinitial *Lavandula angustifolia* materials were produced through different polycross hybridization methods to develop clonal varieties Moldoveanca 4, Vis magic 10, and Alba 7. Promising hybrid forms were used to produce clonal varieties by vegetative multiplication. The *Salvia officinalis* variety Miracol represents a simple hybrid between two genotypes originating from Moldova and Crimea. The *Calendula officinalis* varieties Nataly and Diana were developed by selection in the F_2 hybrid population. The population was obtained through hybridization between inbreeding lines S_{2-12} of different provenience. Selections were carried out for quantitative traits. The varieties of the *Anethum graveolens, Glaucium flavum,* and *Silybum marianum* species were developed using individual selection methods.

The essential oil content was assessed by hydrodistillation and recalculated for dry matter. The qualitative and quantitative analyses were performed using Gas Chromatography (GC) coupled with Mass Spectrometry (GC-MS): gas chromatograph - Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with quadruple, capillary column (30m x 0.25mm i.d., film thickness 0.25 μм) with the HP-5ms non-polar stationary phase. The injector and detector temperatures were 250°C and 280°C, respectively, with a temperature gradient from T_1 = 70°C (2 min), T_2 = 200°C (5°C/min) to T_3 = 300°C (20°C/min, 5 min). Mobile phase: helium 1ml/min, the injected volume of essential oil - 0.03 μl, split rate - 1:100. The identification of the chromatographic peaks was performed using the software package AMDIS ™, coupled with NIST database. Flavones and polyphenols concentrations in *Calendula officinalis* inflorescences were determined by the methods described in the European Pharmacopeia.

The validation of the agronomic attributes, quantitative characters of the new varieties was done by testing in the comparative competitive crops by State Commission for Plant Cultivars Testing methods.

RESULTS AND DISCUSSIONS

The studies carried out in the area of genetics and breeding of medicinal and aromatic plants in the Republic of Moldova have resulted in the development, registration and patenting of the new varieties. The researches have been more profound and efficient in Sage Clary (*Salvia sclarea*) (GONCEARIUC, 2002; GONCEARIUC, 2008; GONCEARIUC, 2010).

The development, evaluation and use of the initial valuable material that includes inbreeding and male sterile lines, the lines that consolidate male sterility, simple hybrids, three line hybrids, double hybrids, backcrosses and stepwise hybrids have resulted in some high-efficient hybrids with an enhanced producing capacity. Among them, there are Dacia-50, Ambra Plus, Balsam, early- and late-ripening varieties named Victor and Nataly-Clary, and a medium-ripening variety named Dacia-99. These varieties reach the ripening state gradually and together with the early-ripening varieties Dacia-50, Ambra Plus, and Balsam, form a conveyer during harvesting, which allows a gradual harvesting of each variety and ensures a substantial reduction in raw material and essential oil losses. The varieties Victor (late), Dacia-50 (early) and Dacia-99 (medium) are simple hybrids, while Nataly-Clay (late) is a triple hybrid; Ambra Plus (early) and Balsam (early) are very complex hybrids, developed through stepwise and backcross hybridization (GONCEARIUC, 2009; GONCEARIUC, 2013).

All the varieties are suitable for both processing of raw material (inflorescences) and production of essential oil through distillation, as well as of concrete through organic solvent extraction. The different vegetative period and gradual ripening allow the expansion of the harvesting period up to 25-27 days. In its turn, this contributes to the increase of the areas occupied with sage and processing of a higher quantity of raw material while expanding industrial processing capacities (GONCEARIUC, 2010; GONCEARIUC, 2013). When the plantations are sown with new sage cultivars, the seed rate is 4-5 kg/ha of the first class as opposed to other varieties, which need a sowing rate of 10-12 kg/ha of seeds.

The plantations of these varieties can be exploited three years. The yield of inflorescences of *S. sclarea* varieties in three years (2011-2013) of cultivation made 18.1-24.8 t/ha (Table 1). It should be mentioned that the varieties listed have a producing capacity of up to 14 -15 t / ha of inflorescences in the second year of vegetation. The results obtained in 2012 were more modest because that year was very dry. Throughout the testing period, all the

varieties accumulated high contents of essential oil (0.825-1.494%), especially in the second year of vegetation (1.253-1.494%), but the early-ripening variety Balsam was the best for that character.

The producing capacity of essential oil in the *S. sclarea* varieties developed is very high ranging between 63.8 and 79.5 kg/ha in three years of plantation exploitation. The early-ripening varieties Ambra Plus and Balsam are the most productive.

Figure 1. *Salvia sclarea* variety Nataly Clary.

Figure 2. *Salvia sclarea* variety Ambra Plus.

Table 1. Inflorescence yield, essential oil content, and productivity of *Salvia sclarea* varieties in three years of vegetation (2011-2013).

Varieties	Yield of inflorescences, t/ha				Content of essential oil, % (dry matter)			Production of essential oil, kg/ha			
	I year 2011	II year 2012	III year 2013	Σ	I year 2011	II year 2012	III year 2013	I year 2011	II year 2012	III year 2013	Σ
Early-ripening varieties											
Dacia-50, st.	3.3	11.2	5.5	20.0	0.926	1.143	0.988	9.0	38.6	16.2	63.8
Ambra Plus	9.0	11.0	4.8	24.8	0.825	1.179	1.029	22.3	39.1	14.7	76.1
Balsam	6.3	10.4	4.4	21.2	1.009	1.494	1.078	18.9	46.5	14.1	79.5
Medium-ripening variety											
Dacia-99,	3.9	10.4	3.8	18.1	1.003	1.411	1.023	11.7	44.1	11.3	67.1
Late-ripening varieties											
Victor	4.8	11.7	3.3	19.8	0.833	1.253	1.187	12.1	43.9	11.8	67.7
Nataly Clary	4.5	10.6	4.1	19.2	0.880	1.291	1.043	13.5	41.0	13.8	68.3

It is very important that the new varieties bloom in the first year of vegetation, while high resistance to wintering allows exploitation of plantation in the third year of vegetation. By the third year, their yielding capacity makes 14.1-16.2 kg/ha of essential oil depending on the variety. The quality of essential oil is high because the concentration of linalyl acetate in essential oil is 63-70% and sclareol – 6-12% (GONCEARIUC, 2010).

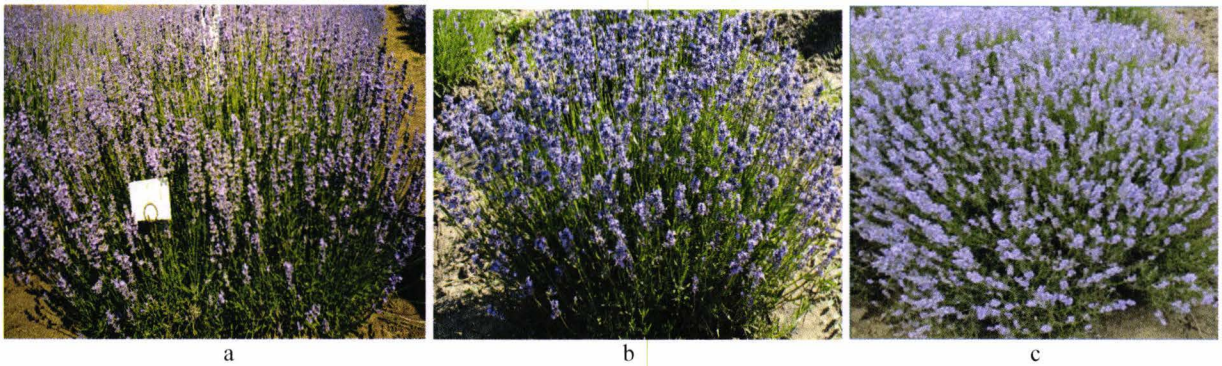
All the *Salvia sclarea* varieties are registered in the Republic of Moldova. The varieties Dacia 99, Victor, and Nataly-Clary were patented in 2011. The variety Ambra Plus was patented in 2013.

The studies carried out in the area of genetics and breeding of lavender (*Lavandula angustifolia* Mill.) have started with the development of new genotypes through polycross hybridization using germ plasma resources from France, Crimea, and Moldova. Three new clone varieties of *L. angustifolia* named Moldoveanca-4, Alba-7, and Vis magic-10 have been developed up to now. They are resistant to frost, wintering and drought (GONCEARIUC, 2005; 2005a; GONCEARIUC & BALMUSH, 2006). All the lavender clone varieties were patented in 2010. Moldoveanca 4 and Vis magic 10 do not actually exceed the clone variety witness for the production of inflorescences and both have an increased content of essential oil (4.491, 4.224%), which provides yields of 178.7 and 125.3 kg/ha of essential oil respectively (Table 2).

Table 2. The average productivity of *Lavandula angustifolia* Mill. clone-varieties.

Cultivars	Yield of inflorescences, t/ha	Essential oil content, % (dry matter)	Essential oil production, kg/ha	Essential oil yield, kg/ha of inflorescences
C-90, standard., late	8.9	2.629	93.5	10.5
Moldoveanca-4,early	8.7	4.491	178.7	14.0
Vis magic-10, medium	9.0	4.224	125.3	16.9
Alba-7, late	12.8	5.376	245.0	21.5

Alba 7 is the most efficient clone variety ensuring an inflorescence production of 12.8 t/ha with an essential oil content of 5.376%, which guarantees a very high production of essential oil of 245 kg/ha. One ton of inflorescences provides between 14 kg and 21.5 kg of essential oil depending on the variety through steam distillation. Thus, the efficiency of the new clone varieties is much higher than that of the witness (GONCEARIUC, 2005).



a b c

Figure 3. *Lavandula angustifolia* Mill. clone-varieties: a- Moldoveanca- 4, b- Vis magic-10, c- Alba-10.

Dill (*Anethum graveolens* L.) is another essential oil containing and medicinal species cultivated in Moldova. During 1996-2000, when the markets of essential oils had been lost by this country and the essential oil crop plantation was annihilated, dill oil was exported every year. An early-ripening dill variety, named Ambassador (Fig. 4), has been developed to increase the efficiency of cultivation and processing of this species. It is characterised by average yields of raw material making 10.5 t/ha, those of essential oil of 88.9 kg/ha with a carvone concentration of 39.8%. The variety was registered in 2004. The dill varieties that have been cultivated up to the present have a producing capacity of only 50-60 kg/ha of essential oil with a concentration of the principal component carvone making only 29.5% (GONCEARIUC & BALMUSH, 2006).

The works performed on *Salvia officinalis* L. have resulted in the development of an early ripening variety, named Miracol that is resistant to drought, frost and wintering (Fig. 5). It was registered in 2005. The variety can be used to produce pharmaceutical raw material of *Folium Salviae* and *Herba Salviae* and essential oil – *Oleum Salviae*. The producing capacity of the variety Miracol is 900 kg/ha of dry leaves (13% of humidity) or 18 kg/ha of essential oil (Table 3). In the case of two yields, the producing capacity of the variety is even higher (GONCEARIUC, 2008; GONCEARIUC et al., 2012).

Table 3. Yield of *Salvia officinalis* L. variety Miracol.

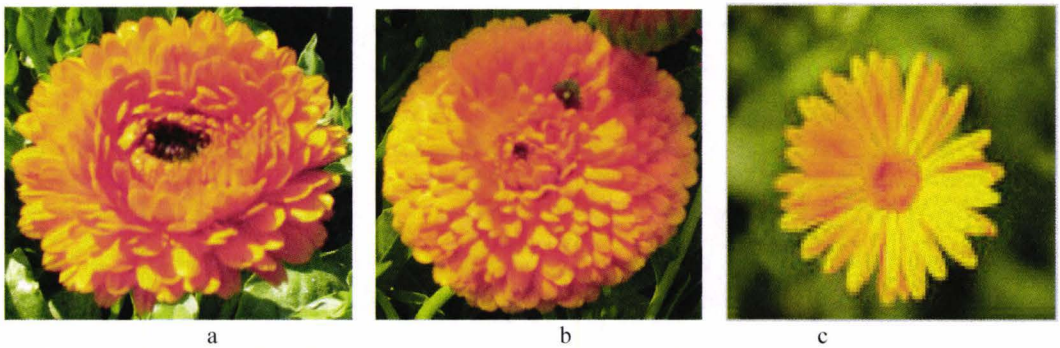
Variety	Years	Yield of raw material, kg/ha		Essential oil content, % (dry matter)	Production of essential oil	
		Humidity 60%	Dry matter		kg/ha	%
Miracol	2012	2990	850	2.500	18.7	183.3
	2013	2930	960	2.240	17.4	153.9
	X	2960	905	2.362	18.0	168.2
Ghineţei, Witness	2012	2090	600	1.280	10.2	100.0
	2013	2010	570	1.691	11.3	100.0
	X	2050	590	1.485	10.7	100.0

Figure 4. *Anethum graveolens*, variety Ambasador at the harvesting phase.

Figure 5. *Salvia officinalis*, variety, Miracol.

Germ plasma resources of different genetic and geographic origin have been used to develop genotypes with new characteristics and properties in *Calendula officinalis* L. (Common marigold). A complex hybridization has produced many varieties, two of them – Nataly and Diana with large inflorescences, 7.0 cm in diameter and a high number of

ligulate (Fig. 6) flowers, with a producing capacity of more than one ton per hectare. The varieties are distinguished by the tubular flower colour. Thus, the tubular as well as ligulate flowers are orange in Nataly variety, while the tubular flowers are brown and the ligulate flowers are orange in Diana variety. The concentration of the active matter in the new marigold variety is much higher than that in the local population cultivated in Moldova, which served as control. The distinction between them, as for this index, is as follows: the flavones concentration in Nataly cultivar is 0.873%, being higher in Diana cultivar, which contains 0.624%, while the content of polyphenols is relatively lower (0.988%) than in Diana cultivar in which the polyphenols concentration is 1.038% (GONCEARIUC, 2008; 2008a; 2011).



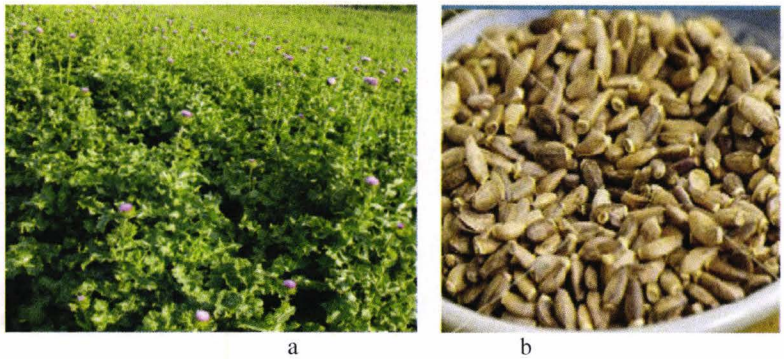
a b c

Figure 6. *Calendula officinalis* inflorescences: a- Diana variety, b- Nataly variety, c- local population.

Genetic and breeding studies have also been conducted on *Glaucium flavum* Cr. (Yellow poppy), a very important species due to the fact that its *herba* contains 15 alkaloids, glaucine being the most important with an action similar to that of codeine but it is cheaper and has no depressing action on respiration, no negative impact on the digestive system and, in contrast to codeine, produces no addiction. The studies have resulted in a new variety of *G. flavum*, named Agat (Fig. 7) with a vegetation period of three years. The average production of Agat fresh *herba* is 3.2 t/ha in the first year and 10.8 t/ha in the second year, while it makes 10.2 t/ha in the third year. Totally, the variety provides an average production of 24.0 t/ha in three years of exploitation.

Among the medicinal plant species under study, there is milk thistle (*Silybum marianum* (L) Gaertn). Individual selections and subsequent hybridization have afforded an early-ripening cultivar named Argintiu homologated in Moldova in 2004 and patented in 2013. The cultivar is resistant to drought, while simultaneous maturation of fruits, in the majority of inflorescences, contributes to mechanical harvesting. The average fruit producing capacity (*Fructus Cardui Marianus*) of Argintiu variety (Fig. 8) is 890-1,000 kg/ha under unfertilized conditions of cultivation (GONCEARIUC, 2009).

Figure 7. *Glaucium flavum*, variety Agat.



a b

Figure 8. *Silybum marianum* variety Argintiu: a-plantation, b-seeds.

CONCLUSION

1. The studies carried out in the area of genetics and breeding of aromatic and medicinal plants in the Republic of Moldova have resulted in the development of new high-efficient cultivars.
2. The new cultivars of *Salvia sclarea* that are early-, medium- and late-ripening allow the production of 18-24.8 t/ha of inflorescences and 67-79 kg/ha of essential oil depending on the variety.
3. New cultivars of *Lavandula angustifolia* named Moldoveanca-4, Alba-7, and Vis Magic-10 have been developed. Their producing capacity is 125-245 kg/ha of essential oil and depends on the variety.
4. The works carried out on breeding of *Anethum graveolens* have resulted in the development of a cultivar named Ambassador with a producing capacity of 10.5 t/ha of raw material and 88.9 kg/ha of essential oil with a carvone content of 39.8%.

5. A new cultivar named Miracol has been developed in *Salvia officinalis*, which contributes to a producing capacity of 900 kg/ha of dry leaves and 18 kg/ha of essential oil for a single harvesting.
6. The breeding works performed on *Glaucium flavum* have provided a variety named Agat with a yield of 24.0 t / ha of fresh *herba* in three years of plantation exploitation.
7. In *Silybum marianum*, the studies have afforded a new cultivar named Argintiu, which is early ripening with a producing capacity of approximately 890-1,000 kg/ha of fruits.
8. Hybridizations and individual selections have resulted in the development of two new cultivars of *Calendula officinalis* – Nataly and Diana with a production of more than 1,000 kg/ha of dry inflorescences, a content of flavones and polyphenols making 0.624-0.873% and 0.988-1.038% respectively.

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RESEARCH REGARDING THE EFFECTS OF THE TREATMENT WITH FUNGICIDES ON THE PHYSIOLOGICAL PROCESSES IN *Lycopersicon esculentum* MILL. PLANTS ATTACKED BY *Phytophthora infestans* (MONT.) DE BARY

NICOLAE Ion, BUŞE-DRAGOMIR Luminiţa

Abstract. Physiological researches were performed at tomato plants grown in the region of Oltenia. After the reporting and identification of the attack produced by the pathogen, tomato culture has been divided into two groups, a first group where fungicide treatment was applied and the second group without treatments. After applying the treatments, physiological analysis were carried out on July 14th 2012, at the attacked plants (plants without treatment), compared to the plants treated with fungicides (plants with treatment). It was found that the intensity of photosynthesis and transpiration intensity vary according to the analysed plants and climatic conditions. In the attacked plants by the pathogen there was registered a lower intensity of photosynthesis, which is correlated with the low chlorophyll content, but also a decreased intensity of transpiration due to the deterioration of leaves and stomata coverage by the mycelium pathogen. It was also recorded a lower water content and a higher content of dry matter, which caused serious hydric and metabolic imbalances in the attacked plants, compared to the plants where fungicide treatments were applied.

Keywords: fungicide, pathogen, physiological processes, tomato plants.

Rezumat. Cercetări privind efectele tratamentului cu fungicide asupra proceselor fiziologice la plantele *Lycopersicon esculentum* Mill. atacate de *Phytophthora infestans* (Mont.) de Bary. Cercetările fiziologice s-au efectuat la plantele de tomate cultivate în zona Olteniei. După semnalarea şi identificarea atacului produs de patogen, cultura de tomate a fost împărţită în două loturi, la un lot s-au efectuat tratamente cu fungicide, iar la al doilea lot nu s-a efectuat tratamente. După aplicarea tratamentelor s-au efectuat analize fiziologice, la data de 14 iulie 2012, la plantele atacate de patogen (plante fără tratament), în comparaţie cu plantele tratate cu fungicide (plante cu tratament). S-a constatat că intensitatea fotosintezei şi intensitatea transpiraţiei variază în funcţie de plantele analizate şi condiţiile climatice. În plantele atacate de patogen s-a constatat scăderea intensităţii fotosintezei, fapt corelat cu conţinutul scăzut în clorofilă, dar şi scăderea intensităţii transpiraţiei ca urmare a deteriorării frunzelor şi acoperirii stomatelor de către miceliu patogenului. S-a înregistrat, de asemenea un conţinut mai scăzut de apă şi un conţinut mai mare de substanţă uscată, fapt ce a determinat grave dezechilibre hidrice şi metabolice în plantele atacate, comparativ cu plantele la care s-au efectuat tratamente cu fungicide.

Cuvinte cheie: fungicid, patogen, procese fiziolgice, plante de tomate.

INTRODUCTION

The physiological research has been carried out after applying treatments with fungicides and consisted in carrying out analysis of the attacked plants, compared to the treated plants with fungicide.

Vegetables are plants known for their nutritional value and therapeutic effects. Vegetable consumption stimulates body hydration, muscle activity, appetite, calcification, enzyme activity, defense ability, inhibits bacterial fermentation, and provides essential amino acids (SUCIU et al., 1987).

Lycopersicon esculentum Mill. (syn. *Solanum lycopersicum* L.) is a herbaceous plant, vegetable, with therapeutic value. Tomatoes have a differentiated tissues structure with different chemical composition and physiological activity (HOROTAN & OANCEA, 2013).

Phytophthora infestans (Mont.) de Bary is an important and destructive disease on tomatoes. Crop losses are very high, the percentage of fruits damaged exceeding 50% in untreated cultures.

In the last decades, the use of fungicides in agriculture for fungi diseases control has become crucial. Fungicide research has produced a diverse range of products with new modes of action. However, the extensive use of these compounds in the agriculture system raises public concern because of the harmful potential of such substances in the environment and human health (DIAS, 2012).

Application of fungicides may affect crop physiology by various disruptions such as growth reduction, perturbation in the development of reproductive organs, alteration of nitrogen, and/or carbon metabolism leading to a lower nutrient availability for plant growth. This former physiological trait is fundamental for plant culture and is reflected by both photosynthetic rate and mobilization of carbohydrate reserves (PETIT et al., 2012).

The diurnal dynamics of photosynthesis and transpiration in the attacked leaves plants is similar to that in the plant leaves analysed after treatments, but the recorded values are lower in the attacked leaves (NICOLAE & BUŞE-DRAGOMIR, 2013).

The intensity of photosynthesis processes in normal conditions tomato plant growth is 12.9 μmol/m 2/s (GALTIER et. al., 1995). Determinations carried out in tomato plant show that the intensity of photosynthesis processes at basal leaves is lower (3.35 μmol/m^2/s); the intensity of this process increases at leaves of the middle plant (10.73 μmol/m^2/s) and falls to the top of the plant leaves (5.19 μmol/m^2/s) - BURZO et. al., 2000.

Positive correlations were established between the intensity of the physiological processes and the photosynthetic active radiation, the leaf temperature and stomatal conductance of CO_2 (NICOLAE, 2010).

MATERIAL AND METHODS

Physiological research on the effects of the treatment with fungicides on the intensity of the physiological processes was performed in tomato plants *(Buzau 50* variety) cultivated in the region of Oltenia (Dolj county).

Buzău 50 variety is an early variety, with an indefinite increase. Plants are vigorous (190 cm) and have rich foliage that protects fruit. The fruits are spherical, have intense red color and have an average weight of 250 g.

After reporting the attack and identification of natural attack produced by *Phytophthora infestans* (Mont.) de Bary, at the beginning of formation of fruits, the tomato crops were divided into two groups (the experimental variants). In case of the first group of plants, there have been conducted three treatments with fungicide *Shavit* F 72 WP at a period of 7 days between treatments (plants with treatment), while in case of the second group, there has been applied no treatment (untreated control plants). After a period of 10 days from the last treatment plants were analysed physiologically. The analyses were performed on the 14th of July 2012 at the attacked plants, compared to the treated plants with fungicide.

Shavit F 72 WP (Triadimenol 20 g/kg + Folpet 700 g/kg), produced by Makhteshim Agan-Israel, is a complex fungicide containing two active substances with systemic and contact action, which ensures a quick effect, preventative and curative of long duration.

The protection period depends on the local conditions and varies from 7 to 14 days. In case of the tomato plants attacked by the pathogen, the dose applied was 0.2% (20 g per 10 liters of water).

The physiological processes intensity was established with the photosynthesis measurement system LCi (Ultra Compact Photosynthesis System - ADC BioScientific Ltd.) and the obtained results were graphically represented and statistically interpreted using the Excel software.

The water and dry substance contents were determined by the gravimetric method.

The chlorophyll content was estimates by Minolta SPAD 502. The estimate of the attack produced by pathogen was made using the calculation formulae elaborated by SĂVESCU & RAFAILĂ, 1978.

RESULTS AND DISCUSSIONS

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is manifested by the appearance of some large spots, grey-green, with downy whitish on the underside of the leaf, on the edge and especially on the tips of the leaves. The attacked tissues get brown and the edges of the leaves get dry and twist towards the top. On the stems and petiole of the leaf, there appear elongated, discoloured spots, which become brown or grey-brown (Fig. 1).

The most common and damaging form of the attack is the one on the fruits. The attack starts with the formation of the fruit and lasts until ripening. Around the fruit peduncle, large, yellowish-green spots form, which then become grey-brown with uneven well, hard and dry surface. The brown spots expand then into the pulp of the fruit (MITREA, 2006).

Phytophthora infestans (Mont.) de Bary presents hyaline sporangiophores, non-septate, fasciculate, monopodial branching; they go out by the ostiole of stomata presenting a terminal unicellular, hyaline, limoniforme sporanges (TĂNASE & ŞESAN, 2006) - Fig. 2.

Figure 1. The *Lycopersicon esculentum* attacked by *Phytophthora infestans* (original).

Figure 2. *Phytophthora infestans* - sporangiophores with sporanges (oc. 10 x ob. 20) (original).

The evolution of temperatures and rainfall during the vegetation period favoured the emergence of the disease. Through the application of the foliar surface treatment with fungicides necrosis was significantly reduced, and the leaves with specific symptoms of the attack produced by the pathogen have maintained green during the vegetation period.

The physiological research was performed on the 14th of July 2012, at the plants attacked by *Phytophthora infestans* (Mont.) de Bary (plants without treatment), compared to the plants that have received fungicide treatments (plants with treatment).

The estimation of the attack (frequency, intensity and degree of attack) produced by the pathogen at the attacked plants is presented in Fig. 3.

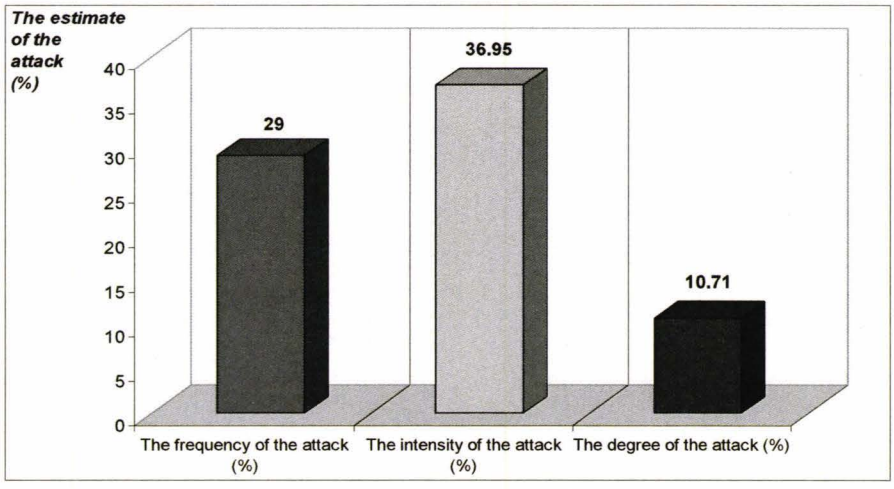

Figure 3. The estimate of the attack produced by *Phytophthora infestans* (Mont.) de Bary in *Lycopersicon esculentum* Mill.

Physiological processes intensity is lower in the tomato plants attacked by the pathogen, compared to the plants analysed after the treatment with fungicides, as a result of the reduction of the assimilation surface due to the reaction of the plants to the pathogen, deterioration of the chlorophyll pigments and stomata coverage by the pathogen (Figs. 4; 5).

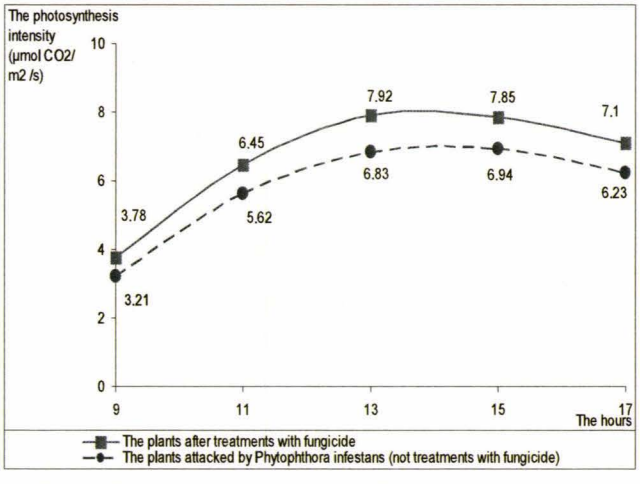

Figure 4. Diurnal dynamics of the photosynthesis intensity in tomato plants (*Lycopersicon esculentum* Mill.).

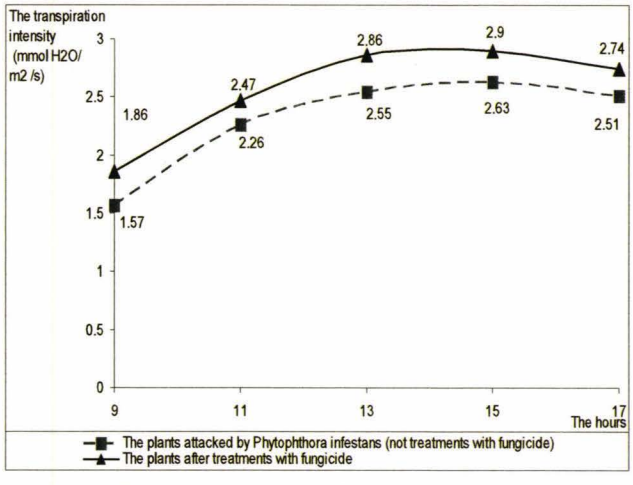

Figure 5. Diurnal dynamics of the transpiration intensity in tomato plants (*Lycopersicon esculentum* Mill.).

The physiological processes intensity (photosynthesis and transpiration intensity) is correlate with the physiological parameters (photosynthetic active radiation present on the surface of the leaves, leaf temperature, stomatal conductance of CO_2), but presents different values in the tomato plants attacked by *Phytophthora infestans* (Mont.) de Bary, in comparison with the tomato plants analysed after the treatments with fungicide. Thus, there was established a strong association between these.

As a result of the physiological research carried out at the tomato plants one can observe an increase of the physiological parameters, during the day, increase starting in the morning; they present higher values at lunch and a gradually decrease towards evening (Table 1).

Table 1. Diurnal dynamics of the physiological parameters registered in tomato plants.

The physiological parameters in the tomato plants		The hours of performed analyses				
		9^{00}	11^{00}	13^{00}	15^{00}	17^{00}
The photosynthetic active radiation (*µmol / m² / s*)	The plants after the treatments with fungicide	1150	1485	1645	1565	1490
	The plants attacked by the pathogen	1135	1470	1634	1548	1475
The leaf temperature (*°C*)	The plants after the treatments with fungicide	28.3	31.4	34.1	33.2	33.1
	The plants attacked by the pathogen	28.4	31.4	34.3	33.5	33.3
The stomatal conductance (*mol / m² / s*)	The plants after the treatments with fungicide	0.06	0.08	0.13	0.12	0.1
	The plants attacked by the pathogen	0.04	0.07	0.1	0.09	0.07

Linear regression carried out between the photosynthesis intensity and photosynthetic active radiations present on the surface of the leaves shows a good positive correlation between these; the coefficient of determination (R^2) is 0.87 for the tomato plants attacked by the pathogen and 0.96 for the tomato plants after the treatments with fungicide. Linear regression carried out between the transpiration intensity and photosynthetic active radiations shows a good positive correlation; the coefficient of determination (R^2) is 0.90 for the attacked tomato plants and 0.92 for the tomato plants after the treatments with fungicide (Figs. 6; 7).

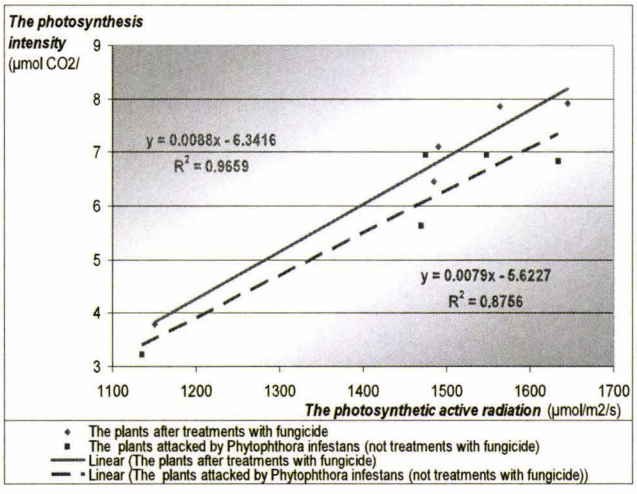

Figure 6. The correlation between the photosynthesis intensity and the photosynthetic active radiation in tomato plants (*Lycopersicon esculentum* Mill.).

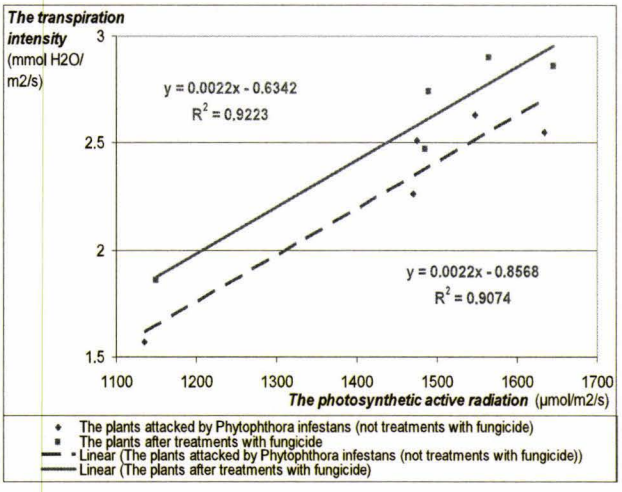

Figure 7. The correlation between the transpiration intensity and the photosynthetic active radiation in tomato plants (*Lycopersicon esculentum* Mill.).

Linear regression carried out between the photosynthesis intensity and leaf temperature shows a good positive correlation; the coefficient of determination (R^2) is 0.95 for the attacked tomato plants and 0.96 for the tomato plants after the treatments with fungicide. Linear regression carried out between the transpiration intensity and leaf temperature shows a good positive correlation between these; the coefficient of determination (R^2) is 0.94 for the attacked tomato plants and 0.96 for the tomato plants after the treatments with fungicide (Figs. 8; 9).

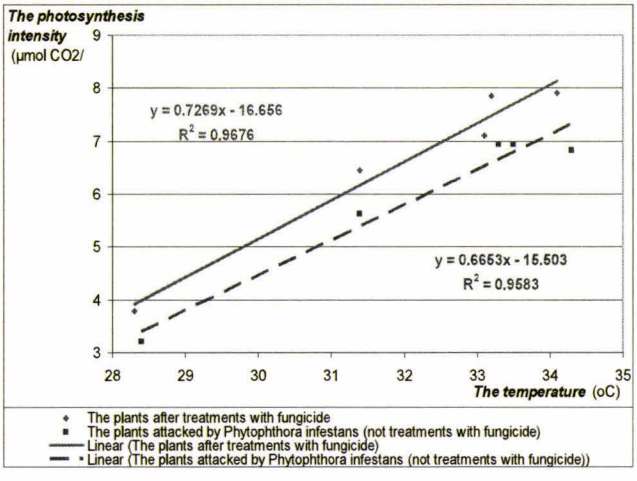

Figure 8. The correlation between the photosynthesis intensity and the leaf temperature in tomato plants (*Lycopersicon esculentum* Mill.).

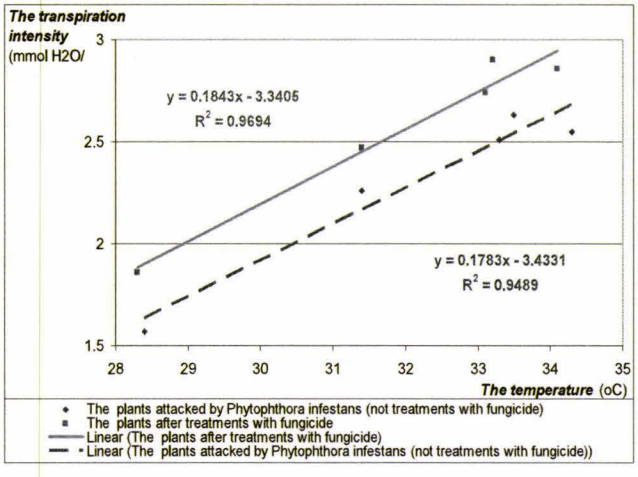

Figure 9. The correlation between the transpiration intensity and the leaf temperature in tomato plants (*Lycopersicon esculentum* Mill.).

Linear regression performed between the photosynthesis intensity and stomatal conductance of CO_2 shows a positive correlation between these; the coefficient of determination (R^2) is 0.76 for the attacked tomato plants and 0.86 for the tomato plants after the treatments with fungicide. Linear regression made between the transpiration intensity and stomatal conductance of CO_2 shows a positive correlation; the coefficient of determination (R^2) is 0.81 for the attacked tomato plants and 0.86 for the tomato plants after treatments with fungicide (Figs. 10; 11).

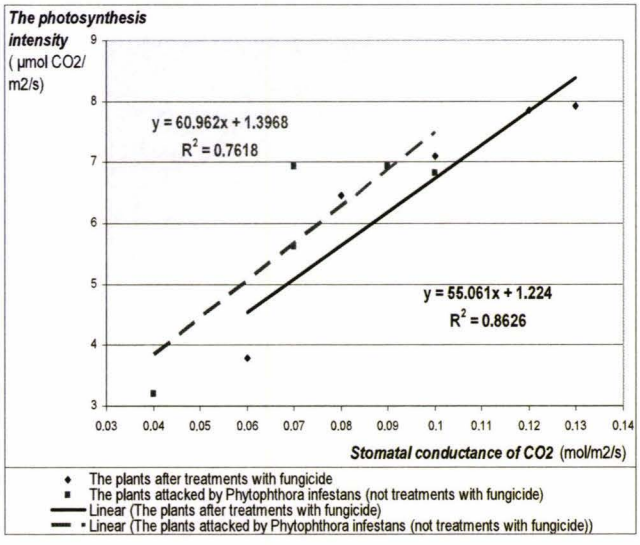

Figure 10. The correlation between the photosynthesis intensity and the stomatal conductance in tomato plants (*Lycopersicon esculentum* Mill.).

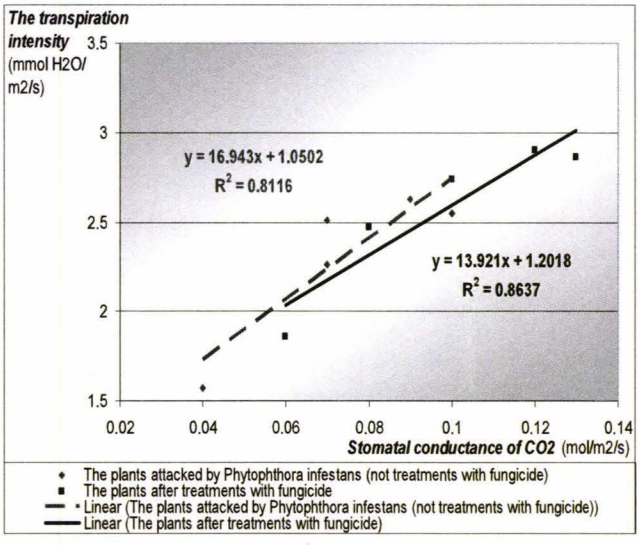

Figure 11. The correlation between the transpiration intensity and the stomatal conductance in tomato plants (*Lycopersicon esculentum* Mill.).

The tomato plants attacked by the pathogen present a 3.02 % lower water content and a 14.97 % higher dry substance content in comparison with the tomato plants after the treatments with fungicide (Fig. 12).

In the attacked tomato plants, it is noticed a 17.88 % lower chlorophyll content as a result of the deterioration of the chlorophyllian pigments under the action the pathogen (Fig. 13).

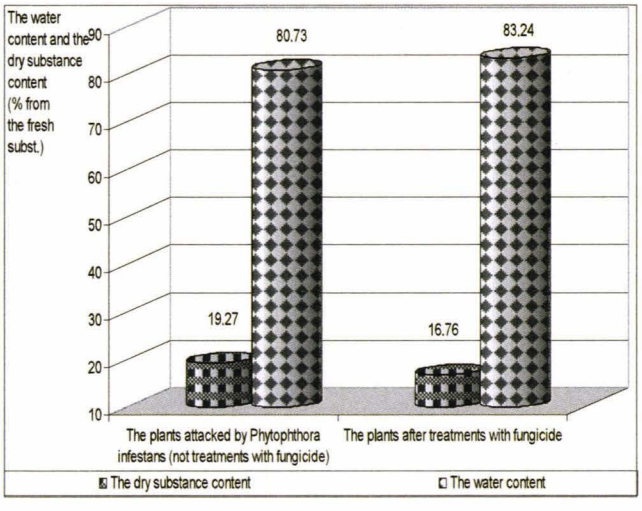

Figure 12. The water content and the dry substance content in the leaves of tomato plants (*Lycopersicon esculentum* Mill.).

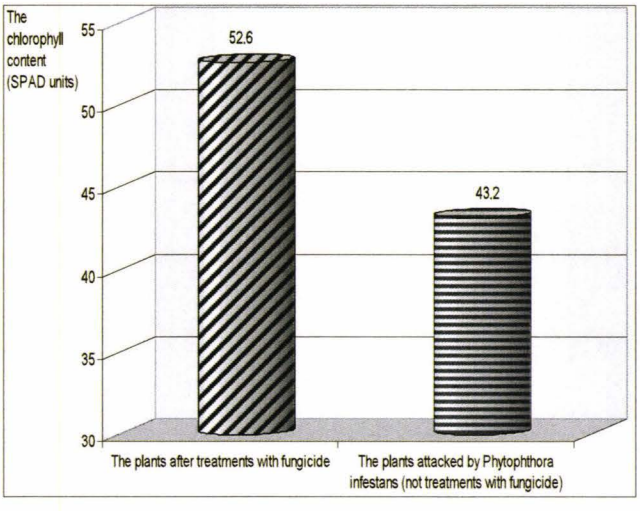

Figure 13. The chlorophyll content in the leaves of tomato plants (*Lycopersicon esculentum* Mill.).

CONCLUSIONS

After the application of the treatments with fungicides in the tomato plants attacked by *Phytophthora infestans* (Mont.) de Bary it has been noticed the significantly reduced degree of diseases, the reduction of the area affected by the pathogen; the applied treatments have also influenced the plant growth and the further development of the plants.

In tomato plants it has been noticed that the dynamics of photosynthesis and transpiration, during the day, in the plants attacked by the pathogen is similar to that in plant analysed after the treatments with fungicide, but the recorded values are lower in the attacked plants.

At the plants attacked by the pathogen, it was noticed that photosynthesis and transpiration intensity is lower as a result of the reduction of the leaf surface due to the formation of large, grey-green or brown spots, deterioration of the chlorophyll pigments and stomata coverage by the pathogen.

The attacked tomato plants present a lower chlorophyll content and a lower water content, in comparison with the tomato plants analysed after the treatments with fungicide, which caused hydric and metabolic imbalances.

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RADIOSENSITIVITY OF MAIZE TO GAMMA RADIATION BASED ON PHYSIOLOGICAL RESPONSES

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Abstract. The present work introduces the study of two maize lines dose-dependent radiosensitivity, based on physiological responses, namely germination, plant growth and development and content of photosynthetic pigments. The experimental material is represented by maize hybrid Turda Star and one of the parental lines. Dry seeds were exposed at doses ranging from 2 to 50 Gy. Our results showed that exposure at doses between 2 to 50 Gy resulted in a significant increase of hybrid growth parameters, expressed through the final germination percentage, speed of germination, root and shoot length. Moreover, plants derived from irradiated seeds exhibited an increased content of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) than non-irradiated ones. In parallel with this findings, the seeds belonging to the parental line showed a different pattern, respectively all studied parameters were increased at doses up to 5 Gy, while at higher doses (15-50 Gy) were significant decreased as compared with the control. The present results highlight the superiority of using maize hybrids over the parental forms. Low gamma-irradiation of maize hybrid seeds can be used to produce seedlings with improved traits. Also, it is confirmed the existence of a threshold between positive and negative effects induced by gamma radiation.

Keywords: gamma radiation, germination potential, growth parameters, maize, photosynthetic pigments.

Rezumat. Radiosensibilitatea porumbului la radiaţii gamma pe baza răspunsului fiziologic. În lucrarea de faţă este prezentat studiul privind radiosensibilitatea a două linii de porumb în funcţie de doza de iradiere. Acesta este evaluat pe baya răspunsului fiziologic, respectiv a procesului de germinare, de creştere şi dezvoltare a plantelor şi a conţinutului de pigmenţi asimilatori. Materialul experimental este reprezentat de hibridul de porumb Turda Star şi de una din formele parentale. Seminţele în stare uscată au fost expuse la o sursa de radiaţii gamma, la doze cuprinse între 2 şi 50 Gy. Pe baza rezultatelor obţinute se observă că expunerea seminţelor hibridului Turda Star la doze cuprinse între 2 şi 5 Gy a dus la o creştere semnificativă a parametrilor de creştere, exprimaţi prin procentul final de germinare, viteza de germinare şi lungimea rădăcinii şi tulpinii. Mai mult, plantele derivate din seminţele iradiate au prezentat un conţinut mai ridicat de pigmentţi asimilatori (clorofila, clorofila b, carotenoizi) faţă de cele neiradiate. În paralel cu aceste observaţii, s-a remarcat ca seminţele aparţinând liniei parentale s+au comportat diferit, mai exact toţi parametrii studiaţi au crescut până la doza de 5 Gy, în timp ce la doze mai ridicate (15-50 Gy) acesteti au fost semnificativ reduşi faţă de martor. Rezultatele obţinute evidenţiază superioritatea hibrizilor faţă de formele parentale. Expunerea seminţelor hibridului de porumb la doze joase de radiaţii gamma poate fi utilizată ca o metodă de obţinere a plantelor cu caracteristici îmbunătăţite. De asemenea, se confirmă existenţa unei doze prag între efectele pozitive şi cele negative induse de radiaţiile gamma.

Cuvinte cheie: capacitate germinativă, parametri de creştere, pigmenţi asimilatori, porumb, radiaţii gamma.

INTRODUCTION

Gamma radiation has been extensively used in plant breeding programs. As a mutagenic agent, gamma radiation can induce both beneficial and harmful effects on plants (KUMAR & KUMAR, 2009). In this regard, the hormesis term has been defined as a dose-response phenomenon characterized by a switchover from low dose stimulation to a high dose inhibition (KIM et al., 2004).

In order to evaluate the effects induced by gamma radiation, a number of parameters indicating plant response have been used. The most widely used indicators are those based on physiological changes such as seed germination and plant growth stimulation or inhibition (BORZOUEI et al., 2010). SINGH & DATTA (2010) observed that low doses of gamma radiation improved plant vigour, grain development and yield attributes of wheat. The positive effects induced by low gamma doses on plant growth and development derived from irradiated seeds have been also reported in grapevine (CHARBAJI & NABULSI, 1999) and rocket (MOUSSA, 2006). In the case of exposure at high doses, CHAOMEI & YANLIN (1993) observed that there is a reduction of the germination capacity with a corresponding decline of plant growth and development at wheat (*Triticum aesticum* L.). Exposure of tabacco (*Nicotiana tabaccum* L.) young plants at doses ranging from 30 to 50 Gy resulted in a significant decrease of the growth process, while at 70 Gy it was completely stropped (CHO et al., 2000). High doses of gamma radiation disturbed the germination process of *Echinacea purpurea* (L.) Moench, leading to a reduction of germination capacity (ICHIM et al., 2006). KIONG et al. (2008) stated that despite the diversity of gamma ray targets in plants, the photosynthetic apparatus is one of the main targets. Studies on mung bean (*Vigna radiata* (L.) R.Wilczek) (SINGH & SHARMA, 1992), alfalfa (*Medicago sativa* L.) (REJILI et al., 2008), rice (*Oryza sativa* L.) (SHEREEN et al., 2009) and okra (*Abelmoschus esculentus* Moench) (HEGAZI & HAMIDELDIN, 2010) revealed that exposure at low doses lead to an increase of the assimilatory pigments content, while at high doses it decreases significantly as compared with non-irradiated ones.

Maize (*Zea mays* L.) is one of the most important crop plants. Due to its high yield, as well as its usage in a variety of human activities, maize is one of the most cultivated species worldwide, ranking the third in cultivated area after rice and wheat, and the first as production (CRISTEA et al., 2004). Beside its economic importance, for over 100 years maize has served as a premiere model organism for biological research (LAWRENCE et al., 2008). Nowadays it is a widely used model organism in many biological investigations such as heterosis, quantitative inheritance, plant

domestication, developmental physiology, genome evolution, epigenetics, pest resistance, comparative genomics and plant response to various environmental factors (STRABLE & SCANLON, 1999).

The aim of the present study is to study the dose-dependent response of two maize line following exposure at gamma radiation based on physiological responses, namely germination, plant growth and development and content of photosynthetic pigments.

MATERIALS AND METHODS

Seeds of maize triliniar hybrid Turda Star (TSh) and one of the parental line (PL), obtained from Agricultural Research and Developmental Station Turda, Romania, were used as experimental material. Dry seeds were exposed at a ^{60}Co gamma source (Gamma Chamber 900 at Faculty of Physics, Babeş-Bolyai University) at a dose rate of 5.72 Gy/h. The applied doses were 0 Gy, 2 Gy, 5 Gy, 15 Gy, 30 Gy and 50 Gy.

The irradiated and non-irradiates seeds (40 seeds/dose) were sown on moistened filter paper and then germinated in laboratory conditions (20°C and 8h photophase). Seeds were considered germinated when the radical extension reached 2 mm in length.

In order to evaluate the effects on the germination process there were calculated two indices, namely the Final germination percentage (FGP) and the speed of germination (SG). They were calculated as described in Table 1.

Table 1. Description of parameters used to study the seed germination.

Germination index	Formula	Description	Reference source
FGP	$FGP = \frac{N_T}{N} * 100$	*N_T: proportion of germinated seeds in each treatment for the final measurement* *N: Number of seeds used in bioassay*	ANJUM & BAJWA 2005
SG	$GS = (N_1 * 1) + \frac{N_2 - N_1}{2} + \cdots + \frac{N_n - N_{n-1}}{n}$	*N_1, N_2, N_3, N_{n-1}, N_n: proportion of germinated seeds observed on day 1, 2, 3,..., N*	CHIAPUSIO et al (1997)

Measurements of seedlings root and shoot length, both for the control and the irradiated samples, were recorded on the 7th day after the start of the experiment.

Chlorophyll a, chlorophyll b and carotenoids content of fresh leaves was determined according to the MORAN & PORATH (1983) method. Leaves of 17 days old plants were cut into small pieces and suspended in DMF (N,N-dimethylformamide), approximately 100 mg vegetal material/2 ml DMF. The samples were left to incubate for 48h in the dark at 4°C. The absorbance was measured with a spectrophotometer (UV/VIS SP8001 Metertech Spectrophotometer at Department of Experimental Biology and Biochemistry, Institute of Biological Research, Cluj-Napoca) at 664 nm, 647 nm and 480 nm. Chlorophyll a, chlorophyll b and carotenoids pigments were calculated according to equations of WELLBURN (1994) and expressed in µg/g fresh weight (FW).

The differences in average of all tested parameters between irradiated and non-irradiates samples was realized by subjecting the experimental data to one-analysis of variance (One-way ANOVA) with Dunnett's post test, at a 5% level of probability. The statistical analysis was performed using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego, USA) and the graphics were realized with Excel program.

RESULTS AND DISCUSSIONS

Seed germination test revealed the dose-dependent response between the two maize lines. As illustrated in Fig. 1, exposure of Turda Star hybrid at 2 Gy lead to a significant increase of the FGP by 8%. At higher doses, it is observed a significant increase by 12% and 14% at 5 Gy, respectively at 15 Gy. As compared with the control, exposure at 30 Gy and 50 Gy resulted in a highly significant increase of the percentage of germinated seeds by 17% and 20%. Unlike these results, the parental line showed a different pattern. It can be noticed that at 2 Gy and 5 Gy the FGP is increased by 2% and 4%, which are no statistically significant. At doses > 5Gy, respectively at 15 Gy, 30 Gy and 50 Gy the final germination percentage was significant decreased by 8%, 12% and 16% than the control.

The speed of the germination process for TSh showed a significant increase with increasing the irradiation dose (Fig.2). Thus, comparing with the control, exposure at 2 Gy, 5 Gy, 15 Gy and 30 Gy resulted in the increase of SG by 135, 17%, 24%, respectively 29%. The highest acceleration of the germination process, by 35%, was recorded at 50 Gy. In seeds belonging to the PL there was observed an insignificant increase by 5 and 7% at 2 Gy and 5Gy. Reported at non-irradiated samples, the seeds exposed at doses ≥ 15 Gy showed a statistically significant delay in the germination process by 13-20%.

Gamma radiation had a positive significant effect on TSh root length (Table 2). Following exposure at doses ranging from 2 to 50 Gy, it was observed an increase of root length by 24-99% as compared with the control. Regarding shoot length it can be seen that at 2 Gy there was an insignificant increase by 3%, while plants derived from seeds exposed at higher doses (5-50 Gy) showed a significant increase of shoot length. Exposure of PL seeds at 2 Gy and 5 Gy resulted in an increase of root (9 and respectively 17%) and shoot (5 and 9%) length, which is not significant in statistical terms. At 15 Gy, plants showed a slightly decrease, by 21% of root length and a significant reduction of shoot

length by 26%. Higher doses of gamma radiation, respectively 30 Gy and 50 Gy, significantly affected plant growth and development, the root length was decreased by 38 and respectively 61% and shoot length by 31 and 42%.

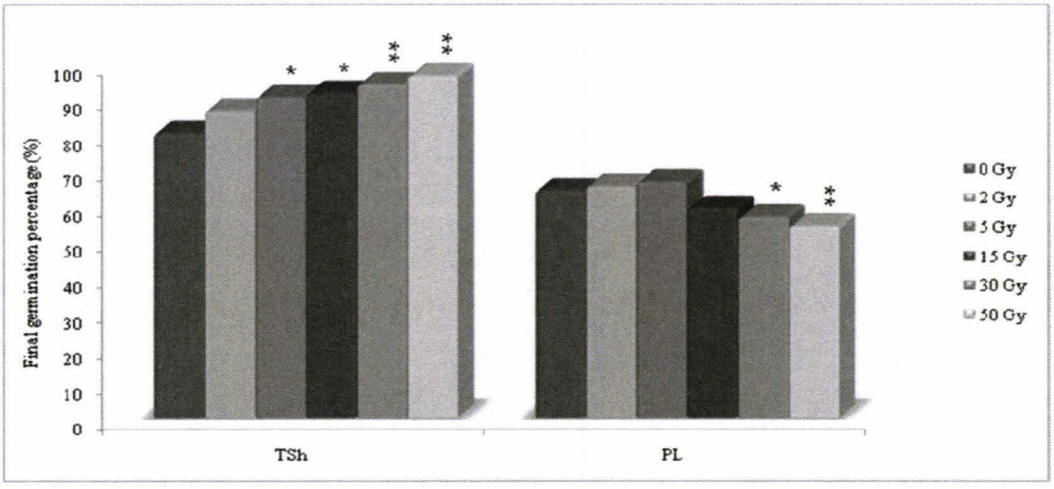

Figure 1. Effect of gamma radiation on final germination percentage

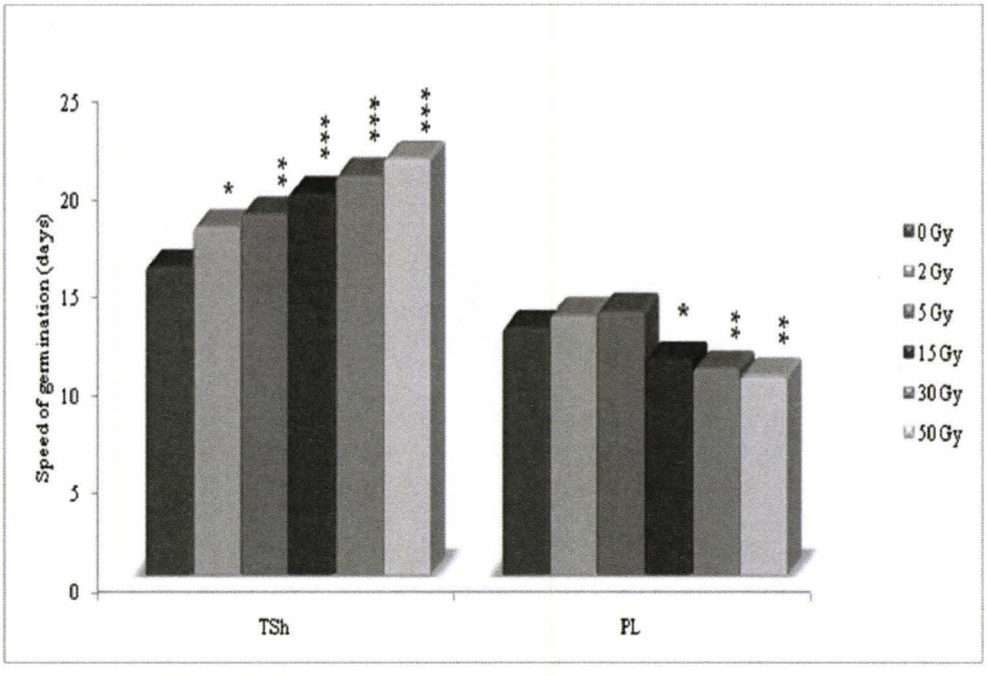

Figure 2. Effect of gamma radiation on maize speed of germination.

Table 2. Effects of gamma radiation on root and shoot length (cm).

	Turda Star Hybrid		Parental line	
Gamma radiation treatment (Gy)	*Root length (cm)*	*Shoot length (cm)*	*Root length (cm)*	*Shoot length (cm)*
Control (0)	1,5	0,6	0,9	0,55
2	1,8*	1,2	0,98	0,52
5	1,9**	1,5**	1,06	0,58
15	2,5***	1,8**	0,7	0,39*
30	2,8***	1,9***	0,5*	0,39*
50	2,9***	2,2***	0,3**	0,35**

The content of the assimilatory pigments in 17-days old TSh leaves is presented in Fig. 3. The statistical analysis revealed that comparing with the control the content of chlorophyll a significantly increased in plants derived from irradiated seeds, reaching the maximum increase, by 44%, at 50 Gy. Chlorophyll b, as well as the carotenoids showed a significant increase up by 105%, respectively 101%, in irradiated samples, as compared with the control.

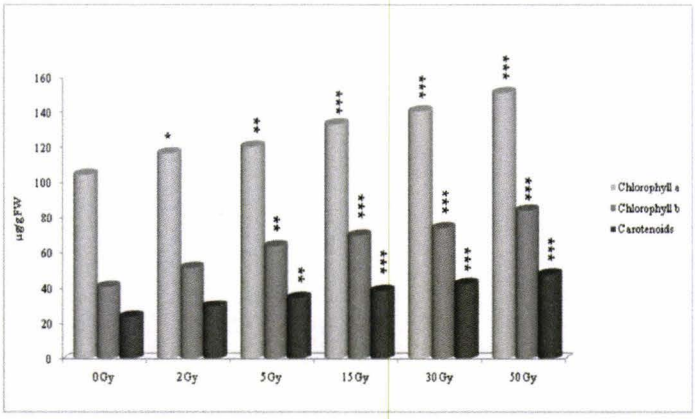

Figure 3. Effects of gamma radiation on assimilatory pigments in maize Turda Star hybrid leaves.

The analysis of the assimilatory pigments content in PL plants (Fig. 4), highlights the positive effects of doses ≤ 5 Gy, where it was recorded the maximum increase by 14% of chlorophyll a, by 38% of chlorophyll b and by 31% of carotenoids. Comparing with the control, doses between 15 and 50 Gy induced a decrease of assimilatory pigments, the minimum values being observed at 50 Gy where leaves showed a reduced content of chlorophyll a, chlorophyll b and carotenoids by 14%, 35%, respectively 32%.

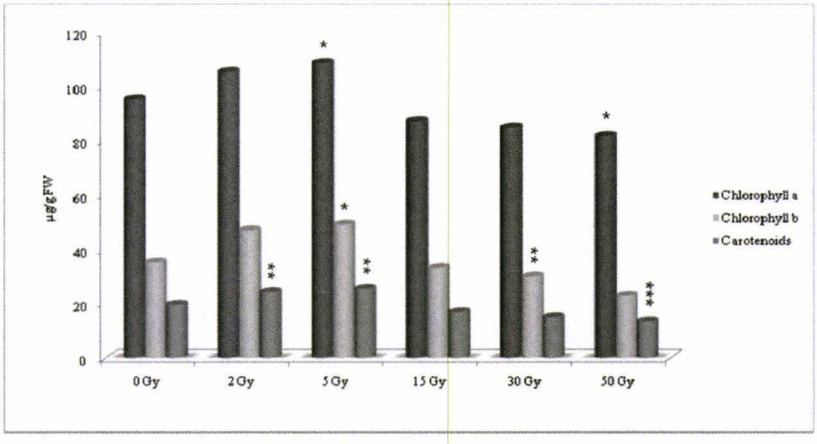

Figure 4. Effects of gamma radiation on assimilatory pigments content in maize Parental Line leaves.

Plant radiosensitivity is dependent upon several factors, some related to plant characteristics (e.g. species/cultivar/variety, plant age, physiology, tissue architecture, morphology and genome organization) and some related to radiation features (e.g. type of radiation, dose rate and time of exposure) (DE MICCO et al., 2011; JAN et al., 2012).

The results obtained in this study highlight the superiority of a maize hybrid unlike the parental form. This phenomenon is called heterosis and it can be defined as the increase in size or growth rate of hybrids over parental line, manifested in quantitative characters and expressed as increased biomass, growth rate, fertility, resistance to diseases and insects as well as tolerance to abiotic factors (CRISTEA et al., 2004).

The stimulating effects induced by gamma radiation on plant growth and development may be attributed to an increase of plant growth regulators (auxins and gibberellins) levels, which enhances the mitotic activity in the meristematic tissues by increasing the number of dividing cells (LATIF et al., 2011). ABDEL-HADY et al. (2008) stated that activation of RNA or protein synthesis during the early stage of germination in irradiated seeds may enhance the germination process. Along with the stimulation of cell division and enzymatic activity, there is an increase of mineral and water uptake, which can explain the increase of assimilatory pigments in plants derive from seeds exposed at low doses (MAJEED et al., 2010).

Gamma radiation exerts its negative effects through the increase amount of free radicals resulted from water radiolysis, which damage important cellular components and perturb vital processes (FAN & SOKORAI, 2005). Reactive oxygen species are believed to be a major contributing factor to stress injuries and cause rapid cellular damage because they are highly reactive to membrane lipids, protein and DNA (EL-BELTAGI et al., 2011). CHATURVEDI et al. (2012) stated that increased radiation doses enhanced the membrane permeability resulting in higher loss of leachates and reduced germination percentage. PREUSS & BITT (2003) attributed the decreased shoot and root length at higher doses of gamma rays to a reduced mitotic activity in meristematic tissues due to cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome. Photosynthesis, an important marker of internal plant metabolism, together with cell growth, is among the primary processes that are in general, affected by gamma

radiation (SINGH et al., 2013). According to STRID et al (1990) the photosynthetic pigments can be destroyed by gamma rays with concomitant loss of photosynthetic capacity. BYUN et al. (2002) explained this by gamma radiation ability to break chlorophyll molecules apart.

CONCLUSIONS

Gamma radiation exposure of maize Turda Star triliniar hybrid at doses ranging from 2-50 Gy resulted in a increase of 8-20% of the final germination percentage, 13-35% of the speed of germination, 24-99% of the root length and 3-38% of shoot length. As compared with the control, the content of chlorophyll a increased by 11-44%, chlorophyll b by 25-105% and carotenoids 25-101. Seeds of the Parental Line submitted to exposed at doses up to 5 Gy showed an increase of the final germination percentage by 4%, of the speed of germination by 6.6%, root length by 18%, shoot length by 9.3% and the content of chlorophyll a increased by 14%, chlorophyll b by 39% and carotenoids by 31% as compared with the control. At higher doses all studied parameters were decreased, the lowest values being recorded at 50 Gy where FGP decreased by 16%, the GS by 19%, root and shoot length by 61%, respectively by 42%, chlorophyll a by 13%, chlorophyll b by 35% and carotenoids by 31%. The present results highlight the superiority o maize hybrid over the parental forms. Concerning the irradiation dose, our results confirm the conclusions of previous studies regarding the hormetic effect induced by gamma radiation. Low gamma-irradiation of maize hybrid seeds can be used to produce seedlings with improved traits.

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ANTIOXIDANT RESPONSE IN SOYBEAN CELL SUSPENSIONS TREATED WITH FUNGAL ELICITORS

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Abstract. Soybean, *Glycine max* (Linnaeus 1737, Merrill 1917), is a cultivated plant with major economic value, so it is very important to maintain this crop-yield and in the same time to minimize the environmental impact, using environmental friendly technologies. The aim of this study was to describe the antioxidant response induced in soybean cell suspensions by fungal elicitors obtained from *Trichoderma viride, Trichoderma harzianum, Penicillium chrysogenum* and *Botrytis cinerea* strains. We used six experimental variants, represented by various combinations of the fungal filtrates, obtained using different procedures (E1, E2, E3, E4, PD-E1, PD-E4, each with 1%, 5% and 10% concentrations (w/v)). Soybean cell suspensions were treated for 24 hrs with the corresponding fungal filtrates, and after 24 hrs, quantitative and qualitative determinations of antioxidant enzymes were performed. Among the tested antioxidant enzymes, the peroxidases (POX) activity was the only one increasing in all experimental variants and for all elicitor concentrations used, except for 10% concentration of E1 variant. In case of catalases (CAT), the enzymatic activity increased only in E2 variant at 1% fungal filtrate concentration and E4 at 1% and 5% fungal filtrate concentration, the other variants presenting a decrease of enzymatic activity compared to the control. Superoxide dismutase (SOD) activity was generally higher in treated variants, with a maximum for 5% fungal filtrate concentrations in E1 and E4 variants. Concluding, some defense mechanisms were activated by fungal filtrate administration, and the E4 variant was the most effective in the activation of soybean cell suspension enzymatic antioxidant system by increasing the activities of the three major enzymes involved in reactive oxygen species detoxification, POX, CAT and SOD, at 1 and 5% concentration.

Keywords: fungal elicitors, cell suspension, soybean, antioxidant enzymes.

Rezumat. Răspunsul antioxidant al suspensiilor celulare de soia tratate cu elicitori fungici. Soia, *Glycine max* (Linnaeus 1737, Merrill 1917), este o plantă de cultură cu o valoare economică deosebită. Astfel, este foarte importantă menţinerea productivităţii acestei culturi şi în acelaşi timp minimizarea impactului asupra mediului înconjurător prin utilizarea unor tehnologii nepoluante. Scopul prezentului studiu este de a descrie modificările biochimice în suspensiile celulare de soia, induse de tratamentul cu filtrate fungice obtinute din tulpini de *Trichoderma viride, Trichoderma harzianum, Penicillium chrysogenum* şi *Botrytis cinerea.* Au fost utilizate şase variante experimentale, reprezentate de diferite amestecuri de filtrate fungice, obţinute utilizând diferite proceduri (E1, E2, E3, E4, PD-E1, PD-E4, fiecare variantă în concentraţie de 1%, 5% şi 10%). Suspensiile celulare de soia au fost tratate timp de 24h cu filtratele fungice în concentraţiile amintite iar după 24h au fost realizate analize calitative şi cantitative ale unor enzime antioxidate. Dintre enzimele testate, activitatea peroxidazelor (POX) a crescut la toate variantele testate, la toate concentraţiile de elicitori utilizate, cu excepţia concentraţiei de 10% a variantei E1. În cazul catalazelor (CAT), activitatea enzimatică a crescut la plantele tratate cu filtratul fungic corespunzător variantei E2 la concentraţia 1% şi la varianta E4 concentraţiile 1% şi 5%. Celelalte variante au prezentat o descreştere a activităţii enzimatice în comparaţie cu varianta martor. Activitatea enzimatică a superoxid dismutazelor (SOD) a fost în general mai ridicată la variantele tratate, înregistrând o valoare maximă la concentraţia de 5% a variantelor E1 şi E4. În concluzie, unele mecanisme de apărare ale celulelor de soia au foste activate în urma administrării filtratelor fungice. De asemenea, administrarea filtratului fungic din varianta E4, având concentraţiile de 1% şi 5%, a fost cea mai eficientă pentru activarea sistemului antioxidant enzimatic la suspensii celulare de soia, prin creşterea activităţii enzimatice ale enzimelor implicate în neutralizarea speciilor reactive de oxigen, POX, CAT şi SOD.

Cuvinte cheie: elicitori fungici, suspensii celulare, soia, enzime antioxidante.

INTRODUCTION

Plant cell suspensions are ideal experimental systems for the study of morphogenetic processes, secondary metabolism and the response of cells towards aggressive environmental agents, including pathogen attack. Cellular suspensions are relatively homogenous systems, axenic, with cells deprived of cuticle, with reduced needs for intercellular transport and almost all are metabolically active.

G. max is a cultivated plant with major economic value, soybean seed being the world main source of vegetable protein and oil. This valuable plant species has numerous pathogens, which can cause significant crop losses, so it appeared the necessity to develop new technologies to improve disease resistance and implicitly to prevent crop losses.

This goal can be achieved by activation of plant defense system, enhancing basal resistance to pathogens. Plants resistance to pathogens can be enhanced by both biotic and abiotic factors (elicitors). Among biotic resistance inducers, microbial cultures were often used to activate different defense responses in plants (TON et al., 2002, BENHAMOU et al., 2003, SIDDIQUI & MEON, 2009, AN et al., 2010). Also, it was studied the utilization of elicitors, both exogenous (SHIMIZU et al., 2013) and endogenous (MOHARAM, 2013) for defense activation against pathogens. Although different aspects of disease resistance activation in plants were intensely studied, there are no reports regarding fungal filtrates utilization for defense induction in soybean cell suspensions. The goal of the present study is to describe the biochemical response of soybean cell suspensions to the treatment with fungal elicitors. The elicitors were represented by a mixture of fungal filtrates obtained from strains of antagonistic species *Trichoderma viride* and *Trichoderma harzianum,* a non-pathogenic species *Penicillium chrysogenum* and a pathogenic species, *Botrytis cinerea.* The elicitors from these microorganisms can activate plant defense responses dependent on various signalling molecules, like

salicylic acid, jasmonic acid or ethene. In our study, combining fungal filtrates from these four different species we intended to activate different defense signalling pathways and to induce defense-specific reactions in our *in vitro* system.

MATERIALS AND METHODS

Soybean cell suspension preparation

We used two-weeks old plantlets obtained from aseptic germinated seeds of the Romanian cultivar Daciana (provided by the NARDI Fundulea). Explants of 3-5 mm from cotyledon, epicotyl, hypocotyl and leaf fragment were cultivated on callusogenesis culture medium. Cell suspensions were obtained on S2-4 liquid medium, from fragments of callus induced on the same growth medium supplemented with agar. The (growth) callusogenesis medium contains essential nutrients, micronutrients and vitamin B5 GAMBORG (1968), to which we added 1 mg/l 2,4D, 0.1 mg/l BAP, 0.3 g/l hydrolysed casein, 7 g/l agar and 30 g/l sucrose. The callus was grown in dark conditions, at a temperature of 25°C ± 2°C.

Treatment protocol

The seven variants used by us were represented by: M - control (no treatment), E1 - treatment with fungal filtrate obtained from *Botrytis cinerea* strains F1 (BcF1), E2 - treatment with fungal filtrate obtained from *Botrytis cinerea* strains Bc F1, Bc F7, Bc S1, Bc P2, E3 - treatment with fungal filtrate from *Trichoderma viride* strains P 456 (TvP456), TvP1, *Trichoderma harzianum* ThP8 and *Penicillium chrysogenum* A2 (Pc A2), E4 - treatment with fungal filtrate obtained from strains Bc F1, Bc F7, Bc S1, Bc P2, TvP456, TvP1, ThP8, Pc A2. PD1-E4 and PD2-E1 represented the technological variants of E4 and E1 fungal filtrate, which differed in concentration of initial fungal inoculums. Soybean cell suspensions were treated for 24 hrs with the six fungal filtrates in 1%, 5% and 10% concentrations (w/v), each with three replicates, and were maintained in dark, on an orbital shaker at 80 rpm. Each variant was.

Total protein extract preparation

The samples used for this experiment consisted of both cellular suspension soybean cells and cellular suspension filtrate. Total cytosolic proteins were extracted by grinding the cells with quartz sand in phosphate buffer 0.05M, pH7, with 2 mM Na_2EDTA and 4% (w/v) PVP, at 40°C. Extracellular proteins were obtained by suspension cell filtration. After centrifugation at 15,000 rpm, for 15 min, the supernatant was stored at 4°C, and the total protein content was measured according to BRADFORD (1976).

Enzyme quantification and electrophoretic analysis of isoenzymes

The quantification of enzymatic activity of peroxidases, catalases and superoxide dismutases was done as described before (HELEPCIUC et al., 2014 – in press).

Isozyme electrophoresis was done in native polyacrylamide gel (10% polyacrylamide for POX and SOD, 8% polyacrylamide for CAT) in 50mM Tris-glycine buffer, pH 7, at 10 mA for migration and 15 mA for concentration for 2 hrs. POX were stained using the method with benzidine in acetate buffer pH 5 (WANG & WANG, 1989). Superoxide dismutase (SOD) was stained with Nitro Blue Tetrazolium according to BEAUCHAMP & FRIDOVICH (1971), and catalase (CAT) was stained according to the method described by IORDĂCHESCU & DUMITRU (1988). For quantification of each enzyme activity we have used three replicates.

RESULTS AND DISCUSSIONS

Callus and cell suspension particularities

In order to obtain cellular suspensions, it is important that the inoculum, represented by callus, to be friable. However, we have obtained a compact, proliferative, non-morphogenic and very adherent callus, characteristics that allowed us only to obtain a cell suspension formed of cell aggregates with a 1-2 mm diameter, rich in chlorophyll. The structure of the obtained callus is probably a result of a very active secondary metabolism and a high content of proteins involved in adhesion (KITABAKE & FUJITA, 2000). Optic microscopy analysis of this callus on squash samples identified small cells, with a weakly developed vacuome and a high rate of division, and of elongated cells, with well-developed vacuoles and a high metabolic rate (Fig 1). In addition, we observed xylematic cells that facilitate the transport of nutrients towards the interior of cell clusters (Fig. 1).

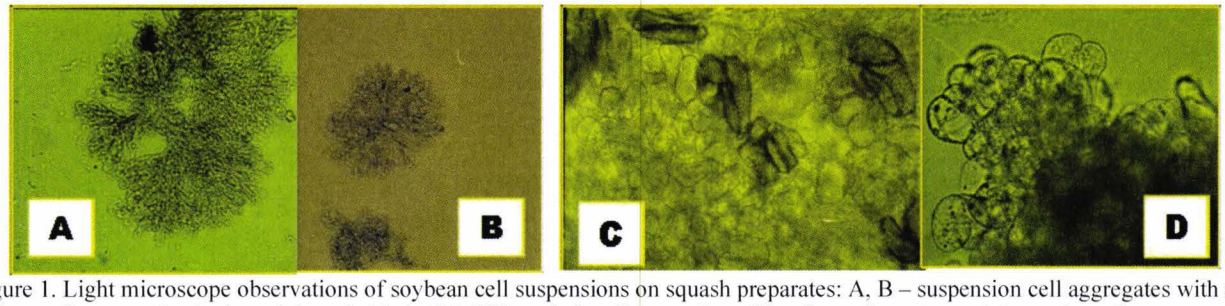

Figure 1. Light microscope observations of soybean cell suspensions on squash preparates: A, B – suspension cell aggregates with a 1-2 mm diameter; C – (xylematic) tracheids (cells) differentiation; D – small proliferating cells at the periphery of a cell aggregate.

Cell suspension response to fungal elicitors

Administration of fungal elicitors did not induce either visually or microscopically changes in the treated variants comparing with the control, but at biochemical level, the analysis of enzymes involved in oxidative stress control, like POX, CAT and SOD indicated a defense response of the soybean cells suspension. SOD activity increased compared to the control, but the response was not uniform, in several experimental variants the enzyme activity decreasing at higher concentrations of the elicitor. The highest SOD activity value was recorded for the 5% elicitor concentration in E1 experimental variant, while in 10% concentration variant the enzyme activity decreased (Fig. 2). The superoxide dismutase spectrum (Fig. 3) reveals five SOD isoenzymes in all variants except for E1 at 5% and 10% concentrations, in these two variants only two isoforms being expressed. The SOD isozymes are not correlated with enzyme activity measurements.

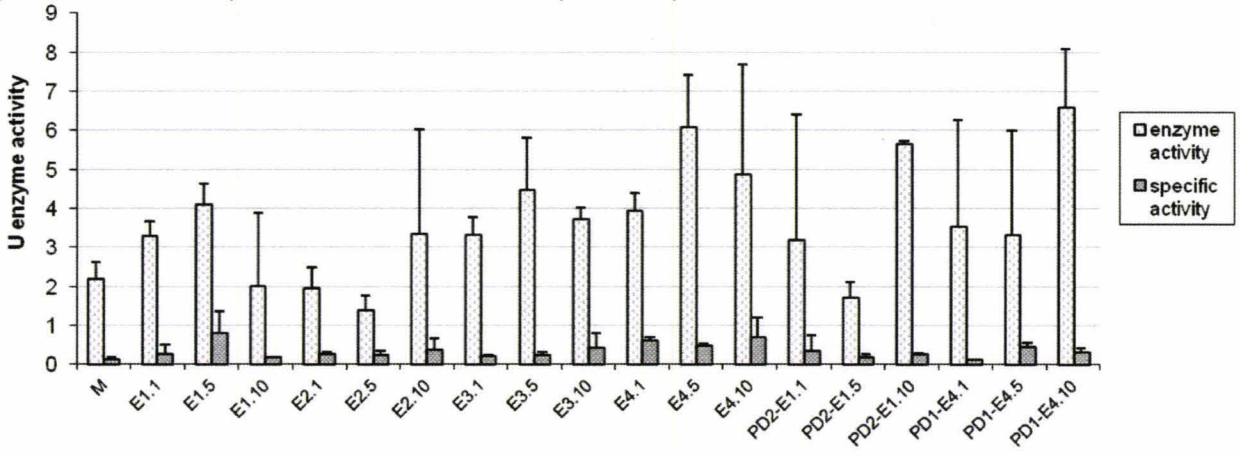

Figure 2. SOD activity in untreated cell suspensions (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

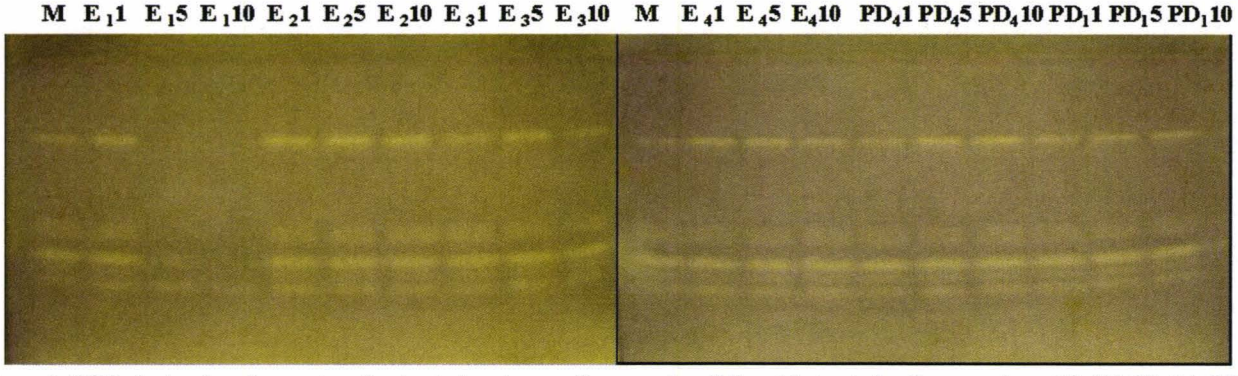

Figure 3. SOD electrophoretic spectra of untreated soybean cell suspension (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

The treatment with fungal filtrates induced an increase in POX activity independent of concentration and the type of elicitor used (Fig. 4). The highest POX activity level was observed for the 1% concentration of the experimental variant E4. The treatments with the lowest concentration of fungal filtrate (i.e. 1%) induced an increase in POX activity in variants E2 and E4.

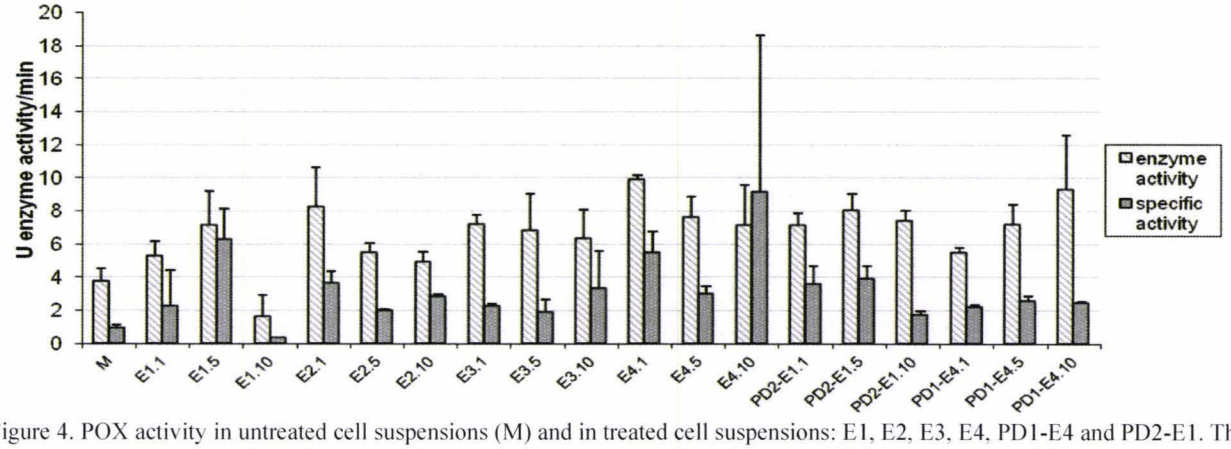

Figure 4. POX activity in untreated cell suspensions (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

Electrophoresis of POX showed differences in the activities of the isozymes, generally correlated with the dynamics of overall POX activity. POX isozymes are concentrated in the slow-migration zone and their activity increases in the E4 experimental variant. In case of E4 variant, new isoforms of POX are present in the samples treated with high concentrations (10%) of fungal filtrate (Fig. 5, see arrow). It can also be observed that some treatments induce an overexpression of some isoforms, for example the isoperoxidase from the last position on the gel in case of E2 variant (Fig. 5, see arrow) and the isoperoxidase from the first position on the gel in case of E4 variant (Fig. 5, see arrow).

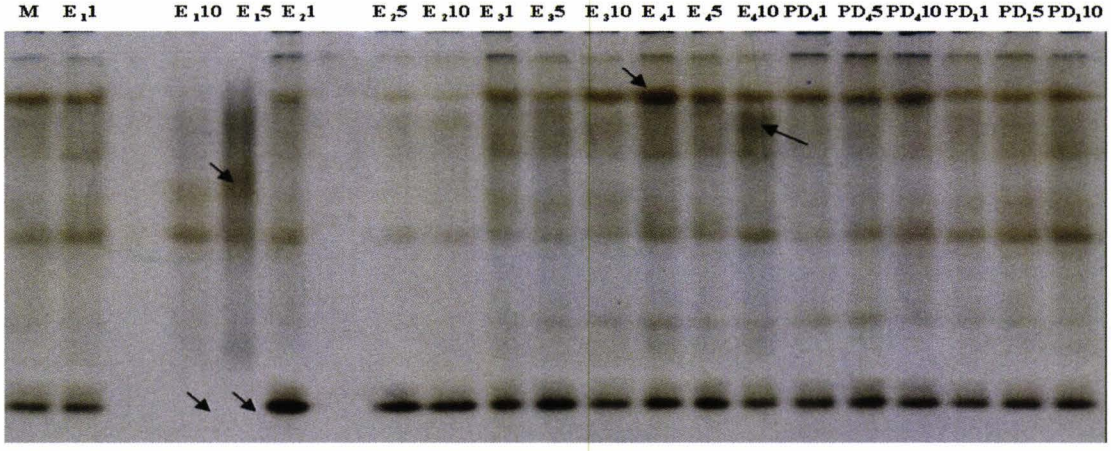

Figure 5. POX electrophoretic spectra of untreated soybean cell suspension (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

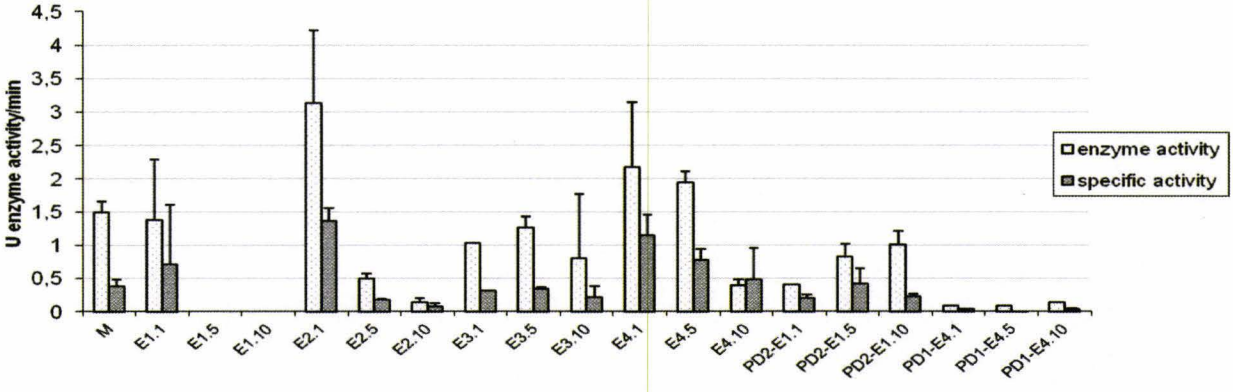

Figure 6. CAT activity in untreated cell suspensions (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

CAT activity is generally decreasing comparing with the control, except for several experimental variants which present an increase: E2 for a 1% fungal elicitor concentration and E4 at 1% and 5% (Fig. 6). In all these variants POX activity was also higher. Electrophoresis of CAT in soybean cell suspensions showed a single isoenzyme (Fig. 7) that is absent in experimental variant E1, in higher concentrations, results which are correlated with the quantitative assay of CAT. Also, in 5% E2 and 10% E3 variants the CAT isoform is not expressed.

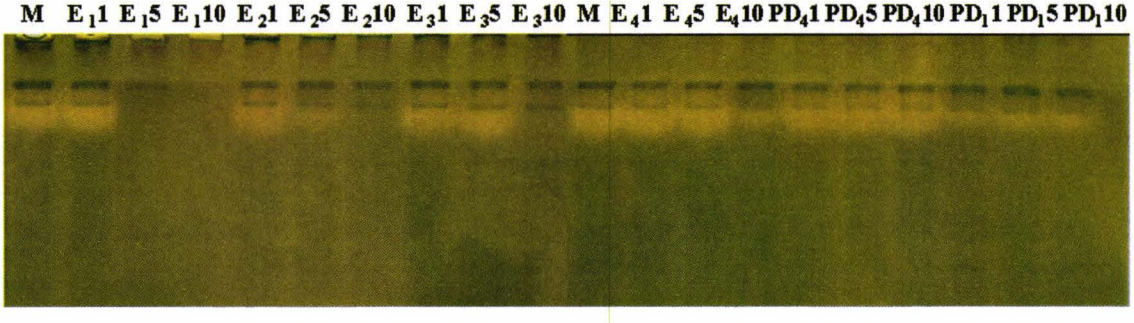

Figure 7. CAT electrophoretic spectra of untreated soybean cell suspension (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

Peroxidase extracellular enzymatic activity in soybean cell suspensions was higher than at cellular level, which indicates an intense excretion of peroxidases in extracellular medium. As in case of intracellular peroxidases, the enzymatic activity in culture medium was generally higher than the control, except for E1 and PD-E1 variants (Fig. 8).

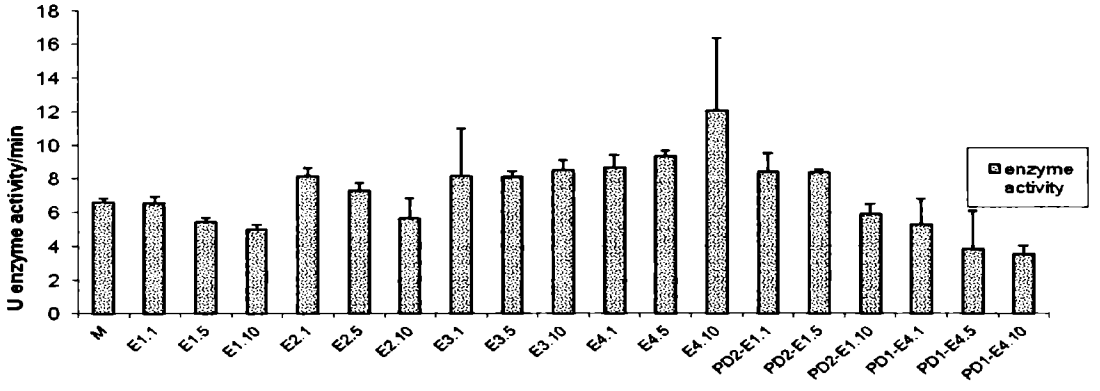

Figure 8. POX extracellular activity in untreated cell suspensions (M) and in treated cell suspensions: E1, E2, E3, E4, PD1-E4 and PD2-E1. The second digit of each sample represents the concentration of the fungal filtrate 1%, 5% or 10%.

Although our previous results showed an induction of catalase-detoxifying H_2O_2 excess pathway (COGĂLNICEANU et al., 2010, HELEPCIUC et al., 2014 in press), the current results indicate an involvement of peroxidase rather than catalase in oxidative stress response of the cell to biotic elicitors. Similar results were mentioned previously in scientific literature, when single elicitors or elicitors from just one species where used. For example, the results obtained by SAISAVOEY et al. (2014) in *Pueraria mirifica* cell suspension treated with a plant endogenous elicitor, methyl jasmonate, showed that catalase also decreased in elicited variants comparing with the control. Also, a study reveals that in *Euphorbia pekinensis* cell culture treated with fungal elicitors from *Fusarium* sp., at 48 hrs catalase activity is slightly higher in elicited variants, but reaches a maximum six days after the elicitor administration (GAO et al., 2011).

However, in our study, the E4 culture filtrate variant succeeded to induce an increase in POX, CAT and SOD enzymatic activities, at 1% and 5% concentration, after 24 hrs treatment. As mentioned before, the E4 variant was obtained by filtration of fungal cultures of four different species, with a total of eight fungal strains, so it contains the highest variety of elicitors comparing with the other variants. Among them, there is a phytopathogenic species *Botritys cinerea*, and three antagonistic species, *Trichoderma viride*, *Trichoderma harzianum* and *Penicllium chrysogenum*. Previous studies reported the activation of various defense responses when mixtures of fungal elicitors were applied (NAVAZIO et al., 2007). It is known that interference of signalling pathways provides a flexibility of plant defense response, conferring improved disease resistance in plants (PIETERSE et al., 2006). Probably our mixtures of fungal filtrates used contained molecules that elicit different signalling pathways which induced antioxidant defense response.

CONCLUSIONS

Fungal filtrates activated the antioxidant defense response in our system, inducing an increase in SOD, CAT and POX antioxidant activity. The best defense response in soybean cell suspensions was obtained by treatment with E4 variant. All three antioxidant enzymes registered higher enzymatic activities comparing with the control. For the rest of the experimental variants, SOD and POX had generally higher enzymatic activities than the control, while CAT showed a lower activity. The mixture of the eight fungal strains from E4 induced the most efficient defense response, activating the antioxidant response reflected by increased enzymatic activity in the experimental system used.

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BIOLOGICAL CHARACTERISTICS AND ACCUMULATION OF POLYPHENOLICS IN *Polygonum sachalinense* INTRODUCED IN THE FLORA OF THE REPUBLIC OF MOLDOVA

IVANOVA Raisa, TITEI Victor

Abstract. This research was designed to study some biological characteristics of autochthonous variety *GIGANT* of *Polygonum sachalinense* F. Schmidt ex Maxim (Giant knotweed) introduced and cultivated in the Republic of Moldova. The accumulation of polyphenolics in both under-ground and aerial parts of a plant was also investigated. The total polyphenolic content was determined by the Folin-Ciocalteu procedure. For chemical analyses we used fresh collected samples of rhizomes, leaves and flowers. The results of biological indexes determination showed that in the natural conditions of R. Moldova the plants developed intensively, the productivity of aerial biomass reaching 8.99 kg/m^2 or 2.69 kg/m^2 of dry matter. Plants were not affected by atmospheric drought, diseases and pests. The flowering phase begins after 135...150 days of vegetation, but the plant does not form fertile seeds. The variety *GIGANT* can be multiplied only by vegetative procedure from rhizomes or cuttings. The accumulation of polyphenolic substances in leaves took place in diverse periods of vegetation at different rates, but the increase of polyphenolics content in rhizomes was constant. In the period of flowering the richest polyphenolics content was determined in flowers about 34.90±0.55 mg per g of fresh weight, which decreased deeply in the period of seed formation (approximately 5 times).

Keywords: *Polygonum sachalinense*, variety *GIGANT*, biological characteristic, polyphenolics.

Rezumat. Particularităţi biologice şi acumularea de polifenoli la specia *Polygonum sachalinense* introdusă în flora Republicii Moldova. Această cercetare a fost concepută pentru a studia unele caracteristici biologice ale soiului autohton *GIGANT* de *Polygonum sachalinense* F. Schmidt ex Maxim (hrişca de Sahalin) cultivat în Republica Moldova. În plus a fost investigată acumularea polifenolilor în părţile atât subterane cât şi aeriene ale plantelor. Conţinutul total de polifenoli a fost determinat prin procedura Folin-Ciocalteu. Pentru analize chimice s-au folosit mostre proaspăt colectate de rizomi, frunze şi flori. Rezultatele determinărilor ale indicilor biologice au arătat că în condiţiile naturale ale R. Moldova plantele se dezvoltă intens, productivitatea biomasei aeriene atinge 8.99 kg/m^2 sau 2.69 kg/m^2 de substanţă absolut uscată. Plantele nu au fost afectate de seceta atmosferică, boli şi dăunători. Faza de înflorire începe după 135...150 zile de vegetaţie, dar planta nu formează seminţe fertile. Soiul *GIGANT* poate fi multiplicat numai prin procedura vegetativă prin rizomi sau butaşi. Acumularea substanţelor polifenolice în frunze a avut loc în diverse perioade de vegetaţie la rate diferite, dar ascensiunea conţinutului de polifenoli în rizomi a fost constantă. În perioada de înflorire cel mai bogat conţinut de polifenoli a fost determinat în flori 34.90±0.55 mg per g de greutate în stare proaspătă, care a scăzut profund în perioada de formare a seminţelor (aproximativ de 5 ori).

Cuvinte cheie: *Polygonum sachalinense*, soiul *GIGANT*, particularităţi biologice, polifenoli.

INTRODUCTION

The family *Polygonaceae Juss* (knotweed) includes about 50 genera and 1,100 species. *Polygonum L.* genus, comprising approx. 200 extra tropical species typically found in areas of the northern hemisphere, presents a special interest. The giant knotweed, native to the Far East of Russia and northern Japan, was introduced in Europe as ornamental and fodder plant in the 19th century. Being highly invasive, these exotic species is recognized as a major environmental management problem in Europe (GERBER et al., 2008; KOVAROVA et al., 2011). However, many species of *Polygonum spp.* are known due to the high rate of accumulation of biologically active substances beneficial to health (FAN et al., 2009; ZHANG et al., 2005; ABD EL-KADER et al., 2013). Nine species of *Polygonum L.* were identified in the flora of R. Moldova, the most common of which is *Polygonum aviculare* L., annual species with medicinal utility as antioxidant and anti-inflammatory preparation (HSU, 2006; GRANICA et al., 2013). Due to the stable production and tolerance to pedoclimatic factors, the perspective species for the Moldavian flora is the giant knotweed *Polygonum sachalinense* F. Schmidt ex Maxim, known also by other synonym names: *Reynoutria sachalinensis* (F. Schmidt) Nakai, *Fallopia sachalinensis* (F. Schmidt) Ronse DECR., *Pleuropterus sachalinensis* (F. Schmidt) H. Gross, *Tiniaria sachalinensis* (F. Schmidt) Janch (STRASIL, 2006). As a result of many years work for the naturalization, introduction and adaptation of *Polygonum sachalinense* F. Schmidt ex Maxim to the conditions of the Republic of Moldova, it has been created a new autochthonous variety named *GIGANT* and registered in the Catalogue of Plant Varieties of R. Moldova in 2012. This species is a natural source of secondary metabolite compounds, which possesses biological activity. From different plant organs there have been isolated the compounds of stilbene group (resveratrol and its glycoside derivates) and polyphenolics group (flavonoids, phenylpropanoids and its glycoside derivates). Resveratrol and related phytocompounds exhibit antioxidant, anti-inflammatory and anticancer effects (GHANIM et al., 2010; LI et al., 2013; YAN et al., 2014). It is known that the content of polyphenolics predetermined the biological activities, in special radical scavenging activities (FU et al., 2008; IVANOVA, 2011). However, the chemical profile of polyphenolic composition of knotweed species cultivated in R. Moldova is not studied. The aim of these researches was to determine the biological characteristics and dynamics of polyphenolics accumulation during the vegetation period in different parts of *Polygonum sachalinense* F. Schmidt ex Maxim, variety *GIGANT*.

MATERIAL AND METHODS

Plant materials: rhizomes, leaves and flowers of *Polygonum sachalinense* F. Schmidt ex Maxim, variety *GIGANT* were collected in Chisinau area of the Republic of Moldova (lat. 47°01', long. 28°52', alt. 173 m above sea level), from the Botanical Garden (Institute) plantation in the season of 2013:

1) in April after 20 days of vegetation (Fig.1a);
2) in June after 60 days, the end of intensive growth (Fig. 1b);
3) in September after 150 days, phase of florescence (Fig. 1c);
4) in October after 180 days, phase of seed formation (Fig. 1d).

The biological characteristics have been studied according to the methodical recommendation (IVANOV, 1985).

The total polyphenolic content has been determined by Folin-Ciocalteu procedure (SINGLETON et al., 1999) and calculated in gallic acid equivalent, in mg per g of fresh weight (FW).

Figure1. *Polygonum sachalinense F.* Schmidt ex Maxim, variety *GIGANT* in different phases of vegetation.

RESULTS AND DISCUSSIONS

In the first year of vegetation in the period between 20 and 25 days (in April) after bud emergence, the aerial part of *Polygonum sachalinense F. Schmidt ex Maxim, variety GIGANT* grown up slowly, formed the rosette comprising only of 3…5 leaves. During the next growth period, the development was accelerated by shoots initiating, the height of which at the end of May reached to 47…63 cm, and had 5…7 formed internodes with leaves of 19…23 cm in length and 8…11 cm in width. There was observed the branching of the central stem from the seventh internode giving rise to shoots of the first, the second and the third degrees. So, at the end of August, the plants reached 164…170 cm and the stems between the first and the sixth internodes became ligneous; the flower buttons were forming. In the underground, there was developed the turning root system, which consists of a main root, lateral roots and viable forms of rhizomes with dormant buds. Next year, new shoots will develop from these buds. The majority of the roots were concentrated in the soil layer of 5…30 cm, but some roots penetrated to a depth of 65…70 cm. Over 5-7 days from the first autumn frosts, the leaves fell completely off shoots.

In the second year of vegetation, the plants revival started in the end of March; one bush contained about 9 shoots, which reached 3 m at the end of the year of vegetation. In the underground, the part of plants the intensely developed root system formed the rhizomes of 2.3…4.2 cm thickness.

In the third year, the development rate was accelerated; one bush already gave about 11…15 shoots with the dispersion area of 40…75 cm from the planting place. The plant height during 20 days of vegetation reached 1.5 m, in mid-June - 3.1…3.6 m and in the flowering period was above of 4.2 m.

Table 1. Biological characteristics of *Polygonum sachalinense* F. Schmidt ex Maxim, variety *GIGANT.*

Month of sampling	Height of stems, m	Biomass productivity, kg/m^2	Dry matter content, kg/m^2	
			leaves	total
April	0.73±0.01	2.63±0.02	0.15±0.01	0.27±0.01
June	3.49± 0.09	7.79±0.14	0.73±0.03	1.93±0.08
September	4.08±0.14	8.99±0.18	0.69±0.05	2.69±0.05
October	3.94±0.07	6.24±0.04	0.30±0.02	2.18±0.04

The results of biological indexes determination in 2013 showed that the plants developed intensively, the productivity of aerial biomass reaching to 8.99 kg/m^2 or 2.69 kg/m^2 of dry matter (table 1). The quota of leaves biomass in total harvested biomass during the vegetation period was decreased, the highest 37% being in June. Plants were not affected by atmospheric drought, diseases and pests. The florescence phase began after 135…150 days of vegetation, but the plant did not form fertile seeds. The variety *GIGANT* must be multiplied only by vegetative procedure from rhizomes or cuttings.

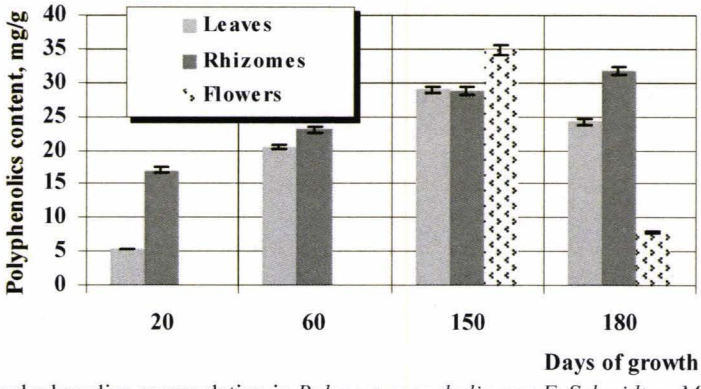

Figure 2. Dynamics of polyphenolics accumulation in *Polygonum sachalinense F. Schmidt ex Maxim, variety GIGANT.*

The dynamics of synthesis and accumulation of polyphenolic substances in leaves and rhizomes of *Polygonum sachalinense* F. Schmidt ex Maxim *variety GIGANT* throughout the growing season is represented in figure 2. In April at the 20th day of growth, the leaves accumulated 5.89±0.05 mg of polyphenolics per g, which was 3 times less than in rhizomes. In the period of 20-60 growing days, there has been observed the fastest accumulation of polyphenolic substances in leaves, when the plants intensively developed. After that, the content of polyphenolics continued to increase in leaves till the flowering period, but more slowly. In the period of seeds formation, the content of polyphenolics in leaves decreased by 1.2 times in comparison with the flowering period. This fact reflected the physiological reconstruction of plants and their preparation for winter. Thus, the accumulations of polyphenolic substances in leaves took place in diverse periods of vegetation at different rates, but the increase of polyphenolics content in rhizomes was constant (Fig. 2). The content of polyphenolics in flowers was 34.90±0.55 mg/g in the period of abundant flowering, and 7.76±0.15 mg/g in the period of seeds formation.

CONCLUSIONS

This study suggests that *Polygonum sachalinense F. Schmidt ex Maxim, variety GIGANT* cultivated in Moldova manifested the highest biological characteristics of development and multiplication, being a promising source of polyphenolics accumulated during the vegetation period; however, the potential properties of biological activity have to be further studied.

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MEDIUM-TERM PRESERVATION OF *Dianthus trifasciculatus* Kit ssp. *parviflorus* THROUGH MINIMAL CULTURES

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Abstract. The species taking in our study. *Dianthus trifasciculatus* ssp. *parviflorus* is a critically endangered taxon. *in vitro* studies being useful for *ex situ* preservation purpose. Owing to *in vitro* rapid growth and proliferation. it is important to establish a medium-term conservation protocol based on minimal cultures. Several factors were tested concerning the ability to reduce the growth of tissues cultures and to maintain regeneration capacity. Mannitol and PEG 4000 were proven to be the most suitable for the establishment of minimal cultures. Regenerants from three years old minimal cultures maintained in the presence of mannitol were analysed concerning isoenzymes spectrum for peroxidase. esterases. alkaline phosphatases and catalases. Biochemical analyses have shown that peroxidases (POX) and catalases (CAT) are sensitive to the culture conditions (the maintenance in presence of osmolytes). The treatment with 6% mannitol influenced the intensity of the bands analysed in the case of rooted plants even after 2 months of culture in the absence of any osmolyte. The analysis of esterases and alkaline phosphatases spectra has shown the regenerants identity.

Keywords: *Dianthus trifasciculatus* Kit ssp. *parviflorus*. medium-term. minimal cultures. mannitol. isoenzymes spectra.

Rezumat. Conservarea pe termen mediu a speciei *Dianthus trifasciculatus* Kit ssp. *parviflorus* în culturi minimale. Specia studiată de noi, *D. trifasciculatus* ssp. *parviflorus* este un taxon critic periclitat. studiile *in vitro* fiind utile pentru conservarea *ex situ*. Datorită creşterii şi proliferării rapide este important să se stabilească un protocol de conservare pe termen mediu bazat pe culturi minimale. Mai mulţi factori au fost testaţi pentru abilitatea de a reduce creşterea culturilor de ţesuturi şi de a menţine capacitatea regenerativă. Manitolul şi PEG 4000 s-au dovedit a fi cei mai potriviţi pentru stabilirea culturilor minimale. Regeneranţi proveniţi din culturi minimale de trei ani. menţinute in prezenţa manitolului au fost analizaţi pentu spectrul peroxidazelor. esterazelor. fosfatazei alcaline şi catalazelor. Analiza biochimică a aratat că peroxidazele şi catalazele sunt sensibilie a condiţiile de cultură (menţinerea în prezenţa osmoliţilor). Tratamentul cu 6 % manitol influenţează intensitatea benzilor analizate în cazul plantelor înrădăcinate chiar după 2 luni de cultură în absenţa oricărui osmolit. Analiza spectrelor esterazei şi fosfatazei alcaline au arătat indentitatea regeneranţilor.

Cuvinte cheie: *Dianthus trifasciculatus* Kit ssp. *parviflorus*. termen-mediu. culturi minimale. manitol. spectrele izoenzimatice.

INTRODUCTION

Biotechnology has proved its role for the plant biodiversity preservation, several results being obtained in the case of cultivated plants, but also in the case of wild species and especially the threatened ones (BENSON, 1999; HOLOBIUC, 2006; SARASAN et al., 2006; ENGELMAN, 2010; REED et al., 2011; BUNN et al., 2011). As a huge progress occurred in this area of research and many achievements are permanently reported, it is very important that the results (technologies) to be recognized as useful and to be effectively integrated in the conservation programs (BENSON, 1999).

Biotechnology alternative based on *in vitro* cultures has not to substitute the classical successful methods, but to complement them or to insure a reliable alternative in the case of threatened taxa with reproduction problems, low variability and/ or small populations.

Tissue cultures ensure the preservation during different periods of time, independent of climate changes, pests, in limited spaces. On the other hand, owing to the requirement of qualified staff, expenses for establishment and maintenance of cultures and energy consumption, it is necessary to evaluate if the proposed aim of the conservation is appropriate to be achieved using *in vitro* cultures. For this reason, it is important to establish optimized, reproducible and low costing technologies, which can be easily implemented in practice.

Depending on the durations and methods used, three levels of conservation are available for *ex situ* preservation: 1) short- term conservation: primary tissues cultures established for multiplication purpose, for temporary storage of plant germplasm, for repopulation of the natural habitats and for international exchanges, 2) medium-term conservation, during months and years, based on growth retardation of the tissues cultures 3) long-term conservation, relied on cryopreservation procedures, which allow the maintenance on an indefinite period of time.

Medium-term conservation is based on the maintenance of tissue cultures in the presence of different restrictive factors. The so called "slow growth" method is the result of the reduction of growth rate and multiplication (WHITERS, 1987). Minimal cultures are obtained owing to this growth reduction and development through the modulation of different factors (LYNCH, 1999). The interval of transfers can be prolonged, the cultures vessels can be smaller and medium consumption and handling are also limited.

Medium-term preservation based on minimal cultures was extensively used especially in the case of cultivated species (MALAURIE, 1993; WYSOTSKAYA, 1994; REED & CHANG, 1997; REED, 1999; SARKAR & NAIK, 1998, SARKAR et al., 1999; GOPAL et al., 2002; CHA-UM et al., 2006) and also of wild threatened plants (REED et al., 2011).

In this field, different methods have already been reported based on the modulation of the physical factor as: temperature, light, oxygen content (MULLIN & SCHLEGEL 1976; WESCOTT, 1981; REED, 1993; MORIGUGHI et al., 1995, CACHIŢĂ & HALMAGY, 1997; SARKAR & NAIK, 1998), a combination of several factors (REED, 1999; NEGASH et al., 2001) or chemical factors as: nutrient reduction (low mineral or sucrose concentrations) (NG & NG, 1991,

MALAURIE et al., 1993), encapsulation in alginate (WITHERS, 1991; ENGELMANN, 1991; MALAURIE et al., 1998, HOLOBIUC et al., 2009b), addition of osmotic active compounds (STARITSKY et al., 1986; NG & NG, 1991; CONSTANTINOVICI, 1996; GOLDMIRZAIE & TOLEDO, 1999, NAGATOME et al., 2000; HOLOBIUC et al., 2009a; 2010) or the use of the growth retardants as flurprimidol or ancymidol (JARRET et al., 1991; SWARUP et al., 2001) or growth inhibitors as Abscisic acid (HALMAGY et al., 2001).

For the *ex situ* preservation of threatened plant taxa, slow growth methods or minimal cultures represent convenient alternatives and they can contribute to the establishment of *in vitro* collections (CONSTANTINOVICI, 1996; PENCE, 1999; HOLOBIUC et al., 2010).

In Romania, several achievements concerning the establishment of primary cultures and multiplication based on short-term protocols in *Dianthus* threatened species were obtained (ZĂPARŢAN, 1995; CRISTEA et al., 2002; MICLĂUS et al., 2003; CRISTEA et al., 2004; MARCU et al., 2006; HOLOBIUC et al., 2006; HOLOBIUC et al., 2009c; HOLOBIUC et al., 2010b; HOLOBIUC et al., 2013; CRISTEA et al., 2013; JARDA et al., 2014).

In the case of medium-term and long- term conservation of threatened *Dianthus* taxa, just few studies were reported in Romania (HOLOBIUC et al., 2009a, b; HOLOBIUC et al., 2010; CATANĂ et al., 2010; JARDA et al., 2011).

The species analysed in our study, *D. trifasciculatus* ssp. *parviflorus* is a critically endangered taxon (DIHORU & NEGREAN, 2009), *in vitro* studies being useful for *ex situ* preservation purpose.

In this taxon, an optimized protocol for short-term preservation and multiplication have already been reported (HOLOBIUC et al., 2013), but as all *Dianthus* species, this plant has an *in vitro* rapid growth and multiplication rate, for this reason being important to establish a medium-term conservation protocol based on minimal cultures.

MATERIALS AND METHODS

For medium-term cultures initiation, we used as explants double node shoots fragments collected from the primary regenerative cultures initiated from one individual. They were inoculated on different media variants based on MURASHIGE & SKOOG formula (1962), added with Gamborg vitamins (GAMBORG et al., 1968) and supplemented with different compounds. Several factors were tested for the induction of minimal cultures as osmolytes (PEG 4000, mannitol, sucrose) and plant growth regulators as Abscisic acid (ABA) and Jasmonic acid (JA) (Table 1).

Table 1. Media composition used for the induction of minimal cultures in *D. trifasciculatus* ssp. *parviflorus*.

Composition		Media variants							
		M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
Macroelements		MS	MS	MS	MS	MS	MS	MS	MS
Microelements		MS	MS	MS	MS	MS	MS	MS	MS
Complex B Vitamins		B 5	B5	B5	B5	B5	B5	B5	B5
Plant regulators (mg/l)	**Abscisic acid**	-	-	-	-	-	-	**20**	-
	Jasmonic acid	-	-	-	-	-	-	-	**3**
Other compounds (g/l)	**Mannitol**	-	**30**	**60**	-	-	-	-	-
	PEG 4000	-	-	-	-	-	**60**	-	-
	Sucrose	30	30	30	**60**	**90**	30	30	30
Agar (g/l)		8	8	8	8	8	8	8	8

For each treatment, there were cultured 5 explants/ glass vessel in 3 repetitions. All the cultures were maintained in the growth chamber at 2000 lux illumination and 16/8 photoperiod and 25^{0}C temperature.

The cultures were evaluated using 2 parameters: the maximum length of the developed shoots/ initial explant (in mm) and the mean number of regenerants/ initial explant scored after different time intervals (40, 80 and 120 days, respectively) to characterize the behaviour and main step of establishment of minimal cultures in this taxon.

Graphic values are expressed as mean values ±SD. The data were statistically analysed using Daniel's XL Toolbox version 6.52. One-way analysis of variance (ANOVA) was applied to calculate the statistical significance at $p<0.05$. Multiple Tukey Comparison test was also used to compare the means at 5% probability level.

The minimal cultures with the best response concerning the growth reduction and regeneration capacity were maintained in the *Dianthus* taxa collection established by us during several years.

From three years old minimal cultures, maintained on 3% and 6% mannitol added media, were regenerated plants after their rooting on IBA (1mg/l) supplemented culture medium. These regenerants were biochemically analysed concerning isoenzymes spectrum for peroxidase, esterases, alkaline phosphatases and catalases to check if the preservation in medium-term culture during several years influenced their stability.

Biochemical analyses of the regenerants from medium-term cultures: rooted plants obtained from 3 years long-term cultures maintained in the presence of mannitol 3 % and 6 % as factor which reduces the growth, but also compatible with the maintenance of regeneration ability and long survival of the cultures were analysed concerning the pattern of several isoenzymes as peroxidases, esterases, catalases and alkaline phosphatases.

Two samples were collected from every 5 jars (I-V) and the number of individuals analysed were 1, 1', 2, 2',3, 3', 4, 4' and 5, 5'. The samples from the first jar (1 an1') derived from medium-term cultures maintained in the presence of 6% mannitol, the others from cultures maintained in the presence of 3% mannitol.

Preparation of total protein extract.

The plant material was ground with quartz sand for obtaining the homogenate of the total protein extract. The extraction of enzymes was carried out in 0.05M phosphate buffer pH 7.2mM EDTA, PVP 4%, at 4°C for 4h. After centrifugation at 18000 rpm, for 20 min, the supernatant was used for electrophoresis. It was prepared the running polyacrylamide gel 7% (for catalases) and 10 % (for esterases, phosphatases and peroxidases) and stacking gel; as buffer we used 0.05M Tris-Gly, pH 8.3. The running marker was bromophenol blue.

Isozymes electrophoretic spectra.

a) For peroxidases detection, a solution of benzidine in acetate buffer and H_2O_2 as substrate was used. The bands were stained in brown (WANG & WANG, 1989). A solution of benzidine in acetate buffer and H_2O_2 was used as substrate.

b) For esterases detection, the substrate α, β–naphthyl acetate and Fast Blue RR dissolved in phosphate buffer 0.1 M, pH=6.5 were used. The bands were stained in red-violet. (BACH, 1989, a modified method).

c) For catalases, 0.003% H_2O_2 prepared in 0.01 M phosphate buffer, pH=7, 2% $K_3(Fe(CN)_6)$ and 2 % $FeCl_3$ were used as substrate. The bands were stained in yellow with a background green-blue (IORDĂCHESCU & DUMITRU, 1988).

d) For phosphatases alkaline, as substrate Na α –naphthyl phosphate, 0.5M $MgCl_2$ and 0.25 M $MnCl_2$ and Fast Blue RR in Tris-citrate, pH=8,3 was used. The bands were stained in brown.

RESULTS AND DISCUSSIONS

As a condition to establish medium-term cultures as *ex situ* preservation tool is necessary to have a reproducible and affordable protocol, which can help to minimize the growth and also to maintain the regeneration ability with a reduction of the interval of cultures transfers.

Using double node-stem fragments as explants, several variants of media were tested concerning the growth and regeneration response of the explants to check which is the most appropriate for our aim to maintain in collection as minimal cultures. The parameters scored after different time intervals (40, 80 and 120 days) showed significant differences concerning the growth and regeneration between M1 (control) and the other variants M2-M7 (Tables 2 and 3).

Concerning the growth reduction, the first parameter scored by us (the maximum growth or length of the shoots developed on the initial explant), comparing to M1 medium, on almost all variant tested (excepting M8 added with Jasmonic acid) was registered a growth retardation effect (Fig. 1).

In the case of the osmolytes addition in the culture media, the reduction of growth was pronounced in the first months. This effect of growth limitation was maintained during the time as effect of osmotic stress imposed by mannitol, PEG or sucrose.

In the case of use of PEG 4000 at the level tested (6%), the reduction of growth registered after 40 days was weaker compared to mannitol and even to sucrose added media. The rooting process occurred more slowly compared to the variants with mannitol.

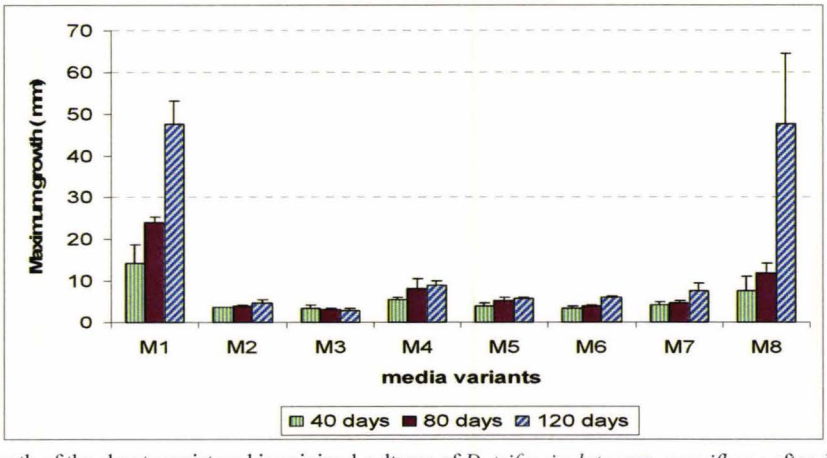

Figure 1. Maximum growth of the shoots registered in minimal cultures of *D. trifasciculatus* ssp. *parviflorus* after different interval of time.

Table 2. Mean values and standard deviation for maximum growth of shoots/explant registered on different media variants after 40, 80 and 120 days.

Variants analysed	M1	M2	M3	M4	M5	M6	M7	M8
40 days	14,33±4,34*	3,6±0,2 *	3,46±0,7*	5,47±0,5*	3,87±0,83*	3,4±0,52*	4,27±0,64*	7,53±3,51*
80 days	24±1,44*	3,87±0,23*	3,2±0,34*	8,27±2,41*	5,27±0,75*	3,93±0,3*	4,73±0,57*	12±2,39*
120 days	47,67±5,47*	4,67±0,94*	3±0,4*	8,87±1,28*	5,8±0,2*	6±0,34*	7,73±1,81*	47,52±16,95

Legend: Values are means of 3 replicates with standard error. * showed that the values are significantly different from the M1 (control) by the Tukey test at ($P<0.05$).

Cultures performed on variants M2, M3, M6 showed a growth limitation similarly after 40 days.

The level of 6% sucrose was less limiting concerning the growth of shoots comparing to the higher level (9%) concerning the growth of shoots. After increasing the period of the culture, sucrose in excess proved to have a toxic effect inducing shoots death, despite of some inductor effect concerning the regeneration, aspect more evident after 3 months of exposure. Some already developed shoots degenerated and some new developed. After 120 days, a generally reduction of the growth of the shoots was noticed in the cultures maintained on the variants M2-M7 (Fig. 1).

Abscisic acid presence (20 mg/l) determined the arrest of the growth and absence of regeneration; the lateral meristem of explants could be maintained in a latent stage during 2-3 months.

Jasmonic acid added in variant M8 determined a good vigour of plants, but did not reduce the growth, which was similarly to the control, allowing the development of 1-2 lateral shoots.

Concerning the induction and the maintenance of the regenerative process, the best values were registered in the presence of mannitol 3% and PEG 4000 at 6% level starting from the first month of culture (Fig. 2).

The regeneration capacity scored as mean number of shoots/ explant showed that after a first slow inhibitory effect after the initiation of the cultures in the first month, lately a stimulatory effect was detected; the variant added with mannitol at 3% level induced the best response after 80 days (Fig. 3a) and 120 days of maintenance, the number of regenerants increasing in time despite their reduced growth. The initial explants degenerated, but new regenerants developed starting form lateral meristems as primary origin.

Also the presence of 6% mannitol had an inductor effect on the regeneration process, with the best rate after 120 days. The stress effect was overcome later, in the second month on this variant, the degeneration of the first regenerants occurred, but the extension of the maintenance in the same conditions allowed the development of a large number of small propagules (regenerants) (Fig. 2) after 120 days.

In the case of PEG 4000, the effect concerning the reduction of the growth was not so strong, but also stimulated the regeneration process after a delay of the morphogenesis in the first month, a resuscitation of the process appeared subsequently.

In the first phase of the establishment of the medium-term cultures, PEG presence inhibited the rooting process, but in the second month of culture, the primary stress effect was overcome by the plants adaptation mechanisms and rhizogenesis process was also induced (Figs. 3e, 3c).

The addition of sucrose at 6 and 9% final concentrations as osmotic factors determined the reduction of the growth (Fig. 1) and sustained the regeneration process, especially in the first two months (Fig. 2).

The mean values of the regenerants/explant were lower than mannitol and PEG 4000 - added variants and decreasing in time owing to the degeneration of the neo-formed shoots. Despite of the positive effect observed in the first month, the signs preceding the necrosis and cellular death were correlated to the activation of the synthesis of anthocyanins (Fig. 3d, f). As result, after 120 days, some of the shoots died, but some new buds developed for a short time.

The extension of the duration of the maintenance of the cultures more than 120 days conducted to the conclusion that minimal cultures in this taxon can be optimally performed in the presence of mannitol 3 %, the reduced growth being associated with very good regeneration, results comparable to other *Dianthus* taxa, studied by us (HOLOBIUC et al., 2010).

In the presence of PEG 6%, the regeneration rate decreased comparing to that registered in the presence of mannitol. In the case of sucrose excess, despite the acceptable effect noticed in the first two months, after 120 days, the shoots started to die, after an activation of the synthesis of anthocyanins before this radical response.

In case of the use of Abscisic acid, as growth inhibitor, the level tested by us (20 mg/l) allowed the maintenance of the explant at the initial state for 3 months (the lateral meristems did not grow and not develop lateral shoots). The presence of Jasmonic acid (3 mg/l) had an opposite effect, conducting to similar results concerning the regeneration to the control, but the shoots were better developed.

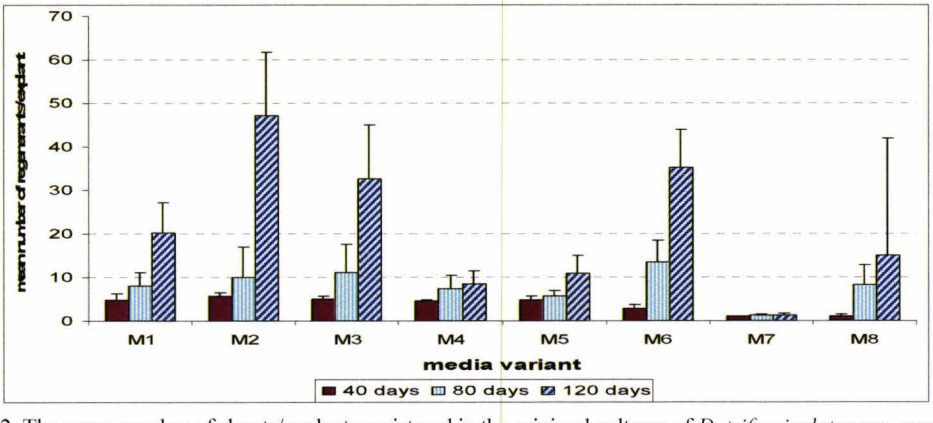

Figure 2. The mean number of shoots/explants registered in the minimal cultures of *D. trifasciculatus* ssp. *parviflorus* after different intervals of time.

Table 3. Mean values and standard deviation for maximum growth of shoots/explant registered on different media variants after 40, 80, and 120 days.

Variants analysed	M1	M2	M3	M4	M5	M6	M7	M8
40 days	4,8±1,4*	5,67±0,9	4,93±0,75	4,67±0,11	4,87±0,8	2,87±0,75	1,07±0,11*	1,08±0,34*
80 days	8±3,04*	9,93±6,98	11±6,53	7,33±3,06	5,6±1,29	13,4±5,06	1,2±0,41*	8,26±4,53
120 days	20,27±6,87*	47,13±14,69	32,66±12,33	8,53±3,04	10,8±4,16	35,13±8,83	1,27±0,45*	15,06±26,81

Values are means of 3 replicates with standard error. * showed that the values are significantly different compared with the M1 (control) by the Tukey test at ($P<0.05$).

Figure 3a. Minimal cultures in *D. trifasciculatus* ssp. *parviflorus* established in presence of 3% mannitol after 80 days (Bar = 1 cm) (original).

Figure 3b. Minimal cultures with direct morphogenesis and activation of the synthesis of anthocyanins in the presence of 6% mannitol after 80 days (Bar = 1 cm) (original).

Figure 3c. Regenerative aggregates with limited growth induced in the presence of 6% PEG 4000 after 80 days (Bar = 1cm) (original).

Figure 3d. Morphogenic minimal cultures with active the synthesis of anthocyanins preceding shoots degeneration in the presence of 9% sucrose after 80 days of culture (Bar = 1 cm) (original).

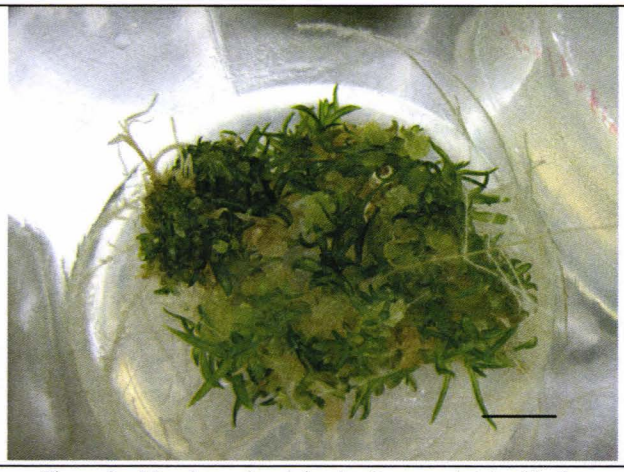

Figure 3e. Morphogenic minimal cultures maintained in the presence of PEG 4000 6% after 6 months (Bar = 1 cm) (original).

Figure 3f. Minimal cultures maintained in the presence of sucrose 6% during 6 months (Bar = 1 cm) (original).

Biochemical analysis of in vitro regenerated plant material from minimal culture.

The genetic stability of the preserved plant material was usually evaluated in the case of cultivated or medicinal plants *in vitro* regenerated and/or maintained in collections. For this purpose, besides molecular markers, biochemical analysis proved to be useful to study the effect of *in vitro* micropropagation and also for the characterization of medium-term cultures maintained *in vitro*.

Isoenzymes spectra are detected by electrophoresis and specific staining of the bands; a certain configuration of the bands can be detected according to the number of the loci, the homo or heterozygous status and the number of subunits (BUTIUC-KEUL, 2006). In fact, isoenzymes are codified by genes situated on different loci but codifying proteins with the same enzymatic activity.

The results concerning the isoenzymes spectra analysed by us in the case of clones of *D. trifasciculatus* ssp. *parviflorus* regenerated from minimal cultures are shown in figure 4.

Electrophoresis spectra of peroxidases (POX).

Peroxidases electrophoretic spectrum in the case of the samples 2,2', 3,3', 4,4' and 5,5' consisted in 5 bands with some differences of intensity in the case of samples 2,2'-5,5'(more intense)- Fig. 4.

Electrophoresis spectra of POX determined in the case of the samples 1 and 1' originated on medium-term cultures maintained in the presence of a higher level of mannitol (6%) showed a different intensity of bands comparing to the samples originated in medium term cultures maintained on lower level of mannitol (3%).

Alkaline phosphatases electrophoretic spectrum showed a single band in all analysed samples, proving the identity of these clones concerning this character (marker).

Isoesterase spectrum showed also the identity in all samples (clones) analysed, with a more intense expression in the case of samples derived of mannitol 6% cultures.

Peroxidases are inducible and more sensitive to the condition of culture and physiological status of the plants, but isoesterases are more stable, being preferred in some cases to asses genetic diversity or variability.

For **catalase spectrum,** in the samples 1 and 1' a low intensity of the bands was detected, but in the case of 3' sample, an intensification of catalase activity and higher intensity of the bands was noticed, meanwhile the clones 2, 2', 3, 4, 4', 5, 5' shown the same pattern of bands.

The use of this biochemical marker is not suitable to check the plants variability, being most susceptible to environmental conditions (the conditions of culture and the developmental stage).

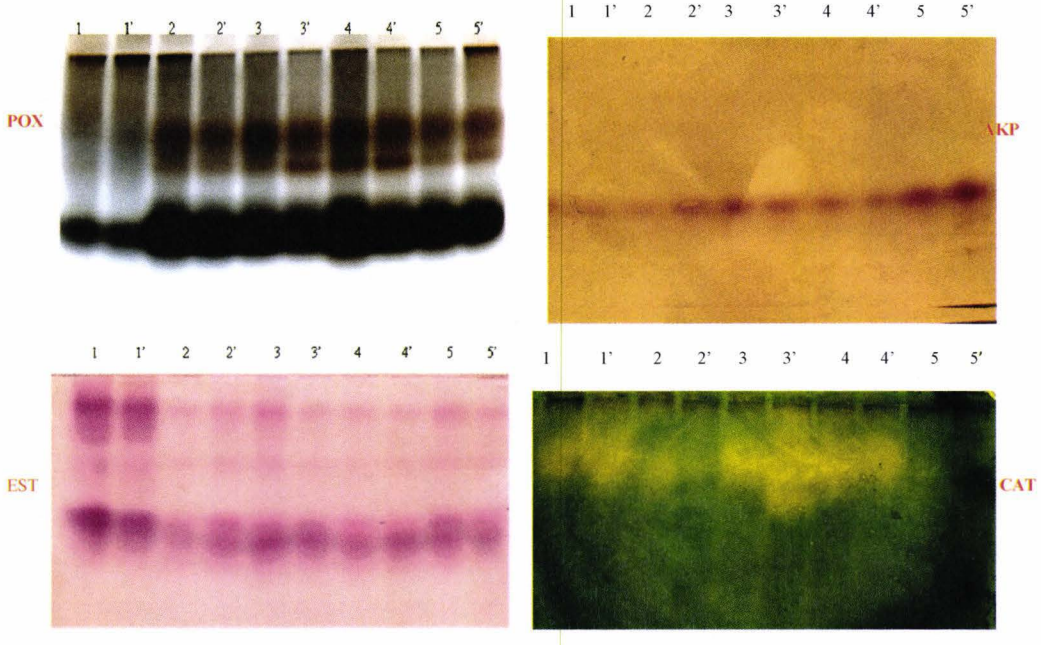

Figure 4. Isoenzymes spectra in the case of regenerants derived from medium-term cultures in *D. trifasciculatus* ssp. *parviflorus* (original).

Isoenzymes were used before to characterize the clones of *in vitro* regenerated *Atropa belladonna* (BUTIUC-KEUL et al., 2005). The aim was to detect lines highly productive of atropine, but isoperoxidases spectrum did not show any differences among the clones.

In the regenerants obtained in the primary cultures of *Arnica montana*, BUTIUC-KEUL & DELIU (2001), reported that isoesterase pattern was the same in all individuals, proved to obtain a clonal propagation.

In sweet potato accessions maintained *in vitro* during 1.6 years, regenerants conserved their isoenzymes profile concerning 5 enzyme systems, showing genetic stability (LAKHANPAUL et al., 1990). KRZAKOWA (1996) reported the use of peroxidase spectrum to detect differences among individuals and populations of *Phragmites australis*.

In the case of two *Dianthus* taxa preserved in medium-term cultures (*D. spiculifolius* and *D. glacialis* ssp.*gelidus*), isoenzymes profiles and activity were used previously to evaluate the cultures maintained in the presence of different levels of mannitol.

The antioxidant enzymes activity and profiles in the first month and after prolonged exposure at osmotic stress (several months) were influenced by the concentration of the osmolytes, the duration of exposure to stress and by the capacity to overcome the stress and to induce developmental processes as morphogenesis and even somatic embryogenesis (HOLOBIUC et al., 2009a; HOLOBIUC et al., 2010). Isoenzymes patterns were correlated to the adaptation to osmotic stress, growth reduction and regeneration process.

CONCLUSIONS

Our studies concerning the induction of minimal cultures in *D. trifasciculatus ssp. parviflorus* proved that taking into account the main purpose, different factors added in culture media can be used to obtain different results: a reduction of growth, but associated to a very high multiplication, a moderate growth and an acceptable multiplication rate or even stopping the development of the explants.

Both mannitol and PEG 4000 can be used for minimal cultures establishment. In case of mannitol, the limitation of growth is stronger and regeneration better. Mannitol at moderate level and PEG 4000 can be used for long-term maintenance of the regenerative cultures. The regeneration rate in the presence of PEG is good, but the rooting of regenerants occurred slowly.

If the aim is just to maintain the explants *per se*, ABA-added medium variant can be used for 2-3 months preservation, the lateral meristems of the explants did not evolve and explants did not root.

On the other hand, the presence of Jasmonic acid in the culture medium improved the plant growth and vigour being suitable to be applied before different selective factors or before acclimatization.

Our results showed that POX and CAT are sensitive to the culture conditions (the previously treatment with osmolytes applied during medium–term cultures). The maintenance of the culture in the presence of 6% mannitol influenced the intensity of the detected bands even after 2 months of culture of regenerated shoots in the absence of any osmolyte.

Esterases and alkaline phosphatases spectra have shown the regenerants plants identity; their origin was the same.

The minimal cultures were successfully established in this taxon and healthy and normal plants can be regenerated even after several years of maintenance.

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FACTORS AFFECTING THE ROOTING OF CUTTINGS OF *Syringa vulgaris* L. CULTIVARS

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Abstract. *Syringa vulgaris* L. is famous for its great diversity of ornamental cultivars and hybrids. The vegetative propagation aims at their conservation and use in the design of gardens and parks. For this reason stem cuttings were collected from old individuals of the species, 'Charles Jolly' and 'Mme Florent Stepman'. One-year old hardwood cuttings were taken in the beginning of spring from dormant individuals and green, softwood cuttings, were collected in the middle of blooming stage. The base of the cuttings was dipped in IBA powder in concentrations 0.3%, 0.5%, and 0.8% and untreated cuttings were used as control. Our results indicated that rooting was not induced on the hardwood cuttings. All treatments with IBA supported the induction of rooting in softwood cuttings and decreased the period of rooting. The cuttings from 'Charles Joly' and *Syringa vulgaris* reached statistically the highest value of rooting (45.0 ± 2.9% and 50.0 ± 5.0%, respectively), but they did not show statistically significant differences between them. The rooting registered the lowest value in the cuttings from 'Mme Florent Stepman' (33.3 ± 13.6%).

Keywords: IBA, lilac, propagation.

Rezumat. Factorii care afectează formarea rădăcinii la butaşii soiurilor de *Syringa vulgaris* L. *Syringa vulgaris* L. este renumit pentru marea sa diversitate de soiuri şi hibrizi ornamentali. Înmulţirea vegetativă are ca scop conservarea şi utilizarea lor în proiectarea de grădini şi parcuri. Din acest motiv, butaşii obţinuţi din tulpină au fost colectaţi de la plantele mature, aparţinând speciilor "Charles Jolly" şi "Doamna Florent Stepman". Butaşii lemnoşi, de un an, au fost prelevaţi la începutul primăverii de la specimenele latente şi butaşii verzi au fost colectaţi la mijlocul stadiului de înflorire. Baza butaşilor a fost scufundată în pudră de IBA, cu concentraţii de 0.3%, 0.5%, 0.8%, dar au fost folosiţi şi butaşi netrataţi, ca probă martor. Rezultatele noastre au indicat că înrădăcinarea nu a fost indusă în cazul butaşilor lemnoşi. Toate tratamentele cu IBA au indicat înrădăcinarea la butaşi obţinuţi din lăstari şi reducerea perioadei de înrădăcinare. Butaşii de la "Charles Joly" şi *Syringa vulgaris* au atins statistic cea mai mare rată de înrădăcinare (45.0 ± 2.9% şi 50.0 ± 5.0%, respectiv), dar nu s-au remarcat diferenţe statistice semnificative între ei. Cea mai redusă rată de înrădăcinare a fost înregistrată la butaşii proveniţi de la "Doamna Florent Stepman" (33.3 ± 13.6%).

Cuvinte cheie: IBA, liliac, înmulţire.

INTRODUCTION

Common lilac (*Syringa vulgaris* L.) is famous for its great diversity of ornamental cultivars and hybrids (KRÜSSMANN, 1984; DIRR & HEUSER,1987; DIRR, 1998; FIALA, 2008) making it very suitable for use in urban areas.

The cloning of its cultivars is performed by grafting on seedlings. However, the production of large quantities of grafts is limited by the season, period duration for rootstock production, and the success depends on the method of grafting (KRÜSSMANN, 1984; DIRR & HEUSER, 1987; DIRR, 1998; FIALA, 2008). Also, the grafting is labour-consuming and needs large areas. That is why the rooting of cuttings is an easier and cheaper method for cloning of the lilac cultivars and hybrids. According to BOJARCZUK (1975) own-root lilacs grow more intensively, exhibit more viability and are considered more resistant to disease than grafted plants. They are also easier to propagate since root sprouts originate from the same cultivar.

Rhizogenesis in cuttings of woody plants can be affected by a variety of factors that include genotype and age of the donor plant, physiological state at the time of excision, age or degree of lignification of the tissues in the cutting and environmental conditions that support the expression of the rooting potential (CAMERON et al., 2003).

Syringa vulgaris cuttings are generally considered difficult to root because they can be rooted only for a short period during the year i.e. only during the phase of full bloom (SCHMIDT, 1978; BOJARCZUK, 1975, 1978a, 1979; DIRR & HEUSER, 1987; DIRR, 1998; HARTMANN et al., 2002; CAMERON et al., 2003). Rooting varies considerably between the cultivars (SCHMIDT, 1978; BOJARCZUK,1979; BASSUK et al., 1984; TYATYUSHKINA, 2007) and depends on their age (WALDENMAIER & BÜNEMANN, 1991), and different part of the shoots (BOJARCZUK & JANKIEWICZ, 1975; SCHMIDT, 1978). It was demonstrated that severe pruning, which promotes rapid growth from relatively few shoots, is beneficial and is widely used since it is considered to restore or maintain juvenile characteristics, including ease of rooting (CAMERON et al., 2003). It was found that etiolation could facilitate the rooting of cuttings (BASSUK et. al.,1984; PATIENCE & ALDERSON, 1984, HOWARD & RIDOUT, 1992). It has been shown that the type of auxin and its concentration is essential for rooting of softwood lilac cuttings; however the percentage of rooted cuttings and the optimal concentrations of auxin are cultivar dependant (BOJARCZUK, 1975; DIRR, 1987).

The aim of this work was to study the effect of the physiological status of the stock plants, cultivar, and concentration of IBA on rooting of *Syringa vulgaris* cuttings.

MATERIAL AND METHODS

Stem cuttings of 8 to 12 cm in length were collected from old individuals of the species, 'Charles Jolly', and 'Mme Florent Stepman'. To study the effect of physiological condition of the donor plant on the rooting, one-year old hardwood cuttings were taken in the beginning of spring from dormant individuals (on March 20 and 21, 2012) and green, softwood cuttings, were collected in the middle of blooming stage (on May 7, 8, and 9, 2012). The leaves from the lower part of the stem (4-8 cm) were removed and discarded and the cuts were made 2-3 mm under the lowest buds. For decreasing of the transpiration, most leaves were eliminated and two-thirds of the leaf blade of all upper leaves was removed. The base of the cuttings was dipped into indole-3-butyric acid (IBA) powder in concentrations 0.3%, 0.5%, and 0.8% and non-treated cuttings were used as control. They were inserted at 3 × 3 cm spacing directly into rooting substrate of peat and perlite (2:1; v/v) on a mist propagation bench in glasshouse (Fig. 1). Air temperature on the mist bench was 20 ± 2°C. The air humidity was controlled by automatic mist system and was 70%. Rooting was defined as the emergence of one or more roots of 3 mm or longer in length. The formation of callus, percentage of rooted cuttings, number of roots per cutting, and root length (total length of all first order roots per cutting was calculated) was recorded after 35, 55, 75, and 95 days.

Figure 1. Cuttings inserted into rooting substrate of peat and perlite (2:1; v/v) on a mist propagation bench in glasshouse.

Each treatment, cultivar, and season contained three replicates and for each of them 20 cuttings were used. The results were analysed by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$, using SPSS 20.0 for Windows. Percentage values were transformed using arcsine square root ($\sqrt{P}$) (COMPTON 1994) to normalize error distribution prior variance analysis.

RESULTS

Callus formation was not observed on the hard-wood cuttings. However, its formation was observed before the rooting of soft-wood cuttings (Fig. 2) after 35 days from the beginning of the experiment and depended on the genotype peculiarities of the investigated cultivars. However, in 'Charles Joly' the rate of cuttings, formed callus increased until the 55th day. In the highest concentrations of IBA (0.5% and 0.8%) the callus formation continued in the cuttings of 'Charles Joly' until the 55th day when 0.5% IBA was used. After these periods, some of the cuttings with callus died but, statistically, their mean rate remained the same (Table 1).

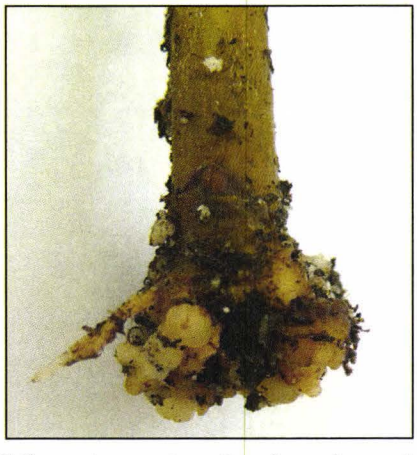

Figure 2. Callus and root primordium formation on the cuttings.

In the end of experiment the size of the callus reached about 1.0 cm. Different treatments did not affect-statistically the rate of the cuttings with callus in 'Charles Joly' and 'Mme Florent Stepman', but in *Syringa vulgaris,* the cuttings formed callus decreased with the increasing of IBA concentration (Table 1). However, a higher rate of cuttings formed callus was observed after treatment with 0.3% IBA and it was statistically higher in the cuttings of 'Charles Joly' and *Syringa vulgaris* (46.7 ± 4.4% and 61.7 ± 9.3%, resp.) in comparison with 'Mme Florent Stepman' (20.0 ± 10.4%) (Table 1).

Table 1. Factors affecting the dynamics of callus formation (%).

Cultivar	Concentration of IBA	35th day	55th day	75th day	95th day
		M ± SE	M ± SE	M ± SE	M ± SE
1	Control	48.3 ± 8.3 c A	76.7 ± 10.9 d C	66.7 ± 8.3 d BC	56.7 ± 8.3 ef AB
	0.3 %	48.3 ± 4.4 c A	73.3 ± 7.3 d B	65.0 ± 7.3 d AB	61.7 ± 9.3 f AB
	0.5 %	40.0 ± 5.0 c A	41.7 ± 4.4 bc A	31.7 ± 1.7 b A	28.3 ± 1.7 abc A
	0.8 %	35.0 ± 5.8 c A	51.7 ± 8.3 bc A	45.0 ± 5.8 bc A	41.7 ± 4.4 cde A
2	Control	10.0 ± 2.9 a A	36.7 ± 1.7 bc B	40.0 ± 5.8 bc B	36.7 ± 1.7 bcd B
	0.3 %	30.0 ± 7.6 abc A	55.0 ± 7.6 c B	51.7 ± 9.3 cd B	46.7 ± 4.4 c-f AB
	0.5 %	30.0 ± 5.8 abc A	50.0 ± 7.6 bc B	53.3 ± 10.1 cd B	53.3 ± 6.0 def B
	0.8 %	35.0 ± 2.9 bc A	36.7 ± 1.7 bc A	43.3 ± 1.7 bc A	45.0 ± 5.0 c-f A
3	Control	13.3 ± 10.9 ab A	11.7 ± 9.3 a A	8.3 ± 3.3 a A	10.0 ± 5.0 a A
	0.3 %	30.0 ± 5.0 abc A	33.3 ± 10.1 b A	25.0 ± 5.0 ab A	20.0 ± 10.4 ab A
	0.5 %	33.3 ± 7.3 bc AB	38.3 ± 7.3 bc B	35.0 ± 2.9 bc AB	16.7 ± 6.0 ab A
	0.8 %	33.3 ± 6.0 bc A	41.7 ± 6.0 bc A	45.0 ± 11.5 bc A	31.7 ± 8.3 bc A

The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$

Legend: 1 – *Syringa vulgaris*, 2 – *Syringa vulgaris* 'Charles Joly', 3 – *Syringa vulgaris* 'Mme Florent Stepman'

Our results indicated that rooting was not induced on the hardwood cuttings (data not shown). The beginning of the adventitious roots formation in softwood cuttings was observed incidentally in some treatments after 35 days from the beginning of the experiment. In the control variant, the rooting began after 55 days and statistically increased until the 75th day. Although all investigated genotypes demonstrated lowest rooting rates of the non-treated cuttings, it could be noticed that cuttings from 'Charles Joly' and *Syringa vulgaris*, demonstrated statistically higher rooting potential in the end of experiment (18.3 ± 4.4% and 20.0 ± 5.8%, resp.) than 'Mme Florent Stepman' (1.7 ± 1.7%), which can be ascribed to the higher potential of the genotype rootability of these cultivars. However, all treatments with IBA supported the induction of rooting and decreased the period of rooting. The cuttings from 'Charles Joly' and *Syringa vulgaris* reached statistically the highest value of rooting (45.0 ± 2.9% and 50.0 ± 5.0%, respectively), but they did not show statistically significant differences between them (Fig. 3).

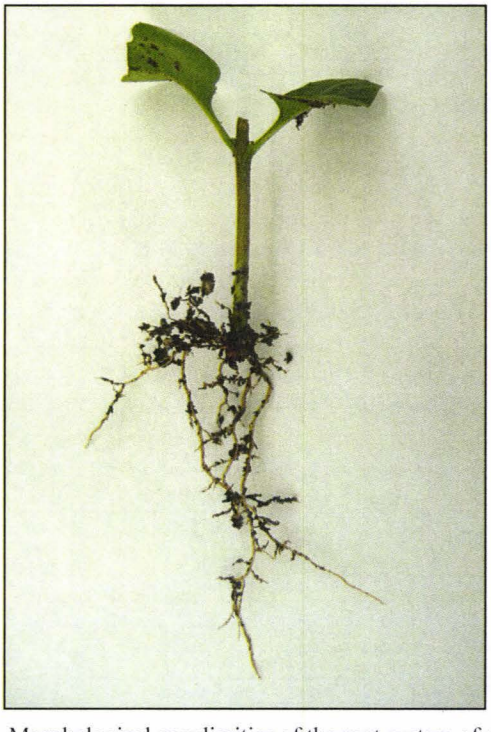

Figure 3. Morphological peculiarities of the root system of a cutting.

The rooting registered the lowest value in the cuttings from 'Mme Florent Stepman' (33.3 ± 13.6%), but it was not statistically different from 'Charles Joly' (Table 2).

Table 2. Factors affecting the dynamics of the rooting process (%).

Cultivar	Concentration of IBA	35th day	55th day	75th day	95th day
		M ± SE	M ± SE	M ± SE	M ± SE
1	Control	0.0 ± 0.0 a A	0.0 ± 0.0 a A	18.3 ± 6.0 bcd B	20.0 ± 5.8 bc B
	0.3 %	3.3 ± 1.7 a A	43.3 ± 1.7 e B	48.3 ± 3.3 f B	50.0 ± 5.0 e B
	0.5 %	5.0 ± 2.9 a A	23.3 ± 1.7 cd B	23.3 ± 1.7 cde B	25.0 ± 0.0 bc B
	0.8 %	3.3 ± 1.7 a A	31.7 ± 4.4 de B	31.7 ± 7.3 de B	33.3 ± 6.0 cd B
2	Control	0.0 ± 0.0 a A	1.7 ± 1.7 ab A	5.0 ± 2.9 ab AB	18.3 ± 4.4 bc B
	0.3 %	0.0 ± 0.0 a A	21.7 ± 9.3 cd B	36.7 ± 6.0 ef BC	41.7 ± 1.7 de C
	0.5 %	0.0 ± 0.0 a A	15.0 ± 5.0 abc AB	30.0 ± 5.0 de BC	45.0 ± 2.9 de C
	0.8 %	0.0 ± 0.0 a A	18.3 ± 8.3 cd AB	28.3 ± 10.1 de B	33.3 ± 8.8 cd B
3	Control	0.0 ± 0.0 a A	0.0 ± 0.0 a A	1.7 ± 1.7 a A	1.7 ± 1.7 a A
	0.3 %	0.0 ± 0.0 a A	8.3 ± 1.7 abc A	8.3 ± 1.7 abc A	13.3 ± 3.3 ab A
	0.5 %	1.7 ± 1.7 a A	16.7 ± 4.4 bcd AB	23.3 ± 8.8 cde B	23.3 ± 8.8 bc B
	0.8 %	0.0 ± 0.0 a A	18.3 ± 13.3 cd B	31.7 ± 14.2 de B	33.3 ± 13.6 cd B

The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$

Legend: 1 – *Syringa vulgaris*, 2 – *Syringa vulgaris* 'Charles Joly', 3 – *Syringa vulgaris* 'Mme Florent Stepman'

The results of the experiments showed conclusively that the rooting potential is determined by all investigated factors. The duration of cultivation was statistically the most significant factor for the rooting of cuttings (Table 3, F = 55.598, $p = 0.000$). Another key factor, also statistically significant, was the concentration of IBA (Table 3, F = 25.528, $p = 0.000$). Adventitious root induction depended also on the genotype of the donor plant (Table 3, F = 16.661, $p = 0.000$) but it was the most insignificant of the investigated factors.

Table 3. Significance of the studied factors and their combinations on the dynamic of rooting estimated by a post hoc LSD test.

Factors	F	Level of significance
CV	16.661	0.000
C	25.528	0.000
DR	55.598	0.000
CV × C	5.191	0.000
CV × DR	2.555	0.024
C × DR	2.465	0.014
CV × C × DR	107.658	0.298

a R Squared = 0.792 (Adjusted R Squared = 0.691), $p < 0.05$.

Legend: CV = Cultivar, C = Concentration of IBA, DR = Duration of rooting

Our investigation showed that the number of roots was statistically least on non-treated cuttings (control). The number of induced roots increased statistically significantly until the 55th day after all treatments with IBA.

In the end of the experiment, the highest number of roots was induced in *Syringa vulgaris*, after the treatment of their cuttings with 0.5% IBA (10.3 ± 1.4), in 'Charles Joly' after the treatment with 0.3% IBA (5.1 ± 0.5), and in 'Mme Florent Stepman' the maximal number of roots was observed after the treatment with 0.8% IBA (8.3 ± 1.4). However, it should be noted that the concentration of IBA did not affect statistically the number of roots within a cultivar. A exception of this tendency was found only in the cuttings of 'Mme Florent Stepman', which formed statistically more roots after the treatment with 0.8% IBA (Table 4).

Table 4. Factors affecting the number of inducted roots.

Cultivar	Concentration of IBA (mg l^{-1})	35th day	55th day	75th day	95th day
		M ± SE	M ± SE	M ± SE	M ± SE
1	Control	0.0 ± 0.0 a A	0.0 ± 0.0 a A	2.1 ± 0.4 a B	3.5 ± 0.7 ab C
	0.3 %	1.5 ± 0.5 b A	4.5 ± 0.6 cdf AB	7.0 ± 0.9 b BC	7.9 ± 1.1 c C
	0.5 %	1.5 ± 0.5 b A	7.6 ± 1.4 g AB	10.6 ± 1.3 c B	10.3 ± 1.4 c B
	0.8 %	5.0 ± 2.0 c A	6.1 ± 1.0 eg A	7.6 ± 1.2 b A	8.3 ± 1.3 c A
2	Control	0.0 ± 0.0 a A	1.0	1.7 ± 0.3 a B	2.0 ± 0.4 a B
	0.3 %	0.0 ± 0.0 a A	5.3 ± 0.9 de B	4.8 ± 0.6 a B	5.1 ± 0.5 b B
	0.5 %	0.0 ± 0.0 a A	3.6 ± 1.0 bcd B	3.9 ± 0.6 a B	4.9 ± 0.5 b B
	0.8 %	0.0 ± 0.0 a A	4.6 ± 0.9 cde B	4.4 ± 0.7 a B	5.0 ± 0.7 ab B

3	Control	0.0 ± 0.0 a A	0.0 ± 0.0 a A	1.0	1.0
	0.3 %	0.0 ± 0.0 a A	2.4 ± 0.7 bc B	2.6 ± 0.7 a B	2.0 ± 0.5 ab B
	0.5 %	2.0	2.0 ± 0.3 b A	2.3 ± 0.5 a A	2.8 ± 0.6 ab A
	0.8 %	0.0 ± 0.0 a A	6.2 ± 1.4 efg B	7.1 ± 1.0 b BC	8.9 ± 1.3 c C

The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The results of some treatments are not included in the statistical comparison because only single cuttings are rooted.

Legend: 1 – *Syringa vulgaris*, 2 – *Syringa vulgaris* 'Charles Joly', 3 – *Syringa vulgaris* 'Mme Florent Stepman'

All investigated factors in our experiment, individually or jointly, had high level of significance at $p \leq 0.05$. The duration of rooting and genotype were statistically the most significant factors for the number of the induced adventitious roots (Table 5, F = 50.282 and 36.637, respectively, $p = 0.000$). Another key factor, also statistically significant, was the concentration of IBA (Table 5, F = 33.513, $p = 0.000$).

Table 5. Significance of the studied factors and their combinations on the number of inducted roots estimated by a post hoc LSD test.

Factors	F	Level of significance
CV	36.637	0.000
C	33.513	0.000
DR	50.282	0.000
CV × C	7.392	0.000
CV × DR	4.210	0.000
C × DR	3.930	0.000
CV × C × DR	1.356	0.143

a R Squared = 0.138 (Adjusted R Squared = 0.124), $p < 0.05$.

Legend: CV = Cultivar, C = Concentration of IBA, DR = Duration of Rooting

The length of the roots increased in the period between the 35th and the 95th day of cultivation. In the end of the experiment, the roots of not treated cuttings (control) had in general the lowest length. The length of roots in each genotype was not affected significantly by different treatments with IBA. In 'Mme Florent Stepman', statistically the highest length of the roots was reached after the treatment of the cuttings with 0.8% IBA (547.4 ± 81.3 mm, resp.) in comparison with the other treatments (Table 6).

All investigated factors in our experiment, individually or jointly, had high level of significance at $p < 0.05$, with exception of the combination Cultivar × Concentration of IBA × Duration of rooting (CV × C × DR). Expectably, the most significant factor, having an effect on the mean length of the induced adventitious roots, was the duration of cultivation of the cuttings (F = 59.129). Another key factor, also statistically significant, was the concentration of the used IBA (F = 31.323). The genotype peculiarities of the cultivar (F = 27.927) exerted the lowest influence (Table 7).

Table 6. Factors affecting the length of inducted roots (mm).

Cultivar	Concentration of IBA (mg l^{-1})	35th day	55th day	75th day	95th day
		M ± SE	M ± SE	M ± SE	M ± SE
1	Control	0.0 ± 0.0 a A	0.0 ± 0.0 a A	60.2 ± 18.2 a B	200.0 ± 50.4 ab C
	0.3 %	7.0 ± 3.0 b A	129.5 ± 22.0 bc AB	287.2 ± 38.3 cd B	416.1 ± 62.5 cd C
	0.5 %	7.0 ± 3.0 b A	211.9 ± 52.3 d A	402.4 ± 60.1 de B	480.3 ± 70.7 d B
	0.8 %	33.5 ± 19.5 c A	193.6 ± 41.8 cd A	407.7 ± 59.3 e B	407.0 ± 58.0 cd B
2	Control	0.0 ± 0.0 a A	23.0	36.0 ± 16.3 ab B	58.8 ± 18.8 a B
	0.3 %	0.0 ± 0.0 a A	125.5 ± 30.0 bc B	170.3 ± 26.6 ab B	210.1 ± 23.0 ab C
	0.5 %	0.0 ± 0.0 a A	67.9 ± 25.5 b B	142.7 ± 30.1 ab C	181.1 ± 23.6 ab C
	0.8 %	0.0 ± 0.0 a A	121.9 ± 38.5 bc B	210.9 ± 54.7 bc C	231.8 ± 33.5 b C
3	Control	0.0 ± 0.0 a A	0.0 ± 0.0 a A	18.0	23.0
	0.3 %	0.0 ± 0.0 a A	58.4 ± 20.1 ab B	99.6 ± 43.5 ab BC	129.8 ± 36.1 ab C
	0.5 %	37.0	90.9 ± 30.4 b A	149.0 ± 36.6 ab AB	255.9 ± 75.2 abc B
	0.8 %	0.0 ± 0.0 a A	196.8 ± 42.9 cd B	356.7 ± 62.6 de C	547.4 ± 81.3 d D

The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The results of some treatments are not included in the statistical comparison because only single cuttings are rooted.

Legend: 1 – *Syringa vulgaris* L., 2 – *Syringa vulgaris* 'Charles Joly', 3 – *Syringa vulgaris* 'Mme Florent Stepman'

Table 7. Significance of the studied factors and their combinations on the length of inducted roots estimated by a post hoc LSD test.

Factors	F	Level of significance
CV	27.927	0.000
C	31.323	0.000
DR	59.129	0.000
CV × C	6.832	0.000
CV × DR	4.328	0.000
C × DR	5.357	0.000
CV × C × DR	1.788	0.021

a R Squared = 0.144 (Adjusted R Squared = 0.129), $p < 0.05$.

Legend:, CV = Cultivar, C = Concentration of IBA, DR = Duration of Rooting

DISCUSSION

It was found that rooting ability of lilac cuttings depended markedly on the physiological status of the donor plant at the time of cutting excision and is of high importance for the rooting process (COGGESHALL, 1962; BOJARCZUK, 1975; BOJARCZUK & JANKIEWICZ, 1975; SCHMIDT, 1978; CAMERON et al., 2003). It was pointed that the rank-growing, succulent tissues are likely to have insufficient or even inappropriate reserves of carbohydrate storage (HARTMANN & KESTER, 2002), and high nitrogen content (HARTMANN & KESTER, 2002; DICK & DEWAR, 1992). Also, softwood cuttings, in comparison with dormant, hardwood cuttings, tend to have higher auxin. They have a moderate light requirement, since some photosynthesis enhances their rooting and their rooting requires more intensive water management, using mist or fog. For this reason softwood cuttings are recommended as the best plant material for rooting of difficult-to-root species (HARTMANN & KESTER, 2002).

The biochemical studies for root formation in common lilac cultivars implies that the better rooting cultivars compared to the poorer rooting cultivars may be caused by their higher content of ortho-dihydroxyphenols, which lower the activity of auxin. These cultivars are also characterized by a low level of monohydroxyphenols, which lower the activity of auxin (BOJARCZUK, 1978, 1979).

In agreement with the results of several authors (BOJARCZUK & JANKIEWICZ, 1975; BOJARCZUK, 1975, 1978, 1979; SCHMIDT, 1978; DIRR & HEUSER, 1987; DIRR, 1998, HARTMANN et al., 2002; FORD et al., 2002; CAMERON et al., 2003) our best results were obtained with cuttings made at flowering time. These results indicate that common lilac is one of the exceptions for the most suitable physiological state at the time of excision of cuttings in woody plants.

Our results are in agreement with the findings of a number of researchers, which reported that the rooting depends on the genotype of the donor plant and vary from 0% to 100% (SCHMIDT, 1978; BOJARCZUK, 1978b, 1979; TYATYUSHKINA, 2007).

Improving the rooting of cuttings of 'Charles Joly' has been obtained by treatment of etiolated shoots with black plastic tape, soaked with 0.1% or 0.8% IBA and wound about the base of the new shoots (BASSUK et al., 1984; MISKE & BASSUK, 1985; MAYNARD & BASSUK, 1987). However, it was found that the annual pruning (CAMERON et al., 2003) or using of etiolated shoots could significantly facilitate the rooting of softwood cuttings of this cultivar.

Some authors have evaluated the rooting ability after 5-14 weeks (BOJARCZUK, 1975, 1978b; BOJARCZUK & JANKIEWICZ, 1975; SCHMIDT, 1978; BASSUK et al., 1984, MISKE & BASSUK, 1985, FORD et al., 2002; CAMERON et al., 2003) but others have not indicated the duration of rooting process (BOJARCZUK 1979; MAYNARD & BASSUK, 1987; TYATYUSHKINA, 2007). Our results showed that the rooting ability of the cuttings of *Syringa vulgaris* is strongly influenced by the duration of cultivation. Although IBA supported the induction of rooting, the period of rooting should not be shorter than 75 days.

It has been reported that the type and concentration of the auxin used are of critical importance for rooting of woody species (SCHMIDT, 1978; BONGA & VON ADERKAS, 1992; ILIEV, 1996; DE KLERK et al., 1997; HARTMANN et al., 2002; DANCHEVA, 2005; ILIEV et al., 2010). It was found that concentration of IBA in the diapason of 0.1% - 0.8% facilitate the rooting of cuttings in common lilac and its cultivars (BASSUK et al., 1984; MISKE & BASSUK, 1985; MAYNARD & BASSUK, 1987). Although the tested concentrations of IBA in our experiment were unable to overcome the influence of the physiological status of the donor plant, it supported the rootability of softwood cuttings and decreased the period of rooting. However, there is always an effect of the particular genotype with respect to the percentage of rooting. This is in agreement with previous findings for common lilac cultivars (SCHMIDT, 1978; BOJARCZUK, 1979; TYATYUSHKINA, 2007) or hybrids (COGGESHULL, 1962; BASSUK et al., 1984).

Root number has been used as an expression of rhizogenesis potential (VAN DR KRIEKEN et al., 1992; GUAN & DE KLERK, 2000; CAMERON et al., 2003; DANCHEVA, 2005; ILIEV et al., 2010; LYUBOMIROVA &

ILIEV, 2013), the assumption being greater root numbers correlate with greater rooting potential. Also, it has been shown that the quality of formed root system is an important factor for successful acclimatization (MCCLELLAND et al., 1990; HARTMANN et al., 2002). Our study showed that once roots reach a certain stage of development, they inhibit further initiation of root primordial. The inhibition might be a result of activation of a gene responsible for the root elongation and synthesis of plant growth regulators, which repress the root primordia induction. Gibberellins have been shown to inhibit rooting directly (KAWAI ,1997) and the action of growth retardants on improving root induction may be associated with their anti-gibberellin properties (WIESMAN & RIOV, 1994; PORLINGIS & KOUKOURIKOU-PETRIDOU, 1996). This consideration seems to be a possible explanation of the results achieved in our study. However, more detailed studies are necessary especially since root number is likely to be used widely as a variable in studies of the genetic control of rooting.

In conclusion, in view of our results and according the classification of HARTMAN et al. (2002) *Syringa vulgaris*, 'Mme Florent Stepman', and 'Charles Joly' could be considered into the class of moderately-easy-to-root.

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ROOTING AND ACCLIMATIZATION OF MICROPROPAGATED SHOOTS OF *FRAXINUS EXCELSIOR* L.

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Abstract. Axillary shoot tips, originated *in vitro* were used as initial explants for the rooting experiments. They were cultured on an inductive, half-strength WPM, supplemented with a combination of 1.0 mg l^{-1} IBA and 1.0 mg l^{-1} NAA for 24, 48 or 72 h, respectively and then on half-strength, plant growth regulator-free (PGRs-free) expressive WPM. For comparison of the results, the shoots were cultivated on inductive or expressive medium without transfer. The rooted shoots were planted in 500 ml pots. In one part of the experiment, the posts contained peat and the plantlets were covered with transparent plastic vessels for 14 days to provide high air humidity. For the other experiment, the pots contained peat, perlite, peat and perlite (1 : 1, v/v), Dystric Alluvial Fluvisol and perlite (1 : 1, v/v) and the plantlets were covered with transparent plastic vessels. After 14 days the cover vessels were consecutively removed for 30 min, 60 min, 90 min, and 120 min, daily. All plantlets were grown in a cultivation chamber and after the removal of covers, were transferred in a greenhouse conditions. The highest rooting rate (90.00 ± 5.77%) was observed when the shoots were pulse treated with auxin for 24 h and decreasing of the mean number of roots was found with the increasing of the duration of inductive phase. High survival rate of the plantlets was manifested on different substrates (93-100%) but significant difference between them was not found. However, higher length was observed by using peat containing substrates (from 12.9 ± 1.0 to 19.4 ± 5.1 cm) but statistical difference between them was not found. By contrast, plants acclimatized on perlite containing substrates were smaller than these planted in peat containing substrates. These results demonstrated that the duration of inductive phase is critical for the rooting rate and quality of the root system and the type of the soil substrate is an important factor for the growth of the plants during the acclimatization.

Keywords: common ash, *in vitro*, tissue culture.

Rezumat. Înrădăcinarea şi aclimatizarea lăstarilor micropropagaţi de *Fraxinus excelsior* L. Vârfurile de lăstari axilari, de origine *in vitro*, au fost utilizate ca explante iniţiale pentru experimente privind înrădăcinarea. Acestea au fost cultivate pe un WPM inductiv, redus la jumătate, suplimentat cu o combinaţie de 1,0 mg l^{-1} IBA şi 1,0 mg l^{-1} NAA timp de 24, 48 sau 72 de ore şi pe WPM expresiv, fără un regulator de creştere a plantelor (PGRs-free). Pentru a compara rezultatele, lăstarii au fost cultivaţi pe mediu inductiv sau expresiv, fără transfer. Lăstarii înrădăcinaţi au fost plantaţi în vase de 500 ml. Pentru o parte a experimentului, substratul a conţinut turbă şi plantulele au fost acoperite cu vase din plastic transparent, timp de 14 zile pentru a menţine umiditatea ridicată a aerului. Pentru celelălt experiment, vasele au conţinut turbă, perlit, turbă şi perlit (1: 1, v / v), fluvisol distric aluvionar şi perlit (1: 1, v / v) şi plantulele au fost acoperite cu vase de plastic transparent. După 14 zile, vasele de acoperire au fost îndepărtate consecutiv timp de 30 min., 60 min., 90 min. şi 120 min., zilnic. Toate plantule au fost păstrate într-o cameră de cultivare şi după îndepărtarea capacelor, au fost transferate în condiţii de seră. Cea mai mare rată de înrădăcinare (90.00 ± 5.77%) a fost observată atunci când lăstarii au fost tratati cu auxin pentru 24 de ore, în timp ce scăderea numărului mediu de rădăcini s-a constatat o dată cu creşterea duratei fazei inductive. S-a constatat o rată ridicată de supravieţuire a plantulelor pe diferite substraturi (93-100%) şi nu s-a observat o diferenta semnificativă intre acestea. Cu toate acestea, s-a constatat o lungime mai mare în cazul substratului care conţine turbă (de la 12,9 ± 1.0 - 19.4 ± 5.1 cm), dar nu s-a observat o diferenţă statistică semnificativă între acestea. Prin contrast, plantele aclimatizate pe substrat care conţine perlit au fost mai mici decât cele cultivate pe substratul care conţine turbă. Aceste rezultate au demonstrat că durata fazei inductive este critică pentru rata de înrădăcinare şi calitatea sistemului radicular şi că de tipul de sol este un factor important pentru creşterea plantelor pe perioada de aclimatizare.

Cuvinte cheie: frasin, *in vitro*, cultură de ţesut.

INTRODUCTION

Rooting of microshoots is critical for *in vitro* plant production systems. The induction of rooting depends on a series of interdependent phases (induction, initiation, and expression) (MONCOUSIN et al., 1988; DE KLERK et al., 1999; GASPAR et al., 1992, 1994).

Depending on the juvenility and auxin used and its concentration, different authors achieved 10 to 100% rooting in common ash (CHALUPA 1983, 1987a, b, 1990; HAMMATT & RIDOUT 1992; TABRETT & HAMMATT 1992; HAMMATT 1994, 1996; SILVEIRA & COTTIGNIES 1994; NOUGARÈDE et al. 1996; THOMPSON et al., 2001; SCHOENWEISS & MEIER-DINKEL 2005; MITRAS et al., 2009). The quality of the root system is one of the important factors for the successful acclimatization. It has been suggested that defects in root system functioning are one of the main reasons for the high mortality rate of plants derived from *in vitro* culture (YIE & LIAW, 1977; DAVID, 1982; PATEL et al., 1986). Furthermore, after the transfer from *in vitro* to *in vivo* conditions the plantlets should adapt to different environmental conditions. Usually the irradiance and air turbulence is much higher, air humidity much lower, there are fluctuating temperatures, the type of soil substrate and its humidity is different in comparison with *in vitro* conditions (ZIV 1986; KOZAI 1991; PREECE & SUTTER 1991; DONNELLY & TISDAL 1993; POSPÍŠILOVA et al., 1999; HAZARIKA 2003; ROHR et al., 2003; KOZAI & ZOBAYED, 2000). Depending on the biological peculiarities and genetic potential of the species, the methods which work for *in vivo* environment of one species are not necessary satisfactory to ensure the survival of another. However, there is only one report for high rate of *Fraxinus excelsior* acclimatization (LEBEDEV & SCHESTIBRATOV, 2013) but the effect of the soil substrate is not investigated.

The goal of this work was aimed at identifying suitable duration of inductive phase on rooting of axillary shoots and conditions of acclimatization of common ash plantlets.

MATERIAL AND METHODS

Effect of the inductive medium on the adventitious root formation.

In vitro originated axillary shoot tips (2 cm), having one or two internodes were cultured on an inductive, half-strength WPM (LLOYD & MCCOWN, 1980) rooting medium, supplemented with combination of 1.0 mg l^{-1} indole-3-butyric acid (IBA) and 1.0 mg l^{-1} α-naphthaleneacetic acid (NAA) for 24, 48 or 72 h, respectively and then on half-strength, plant growth regulator-free (PGRs-free) expressive WPM. For the comparison of the results, the shoots were cultivated on inductive or expressive medium without transfer.

Tree replications, each containing ten explants, were used for each variant. After 45 days, the percentage of rooted plants, as well as the number and length of induced roots were determined.

Conditions of the cultivation.

Each variant of the medium contained 20 g l^{-1} sucrose and 7 g l^{-1} agar (Sigma) and pH was adjusted to 5.6 - 5.7 before autoclaving (under pressure of 118 kPa and 120°C for 20 min). The cultures were grown in a cultivation chamber at 25 ± 0.5°C with 16 hrs of cool white fluorescent light at a photosynthetic photon flux density of 40 μmol m^{-2} s^{-1}, daily.

Acclimatization

The rooted shoots were thoroughly washed to remove adhering gel and planted in 500 ml (∅ 95 mm, h = 110 mm) pots. In one part of the experiment the posts contained peat and the plantlets were covered with transparent plastic vessels (250 ml, ∅ 75 mm, h= 95 mm) for 14 days to provide high air humidity (Experiment 1). For the other experiment the pots contained peat, perlite, peat and perlite (1 : 1, v/v), Dystric Alluvial Fluvisol and perlite (1 : 1, v/v) were covered with transparent plastic vessels (Fig. 1). After 14 days the cover containers were consecutively removed for 30 min, 60 min, 90 min, and 120 min, daily (Experiment 2). All plantlets were grown in a cultivation chamber at 25 ± 0.5°C with 16 hrs of cool white fluorescent light at a photosynthetic photon flux density of 40 μmol m^{-2} s^{-1}, daily. After the removal of covers, all plantlets were transferred in a greenhouse with temperature of 20-25°C and regular irrigation.

Figure 1. Pre-acclimatization with covering of the plantlets in the cultivation chamber.

Ten plantlets were used per treatment and the experiment was repeated three times. After 60 days, the percentage of acclimatized plants, as well as the mean length of stem was determined.

The results were analysed by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$, using SPSS 10.0 for Windows (SPSS for Windows 1999). Percentage values were transformed using arcsine square root ($\sqrt{P}$) (COMPTON 1994) to normalize error distribution prior variance analysis.

RESULTS AND DISCUSSION

In many species, the application of endogenous auxin is required to achieve rooting (DE KLERK, 2001, 2002; DE KLERK et al., 1997, 1999; KUREPIN et al., 2011).

In the absence of auxin, the rooting rate in our experiment was low (Table 1), the emergence of new roots progressed slowly and callus formation was not observed on the base of the shoots in the end of the rooting period. On auxin enriched medium without transfer of the shoots, in the end of experiment, significant amount of callus and thicker roots were formed on the base of the shoots, in comparison with all other treatments (Fig. 2). However, statistical significant difference with the auxin-free medium was not found in the rooting rate (Table 1).

Figure 2. Significant amount of callus and formation of thick roots after cultivation on auxin enriched medium without transfer on expressive medium.

Figure 3. Rooted shoots, formed after the application of inductive medium for 24 h and evaluated after 45 days of cultivation.

Smaller amounts of callus and slenderer roots were formed on the base of shoots in the end of the experiment, after all pulse treatments. Our results demonstrated that auxin pulse treatments significantly improve the rooting of shoots *in vitro.* These results are in agreement with the findings of DE KLERK (1996, 2001, 2002), DE KLERK et al. (1999), MITRAS et al. (2009), LYUBOMIROVA & ILIEV (2013). The highest rooting rate (90.00 ± 5.77%) was observed when the shoots were pulse treated with auxin for 24 h (Table 1, Fig. 3). It was pointed that during the initial 24 h after the shoots have been taken, they are not yet very sensitive to auxin. It is considered that during this lag period, dedifferentiation occurs during which cells become competent to respond to the rhizogenic stimulus, auxin. Then up to 72 h, certain previously activated cells become committed to the formation of root primordia by the rhizogenic action of auxin in the induction phase (DE KLERK et al., 1999). However, statistical difference in the rooting rate between different pulse treatments was not found in our experiment. It was found that during this period, auxin pulses strongly increases the number of roots in pea (NORDSTRÖM et al., 1991) and *Helianthus annuus* (LIU & REID, 1992). Despite of that, decreasing of the mean number of roots was found with the increasing of the duration of inductive phase, but statistical difference between all treatments was not ascertained. The longest roots were observed after pulse treatment for 72 h and on auxin free medium without transfer to expressive medium (Table 1).

Our results showed that pulse treatment supports the rooting and the rate of rooting depends on the duration of inductive phase. After the cultivation of shoots to a root induction medium, auxin is no longer required for the rooting and improvement of the root system. These results are in agreement with the findings of other authors (BERTHON et al., 1989, 1990; HAMMATT & RIDOUT, 1992; HAMMATT, 1996; ILIEV & ILIEV, 1997, LYUBOMIROVA & ILIEV, 2013) but the duration of the inductive phase probably depends on the genotype peculiarities of the species, rate of the juvenility of the shoots, type and concentration of the used auxin.

Table 1. Effect of the duration of inductive phase on the rooting of shoots.

Duration of the inductive phase (h)	Rooted shoots (%)	Mean number of the roots	Mean length of the roots (mm)
Auxin enriched medium without transfer	30.0 ± 5.8 c	4.3 ± 1.4 ab	23.5 ± 4.1 b
PGRs-free medium without transfer	53.7 ± 6.7 bc	2.1 ± 0.2 b	32.4 ± 2.1 ab
24	90.0 ± 5.8 a	3.8 ± 0.5 a	29.1 ± 1.3 b
48	80.0 ± 5.8 a	3.1 ± 0.4 ab	29.4 ± 2.0 b
72	76.7 ± 3.3 ab	2.7 ± 0.4 ab	38.9 ± 4.6 a

Legend: Values are mean (M) ± standard error (SE). Means in the column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$.

Different systems for acclimatization are recommended (DEBERGH, 1991; CLAPA et al., 2013). It was reported that the effect of the type of soil substrate is an important factor for the acclimatization of plantlets to *in vivo* conditions (ILIEV et al., 2001; PINKER et al., 2007; JIMÉNEZ et al., 2011; RAGONEZI et al., 2012). High survival rate of the plantlets was manifested on different substrates (93-100%) but significant difference between them was not found (Fig. 4).

Our results indicated that significant elongation of the shoots on some substrates was noticed even in the cultivation room (data are not shown). The substrates studied can be divided into two groups according their effect on the elongation of micropropagated plants in the end of the investigated acclimatization period. Higher length was observed by using peat containing substrates (from 12.9 ± 1.0 to 19.4 ± 5.1 cm) but statistical difference between them was not found. By contrast, plants acclimatized on perlite containing substrates were smaller than these planted in peat containing substrates and statistical difference between them also was not found (Table 2, Fig. 4). The reason for this tendency could be not only the better retention of water and aeration of the peat, but also the higher concentration of nutritive substances in it.

Table 2. Effect of the substrate on acclimatization.

Substrate used	Acclimatized plants (%)	Mean length of the steam (cm)
Peat (Experiment 1)	93.3 ± 6.7 a	18.6 ± 1.9 a
Peat (Experiment 2)	93.3 ± 6.7 a	12.9 ± 1.0 ab
Peat and perlite (Experiment 2)	100.0 ± 0.0 a	19.4 ± 5.1 a
Perlite (Experiment 2)	93.3 ± 3.3 a	5.9 ± 0.5 b
Perlite and soil (Experiment 2)	93.3 ± 3.3 a	8.4 ± 0.6 b

Legend: Values are mean (M) ± standard error (SE). Means in the column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$.

Figure 4. Acclimatized plants in greenhouse conditions. Left: planted on perlite and Right: planted on mixture of peat and perlite.

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SUBCONTINENTAL PERI-PANNONIC SCRUB FROM GEOPARK PLATEAU MEHEDINŢI (ROMANIA)

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Abstract. This paper presents one priority natural habitat from Geopark Plateau Mehedinţi: 40A0* Subcontinental peri-Pannonic scrub. The research was conducted with the occasion of the monitoring of species and habitats from Geopark Plateau Mehedinţi, with the purpose of improving management to achieve the biodiversity conservation objectives. The presentation used as diagnostic elements: code and name NATURA 2000, correspondence with the Romanian habitats, EMERALD, CORINE, PALAEARCTIC HABITATS, and EUNIS classification, general description, stationary particularities, variability and distribution in the territory, phytosociological correspondence, physiognomy and structure, contact habitats, ecological and biological value. It also presents the habitat status, the disturbing factors, the potential threats and the management.

Keywords: priority habitats, Mehedinţi, Oltenia, Romania.

Rezumat. Tufărişuri subcontinentale peripanonice din Geoparcul Platoul Mehedinţi (România). Articolul prezintă un habitat natural prioritar din Geoparcul Platoul Mehedinţi: 40A0* Tufărişuri subcontinentale peripanonice. Cercetările s-au efectuat cu ocazia monitorizării speciilor şi habitatelor din Geoparcul Platoul Mehedinţi, în scopul îmbunătăţirii managementului şi atingerii obiectivelor de conservare a biodiversităţii. Prezentarea utilizează ca elemente de diagnoză: cod şi nume NATURA 2000, corespondenţă a habitatului românesc şi clasificările EMERALD, CORINE, PALAEARCTIC HABITATS şi EUNIS, descriere generală, particularităţi staţionale, variabilitate şi distribuţie în teritoriu, corespondenţe fitosociologice, fizionomie şi structură, habitate în contact, valoarea ecologică şi biologică. Se prezintă, de asemenea, starea habitatului, factorii perturbatori, ameninţările potenţiale şi cadrul de management.

Cuvinte cheie: habitat prioritar, Mehedinţi, Oltenia, România.

INTRODUCTION

The studied area is represented by the calcareous area and "cornete" from Geopark Plateau Mehedinţi. The subcontinental peri-Pannonic shrub are installed at the limestone hillocks called "cornete" (which marks the Jurassic limestone alignment of the Danubian Autochthonous, developed in the northeast - southwest direction) placed between the plateau-unit (east) and the depression (centre) of Mehedinţi Plateau. This limestone area is crossed by many rivers, such as the Coşuştea, the Topolniţa, the Camena, which formed gorges and other limestone formations, known as "cornets", the most impressive and most popular being: Cornetul Cerboanii (810 m), Cornetul Babelor (770 m) and Cornetul Bălţii (701 m); others have lower altitude (about 575 m), and we mention here the formations from Ponoarele: Răiculeşti, Băluţa and Gărdăneasa. The vegetation of these hillocks is represented by the southeast Carpathian lilac (*Syringa vulgaris*) and manna ash (*Fraxinus ornus*) thickets along with the dominant species, *Cornus mas*, which also gave the name of these formations – the species Romanian name is "corn" and, thus, it resulted "cornete".

MATERIAL AND METHODS

The research concerning the inventory and monitoring of the natural habitats were performed in 2010-2013. We performed a Level III monitoring, monitoring which applies only to priority habitats and effectively capitalizes data collected in the field (CANDREA BOZGA et al., 2009).

The study methods are the classical ones. In order to identify and characterize habitats we used the methodology proposed by CRISTEA et al. (2004), DONIŢĂ et al. (2005, 2006), GAFTA & MOUNTFORD (2008).

The presentation used as diagnostic elements: code and name NATURA 2000, correspondence with the Romanian habitats, EMERALD, CORINE, PALAEARCTIC HABITATS, and EUNIS classification, general description, stationary particularities, variability and distribution in the territory, phytosociological correspondence, physiognomy and structure, contact habitats, ecological and biological value. It also presents the habitat status, the disturbing factors, the potential threats and the management.

There have been used previously published data related to this type of vegetation (MALOŞ & FIRESCU, 1971; MALOŞ, 1976; MĂGĂLIE, 1970; POPESCU et al., 2006; POPOVA-CUCU, 1970, 1971; POPOVA-CUCU & POPESCU, 1975), previously published data by the authors of this article, related to the flora of the Geopark (CIORTAN & NEGREAN, 2012; NEGREAN & CIORTAN, 2012, 2013), and data from the Final Report 2007. We also used National Red Lists: BOŞCAIU & al., 1994; DIHORU & DIHORU, 1994; OLTEAN et al., 1994. Generally, the nomenclature of the species was given after Flora of Romania (SĂVULESCU, 1952-1976), Flora Europaea (TUTIN et al., 1964-1980, TUTIN et al., 1996).

RESULTS

Habitat 40A0* Subcontinental peri-Pannonic scrub, represented in the area by the Romanian habitats, that **corresponds to the European classification systems** as it follows:

- R3116 Southeast Carpathian elm-leaf *Spiraea* (*Spiraea chamaedryfolia*) deciduous thickets: EMERALD - 31.8B South-eastern deciduous thickets; CORINE - 31.8B South-eastern sub-Mediterranean deciduous thickets (schibljak); PAL. HAB. 31.8B142 Carpathian elm-leaf *Spiraea* thickets; EUNIS F3.17 *Corylus* thickets.

- R3123 Southeast Carpathian lilac (*Syringa vulgaris*) with *Genista radiata* thickets and R3124 Southeast Carpathian lilac (*Syringa vulgaris*) with *Asplenium ruta-muraria* thickets: EMERALD - 31.8B South-eastern deciduous thickets; CORINE - 31.8B3 Greek sub-Mediterranean deciduous thickets; PAL. HAB. - 31.8B322 Danubian lilac thickets; EUNIS – Moesian lilac thickets.

- R3126 Southeast Carpathian manna ash (*Fraxinus ornus*) thickets: EMERALD - 31.8B South-eastern deciduous thickets; CORINE - 31.8B South-eastern sub-Mediterranean deciduous thickets (schibljak); PAL. HAB. - 31.8.B Balkano - Hellenic deciduous thickets, EUNIS - F3.2431 Moesian oriental hornbeam thickets.

- R3127 Southeast Carpathian lilac (*Syringa vulgaris*) and manna ash (*Fraxinus ornus*) thickets: EMERALD - 31.8B South-eastern deciduous thickets; CORINE - 31.8B South-eastern sub-Mediterranean deciduous thickets; PAL. HAB. - 31.8B3222 Lilac manna ash thickets, EUNIS - F3.2431 Moesian oriental hornbeam thickets.

General description. According to the Interpretation Manual of Natura 2000 habitats in Romania, the habitat is represented by the deciduous scrub with continental and sub-Mediterranean affinities of the Pannonia basin and neighbouring regions including the eastern Alpine periphery, the southern periphery of the Northwestern Carpathians, the Transylvanian plateau and the adjacent foothills and valleys of the Eastern and Southern Carpathians and the Apuseni Mountains, the southern periphery of the Pannonia basin, the Moravian plateau and the hills and valleys of the northern Balkan Peninsula. The habitat forms a mosaic-like vegetation with steppe grassland (6210) and forest-steppe elements or plants of the rupicolous Pannonian grasslands (6190) often along the fringes of woodlands, as it can be noticed from the lists of species that we present for each of the habitat area variants.

Stationary particularities. The habitat is well represented, accompanying the Jurassic limestone of the Danubian Autochthonous, oriented northeast-southwest. The substrate it installs on is represented by limestone [sedimentary structures of Mesozoic age, and Miocene age (Ponoarele) - in the form of a narrow strip (STROE & PEPTEANU, 2011), marl-limestone intercalation], with carbonate humic soils:

a) Southeast Carpathian elm-leaf *Spiraea* (*Spiraea chamaedryfolia*) deciduous thickets are installed on the rocky steeply inclined slopes, with sunny or shady exhibition; the substrate is represented by calcareous conglomerates in large blocks with superficial soils formed between the scattered blocks (e.g. at Coșuștea, Camena);

b) Southeast Carpathian lilac (*Syringa vulgaris*) with *Genista radiata* thickets are installed on the sunny rocky slopes, steeply to moderately inclined; the substrate is represented by ~~of~~ gray limestone; neutral to weakly alkaline soils - rendzinas - often developed only in the cracks of the rocks;

c) Southeast Carpathian lilac (*Syringa vulgaris*) with *Asplenium ruta-muraria* thickets are installed on the steep slopes, rocky vertical walls, with S, SW, SE orientation, along the rivers; the substrate is represented by the calcareous rocks at the surface of which it may appear protorendzina in reduced proportion;

d) Southeast Carpathian manna ash (*Fraxinus ornus*) thickets are installed on the slopes with high inclination and southern (southeastern and southwestern) exhibition; the substrate is represented by limestone cliffs with varying sizes, with thickness reduced and sometimes even absent; soils are rendzinas rich in calcic humus;

e) Southeast Carpathian lilac (*Syringa vulgaris*) and manna ash (*Fraxinus ornus*) thickets are located at the bottom of the slope (e.g. at Ponoarele) or of plates, immediately above them (e.g. at Camena); the substrate is represented by limestones, calcareous sandstones; soils are preluvisols, terra rossa, type rendzina, in a thin but continuous layer, with a large amount of skeleton.

Phytosociological correspondence, variability and distribution in the territory. The habitat is well represented, accompanying the Jurassic limestone strip of the Danubian Autochthonous, oriented northeast-southwest.

We describe the most representative phytocoenosis of the habitat taking into account the richness in rare and endemic species from the sub-Mediterranean type, namely:

a) R3116 Southeast Carpathian elm-leaf *Spiraea* (*Spiraea chamaedryfolia*) deciduous thickets edified by as. *Calamagrostio-Spiraeetum ulmifoliae* Resmeriță et Csűrös 1966. Identified at Camena, Coșuștea Piedmont, and Vârtoape Forest;

b) R3123 Southeast Carpathian lilac (*Syringa vulgaris*) with *Genista radiata* thickets and R3124 Southeast Carpathian lilac (*Syringa vulgaris*) with *Asplenium ruta-muraria* thickets edified by as. *Syringo-Genistetum radiatae* Maloș 1972. Identified at Camena and Piatra Pinului;

c) R3124 Southeast Carpathian lilac (*Syringa vulgaris*) with *Asplenium ruta-muraria* thickets edified by as. *Asplenio-Syringetum vulgaris* Jakucs et Vida 1959. Identified at Ponoarele from Cornetul Răiculești, at the top of the slope of Cornetul Gărdăneasa, at Râienilor Valley (POPOVA-CUCU & POPESCU, 1975), at Topolnița Gorges, Gura Prosăcului, Găurinți.

d) R3126 Southeast Carpathian manna ash (*Fraxinus ornus*) thickets edified by as. *Corno-Fraxinetum orni* Pop et Hodişan 1964 [described at Ponoarele by MĂGĂLIE, (1970), with the lack of the Oriental Hornbeam]. Identified at Ponoarele, between Giurgiani and Vintilani - Balta, Cornetul Babelor and Cornetul Cerboaniei;

e) R3127 Southeast Carpathian lilac (*Syringa vulgaris*) and manna ash (*Fraxinus ornus*) thickets edified by as. *Syringo-Fraxinetum orni* Borza 1958 m. Resmeriţă 1972 (syn. *Syringo-Fraxinetum orni coryletosum colurnae* Borza 1958)]. Identified at Topolniţa – Cornetul Prosăcului, Cornetul Coşuştei, Piatra Coşuştei ridge, Cornetul Balta, Cornetul Piatra Încălecată, Piatra Pinului, Camena, and Isverna).

Physiognomy and structure

a) R3116 Southeast Carpathian elm-leaf *Spiraea* (*Spiraea chamaedryfolia*) deciduous thickets are fragmented throughout the territory of the Geopark. The phytocoenosis identified at Piatra Coşuştei ridge – the species from the phytocoenosis structure are characteristic to the beech forests that encompass this phytocoenosis. The edifying species are: *Spiraea chamaedryfolia, Lonicera xylosteum, Rosa pendulina*, and characteristics: *Spiraea chamaedryfolia* and *Calamagrostis arundinacea.*

The trees layer: sporadic trees species in young stages: *Abies alba, Betula pendula, Fagus sylvatica.*

The shrubs layer: *Spiraea chamaedryfolia, Syringa vulgaris*, alongside that is also growing: *Corylus avellana, Cotoneaster pyrenaicus* (*integerrimus*), *Fraxinus ornus, Lonicera xylosteum, Rosa pendulina, Salix caprea.*

The herbaceous layer: *Angelica sylvestris, Athyrium filix-femina, Calamagrostis arundinacea, Campanula rapunculoides, Carlina vulgaris* s. l., *Digitalis grandiflora, Dryopteris filix-mas, Epilobium collinum, Fragaria vesca, Glechoma hirsuta, Luzula luzuloides, Solidago virgaurea, Poa nemoralis, Sedum telephium* subsp. *maximum, Polystichum setiferum.*

The phytocoenosis identified in Vârtoape Forest is installed in the beech forest with *Geranium macrorhhizum*, on a slope with northeastern exhibition, overshadowed most of the day; it has the following structure and physiognomy:

The trees layer: *Abies alba, Acer platanoides, Acer pseudoplatanus, Fagus sylvatica, Tilia platyphyllos, Ulmus glabra.*

The shrubs layer: *Fraxinus ornus, Spiraea chamaedryfolia, Rubus hirtus.*

The herbaceous layer: *Aconitum moldavicum, Anemone nemorosa, Asarum europaeum, Aremonia agrimonoides, Arum maculatum, Asperula taurina, Brachypodium sylvaticum, Calamagrostis arundinacea, Chaerophyllum aromaticum, Dentaria bulbifera, Digitalis grandiflora, Galium odoratum, Geranium macrorrhizum, Euphorbia amygdaloides, E. platyphyllos, Helleborus purpurascens, Heracleum sphondylium* s. l., *Melica uniflora, Mercurialis perennis, Parietaria officinalis, Symphytum tuberosum, Tanacetum macrophyllum, Urtica dioica.*

At Camena, at altitudes between 650 and 1100 m, the phytocoenoses are installed on the sunny slopes (with NE exhibition), the major habitat being embedded from the southeast Carpathian lilac (*Syringa vulgaris*) and manna ash (*Fraxinus ornus*) thickets.

The trees layer: absent, only *Sorbus aucuparia*, rarely of average heights, more than younger stages.

The shrubs layer: *Spiraea chamaedryfolia*; there also grow *Cotoneaster tomentosus, Sorbus graeca, S. borbasii, Syringa vulgaris, Rosa pendulina.*

The herbaceous layer: *Alyssoides utriculata, Leontodon crispus* subsp. *crispus, Poa nemoralis, Sesleria rigida, Polygala comosa.*

b) R3123 Southeast Carpathian lilac (*Syringa vulgaris*) with *Genista radiata* thickets - phytocoenoses are fragments of the sub-Mediterranean vegetation, edified from mostly southern and south-eastern Dacian subendemic, thermophilic, xerophilic, calcifile species, located at high altitude for them. The edifying species are: *Syringa vulgaris, Genista radiata,* and characteristic: *Syringa vulgaris, Genista radiata, Cotinus coggygria.*

At Piatra Pinului, at altitudes above 860 m, the habitat has the following structure and physiognomy:

The trees layer: *Acer campestre, Betula pendula* (sporadic), *Fraxinus ornus, Juglans regia, Pinus nigra* subsp. *banatica.*

The shrubs layer: *Cotinus coggyria, Cotoneaster tomentosus, Euonymus verrucosus, Lonicera xylosteum, Prunus mahaleb, Rhamnus saxatilis* subsp. *tinctorius, Sorbus borbasii, S. cretica, S. torminalis, S. umbellatta* subsp. *banatica, Syringa vulgaris.*

Undergrowth layer: 20–40 cm: *Genista radiata, Teucrium chamaedrys, T. montanum, Micromeria pulegium.*

The herbaceous layer: *Achnatherum calamagrostis, Acinos arvensis, Allium flavum* subsp. *flavum, A. oleraceum, Alyssum petraeum, Arabis procurrens, A. turrita, Asplenium ruta-muraria, A. scolopendrium, Asperula tinctoria* subsp. *ciliata, Atamantha turbitt* subsp. *hungarica, Bromus riparius, Calamagrostis arundinacea, Campanula rapunculoides, Carex digitata, Clematis vitalba, C. recta, Cystopteris fragilis, Dianthus petraeus, Draba lasiocarpa, Erysimum odoratum, Euphorbia amygdaloides, Festuca xanthina, Galium album* subsp. *album, Geranium macrorrhizum, Hedera helix, Laserpitium latifolium, Linum uninerve, Moehringia muscosa, Neottia nidus-avis, Peucedanum oreoselinum, Piptatherum virescens, Poa nemoralis, Polygonatum multiflorum, P. odoratum, Prenanthes purpurea, Primula auricula* subsp. *serratifolia, Quercus petraea, Rubus canescens, R. idaeus, Saxifraga adscendens* f. *ramosissima, S. rotundifolia, Scabiosa columbaria* subsp. *columbaria, Seseli libanotis, S. rigidum* s. l., *Silene flavescens, Solidago virgaurea, Stachys patula, Tamus communis* subsp. *communis, Taraxacum officinale, T. hoppeanum, Teucrium montanum, Veronica spicata* s. l., *Viburnum lantana, Vincetoxicum hirundinaria.*

At Camena - eastern wall of the first two ridges (E→W) and the plateau from the last ridges from the W, at altitudes between 800 and 950 m.

The trees layer: *Sorbus aucuparia, S. borbasii.*

The shrubs layer: *Abies alba* (juvenile), *Cotinus coggygria, Cotoneaster tomentosus, Fraxinus ornus, Juniperus sabina, Euonimus verrucosus, Rhamnus saxatilis* subsp. *tinctorius, Rosa pendulina, Sorbus aria, S. graeca* (*S. cretica*), *Syringa vulgaris, Viburnum lantana.*

The shrubs layer: *Clematis vitalba, Cytisus ratisbonensis, Genista radiata.*

The herbaceous layer: *Alyssodes utriculata, Asperula capitata, Asplenium ceterach, A. ruta-muraria, Atamantha turbitt* subsp. *hungarica, Carex digitata, Centaurea atropurpurea, C. triniifolia, Cerastium banaticum, Dactylorhiza sambucina* f. *bracteata, D. sambucina* f. *purpurea, Delphinium fissum* subsp. *fissum, Dianthus banaticus, Draba lasiocarpa, Edraianthus graminifolius, Erysimum odoratum, Euphrasia illyrica, Euphorbia epithymoides, Festuca xanthina, Galium album* subsp. *album, G. rubioides, Geranium macrrorhizum, Helianthemum nummularium* s. l., *Hypericum rochelii, Iris* sp., *Isatis tinctoria, Jovibarba heuffelii, Jurinea glycacantha, Orchis mascula* subsp. *signifera, Peucedanum oreoselinum, Polygala vulgaris, Polygonatum multiflorum, Potentilla reptans, Saxifraga paniculata, Seseli libanotis* subsp. *intermedium, S. rigidum* subsp. *rigidum, Sesleria rigida, Senecio jacobaea* subsp. *jacobaea, Silene pusilla, Teucrium chamaedrys, T. montanum, Thlaspi dacicum* subsp. *dacicum, Thalictrum aquilegifolium, T. foetidum, Thymus comosus, Trifolium montanum, Veronica austriaca, V. crassifolia.*

c) R3124 Southeast Carpathian lilac (*Syringa vulgaris*) with *Asplenium-ruta-muraria* thickets - pioneer phytocoenosis, edified from Eurasian species with many southern, sub-thermophilic, mesoxerophilic, calcifile elements. The edifying species are: *Syringa vulgaris, Fraxinus ornus, Carpinus orientalis*, and characteristics: *Syringa vulgaris, Asplenium ceterach, A. ruta-muraria, Cotinus coggygria, Micromeria pulegium.*

The trees layer: phytocoenosis grows as a thicket dominated by scattered bushes of *Fraxinus ornus* and *Carpinus orientalis* to 2–3 m height, which provides a covering to 40–60%.

The shrubs layer: *Cotinus coggygria, Syringa vulgaris.*

Undergrowth layer: is characteristic of the limestone rocks exposed to sunlight: *Alyssum petraeum, Asplenium ceterach, A. ruta-muraria, A. trichomanes* subsp. *qudrivalens, Cerastium banaticum, Chondrilla juncea, Draba lasiocarpa, Euphorbia epithymoides, Galium album* subsp. *album, Koeleria macrantha, Mercurialis ovata, Micromeria pulegium, Moehringia muscosa, Mycelis muralis, Piptatherum virescens, Poa nemoralis, Peucedanum oreoselinum, Scabiosa columbaria* subsp. *columbaria, Seseli rigidum* subsp. *rigidum, Silene vulgaris.*

d) R3126 Southeast Carpathian manna ash (*Fraxinus ornus*) thickets, edified by the *Fraxinus ornus*, and characteristic species *Cornus mas.*

The trees layer: *Fraxinus ornus*

The shrubs layer: *Cotinus coggygria, Cornus mas, Crataegus monogyna, Cytisus nigricans, Juniperus communis, Prunus spinosa, Rhamnus saxatilis* subsp. *tinctorius, Syringa vulgaris, Viburnum lantana.*

At Cornetul Babelor, Cornetul Cerboaniei and Cornetul Piatra Încălecată, *Cornus mas* form a belt at the basal part of the hillocks. At Ponoarele (in the northern part of Zaton Lake) the observed three layers are: the first consists of *Prunus spinosa,* the following of *Cornus mas*, while the hillock peak is covered by *Fraxinus ornus*. On Cornetul Babelor and Cornetul Cerboaniei, the shrub layer is so dense that it forms an impenetrable thicket. These hillocks are the greatest centres for the wildness of the Geopark.

The herbaceous layer: *Achillea millefolium, Alliaria petiolata, Anemone ranunculoides, Asplenium trichomanes, Brachypodium sylvaticum* subsp. *sylvaticum, Capsella bursa-pastoris, Carex caryophyllea, Corydalis intermedia, Eryngium campestre, Galanthus nivalis, Geranium rotundifolium, Geum urbanum, Glechoma hederacea, Helleborus purpurascens, Hepatica nobilis, Isopyrum thalictroides, Lathyrus vernus, Oxalis stricta, Pteridium aquilinum.*

e) R3127 Southeast Carpathian lilac (*Syringa vulgaris*) and manna ash (*Fraxinus ornus*) thickets located at the base of the slopes or above them, on the plateaus. The edifying species are *Syringa vulgaris* and *Fraxinus ornus,* and characteristic: *Fraxinus ornus, Cotinus coggygria, Syringa vulgaris.*

At Piatra Coșuștei it was identified the habitat with the following composition:

The trees layer: *Fagus sylvatica, Fraxinus ornus, Juglans regia, Prunus cerasus, Prunus mahaleb, Quercus dalechampii* (rare).

The shrubs layer: *Cornus mas, Corylus avellana, Juniperus communis, Euonymus europaeus, E. verrucosus, Lonicera xylosteum, Syringa vulgaris.*

The herbaceous layer: *Achnatherum calamagrostis, Allium oleraceum, Arabis hirsuta, Asparagus tenuifolius, Asplenium ceterach, Campanula grossekii, Carduus candicans, Carex polyphylla, Convolvulus arvensis, Echinops bannaticus, Erysimum odoratum, Galanthus nivalis, Inula conyza, Lychnis coronaria, Micromeria pulegium, Moehringia muscosa, Mycelis muralis, Peltaria alliacea, Phleum phleoides, Poa nemoralis, Rosa* sp., *Scabiosa columbaria, Seseli libanotis, Tamus communis* subsp. *communis, Veronica austriaca.*

At Camena one such habitat has the following physiognomy and structure:

The trees layer: *Acer pseudoplatanus, Fraxinus ornus,*

The shrubs layer: *Chamaecytisus ratisbonensis, Cotinus coggygria, Cotoneaster tomentosus, Juniperus sabina, Euonymus europaeus, E. verrucosus, Sorbus borbasii, Syringa vulgaris, Viburnum lantana.*

The herbaceous layer: *Achnatherum calamagrostis, Allium oleraceum, Alyssoides utriculata, Anthriscus nemorosa, Arabis hirsuta, Asplenium ceterach, A. ruta-muraria, A. trichomanes quadrivalens, Aster amellus, Athamanta turbith, Campanula grossekii, Carduus candicans, Carex polyphylla, Cerastium banaticum, Centaurea triniifolia, Clematis vittalba, Convolvulus arvensis, Delphinium fissum* subsp. *fissum, Draba lasiocarpa, Echinops bannaticus, Euphrasia illyrica, Erysimum odoratum, Ferula heuffelii, Festuca xanthina, Galium album* subsp. *album, Galium purpureum, Hypericum rochelii, Inula conyza, Juniperus sabina, Jovibarba heuffelii, Jurinea glycacantha, Laserpitium latifolium, Linum uninerve, Lunaria annua* subsp. *pachyrhiza, Micromeria pulegium, Moehringia muscosa, Mycelis muralis, Peltaria alliacea, Peucedanum oreoselinum, Phleum phleoides, Poa nemoralis, Polygala vulgaris, Potentilla chrysantha, Rosa canina, Saxifraga paniculata, Scabiosa columbaria, Scutellaria altissima, Senecio jacobaea* subsp. *jacobaea, Seseli libanotis* subsp. *sibiricum, Silene flavescens, S. pusilla, Sisymbrium strictissimum, Symphyandra wanerii, Tamus communis* subsp. *communis, Teucrium montanum, Thalictrum aquilegifolium, Veronica austriaca, V. crassifolia, Vincetoxicum hirundinaria.*

At Cornetul Prosăcului.

The trees layer: *Carpinus betulus, Fraxinus ornus.* It is also encountered sporadically: *Quercus cerris, Sorbus torminalis, Tilia cordata, T. platyphyllos.*

The shrubs layer: *Carpinus orientalis, Cornus sanguinea, Corylus avellana, Cotinus coggygria, Crataegus monogyna, Clematis vitalba, Euonymus verrucosus, Hedera helix, Rhamnus saxatilis* subsp. *tinctorius, Rosa canina, R. obtusifolia, R. tomentosa, Spiraea cana, Syringa vulgaris.*

The herbaceous layer: *Achillea crithmifolia, Allium flavum, A. fuscum, Alyssum petraeum, Arabis turrita, Anthriscus nemorosa, Aremonia agrimonoides, Asplenium ceterach, A. ruta-muraria, A. trichomanes* subsp. *quadrivalens, Botriochloa ischaemum, Brachypodium sylvaticum, Campanula grossekii, C. persicifolia, Cardaminopsis arenosa, Carthamus lanatus, Centaurea apiculata* subsp. *spinulosa, Chondrilla juncea, Draba lasiocarpa, Dentaria bulbifera, Fagopyrum convolvulus, Festuca rupicola, Galium album, Galium schultesii, Galium verum, Geranium robertianum, Erophila verna, Erysimum odoratum, Euphorbia myrsinites, Festuca rupicola, Hepatica nobilis, Himantoglossum jankae, Linaria genistifolia, Inula coniza, Isopyrum thalictroides, Lamium galeobdolon, Lathyrus venetus, L. vernus, Lilium martagon, Lithospermum purpureocaeruleum, Lunaria annua* subsp. *pachyrrhiza, Lychnis coronaria, Melica ciliata, M. uniflora, Melittis melissophyllum, Mercurialis perennis, Micromeria pulegium, Mycelis muralis, Origanum vulgare, Petrorhagia saxifraga, Phyllitis scolopendrium, Piptatherum virescens, Poa nemoralis, Polypodium vulgare, Polygonatum odoratum, Primula veris, Sedum hispanicum, S. maximum, S. sexangulare, Seseli rigidum, Sesleria rigida, Silene flavescens, S. vulgaris, Teucrium chamaedrys, Tragopogon balcanicus, Vincetoxicum hirundinaria.*

At Isverna - "Potcoavă" the habitat has the following physiognomy and structure:

The trees layer: *Fraxinus ornus, Prunus mahaleb, Sorbus aucuparia, S. torminalis.*

The shrubs layer: *Cornus sanguinea, Corylus avellana, Cotinus coggygria, Cotoneaster integerrimus, Crataegus monogyna, Clematis vitalba, Euonymus verrucosus, Genista radiata, Juniperus sabina, Lonicera xylosteum, Rhamnus saxatilis* subsp. *tinctoria, Rosa pendulina, R. tomentosa.*

The herbaceous layer: *Acinos alpinus, A. rotundifolius, Allium fuscum, Asperula capitata, Asplenium ceterach, A. ruta-muraria, Athamanta turbith* subsp. *hungarica, Campanula trachelium, Centaurea atropurpurea, Delphinium fissum* subsp. *fissum, Dianthus petraeus, Draba lasiocarpa, Edraianthus graminifolius, Epipactis atrorubens, E. helleborine, Ferula heuffeli, Festuca xanthina, Geranium macrorrhizum, Himantoglossum jankae, Hordelymus europaeus, Inula conizae, I. ensifolia, I. hirta, Jovibarba heuffeli, Lathyrus hallersteinii, L. vernus, Libanotis montana, Lunaria annua* subsp. *pachyrhiza, Micromeria pulegium, Scutellaria altissima, Sedum hispanicum, S. maximum, Seseli rigidum, Silene pussila, Peltaria alliacea, Phleum phleoides, Primula veris* subsp. *columnae, P. auricula* subsp. *serratifolia, Rosa gallica, Teucrium chamaedrys, Thymus pulegioides, Tragopogon balcanicus.*

At Epuran Cave, such a phytocoenosis preserves species as *Micromeria pulegium, Kengia serotina, Allium flavum, Linum uninerve.*

At Ponoarele, it is located one of the largest and most beautiful habitats with lilac of the Geopark – 20 ha. The Cornetul Răiculeşti, Cornetul Băluţa, Cornetul Gărdăneasa presents a representative phytocoenosis of as. *Syringo-Fraxinetum orni* (POPOVA-CUCU & POPESCU, 1975). Also, POPESCU et al. (2004) published the species lists from Lilac Ponoarele Forest.

The trees layer: *Acer campestre, A. tataricum, Carpinus orientalis, Fagus sylvatica* (rare), *Fraxinus ornus, Quercus frainetto, Q. dalechampii, Prunus mahaleb.*

The shrubs layer: *Cornus mas, Crataegus monogyna, Euonymus verrucosus, Rosa corymbifera, Syringa vulgaris, Viburnum lantana.*

The herbaceous layer: *Achillea crithmifolia, A. millefolium, Acinos alpinus* subsp. *majoranifolius, A. arvensis, Agrostis capillaris, Ajuga genevensis, Alyssum alyssoides, A. desertorum, Arenaria serpyllifolia, Asperula taurina* subsp. *leucanthera, Asplenium ceterach, A. ruta-muraria, A. trichomanes* subsp. *quadrivalens, Astragalus glycyphyllos, Bellis perennis, Brachypodium sylvaticum, Calystegia sylvatica, Cardamine hirsuta, Cardaminopsis arenosa, Carex caryophyllea, Carlina biebersteinii* subsp. *brevibracteata, Carduus nutans, Centaurium erythraea, Cerastium fontanum* subsp. *triviale, Cerastium pumilum* subsp. *glutinosum, Clinopodium vulgare, Convolvulus arvensis, Coronilla varia, Corydalis solida, Crepis foetida* subsp. *rhoeadifolia, Cruciata laevipes, C. pedemontana, Cystopteris fragilis, Digitalis*

grandiflora, Epilobium angustifolium, Erodium cicutarium, Erophila verna, Erythronium dens-canis, Eryngium campestre, Festuca rupicola subsp. *saxatilis, F. valesiaca, Filipendula vulgaris, Fragaria vesca, F. viridis, Galium verum* subsp. *verum, Geranium columbinum, G. lucidum, Geum urbanum, Glechoma hederacea, Isopyrum thalictroides, Lactuca serriola, Lapsana communis, Lathyrus sphaericus, Luzula campestris, Lychnis coronaria, Hypochaeris maculata, Medicago minima, Melica uniflora, Melittis melissophyllum, Moehringia muscosa, Myosotis stricta, Mycelis muralis, Pimpinella saxifraga, Poa bulbosa* subsp. *bulbosa, Potentilla micrantha, Prunella laciniata, Pteridium aquilinum, Ranunculus acris, Rubus canescens, R. idaeus, Scleranthus annuus, S. perennis, Sedum acre, S. hispanicum, Saxifraga rotundifolia, S. tridactylites, Scutellaria altissima, Sherardia arvensis, Silene italica, Stachys germanica, Polygonatum odoratum, Rorippa sylvestris, Viola tricolor* subsp. *tricolor, Tamus communis, Teucrium chamaedrys, Thymus comosus, Trifolium arvense, T. aureum, T. montanum, Valerianella locusta, Verbena officinalis, Veronica arvensis, V. chamaedrys, V. serpyllifolia, V. triphyllos, Vincetoxicum hirundinaria, Vulpia myuros.*

Contact habitats: 6190 Rupicolous Pannonic grasslands; 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*); 8160* Medio-European calcareous scree of hill and mountain levels; 8210 Calcareous rocky slopes with chasmophytic vegetation; 9150 Medio-European limestone beech forest of the *Cephalanthero-Fagion*; 91K0 Illyrian *Fagus sylvatica* forests (*Aremonio-Fagion*); 91L0 Illyrian oak hornbeam forest (*Erytronio-Carpinion*); these habitats have in common a series of species, for example:

- common species from rupicolous Pannonic or semi-natural dry grasslands and scrubland facies on calcareous substrates (like is the case at Topolnița, Ponoarele, Camena): *Achillea crithmifolia, Acinos arvensis, Alyssum alyssoides, A. desertorum, Anthemis tinctoria, Brachypodium sylvaticum* subsp. *sylvaticum, B. pinnatum, Bromus riparius, Carex caryophyllea, Chondrilla juncea, Dactylorhiza sambucina* f. *bracteata, D. sambucina* f. *purpurea, Dianthus giganteus, Erysimum odoratum, Festuca rupicola* subsp. *saxatilis, F. valesiaca, Galium album* subsp. *album, G. verum* subsp. *verum, Koeleria macrantha, Lolium perenne, Dianthus giganteus, Himantoglossum jankae* (Mount Fața Satului-Isverna, Topolnița-La Varnițe), *Hypericum perforatum, Hypochaeris maculata, Isopyrum thalictroides, Lychnis coronaria, Orchis mascula* subsp. *signifera* (Mount Fața Satului-Isverna), *O. militaris* (Mount Fața Satului), *Prunella laciniata, Ranunculus acris, Pteridium aquilinum, Stachys germanica, Teucrium chamaedrys, Tragopogon balcanicus, Trifolium arvense, T. aureum, T. montanum, Verbascum phlomoides, Verbena officinalis, Veronica arvensis, V. austriaca.*

- transgressive species from the nearby forests: *Acer campestre, A. platanoides, A. pseudoplatanus, Anemone ranunculoides, Angelica sylvestris, Athyrium filix-femina, Calamagrostis arundinacea, Campanula rapunculoides, Corydalis* sp., *Isopyrum thalictroides, Lathyrus vernus, Lithospermum purpurocaeruleum, Luzula luzuloides, Melittis melissophyllum, Neottia nidus-avis, Mycelis muralis, Phyllitis scolopendrium, Piptatherum virescens, Poa nemoralis, Polygonatum odoratum, P. multiflorum, Polystichum setiferum, Potentilla micrantha, Prenanthes purpurea, Pteridium aquilinum, Rubus canescens, R. idaeus, Saxifraga rotundifolia, Scabiosa columbaria* subsp. *columbaria, Sorbus aucuparia, Stachys patula, Tamus communis* subsp. *communis, Veronica spicata* s. l., *Viburnum lantana, Vincetoxicum hirundinaria.*

- characteristic species from calcareous rocky slopes with chasmophytic vegetation: *Asplenium ceterach, A. ruta-muraria, Atamantha turbitt* subsp. *hungarica, Cerastium banaticum, Delphinium fissum* subsp. *fissum, Dianthus petraeus, Draba lasiocarpa, Primula auricula* subsp. *serratifolia, Seseli rigidum* s. l., *Sesleria rigida, Silene flavescens.*

- characteristic species from Medio-European calcareous scree of hill and mountain levels: *Achnatherum calamagrostis, Micromeria pulegium, Moehringia muscosa.*

Ecological and biological value – high conservation value; habitat with many endemic and rare species.

The criterion Aii corresponding plant species for selecting types of Natura 2000 sites: European endangered species listed in the Habitats Directive Annexes IIb and IVb + Bern Convention – App I, whose conservation requires the designation of Special Areas of Conservation – SAC: *Galanthus nivalis* and *Himantoglossum jankae.*

The criterion Aiii corresponding plant species for selecting types of Natura 2000 sites: endemic and endangered species – National Red Lists (CR, EN, and V), not included in the Ai and Aii categories: *Atamantha turbitt* subsp. *hungarica, Edraianthus graminifolius, Pinus nigra* subsp. *banatica, Primula auricula* subsp. *serratifolia, Sorbus borbasii, Thlaspi dacicum* subsp. *dacicum.*

The criterion Aiv b corresponding plant species for selecting types of Natura 2000 sites: plant species listed in Annex II b - sub-endemic and endangered species - **National Red Lists** (CR, EN, and V) not included in the Ai, Aii and Aiii categories: *Micromeria pulegium.*

Species from the National Red Lists: *Abies alba, Alyssoides utriculata, Centaurea atropurpurea, C. triniifolia, Cerastium banaticum, Dactylorhiza sambucina, Delphinium fissum* subsp. *fissum, Dianthus banaticus, Draba lasiocarpa, Euphrasia illyrica, Festuca xanthina, Genista radiata, Hepatica nobilis, Hypericum rochelii, Jovibarba heuffelii, Juniperus sabina, Jurinea glycacantha, Linum uninerve, Mercurialis ovata, Neottia nidus-avis, Orchis mascula* subsp. *signifera, O. militaris, Peltaria alliacea, Petrorhagia saxifraga, Piptatherum virescens, Seseli rigidum* subsp. *rigidum, Silene flavescens, S. saxifraga, Sorbus graeca, Teucrium montanum, Tragopogon balcanicus, Veronica crassifolia.*

DISCUSSIONS

The association *Syringo-Genistetum radiatae* Maloş 1972 is considered by SANDA et al. (2001) as sub-association of *Syringo-Fraxinetum orni* Borza 1958 m. Resmeriţă 1972, and associations that edify habitats R3124, R3126 and R3127 are treated as synonyms of it. Also, for the subas. *cotinetosum* Ciocârlan 1968, Schrött 1968 the same authors indicate the following syn.: *Syringo-Cotinetum coggygriae* (Borza 1931 n.n.) Resmeriţă 1972; for subas. *juniperetosum sabinae* (Gergely 1958) Jakucs 1959 same SANDA et al. (2001) indicates syn. as. *Syringeto-Juniperetosum sabinae* Gergely 1958) and subas. *ceterosum* Jakucs et Vida 1959. The *Genista radiata* species is present in all of the three associations.

In the area we noticed the following situation: *Genista radiata* not so frequent (only two choronyms in the Geopark). On the northeastern side of the first ridge of Camena it occurs with high constancy, on fairly large surface, sometimes resulting 90% coverage (similar situation observed on the Ţesna Valley). These phytocoenoses, where *Genista radiata* is dominant, and *Fraxinus ornus* is rare, may be considered under as. *Syringo-Genistetum radiatae* Maloş 1972; on the second ridge of Camena Mount, on the eastern slope, the situation is different: *Genista radiata* occurs quite often, but the bushes are scattered. Here, *Fraxinus ornus* constitutes a shrub layer; those phytocoenoses could be considered a subas. of the as. *Syringo-Fraxinetum orni* Borza 1958 m. Resmeriţă 1972, namely subas. *genistetosum radiatae* (Maloş 1972) Popescu & Sanda 1990.

Regarding the other phytocoenoses, where the *Genista radiata* does not appear, we believe that it could be treated as subas. of the large as. *Syringo-Fraxinetum orni* Borza 1958 m. Resmeriţă 1972, namely:

- subas. *cotinetosum* Ciocârlan 1968 to which shall be assignable some phytocoenoses from Camena (plateau), Topolniţa Gorges and Cornetul Coşuştei. Those phytocoenoses occur as clumps on the rocky limestone. Around these rocks, there are many species such as *Bromus riparius, Achnatherum calamagrostis, Festuca rupestris, Teucrium chamaedrys* and numerous characteristic species of the communities from the Southeast Carpathian limestone cliffs with *Asplenium trichomanes* subsp. *quadrivalens* and *Poa nemoralis*.

- subas. *juniperetosum sabinae* (Gergely 1958) Jakucs 1959 to which shall be assignable some phytocoenoses from Camena.

Data from the specialized literature, field observations (structure and physiognomy of the associations, the fact that the main edifying species is *Fraxinus ornus*, and *Genista radiata*, *Cotinus coggygria* and *Juniperus sabina*, which are characteristic species, are at least present in the phytocoenosis that edifies their associations or subassociations) only serve to strengthen our conviction that the classification of these types of vegetation in one type of habitat is justified. Also, the substrate type, altitude and slope orientation these species prefer are similar in all areas, and the phytocoenoses of different Romanian subtypes of habitat are intercalated, sometimes indistinguishable. For e.g. on the Mount Camena, there can be met all the variants of the habitat 40A0, the phytocoenosis are intermingled, succeeding according to altitude and slope aspect. The same thing happens to Topolniţa and Cornetul Coşuştei, where the variant with *Genista radiate* is not present.

Disturbing factors of such a habitat are represented by the degradation from grazing, when the habitat is in contact with grassland, deforestation for increasing the pasturelands areas or cultures, arson, the entrance in succession (in the case of secondary habitat), the harvesting blooming lilac twigs for marketing, uncontrolled tourism.

CONCLUSION

1. From the above mentioned aspects it is confirmed that the habitat is represented by the mosaic vegetation composed of steppe meadows (6210) and forest steppe floristic elements or species of plants from rupicolous Pannonian grasslands (6190).

2. The habitat is well represented, accompanying the Jurassic limestone alignment of the Danubian Autochthonous.

3. The status of this habitat is favourable in the examined area. There have not been identified potential threats to the habitat, have not been noticed any parasitic fungi attacks.

4. Under natural conditions the habitat, with its various subtypes, does not require special management. Natural habitats are located on the Natura 2000 site - protected areas.

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DATA REGARDING THE QUALITATIVE COMPOSITION OF THE CILIATES FROM THE MURAT RIVER (AĞRI REGION, TURKEY) ACCORDING TO THE NEW TAXONOMIC SYSTEM PROPOSED BY LYNN

KERKMANN Gina Raluca

Abstract. The ciliates from the aquatic ecosystems of Turkey are poorly known; however, there have been identified 164 forms so far. Protistological literature does not offer information regarding the ciliate species from the water and sediments of the Murat River. We started our researches in 2011 and there have been identified 31 species till now; 13 forms were identified only to genus. The ciliate species have a cosmopolite origin; 26 are also found on the list proposed by Çapar for the Turkish fauna; 12 species are also common with the ciliates from the paralitoral lakes of the Black Sea coast.
In the present scientific paper, it is presented the actual qualitative composition of the ciliates from the Murat River (Ağrı region) according to the new taxonomical system proposed by Lynn, as well as some considerations regarding the former and actual taxonomical system of Protozoa.

Keywords: Murat River, Ciliates, Taxonomy.

Rezumat. Date privind compoziţia calitativă a ciliatelor din Râul Murat (regiunea Ağrı, Turcia) conform noului sistem taxonomic propus de Lynn. Ciliatele din ecosistemele acvatice ale Turciei sunt puţin cunoscute, fiind identificate până în prezent 164 de forme. Literatura protistologică nu oferă informaţii privind speciile de ciliate din apă şi sedimentele râului Murat; cercetările noastre au demarat în 2011 şi au fost identificate până în prezent 31 de specii; 13 forme au fost identificate până la gen. Speciile de ciliate au o origine comopolită. 26 se regăsesc de asemenea pe lista propusă de Çapar pentru fauna Turciei; 12 specii sunt de asemenea comune cu ciliatele din lacurile paralitorale de pe coasta Mării Negre. În actuala lucrare este prezentată compoziţia calitativă actuală a ciliatelor din râul Murat conform sistemului taxonomic propus de Lynn ca si câteva consideraţii privind sistemele taxonomice vechi şi noi ale protozoarelor.

Cuvinte cheie: Râul Murat, ciliate, taxonomie.

INTRODUCTION

The Protozoa fauna of the aquatic ecosystems, especially ciliates, dominate the other groups by species richness and density.

In most of the European countries, ciliate, fauna of aquatic and terrestrial ecosystems was rigorously investigated: in France by DRAGESCO (DRAGESCO, 1998), in Germany by Bick (BİCK, 1972a; 1972b), Wilbert (WİLBERT, 1975) which discovered a special variant for silver impregnations; other scientists, in Romania, in the last 30 years, made researches regarding the ciliates fauna of the Black Sea coast and paramarine lakes: Petran (PETRAN, 1976a; 1976b) and KERKMANN (KERKMANN 2003; 2007; 2011). However, there is another situation for the countries of Asia, especially for Turkey, where the researches about benthic ciliates intensified during the last 20-30 years. The most detailed studies were performed by ŞENLER et al. (1998), ŞENLER & YILDIZ (1998, 1999, 2004) who worked especially on rivers, small ponds and sewage treatment plants and by ÇAPAR (1997, 2005, 2007) on free living pond and wetland ciliates (ÇAPAR, 2007).

ÇAPAR proposed a list of ciliates including 164 species (ÇAPAR, 2007). In her list, there are not given information-about the free ciliates of the Murat River; it is a poorly known ecosystem, except for some geological and fish parasites (ASLAN, 2009; DEMİR et al., 2008). The investigations about the ciliate fauna of the river were initiated in 2011 by Kerkmann (KERKMANN, 2012; KERKMANN et al., 2012).

MATERIAL AND METHODS

Since the summer of 2011 water and sediments samples have been monthly collected from the stations situated along the Murat River, in Ağrı town and outside (KERKMANN, 2012; KERKMANN et al., 2012).

The collected samples were thermally conditioned analysed in the laboratory. The extraction of the ciliates from sediments was made by Uhlig and Webb method (UHLİG, 1964; WEBB, 1956) or directly collected from Petri dishes using fine glass handmade capillary tubes by Bunsen burner (DRAGESCO & DRAGESCO-KERNEİS, 1986) (Photo 1-5).

Some species were determined 'in live', another were subject to vital colorations with red-neutral and postvital with methyl green. For most of the species, there were applied special techniques to obtain permanent slides (Bodian method – Wilbert version and Chatton - Lwoff method) (DRAGESCO & DRAGESCO-KERNEİS, 1986; WİLBERT, 1975). The importance of these techniques consists in emphasizing some infrastructural elements with taxonomic relevance.

In order to establish the taxonomical diagnosis, there were used the main protistological determinators of DRAGESCO, CORLİSS, FOİSSNER and colab. (DRAGESCO & DRAGESCO-KERNEİS, 1986; CORLİSS & LOM, 1985; CURDS, 1982; FOİSSNER, 1984; FOİSSNER et al., 1991).

To compare the qualitative composition of ciliates from many ecosystems, there were also consulted the articles published by the Turkish protistologists especially ÇAPAR (ÇAPAR, 2007).

RESULTS AND DISCUSSIONS

The research activity regarding the ciliate fauna of the Murat River during the years led to the elaboration of a list, which contains so far 31 species; however, we mention that their number enriched during the last years (KERKMANN, 2012; KERKMANN et al., 2012) (Table 1). From 31 species, a number of 13 forms were identified only to genus.

The ciliates from the Murat River appear to have a cosmopolite origin; so, 26 are common to the list proposed by ÇAPAR for the Turkish fauna and 12 are identical with the ciliates from the Romanian paramarine lakes of the Black Sea (DUMİTRACHE-KERKMANN, 2004).

The distribution of the ciliate species into the sediments of the two stations is relatively balanced, as 20 respectively 23 species were found; 11 forms are common to both stations (Table 1). The arrangement of species in table 1. was made according to the new taxonomic system proposed by LYNN (LYNN, 2003 in: ÇAPAR, 2007). Concerning the different taxonomic categories, 7 of the ciliate species identified in the Murat River belong to the class SPİROTRİCHEA (BÜTSCHLI, 1859) and PROSTOMATEA (SCHEWIAKOFF, 1896) and 9 species to a superior class OLİGOHYMENOPHORA (de PUYTORAC *et al.*, 1974). In terms of order, most of the species belongs to the order PRORODONTİNA (CORLISS, 1974).

The old taxonomic systems for ciliates relied exclusively on taxonomic criteria, most of them established after performing some sketches 'in vivo' of the ciliates. They were gradually completed with infrastructural elements emphasized after applying the techniques of obtaining permanent preparations (CHATTON - LWOFF and BODIAN techniques and their further variants) (DRAGESCO & DRAGESCO-KERNEİS, 1986).

The comparative analysis of these different taxonomic systems (Table 2) shows the maintenance of the main groups of ciliates according to the specific arrangement of the somatic and cytostomial ciliature with some variations of the taxonomic rank; for example, the Order SPİROTRİCHA from KAHL system became Subclass SPİROTRİCHA in the system proposed by HONIGBERG *et al.* (HAUSMANN, 1985; LEVİNE *et al.*, 1980).

The penultimate taxonomic system accepted by Protistological Society represents an interesting interference between other systems proposed by de PUYTORAC, CORLISS, LEVINE and it is well synthetized in the monograph of DRAGESCO (PUYTORAC *et al.*, 1974; CORLİSS, 1979; LEVİNE *et al.*, 1980; DRAGESCO & DRAGESCO-KERNEİS, 1986). However, there are a lot of differences between them and the new taxonomic system, taxonomic categories being completed by genetic criteria.

Thus, the number of classes was extended into the LYNN system from 3 to 11 and the number of subclasses from 5 to 10. Some taxonomic categories were not changed: Class OLİGOHYMENOPHORA (PUYTORAC *et al.*, 1974), Subclass HYMENOSTOMATA (TİA) (DELAGE *et al.*, HEROUARD, 1986).

Other taxonomic categories acquired a higher taxonomic rank: Order KARYORELİCTİDA (CORLISS, 1974); Class KARYORELİCTEA (CORLISS, 1974); Order HETEROTRİCHİDA (STEIN, 1859); Subclass HYPOTRİCHİA (STEIN, 1859) (Table 3).

In the taxonomic system proposed by LYNN (2003 in ÇAPAR, 2007), there is 1 Subphylum, 3 classes, 2 subclasses, 3 orders, 3 suborders and 2 families that were described or re-described by Lynn and his colleagues.

CONCLUSIONS

1. In the aquatic ecosystems of Turkey, there were identified 164 ciliates according to the list proposed by Çapar.
2. The qualitative composition of the ciliate fauna from the Murat River (Ağrı region) was for the first time researched by Kerkmann, who proposed a list containing 31 species so far.
3. Most of them have a cosmopolite origin; 12 species are common with the fauna of the paramarine lakes of the Romanian Black Sea coast.
4. The actual taxonomic system for ciliates is characterized by a higher complexity, as the taxonomic infrastructural elements were completed by genetic researches.

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Photo 1 - Station 2 Sewerage system.

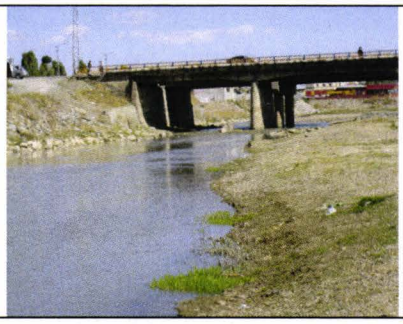

Photo 2 - Station 1 The Murat River bridge.

Photo 3 - The Murat River in winter (Ağrı town).

Photo 4 - Detail of the Murat River sediments.

Photo 5 – The Murat River outside of Ağrı town.

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Table 1. The qualitative composition of ciliates from the Murat River (Ağrı Region).

NO	SUBPHYLUM	CLASSİS	SUBCLASSİS	*ORDO*	SUBORDO	FAMİLİA	KERKMANN (2014)	1	2	ÇAPAR (2007)
1	POSTCİLİODESMATOPHORA	*HETEROTRİCHEA*	-	*HETEROTRİCHİDA*	-	Blepharismidae	*Spirostomum teres* CLAPAREDE et LACHMANN, 1858-1859	+	+	+
2	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	HYPOTRİCHİA	*EUPLOTİDA*	*EUPLOTİNA*	Euplotidae	*Euplotes* sp.	+	-	+
3	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	HYPOTRİCHİA	*EUPLOTİDA*	*EUPLOTİNA*	Aspidiscidae	*Aspidisca* sp.	+	+	+
4	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	STİCHOTRİCHİA	*SPORADOTRİCHİNA*	-	Oxytrichidae	*Oxytricha saprobia (pelionella) (?)* O. F. MÜLLER,1786	+	+	-
5	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	STİCHOTRİCHİA	*SPORADOTRİCHİNA*	-	Oxytrichidae	*Oxytricha* sp.1	-	+	+
6	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	STİCHOTRİCHİA	*SPORADOTRİCHİNA*	-	Oxytrichidae	*Oxytricha* sp.2	+	+	+
7	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	STİCHOTRİCHİA	*SPORADOTRİCHİNA*	-	Oxytrichidae	*Stylonychia* sp.	+	+	+
8	İNTRAMACRONUCLEATA	*SPİROTRİCHEA*	STİCHOTRİCHİA	*UROSTYLİDA*	-	Urostylidae	*Holosticha* sp.	+	+	+
9	İNTRAMACRONUCLEATA	*ARMOPHOREA*	-	*ARMOPHORİDA*	-	Metopidae	*Metopus* spp.	+	+	+
10	İNTRAMACRONUCLEATA	*LİTOSTOMATEA*	HAPTORİA	*HAPTORİDA*	-	Lacrymariidae	*Lacrymaria* sp.	-	+	+
11	İNTRAMACRONUCLEATA	*LİTOSTOMATEA*	HAPTORİA	*HAPTORİDA*	-	Tracheliidae	*Lagynophrya rostrata* KAHL, 1930	-	+	-
12	İNTRAMACRONUCLEATA	*LİTOSTOMATEA*	HAPTORİA	*PLEURONEMATIDA*	-	Litonotidae	*Litonotus lamella (*EHRENBERG, 1838) SCHEWIAKOFF, 1896	-	+	+
13	İNTRAMACRONUCLEATA	*LİTOSTOMATEA*	HAPTORİA	*PLEURONEMATIDA*	-	Litonotidae	*Loxophyllum* sp.	+	-	+
14	İNTRAMACRONUCLEATA	*COLPODEA*	-	*COLPODIDA*	-	Colpodidae	*Colpoda steinii* (MAUPAS,1883)	-	+	+
15	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTID*	-	Prorodontidae	*Prorodon viridis* EHRENBERG, 1840			
16	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTIDA*	-	Prorodontidae	*Prorodon* sp.	-	+	-
17	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTIDA*	-	Prorodontidae	*Plagiocampa rouxi KAHL, 1932*	-	+	-
18	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTIDA*	-	Prorodontidae	*Coleps* sp.	+	-	+
19	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTIDA*	-	Urotrichidae	*Urotricha globosa* CLAPAREDE et LACHMANN, 1857	+	+	+
20	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTIDA*	-	Urotrichidae	*Urotricha* sp.1	+	-	+
21	İNTRAMACRONUCLEATA	*PROSTOMATEA*	-	*PRORODONTIDA*	-	Urotrichidae	*Urotricha* sp.2	+	-	+
22	İNTRAMACRONUCLEATA	*PLAGIOPYLEA*	-	*PLAGIOPYLIDA*	-	Plagyopilidae	*Plagioyla nasuta* STEIN, 1860	+	-	+
23	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	PENICULIA	*PENİCULİDA*	*PARAMECINA*	Paramecidae	*Paramecium aurelia-complex* EHRENBERG, 1838	+	+	+
24	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	PENICULIA	*PENİCULİDA*	*PARAMECINA*	Paramecidae	*Paramecium caudatum* EHRENBERG, 1838	+	+	+
25	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	PENICULIA	*PENİCULİDA*	-	Paramecidae	*Paramecium putrinum* (HILL, 1752) CLAPAREDE and LACHMANN, 1859	+	+	+
26	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	SCUTICOCILIATIA	*PHILASTERIDA*	-	Uronematidae	*Uronema nigricans* (MÜLLER, 1786) FLORENTIN, 1901	-	+	+
27	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	SCUTICOCILIATIA	*PLEURONEMATIDA*	-	Cyclidiidae	*Cyclidium glaucoma* MÜLLER, 1773	-	+	+
28	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	HYMENOSTOMATIA	*HYMENOSTOMATIDA*	*TETRAHYMENINA*	Turaniellidae	*Colpidium colpoda* (LOSANA, 1829) STEIN, 1860	+	-	+
29	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	HYMENOSTOMATIA	*HYMENOSTOMATIDA*	*TETRAHYMENINA*	Turaniellidae	*Dexiostoma campylum* FOCKE, 1836 DUJ. 1841,STOKES,1886	+	-	+
30	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	PERITRICHIA	*SESSILIDA*	-	Vorticellidaee	*Vorticella campanula* EHRENBERG, 1833	+	+	+
31	İNTRAMACRONUCLEATA	*OLIGOHYMENOPHOREA*	PERITRICHIA	*SESSILIDA*	-	Vorticellidae	*Vorticella microstoma –complex* EHRENBERG, 1830	-	+	+
32	TOTAL = 2	*8*	7	*15*	*3*	18		26	20	23

Legend:
1 – Station 1 The Murat River bridge ; 2 - Station 2 Sewerage system

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Table 2. Comparative analysis between different taxonomic systems.

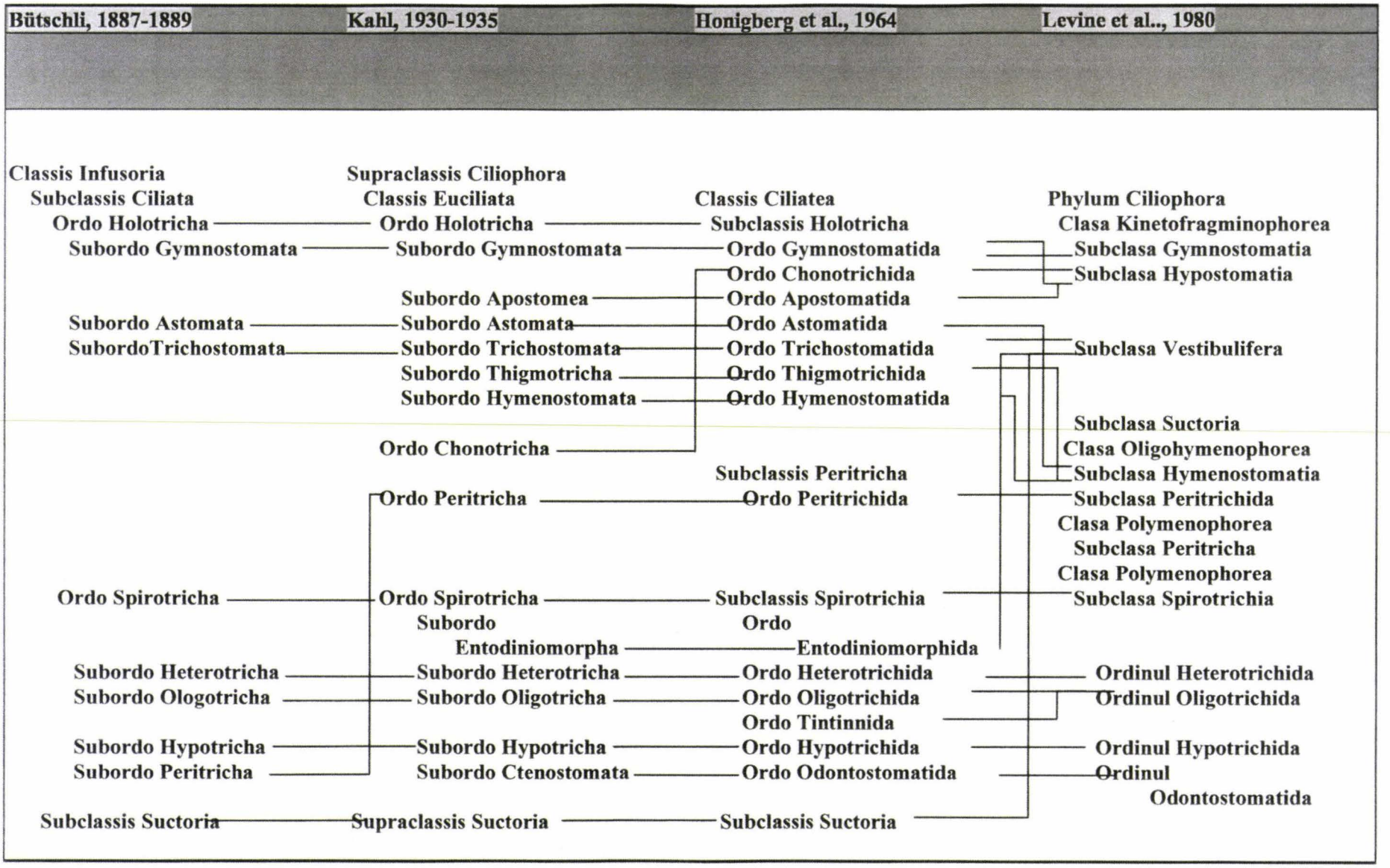

Table 3. Comparative analysis between the last taxonomic system Lynn (LYNN,2003) and old taxonomic system (de PUYTORAC and colab. in: DRAGESCO et DRAGESCO – KERNEIS, 1986).

1 **PHYLUM CILIOPHORA (in DRAGESCO et DRAGESCO – KERNEIS, 1986)**
2 ***CLASSIS KINETOFRAGMINOPHORA*** de Puytorac et al., 1974
3 **Subclassis GYMNOSTOMATA** Bütschli, 1885
4 *Ordo PROSTOMATIDA* Schewiakoff, 1896
5 *Ordo PLEUROSTOMATIDA* Schewiakoff, 1896
6 Incertae sedis: *Ordo KARYORELICTIDA* Corliss, 1974
7 **Subclassis VESTIBULIFERA** de Puytorac et al.,1974
8 *Ordo TRICHOSTOMATIDA* Bütschli, 1883
9 *Ordo COLPODIDA* de Puytorac et al.,1974
10 ***CLASSIS OLIGOHYMENOPHORA*** de Puytorac et al.,1974
11 **Subclassis HYMENOSTOMATA** Delage et Herovard, 1896
12 *Ordo HYMENOSTOMATIDA* Delage et Herovard, 1896
13 *Ordo SCUTICOCILIATIDA* Small, 1967
14 **Subclassis PERITRICHA** Stein, 1859
15 ***CLASSIS POLYHYMENOPHORA*** Jankowski, 1967
16 **Subclassis SPIROTRICHA** Bütschli, 1889
17 *Ordo HETEROTRICHIDA* Stein,1839
18 Ordo ODONTOSTOMATIDA Sawaya, 1940
19 *Ordo HYPOTRICHIDA* Stein,1859
20 *Ordo OLIGOTRICHIDA Bütschli, 1997*
21 **Subclassis HYPOSTOMATA** Schewiakoff, 1896
22 Supraordo NASSULIDA Jankowski, 1967
23 *Ordo CYRTOPHORIDA* Faure – Fremiet (in Corliss, 1956)

1 **PHYLUM CILIOPHORA (in LYNN, 2003)**
2 SUBPHYLUM POSTCILIODESMATOPHORA Gerassimova and Seravin, 1976
3 ***CLASSIS KARYORELICTEA*** Corliss,1974
4 *Ordo LOXODIDA* Jankowski, 1980
5 ***CLASSIS HETEROTRICHEA*** Stein, 1859
6 *Ordo HETEROTRICHIDA* Stein, 1859
7 SUBPHYLUM INTRAMACRONUCLEATA Lynn, 1996
8 ***CLASSIS SPIROTRICHEA*** Bütschli, 1859
9 **Subclassis HYPOTRICHIA** Stein,1859
10 *Ordo EUPLOTIDA* Small and Lynn, 1985
11 ***Subordo EUPLOTINA*** Small and Lynn, 1985
12 **Subclassis CHOREOTRICHIA** Small and Lynn, 1985
13 *Ordo TINTINNIDA* Koloid and Campbell,1929
14 *Ordo CHORFOTRICHIDA* Small and Lynn, 1929
15 **Subclassis STICHOTRICHIA** Small and Lynn, 1929
16 *Ordo STICHOTRICHIDA* Faure-Fremiet, 1961
17 *Ordo SPORADOTRICHINA* Faure-Fremiet, 1961
18 *Ordo UROSTYLIDA* Jankowski, 1979
19 **Subclassis OLIGOTRICHIA** Bütschli, 1887
20 *Ordo HALTERIDA* Petzund -Foissner, 1982
21 *Ordo STROMBIDIIDA* Petzund -Foissner, 1970
22 ***CLASSIS ARMOPHOREA*** Jankowski, 1964
23 *Ordo ARMOPHORIDA* Jankowski, 1964
24 ***CLASSIS LITOSTOMATEA*** Small and Lynn, 1981
25 **Subclassis HAPTORIA** Corliss, 1974
26 *Ordo HAPTORIDA* Corliss, 1974
27 ***CLASSIS PHYLLOPHARINGEA*** de Puytorac et al., 1974
28 **Subclassis PHYLLOPHARINGIA** de Puytorac et al., 1974
29 *Ordo CHLAMYDODONTINA* Deroux, 1976
30 *Ordo DYSTERIIDA* Deroux, 1976
31 **Subclassis SUCTORIA** Claparede et lachmann, 1858
32 *Ordo EXOGENID* Colin, 1912
33 ***CLASSIS NASSOPHOREA*** Small and Lynn, 1987
34 *Ordo MICROTHORACIDA* Jankowski, 1967
35 ***CLASSIS COLPODEA*** Small and Lynn, 1981
36 *Ordo BRYOMETOPIDA* Foissner, 1985
37 *Ordo COLPODIDA* de Puytorac et al., 1974
38 *Ordo CYRTOLOPHOSIDIDA* Foissner, 1978
39 ***CLASSIS PROSTOMATEA*** Schewiakoff,1896
40 *Ordo PROSTOMATIDA* Schewiakoff,1896
41 *Ordo PRORODONTIDA* Corliss, 1974
42 ***CLASSIS PLAGIOPYLEA*** Small and Lynn, 1985
43 *Ordo PLAGIOPYLIDA* Small and Lynn, 1985
44 ***CLASSIS OLIGOHYMENOPHOREA*** de Puytorac et al., 1974
45 **Subclassis PENICULIA** Faure – Fremiet in Corliss, 1956
46 *Ordo PENICULIDA* Faure – Fremiet in Corliss, 1956
47 ***Subordo FRONTONINA*** Small and Lynn, 1985
48 ***Subordo PARAMECINA*** Jankowski in Small and Lynn, 1985
49 **Subclassis SCUTICOCILIATIA** Small, 1967
50 *Ordo PHILASTERIDA* Small, 1967
51 *OrdoPLEURONEMATIDA* Faure – Fremiet in Corliss, 1956
52 **Subclassis HYMENOSTOMATIA** Delage and Herouard, 1896
53 *Ordo HYMENOSTOMATIDA* Delage and Herouard, 1896
54 ***Subordo TETRAHYMENINA*** Faure – Fremiet (in Corliss, 1956)
55 **Subclassis PERITRICHIA** Stein, 1859
56 *Ordo SESSILIDA* Kahl, 1933 *Ordo MOBILIDA* Kahl, 1933

THE CATALOGUE OF ORTHOPTERA (INSECTA: ORTHOPTERA) FROM THE "I. FIRU" ENTOMOLOGICAL COLLECTION HOUSED AT OLTENIA MUSEUM CRAIOVA

CHIMIŞLIU Cornelia

Abstract. The paper analyses the Orthoptera preserved in the "I.Firu" Entomological Collection acquired in 1982 and 1991. The 775 specimens belong to a total of 40 species included in 26 genera. 7 subfamilies. 8 families and 5 superfamilies. The best represented in numerical terms is the Acrididae family. which has 525 specimens of the 775 representing 67.74%. It is followed by the Tridactylidae family with 121 specimens (15.61%) and Tetrigidae with 74 specimens (9.55%). The other five families sums up 55 samples (7.10%). The species were collected in 23 sites in 4 counties of Oltenia and one in Brasov County. 13 sites are mentioned for the first time. The new sites together with the 23 sites mentioned previously (TOGĂNEL & CHIMIŞLIU. 2005). contribute to a better knowledge of the Orthoptera distribution in this area of Romania. The 40 species represent 21.98% of the species of Orthoptera (182) reported from Romania (IORGU et al.. 2008). and 15 of these are new species in the museum patrimony.

Keywords: catalogue. Orthoptera. patrimony Oltenia Museum Craiova. Oltenia fauna.

Rezumat. Catalogul orthopterelor (Insecta: Orthoptera) din Colecţia entomologică "I. Firu", conservată la Muzeul Olteniei Craiova. Lucrarea analizează ortopterele conservate în Colecţia entomologică "I. Firu" achiziţionată în anul 1982 şi 1991. Cele 775 exemplare aparţin unui număr de 40 de specii. incluse în 26 de genuri. 7 subfamilii. 8 familii şi 5 suprafamilii. Familia cel mai bine reprezentată din punct de vedere numeric este familia Acrididae. care însumează 525 exemplare din cele 775 reprezentând puţin peste 67.74%. Este secondată de familia Tridactylidae. cu 121 exemplare (15.61%) şi Tetrigidae. cu 74 exemplare (9.55%). Celelalte 5 familii însumează 55 exemplare (7.10%). Speciile au fost colectate în 23 de situri din 4 judeţe din Oltenia şi unul din judeţul Braşov. Treisprezece situri sunt menţionate pentru prima dată. Acestea se alătură celor 23 de situri de colectare din Oltenia. menţionate anterior (TOGĂNEL & CHIMIŞLIU. 2005). contribuind la o mai bună cunoaştere a distribuţiei ortopterelor în aceasta zonă a României. Cele 40 de specii reprezintă 21.98% din numărul speciilor de ortoptere (182) semnalate din România (IORGU et al.. 2008). iar 15 specii sunt noi semnalări din patrimoniul muzeului.

Cuvinte cheie: catalog. ortoptere. patrimoniul Muzeului Olteniei Craiova. fauna Olteniei.

INTRODUCTION

"I. Firu" Entomological Collection includes 6,871 specimens belonging to 18 orders of insects, the majority being collected in natural and anthropogenic ecosystems of Oltenia, in the period 1951-1982. The collection was acquired in two stages. The first part of the collection (6,000 specimens) was acquired in 1982, and the second part (871 specimens) came into the possession of the museum in 1991. The Coleoptera order (1680 specimens) has the largest percentage being followed by the order Lepidoptera.

Most insects are not published. So far, from this collection were published Lepidoptera (CHIMIŞLIU, 1996, 2006) and partially beetles: Scarabaeoidea (CHIMIŞLIU, 2000) Cerambicidae (SERAFIM et al., 2004) and Elateridae (ZAHARIA (CIUCĂ) & CHIMIŞLIU, 2004) and Diptera (CHIMIŞLIU, 2014)

From the species of the Orthoptera order preserved in the museum entomological patrimony, there were published 54 species collected during the years 1964 to 2005 from 32 collection sites, most in Dolj County (TOGĂNEL & CHIMIŞLIU, 2005).

The first data on the Orthoptera of Oltenia are recorded since the mid-nineteenth century (FISCHER (1853) cit. TOGĂNEL & CHIMIŞLIU (2005). Other authors that have reported Orthoptera species from the fauna of Oltenia are: ZOTTU (1909); MARCU (1929); GEORGESCU (1930); MÜLLER (1932); ELIESCU (1937); KIS (1960); PÎRVESCU (1965); KIS & PÎRVESCU (1966); KIS & VASILIU (1967); BOBÎRNAC (1972);. FIRU et al. (1988) (cit. TOGĂNEL & CHIMIŞLIU, 2005).

The purpose of this paper is to continue the publication of species preserved in the "I. Firu" Entomological Collection for a better understanding of the diversity and the habitat of the insect from Oltenia.

MATERIAL AND METHODS

The analysed material is represented by the species of the Orthoptera order preserved in the "I. Firu" Entomological Collection.

The species were determined by I. Firu and reviewed by B. Kis. The nomenclature and taxonomy of the species used in this work are consistent with the taxonomic system of Fauna Europaea.

For each species, tehre were recorded: the examined material, the number of specimens, the sites, the collection dates and the inventory number. Collection sites are listed in alphabetical order, and the collection dates, in chronological order of the years and months of collection.

Abbreviations: spec(s). – specimen(s); inv. no. – inventory number; * – new species reported from the museum patrimony; counties abbreviations: BV – Braşov; DJ – Dolj; GJ – Gorj; MH – Mehedinţi; VL – Vâlcea.

RESULTS

After analysing the 775 specimens, we identified 40 species included in 26 genera, 7 subfamilies, 8 families and 5 superfamilies. The distribution of specimens, species and genera in the 8 identified families is uneven (Table 1).

The specimens were collected in 23 sites in five counties, but mainly in Dolj County. Of these, four counties are in Oltenia. The only county that is not part of this historic province is Brasov, so that the material examined reflects the diversity of the Orthoptera in Oltenia.

Table 1. The numerical spectrum of the analysed Orthoptera.

Superfamily	Family	Subfamily	No. of genera	No. of species	No. of specs.
ACRIDOIDEA	Acrididae	Acridinae	1	1	44
		Calliptaminae	1	2	15
		Catantopinae	3	3	14
		Gomphocerinae	3	8	214
		Oedipodinae	4	6	238
TETRIGOIDEA	Tetrigidae	-	2	6	74
TRIDACTYLOIDEA	Tridactylidae	-	1	2	121
GRYLLOIDEA	Gryllidae	Gryllinae	2	2	12
		Nemobiinae	1	1	6
	Gryllotalpidae	-	1	1	6
TETTIGONIOIDEA	Conocephalidae	-	1	1	10
	Phaneropteridae		2	2	4
	Tettigoniidae		4	5	17
5	**8**	**7**	**26**	**40**	**775**

Collection sites list:

Albeşti DJ
Braşov BV
Bucovăţ DJ
Bugiuleşti VL
Căpreni GJ
Cernele DJ
Ciuperceni DJ
Craiova DJ
Craiova-Obedeanu DJ
Dănciuleşti
Desa DJ
Drobeta Turnu Severin
Ghindeni DJ
Irimeşti
Malu Mare DJ
Melineşti DJ
Pleniţa DJ
Preajba DJ
Prunet DJ
Rînca GJ
Secui DJ
Ţînţăreni GJ
Vârful Păpuşa (Parâng) GJ

List of identified species:

ORDER ORTHOPTERA
Suborder Caelifera
Superfamily Acridoidea
Family Acrididae
Subfamily Acridinae

Acrida bicolor (Thunberg 1815)

Examined material - 44 specs.: Bucovăţ 1 spec. 01.X.1975 inv. no. 11535; Căpreni 3 specs. 25.VIII.1964 inv. no. 11558, 11561, 11579; Craiova 3 specs. 01.IX.1972 inv. no. 11562, 11565, 11567; Craiova-Obedeanu 4 specs. 15.VIII.1972 inv. no. 11539, 11551, 11563, 11578; 2 specs. 12.VIII.1973 inv. no. 11536, 11538; 1 spec. 17.VIII.1973 inv. no. 11576; 4 specs. 23.IX.1973 inv. no. 11543, 11552, 11555, 11571; 1 spec. 15.IX.1974 inv. no. 11541; 1 spec. 16.IX.1974 inv. no. 11554; 1 spec. 30.IX.1974 inv. no. 11577; 2 specs. 15.VIII.1975 inv. no. 11572, 11573; 2 specs. 16.IX.1975 inv. no. 11545, 11569; Prunet 2 specs. 15.IX.1972 inv. no. 11564, 11566; 6 specs. 26.VIII.1974 inv. no. 11542, 11544, 11546, 11547, 11549, 11574; 2 specs. 16.VIII.1975 inv. no. 11537, 11568; Secui 2 specs. 16.VIII.1965 inv. no. 11553, 11557; 1 spec. 10.IX.1974 inv. no. 11540; 1 spec. 15.VIII.1975 inv. no. 11575; 5 specs. 16.VIII.1975 inv. no. 11548, 11550, 11556, 11559, 11560.

Subfamily Calliptaminae

Calliptamus barbarus (Costa 1836)

Previous reports from the museum patrimony: Craiova-Obedeanu (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 6 specs.: Craiova 1 spec. 27.VII.1965 inv. no. 11527; 1 spec. 12.VIII.1966 inv. no. 11525; 1 spec. 10.VII.1967 inv. no. 11526; Craiova-Obedeanu 1 spec. 15.VIII.1973 inv. no. 11524; 1 spec. 24.VIII.1973 inv. no. 11522; Prunet 1 spec. 27.VIII.1973 inv. no. 11523.

Calliptamus italicus (Linnaeus 1758)

Previous reports from the museum patrimony: Baia de Aramă, Mischii, Negoi, Poiana Mare (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 9 specs.: Căpreni 1 spec. 25.VIII.1964 inv. no. 11514; Craiova 1 spec. 27.VI.1966 inv. no. 11515; 1 spec. 15.VIII.1966 inv. no. 11518; 1 spec. 20.VIII.1979 inv. no. 11513; Craiova-Obedeanu 1 spec. 10.VIII.1973 inv. no. 11519; 2 specs. 25.VIII.1973 inv. no. 11520, 11521; Prunet 1 spec. 15.IX.1972 inv. no. 11517; 1 spec. 15.IX.1975 inv. no. 11516.

Subfamily Catantopinae

****Miramella ebneri*** Galvagni 1953

Examined material - 1 spec.: Vârful Păpuşa 24.VIII.1963 inv. no. 11881.

****Odontopodisma rubripes*** (Ramme 1931)

Examined material - 7 specs.: Pleniţa 1 spec. 25.VII.1973 inv. no. 11534; Prunet 1 spec. 15.IX.1973 inv. no. 11533; 2 specs. 26.VIII.1974 inv. no. 11529, 11530; 1 spec. 21.VIII.1975 inv. no. 11531; 1 spec. 15.IX.1975 nr. inv.11528; Secui 1 spec. 27.VII.1975 inv. no. 11532.

Pezotettix giornae (Rossi 1794)

Previous reports from the museum patrimony: Craiova-Obedeanu, Pisculeţ (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 6 specs. Bucovăţ 27. X.1975 inv. no. 11507- 11512.

Subfamily Gomphocerinae

Chorthippus albomarginatus (De Geer 1773)

Previous reports from the museum patrimony: Lăzarea (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 19 ex.: Bucovăţ 1 spec. 20.X.1975 inv. no. 11637; 1 spec. 27.X.1975 inv. no. 11641; Craiova-Obedeanu 2 specs. 12.VIII.1973 inv. no. 11644, 11645; 4 specs. 23.IX.1973 inv. no. 11642, 11643, 11646, 11647; 3 specs. 30.IX.1974 inv. no. 11631, 11635, 11636; 2 specs. 10.VIII.1975 inv. no. 11638, 11639; Drobeta Turnu Severin 1 spec. 16.VIII.1962 inv. no. 11630; Melineşti 1 spec. 01.VIII.1974 inv. no. 11640; 1 spec. 05.VII.1975 inv. no. 11632; Rînca 1 spec. 26.VIII.1969 inv. no. 11629; Secui 1 spec. 15.IX.1972 inv. no. 11634; 1 spec. 16.VIII.1975 inv. no. 11633.

Chorthippus biguttulus (Linnaeus 1758)

Previous reports from the museum patrimony: Jolotca (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 3 specs.: Craiova 1 spec. 26.VI.1964 inv. no. 11617; 1 spec. 10.VIII.1964 inv. no. 11616; Prunet 1 spec. 21.VII.1973 inv. no. 11618.

Chorthippus brunneus (Thunberg 1815)

Previous reports from the museum patrimony: Ciuperceni, Craiova, Irimeşti, Izvor, Pisculeţ, Ţînţăreni (TOGĂNEL & CHIMIŞLIU 2005).

Examined material - 109 specs.: Bucovăţ 2 specs. 27.X.1975 inv. no. 11736, 11782; Bugiuleşti 1 spec. 05.VII.1964 inv. no. 11718; Ciuperceni 5 specs. 20.VII.1976 inv. no. 11701, 11708, 11717, 11764, 11766; Craiova 1 spec. 10.VII.1954 inv. no. 11737; 1 spec. 27.VI.1963 inv. no. 11778; 5 specs. 05.IX.1972 inv. no. 11684, 11689, 11746, 11748, 11731; 1 spec. 15.VII.1975 inv. no. 11714; 1 spec. 01.X.1975 inv. no. 11745; Craiova-Obedeanu 1 spec. 08.VII.1973 inv. no. 11730; 2 specs. 10.VII.1973 inv. no. 11694, 11703; 1 spec. 11.VII.1973 inv. no. 11695; 1 spec. 12.VII.1973 inv. no. 11693; 1 spec. 24.VII.1973 inv. no. 11721; 1 spec. 10.VIII.1973 inv. no. 11777; 2 specs. 12.VIII.1973 inv. no. 11734, 11744; 1 spec. 15.VIII.1973 inv. no. 11702; 1 spec. 24.VIII.1973 inv. no. 11740; 2 specs. 25.VIII.1973 inv. no. 11705, 11743; 1 spec. 23.IX.1973 inv. no. 11704; 2 specs. 08.X.1973 inv. no. 11738, 11774; 3 specs. 23.VI.1974 inv. no. 11735, 11741, 11769; 3 specs. 30.VI.1974 inv. no. 11728, 11729, 11760; 4 specs. 07.VII.1974 inv. no. 11767, 11768, 11770, 11772; 2 specs. 21.VII.1974 inv. no. 11732, 11771; 1 spec. 18.VIII.1974 inv. no. 11733; 7 specs. 30.IX.1974 inv. no. 11683, 11690, 11696, 11711, 11739, 11754, 11779; 1 spec. 15.VI.1975 inv. no. 11709; 1 spec. 10.VII.1975 inv. no. 11723; 1 spec. 20.VII.1975 inv. no. 11688; 5 specs. 10.VIII.1975 inv. no. 11697, 11698, 11719, 11722, 11765; 1 spec. 20.VIII.1975 inv. no. 11713; 1 spec. 30.VI.1976 inv. no. 11710; Malu Mare 2 specs. 01.VII.19751 inv. no. 1700, 11763; 3 specs. 01.VIII.1975 inv. no. 11687, 11707, 11716; Ghindeni 1 spec. 23.VI.1973 inv. no. 11747; Irimeşti 1 spec. 03.VI.1975 inv. no. 11783; 12 specs. 08.VI.1975 inv. no. 11752, 11762, 11775, 11781, 1178-85; Melineşti 1 spec. 05.VI.1975 inv. no. 11724; Prunet 1 spec. 05.IX.1972 11749; 3 specs. 15.IX.1972 inv. no. 11720, 11750, 11751; 1 spec. 27.VII.1973 inv. no. 11758; 3 specs. 26.VIII.1974 inv. no. 11699, 11756, 11757; 2 specs. 10.IX.1974 inv. no. 11692, 11761; 3 specs. 21.VII.1975 inv. no. 11686, 11706, 11759; Secui 4 specs. 10.VII.1974 inv. no. 11725-11727, 11776; 1 spec. 27.VII.1975 inv. no. 11685; 1 spec. 10.IX.1974 inv. no. 11742; 3 specs. 16.VIII.1975 inv. no. 11712, 11755, 11773, 1 spec. 11780 inv. no. 11691; 2 specs. 15.IX.1975 inv. no. 11715, 11753; 1 spec. 15.X.1975 inv. no. 11691.

Chorthippus loratus (Fischer de Waldheim 1846)

Previous reports from the museum patrimony: Craiova-Obedeanu, Pisculeţ (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 33 specs.: Ciuperceni 2 specs. 20.VII.1976 inv. no. 11664, 11670; Craiova 1 spec. 10.VII.1966 inv. no. 11663; 2 specs. 05.IX.1972 inv. no. 11659, 11666; 1 ex 15.X.1972 inv. no. 11652; 2 specs. 15.VII.1975 inv. no. 11648, 11654; Craiova-Obedeanu 1 spec. 10.VIII.1973 inv. no. 11657; 3 specs. 25.VIII.1973 inv. no. 11669, 11678, 11679; 1 spec. 12.IX.1973 inv. no. 11651; 5 specs. 23.IX.1973 inv. no. 11650, 11658, 11665, 11668, 11671; 1 spec. 18.VIII.1974 inv. no. 11674; 1 spec. 15.IX.1974 inv. no. 11667; 3 specs. 30.IX.1974 inv. no. 11649, 11681, 11682; 1 spec.

15.VII.1975 inv. no. 11672; 3 specs. 16.IX.1975 inv. no. 11660, 11676, 11680; Malu Mare 1 spec. 01.VIII.1975 inv. no. 11653; Prunet 1 spec. 15.IX.1972 inv. no. 11661; 1 spec. 10. X.1974 inv. no. 11662; 1 spec. 16.VIII.1975 inv. no. 11675; Secui 2 specs. 15.IX.1975 inv. no. 11673, 11677.

Chorthippus parallelus (Zetterstedt 1821)

Previous reports from the museum patrimony: Craiova, Rînca, Leamna (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 10 specs.: Rînca 1 spec. 23.VII.1963 inv. no. 11621; 2 specs. 23.VIII.1963 inv. no. 11627, 11628; 2 specs. 24.VIII.1963 inv. no. 11622, 11626; 5 specs. 25.VIII.1963 inv. no. 11619, 11619, 11620, 11624, 11625.

Omocestus haemorrhoidalis (Charpentier 1825)

Previous reports from the museum patrimony: Craiova, Rînca (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material: 2 specs.: Rînca 26.VIII.1963 inv. no. 11614, 11615.

Omocestus minutus (Brulle 1832)

Previous reports from the museum patrimony: Craiova-Obedeanu, Desa (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 34 specs.: Craiova 1 spec. 01.IX.1972 inv. no. 11593; 2 specs. 04.IX.1972 inv. no. 11590, 11591; 2 specs. 05.IX.1972 inv. no. 11585, 11595; 2 specs. 05.IX.1972 inv. no. 11586, 11607; 1 spec. 24.VII.1973 inv. no. 11609; 2 specs. 10.VIII.1973 inv. no. 11594, 11598; 1 spec. 12.VIII.1973 inv. no. 11584; 1 spec. 23.IX.1973 inv. no. 11582; 1 spec. 10.VIII.1974 inv. no. 11612; 7 specs. 18.VIII.1974 inv. no. 11581, 11588, 11589, 11599, 11603, 11604, 11608; 1 spec. 30.VIII.1974 inv. no. 11597; 1 spec. 30.IX.1974 inv. no. 11613; 1 spec. 15.VII.1975 inv. no. 11606; Desa 1 spec. 25.VII.1974 11611; Drobeta Turnu Severin 1 spec. 14.X.19723 inv. no. 11587; Malu Mare 2 specs. 01.VIII.1975 inv. no. 11596, 11601; Prunet 2 specs. 15.IX.1972 inv. no. 11580, 11592; 1 spec. 26.VIII.1974 inv. no. 11583; 2 specs. 10.IX.1974 inv. no. 11602, 11610; 1 spec. 21.VIII.1975 inv. no. 11600; 1 spec. 06.VII.1977 inv. no. 11605.

Stenobothrus lineatus (Panzer 1796)

Previous reports from the museum patrimony: Bistra Mureşului (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material: 4 specs.: Drobeta Turnu Severin 1 spec. 10.VI.1962 inv. no. 11883; Rînca 1 spec. 23.VIII.1963 inv. no. 11885; 1 spec. 25.VIII.1963 inv. no. 11884; 1 spec. 26.VIII.1963 inv. no. 11882.

Subfamily Oedipodinae

****Acrotylus insubricus*** (Scopoli 1786)

Examined material - 67 specs.: Craiova 1 spec. 20.V.1963 inv. no. 11847; 1 spec. 01.X.1972 inv. no. 11851; Craiova-Obedeanu 2 specs. 15.VIII.1972 inv. no. 11788, 11841; 1 spec. 15.IX.1972 inv. no. 11798; 1 spec. 15.VI.1973 inv. no. 11787; 1 spec. 21.VII.1974 inv. no. 11846; 1 spec. 24.VII.1973 inv. no. 11838; 2 specs. 12.VIII.1973 inv. no. 11789, 11796; 2 specs. 25.VIII.1973 inv. no. 11805, 11809; 5 specs. 23.IX.1973 inv. no. 11786, 11799, 11836-11837, 11839; 1 spec. 10.VI.1974 inv. no. 11802; 1 spec. 17.VI.1974 inv. no. 11793; 3 specs. 18.VIII.1974 inv. no. 11812, 11849, 11853; 29 specs. 30.IX.1974 inv. no. 11800-118801, 11803-118804, 11806-118808, 11811, 11813-11819, 11821-118823, 11825-11827, 11829-11834, 11848, 11850; 1 spec. 15.VII.1975 inv. no. 11845; 1 spec. 20.VII.1975 inv. no. 11835; 1 spec. 10.VIII.1975 inv. no. 11820; 2 spec. 16.IX.1975 inv. no. 11810, 11843; Prunet 4 specs. 15.IX.1972 inv. no. 11792, 11794, 11797, 11852; 2 specs. 27.VII.1973 inv. no. 11791, 11795; 2 specs. 26.VIII.1974 inv. no. 11840, 11842; Pleniţa 1 spec. 25.VIII.1973 inv. no. 11824; Secui 1 spec. 06.X.1974 inv. no. 1 spec. 15.IX.1975 inv. no. 11844.

Acrotylus longipes (Charpentier 1845)

Previous reports from the museum patrimony: Craiova-Obedeanu, Mischii, Prunet (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 81 specs.: Craiova 1 spec. 20.V.1967 nr. inv.1196; 5 spec. 01.IX.1972 inv. no. 11953-11954, 11959, 11972; 1 spec. 15.IX.1972 inv. no. 11957; Craiova-Obedeanu 5 specs. 15.VIII.1972 inv. no. 11956, 11968, 12010, 12012, 12016; 1 spec. 05.IX.1972 nr. inv.12013; 1 spec. 24.VII.1973 nr. inv.11961; 5 specs. 10.VIII.1973 nr. inv.11964, 11965, 11976, 11996, 12015; 3 specs. 23.IX.1973 nr. inv.11970, 11977, 11995; 1 spec. 21.VII.1974 inv. no.11950; 4 specs. 18.VIII.1974 inv. no.11986, 11989, 12017, 12019; 1 spec. 15.IX.1974 inv. no.12007; 37 specs. 30.IX.1974 inv. no. 11951-11952, 11955, 11963, 11966, 11969, 11971, 11973-11974, 11978-11982, 11984, 11988, 11990-11991, 11998-12004, 12009, 12011, 12014, 12020-24, 12026; 2 specs. 30.III.1975 nr. inv.11967, 11994; 2 specs. 20.VII.1975 inv. no. 11983, 11993; 2 specs. 10.VIII.1975 inv. no.11962, 12025; 2 specs. 15.VIII.1975 nr. inv. 11985, 11997; 1 spec. 20.VIII.1975 inv. no. 12008; 1 spec. 16.IX.1975 inv. no. 11992; Desa 2 specs. 25.VII.1974 inv. no. 12005, 12006; Prunet 1 spec. 15.IX.1972 inv. no. 11958; 1 spec. 10.IX.1974 inv. no. 11987; Secui 2 specs. 15.IX.1975 inv. no. 11949, 11975.

Aiolopus strepens (Latreille 1804)

Previous reports from the museum patrimony: Craiova, Craiova-Obedeanu, Negoi, Pisculeţ, Preajba (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 13 specs.: Căpreni 1 spec. 01.IX.1964 inv. no. 11875; Craiova 1 spec. 20.VI.1964 inv. no. 11872; 3 specs. 10.VII.1964 inv. no. 11868, 11879, 11880; 1 spec. 08.VIII.1964 inv. no. 11877; 6 specs. 10.VIII.1964 inv. no. 11870, 11871, 11873-11874, 11876, 11878; 1 spec. 15.VIII.1964 inv. no. 11869.

****Aiolopus thalassinus*** (Fabricius 1781)

Examined material - 13 specs.: Craiova 1 spec. 26.VI.1964, inv. no. 11860; 2 specs. 10.VII.1964 inv. no. 11863, 11864; 1 spec. 08.VIII.1964 inv. no. 1856; 3 specs. 10.VIII.1964 inv. no. 11858, 11866, 11867; 1 spec.

19.IX.1964 inv. no. 11859; 1 spec. 27.VI.1965 inv. no. 11862; 1 spec. 25.VII.1970 inv. no. 11865; Craiova-Obedeanu 1 spec. 10.XI.1975 inv. no. 11855; Prunet 2 specs. 21.VII.1975 inv. no. 11857, 11861.

Oedaleus decorus (Germar 1826)

Previous reports from the museum patrimony: Ciuperceni, Craiova-Obedeanu (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 15 specs.: Ciuperceni 1 spec. 20.VII.1976 inv. no. 11898; Craiova-Obedeanu 3 specs. 12.VIII.1973 inv. no. 11889, 11894, 11895; 1 spec. 18.VIII.1973 inv. no. 11888; Malu Mare 1 spec. 01.VIII.1975 inv. no. 11897; Pleniţa 2 specs. 25.VII.1973 inv. no. 11891, 11892; Prunet 2 specs. 10.IX.1974 inv. no. 11893, 11896; 3 specs. 27.VII.1975 inv. no. 11887, 11899, 11890; Secui 1 spec. 10.IX.1974 inv. no. 11886; 1 spec. 16.VIII.1975 inv. no. 11890.

Oedipoda caerulescens (Linnaeus 1758)

Previous reports from the museum patrimony: Craiova-Obedeanu, Cheile Galbenului, Prunet (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 49 specs.: Bugiuleşti 1 spec. 10.VII.1964 inv. no. 11946; Căpreni 1 spec. 01.IX.1964 inv. no. 11945; Craiova 1 spec. 01.IX.1972 inv. no. 11906; 1 spec. 15.VII.1975 inv. no. 11918; Craiova-Obedeanu 1 spec. 23.IX.1963 inv. no. 11948; 1 spec. 15.VII.1973 inv. no. 11904; 2 specs. 24.VII.1973 inv. no. 11931, 11938; 1 spec. 10.VIII.1973 inv. no. 11930; 3 specs. 12.VIII.1973 inv. no. 11920, 11928, 11934; 1 spec. 23.IX.1973 inv. no. 11942; 1 spec. 07.VII.1974 inv. no. 11947; 3 specs. 21.VII.1974 inv. no. 11901, 11915, 11932; 2 specs. 18.VIII.1974 inv. no. 11909, 11929; 3 specs. 30.09.1974 inv. no. 11907, 11908, 11923; 1 spec. 20.VII.1975 inv. no. 11933; Dănciuleşti 1 spec. 08.IX.1974 inv. no. 11939; Desa 1 spec. 25.VII.1974 inv. no. 11916; Malu Mare 2 specs. 01.VIII.1975 inv. no. 1905, 11935; Pleniţa 1 spec. 25.VII.1973 nr. inv.11940; Prunet 1 spec. 15.IX.1972 inv. no. 11936; 1 spec. 10.IX.1974 inv. no. 11903; 8 specs. 27.VII.1975 inv. no. 11911-11914, 11919, 11924, 11926, 11941, 11943, 11944; 1 spec. 27.VIII.1975 inv. no. 11937; Secui 3 specs. 15.VII.1975 inv. no. 11917, 11925, 11927; 2 specs. 15.IX.1975 inv. no. 11910, 11922; 2 specs. 15.X.1975 inv. no. 11902, 11921.

Superfamily Tetrigoidea

Family Tetrigidae

****Depressotetrix depressa*** (Brisout de Barneville 1848) syn. *Tetrix depressa* Brisout de Barneville 1849

Examined material - 12 specs.: Craiova 1 spec. 14.IV.1974 inv. no. 11463; Craiova-Obedeanu 1 spec. 15.IX.1970 inv. no. 11459; 1 spec. 23.IV.1973 inv. no. 11468; 1 spec. 08.VII.1973 inv. no. 11470; 1 spec. 05.VIII.1973 inv. no. 11460; 2 specs. 25.VIII.1973 inv. no. 11462, 11469; 2 specs. 15.VIII.1975 inv. no. 11464, 11466; 1 spec. 18.VIII.1975 inv. no. 11465; Secui 2 specs. 01.VIII.1975 inv. no. 11461, 11467.

****Tetrix bipunctata*** (Linnaeus 1758)

Examined material - 36 specs.: Ciuperceni 1 spec. 20.VI.1976 inv. no. 11476; 1 spec. 20.VI.1976 inv. no. 11498; Craiova: 1 spec. 10.VII.1966 inv. no. 11486; 1 spec. 15.IX.1972 inv. no. 11502; Craiova-Obedeanu: 1 spec. 15.IX.1952 inv. no. 11493; 1 spec. 10.VII.1966 inv. no. 11486; 1 spec. 15.IX.1970 inv. no. 11488; 1 spec. 21.VII.1974 inv. no. 11503; 2 specs. 15.IV.1973 inv. no. 11481, 11499; 1 spec. 23.IV.1973 inv. no. 11505; 1 spec. 25.V.1973 inv. no. 11487; 1 spec. 10.VII.1973 inv. no. 11489; 3 specs. 25.VIII.1973 inv. no. 11471, 11494, 11495; 1 spec. 23.IX.1973 inv. no. 11492; 1 spec. 21.VII.1974 inv. no. 11503; 1 spec. 07.VIII.1974 inv. no. 11496; 3 specs. 18.VIII.1974 inv. no. 11474, 11483, 11497; 1 spec. 15.IX.1974 inv. no. 11473; 1 spec. 25.IX.1974 inv. no. 11491; 2 specs. 14.VI.1975 inv. no. 11482, 11506; 2 specs. 18.VII.1975 inv. no. 11478, 11479; 1 spec. 15.VIII.1975 inv. no. 11472; 1 spec. 15.IX.1975 inv. no. 11475; 1 spec. 16.X.1975 inv. no. 11484; Preajba: 1 spec. 22.VI.1973 inv. no. 11504; 1 spec. 29.IX.1973 inv. no. 11490; Secui: 2 specs. 10.IX.1974 inv. no. 11480, 11501; 1 spec. 16.IX.1975 inv. no. 11500.

Tetrix bolivari Saulcy 1901

Previous reports from the museum patrimony: Craiova, Craiova-Obedeanu, Preajba (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 4 specs.: Craiova-Obedeanu 1 spec. 15.IV.1973 inv. no. 11444; 1 spec. 18.VIII.1974 inv. no. 11442; Preajba 1 spec. 15.IV.1973 inv. no. 11445; Secui 1 spec. 10.IX.1974 inv. no. 11443.

Tetrix ceperoi Bolivar 1887

Previous reports from the museum patrimony: Craiova-Obedeanu (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 7 specs.: Ciuperceni 2 specs. 20.VII.1976 inv. no. 11447, 11450; Craiova-Obedeanu 1 spec. 15.VII.1973 inv. no. 11451; 1 spec. 18.VIII.1974 inv. no. 11446; 1 spec. 30.IX.1974 inv. no. 11449; 1 spec. 18.VI.1975 inv. no. 11448; Preajba 1 spec. 30.IX.1973 inv. no. 11452.

Tetrix subulata (Linnaeus 1758)

Previous reports from the museum patrimony: Preajba (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 9 specs.: Craiova-Obedeanu 1 spec. 15.IV.1973 inv. no. 11434; 1 spec. 01.V.1973 inv. no. 11435; 2 specs. 10.V.1974 inv. no. 11436, 11437; 1 spec. 30.VIII.1974 inv. no. 11441; 2 specs. 15.IX.1974 inv. no. 11433, 11440; 1 spec. 30.IX.1974 inv. no. 11438; Preajba 1 spec. 23.IX.1973 inv. no. 11439.

Tetrix tuerki Krauss 1876

Previous reports from the museum patrimony: Craiova-Obedeanu (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 6 specs.: Craiova-Obedeanu 1 spec. 10.VIII.1973 inv. no. 11453; 1 spec. 10.V.1974 inv. no. 11458; 1 spec. 01.VII.1974 inv. no. 11455; 2 ex.15.IX.1974 inv. no. 11454, 11456; 1 spec. 30.IX.1974 inv. no. 11457.

Superfamily **Tridactyloidea**
Family **Tridactylidae**

Xya pfaendleri (Harz 1970) syn. *Tridactylus pfaendleri* Harz 1970

Previous reports from the museum patrimony: Ciuperceni, Craiova-Obedeanu, Preajba (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 40 specs.: Craiova-Obedeanu 3 specs. 23.IX.1973 inv. no. 11429, 11430, 11432; 20 specs. 10.V.1974 inv. no. 11398-113403, 11408-114415, 11422-11427; 6 specs. 30.VI.1974 inv. no. 11395, 11396, 11404-11407; 6 specs. 06.VII.1974 inv. no. 11416-11421; 1 spec. 07.VII.1974 inv. no. 11394; 2 specs. 30.III.1975 inv. no. 11393, 11397; 1 spec. 15.VI.1975 inv. no. 11428; Prunet 1 spec. 12.VIII.1973 inv. no. 11431.

Xya variegata Latreille 1809 syn *Trydactilus variegata* Latreille 1809

Previous reports from the museum patrimony: Craiova-Obedeanu (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 81 specs.: Albeşti 1 spec. 11.IX.1973 inv. no. 11341; 2 specs. 14.IX.1973 inv. no. 11357, 11358; Cernele 1 spec. 15.IX.1977 inv. no. 11340; Craiova-Obedeanu 1 spec. 15.VII.1967 inv. no. 11355; 1 spec. 15.IV.1973 inv. no. 11339; 1 spec. 01.V.1973 inv. no. 11359; 4 specs. 15.VI.1973 inv. no. 11388, 11389, 11342, 11391; 1 spec. 18.VII.1973 inv. no. 11353; 1 spec. 24.VII.1973 inv. no. 11386; 2 specs. 23.IX.1973 inv. no. 11325, 11392; 39 specs. 10.V.1974 inv. no. 11312-11323, 11327-11338, 11370-11384; 1 spec.17.VI.1974 inv. no. 11385; 2 specs. 21.VI.1974 inv. no. 11366-11367; 3 specs. 30.VI.1974 inv. no. 11343, 11344, 11387; 3 specs. 07.VII.1974 inv. no. 11356, 11368, 11369; 6 specs. 07.VIII.1974 inv. no. 11360-11365; 7 specs. 30.III.1975 inv. no. 11345-11351; 1 spec. 30.IX.1975 inv. no. 11352; Ghindeni 1 spec. 12.VIII.1973 inv. no. 11326; Preajba 1 spec. 22.VI.1973 inv. no. 11354; Prunet 1 spec. 12.VIII.1973 inv. no. 1132; Secui 1 spec. 10.VIII.1973 inv. no. 11390.

Suborder ENSIFERA
Superfamily Grylloidea
Family Gryllidae
Subfamily Gryllinae

****Eumodicogryllus bordigalensis*** (Latreille 1804) syn. *Gryllus bordigalensis* Latreille 1804

Examined material - 4 specs.: Craiova 2 specs. 15.VI.1964 inv. no. 11302, 11303; 1 spec. 12.VIII.1964 inv. no. 11304; 1 spec. 10.VIII.1964 inv. no. 11305.

****Melanogryllus desertus*** (Pallas 1771) syn. *Gryllus desertus* Pallas 1771

Examined material - 8 specs.: Braşov 1 spec. 25.VI.1965 inv. no. 11294; Bucovăţ 1 spec. 10.VI.1977 inv. no. 11301; Bugiuleşti 1 spec. 12.V.1964 inv. no. 11300; Craiova 1 spec. 15.VI.1965 inv. no. 11299; Ţînţăreni 4 specs. 12.VII.1974 inv. no. 11295 – 11298.

Subfamily Nemobiinae

Pteronemobius heydenii (Fischer 1853)

Previous reports from the museum patrimony: Craiova-Obedeanu (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 6 specs.: Craiova-Obedeanu 1 spec. 11.VIII.1974 inv. no. 11288; 1 spec. 20.VI.1975 inv. no. 11291; Secui 1 spec. 15.IX.1975 inv. no. 11293; 3 specs. 16.IX.1975 inv. no. 11289, 11290, 11292.

Family Gryllotalpidae

Gryllotalpa gryllotalpa (Linnaeus 1758)

Previous reports from the museum patrimony: Craiova (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 6 specs.: Craiova 1 spec. 21.VII.1964 inv. no. 11310, 1 spec. 10.VI.1971 inv. no. 11311; Craiova-Obedeanu 1 spec. 30.VI.1974 inv. no. 11309; 2 specs. 21.VII.1974 inv. no. 11307, 11308; 1 spec. 25.V.1975 inv. no. 11306.

Superfamily Tettigonioidea
Family Conocephalidae

****Conocephalus fuscus*** (Fabricius 1793)

Examined material - 10 specs.: Craiova 1 spec. 10.VI.1964 inv. no. 11273; 1 spec. 27.VII.1975 inv. no. 11268; Craiova-Obedeanu 1 spec. 18.VIII.1974 inv. no. 11274; 1 spec. 28.V.1975 inv. no. 11272; Prunet 2 specs. 26.VIII.1974 inv. no. 11271, 11275; 1 spec. 21.VII.1975 inv. no. 11267; Secui 1 spec. 10.IX.1974 inv. no. 11276; 2 specs. 27.VII.1975 inv. no. 11269, 11270.

Family Phaneropteridae

****Barbitistes constrictus*** Brunner von Wattenwyl 1878

Examined material - 1 spec.: Rînca 26.VIII.1963 inv. no. 11287.

****Phaneroptera nana*** Fieber 1853

Examined material - 3 specs.: Craiova 1 spec. 25.VIII.1964 inv. no. 11265; 1 spec. 10.VIII.1966 inv. no. 11264; 1 spec. 05.IX.1972 inv. no. 11263.

Family Tettigoniidae

Pholidoptera transsylvanica (Fischer 1853)

Previous reports from the museum patrimony: Leamna (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 6 specs.: Rînca 4 specs. 23.VIII.1963 inv. no. 12027, 12028, 12031, 12032; 2 specs. 25.VIII.1963 inv. no. 12029, 12030.

Platycleis (Platycleis) albopunctata grisea (Fabricius 1781)

Previous reports from the museum patrimony: Bistra Mureşului (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 2 specs.: Căpreni 1 spec. 25.VIII.1964 inv. no. 11281; Craiova 1 spec. 05.VIII.1966 inv. no. 11282.

Platycleis affinis Fieber 1853

Previous reports from the museum patrimony: Izvor, Mischii (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material - 4 specs.: Craiova 1 spec. 10.VII.1964 inv. no. 11285; 1 spec. 20.VII.1964 inv. no. 11286; 1 spec. 15.VII.1966 inv. no. 11284; 1 spec. 13.VII.1967 inv. no. 11283.

Ruspolia nitidula (Scopoli 1786) syn. *Homorocoryphus nitidula* (Scopoli 1786)

Examined material - 1 spec.: Craiova 25.VIII.1970 inv. no. 11266.

Tettigonia viridissima (Linnaeus 1758)

Previous reports from the museum patrimony: Craiova (TOGĂNEL & CHIMIŞLIU, 2005).

Examined material – 4 specs.: Bucovăţ 1 spec. 20.IX.1981 inv. no. 11278; Craiova 1 spec. 10.VII.1964 inv. no. 11280; 2 specs. 03.VIII.1964 inv. no. 11277, 11279.

DISCUSSIONS

The best represented family in terms of numbers (Fig. 1) is the Acrididae family, which sums up to 525 specimens of the 775 (Fig. 2), representing 67.74%. It is followed by the Tridactylidae family with 121 specimens (15.61%) and Tetrigidae with 74 specimens (9.55%). The other five families totalize 55 samples (7.10%).

Regarding the distribution of species in 8 families, the Acrididae family still owns most species – 20, followed by the families: Tetrigidae (6) Tettigoniidae (5) and Gryllidae (3). The Tridactylidae and Phaneropteridae families have 2 species each, and the Gryllotalpidae and Conocephalidae families are represented by a single species (Fig. 2). A similar situation is with the identified genera in the 8 families (Fig. 3).

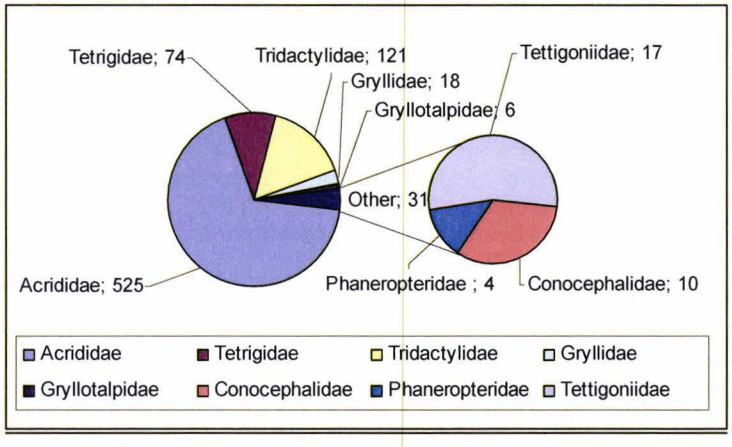

Figure 1. The numerical spectrum of the analysed specimens of Orthoptera.

The 13 newly identified collecting sites from Oltenia (Ciuperceni, Craiova, Craiova-Obedeanu, Desa, Irimeşti, Preajba, Prunet, Rînca, Secui, Ţînţăreni) are added to the 22 previously mentioned sites (TOGĂNEL & CHIMIŞLIU, 2005). Presently, in the museum patrimony, we have specimens of Orthoptera collected in 45 sites from Oltenia. Thus, our data contribute to the extension of the area known to be inhabited by Orthoptera in Oltenia.

Comparing the 54 species previously reported from the museum patrimony with 40 identified species in the analyzed material, 27 species have been found again and 13 species are newly reported from the entomological heritage: *Miramella ebneri*, *Odontopodisma rubripes*, *Acrotylus insubricus*, *Aiolopus thalassinus*, *Depressotetrix depressa*, *Tetrix bipunctata*, *Eumodicogryllus bordigalensis*, *Melanogryllus desertus*, *Pteronemobius heydenii*, *Conocephalus fuscus*, *Barbitistes constrictus*, *Phaneroptera nana*, *Ruspolia nitidula*.

Compared to the total number of the identified species (182) in the fauna of Romania (IORGU et al., 2008), the 40 identified species represent 21.98%.

Adding these species to those previously reported (TOGANEL & CHIMIŞLIU), the number of species of Orthoptera known from the museum patrimony amounts to 67 species from a total of 114 species listed in fauna of Oltenia (IORGU et al., 2008), which means 58.77%. Compared to the 182 species listed in the Romanian fauna (IORGU et al., 2008), 67 species are approx. 36.81%.

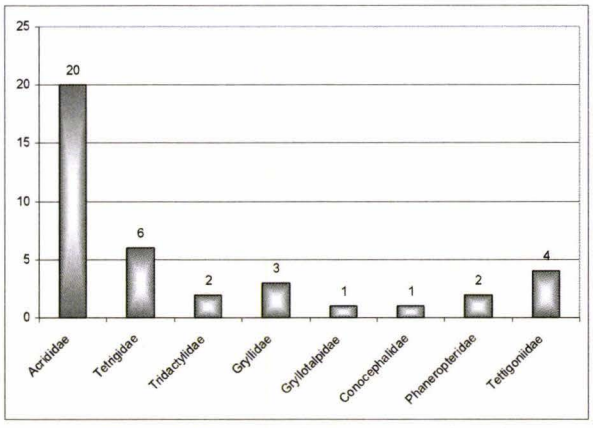

Figure 2. The numerical spectrum of species.

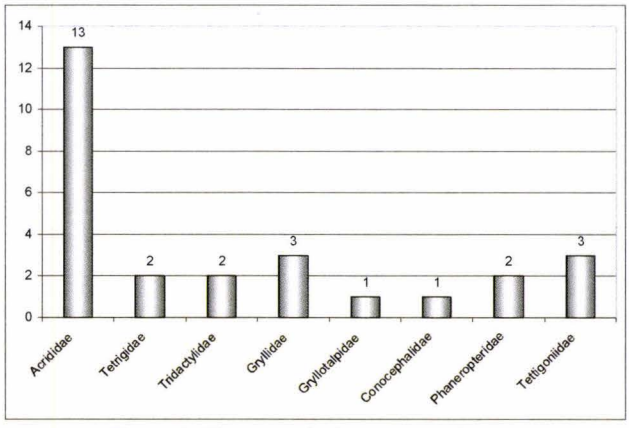

Figure 3. The numerical spectrum of genera.

Note that in the analysed material we found two rare species: *Pholidoptera transsylvanica* – Carpathian element rarely seen at the smaller heights and the *Tetrix tuerki* species – less widespread species that occurs localized.

The invasive species *Calliptamus italicus* was also identified. It was collected during the years 1964-1979, in 4 sites located in Dolj (Craiova, Craiova-Obedeanu, Prunet) and Gorj (Căpreni) Counties.

CONCLUSIONS

Currently (2014), in the museum patrimony we have 67 species of Orthoptera from the fauna of Oltenia, representing 58.77% of the species identified in the fauna of Oltenia and approx. 36.81% of the Orthoptera fauna of Romania (IORGU et al., 2008).

Given that Oltenia represents approx. 11% of Romania-surface and the fact that in the museum patrimony we still have specimens of Orthoptera that have not yet been determined and published, we believe that their diversity is higher.

The results contribute to a better understanding of the Orthoptera diversity and their habitat in Oltenia fauna and implicitly in Romania.

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PREDATORY STINK BUG *Perillus bioculatus* Fabricius 1775 (HEMIPTERA, PENTATOMIDAE) IN THE REPUBLIC OF MOLDOVA

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Abstract. In the central and northern part of the Republic of Moldova, at potato plantations, there was detected the stink bug *Perillus bioculatus*, which is considered as the main entomophage of the Colorado potato beetle. According to preliminary data, the conclusion is made that the North American bug has acclimatized spontaneously in the south-east of Europe. It is assumed that the ecological integration of this harmful species in agrocoenosis will reduce essentially the quantitative effect of the phytophage *Leptinotarsa decemlineata*, but excluding the chemical treatments there will be obtained the ecologically pure agricultural products.

Keywords: Colorado potato beetle *Leptinotarsa decemlineata*, predatory stink bug *Perillus bioculatus*, ecological integration.

Rezumat. Ploşniţa răpitoare *Perillus bioculatus* Fabricius 1775 (Hemiptera, Pentatomidae) în Republica Moldova. În partea centrală şi nordică a Republicii Moldova în plantaţii de cartofi a fost descoperită ploşniţa *Perillus bioculatus* care este considerată ca entomofag principal al gândacului de Colorado. Conform datelor preliminare se conchide, că heteropterul nordamerican s-a aclimatizat spontan în sud-estul Europei. Se estimează că integrarea ecologică a acestei specii prădătoare în agrocenoze va reduce esenţial efectivul numeric al fitofagului *Leptinotarsa decemlineata*, iar prin excluderea tratamentelor chimice vor fi obţinute produse agricole ecologic pure.

Cuvinte cheie: gândacul de Colorado *Leptinotarsa decemlineata*, ploşniţa prădătoare *Perillus bioculatus*, integrarea ecologică.

INTRODUCTION

Yearly, in the Republic of Moldova, potato is cultivated over an area of 25-28 thousand hectares. In 2013, potato has been planted on a total surface of 23.8 thousand hectares. The harvest has amounted to 320-330 thousand tons, the country average reaching 13 tons a hectare (MAIA, 2013).

For example, in Romania potato is considered as a strategic food, being a component of the food safety insurance system. Energy, protein and dry substance contribution per unit of cultivated area is similar to that one obtained from the major crops – wheat and corn. That is why during the past 50 years the potato-cultivated area has been of 250-316 thousand hectares, granting to Romania the second place in Europe (CHIRU et al., 2006).

Colorado potato beetle is considered the main pest of potato crop on the European continent. Harvest losses due to this species are enormous and potato cultivation without protective measures is impossible.

MATERIALS AND METHODS

As the material for this article was the natural population (108 larvae and adults) of the stink bug *Perillus bioculatus* has been detected near of the Chişinău city on the potatoes. Were also taken into account the data on the registration of this species of other localities – Slobozia Dusca village (Criuleni district); Drochia village, (Drochia district) and Parcova village (Edinet district).

RESULTS AND DISCUSSIONS

Colorado potato beetle *Leptinotarsa decemlineata* Say 1824 (Coleoptera, Chrysomelidae) – an invasive species in Europe.

The North American species *Leptinotarsa decemlineata* appeared in Europe in the thirties of the 20th century and has become the most dangerous pest of solanaceous crops: potatoes, eggplants, tomatoes, etc. For the first time in Moldova, the Colorado potato beetle has been detected in 1960, in 1964 this pest being already present in all of the republic districts (VOROTYNTSEVA, 1971).

The pest biological cycle is characterized by a long duration of development stages. For example, the hibernated females laying period and larvae occurrence takes 3-3.5 months with numerical maximum level in late May - first half of June. The laying period and larvae hatching of the second generation, is also extended to 3 months with the maximum level in the second half of July - beginning of August.

Due to the large ecological plasticity and to the lack in local fauna of the specialized entomophages, the control of the Colorado potato beetle is based mainly on chemical method (GUSEV, 1991). In the Republic of Moldova, potato plantations are treated with different approved insecticides – Fastac, Arrivo, Confidor, Actelic, etc. (PAMUJAC et al., 2009). The average number of plantation works is of 2-3, but in some cases (torrential rain, influence of ultraviolet solar rays, etc.) can also amount to 4-5 during the crop growing season (FILIPPOV et al., 1986).

In order to protect human health and environment, the future in this area belongs to biological method by the use of entomophages. The study of natural predators and parasites of the Colorado potato beetle has shown that in Europe it is attacked by 270 species of insects (GUSEV, 1991). However, the most effective and voracious (a stink bug

destroys up to 2,500 of the beetle eggs) are the species of North American bug – *Podisus maculiventris* Say 1832 and *Perillus bioculatus*, trophically specialized on Coleopterans of Chrysomelidae family. The species *P. bioculatus* has been considered better for acclimatization, having a vital cycle well harmonized with that of the pest (MOENS, 1963).

Brief history of works concerning the stink bug *Perillus bioculatus* acclimatization in Europe. The first attempts to acclimatize the *P. bioculatus* species were conducted by French researchers in the 30's of the 20th century – immediately after the Colorado potato beetle emergence (1927) in the province of Bordeaux (MOENS, 1963; TROUVELOT, 1932). However, due to the commencement of the World War II, the investigations stopped and resumed only in 50-60 years, but already in several European countries: France, Germany, Belgium, Yugoslavia, Czechoslovakia, Hungary, Bulgaria, Poland, and USSR (GUSEV, 1991).

The researches with purpose of reproduction, application and acclimatization of *P. bioculatus* species in ex-USSR were conducted during 1960-90 in Lviv, Chernovtsy, Voronezh, Transcarpatia, Krasnodar regions and Moldavian SSR. The biological material was brought from Hungary in 1961. In August, the Quarantine Laboratory of Lviv received for experiments 34 larvae of first and second age (STRADIMOVA, 1967, 1973), and the Ukrainian Institute for Plant Protection of Uzhgorod – 100 eggs, of which 65 stink bug larvae hatched (SIKURA & SMETNIK, 1967). In 1973, a number of 2200 stink bug eggs were brought from Lviv to the Krasnodar region (Lazarevsk district) (GUSEV & ZAYATS, 1978). As a result of multiannual investigations, it was established that in the North and mountain areas, stink bug develops during 2 generations, but in the South – during 3-4 (GUSEV, 1991). It would be logical that, according to all the physiological and biological properties of stink bug (SHAGOV, 1967, 1968, 1969, 1977; SHAGOV & CHESNEK, 1978) and to the climatic parameters of the selected regions, species hibernation finalizes with a high rate of adults survival. However, all attempts failed, demonstrating a successful wintering of the North American stink bug within 7.3-15.7%. The final conclusion was that the stink bug *P. bioculatus* cannot be acclimatized in the South Western part of the former USSR (STRADIMOVA, 1967; ZAYATS, 1968; GUSEV, 1991). Equally, the idea of stink bug acclimatization was abandoned in other European countries, in which this issue was studied and during already 25-30 years anything concerning this matter has not been published in scientific literature.

In the Republic of Moldova investigations concerning *Podisus maculiventris* and *P. bioculatus* species started by elaborating mass reproduction methods (KOLESNICHENKO, 1981) and by applying them in order to control the Colorado beetle within potato and eggplant cultures (FILIPPOV et al., 1986; GUSEV & KOVALI, 1990). Information about the acclimatization attempts in Moldova of the species named above has not been found in special literature.

Colonization by the species *Perillus bioculatus* of the countries of South Eastern Europe. First accounts of the species *P. bioculatus* in natural conditions appeared in 2004, when it was detected on potato plants in the European part of Turkey and Greece (KIVAN, 2004; PERICART, 2010).

In 2008, in the Krasnodar region (Russia), some populations of stink bug *P. bioculatus* have been found. Their density on the plants of *Ambrosia artemisiifolia* Linnaeus 1753 (colonized with North American beetle *Zygogramma suturalis* Fabricius 1775 – food for the predatory bug) reached 10-20 larvae and adults per 1 m^2, in summer 3 generations having developed. The researchers' conclusion was that, for the first time in the history of plants biological protection, a simultaneous acclimatization of two species occurred: of herbivore beetle *Zygogramma suturalis* and predatory stink bug *P. bioculatus* (ISMAILOV & AGASIEVA, 2010).

During the last years, faunistic reports have appeared (based on a few samples of *P. bioculatus*) of Bulgaria (SIMOV et al., 2012) and Serbia (PROTIC & NEBOJSA, 2012).

On July 2013, the stink bug *P. bioculatus* was detected in the Republic of Moldova on potatoes, attacked by the Colorado potato beetle near Chişinău city. The bug population constituted more than 100 larvae and adults, having a viable and very active state (Figs. 1; 2).

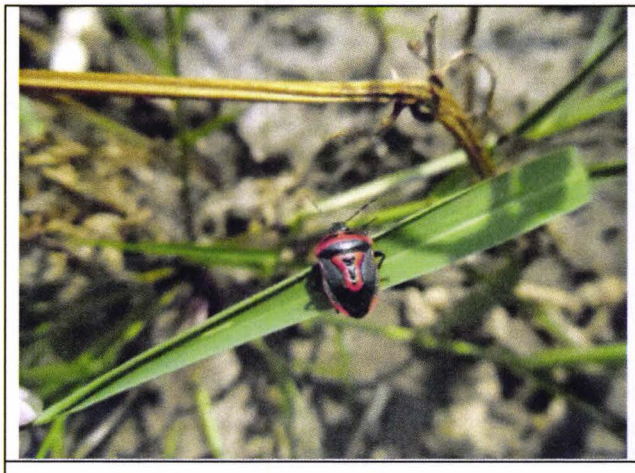

Figure 1. A stink bug adult *Perillus bioculatus* (original).

Figure 2. A stink bug larva of III age attacking a Colorado potato beetle larva (original).

As a result of subsequent investigations, other localities have also been revealed (Slobozia Dusca village, Criuleni district; Drochia village, Drochia district; Parcova village, Edinet district), where larvae and adults of stink bug *Perillus bioculatus* have been detected on potatoes. But this information requires confirmation, because of the lack of samples.

The first reports concerning this discovery (DERJANSCHI & ELISOVEŢCAIA, 2013; DERJANSCHI et al., 2013) have incited a strong interest within the international scientific groups, because the bug population in the Republic of Moldova is the northernmost and can serve as initial colonization culture in European countries. At the same time, it should be mentioned that the invasive plant *Ambrosia artemisiifolia* is present in our country, but the coleopteran *Zygograma suturalis* has not been recorded, with which *Perillus bioculatus* is trophically related in Krasnodar region. This is why there are necessary bio ecological researches of the peculiarities of the stink bug *Perillus bioculatus* populations in the Republic of Moldova conditions, which will form the basis for introduction of material collected from nature into laboratory culture and concluding of stink bug mass reproduction methods.

CONCLUSIONS

The stink bug *Perillus bioculatus* detection in natural conditions on the territory of the Republic of Moldova and other countries (Turkey, Greece, Krasnodar region of the Russian Federation, Bulgaria and Serbia) demonstrates that this North American species has acclimatized spontaneously in the South Eastern Europe.

The integration of predatory bug *Perillus bioculatus* species in agrocoenosis will allow naturally to reduce the Colorado potato beetle numerical effective, to avoid polluting the environment with insecticides and to obtain ecologically pure agricultural products.

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SURVIVAL LENGTH AND STRATEGIES OF THE LIGHT ATTRACTED CARABIDS IN THE CENTER OF A LARGE CITY

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Abstract. The excessive illumination of some places in the interior of large cities, especially of advertisements and some historical buildings, causes massive immigration of insects into such places. In Carabids, the most intensive migrations take place approximately from mid-July to mid-September, reaching the peak by the end of July – the beginning of August. In 1998 and 1999, marking and recapturing of medium sized and autumn-breeding species (*Pseudoophonus rufipes, P. calceatus* and *Dolichus halensis*) representing the major part of the light attracted Carabids was carried out in one such place in the centre of Bratislava. It was showed that the major part of the attracted individuals live the illuminated place within several hours. Only a small part of them remain there and form temporal aggregations, in which some individuals are able to survive for about 4-6 weeks. The proportion of the remaining individuals is very variable and independent on the number of the attracted individuals. There is only a weak tendency that the proportion of the remaining individuals is larger in the days with low intensity of migration. Beetles occupy different covers (ant galleries, armature of rain water pipes, ventilation openings) and use supplementary food resources (human food wastes, other light attracted insects, dead congeneric Carabids, dog excrements). The species show different movement and "hunting" strategies according to their life form – the stratochortobionts mostly walk slowly along the building bases and sometimes undertake trips into the centre of the pavement, with irregular trajectories and many pauses. They are often trampled by pedestrians. In contrast, the stratobionts hide in galleries in the bases of buildings and undertake quick attacks into the pavement in direct loop-like paths and rapidly return to the initial place.

Keywords: light pollution, migration, Carabids, survival strategies, large cities.

Rezumat. Durata şi strategii de supravieţuire ale Carabidelor atrase de lumină în centrul unui oraş mare. Iluminarea exagerată a unor locuri din centrele oraşelor mari, în primul rând a reclamelor şi a dominantelor istorice, provoacă imigrarea masivă a insectelor în astfel de locuri. La carabide, aceste migraţii au loc aproximativ de la jumătatea lunii iulie până la jumătatea lunii septembrie, cu vârful la sfârşitul lunii iulie şi la începutul lunii august. În anii 1998 şi 1999, în centrul oraşului Bratislava a fost efectuată marcarea şi recapturarea speciilor cu talie mijlocie şi cu reproducere de toamnă (*Pseudoophonus rufipes, P. calceatus* şi *Dolichus halensis*), care reprezintă cele mai abundente specii dintre carabidele atrase de lumină în aceste locuri. S-a arătat că cea mai mare parte a indivizilor atraşi părăsesc aceste locuri în cursul câtorva ore. Numai o mică parte din ele rămâne pe loc şi formează agregaţii temporare în care unii indivizii sunt capabili să supravieţuiască chiar 4-6 săptămâni. Proporţia indivizilor care rămân pe loc este extrem de variabilă şi independentă de numărul indivizilor atraşi. Există însă o tendinţă slabă, ca proporţia indivizilor stabiliţi să fie mai mare în zile cu migraţie puţin intensă. Gândacii ocupă diferite ascunzişuri (galerii de furnici, armătură de conducte de apă de ploaie, deschideri de aerisire) şi se hrănesc cu diferitele surse suplimentare (resturi de mâncare aruncate, celelalte insecte atrase de lumină, carabide congenere moarte, excrementele câinilor). Speciile folosesc diferite „strategii de vânat" după forma lor de viaţă. Stratohortobiontele se târăsc cel mai mult pe lângă baza clădirilor şi uneori fac „excursii" în centrul trotuarului pe o cale neregulată, cu multe pauze. Adesea sunt călcate de pietoni. Spre deosebire, stratobiontele se ascund în diferite galerii la baza clădirilor şi fac atacuri rapide în centrul trotuarului, pe o cale directă, în forma unei bucle. După atac, se întorc la locul iniţial.

Cuvintele cheie: poluarea cu lumină, migraţie, carabide, strategie de supravieţuire, oraşe mari.

INTRODUCTION

The excessive illumination of the cities, advertisements and the roadways considerably change life conditions of animals in the landscape and modifies their migration paths, in particular of insects, which are strongly attracted to light and even contribute to the spreading of their geographical distribution (HALLQUIST et al., 2011). Light pollution has become a serious problem of nature and environment protection and is taken into consideration in landscape management (FRANCIS & CHADWICK, 2013; LONGCORN & RICH, 2008; NOWINSZKY, 2006; PATRIARCA & DEBERNARDI, 2010; POVOLNÝ, 2004).

Although insect attraction to light is known from the remote past and the collection of insects on light is recommended in different entomological manuals since the turn of the 19th and the 20th century, this phenomenon got a completely new dimension in the late 1920-s, when the electric illumination of streets and houses became a-standard. Just at that time, *Agonum gracilipes* (Duftschmidt 1812), an eurytopic, but usually rare species, was surprisingly recorded in cities centres (DELAHON, 1931). The special affinity of this species to light was also shown by HONĚK & PULPÁN (1983). However, the classical bulbs used to illuminate public places did not act as a stronger attractant at night migrations of carabids. The intensity of illumination of public places considerably increased in early 1960-s, when mercury discharge lamps began to be used, being gradually replaced by the high-pressure sodium lamps. In such conditions, in the late 1970-s, it was observed that night migrations of Carabids in city centre depends on the intensity of local illumination and direction of streets in relationship to an area of urban greenery (ŠUSTEK, 1981; ŠUSTEK & VAŠÁTKO, 1982). A radical change came in the 1990-s, when strong halogen discharge lamps with high light temperature around 5000°K (OSRAM, 1994) and producing an enormously bright white light were introduced to increase attractiveness of advertisement or to illuminate significant historical dominants. Unlike the mercury discharge

lamps, they almost do not have the UV component in their spectre, but their intensive white light strongly attracts attention of the human kind and, obviously, also of the insects.

Due to it, insects are attracted into city centres in enormous amounts, penetrating even in the interior of supermarkets with permanently open entries or into flats. The local aggregations of huge amounts of "unknown" insects disturb pedestrians, draw attention of the public, provoke fear from an unusual phenomenon and cause hygienic problems. Such cases are commented even in the press (Fig. 16) or discussed in other mass-media.

The species diversity of Carabids and other beetles attracted to light in intensively illuminated places in the centre of big cities in warm evenings, especially from mid-July till mid-August can be very high (ŠUSTEK, 1999, 2002). Their temporal aggregations consist mostly in the species living in arable land in city surroundings, but also include many ripicolous or aquatic beetle species.

There arise questions – how the Carabids are able to survive in the city, how long they stay in places which essentially differ from their natural habitat and do not offer them corresponding food basis, and what strategies the Carabids use to survive in such unsuitable habitats. An attempt at answering this question is the objective of this study.

MATERIAL AND METHODS

The study was carried out since mid-July to late October 1998 and from mid-July to early November 1999. It focused just on three medium sized Carabids – *Pseudoophonus rufipes* (De Geer 1774), *Pseudophonus calceatus* (Duftschmid 1812) and *Dolichus halensis* (Schaller 1783). They occurred sufficiently abundantly (Table 1) and their body size allowed marking them by complex pictorial codes allowing recording the date the individuals were captured for the first time. At the same time, activity of other medium sized species, represented in this site by few individuals, but constantly occurring, like *Chlaenius spoliatus* (P. Rossi 1792) was also observed. However, they were not marked to prevent their stressing or wounding. This allowed observing the behaviour of relatively rare species in conditions completely different from their natural habitats.

Table 1. Number of marked individuals of three Carabid species attracted to light in the centre of Bratislava.

Species	Year		Total
	1998	1999	
Pseudoophonus rufipes (De Geer 1774).	1,114	2,739	3,853
Pseudophonus calceatus (Duftschmid 1812)	17	446	463
Dolichus halensis (Schaller 1783)	20	108	128
Total	1,151	3,293	4,444

The study site was situated in the western part of the Hodžovo námestie square in Bratislava, where a perfumery shop had extremely illuminated windows (11 halogen lamps of 50 W) and the shop sign above them (six halogen lamps of 70 W). In addition, on the shield of the close house between the Panenská ulica and Palisády streets, a huge billboard illuminated by three 440 W halogen reflectors was placed (Fig. 1). The close vicinity of this place was illuminated by sodium discharge lamps of 400 W. In this way, a strongly illuminated place arose there. Isolines of illumination intensity are given in Figures 1 and 2. Immediately in the front of the shop, there was an about 15 meters wide asphalted footpath separated from the road by a grassy bad with several low pines. On left of the shop, as a continuation of the Panenská ulica street, an asphalted area serving as a bus stop was situated. This area was extremely busy, even in the late evening hours.

The study site was situated at the foothill of Little Carpathians at the altitude of 156.5 m a. s. l. Thus, it lied approximately at a level almost identical with the level of roofs of the houses in the southeastern part of Bratislava with the altitudes of 135-136 m a. s. l. Owing to it, the illuminated place was visible from a large distance and attracted the flying beetles from a large area. Due to it, their concentration in this place was much higher than in other strongly illuminated plates in the city centre (ŠUSTEK, 1999). The described form of this locality did not last long because the construction of an administrative building started in 2000.

The illumination was measured by the luxmeter Metra LUX PU 150. The beetles were marked by means of commercially available acetone-dissoluble colours used in apiculture for marking of bee mothers. In each day, other combination of dots on elytra was used. The colours were changed when the possibility to create a new code of dots combination was exhausted. The codes and colours used in those respective dates were recorded on carton cards, which made possible to identify the date, in which the recaptured beetles were marked and released. At each visit, the number of marked beetles was recorded. This mode of marking allowed to rapidly distinguishing the individuals, but it has a disadvantage that some individuals removed the dots from their back part of their elytra by cleaning it with the row of thorns on the inner side of the hinder tibia. This mode of cleaning was even observed *in situ*. Owing to it, some individuals might be marked repeatedly considered as new incomers and the recorded values of survival length can be lower than the real ones. This can be especially the case of *Dolichus halensis* and *Pseudoophonus calceatus* with smooth surface of elytra. In contrast, on the pubescent elytra of *P. rufipes* the marks held better.

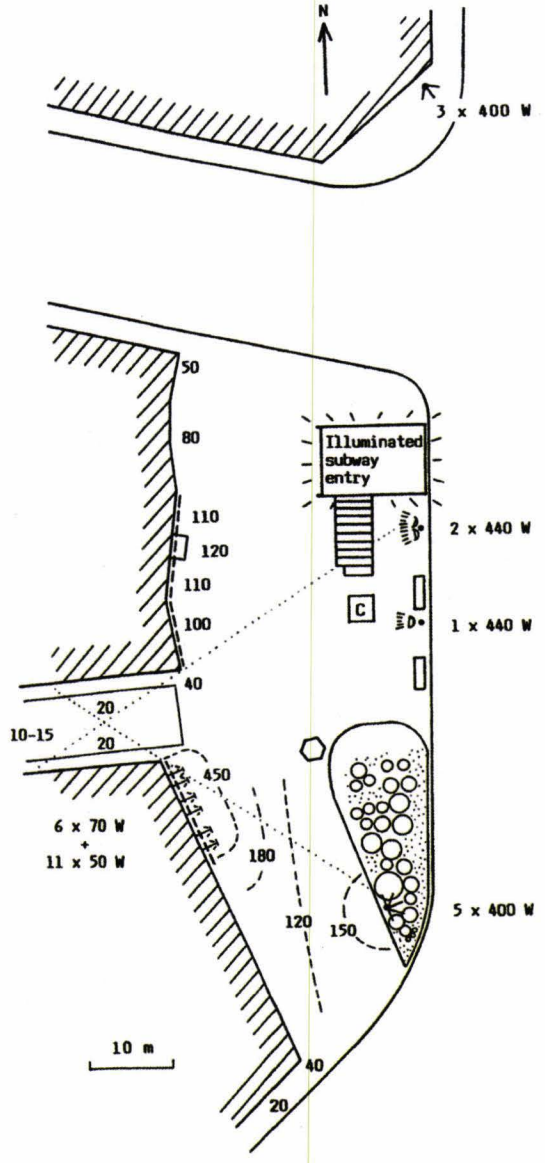


Figure 1. Situation of study site in the western side of the Hodžovo námestie square in Bratislava and isolines of illumination intensity in its individual parts, the 440 W lamps were halogen discharging lamps with the light temperature of about 5000 °K, while the 400 W lamps were sodium discharging lamps with typical yellow light.

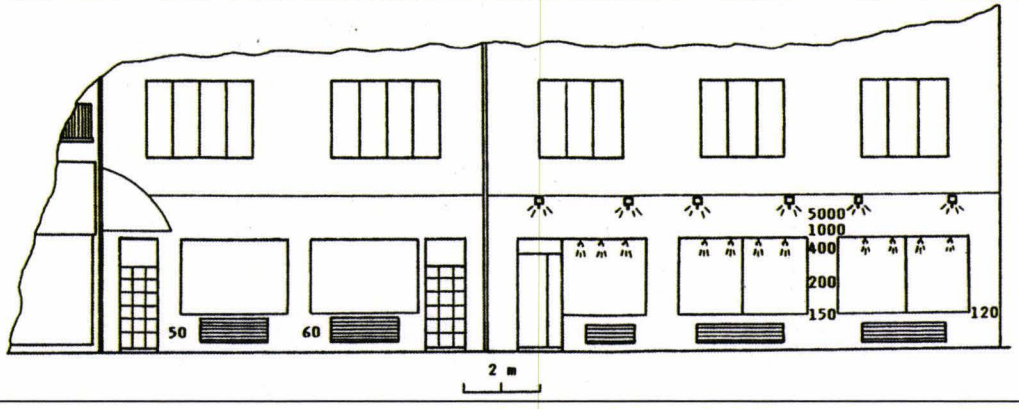
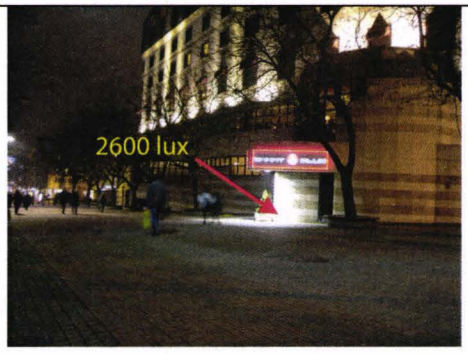
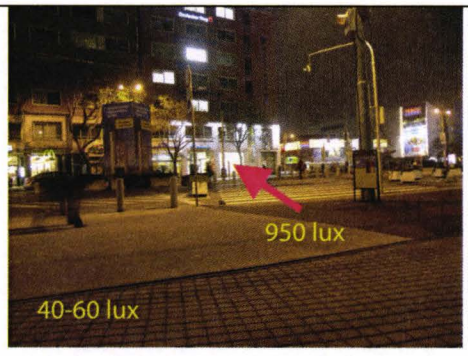
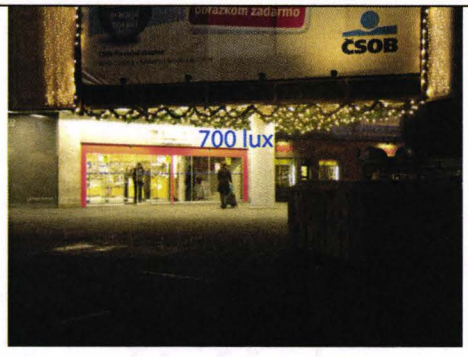
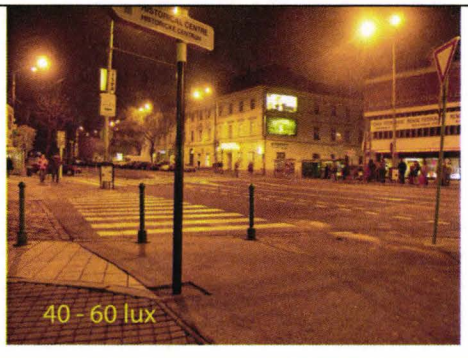


Figure 2. Illumination intensity in lux in the vicinity of the shop windows, on which the beetles were attracted and along which they were marked and recaptured.









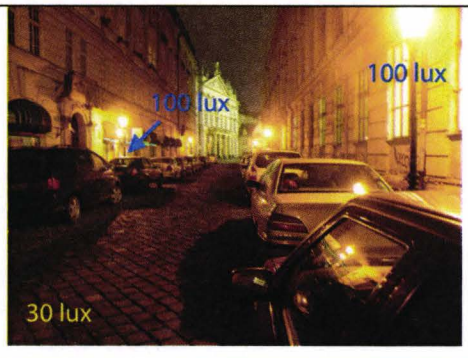


Figure 3. Illumination intensity in streets and squares in the centre of Bratislava.

The sites were visited at least every second-third day, after nightfall, from the second half of July till the beginning of November, when the last individuals of the three studied species were found. The length of visit and marking new individuals varied from 1 to 6 hours, according to the momentary temperature and the flight activity of beetles. The flight activity was especially high in the nights preventing passing a frontal system, when the number of marked individuals reached even several hundred.

By night, the illumination intensity in the darkest places of the city centre reaches only 4-6 lux, while in the most places illuminated by sodium lamps it varies between 30-60 lux, only rarely reaches to about 100 lux. In the places near shop windows or entries, illumination varies around 300-700 lux. In the particularly strongly illuminated places close to the light sources it reaches about 2,500 lux (Fig. 3), but at the immediate vicinity of the lamps on shop banners the light intensity can reach even 5000 lux (Fig. 2), which represents about half the intensity of the sun light in sunny summer days. The gradient of illumination intensity is however very steep, as shown on the illuminated wall around the perfumery windows (Figs. 1; 2). For the concentration of attracted beetles, the contrast of illumination intensity was important.

RESULTS AND DISSCUSIONS

Survival strategies of beetles attracted to light

The attracted beetles concentrated at the foot of walls, but in the especially warm days they also occurred on the whole surface of the footpath, at a larger distance from the strongest sources of light. In general, the attracted beetles, especially in the days of intensive nocturnal migrations, divided into two parts. The larger part stayed in this place only for one or three hours and then flew away. A smaller part colonized this place and searched there for covers.

As covers, the beetles mostly used the existing fissures in the pavement and between the pavement and the wall foot, in ventilation windows of cellars, around or between rain-water pipe armatures (Fig. 4), especially if some plants grew around them. They also hid in the sand filling spaces between stone tiles and often used the galleries built by ants (*Lasius* sp.).

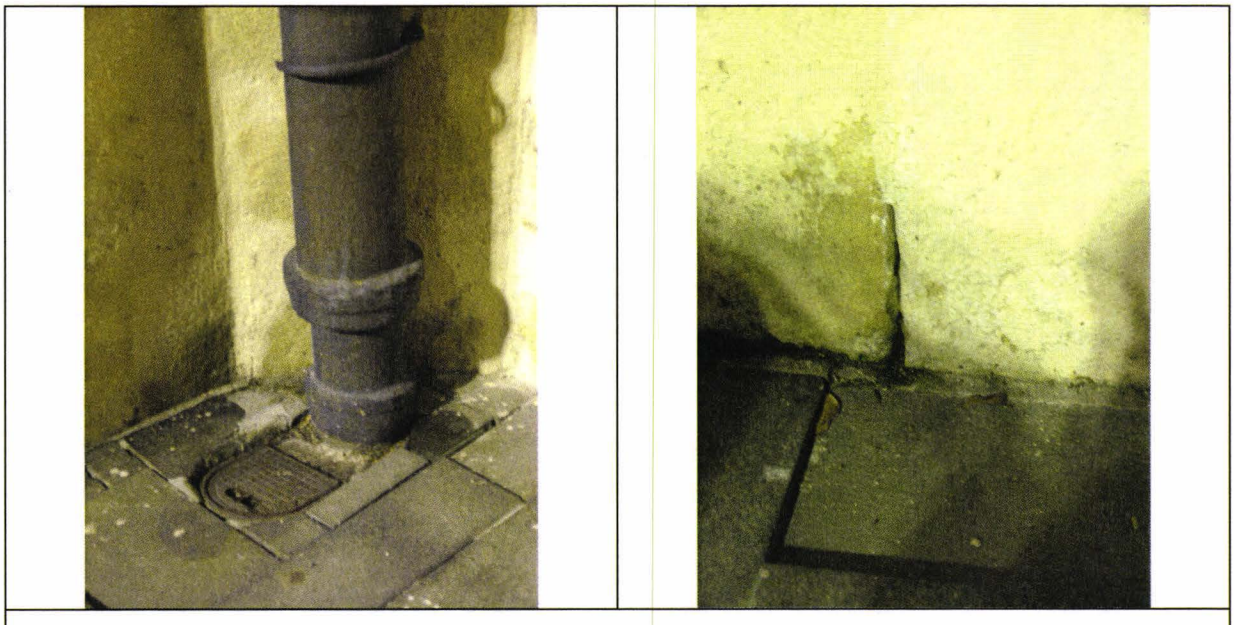


Figure 4. Typical covers used by Carabids attracted to light into the city centre and remaining there forming temporary aggregations in the "asphalt desert".

There can be distinguished two movement and behaviour strategies of the Carabids forming temporary aggregations in the city centre. They are correlated with the life forms defined by SHAROVA (1981). One is used by the stratochortobionts including mostly the representatives of the genera *Ophonus, Pseudoophonus* and *Harpalus*. They mostly walked along the walls in a 2-3 cm wide strip of sandy substrate filling the fissure between the wall and asphalt. In warm days, they also tended to ascend on the walls toward the light sources up to the height of 2-3 m, only rarely higher. Some individuals running along the walls left this "secure" zone and slowly walked toward the centre of the adjacent pavement, in an irregular trajectory (Fig. 5), searching there for pray or food. They often broke the walking and stayed on a place for several minutes. During such walks, they often become victims of trampling by pedestrians. However, after such "trip" a part of them succeeded to return to the "secure" zone at the wall and continued to run along it. This movement was not continuous. They often rested in a place or hid for some minutes in ant galleries or other covers. After a time, they appeared again. There were no conflicts between beetles.

The second strategy was used by the "stratobionts inhabiting crutches" (SHAROVA, 1981). In this study they were represented by *Dolichus halensis* and three individuals of *Chlaenius spoliatus* (P. Rossi 1792). They usually stayed in the mouth of a gallery dug in sandy material at the wall foot and sometimes undertook short, but quick attacks toward the centre of the pavement. The trajectory had the shape of a smooth curve and rapidly returned to their starting point at the gallery entrance (Fig. 6). Only rarely they rested in the centre of pavement. They manifested an outstanding space memory and ability to orientate in this microhabitat. Due to such strategy, they only rarely become victims of pedestrians. In this way, one *Chlaenius spoliatus* was able to survive in one of the busiest places in Bratislava for almost three weeks.

The similar movement strategy of *Dolichus halensis* and *Chlaenius spoliatus* is remarkable from the point of view of their different habitat preference in western Palaearctic. *D. halensis* is here a typical mesohydrophilous species of arable land, while *Chlaenius spoliatus* is a ripicolous and polyhydrophious species. However, the east palaearctic populations of *Dolichus halensis* inhabit vegetation on the loamy banks of brooks (personal observations in North Korea) and co-occur there with several eastern palaearctic species of the genus *Chlaenius*. Thus, the similar behaviour of *Dolichus halensis* with *Chlaenius* spp. may indicate that *Dolichus halensis* in west Palaearctic also originally inhabited dry shore terraces and preserved some behaviour features of the ripicolous species.

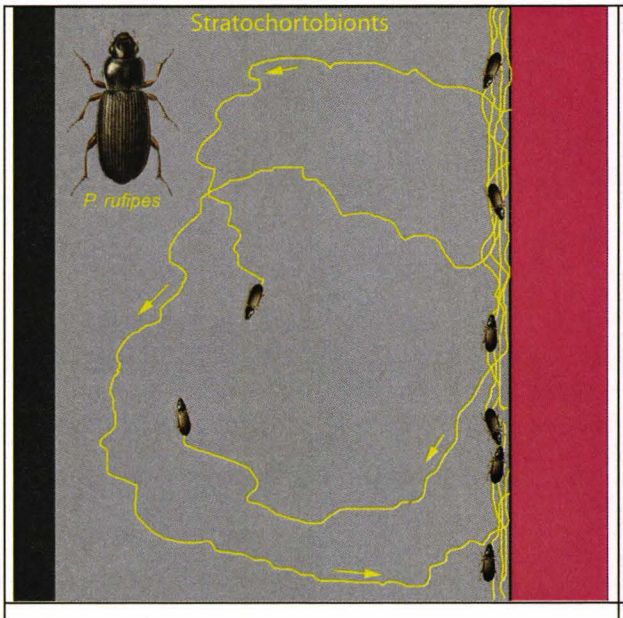


Figure 5. Character of movement of stratochortobionts on footpaths at building foundations in the city centre.

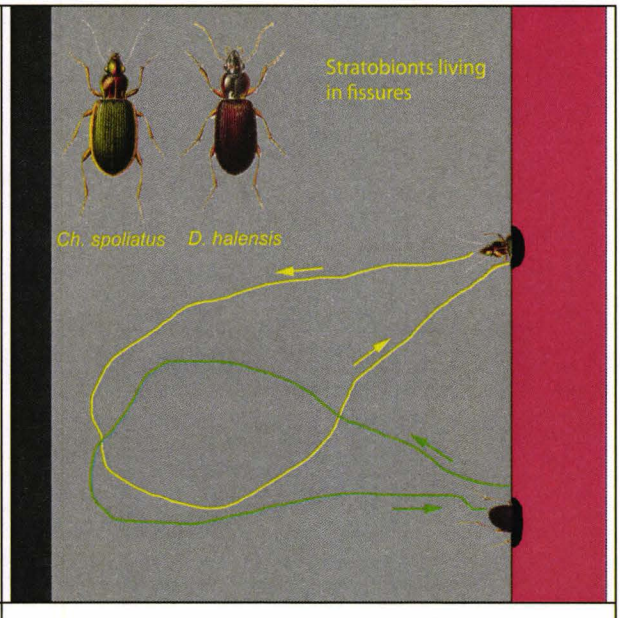


Figure 6. Character of movement of stratobionts on footpaths at building foundation in the city centre.

The beetles colonizing such unsuitable and unnatural places found there a relatively rich food offer. It permanently consisted of ants (*Lasius* sp.) cohabiting in the same place; in warm days, it also consisted of other insects (mainly Diptera, Homoptera and Neuroptera) attracted to light and falling down to the ground. However, the Carabids often ate pieces of fallen apricots or peaches, ice-cream drops or even dog excrements. They also ate the bodies of dead or trampled carabids, often conspecific individuals. This very wide food spectrum was made possible by panthophagy that is characteristic to the species of the genera *Pseudoophonus* and *Harpalus*.

Survival length of three Carabid species in the asphalt desert in the city centre

The number of beetles marked in individual evenings varied strongly ranging from 0 to 350 individuals (Figs. 7-14). The flight activity was quite independent on the momentary temperature, but in accordance with the observations of KÁDÁR & LŐVEI (1992) and KÁDÁR & SZÉL (1995), the extreme activity peaks occurred in the nights before passing a frontal system. The strong migrations occurred especially from mid-July to the first decade of August. Later, the activity declined considerably. Since mid-September, the three studied beetles flew to light only sporadically. However, strong night migrations of *Trechus quadristriatus* (Schrank 1781) were observed in early September, with culmination on the 8th of September 1998 (Fig. 15). This migration corresponds with the late autumnal peaks of occurrence of this species in different field ecosystems and to certain degree also in the damaged floodplain forests (ŠUSTEK, 1999).

The length of the flying activity of the three studied species was much longer in 1999 than in 1998. In 1999, the last individuals of *Pseudoophonus rufipes* were marked on October 15, whereas in 1998 on September 25. In general, in the days of high flight activity, the portion of beetles colonizing the study sites was much lower than in the days of moderate or low flying activity. On August 8-9, 1999, when 250-300 individuals of *Pseudoophonus rufipes* were marked each day, the marked individuals flew away within 2-3 hours after marking, being replaced by invasion of further individuals. Their departure was directly observed while marking other individuals.

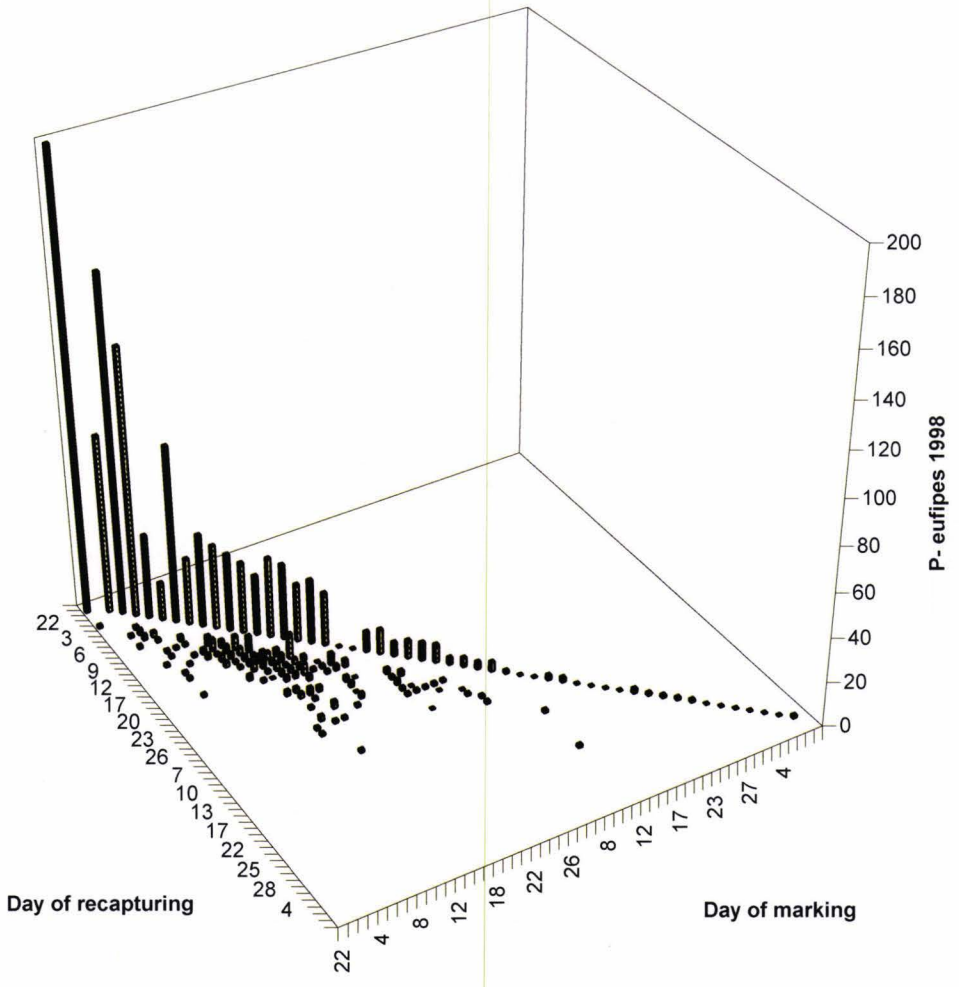


Figure 7. Number of marked and recaptured individuals of *Pseudopohonus rufipes* attracted by light in Hodžovo námestie square in Bratislava in the period July 22 - October 7, 1998.

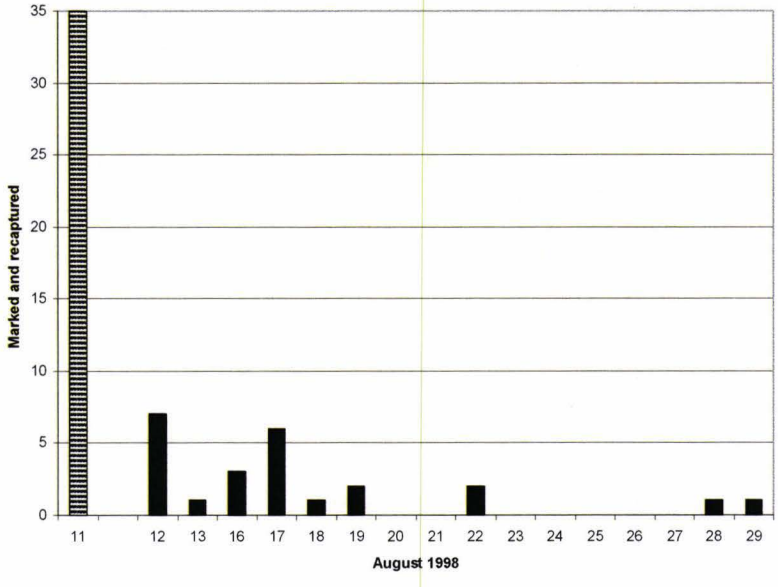


Figure 8. The longest period of survival (18 days) of *Pseudoophonus rufipes* in 1998 (35 marked individuals on August 11).

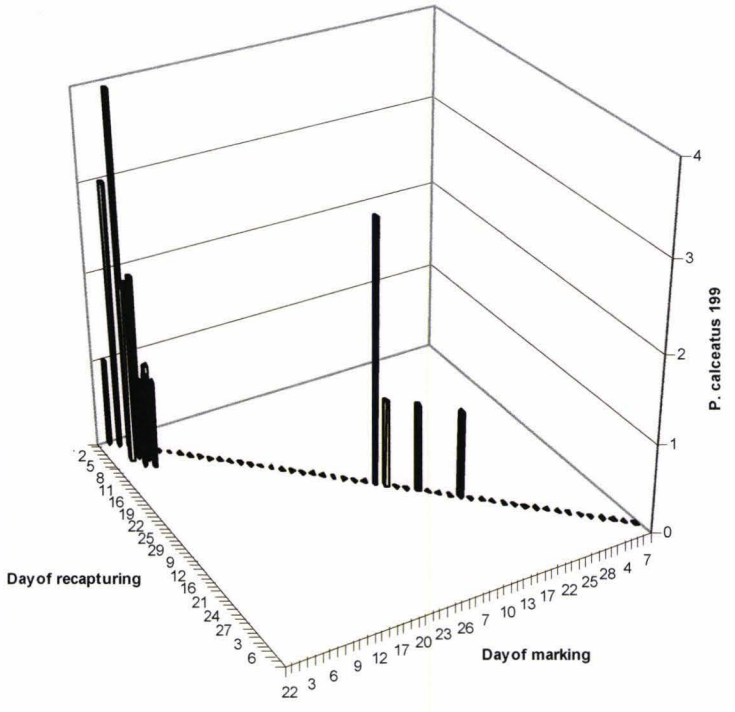


Figure 9. Number of marked and recaptured individuals of *Pseudoophonus calceatus* attracted by light in Hodžovo námestie square in Bratislava in the period July 22 - October 7, 1998.

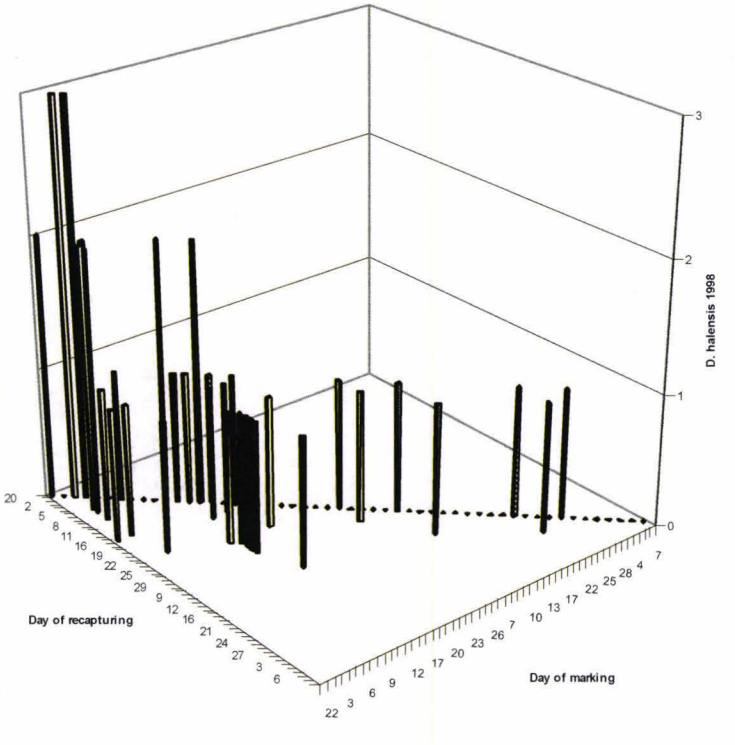


Figure 10. Number of marked and recaptured individuals of *Dolichus halensis* attracted by light in Hodžovo námestie square in Bratislava in the period July 22 - October 7, 1998.

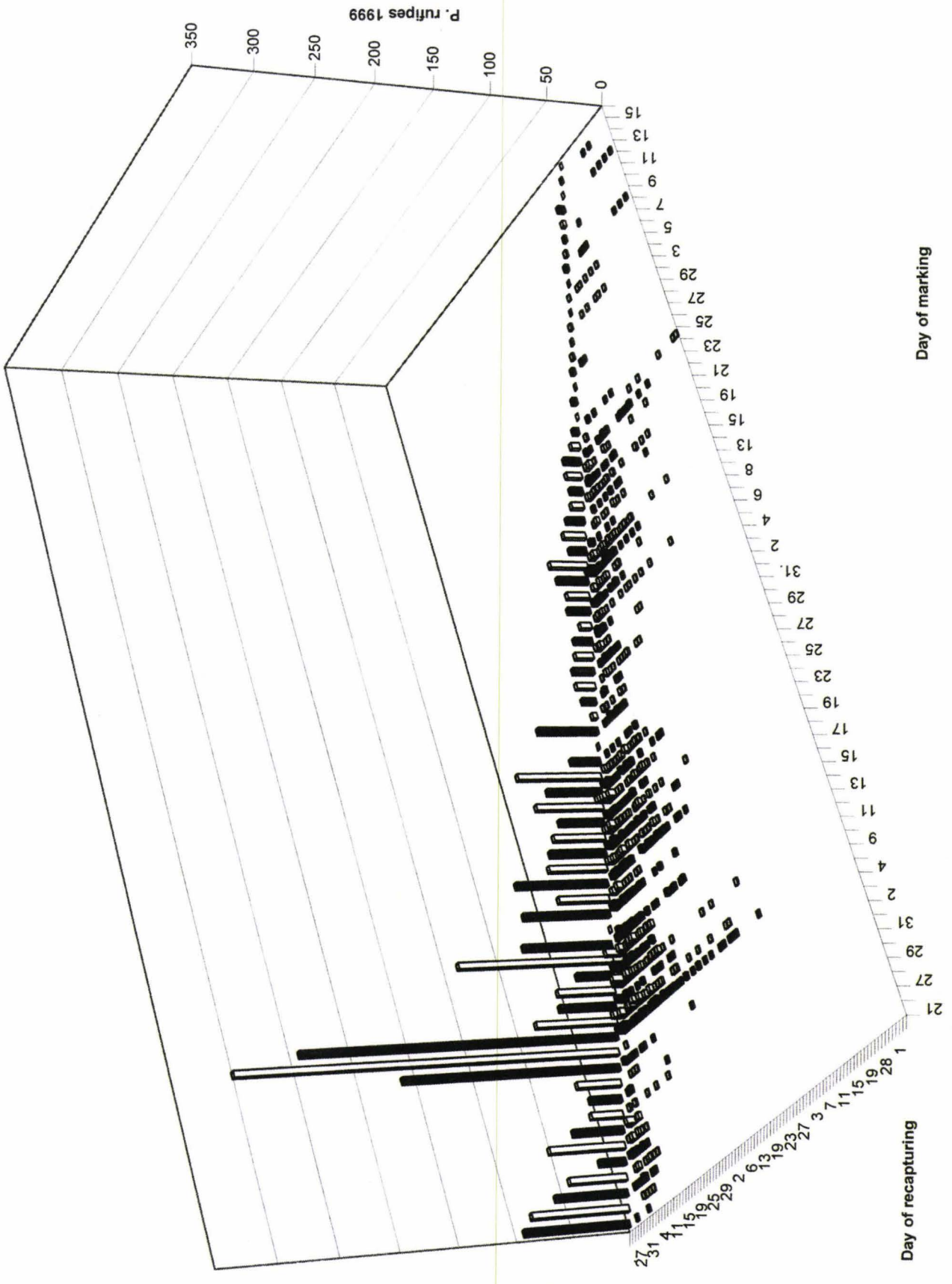


Figure 11. Number of marked and recaptured individuals of *Pseudoophonus rufipes* attracted by light in Hodžovo námestie square in Bratislava in the period July 21 - October 20, 1999.

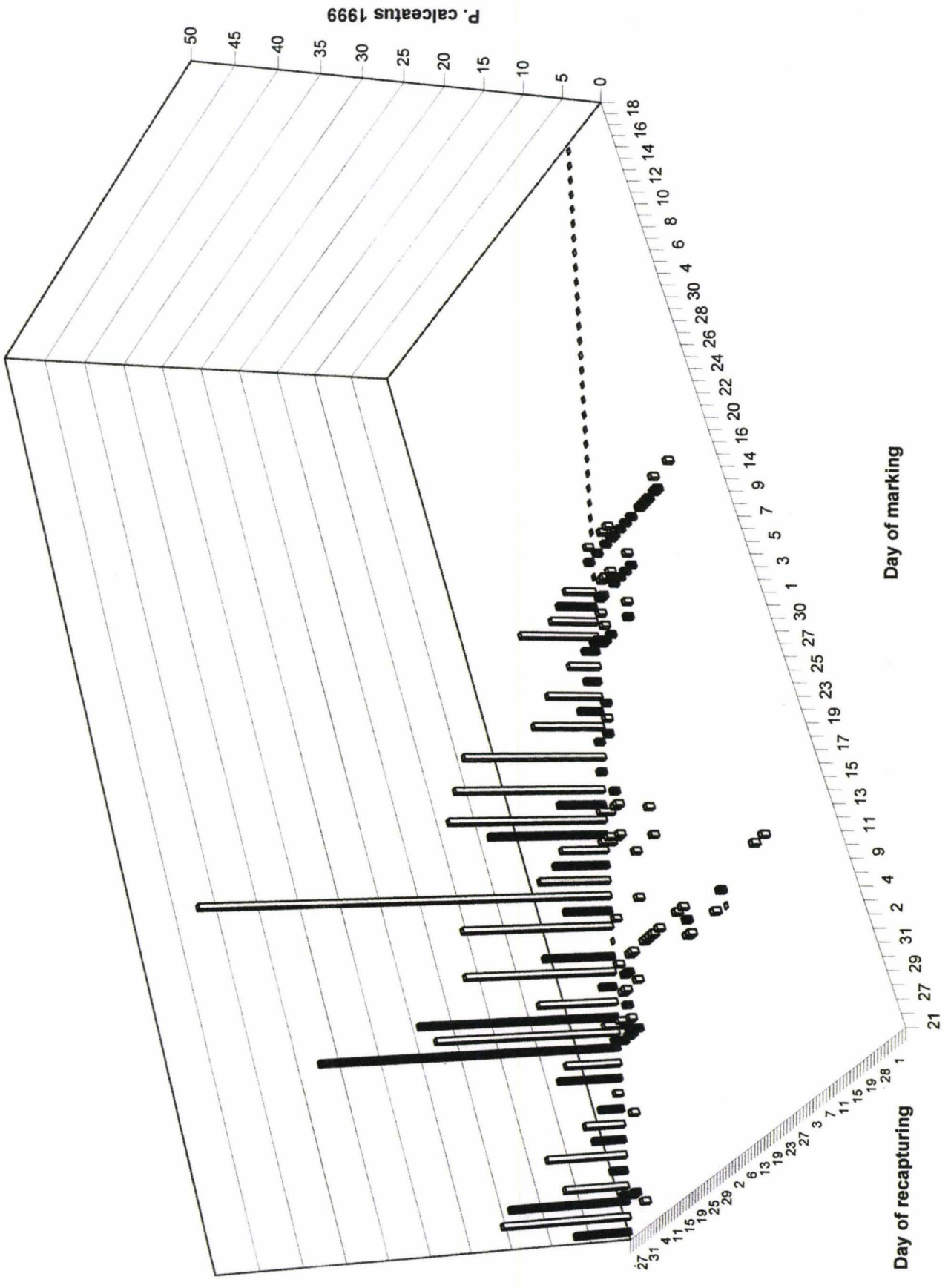


Figure 12. Number of marked and recaptured individuals of *Pseudoophonus calceatus* attracted by light in Hodžovo námestie square in Bratislava in the period July 21 - October 20, 1999.

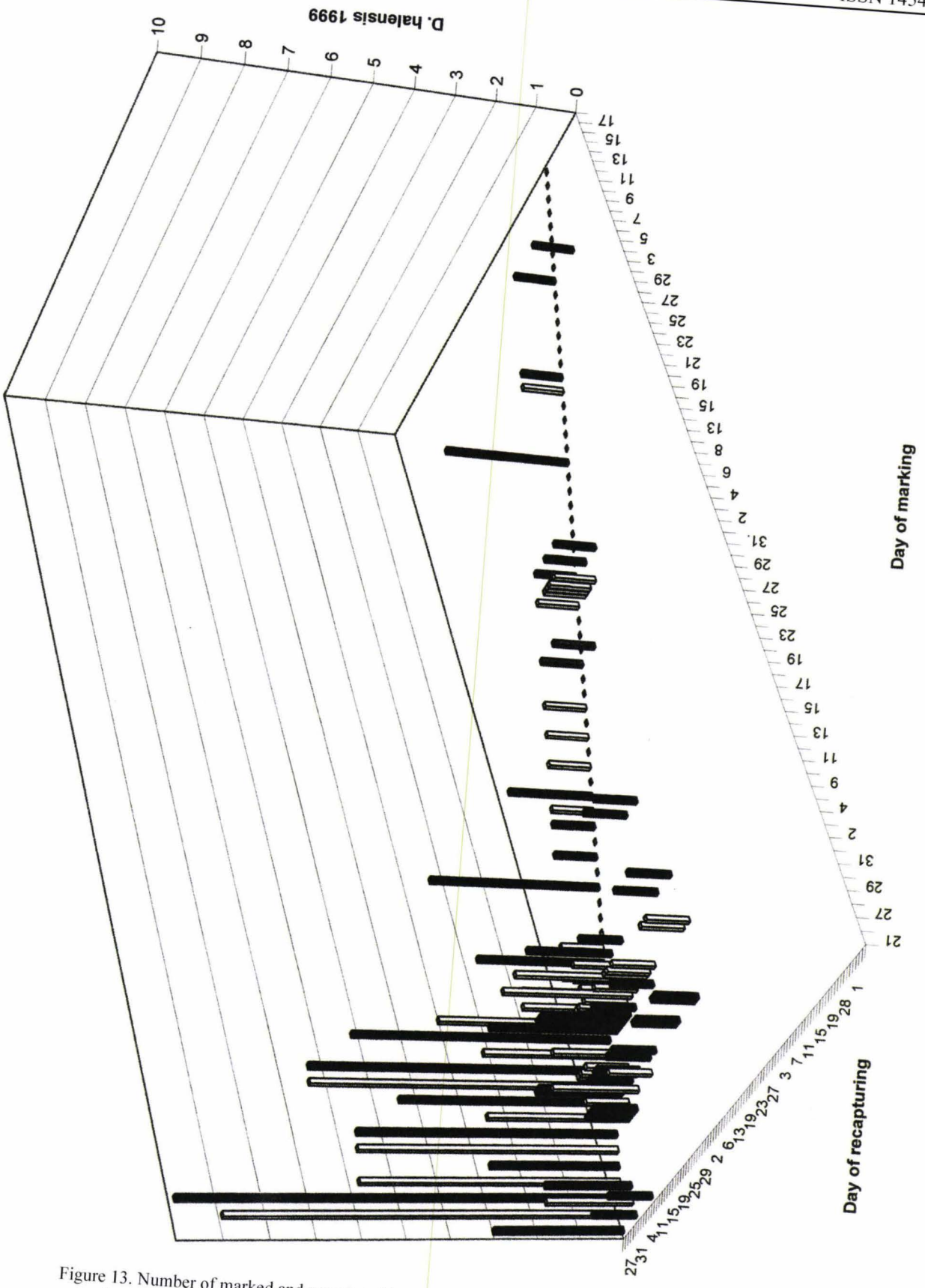


Figure 13. Number of marked and recaptured individuals of *Dolichus halensis* attracted by light in Hodžovo námestie square in Bratislava in the period July 21 - October 20, 1999.

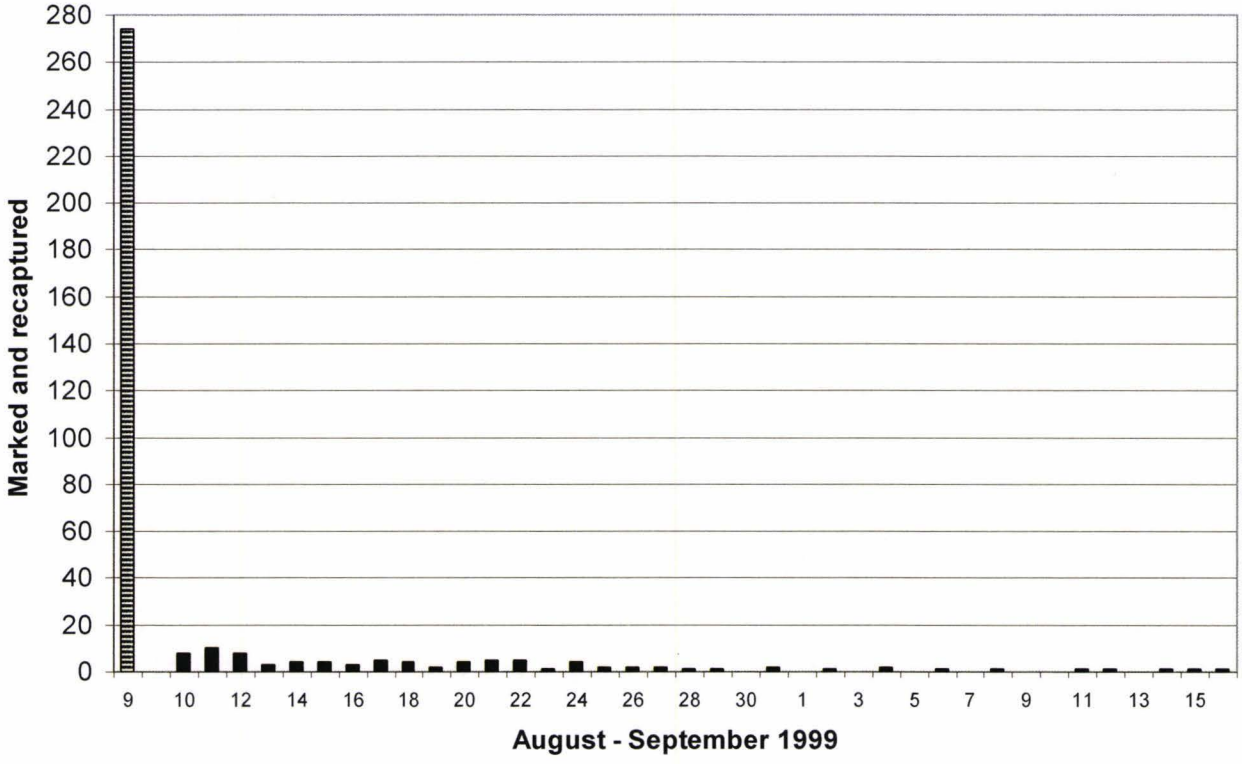


Figure 14. The longest period of survival (18 days) of *Pseudoophonus rufipes* in 1998 (35 individuals of marked on August 11).

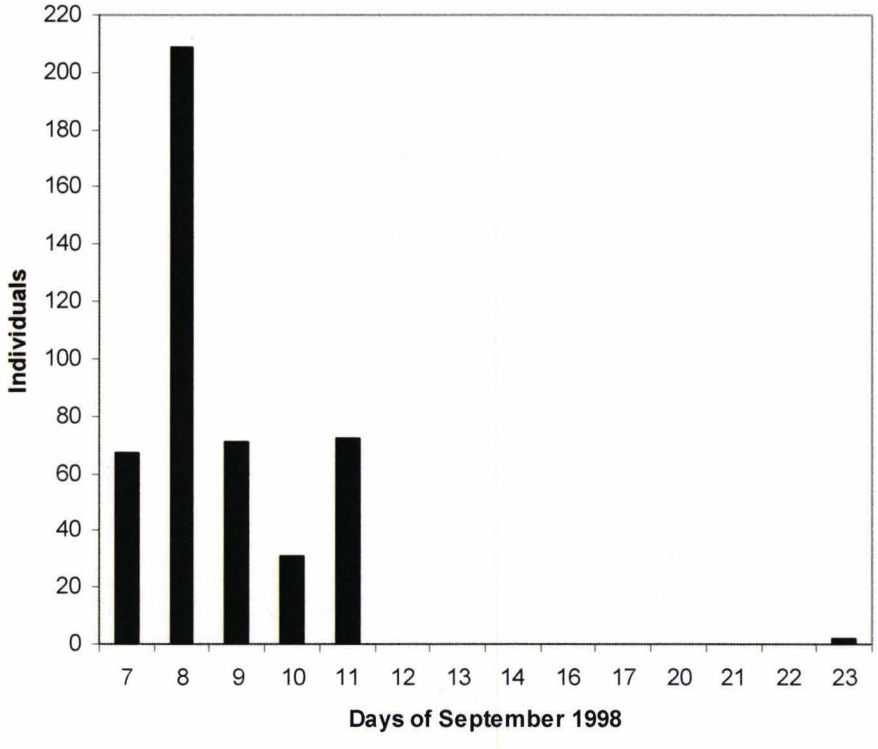


Figure 15. Flight activity of *Trechus quadristriatus* in September 1998 with a strong peak on September 8.

The relation between the number of marked and recaptured individuals of *Pseudoophonus rufipes* was very variable. This variability is best documented by the situation from early August 1999. Among 328 individuals marked on August 8, 1999 only one individual (!!!) was recaptured on the next evening and any other individual marked in

evening of August 8 was not found in the next period (Fig. 11). In contrast, among 190 individuals marked on August 4, 4 individuals were recaptured and one individual was recaptured during next 12 evenings, with several pauses.

Similarly, among the 274 individuals marked on August 9, even 10 individuals were found in the second next evening, while in the subsequent 16 days the number of recaptured colonists stabilized on 4-6 and in the period of further 24 days the number of recaptured individuals declined to 1. In this way, the longest survival time of 42 days was obtained (Fig. 14). In the movement activity of the beetles colonizing the study area differently long pauses are visible. These pauses occurred usually at the end of the life of marked beetles and their length was probably connected with the decline of vitality of individual beetles.

Less conspicuously, the same situation also occurred in August 1998 (Fig. 7). The high numbers of *Pseudoophonus rufipes* marked on August 2-4 are associated with the small numbers of individuals colonizing the study site and with a shorter period of their survival. On the contrary, relatively lower numbers of individual marked later, until mid-September, are associated with larger numbers of colonizing individuals and their longer survival. The longest period of survival (18 days) was recorded in the beetles marked on August 11 (Fig. 8).

The numbers of marked and recaptured *Pseudoophonus calceatus* and *Dolichus halensis* were always much lower (Table 1, Figs. 9, 10, 12, 13). In both years, most individuals of these species were marked in the first decade of August. Their flight activity lasted shorter than in *Pseudoophonus rufipes* and the survival of recaptured individuals also was shorter. Only among 5 individuals of *Dolichus halensis* marked on August 2, 1999 one individual survived 28 days. Similarly, in *Pseudoophonus calceatus*, the longest survival was observed in 10 individuals marked on August 14, 1999. Among them, only one individual was recaptured after 24 days. The pattern of survival of *Pseudoophonus calceatus* and *Dolichus halensis* differed from that of *Pseudoophonus rufipes* by a higher proportion (about 10 %) of individuals colonizing the study site. Similarly as in *Pseudoophonus rufipes*, there were observed pauses in the occurrence of the recaptured colonists at the second half of their survival period. The observed maximum survival of both species was shorter than that of *Pseudoophonus rufipes.* However, the observed values could be influenced by the worse adhesion of colours on smooth elytra of both species, from which the marks could be easier removed. However, the potential survival of *Pseudoophonus calceatus* can be similar as in *P. rufipes* and in *Dolichus halensis* it can be still longer, because of other movement strategy (see above).

Among other Carabids, a mass flight activity and attraction to light was observed only in *Trechus quadristriatus* (Fig. 15). That occurred only during five days in early September 1998, with an expressive peak on September 8. Later, only one individual was observed on September 23. Obviously, all attracted individuals left the study site quickly.

Kvapníci jdou za chladem. Nový domov našli v nemocnici

Brouk plní prostory bohunické nemocnice. Neškodí, ale personál neví, jak se hmyzu zbavit.

NELA MAŤAŠEJE

Brno/ Zapíjnuje chodby, pokoje i operační sály Fakultní nemocnice Bohunice a nikdo si s ním neví rady. Brouk kvapník plistnatý se tu stal nevítaným hostem, přestože nikomu neubližuje. Hmyz láká do budov příjemnější klima, protože i na něj je venku příliš horko.

Problémy s patnáct milimetrů velkým kvapníkem má nemocnice každý rok. „Potýkáme se s ním už patnáct let, ale letošní rok je nejhorší. Mohou za to vysoké teploty, před kterými brouci prchají do chladu," popsal situaci externí pracovník nemocnice a deratizátor Jaroslav Báňa, který má likvidaci kvapníka na starosti.

Má léto rád

Brouk je v teplých letních měsících nejvíce aktivní. Děje tvající tropy ho teď vyhánějí z polí a lak. „V noci se vchodem a okny dostává do bohunické nemocnice. Kromě chladu ho láká i světlo. Je jich tu pořád několik desítek," poznamenal Báňa.

Podle něj je však zbytečné proti kvapníkovi nasadit chemii. „Brouk do druhého dne umírá. Zbytečně bychom postříkem zamořili prostor nemocnice," myslí si deratizátor.

Mrtvá hmyzí těla tak musí personál nemocnice neustále odklízet. „Je to blízkinec. Jsme domluvení s úklidovou službou, která pulíruje každou hodinu chodby nemocnice. Nevypadá dobře, když se ve zdravotnickém zařízení povalují mrtví brouci," připustil Báňa.

Pacienti i návštěvníci bohunické nemocnice si totiž často myslí, že jde o rusy nebo šváby, kteří jsou symbolem špatné hygieny. Kvapník je ale podle odborníků nezávadný. „Na člověka tito brouci nepřenášejí žádnou nákazu, spíše jej pouze obtěžují svou přítomností. Je velmi nepříjemné, když lezou ve vlasech nebo po kůži," vyličil vedoucí oddělení environmentální epidemiologie Zdravotního ústavu Brno Milan Pejčoch, který doporučuje proti broukovi nasadit sítě do oken. Podle Jaroslava Báni to ale nemá smysl. „Musely by se zbarikádovat všechny vchody, a to zkrátka v nemocnici nejde," uvedl deratizátor.

Většina lékařského personálu s broukem navíc

žádné problémy nemá. „Byli jsme informováni o tom, že je kvapník nezávadný, takže nám nevadí. Pokud se člověk neštítí běžného neškodného hmyzu, nemůže mu vadit ani tento brouk. Je plachý," vysvětlil svůj postoj hematolog Jaroslav Petřík, který na malého brouka nedávno narazil při procházení nemocničními chodbami.

Kromě nemocnice se ale kvapník objevuje i v dalších zařízeních. „Odstraňujeme ho z nádraží, hypermarketů i třeba z budovy pošty. Za posledních pět let je to problém nejen Brna, ale i celé republiky," tvrdí Báňa.

S tím souhlasí také deratizátor Stanislav Kopr. Brouci hledají klimatizaci a místa, kde nebude šedesát stupňů. Není možné se divit, když nalétávají do hypermarketů a velkých obchodních středisek. Je roztříšen i v centru v Dolních Heršpicích," uvedl Kopr.

Klimatizované obchodní prostory tu podle něj vznikly prakticky v poli, kde předtím brouci mnoho let žili. „V jejich přirozeném prostředí se tak objevila chladná místa, oni je pouze využívají," myslí si Kopr.

Přemnožený není

Kvapník plistnatý, který ve svém přirozeném prostředí žije až třicet dnů, prý ale v Brně přemnožený není. „Jeho počty jsou v normálu. Spíše se jen snaží ukrýt před příliš teplým počasím, proto na něj člověk může častěji narazit. Není problém ho potkat třeba v bytě nebo rodinném domě," dodal brněnský entomolog Václav Pižl.

Figure 16. An article from the newspaper Rovnost from July 2000 about the occurrence of *Pseudoophonus rufipes* in the hospital in Brno – Bohunice (Moravia). The title translation: "The beetles search for cold. They have found a new home in the hospital. The beetle fills the interior of Bohunice Hospital. It does not cause any damage, but the personnel does not know, how to get rid of the insect" The text contains an interview with Václav Pižl, an entomologist, about the problem. The interviewed man is not a specialist in carabids. Therefore some information is not accurate.

CONCLUSIONS

The attraction to extremely illuminated sites in centres of cities results in formation of temporary aggregations of Carabids, but most of the attracted beetles leave the illuminated sites within a few hours. Thus, such sites do not represent a fatal trap for most of them. The individuals, which colonize such places, show a remarkable adaptability to a highly unfavourable habitat. They have a survival chance of 2-3 weeks, but in extreme cases even of six weeks. However, the presence of the extremely illuminated places in urban or free landscape (advertisement billboards along highways) can considerably influence the migration paths of Carabid beetles and can reduce their chance to find new suitable habitats. In this way, the diversity and structure of the ecosystem can be reduced or unnaturally modified. At the same time the light attraction of Carabids can support the metapopulations inhabiting the plots of urban greenery, parks and gardens in residential quarters.

The most negative aspect of the attraction of Carabids (and other insects) to the extremely illuminated places in the cities is the producing of conflicts with the public (Fig. 16) and hygienic problems in shops or some public institutions. In the private sphere, the most affected social group are the owners of flats situated near to the huge illuminated advertisement tables placed on walls of living houses. The beetles fly in considerable numbers into the opened windows and disturb the inhabitants, who turn with claims and questions to the radio or TV. This experience should be used for formulation of hygienic criteria for the regulation of the placement of illuminated advertisements in the cities, as well as to regulate the excessive energy consuming illumination as such.

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ASPECTS OF TAXONOMIC AND ECOLOGICAL STRUCTURE OF THE COENOSES OF CARABIDAE (COLEOPTERA) IN FIVE ALFALFA CROPS, MOLDOVA (ROMANIA)

VARVARA Mircea

Abstract. The paper is based on quantitative collectings of the individuals belonging to the epigeic species of Carabidae from five crops of alfalfa, Moldova (1977, 1978 1981, 1983). For unitary collectings of the carabidological material, there were used 12 Barber pit-falls in every crops and year. In total, there were made 75 collectings, analysed 900 samples and collected 11.618 specimens of Carabidae belonging to 13 subfamilies, 20 genera and 45 species. In total, the Harpalinae subfamily is represented by four genera (20%), 11 species (24.44%) and 1.486 individuals (12.79%). The Pterostichinae subfamily is represented by five genera (25.00%), 10 species (22.22%) and 9.651 (83.06%). The average number of species present in the alfalfa crops was 9, with a variation between 7 species (Hemeiuş, 1981, Bacău County) and 25 (Suceava, 1978, Suceava County). The mean number of the individuals present in crops was 2.323, with a large variation between 636 (Pogoneşti, 1983) and 5.399 (Suceava, 1978). Three species had special relative abundances: *Harpalus (Pseudophonus) rufipes* 1.051 individuals (9.05% of total), *Poecilus cupreus* 3.585(30.86%) and *Pterostichus melanarius* 5.969 (51.38%). The average value of the Shannon diversity index was 1.66 with a variation between 0.92 (Suceava, 1977) and 3.24 (Suceava, 1978) and evenness 42%, with a variation between 29% (Suceava, 1978) and 71% (Căbeşti, 1983).

Keywords: Alfalfa crop, carabids, species, taxonomic structure, abundance, dominance, ecological requirements, Shanon index, evenness.

Rezumat. Aspecte ale structurii taxonomice şi ecologice ale cenozelor de Carabidae (Coleoptera) în cinci culturi de lucernă, Moldova (România). Lucrarea se bazează pe colectările cantitative ale indivizilor speciilor epigee de Carabidae din cinci culturi de lucernă, Moldova (1977, 1978, 1981, 1983). Pentru colectarea unitară a materialului carabidologic s-au folosit câte 12 capcane Barber în fiecare cultură şi an. În total, s-au efectuat 75 colectări, analizat 900 probe şi colectat 11.618 exemplare de Carabidae care aparţin la 13 subfamilii, 20 genuri şi 45 specii. În total, subfamilia Harpalinae este reprezentată prin 4 genuri (20%), 11 specii (4,44%) şi 1486 indivizi (12.79%). Subfamilia Pterostichinae este reprezentată prin 5 genuri (25 %), 10 specii (22,22%) şi 9651 (83.06 %).Numărul mediu de specii prezente în culturi de lucernă a fost 9, cu o variaţie între 7 specii (Hemeiuş, 1981, judeţul Bacău) şi 25 (Suceava, 1978, judeţul Suceava). Valoarea medie a numărului de indivizi prezenţi în culturi a fost de 2323, cu o amplă variaţie, între 636 (Pogoneşti, 1983) şi 53. 99 (Suceava, 1978). Trei specii au avut abundenţe relative deosebite: *Harpalus (Pseudophonus) rufipes* 1051 indivizi (9.05% din total), *Poecilus cupreus* 3585 (30.86%) şi *Pterostichus melanariuis* 5969 (51,38%). Valoarea medie a Indicelui de diversitate Shannon a fost 1,66, cu o variaţie între 0.92 (Suceava, 1977) şi 3,24 (Suceava, 1978), iar echitabilitatea, 42 %, cu o variaţie între 29 % (Suceava, 1978) şi 71 % (Căbeşti, 1983).

Cuvinte cheie: cultura de lucernă, carabide, specii, structură taxonomică, abundenţă, dominanţă, cerinţe ecologice, indice de diversitate Shanon, echitabilitate.

INTRODUCTION

Coenosis structure and especially the density and dominance (relative abundance) of carabids populations in the agroecosystems (wheat, potatoes, sugar beet, maize, alfalfa, clover, etc.) are influenced by many synergically acting factors, like pedological and hydrological conditions, microclimatic conditions specific for each crops, stand duration and timing of crop presence on the field, agro technical measures, and chemical pest control (VARVARA & SUSTEK, 2011).

Moldova is a zoogeographical region of Romania (KIS, 1970) characterized by a temperate continental climate, with the annual average temperature between 7.0 and 9.0°C and annual average precipitations of 450-650 mm. Within Moldova, (Romania) three climatic districts are distinguished: **(1)** The northern one with the southern limit southerly of Iaşi city (annual precipitations 500-600 mm, average annual temperature 9.4°C; **(2)** the central one with the southern limit south of Huşi town (annual precipitations 400- 500 mm, average annual temperature 9.5°C and **(3)** the southern one (annual precipitations 400-500 mm, average annual temperature 10.5°C) (according to www.meteoromania.ro).

Alfalfa (*Medicago sativa*) is a perennial plant that normally lives 4-8 years. It is a valuable forage legume plant for cows, cattle, beef cattle, sheep, goats, horses, rabbits, pigs because it is rich in proteins, calcium, minerals and vitamins (B group, C, D, K). Also, *Medicago sativa* is important for agriculture. It prevents erosion, enriches the soil with nitrogen due to symbiotic bacteria, provides food for pollinators, offers shelter for predators and parasitoids.

In Moldova, in some agricultural crops, ecological research were made by VARVARA & BRUDEA (1983); TURCULEŢ & VARVARA (2006) (clover); VARVARA et al. (1999) (potatoes), VARVARA & BULIMAR (2003); VARVARA (2005) (wheat); VARVARA (2001), VARVARA et al. (2001) (potatoes); VARVARA & GĂLUŞCĂ (2007); VARVARA (2008) (sugar beet); VARVARA & APOSTOL (2008) (sun flower).

MATERIAL AND METHODS

The carabidological material for this paper is original. The material was collected from alfalfa crops, from three counties, five localities (Moldova) in the interval 1977-1983: Suceava, 1977, 1978 (Suceava County), North of Moldova, Hemeiuş, 1981, Căbeşti, 1983 (Bacău County), Pogoneşti, 1983 (Vaslui County).

For collecting, there was used the Barber pit-falls method, a statistic, unitary, objective standard method that permits the continuous qualitative and quantitative collecting and dynamics of the epigeic arthro-fauna. In every crop and season of

collecting, there were used 12 pit-falls to collect all kinds of species in the structure of dominance (subrecedent-eudominant). The canisters contained a 4% formalin solution for preserving of the entomological material; the pit-falls were protected from precipitations. Each pit-fall had a capacity of 385 cubic centimetres, 7 centimetres in diameter, 10 centimetres in height. The pit-falls were set on three rows, each row having four pit-falls. The distance among rows and pit-falls was five meters.

The collecting was conducted for five months, from May to September. In crops, there were carried out between 12 and 15 calendar dates of collectings. The individuals collected from a pitfall constituted a sample (Table 1).

The paper presents the coenosis taxonomic structure of Carabidae and the variation of several ecological parameters: relative abundance, dominance, the main ecological requirements of the species of Carabidae in the ecological conditions of the alfalfa crop in the years 1977, 1978, 1981, 1983. The material collecting in the field and in the respective localities were made by BRUDEA (1983), PROCA (1984), MĂRCUŢĂ (1984), the last two being biology teachers in the general education in the localities mentioned for obtaining the first didactic degree in the preuniversity education. All the methodical instructions for collecting the material were given by me, Varvara Mircea, the advisor of the works for obtaining the first degree in the preuniversity teaching. The determination of the species of Carabidae was made in accordance with determination books in Romanian, Russian, German, English languages.

The objectives of the paper concern the knowledge of the numerical taxonomic structure (subfamilies, genera, species, specimens of the coenosis of Carabidae; specific diversity, the Index of diversity, the structure and variation of dominance), and the characterization of the species and the coenosis of Carabidae as reproduction of species, preferences for moisture, biotope, food regime and geographical distribution. Data for ecological characterization of the species were taken from different papers (NECULISEANU, 2003; PETRUSENKO, 1970; PETRUSENKO & PETRUSENKO, 1972; SUSTEK, 2000; VARVARA, (2005a, 2008); VARVARA & BRUDEA, 2012).

RESULTS

In those four years of collecting, there were used 60 Barber's pit-falls in crops for 723 days, were performed 75 collectings, analysed 900 samples in which there were numbered 11,618 individuals belonging to 13 subfamilies, 20 genera and 45 species (Carabidae) (Table 1).

Table 1. General information on the collectings in the alfalfa crops, Moldova, (Romania).

	Localities	County	Collecting limits	Days	Collectings	Samples	Individuals
1	Suceava, 1977	Suceava	April 28 – Sept. 30	156	15	180	2711
2	Suceava, 1978	Suceava	April 29-Sept. 30 .	155	15	180	5399
3	Hemeiuş, 1981	Bacău	May 11 – Oct. 18	142	17	204	2350
4	Pogoneşti, 1983	Vaslui	April 25 –August 20	117	12	144	636
5	Căbeşti, 1983	Bacău	May 1 – Sept. 30	153	16	192	522
			Total	723	75	900	11.618

Numerical variation of taxa is given in Table 2. Subfamilies, variation between 3 and 9; genera, 5-13; species, 7-25; individuals, 636-5,399 (Table 2).

Table 2. Taxonomic and numerical structure of the coenosis of Carabidae in five alfalfa crops from Moldova (1977, 1978, 1981, 1983).

	Taxa	1	2	3	4	5	Total
1	Subfamilies	6	7	3	5	9	13
	%	46.15	53.84	23.07	38.46	69.23	
2	Genera	7	11	5	6	13	20
	%	35.0	55.0	25,0	30	65.0	
3	Species	9	23	7	17	25	45
	^%	20.0	51.11	15.55	37.77	55.55	
4	Individuals	636	522	2350	2711	5399	11.618
	%	5.47	4.49	20.23	23.33	46.47	99.99

Legend = **1**. Pogoneşti, 1983; **2**. Căbeşti, 1983; **3**. Hemeiuş, 1981; **4**. Suceava, 1977; **5**. Suceava, 1978.

Subfamilies, number of localities in which they were found and the numerical and percentage of individuals capturers are given in Table 3.

Table 3. The subfamilies of Carabidae present in alfalfa crops, Moldova.

No	Subfamilies	Localities	Individuals	%
1	Cicindelinae	1	9	0.07
2	Carabinae	5	243	2.09
3	Loricerinae	1	8	0.06
4	Scaritinae	1	46	0.39
5	Broscinae	1	2	0.01
6	Anisodactilinae	2	42	0.36
7	Harpalinae	4	1,486	12.79
8	Stenolophinae	2	4	0.03
9	Pterostichinae	5	9,651	83.06
10	Callistinae	1	2	0.01

11	Zabrinae	4	117	10.07
12	Dromiinae	2	3	0.02
13	Brachininae	1	5	0.04
	Total		11.618	

The subfamilies Harpalinae and Pterostichinae are eudominant as number of individuals and percentages. The numerical land percentage variation of the species collected, on localities, is detailed in Table 4.

Table 4. The species of carabids found in alfalfa crops from Moldova. their relative abundance and dominance.

No	Species	Pogoneşti	Căbeşti	Hemeiuş	Suceava -77	Suceava-78	Total
1	*Cicindela germanica*	-	-	-	-	9	**9**
	%	-	-	-	-	0.17	0.08
2	*Calosoma auropunctatum*	**20**	**11**	-	-	-	**31**
	%	3.14	2.11	-	-	-	0.27
3	*Carabus violaceus*	-	-	**35**	**51**	**19**	**105**
	%	-	-	1.49	1.88	0.35	0.90
4	*C. cancellatus*	-	-	**3**	-	-	**3**
	%	-	-	0.13	-	-	0.03
5	*C. granulatus*	-	-	**10**	**1**	**6**	**17**
	%	-	-	0.43	0.04	0.11	0.15
6	*C. scabriusculus*	-	**1**	-	**2**	-	**3**
	%	-	0.19	-	0.07	-	0.03
7	*C. excellens*	-	-	-	**5**	**16**	**21**
	%	-	-	-	0.18	0.30	0.18
8	*C. besseri*	-	**63**	-	-	-	**63**
	%	-	12.07	-	-	-	0.54
9	*Loricera pilicornis*	-	-	-	-	**8**	**8**
	%	-	-	-	-	0.15	0.07
10	*Clivina fossor*	-	-	**46**	-	-	**46**
	%	-	-	1.96	-	-	0.40
11	*Broscus cephalotes*	-	-	-	-	2	2
	%	-	-	-	-	0.04	0.02
12	*Anisodactylus poeciloides*	-	-	-	-	**2**	**2**
	%		-	-		0.04	0.02
13	*A. signatus*	-	-	-	**11**	**15**	**26**
	%	-	-	-	0.41	-	0.22
14	*A.binotatus*	-	-	-	**6**	**8**	**14**
	%	-	-	-	0.22	0.15	0.12
15	*Ophonus sabulicola*	-	**1**	-	-	-	**1**
	%	-	0.19	-	-	-	0.01
16	*O. diffinis*	-	**1**	-	-	-	**1**
	%	-	0.19	-	-	-	0.01
17	*Metophonus azureus*	-	**28**	-	-	-	**28**
	%	-	5.36	-	-	-	0.24
18	*Harpalus rufipes*	**417**	**57**	-	**332**	**245**	**1051**
	%	65.57	10.92		12.25	4.54	9.05
19	*H. griseus*	-	-	-	**3**	**14**	**17**
	%	-	-	-	0.11	0.26	0.15
20	*H. aeneus*	-	**3**	-	**9**	**4**	**16**
	%	-	0.57	-	0.33	0.07	0.14
21	*H. rubripes*	-	**9**	-	-	**2**	**11**
	%	-	1.72	-	-	0.04	0.09
22	*H. distinguendus*	**120**	**171**	-	**3**	**2**	**296**
	%	18.87	32.76	-	0.11	0.04	2.55
23	*H. latus*	**9**	**5**	-	**2**	-	**16**
	%	1.42	0.96	-	0.07	-	0.14
24	*H. calceatus*	-	-	-	-	**2**	**2**
	%	-	-	-	-	0.04	0.02
25	*H. tardus*	-	**47**	-	-	-	**47**
	%	-	9.0	-	-	-	0.40
26	*Acupalpus meridianus*	**2**	**2**	-	-	-	**4**
	%	0.31	0.38	-	-	-	0.03
27	*Poecilus cupreus*	-	**10**	**1539**	**461**	**1575**	**3585**
	%	-	1.92	65.49	17.00	29.17	30.86
28	*Pterostichus melanarius*	-	**1**	**713**	**1817**	**3438**	**5969**
	%		0.19	30.34	67.02	63.689	51.37
29	*Pt. ovoideus*	-	-	-	**2**	**7**	**9**
	%	-	-	-	0.07	0.13	0.08
30	*Pt. melas*	-	**4**	-	-	-	**4**
	%		0.77	-	-	-	0.03
31	*Pt. nigrita*	-	-	-	**2**	-	**2**

	%	-	-	-	0.07	-	0.02
32	*Calathus fuscipes*	-	**52**	-	**2**	-	**54**
	%	-	9.96	-	0.07	-	0.46
33	*C. ambiguus*	**10**	**7**	-	-	-	**17**
	%	1.57	1.34	-	-	-	0.15
34	*C. melanocephalus*	-	-	-	-	**2**	**2**
	%	-	-	-	-	0.04	0.02
35	*Dolichus halensis*	-	-	**4**	-	**3**	**7**
	%	-	-	0.17	-	0.06	0.06
36	*Agonum sexpunctatum*	-	-	-	-	**2**	**2**
	%	-	-	-	-	0.04	0.02
37	*Amara equestris*	-	-	-	**2**	-	**2**
	%	-	-	-	0.07	-	0.02
38	*A.ovata*	**52**	**1**	-	-	**2**	**55**
	%	8.18	0.19	-	-	0.04	0.47
39	*A.similata*	-	**8**	-	-	**12**	**20**
	%	-	1.53	-	-	0.22	0.17
40	*A.familiaris*	-	-	-	-	**2**	**2**
	%	-	-	-	-	0.04	0.02
41	*A.aenea*	-	**33**	-	-	-	**33**
	%	-	6.32	-	-		0.28
42	*Chlaenius vestitus*	-	-	-	-	**2**	**2**
	%	-	-	-	-	0.04	0.02
43	*Zabrus tenebrioides*	**5**	-	-	-	-	**5**
	%	0.79	-	-	-	-	0.04
44	*Brachinus crepitans*	-	**5**	-	-	-	**5**
	%	-	0.96	-	-	-	0.04
45	*Microlestes maurus*	**1**	**2**	-	-	-	**3**
	%	0.16	0.38	-	-	-	0.03
	Total species	**9**	**23**	**7**	**17**	**25**	**45**
	%	20.0	51.11	15,55	37.77	55.56	
	***Total* individuals**	**636**	**522**	**2350**	**2711**	**5399**	**11.618**
	%	5.47	4.49	20.23	23.33	46.47	99.99.
	No. of eudominant species	2	3	2	3	2	
	H' (Shannon index of diversity)	1.18	1.58	3.24	0.92	1.38	
	E (Evenness index)	0.42	0.49	0.71	0.23	0.29	

The main ecological requirements of the species found are presented in Table 10. The main synthetic ecological requirements of the coenosis of Carabidae referring to types of reproduction, preferences for moisture, biotopes, food and geographical distribution are presented in Tables 5-9 and Figs. 1-5.

Table 5. Numerical and percentage variation of the types of reproduction in the species of Carabidae from five alfalfa crops. Moldova. (Romania).

No.	Seasons	No of species	%
1	Spring	26	57.77
2	Spring, Summer	1	2.22
3	Summer	1	2.22
4	Autumn	15	33.38
5	Autumn, Spring	1	2.22
6	Plastic	1	2.22
	Total	45	100

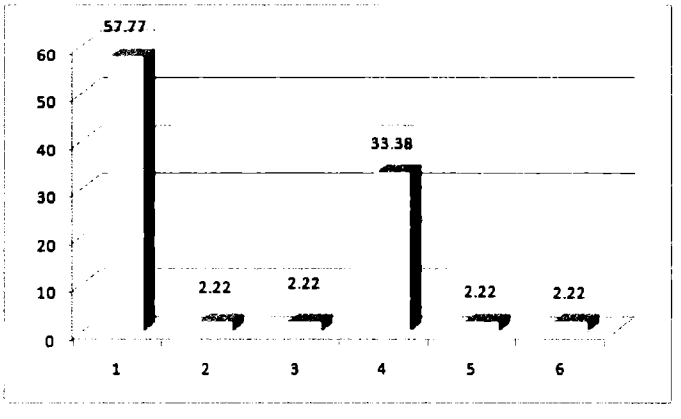

Figure 1. Percentage ratios of the types of reproduction of Carabidae in five alfalfa crops. Moldova.

Legend: 1-6 in Table 5.

The spectrum of moisture preferences of the species of Carabids from five alfalfa crops, Moldova (Romania) are presented in Table 6 and Figure 2.

Table 6. The spectrum of moisture preferences of the species of Carabids from five alfalfa crops. Moldova (Romania).

No.	Preference for moisture	No of species	%
1	Hygrophilous	3	6.66
2	Hygro-mesophilous	2	4.44
3	Mesophilous	28	62.22
4	Meso-xerophilous	10	22,22
5	Xerophilous	2	4.44
	Total species	**45**	99.98

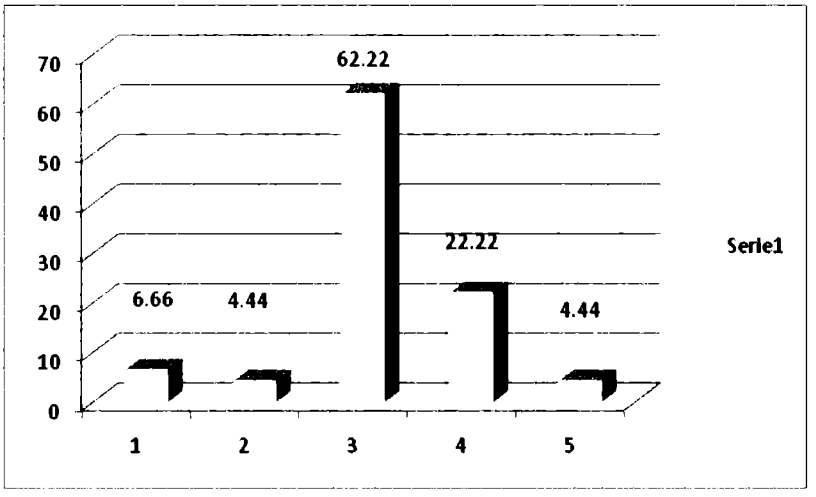

Figure 2. Percentage ratios of preferences for moisture of Carabidae from five alfalfa crops. Moldova.

Table 7. The spectrum of biotope preferences of the species of Carabidae from five alfalfa crops. Moldova (Romania).

No.	Biotopes	No of species	%
1	Forest	6	13.33
2	Forest, steppe	3	6.67
3	Forest, crops	3	6.67
4	Steppe	1	2.22
5	Steppe, crops	11	24.44
6	Crops	15	33.33
7	Open land	3	6.67
8	Riparian	1	2.22
9	Eurytopic	2	4.44
	Total	**45**	**99.99**

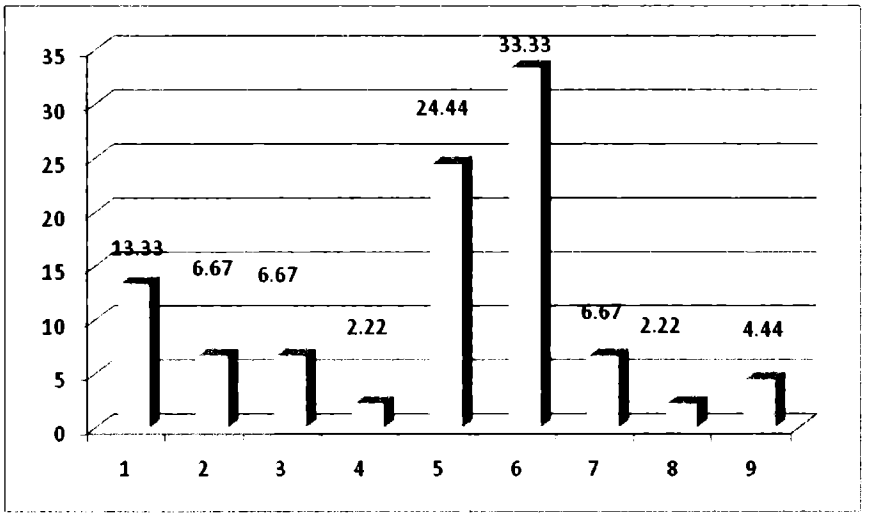

Figure 3. Percentage ratios of the preferences for biotopes of Carabidae from five alfalfa crops. Moldova (Romania).
Legend: 1-9 in Table 7.

Table 8. The spectrum of food regime of the species of Carabidae from five alfalfa crops. Moldova.

No.	Food regime	No of species	%
1	Zoophagous	20	44.44
2	Mixophagous	1	2.22
3	Phytophagous	7	15.56
4	Pantophagous	17	37.78
	Total	45	100

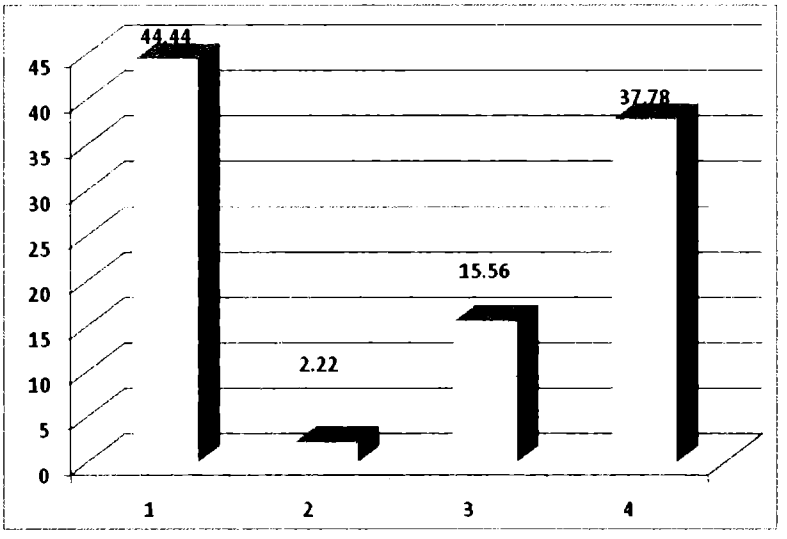

Figure 4. Percentage ratios of the trophic spectrum of the Carabidae from five alfalfa crops . Moldova.

Table 9. The numerical and percentage spectrum of the geographical distribution of the species of Carabidae from five alfalfa crops of Modova (Romania).

No	Geographical distribution	Species	%
1	Palaearctic	14	31.11
2	West palaearctic	15	33.33
3	Holarctic	1	2.22
4	European	4	8.89
5	Euro-Mediterranean	2	4.44
6	Euro-Siberian	5	11.11
7	Euro-Caucasian	3	6.67
8	East-European	1	2.22
	Total	**45**	**99.99**

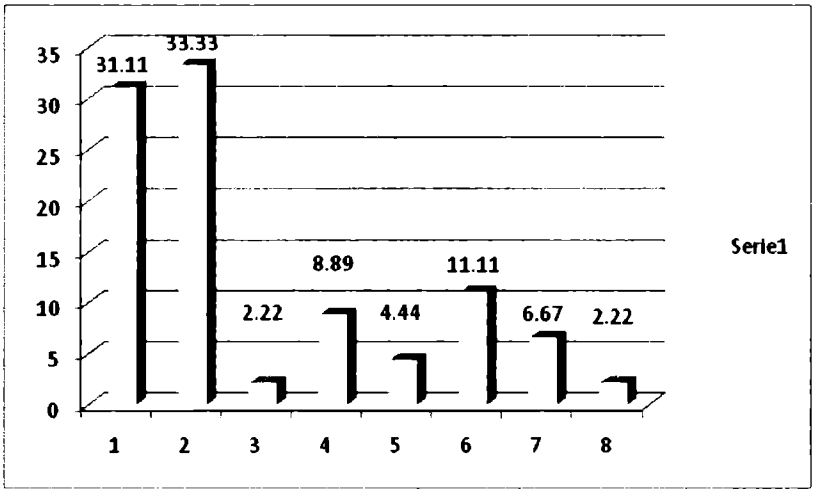

Figure 5. The percentage spectrum of the geographical distribution of Carabidae in five alfalfa crops from Moldova (Romania).

The main ecological requirements, numerical and percentage of the species are presented in Table no. 10.

Table 10. General ecological requirements of the species found in the alfalfa crops of Moldova.

No.	Species	1	2	3	4	5
1	*Cicindela germanica* Linne 1758	Sp	M	St,Cr	Z	Wp
2	*Calosoma auropunctatum* Herbst 1784	Sp	M	St,Cr	Z	Wp
3	*Carabus violaceus* Linne 1758	A	M-X	F,Cr	Z	Es
4	*C. cancellatus* Illiger 1798	Sp	M	F	Z	Pl
5	*C.granulatus* Linne 1758	A	H-M	Cr	Z	Pl
6	*C. scabriusculus* Oliver 1795	Sp	M-X	St,Cr	Z	E
7	*C. excellens* Fabricius 1798	Sp	M	F	Z	E
8	*C. besseri* Fischer Von Waldheim 1822	A	M	St,Cr	Z	EstE
9	*Loricera pilicornis* Fabricius 1775	Sp	H	F,Cr	Z	Hol
10	*Clivina fossor* Linne 1758	Sp	M	Rip.	Z	PL
11	*Broscus cephalotes* Linne 1758	Sp	X	St,Cr	Z	E
12	*Anisodactylus poeciloides* Stephens 1828	Sp	M	Cr	Mixofit	Em
13	*A. signatus* Panzer 1797	Sp	M	Cr	P	Pl
14	*A. binotatus* Fabricius 1787	A	M	St,Cr	Fit	Es
15	*Ophonus sabulicola* Panzer 1796	A	M	St, Cr	Fit.	Es
16	*O. diffinis* Dejean 1829	A	M	St.Cr	Fit.	Em
17	*Metophonus azureus* Fabricius 1775	A	M	St,Cr	Fit.	Wp
18	*Harpalus rufipes* De Geer 1774	A	M-X	OLS	P	Wp
19	*H. griseus* Panzer 1767	A	M-X	OLS	P	Pl
20	*H. aeneus* Fabricius 1775	Sp	M-X	Cr	P	Pl
21	*H. rubripes* Duftschmid 1812	A	M	F	P	Wp
22	*H. distinguendus* Duftschmid 1812	Sp,S	M	OLS	P	PL
23	*H. latus* Linne 1758	A	M	F,St	P	PL
24	*H. calceatus* Duftschmid 1812	Sp	M-X	Cr	P	PL
25	*H. tardus* Panzer 1797	Sp	M-X	St	P	Es
26	*Acupalpus meridianus* Linne 1767	Sp	M	Cr	P	E
27	*Poecilus cupreus* Linne 1758	Sp	M	Cr	Z	Wp
28	*Pterostichus melanarius* Illiger 1798	Sp	M	F,Cr	Z	Wp
29	*Pt. ovoideus* Sturm 1824	Sp	M	F	Z	Ec
30	*Pt. melas* Creutzer 1799	Sp	M-X	F	Z	Ec
31	*Pt. nigrita* Paykull 1790	S	H	F,St	Z	PL
32	*Calathus fuscipes* Goeze 1777	A	M	Eu	P	Wp
33	*C. ambiguus* Paykull 1790	A	M-X	St,Cr	P	Wp
34	*C. melanocephalu* Linne 1758	A,Sp	M	Eu	P	Pl
35	*Dolichus halensis* Schaller 1783	A	M	Cr	P	PL
36	*Agonum sexpunctatum* Linne 1758	Sp	H-M	Cr	Z	Pl
37	*Amara equestris* Duftschmid 1812	Sp	M	Cr	P	Es
38	*A. ovata* Fabricius 1792	Sp	M	F	P	Pl
39	*A. similata* Gyllenhal 1812	Sp	M	F,St	P	Wp
40	*A. familiaris* Duftschmid 1812	Sp	M	Cr	Fit	Wp
41	*A. aenea* De Geer 1774	Sp	M	Cr	Fit	Wp
42	*Chlaenius vestitus* Paykulll 1790	Sp	H	Cr	Z	Wp
43	*Zabrus tenebrioides* Goeze 1777	A	M	Cr	Fit	Ec
44	*Brachinus crepitans* Linne 1758	Sp	M-X	St,Cr	Z	Wp
45	*Microlestes maurus* Sturm 1827	Plastic	X	Cr	Z	Wp

Legend: 1 = Reproduction type; **2** = Moisture preference; **3** = Biotope preference; **4** = Food regime; **5** = Zoogeographical distribution; **Sp.** = Spring; **A** = Autumn; **S** = Summer; **H** = Hygrophilous; **H-M** = Hygro-mesophilous; **M** = Mesophilous; **M-X** = Meso-Xerophilous; **Cr** = Crops; **F** = Forest; **OLS** = Open landscape; **St** = Steppe; **Eu** = Eurytopic; **Rip.** = Riparian; **Fit** = Phytophagous; **Mixofit** = Myxo-phytophagous; **P** = Pantophagous; **Z** = Zoophagous; **E** = European; **Ea** = Euro-Asiatic **Ec** = Euro-Caucasian; **East E.**= East European; **Em** = Euro-Mediterranean; **Es** = Euro-Siberian; **Hol** = Holarctic; **Pl** = Palaearctic; **Wp** = West palaearctic; **Pla** = Plastic.

DISCUSSIONS

In the ecological conditions of the alfalfa crop (1977, 1978,1981,1983) from Moldova, the coenosis structure of Carabidae was characterized by the variation of the taxon number, number of specimens, the structure of dominance, the values of the two ecological indexes, due to the type of ecosystem and pedoclimatic conditions.There were collected 11,618 specimens belonging to 13 subfamilies of Carabidae, 20 genera and 45 species. The concrete variations on localities and years are shown in Table no 2. The year 1978 was the most favourable when it there were collected 5,399 individuals (46.47% of total), that is 8-10 times more than in the alfalfa crops from Căbeşti (Bacău County) and Pogoneşti (Vaslui County). Soil moisture favoured the increase of the number of individuals, an ecological fact indicated by the presence of the specimens of *Loricera pilicornis* and *Chlaenius vestitus*, hygrophilous species, only in 1978. These two species were not collected in the other four localities (Table 4). *Pterostichus melanarius* (a mesophilous species) was collected two times more in 1978 than in 1977 (Suceava), four times than in the locality Hemeiuş, 1981 (Bacău County). The species was not collected in Căbeşti locality (1983) and only one individual in Pogoneşti (1983). Sure, it was because of the variation of soil moisture determined by the geographical position of those two localities. *Poecilus cupreus* (mesophilous to moderately hygrophilous) was collected three times more in 1978 than 1977 in the same locality, Suceava, in the same crop (alfalfa). It was not collected in Căbeşti locality and only 10 specimens in Pogoneşti locality. Comparing our data with those from the Republic of Moldova,

there were collected in total 98 species of which: 45 (45.91%) (VARVARA, 2014) – (in the present paper); Republic of Moldova, 48 (48.97 %), (NECULISEANU, 2003); 57 (58.16%), DĂNILĂ, 2005); 3 (3.06%), (BUŞMACHIU & BACAL, 2012). Out of the total number of 98 species, 13 species (13.26 %) are common in Moldova and the Republic of Moldova; 20 species (20.40 %) were common within the Republic of Moldova; 13 species (13.26%) were found only in Moldova (Romania); 29 species (29.59 %) were found only in the Republic of Moldova; 24 species were found only by NECULISEANU (2003); 34 species (34.69%) were collected only by DĂNILĂ (2005).

Among common species in Moldova and the Republic of Moldova, we cite: *Anisodactylus signatus*, *Harpalus rufipes, H. riseus, H.rubripes, H. distinguendus, Poecilus cupreus, Pterostichus melanarius, Calathus ambiguus, Amara aenea* and *Brachinus crepitans*. Out of the total common species in Moldova and the Republic of Moldova, 69% are mesophilous and 31% meso-xerophilous. In Moldova, (Romania), the subfamilies Harpalinae and Pterostichinae are well represented as species and specimens. The specimens percentages of Harpalinae varied between 4.98% (1978) and 12.87% (1977), a difference of 7.89% between years, knowing the fact that within this subfamily mesoxerophilous species are comprised. The specimens percentages of Pterostichinae varied between 84.25% (1977) and 93.11% (1978), a difference of 8.86%. These differences are another proof that the year 1978 was more humid than the year 1977. Three species, *Pterostichus melanarius*, *Poecilus cupreus* and *Harpalus rufipes* are euconstant and eudominant in both years, except *Harpalus rufipes*, which was dominant in 1978. Normal variation values of the diversity index (Shannon-Wiever) are between 1.50 and 3.50. Our data show that this index had the value of 0.92 (Suceava, 1977) and 3.24 (Hemeiuş) and the value of evenness was 0.23 (Suceava, 1977) and 0.71 (Hemeiuş, 1981). The values were influenced by the exaggerated dominance of those three species. It meant that there were very good conditions for these species in the alfalfa crop.

Survival and reproduction are the two main features of species. All the adaptations converge towards these targets. Reproduction types and their percentages are given in Table 5 and illustrated in Fig. 1. Two reproduction types predominate. Spring species (26 species, 57.77%) and autumn species (15 species, 33.33). Based on our published results these two groups of species predominate in the coenosis of carabids in wheat crops, potato crops, clover crops, deciduous and coniferous ecosystems from Moldova (according to VARVARA, 2005, 2008; VARVARA & BRUDEA, 2012).

According to NECULISEANU (2003: 131-137) species breeding in the spring-summer winter as adults, their life cycle lasts one or two years. The egg-laying takes place in April-August and the appearance of the larvae, pupae and young adults in May-September. Species breeding in the autumn-summer winter as larvae or partly as adults present a life cycle that lasts one year, two years or three years.

The results referring to moisture preferences are given in Table 6 and illustrated in Fig 2. According to the law of tolerance most individuals of a species are found in the optimum preferrendum. The mesophilous and meso-xerophilous species predominate: 62.22 % are mesophilous and 20.22 % are meso-xerophilous. According to our published results the mesophilous and mesoxerophilous species predominate in the ecosystems of winter wheat, potatoes, clover, sugar beet, deciduous forests in various proportions according to local conditions (VARVARA, 2008).

The coenosis of carabids in the alfalfa crop as concerns the preference for biotopes is formed of a mixture of species. There were found 9 classes of species (Table 7 and Fig. 3). The species which prefer crops are in proportion of 33% followed by species which live in steppe, crops (24.44%) and forest 13.33%. Referring to the food regime, the coenosis of carabids in the alfalfa crop is characterized by the predominance of zoophagous and pantophagous species (Table 8, Fig. 4), in total, 82.28%, like in sugar beet and clover crops (VARVARA & BRUDEA, 1983; TURCULEŢ & VARVARA, 2006). Zoogeographically, the species in the alfalfa crop belong to 8 geographical regions (Table 9, Fig. 5). The Palaearctic and West Palaearctic species (64.44%) characterize the coenosis of Carabidae.

CONCLUSIONS

In the local conditions of the alfalfa crops Moldova (1977,1978, 1981, 1983), from North, Centre and South of Moldova, the coenosis of ground beetles comprised 13 subfamilies, 20 genera and 45 species with local and annual variations. The dominant subfamilies are: Harpalinae, Pterostichinae; the dominant species are: *Pterostichus melanarius*, *Poeciluscupreus* and *Harpalus rufipes*. Their specimens represented 91.28% (out of total). The majority of species are subrecedent. The Shannon index had the value of 0.92 (Suceava, 1977) and 3.24 (Hemeiuş, 1981). Evenness was 0.23 (Suceava, 1977) and 0.71 (Hemeiuş, 1981).

Ecologically, the coenosis of Carabidae is dominated by the species with reproduction in Spring (57.77%) and Autumn (33.38 %), mesophilous (62.22 %) and meso-xerophilous (22.22%), pantophagous 37.78%, phytophagous 15.56%, distributed in crops (33.33%), steppe, crops 24.44%, forest 13.33, zoophagous (44.44%), pantophagous 37.78%, phytophagous 15.56% spread in West Palaearctic 33.33% and Palaearctic (31.11%).

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Goniozus claripennis (Förster 1851) (HYMENOPETRA: BETHYLIDAE) AS PARASITOID OF GRAPE LEAF-ROLLER *Sparganothis pilleriana* (Den. et Schiff.) (LEP: TORTRICIDAE) LARVAE IN SOUTHERN VINEYARDS OF ROMANIA

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Abstract. Research conducted in two southern vineyards of Romania, Ştefăneşti (Argeş County) and Dăbuleni (Dolj County) highlighted the *Goniozus claripennis* species as primary, larval and gregarious ectoparasitoid of *Sparganothis pilleriana*. Parasitoid adults can be found in vineyards in late April, early May, depending on local and annual microclimate and parasitize host larvae of different ages. In this study, 11 individuals of *G. claripennis* on the host larva is a new record. The contribution of the parasitoid in parasitizing the grape leaf-roller larvae is 3.37%. Parasitoid activity manifests itself more strongly in the Dăbuleni vineyards (5.2%) where the pest population is larger in number. The host-parasitoid relationship is new to Romania.

Keywords: vineyards, host larvae, ectoparasitoid, percentage of parasitation.

Rezumat. *Goniozus claripennis* (Förster 1851) (Hym: Bethylidae), un parazitoid al larvelor de *Sparganothis pilleriana* (Den. et Schiff.) (Lepidoptera: Tortricidae) în viile din sudul României. Cercetări efectuate în două podgorii din sudul României, Ştefăneşti (Judeţul Argeş) şi Dăbuleni (Judeţul Dolj) au evidenţiat specia *Goniozus claripennis* ca ectoparazitoid primar, larvar şi gregar al larvelor de *Sparganothis pilleriana*. Adulţii parazitoidului se întâlnesc în vii la sfârşitul lunii aprilie, începutul lunii mai, în funcţie de microclimatul local si anual şi parazitează larve ale gazdei de vârste diferite. În acest studiu, prezenţa a 11 parazitoizi pe o larvă gazdă este un nou record. Contribuţia parazitoidului în parazitarea larvelor moliei frunzelor viţei de vie este de 3,37%. Activitatea parazitoidului se manifestă în procent mai mare în viile din Dăbuleni (5,2%), unde populaţia dăunătorului prezintă efective mai mari. Relaţia parazitoid-gazdă este nouă pentru România.

Cuvinte cheie: podgorie, larva gazdă, ectoparazitoid, procentaj de parazitare.

INTRODUCTION

Goniozus claripennis (Förster 1851) (= *Bethylus formicarius* Audouin; = *Goniozus audouini* Westwood) is a primary, larval and gregarious ectoparasitoid, known from hosts such as *Tortrix viridana* Linnaeus 1758, *Sparganothis pilleriana* (Denis & Schiffermüller 1775) and *Eupoecilia ambiguella* (Hübner 1796) (TRJAPIŢÂN, 1978). The first to report the species as a parasitoid of *S. pilleriana* larvae was AUDOUIN in 1842 (in VOUKASSOVICH, 1924), who observed and described its development. Subsequently, the species was also reported in other European regions, but it was only VOUKASSOVICH (1924) who devoted it a detailed study on the occasion of his research on the biology and parasitoids of microlepidoptera *S. pilleriana*. Observations on the biology of this species are also performed by ZEROVA et al. (1989).

In Romania, the species has been also obtained from two hosts: *Archips rosanus* Linnaeus 1758 and *Adoxophyes orana* (Fischer von Roslerstamm 1834) (DIACONU, 1999). Parasitation of larvae of different ages and gregarious behaviour turns this species into a particularly attractive one to the biological control programs.

In the present study, we observed aspects of biology and the importance in reducing of the host populations.

MATERIAL AND METHODS

The observations were carried out in two vineyards in southern Romania namely Ştefăneşti and Dăbuleni. The vineyards of Ştefăneşti located in the central southern region of the Wallachian hills, and characterized, over the period of the observations, by a rather wet and cool climate, present a small *S. pilleriana* population. This microlepidopterian was identified and observed in a vineyard of about 3 hectares in the plot growing the "Fetească regală "variety. In the vineyards of Dăbuleni, located in southern Oltenia near the Danube, with a climate characterized by Mediterranean influences, the *S. pilleriana* population is well developed. The observations were carried out, in a vineyard of about 2 hectares, on the variety called "Roşioară". The larvae host was collected in May-July period, 2000-2003 in Ştefăneşti, and 2000-2002 in Dăbuleni. The caterpillars were reared isolatedly up to the apparition of tortricid or parasitoid adults, their food being the vine leaves. 87 individuals of *G. claripennis* were achieved under laboratory conditions.

RESULTS AND DISCUSSIONS

The data about the recorded parasitoids have been arranged in the following order: collecting date/stage of host/stage of collected parasitoid/individuals (♀ and ♂) obtained.

It was obtained as a larval, gregarious, primary endoparasitoid in:

Ştefăneşti: June 9, 2000/mature larva/larva/2♀♀; June 16, 2001/immature larva/larva/2♀♀; June 26, 2001/ larva remains/cocoon/ 2♀♀,1♂; June 29, 2001/ mature larva/larva/3♀♀, 2♂♂; June 28, 2002/mature larva/larva/1♀,

1♂, 1 hyperparasitoid;

Dăbuleni: May 9, 2000/immature larva V2/larva/1♀, 1♂, 1 hyperparasitoid; May 26, 2000/immature larva V3/larva/2♀♀, 1♂; June 27, 2000/mature larva/cocoon/2♀♀; June 27, 2000/larva remains/cocoon/2♀♀; 1 hyperparasitoid; June 27, 2000/ larva remains/cocoon/1♀, 1♂; May 26, 2001/ immature larva V2/two eggs/2♀♀; May 26, 2001/immature larva V3/larva/2♀♀, 1♂; May 26, 2001 immature larva V3/larva/2♀♀; June 19, 2001/larva remains/cocoon/ 1♀, 1♂; June 19, 2001/ larva remains/cocoon/ 3♀♀, 1♂; June 19, 2001/ larva remains/cocoon/ 2♀♀; July 5, 2001/ larva remains/cocoon/ 5♀♀, 2♂♂; July 5, 2001/ larva remains/cocoon/6♀♀, 2♂♂; July 5, 2001/ mature larva/larva/ 7♀♀, 3♂♂, 1 hyperparasitoid; July 5, 2001/ immature larva V3/ larva/2♀♀, 1♂; July 5, 2001/ larva remains/cocoon/ 3♀♀; July 5, 2001/ larva remains/cocoon/ 2♀♀; July 5, 2001/ mature larva /larva/4♀♀, 1♂; May 27, 2002/immature larva V4/larva/1♀, 2 hyperparasitoids; June 17, 2002/larva remains/cocoon/3♀♀; June 17, 2002/ mature larva /larva/2♀♀, 1♂; June 17, 2002/larva remains/ cocoon/ 2♀♀.

Some aspects of the biology of *Goniozus claripennis*

Period of activity of the parasitoid

According to our observations, the period during which the *S. pilleriana* larvae can be parasitized is about a month and a half, parasitoid adults occurring in southern vineyards of Romania since late April, early May. Thus, in Dăbuleni, where the species was more frequent, in 2000, the first parasitized larvae were collected the earliest at the beginning of May, on the 9th of May, and the latest on the27th June, and in 2001 (colder thermally), the first parasitized larvae were collected on the 26th May, while the last on of 5th July.

G. claripennis parasitized both young and fully developed caterpillars, larvae of the 2nd, 3rd, 4th and 5th ages, respectively.

The eggs are laid on the caterpillar body, dorsal, on the previous segments and rarely on the posterior. PETER & DAVID (1991) observed a closed species, *G. sensorius* Gordh 1988, and the maximum number of eggs are laid on the 6th and 7th segment, and none in the terminal segments. As for the collected specimens, the number of laid eggs varied between 2 and 11. After hatching, parasitized larvae remain attached to the body of the *S. pilleriana* larva, the most forward part narrowing into a sucking organ deeply stuck into the host larva body.

The mobility of parasitized caterpillars depends on the age, size and number of parasitic larvae. Thus, fully grown *Sparganothis pilleriana* caterpillars which had on them 2-3 *G. claripennis* larvae of about 1-1.5 mm in length were still active, while young caterpillars, parasitized by larvae of 1-1.5 mm, were lumbered

The number of parasitoid larvae growing on a host is in relation to its size. On the 5th of July, 2001, in Dăbuleni a fully grown *S. pilleriana* caterpillar was collected, which presented on its body 11 parasitoid larvae, of which only 10 have reached maturity, the eleventh being hyperparasitized. In his research, VOUKASSOVITCH (1924) mentions a maximum of eight *G. claripennis* larvae on the host caterpillar. In our study, young aged caterpillars were parasitized by 2-3 *G. claripennis* larvae.

Parasitic larvae, after having completed their development, leave the host larva of which only the tegument remains and spin a globulous cocoon of a lighter braid, where they turn into nymphs, as highlighted in the research undertaken. Thus, individuals which have turned into nymphs shortly after collection required under laboratory conditions 7-8 days to turn into adults. For example, on the 5th July 2001 in Dăbuleni, 7 yellowish fully grown larvae were collected, located next to the remnants of a *S. pilleriana* larva, on 7th July they wove the cocoons and on 15th July the *G. claripennis* adults hatched.

Sex-ratio

From the literature it can be gathered that the number of the females is much larger than that of the males. In full agreement with the above data, in the year 2001 in Dăbuleni, from 13 larvae of *S. pilleriana* parasitized, 53 individuals of *G. claripennis* developed, from which 41 females, and 12 males. Males had a percentage of 22.64%, while females covered 77.35% (Table 1).

Table 1. *Goniozus claripennis* individuals obtained in 2001.

Locality	Date of collecting	No. of larvae parasitized	♂♂, ♀♀
Dăbuleni	May 26	3	6♀♀, 1♂
	June 19	3	6♀♀, 2♂♂
	July 5	7	29♀♀, 9♂♂
Ştefăneşti	June 16	1	2♀♀
	June 26	1	3♀♀
	June 29	1	3♀♀, 2♂♂
Total		**16**	**49♀♀, 14♂♂**

In Dăbuleni, on the 5th of July 2001, out of 10 *G. claripennis* larvae which had parasitized a *S. pilleriana* caterpillar of last age collected, 7 ♀♀ and 3 ♂♂ were obtained.

During the research, the females are much more numerous than the males, 67 ♀♀ and 20 ♂♂, so the sex-ratio has a sub-unit value: 0.29.

The importance of the species *Goniozus claripennis* in the larval parasitism of the host

Due to gregarious behaviour and parasitation of larvae of different ages, this Hymenoptera, *G. claripennis* has great importance in controlling the populations of the species that it parasitizes. In this study, the parasitoid contributes with 3.37 % to reducing the grape leaf-roller larvae (Table 2). Thus, within the complex of larval parasitoids of *Sparganothis pilleriana, G. claripennis* species, it has a greater role as compared to another larval gregarious ectoparasitoid, *Colpoclypeus florus* (Walker 1839) (BĂRBUCEANU & ANDRIESCU, 2010).

Table 2. Importance of *Goniozus claripennis* in parasitizing *Sparganothis pilleriana* larvae.

Locality	Year	No. of larvae collected	No. of larvae parasitized	%	*Goniozus claripennis*	
					No. of larvae parasitized	%
Ştefăneşti (Ag)	2000	48	9	18.75	1	2.08
	2001	103	10	9.71	3	2.91
	2002	118	13	11.02	1	0.85
	2003	107	24	22.43	-	-
Subtotal		376	56	14.9	**5**	**1.33**
Dăbuleni (DJ)	2000	152	33	21.71	5	3.29
	2001	140	31	22.14	13	9.29
	2002	131	13	9.92	4	3.05
Subtotal		423	77	18.2	22	**5.2**
Total		**799**	**133**	**16.65**	27	**3.37**

Host parasitizing percentages have higher values in Dăbuleni vineyards than in those from Ştefăneşti (Fig.1), situation due to host development conditions in this vineyard. During the study period, parasitoid participation in parasitizing host larvae was 5.2% in Dăbuleni, while in Ştefăneşti, larva parasitization was only 1.33%.

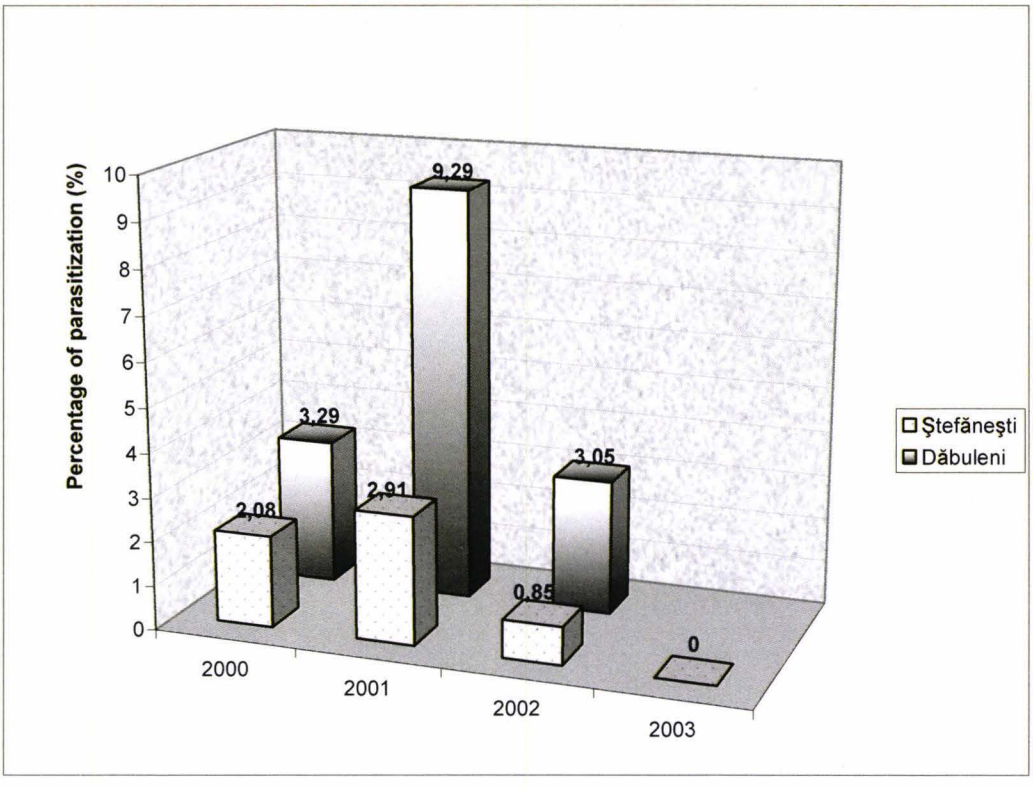

Figure 1. The role of *Goniozus claripennis* in parasitizing *Sparganothis pilleriana* caterpillars.

From the literature it appears that another host preferred by this parasitoid is *Eupoecilia ambiguella*, which grows well in moist and cool climate regions (TRJAPIŢÂN, 1978). Probably, parasitoid activity is also favoured by this climate. This behaviour would be supported by the higher parasitation percentages achieved in the two locations in

2001 (Fig.1), a year characterized by lower temperatures and high humidity.

On the contrary, in 2000 and 2002, years characterized by high temperatures and dryness, *G. claripennis* species had a lower participation in host parasitation.

The absence of *G. claripennis* species in 2003 may be determined on the one hand, by the fact that high temperatures in May led to a rapid development of *S. pilleriana* larvae, so that young aged larvae preferred by the parasitoid were present a too short time to be parasitized, and on the other hand, the number of samples was insufficient to reveal the presence of the species.

CONCLUSIONS

Goniozus claripennis (Förster) is a primary, larval and gregarious ectoparasitoid, obtained from larval of *Sparganothis pilleriana* in two vineyards in southern Romania.

The number of the adults of *G. claripennis* that develop on a larva of *Sparganothis pilleriana* varies in keeping with the size of the host, as the mature hosts can provide as many as 10-11 individuals. In this study, 11 individuals of *G. claripennis* on the host larva is a new record.

The females are much more numerous than the males, so the sex-ratio has a sub-unit value: 0. 29

The contribution of that parasitoid to the limitation of grape leaf-roller larvae is of 3.37%. The higher value of the parasitation ratio (5.2%) was recorded in Dăbuleni vineyards, where the local climate is more favourable to the host.

The host-parasitoid relationship is new to Romania.

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THE EVALUATION OF THE REPRODUCTIVE INDICATORS OF THE NEW GENERATIONS OF SELECTION OF THE MOLDAVIAN CARP BREEDS *Cyprinus carpio* Linnaeus 1758 (MOLDOVA)

CURCUBET Galina, DOMANCIUC Vasili, FULGA Nina

Abstract. The present paper renders the evaluation of the reproductive indicators of the new generations of the Moldavian carp breeds and their conformity to the breed standard. It was emphasized that the diameter of small and large yolk granules, as well as of cortical vacuoles changed depending on carp breed and fish age. The results of the studies also showed degenerative modifications in ovulated spawn.

Keywords: breed, carp, female, fecundity, oocyte.

Rezumat. Evaluarea indicilor reproductivi a noilor generaţii de selecţie a raselor de crapi moldoveneşti *Cyprinus carpio* Linnaeus 1758 (Moldova). Este prezentată evaluarea indicilor reproductivi a noilor generaţii de selecţie a raselor de crapi moldoveneşti şi conformitatea lor standardului rasei. A fost subliniat faptul că diametrul de mici şi mari granule a gălbenuşului, precum şi de vacuole corticale se schimbă în funcţie de rasa de crap şi de vârsta peştelui. Rezultatele studiilor au arătat, de asemenea, modificări degenerative în icre ovulate.

Cuvinte cheie: rasă, crap, femele, prolificitate, oocite.

INTRODUCTION

Carp represents the traditional object of breeding and rearing in Moldova's Aquaculture, enhancing the productive qualities, which play an important role in increasing its production. For increasing the production of pond fish, it is very important to use high productive fish breeds, lines and interbreeding.

The works in this direction include the task of originating and introducing new selective achievements with increased productivity and high quality.

As a result of focused selection, three new Moldovan breeds were created and approved: Teleneshtskiy Scaly Carp and Teleneshtskiy Frame Carp, the fourth generation of selection; and Kuboltskiy Scaly Carp, the sixth generation of selection (DOMANCIUC, 1993; DOMANCIUC & CURCUBET, 2002). The selection was done in the direction of increasing the resistance to infectious diseases, the growth rate, improvement of tolerance to cold and fecundity (CURCUBET, 1994). These breeds of carp are remarkable because their productive, reproductive characteristics exceed present norms (CURCUBET & DOMANCIUC, 2005). Their good body indices and increased resistance to infectious diseases (Teleneshtskiy Carp), hardiness, high survival rate and advanced ability to utilize natural fish food resources (Kuboltskiy Scaly Carp) are also among those characteristics which make them so productive.

Since 2003, the carp breeds Teleneshtskiy Scaly, Teleneshtskiy Frame and Carp Kuboltskiy Scaly have been widely introduced into production. These carp breeds form more than 95% of the carp bloodstocks in the Republic of Moldova.

At present, there has been created the fifth generation of Teleneshtskiy Carps and the seventh generation of Kuboltskiy Scaly Carp.

One of the most significant indicators of productivity is fecundity that depends on both the environmental conditions as well as on the hereditary factors (MASLOVA, 2005).

The aim of the present work is the evaluation of the reproductive indicators of the new generations of indigenous breeds and their conformance to the breed standard.

MATERIAL AND METODS

The works on artificial reproduction were carried out in the certified selection fish farm – Teleneshty Branch of the State Enterprise in the Researches and Production of Aquatic Biological Resources "Aquaculture-Moldova" (fish hatchery Verezheny).

For the analyses of the reproductive characteristics of the new generations of three approved Moldavian carp breeds: Teleneshtskiy Scaly Carp and Teleneshtskiy Frame Carp, the fifth generation (5) and Kuboltskiy Scaly Carp, the seventh generation (7) of selection, there were used females aged ~~of~~ six years.

As indices, there were identified: number of spawned females (%), eggs per female (g), maturity rate (%), working fecundity (thousand), relative fecundity (thousand/kg), fertilization (%), development of eggs (%), yield the three-day larvae per female (thousand), survival rate of larvae from eggs (%), body weight of fingerlings (g), survival rate of yearlings from eggs (%).

For the histological examination of the ovulated eggs, there were used two groups of females of different ages from the three breeds of the new generations-five and seven years old.

The samples of sexual cells were fixed in Bouin liquid and filled in paraffin wax with further histological processing by the standard technique. All the studied females were subjected to the general biological analysis with calculation of gonad-somatic index (GSI). The stages of gonad maturity were determined according to the recommendation of SAKUN & BUTSKAIA (1963) and development degree of oocytes – after the classification of KAZANSKII (1949) with additions proposed by MAKEEVA & EMELIANOVA (1989). The cuts of gonads were coloured after Mallory's method (ROSKIN & LIVENSON, 1957). The diameter of oocytes was determined using the ocular-micrometre. All data were processed statistically (LAKIN, 1980). The microphotos were taken with the help of the microscope with videocamera "Lomo, Mikmed-2".

RESULTS AND DISCUSSIONS

The reproducers of the three breeds of the 5th, 7th generations of selection used for reproduction process had a positive reaction to hormonal stimulation. The percentage of spawning females of Teleneshtskiy Scaly Carp, Teleneshtskiy Frame Carp and Kuboltskiy Scaly Carp was 86.7, 90.0, 88.9 %; the working fecundity of females - 910, 930, 870 thousand units of eggs, respectively to the breeds. The values of the coefficient at maturity of Teleneshtskiy Carps were similar, while in case of Kuboltskiy Scaly Carp were slightly lower.

The realized fecundity, the yield of the three-day larvae, was considerable: 500, 495, 470 thousand units per female that is 1.88-2.0 times higher than the norms. The survival rates of larvae from eggs were 55, 53, 54%, correspondingly.

Re-spawning females of the new generations of selection had high reproductive characteristics and a number of indicators considerably exceed normative parameters: on working fecundity – 74-88%; yield of the three-day larvae per female – 88-100% (Fig. 1).

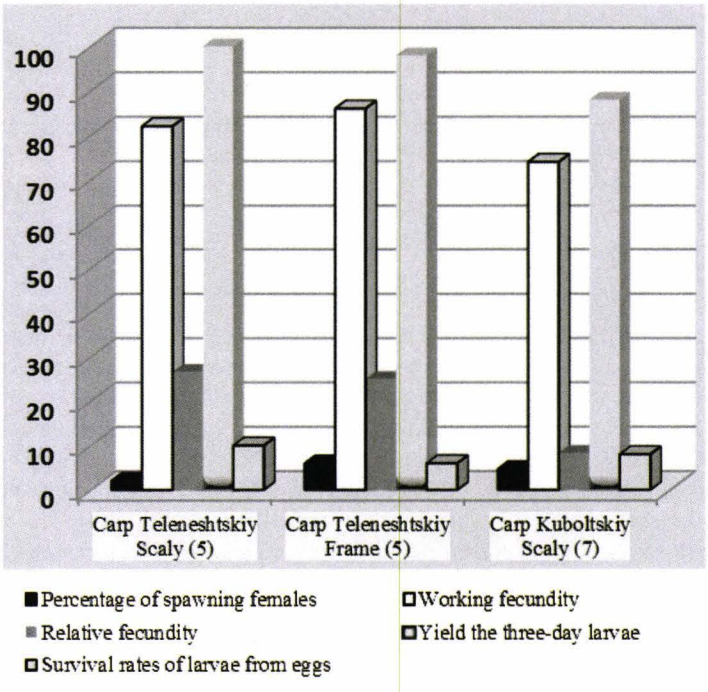

Figure 1. Comparative evaluation of productivity indicators of the new generation of carp breeds with the norms.

By comparing the breed standards, it was noted not only their conformity, but also a clear advantage in terms of females productivity: Teleneshtskiy Scaly Carp (5) – 91.6 % higher, Teleneshtskiy Frame Carp (5) –-138.8% higher both by increasing the average weight of fingerlings and their survival, Kuboltskiy Scaly Carp (7) – 132.8% higher – mainly due to the survival rate of three-day larvae, yearlings from eggs (Fig. 2).

In order to define the state of the gonads of the native-born breeds of carp female in the period of artificial reproduction, there were performed histological studies of ovulated eggs.

In preparations, the ovulated eggs have weakly coloured their own covering with conspicuous radial striations. The animal pole covering has a pronounced funnel micropyle (Fig. 3).

Directly under the covering, there are located 2-3 rows of cortical vacuoles, the diameter of which in the studied species of carp has a different value. At the animal pole, the number of rows of cortical vacuoles is reduced and close to the micropyle, or they are absent at all. Almost the entire amount of eggs is filled with the granules of yolk in different shapes and sizes, the diameter of which varies depending on the species of carp and body weight (Table 1).

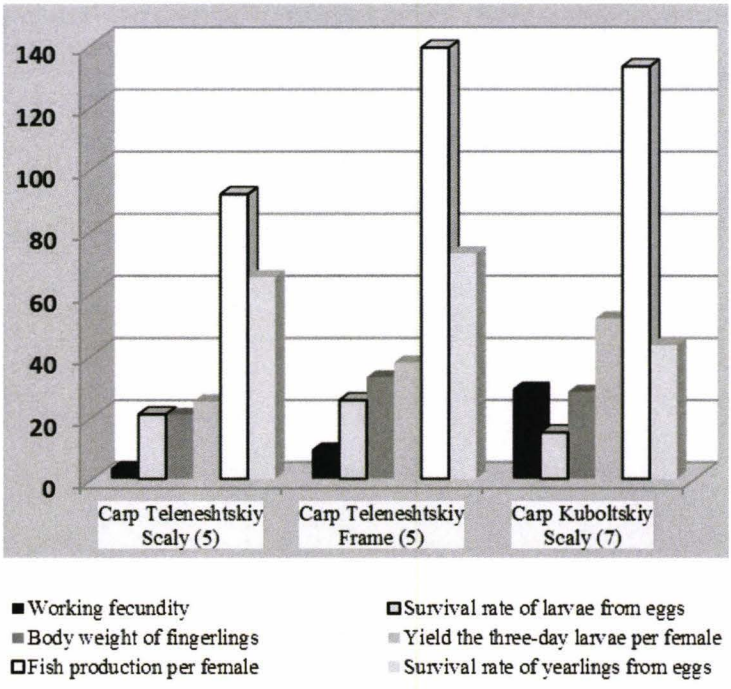

Figure 2. Comparative evaluation of the productivity indicators of the new generation of carp breeds on with the breed standard.

At the same carp females, regardless of their breed, microscopic picture of the ovulated eggs has distinctive features. In certain number of eggs, the plasma and trophic inclusions polarization have not been completed yet and the animal pole is poorly showed (Fig. 4).

Table 1. Dimensions of trophic inclusions in ovulated oocytes of different breeds of carp.

Breeds of carp, generations	Diameter of yolk granules, um		Diameter of cortical vacuoles, um
	large	small	
females aged five (5.5- 5.6 kg)			
Kuboltskiy Scaly Carp, the seventh generation of selection	25.5 18.6-31.0	17.3 12 .4-21.7	17.4 15.5-21.7
Teleneshtskiy Frame Carp, the fifth generation of selection	23.4 18.6-27.9	16.1 12.4-21.7	14.6 12.4-18.6
Teleneshtskiy Scaly Carp, the fifth generation of selection	23.6 18.6-27.9	15.8 12.4-21.7	16.8 12.4 .4-21.7
females aged seven (7.5-8.0 kg)			
Teleneshtskiy Scaly Carp, the fifth generation of selection	27.0 24.8-31.0	17.5 15.5-21.7	19.6 15.5-24.8

In another group of gametal cells, there occurs the sticking of yolk granules in large conglomerates (Fig. 5). Between yolk granules in the centre of the eggs, there are observed some portions of the cytoplasm, which later would join the animal pole and form the blastodisc.

The histological studies showed heterogeneity of ovulated eggs. Among them, there are gametal cells, the morphological condition of which show the different degree of their degenerative changes. For some eggs, the swelling of their own covering, destroying the integrity of cortical vacuoles, yolk granules with the following sticking of their contents into a homogeneous mass is typical, (Figs. 6, 7), for others - penetration of vacuole content into the yolk mass (Figs. 8 , 9).

In the female ovulated eggs of Teleneshtskiy Scaly Carp in age of seven, there were also revealed some destructive changes that are expressed in the destruction of yolk granules, followed by homogenization of their contents (Fig. 10).

The similar state of cellular structures was observed in the ripe roe of Ropsha Carp during its artificial reproduction (SACUN & LEMANOVA, 1974).

In even-aged females, there were revealed the dimensional distinction of trophic inclusions by breeds and different quality of ovulated spawn of the same female: there are gametal cells, the morphological state of which shows different degrees of degenerative changes.

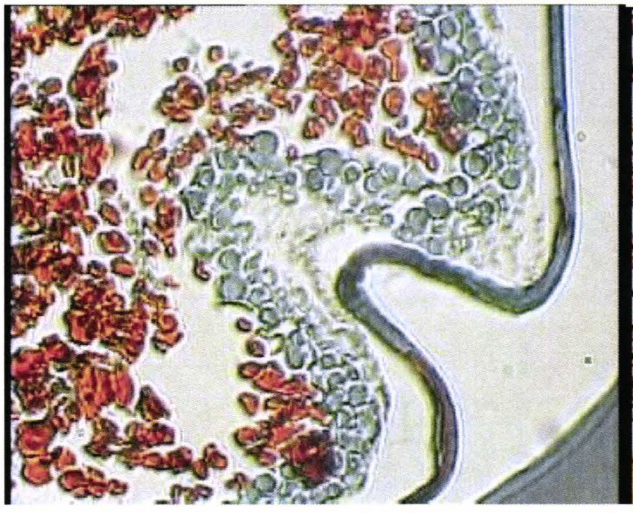

Figure 3. The micropyle of ovulated oocyte (orig.).

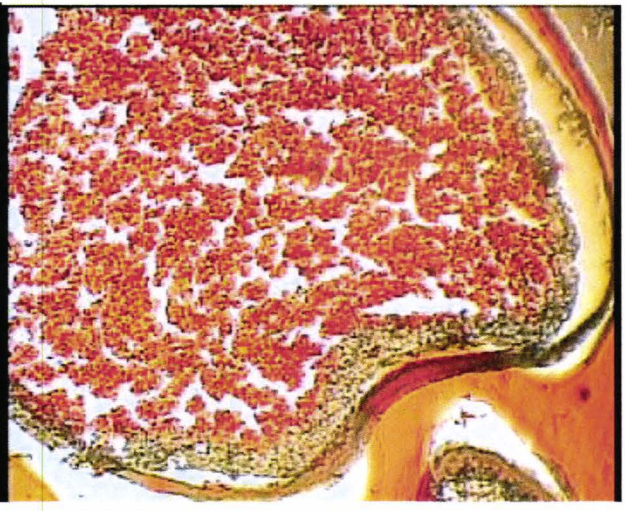

Figure 4. The mature oocyte at the female of Kuboltskiy Scaly Carp. Polarization of yolk is not completed. The micropyle expressed weakly (orig.).

Figure 5. The oocyte at the female of Teleneshtskiy Frame Carp. Yolk granules clumped into large conglomerates (orig.).

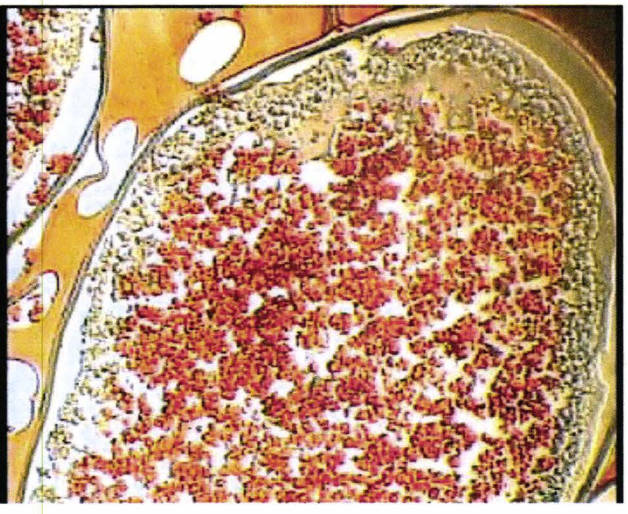

Figure 6. The oocyte the female of Teleneshtskiy Frame Carp. Merging the contents of cortical vacuoles into a homogeneous mass. The swelling of own membrane (orig.).

Figure 7. The fragment of the oocyte at the female of Teleneshtskiy Scaly Carp (7. 5 kg). Destruction of cortical vacuoles the contents of yolk granules in the animal pole. (orig.).

Figure 8. Degeneration of the oocyte at the female of Teleneshtskiy Scaly Carp (7. 5 kg). Destruction and merging and moving them to the centre of the cell. (orig.).

In old aged females of Teleneshtskiy Scaly Carp, there were also revealed destructive changes that are expressed in the destruction of yolk granules, followed by homogenization of their contents.

According to the data of some authors (STATOVA et al., 1982), irregularities in the structure of egg cell do not detain ovulation and spawning but lead to decreasing of its fecundating ability and further healthy embryo development.

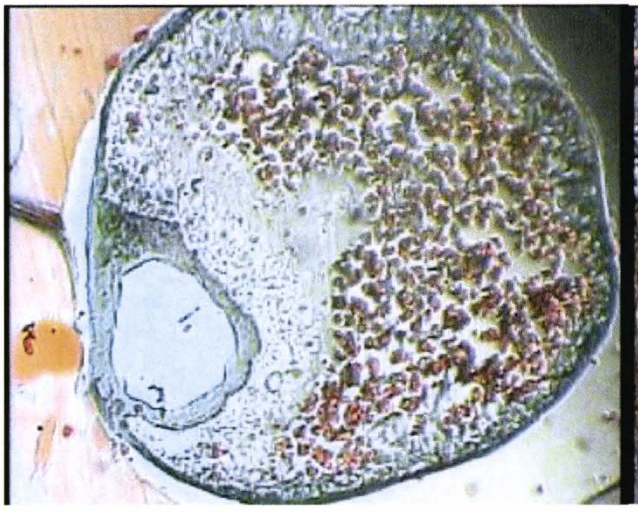

Figure 9. Degenerative changes of the oocyte at the female of Carp Teleneshtskiy Scaly Carp (5. 5 kg). The formation of vacuoles in animal pole cells. Destruction of vacuoles and penetration their contents in the mass yolk granules (orig.).

Figure 10. The fragment resorption of the oocyte at the six years old female of Teleneshtskiy Scaly Carp (orig.).

The changes revealed in the gametal cells of the females of the three breeds are caused by asynchronous growth of oocyte in the period of vitellogenesis that in a particular way affects the technological characteristics of carp during artificial reproduction at different stages of ontogeny, in some way reducing the percentage of fertilization, egg development and yield of the three-day larvae. These should be taken into account during selection works with the target program - fish fecundity increase.

CONCLUSION

1. Re-spawning females of the new generations of selection have high reproductive characteristics and a number of indicators considerably exceed normative parameters: working fecundity; yield of the three-day larvae per female. It was noted not only conformity, but also the advantage over the breed standards.
2. In even-aged females, there were revealed dimensional contrast of trophic inclusions by breeds and different quality of ovulated eggs due to different degrees of degenerative changes in the cells of the same female.
3. In old aged females of Teleneshtskiy Scaly Carp, there were also revealed destructive changes that are expressed in the destruction of yolk granules, followed by homogenization of their contents.
4. The changes in the gametal cells of females occurred due to asynchronous growth of oocyte in the period of vitellogenesis that reduces the percentage of fertilization, egg development and yield of the three-day larvae. It has to be taken into account in the selection works with the aim of fish fecundity increasing.

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CHARACTERISTICS OF THE REPRODUCTIVE SYSTEM IN SEXUALLY ADULT FEMALES OF *Sander lucioperca* (L.) FROM DIFFERENT POPULATIONS IN THE PERIOD OF THE REPRODUCTIVE CYCLE

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Abstract. Histological studies of the reproductive system in *Sander lucioperca* females from different populations that live in the water bodies of the Dniester and the Prut basin were performed. It was found that in the females of Dubăsari reservoir the gonads contain two generations of oocytes, whereas in the individuals from Costesti - Stânca reservoir the ovary is filled with only one generation of eggs. In the individuals from Dubăsari reservoir with asynchronous oocyte development throughout the annual reproductive cycle, only one portion of the eggs is spawned, while the second generation of eggs undergoes resorption. As a result, *S. lucioperca* in both reservoirs spawns eggs only one time.

Keywords: oocyte, eggs, gonads, resorption, vitellogenesis, vacuolization, *Sander lucioperca*.

Rezumat. Caracterizarea sistemului reproducător la femelele sexual mature de *Sander lucioperca* (L) din diferite populaţii în perioada ciclului reproductiv. A fost efectuat studiul histologic al sistemului de reproducere la femele din diferite populaţii de *Sander lucioperca*, din bazinele acvatice are râurilor Nistru şi Prut. S-a constatat că gonadele femelelor din lacul de acumulare Dubăsari conţin două generaţii de oocite, în timp ce la cele din Costeşti – Stânca, ovarele conţin doar o generaţie de icre. La indivizii din rezervorul Dubăsari cu dezvoltarea asincronă a oocitelor de-a lungul ciclului de reproducere anual se generează doar o pontă, iar a doua generaţie de oocite este supusă resorbţiei. Astfel, *S. lucioperca* din ambele rezervoare depune icre o singură dată.

Cuvinte cheie: oocit, icre, gonade, resorbţie, vitelogeneză, vacuolizare, *Sander lucioperca*.

INTRODUCTION

Detailed studies on the elucidation of gametogenesis features in *Sander lucioperca* have been performed by TRUSOV (1947; 1949). According to the author, in the females from Lake Ladoga and the Gulf of Pärnu there is some asynchrony in the oocytes growth in phases of vitellogenesis. However, it does not lead to a portioned spawning and it was noted synchronic development of eggs in *Sander lucioperca* individuals from the Don, the Volga and the Kuban rivers. On the basis of comparative histological examination of the gonads during the annual reproductive cycle, TRUSOV established the dependence of sexual cells development on the environmental conditions of their habitat. In the water basins, at different latitudes, in females there were found not only different degree of sexual cells development asynchrony, but different duration of the particular phases of development during the annual cycle (KOSHELEV, 1984).

The gametogenesis of *S. lucioperca*, inhabiting water bodies of the Dniester basin, was described in detail in the papers of many authors. According to the data of STATOVA (1962), in sexually mature individuals from Dubăsari reservoir before the construction of Novo-Dnestrovsc hydroelectric station, the development of germ cells in the initial phases of trophoplasmic growth in the period from August to October is asynchronous, but in the process of vitellogenesis, the oocytes became equal in their development and toward spawning produced a single generation of eggs. In more recent studies, KARLOV (1975) pointed to the emergence in the given water basin of up to 36% of the individuals with asynchronous oocyte development, which lasted until their ovulation.

At the studies of *S. lucioperca* oogenesis in Cuchurgan reservoir STATOVA (1973) emphasized the differentiation of the females after the character of gametogenesis: in some individuals the sexual cells developed asynchronously during the whole reproductive cycle, while in other individuals they developed synchronically. The author connected the differences in gametogenesis with the individuals got in the reservoir from the Dniester River when floods occurred.

In her work on the lower sector of the Dniester, CEPURNOVAVA (1975, 1991) notes a one-time spawning *S. lucioperca*, despite some asynchrony in the development of oocytes in vacuolization phase, which is smoothed in subsequent phases of trophoplasmic growth.

The present paper includes studies of the reproductive system of *S. lucioperca* females inhabiting in the anthropogenically modified conditions of Dubăsari and Costeşti-Stânca reservoirs, which are located in the Dniester basin and the Prut basin respectively.

MATERIAL AND METHODS

For the histological studies, mature females of *S. lucioperca* were used, caught during the spring – winter period (April-February) 2006-2011 from Dubăsari and Costeşti- Stânca reservoirs in amount of 56 and 42 individuals, respectively. The gonad samples were fixed in Bouin's fluid, followed by treating according to standard methods. Gonad maturity stages and phases of oocyte development were determined according to MEIEN (1939), developed for teleosteans, with additions introduced by TRUSOV (1949) for Zander, as well as according to the recommendations of SAKUN & BUTSKAIA (1963). Sections with a thickness of 7 μ were stained after Mallory (ROSKIN & LIVENSON, 1957). Micrographs were made using a microscope "Lomo, Mikmed-2" with video camera, using 10x magnification of eyepiece, 15x of lens.

RESULTS AND DISCUSSIONS

Dubăsari reservoir. According to the histological studies of the gonads in Dubăsari reservoir there inhabit *S. lucioperca* females with one, as well as with two generations of oocytes. In previous studies (TOMNATIK, 1964; TOMNATIK & KARLOV, 1971), there were also observed females with asynchronous vitellogenesis. However, the authors believed that asynchrony in the development of oocytes does not lead to portioned spawning and, eventually, only one generation of eggs ovulates. When forming two generations of oocytes, our studies also indicate the spawning of only the first portion of the eggs, while the second generation of germ cells undergoes resorption (Fig. 1).

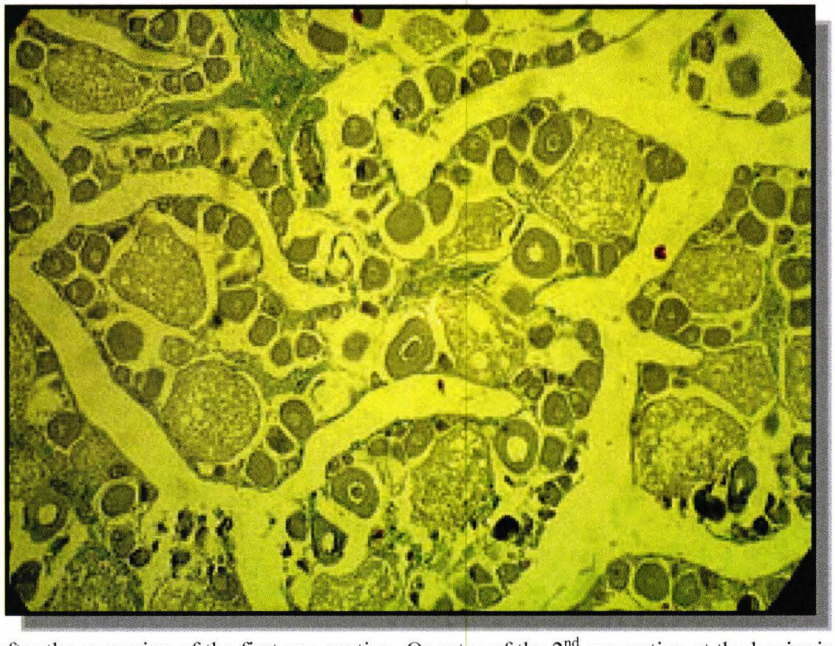

Figure 1. Gonads after the spawning of the first egg portion. Oocytes of the 2nd generation at the beginning stage of resorption (Ocular 10x, objective 15x).

In females with simultaneous development of germ cells in the gonads after spawning, the whole complex of oocytes of the 2nd maturity stage remains that characterizes the one time spawning fish.

According to our earlier studies (FULGA & BODAREU, 1992), the spawning in *S. lucioperca* began in the first and second decades of May. In modern conditions of Dubasari reservoir the females spawn eggs a month earlier than usual. In control catches there are individuals with gonads at stage five in the second decade of April. In this case the crucial role is played by the temperature factor. According to some researchers (STATOVA, 1959; TRUSOV, 1949) the transition of *S. lucioperca* ovary in the fifth maturity stage occurs quickly and lasts a few hours, so it is difficult to determine the spawning time. As the authors point out, the time and duration of the spawning period may vary depending on the temperature of the reservoir. As a result of increased water temperature in winter and spring, the females caught at the beginning of the second decade of May had already the second after spawning stage of gonads. In this state the ovaries remain until September and then move in the third stage of maturity. During this period, the development of eggs is asynchronous. According to the results of previous years (STATOVA, 1962; FULGA & BODAREU, 1992) the oocytes transition to the original accumulation of fat droplets was accomplished in late October and, by the spring (March-April), the oocytes have completed the process of accumulation of yolk granules and moved into the forth stage of gonad maturity. The authors noted the uneven growth of germ cells in the initial phases of trophoplasmic growth period. When gonads passing into the three-A maturity stage the asynchrony in the development of eggs begins to flatten.

Currently, in females with a single generation of oocytes, it can be still observed the unevenness in their development until the transition into a phase of initial accumulation of fat. In catches there are caught females with gonads at stages three-A of maturity in early October. In February, the oocytes in these individuals results in the accumulation of yolk granules and their gonads correspond to three-A-four stage of maturity (Table 1). The oocyte transition in the phase of filled yolk oocyte in the winter months also was noted in previous studies (KARLOV, 1975).

Costeşti-Stânca reservoir. In Costeşti-Stânca reservoir *S. lucioperca* is a single time spawning fish. Its gonads contain one generation of oocytes, as evidenced by the histological studies of female ovaries after they have completed their spawning. Females, in the ovaries of which were found traces of spawning fall in catches in early April. Among the spawned fish, there are also present individuals with gonads at four-A and five stages of maturity that prove the asynchronous maturation of individuals and extended spawning period, which lasts until the second decade of May.

After spawning the female gonads become the six-second stage of maturity and contain only oocytes of protoplasmic growth and follicular membrane in the process of resorption (Fig. 2). It is known that after the resorption of the follicular membrane the ovary passes in the second stage of maturity. According to the studies performed in the past century (STATOVA, 1959; CEPURNOVA, 1975) in *S. lucioperca* from Dubăsari reservoir this process lasts about a month. According to our data, the transition of gonads of sexually mature females from Costeşti – Stânca reservoir into the second stage of maturity occurs within a month and the duration of the protoplasmic growth of oocytes in repeatedly maturing fish is about three months (Table 1).

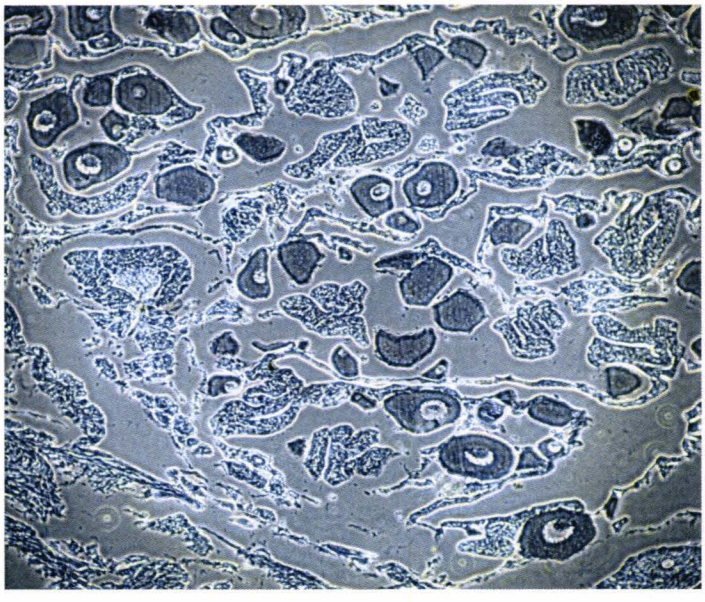

Figure 2. Fragment of zander ovary after spawning (Ocular 10x, objective 15x).

Table 1. State of the reproductive system of sexually mature females of zander from different populations.

Time	Stages of gonad maturity	Phases of oocyte development
Dubăsari reservoir		
2nd decade of April	V VI	Maturation Ovulation
May	VI-II	Resorption of the empty follicular membranes and of second generation oocytes
June	II	Protoplasmic growth
July	II	Protoplasmic growth
August	II-III	Vacuolization of cytoplasm
September	III	Primary yolk accumulation
October 1st decade	III-A	Initial fat accumulation
February	III-A-IV	Completion yolk granules accumulation and fat droplets merge into larger formations
Costeşti-Stânca reservoir		
1st decade of April	IV-A V VI	Finishing of trophoplasmic growth Maturation Ovulation
2nd decade of May	VI- II	Resorption of the empty follicular membranes
June	II	Protoplasmic growth
July	II	Protoplasmic growth
2nd decade of August	II-III	Vacuolization of cytoplasm
September	III	Primary yolk accumulation
October 2nd decade	III-A	Initial fat accumulation

The transition of the ovaries from the second to the third stage of maturity in females from Costeşti-Stânca reservoir occurs in the second decade of August till October and lasts about two months. In *Sander lucioperca* individuals from Dubăsari reservoir the transition duration in stage three of gonad maturity lasts two months (STATOVA, 1962), while and in the Dniester estuary – four months, from June until late September (CEPURNOVA, 1991).

In the females of *Sander lucioperca* from Costeşti-Stânca reservoir as well as in the individuals from Dubăsari reservoir the development of eggs in the process of vacuolization occurs asynchronously (Fig. 3) and also continues during filling vacuoles with yolk. In *Sander lucioperca* individuals at the northern boundary of the range, in the Gulf of Pärnu and Lake Ladoga, uneven growth of oocytes is observed up to their transition to ovulation (KOSHELEV, 1984).

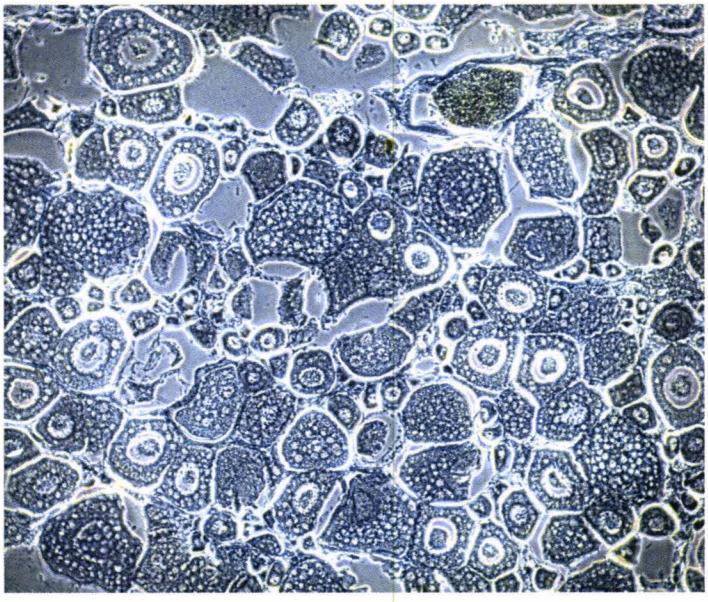

Figure 3. Asynchronous development of oocytes in the phase of vacuolization (Ocular 10x, objective 15x).

The phase of primary accumulation of yolk is characterized by the appearance of vacuoles in the cytoplasm of oocytes filled with yolk. The presence of a significant amount of such cells indicates the gonad transition to stage three of maturity. Such females were caught in early October. The initial phase of fat accumulation is characterized by the presence in the oocyte cytoplasm of clumps of yolk and scattered fat droplets between them. Oocyte membranes progressed in their development. In the radial membrane the striation is clearly visible. Between the cells of the follicular epithelium there are visible borders. In late October, during the transition of the gonads in the three-A stage of maturity and of oocytes to the original accumulation of fat droplets (end of October), the asynchrony in their development is smoothed. As a result, in females, starting with stage three-A of maturity the gonads contain a single generation of eggs (Fig. 4). In some individuals 60% of the oocytes filled with yolk are affected by the resorption process (Fig. 5).

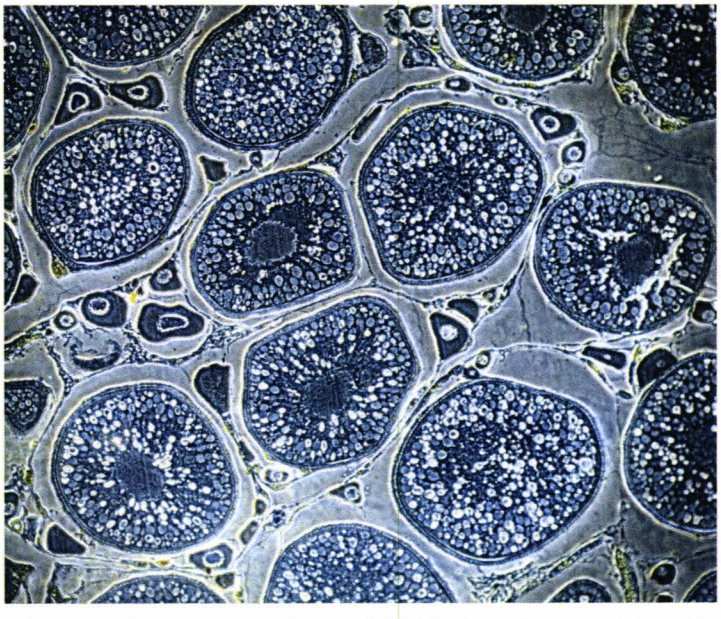

Figure 4. Synchronic development of oocytes in the phase on initial fat droplets accumulation (Ocular 10x, objective 15x).

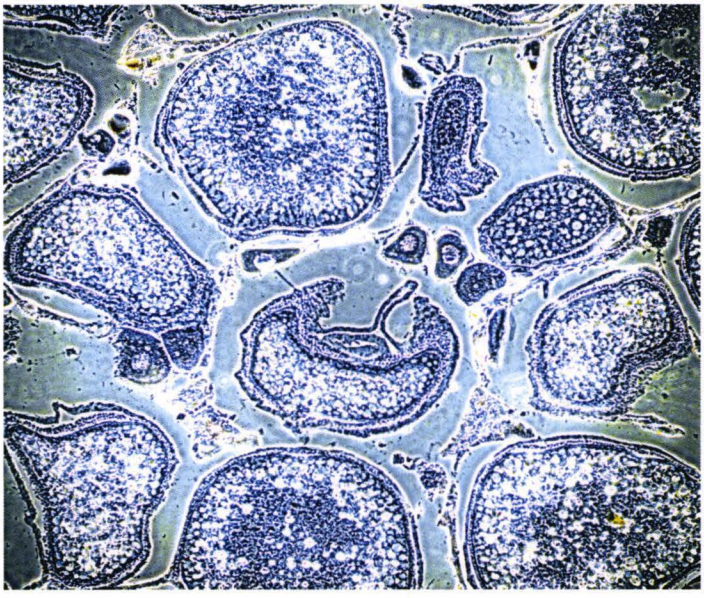

Figure 5. Ovary on the third maturity stage containing oocytes in the process of resorption (Ocular 10x, objective 15x).

In the seventies of the last century cases of mass resorption of oocytes in *Sander lucioperca* from Dubăsari reservoir were not observed (CEPURNOVA, 1975). But in subsequent years of studies, in this water basin, there were identified females with destructive changes in the development of germ cells that have completed vitellogenesis (FULGA & USATYI, 2008). Abnormalities in the structure of yolk oocytes in the absence of conditions for spawning have also been reported for zander in the Danube (KUKURADZE, 1969) and the Volga delta (KOSHELEV, 1984).

CONCLUSIONS

1. In Dubăsari reservoir, there inhabit *Sander lucioperca* females with one as well as with two generations of oocytes. In individuals with asynchronous development of oocytes during the whole year, in the reproduction cycle only one portion of eggs is spawn, while the second generation is subjected to resorption.

2. Due to the increased temperature of the water in the winter-spring period, in *S. lucioperca* from Dubăsari reservoir, it has been a shift of the particular phases of sexual cells development at earlier terms and as a result the zander spawning starts a month earlier than it was noted before the functioning of the Novo-Dniester hydroelectric station.

3. In Costeşti-Stânca reservoir the gonads *S. lucioperca* females contain one generation of oocytes. The duration of spawning season lasts about six weeks (the first decade of April – the second decade of May). The state of ovaries (the sixth-second stage of maturity) lasts one month and a half. The gonads of spawned females contain the entire oocyte complex corresponding to the second stage of maturity that characterizes single time spawning fish.

4. In *S. lucioperca* from Costeşti-Stânca reservoir as well as in individuals from Dubăsari reservoir with a single generation of oocytes the growth in the period vacuolization and of initial accumulation of yolk granules is asynchronous. Unevenness in the development of oocytes begins to flatten in the phase of primary fat accumulation. Only starting from stage three-A of maturity their gonads comprise a single generating of oocytes.

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OVERALL ANALYSIS OF NATURE 2000 SITE ON AVIFAUNA ROSPA0137 PĂDUREA RADOMIR

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Abstract. The study presents the results of the avifaunistic investigations carried out on the site ROSPA0137 Pădurea Radomir in April-July 2010 and January-August 2014. After a brief overview of the geomorphological and hydrographic characteristics of the area and the biotope types of the site's perimeter, we bring new data of the avifaunistic diversity and numerical estimations of the populations of bird species nesting in the site or stationing during spring-autumn migration. The avifauna also includes bird species transiting the site. Since many species of birds found in the protected area are part of the european conservation interest, for each we have presented a protection status established by national and european legislation. The survey data can be used as a scientific support for updating or improving safety standard site and database required to draw up an area plan management.

Keywords: avifauna, Natura 2000, ROSPA0137 Pădurea Radomir.

Rezumat. Analiză de ansamblu asupra avifaunei din situl Natura 2000 ROSPA0137 Pădurea Radomir. Studiul prezintă rezultatele investigaţiilor avifaunistice realizate în situl ROSPA0137 Pădurea Radomir în lunile aprilie - iulie 2010 şi ianuarie - august 2014. După o prezentare succintă a caracteristicilor geomorfologice şi hidrografice ale ariei, precum şi a tipurilor de biotopuri din perimetrul sitului, aducem date noi privind diversitatea avifaunistică şi estimările numerice ale populaţiilor de specii de păsări care cuibăresc în sit sau staţionează în timpul migraţiilor de primăvară - toamnă. În tabloul avifaunistic sunt cuprinse, totodată, şi speciile de păsări care tranzitează situl. Întrucât multe din speciile de păsări identificate în aria protejată sunt de interes conservativ european, pentru fiecare am prezentat statutul de protecţie instituit prin legislaţia naţională şi europeană. Datele studiului pot constitui suportul ştiinţific pentru reactualizarea sau îmbunătăţirea fişei standard a sitului şi pentru baza de date necesare la întocmirea planului de management al ariei.

Cuvinte cheie: avifauna, Natura 2000, ROSPA0137 Pădurea Radomir.

INTRODUCTION

ROSPA0137 Pădurea Radomir is located in the southwest of the country, on the level of the Romanaţi Plain subunit Leu-Rotunda Field, about 12-14 km west of Caracal and 40-42 km east of Craiova, between the railway Craiova - Caracal, to the north and Bucureşti - Craiova european road (E70), to the south. Site area is 1233 ha (Fig. 1).

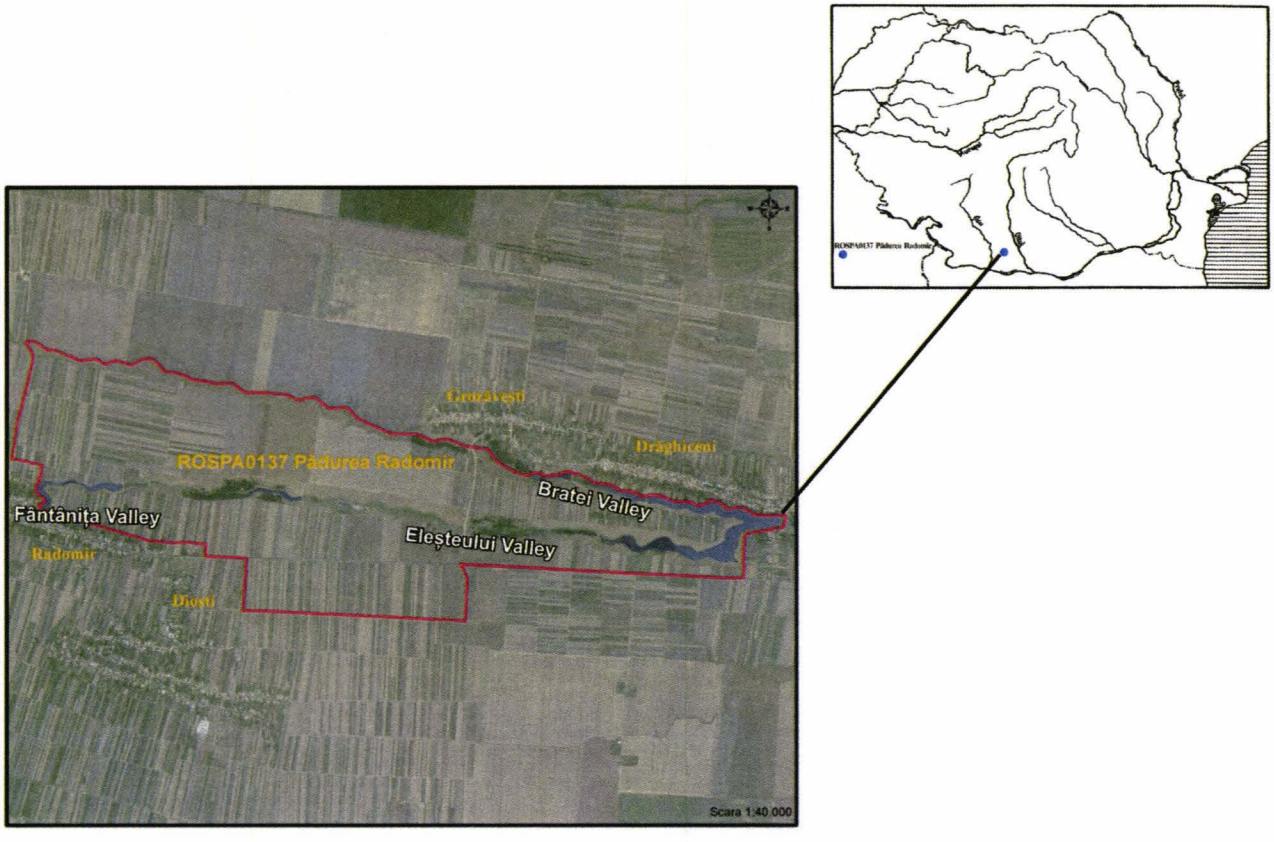

Figure 1. Perimeter of Nature 2000 site ROSPA0137 Pădurea Radomir (www.padurearadomir.ro).

In the Leu-Rotunda Field there are two main subunits: the dunes landscape to the west and the loess plains with torrential valleys which are tens of kilometres long to the east that often intersected the groundwater. The valleys are parallel, with a northwest - southeast orientation that corresponds to the west winds direction, which are a characteristic of Oltenia Plain (COTEŢ, 1957). The valleys' depth of Leu Field - Dioşti is 30-50 m. Nowadays, the winds play an important role in shaping the landscape, due to their direction and steppe regime with low rainfall.

As a result of the suffusion followed by compaction, on the loess arise closed small excavations with circular or oval shapes called "kroves or gavans". The continuous broadening and deepening of the "kroves and gavans" leads to the formation of large depression spaces, called "padinas" (like those in the west and southeast of Dioşti). The perimeter of Nature 2000 site ROSPA0137 Pădurea Radomir includes the upper and medium hydrographic basin of the Gologanu Creek (Caracal) and its tributary the Eleşteului Valley (Fig. 1).

The Gologanu Creek is about 16 km long and it is known in the upper course of the creek as the Brata Valley and in the lower course, as the Caracal Creek. The Eleşteu Valley is 6.5 km long and it flows into the Gologanu Creek at Drăghiceni. In the upper course, west of Radomir Village, the tributary is called the Fântâniţa Valley. The existing lakes have different origins, most natural lakes being formed in kroves; others - like the ones in the Gologanu Valley and the Eleşteului Valley - are man-made dam reservoirs. The hydric load in the Gologanu Creek basin is made of rainfall water and surface runoff. The groundwater from the sand dunes drains in the Gologanu and the Eleşteului Valley. The krove water, padina and streams are dependent on the rainfalls. In dry periods the evaporation is intense and the groundwater level decreases (BADEA et al., 2011).The climate is continental dry with Mediterranean influences. The average yearly temperature is 10.9°C and the average annual precipitations are 522.8 mm. In summer, maximum temperature is about 36°C, only exceptionally increasing to 40°C. The amount of precipitations varies between 400-600 mm. The most frequent winds are Crivăţul and Austrul. Crivatul wind blows during winter from the north-east and Austrul blows during summer from the west. The first is cold and wet and the second is dry and warm. In terms of pedological aspects, most of the deposits are represented by loams, clays, loess, and loess and fluvial deposits including alluvial and proluvial and colluvial. The most widespread soils are the reddish-brown soil, brown luvic soil (red luvisol), brown mesobasic soil, cambic chernozem, psamosoil, marshy grounds (GRIGORAŞ et al., 2009). The plain field is particularly favourable for agricultural crops, especially cereals.

The geomorphological and hydrographic features of the area generate the biotope types of the site perimeter.

The main biotopes of the site are:

- terrestrial biotopes represented by: arable land (76%); pasture (14.6%); forest vegetation areas (1.6%) relatively young plantations of acacia - *Robinia pseudacacia* (Radomir Forest, Drăghiceni Forest) and oak species composing Grozăveşti Oak Forest (located in the southeastern half of the site), which is characterized by pedunculate oaks - *Quercus robur* and gray oak - *Q. pedunculiflora* specimens exceeding 100 years of age (Photo 1 and 2); between the species of oaks there are other tree species interfering as: *Populus* sp, *Ulmus* sp. and *Fraxinus* sp., and various species of shrubs (*Crataegus monogyna, Rosa canina* etc.);
- aquatic biotopes: ponds, streams (4.76);
- amphibious or semi-aquatic biotopes represented by reed and wetlands (1.12%).

In phyto-graphic terms, the site area is part of the steppe. The less rich vegetation and few places of refuge are the parameters that determine a restricted biodiversity (*** 1960; BĂLĂIANU, 1980). However, PAPP & FÂNTÂNĂ (2008) emphasized the importance of the avifaunistical area of Pădurea Radomir (1031 ha) due to the presence of large colonies of *Falco vespertinus* and *F. tinnunculus*, mentioning the other prey species such as *Circus cyaneus C. pygargus, Buteo buteo* and *Falco subbuteo*. Later, the research conducted by us in 2010, the A. I. A. Pădurea Radomir has confirmed the existence of *Falco vespertinus* and *F. tinnunculus* colonies favoured by the availability of food resources (especially rodents) and nesting places (many corvid nests). In addition, the surrounding areas proved to be populated by migratory bird species with unfavourable worldwide / European Conservatory Status like: *Aythya nyroca, Ciconia ciconia, Egretta garzetta, Chlidonias hybridus, Lanius collurio, L. minor, Emberiza hortulana* etc, whose presence has strengthened the argument for its designation as a special Protection Area (SPA) and its integration into the ecological network Natura 2000 (H. G. No. 971 of 5 October 2011). SPA area was, however, extended to 1,233 ha, to include aquatic and terrestrial biotopes in the eastern area, which are important for nesting species of conservation interest mentioned above.

The SPA standard sheet includes 39 species, of which 27 are confirmed in our study. The species that we do not have information about are: *Circus pygargus, Caprimulgus europaeus, Jynx torquilla, Dendrocopos medius, Riparia riparia, Sylvia atricapilla, S. borin, Phylloscopus trochilus, Regulus ignicapillus, Ficedula albicollis, Lanius excubitor, Carduelis spinus*. Instead, however, in our research we have identified species of European conservation interest, which are not cited in the text of the site. Therefore, the aim of this study is to add new data to avifaunistic diversity of the site and estimate the number of populations of species that nest and / or station in the site during the spring-autumn migrations. The data can be used as a scientific support for updating or improving safety standard of the site and database required to draw up an area plan management.

MATERIAL AND METHOD

As research material we used: binoculars (Zeiss Jena 10x50 and 12x40 Buchnell), Bird field Guide (PETERSON et al., 1989; BRUUN et al., 1999), a camera (Sony 15 x) and for the scientific processing data (systematic, ecological, phenological classification) we have documented from the literature: SZABÓ-SZELEY & BACZO, 2006:

CĂTUNEANU et al., 1978; MUNTEANU, 1992). The protection status of the species was supported by the european and national legislation in force.

The study methods consisted in direct observations - made at fixed locations (near feeding or resting places of the birds) or move on predetermined paths and counting in the points with groups of birds. The avifauna research of the site was made at the following dates: the 10th of April 2010, the 8 to the 9th of May 2010, the 5th of July 2010, the 20th of December 2013, the 11th of January 2014, the 22nd of February 2014, the 15th and the 24th of March 2014, the 22nd and the 29th of April 2014, the 19th and the 26th of May, the 6th and the 21st of June, the 14th and the 22nd of July, covering all environmental aspects (hiemal: the 1st of November to the 1st of March; prevernal: the 1st of March to the 1st of May; vernal: the 1st of May to the 15th of June; aestival: the 15th of June to the 15th of July and serotonin: the 15th of July to the 15th of September), except for the appearance of the autumn aspect (the 1th of September - the 1st of November).

Please note that the research was carried out in 2010 and it was part of the development scientific project Nature 2000 network, coordinated by N. I. R. D. "Delta" from Tulcea.

Along with the avifaunistical field data, we have also collected some bird ethological aspects (breeding behaviour, territorial defense), ecological (interspecific relationships and the relationship between birds and environmental factors), as well as human activities aspects, etc.

RESULTS AND DISCUSSIONS

Based on our personal research results, we have accomplished an avifauna picture of the ROSPA0137 Pădurea Radomir, presented in Table 1. For each of the species we mentioned the affiliation belong to biotope type, phenological category, and some observations and numerical estimations, the threat and protection status at the national and european level. The avifaunistical list totals 69 species, classified in 34 Families and 15 systematic Orders. The best represented are the Orders: the Passeriformes (26 species), Ciconiiformes (6 species) and Anseriformes (5 species) followed by Accipitriformes, Galliformes, Columbiformes and Coraciiformes Orders, each with 3 species. In ecological terms, the dominance is represented by terrestrial species due to the weight of terrestrial habitats (grassland, arable land, woodland, etc.) compared with aquatic species that inhabit wetlands (ponds and amphibian habitats: reed, wetlands), much smaller in size.

Table 1. The avifauna list from the ROSPA0137 Pădurea Radomir site, the ecologic type, the phenological type and the protection statute of the birds species.

No.	Species	Ecologic type	Phenological type	Own observation		Protection statute				
				Breeding (pairs estimated)	Estimated effectives (number of specimens)	Birds Directive	Berna Conv.	Bonn Conv.	G.E.O. 57	Hunting Law 407/2006
ORD. PODICIPEDIFORMES										
Fam. Podicipedidae										
1.	*Podiceps cristatus*	Aqu.	SV	6-8	+ 30	-	AIII	-	-	AII
ORD. PELECANIFORMES										
Fam. Phalacrocoracidae										
2.	*Phalacrocorax carbo*	Aqu.	P	-	2	-	AIII	-	-	AI
ORD. CICONIIFORMES										
Fam. Ardeidae										
3.	*Ixobrychus minutus*	Aqu.	SV	4-6	16 – 24	AI	AII	AII	A III	AII
4.	*Nycticorax nycticorax*	Aqu.	SV	8-10	40 - 50	AI	AII	-	A III	AII
5.	*Ardeola ralloides*	Aqu.	SV	?	3 – 5	AI	AII	-	A III	AII
6.	*Egretta garzetta*	Aqu.	SV, P	10-12	50 - 60	AI	AII	-	A III	AII
7.	*Ardea cinerea*	Aqu.	SV, P	8-10	+ 30	-	AIII	-	-	AII
8.	*Ardea purpurea*	Aqu.	SV	4-7	16 - 20	AI	AII	AII	A III	AII
Fam. Ciconiidae										
9.	*Ciconia ciconia*	Aqu.	SV	3-5	+ 12	AI	AII	AII	A III	AII
10.	*Ciconia nigra*	Ter.	P	-	1	AI	AII	AII	A III	AII
ORD. ANSERIFORMES										
Fam. Anatidae										
11.	*Cygnus olor*	Aqu..	WV, P	-	5	-	AIII	A II	-	AII
12.	*Anas platyrhynchos*	Aqu.	PM	5-8	+ 140	-	AIII	AII	-	AI
13.	*Anas querquedula*	Aqu.	SV	2-4	+ 26	-	AIII	AII	-	AI
14.	*Aythya ferina*	Aqu.	P, WV, SV	?	2 - 5	-	AIII	AII	-	AI
15.	*Aythya nyroca*	Aqu.	SV	1 – 2	3 - 6	AI	AIII	AII	A III	AI
ORD. ACCIPITRIFORMES										
Fam. Accipitridae										
16.	*Circus cyaneus*	Ter.	WV, P	-	1 - 2	AI	AII	AII	A III	AII
17.	*Accipiter nisus*	Ter.	WV, P	-	1 - 2	-	AII	AII	-	AII
18.	*Buteo buteo*	Ter.	R	?	2 - 4	-	AII	AII	-	AII
ORD. FALCONIFORMES										
Fam. Falconidae										
19.	*Falco tinnunculus*	Ter.	PM	10-12	+ 40	-	AII	AII	-	AII
20.	*Falco vespertinus*	Ter.	SV	15-17	+ 55	AI	AII	AII	A III	AII

ORD. GALLIFORMES										
Fam. Phasianidae										
21.	*Perdix perdix*	Ter.	R	+ 18		-	AIII	-	-	AI
22.	*Phasianus colchicus*	Ter.	R	+ 10		-	AIII	-	-	AI
23.	*Coturnix coturnix*	Ter.	SV	+ 15		-	AIII	AII	-	AI
ORD. GRUIFORMES										
Fam. Rallidae										
24.	*Gallinula chloropus*	Aqu.	SV	+ 20	+ 100	-	AIII	-	-	AI
25.	*Fulica atra*	Aqu.	PM	15-20	25 - 50	-	AIII	-	-	AI
ORD. CHARADRIIFORMES										
Fam. Recurvirostridae										
26.	*Himantopus himantopus*	Aqu.	SV, P	2-3	4 - 8	AI	AII	AII	A III	AII
Fam. Charadriidae										
27.	*Vanellus vanellus*	Aqu.	SV, P	+ 12	+ 60	-	AIII	AII	-	AII
Fam. Scolopacidae										
28.	*Tringa ochropus*	Aqu.	P	?	+ 14	-	AII	AII	-	AII
Fam. Laridae										
29.	*Larus ridibundus*	Aqu.	P	-	12-18	-	AIII	-	-	AII
30.	*Larus cachinans*	Aqu.	P	-	2 – 4	-	-	-	-	AII
Fam. Sternidae										
31.	*Sterna hirundo*	Aqu.	P, SV	-	25-30	AI	AII	AII	A III	AII
32.	*Chlidonias hybrida*	Aqu.	P, SV	7 - 10	35-40	AI	AII	-	A III	AII
ORD. COLUMBIFORMES										
Fam. Columbidae										
33.	*Columba palumbus*	Ter.	SV	6 - 8		-	-	-	-	AI
34.	*Streptopelia decaocto*	Ter.	R	+ 15		-	AIII	-	-	AI
35.	*Streptopelia turtur*	Ter.	SV	2 - 4		-	AIII	-	-	AI
ORD. CUCULIFORMES										
Fam. Cuculidae										
36.	*Cuculus canorus*	Eur.	SV	+ 4		-	AIII	-	-	AII
ORD. STRIGIFORMES										
Fam. Strigidae										
37.	*Athene noctua*	Ter.	R	2 - 4		-	AII	-	-	AII
38.	*Asio otus*	Ter.	R	1 - 3		-	AII	-	-	AII
ORD. CORACIIFORMES										
Fam. Meropidae										
39.	*Merops apiaster*	Ter.	SV	+ 7	+ 28	-	AII	AII	-	AII
Fam. Coraciidae										
40.	*Coracias garrulus*	Ter.	SV	?	6 - 8	AI	AII	AII	A III	AII
Fam. Upupidae										
41.	*Upupa epops*	Ter.	SV	4 - 6		-	AII	-	-	AII
ORD. PICIFORMES										
Fam. Picidae										
42.	*Dendrocopos syriacus*	Ter.	R	1-2	2 - 3	AI	AII	-	A III	AII
43.	*Dendrocopos major*	Ter.	R	1-2	1- 3	-	AII	-	-	AII
ORD. PASSERIFORMES										
Fam. Alaudidae										
44.	*Alauda arvensis*	Ter.	PM, SV	+ 35	+ 100	-	AIII	-	-	AII
45.	*Galerida cristata*	Ter.	R	+ 15		-	AIII	-	-	AII
Fam. Hirundinidae										
46.	*Hirundo rustica*	Ter.	SV	zeci	+ 200	-	AIII	-	-	AII
Fam. Motacillidae										
47.	*Anthus campestris*	Ter.	SV	+ 8		AI	AII	-	A III	AII
48.	*Motacilla flava*	Ter.	SV	+ 16		-	AII	-	-	AII
49.	*Motacilla alba*	Eur.	SV	+ 5		-	AII		-	AII
Fam. Turdidae										
50.	*Luscinia megarhynchos*	Ter.	SV	+ 4		-	AII	-	-	AII
Fam. Sylviidae										
51.	*Acrocephalus arundinaceus*	Aqu.	SV	+ 6			AII	-	-	AII
52.	*Sylvia communis*	Ter.	SV	+ 4		-	AII	-	-	AII
Fam. Paridae										
53.	*Parus major*	Ter.	R	+ 20		-	AII	-	-	AII
Fam. Oriolidae										
54.	*Oriolus oriolus*	Ter.	SV	+ 5		-	AII	-	-	AII
Fam. Laniidae										
55.	*Lanius collurio*	Ter.	SV	6-10		AI	AII	-	A III	AII
56.	*Lanius minor*	Ter.	SV	5 - 8		AI	AII	-	A III	AII
Fam. Corvidae										
57.	*Garullus glandarius*	Ter.	R	+ 10		-	-	-	-	AI
58.	*Pica pica*	Ter.	R	tens	tens /hundreds	-	-	-	-	AII
59.	*Corvus monedula*	Ter.	R	tens	hundreds	-	-	-	-	AI
60.	*Corvus frugilegus*	Ter.	R	tens	hundreds	-	-	-	-	AI

61.	*Corvus cornix*	Ter.	R	+ 18	tens	-	-	-	-	AI
Fam. Sturnidae										
62.	*Sturnus vulgaris*	Ter.	R	tens	hundreds /thousands	-	-	-	-	AI
Fam. Passeridae										
63.	*Passer domesticus*	Ter.	R	tens	hundreds	-	-	-	-	AI
64.	*Passer montanus*	Ter.	R	tens	hundreds	-	AIII	-	-	-
Fam. Fringillidae										
65.	*Fringilla coelebs*	Ter.	R	+ 7		-	AIII	-	-	-
66.	*Carduelis chloris*	Ter.	R	+ 14		-	AII	-	-	AII
67.	*Carduelis carduelis*	Ter.	R	?	tens	-	AII	-	-	AII
Fam. Emberizidae										
68.	*Emberiza hortulana*	Ter.	SV	+ 12		AI	AIII	-	A III	AII
69.	*Emberiza calandra*	Ter.	R	+ 28		-	AIII	-	-	AII

Legend:

Phenological type: R – resident; PM – partial migrant. P – passage visitors; SV – summer visitors. WV – winter visitors.

Ecologic type (Habitat): Aqu. – aquatic. Ter. – terrestrial, Eur. – eurytopic.

Protection statute:

Birds Directive: European Council Directive 79/409 E.E.C. regarding wild birds conservation, adopted on the 2nd of April 1979, AI/Annex 1 – species that are the subject of special conservation measures regarding the habitat.

Berna Convention: No. 13 Law from the 11th of March 1993 for Romania adhesion to the Convention regarding the conservation of wildlife and natural habitats from Europe, adopted at Berne on the 19th of September 1979; AI/Annex II – strictly protected fauna species; AIII/Annex III – protected fauna species.

Bonn Convention: 13/1998 Law for Romania adhesion to the Convention regarding the conservation of the migratory species from wild animals, adopted at Bonn on the 23rd of June 1979; AI/Annex I migratory species with unfavourable conservation statute, for which the countries must assure immediate protection; AII/Annex II - migratory species with unfavourable conservation statute, for which the countries must assure conservation and management.

G. E. O. 57: Government Emergency Ordinance no. 57 from the 20th of June 2007 regarding the regime of the protected natural areas, conservation of the natural habitats, flora and wild fauna (modified and completed through O.U. from M. Of. No. 787/25.XI.2008); AIII/ Annex 3 – plants and animals species, whose conservation requires the designation of special conservation areas and of special avifaunistic protection areas.

Hunting Law 407/2006 (modified and completed through O.U din M. Of. no. 787/25.XI.2008): AII/Annex II – wild fauna of hunting interest, at which hunting is forbidden; Annex I / wild fauna of hunting interest at which hunting is allowed in some periods.

Among the largest terrestrial species, we recorded the majority of them to Passeriformes (*Alauda arvensis* and *Hirundo rustica* - dozens (hundreds), *Corvus frugilegus* - over 300-400 specimens, *Pica pica* and *C. monedula* - tens / hundreds of specimens, *Sturnus vulgaris* - hundred / thousand specimens, etc.); diurnal predatory species: *Falco tinnunculus* and *F. vespertinus* were also an important avifaunistic component, which was noted especially during the breeding process.

The aquatic species which revealed to be numerous over the study period are: *Podiceps cristatus* - about 35 specimens, *Nycticorax nycticorax* 40-50 specimens (ad., and juv.), *Egretta garzetta*, 50-60 specimens (ad., and juv.), *Ardea cinerea* - over 35 specimens (ad., and juv.). During spring migration, we recorded significant numbers of anatides, namely *Anas platyrhynchos* - 150 specimens and *A. querquedula* - over 35 specimens and charadriide: *Vanellus vanellus* - over 40 specimens.

In terms of phenology, the majority was represented by summer visitor species, largely nesting in the area, followed by partially-migratory and sedentary species.

The first concerns regarding the nesting process, we have recorded at *Corvus frugilegus*, which came back to the old nests installed in Grozăveşti Oak Forest from the Eleşteului Valley, in the first half of March, where it made a large colony (over 150 nests).

Since the second half of March, we noticed increased prenuptial events (territoriality songs, "dance" calls, ornamental plumage) at several species, such as *Podiceps cristatus, Anas platyrhynchos, Falco tinnunculus, Fulica atra, Vanellus vanellus, Alauda arvensis*, etc.

In early April (01.04), we registered species competitiveness between *Nycticorax nycticorax* and *Ardea cinerea*, in order to occupy about. 8-10 nests, which were built in *Populus alba* trees near the colony of *C. frugilegus* (the Eleşteului Valley - Grozăveşti), finally the nests were occupied by the Grey Heron.

During the second half of April (22.04 and 29.04), we caught the most spectacular events of the ardeide species: *Egretta garzetta* (10-12 pairs) and *Nycticorax nycticorax* (8-10 pairs) which joined the *C. frugilegus* colony. 10-12 pairs of *Falco vespertinus* species joined the former ones. Red-footed Falcon, however, began nesting in late May and the first half of June, when the above mentioned species already had baby birds in various stages of development. In the research conducted in July (14.07 and 22.07) we noticed that the red-footed falcon was still in the nest feeding chick period.

As the nesting avifauna attests the best quality and ornithological importance of the area, we will continue to report further details on species nesting in the territory of ROSPA0137 Pădurea Radomir site.

Podiceps cristatus nests in deep, large swamps with growing paludous vegetation (Marioara Swamp - Drăghiceni) from the Eleşteului Valley, in low amounts.

Ciconiiformes are found on nearly all swamps in the study area; however, the frequency and density of the recorded species are different. *Ixobrychus minutus* was frequently reported by us during the vernal and aestival aspects on the Eleşteului Valley, wetlands with dense reed from Drăghiceni area; livestock species cannot be assessed with certainty because of their hidden living in the dense macrophytes of the lakes. *Nycticorax nycticorax* is a summer visitor in the mixed colony stationed in the grove forest with tall and old trees from the Eleşteului Valley (Photo 3 and 4). *Ardeola ralloides* was reported by few specimens (3-5) in isolated wetlands with rich vegetation and shallow waters of the western end of the site known as the

Fântâniţa Valley, only in the second decade of April. We have no indication of nesting species in the area. Few specimens of *Egretta garzetta* are present throughout the warm season, almost in all shallow wetlands. In the latter part of April (29.04) the breeding pairs settled in nests built in high tree vegetation (*Quercus* sp. etc.) from the Eleşteului Valley (Oak Forest Grozăveşti) - Photo 5 and 6. Here, they have received trophic resources available in the swamps near the colony or from the eastern area, and the security offered by the cohabitation with other species of ardeides, carrion crows and the Red-footed Falcon. *Ardea cinerea* has often settled at the swamp shores, especially in the Eleşteului Valley and the meadows surrounding them. During the second half of June, the 8-10 breeding pairs have ended their nesting period - Photo 7. *Ardea purpurea* was reported by us in the Eleşteului Valley - in the eastern half of the site, on the storage ponds, covered by dense reed from Drăghiceni area - Photo 8. The hiding living of these species does not allow us a real estimate, but the number of juvenile specimens captured in mid-July, indicates a population of at least 4-7 pairs. *Ciconia ciconia* is a species often observed in the site, during feeding trips, but the number of specimens reported was generally modest. Their nests are placed on electric poles located in the surrounding town area.

The low numbers of Anseriformes identified during the vernal - aestival season are mostly brooding. *Anas plathyrhynchos* is the most common and largest hatchery flocks of Anseriformes. *Anas querquedula*, *Aythya nyroca* and *A. ferina* were rarely reported by us in the vernal - summer season and few pairs (2-3), especially in storage swamps, with rich paludous vegetation in the eastern area.

Diurnal raptors (Order Falconiformes), which nest and also uses terrestrial trophic biotope resources of the site and its surroundings, more precisely from the agroecosystems are: *Falco tinnunculus* - sedentary, migratory species common in the study sector, which, starting the end March until the second decade of April we observed her often occupying old nests of *Pica pica* and *Corvus frugilegus* from the Acacia plantations of Radomir and Drăghiceni; *Falco vespertinus* is a summer visitor, currently on the site since the second half of April - Photo 9, 10 and 11. The most representative colony of the Red-footed Falcon is located in the Oak Forest Grozăveşti (10-12 pairs), from the Eleşteului Valley and another colony, less numerous, was located in the Acacia plantation of Radomir (about 5 pairs).

The Galliformes are represented by three species common in the agroecosystems; one of them is the summer visitor species - *Coturnix coturnix* and two sedentary species - *Phasianus colchicus, Perdix perdix*. All three are terricolous species that nest and feed on the ground and have dozens of copies in the area.

In the Gruiformes order, we know two species - *Gallinula chloropus* and *Fulica atra*, which are more numerous on the storage ponds with rich vegetation covered ponds from the Eleşteului Valley (Drăghiceni) and the ones from the Fântâniţa Valley (Radomir).

Charadriiformes, which nest in the site, are represented by two species (assigned to 2 families) whose frequency and distribution are influenced by the hydroclimatic conditions. *Himantopus himantopus* is rarely a summer visitor in the site; only 2-3 nesting pairs were recorded by us during the vernal-summer season on some swamps formed in the eastern sector of the Eleşteului and Bratei Valleys, located at the northern limit of the site. *Vanellus vanellus* is a summer visitor much more often seen than the previous species. At the end of February and in July we have recorded the most important lots (+ 60 specimens), on meadows and farmland near to the ponds from the Eleşteului Valley. The prenuptial events, as well as the presence of the juvenile specimens observed at these two species by the end of the summer season are also an indication that they were nesting in the area. The Sternides are represented by two species summer visitors: *Sterna hirundo* and *Chlidonias hybrida*. *Chlidonias hybrida* forms small colonies on the ponds with vegetation from the Fântâniţa Valley (Radomir).

There are four nesting species of Columbiformes present: *Columba palumbus* has a large area of distribution and a constant presence in the area, at least 6-8 pairs observed in Acacia plantations in Radomir and Drăghiceni, especially in the Oak Forest Grozăveşti. *Streptopelia decaocto* is a sedentary specie, common in the in the vicinity of the studied site. *Streptopelia turtur* is a summer visitor species, which is relatively rare in the site; we have noticed, either by sound or by direct observations, especially in the southwest of the site, in the arboreal vegetation of the Radomir area. *Cuculus canorus* is an usually summer visitor, part of the Cuculiformes Order, it was frequently reported in reed areas, which leads us to assume that they lay their eggs in the Warbler nests installed in these habitats.

We have identified two sedentary species of the night group raptors (Strigiformes Order), which were directly observed and relatively rare: *Athene noctua*, in the neighbourhood of Drăghiceni and *Asio otus* in Acacia plantation of Radomir.

The Coraciiformes are represented by three species summer visitors: *Merops apiaster* was a small colony in the eastern part of the immediate vicinity of the protected area; *Coracias garrulus* was observed by us in the Oak Forest Grozăveşti only at the end of nesting, which is why we attribute the passage status to this species in the site; *Upupa epops* is spread within the entire studied area but it is not numerous.

The most frequent events of piciformes we have noticed at *Dendrocopos syriacus* (common species found in gardens with shrubs) and, more rarely, *D. major*.

The Passeriformes are mostly nesting species in all habitats of the researched area (forests, trees and isolated shrubs, reed, cropland or grassland). We have recorded a significant presence in terms of frequency and herds at the agroecosystems species (*Galerida cristata, Alauda arvensis, Motacilla flava, Corvus frugilegus, Sturnus vulgaris, Passer montanus, Miliaria calandra*), which are mostly sedentary species. In the vernal and summer seasons there were also highlighted the following species: *Lanius minor, L. collurio* and *Emberiza hortulana*, summer visitor species with a special protection status, which we recorded mostly in open spaces, with shrubs / trees and at the selvage of the Acacia plantations of Grozăveşti and Radomir. Since many of the bird species identified in the site are of European

Conservation Interest, in Table 1 we have mentioned the present protection status of each species from the ROSPA0137 Pădurea Radomir, according to the european legislation in force. Statistically, the 69 species recorded are:

- 19 species of preserved bird habitats are covered by the 1st Annex of the Birds Directive: *Ixobrychus minutus, Ardeola ralloides, Nycticorax nycticorax, Egretta garzetta, Ardea purpurea, Ciconia ciconia, C. nigra, Aythya nyroca, Circus cyaneus, Falco vespertinus, Himantopus himantopus, Sterna hirundo, Chidonias hybridus, Dendrocopos syriacus, Coracias garrulus, Anthus campestris, Lanius collurio, L. minor, Emberiza hortulana*;
- 35 species are also strictly protected by the 2nd Annex of the Bern Convention and the other 25 species are under the protection of the 3rd Annex of the same Convention;
- 21 species are listed in the 2nd Annex of the Bonn Convention (migratory species with unfavourable conservation status, which must be ensured by the states regarding the conservation and management);
- 19 species are the aim for designating the area as a Special Protection Area, according to the 3rd Annex of G. E. O. 57;
- 48 species are banned to be hunted according to the 2nd Annex of the Hunting' law (Fig. 2).

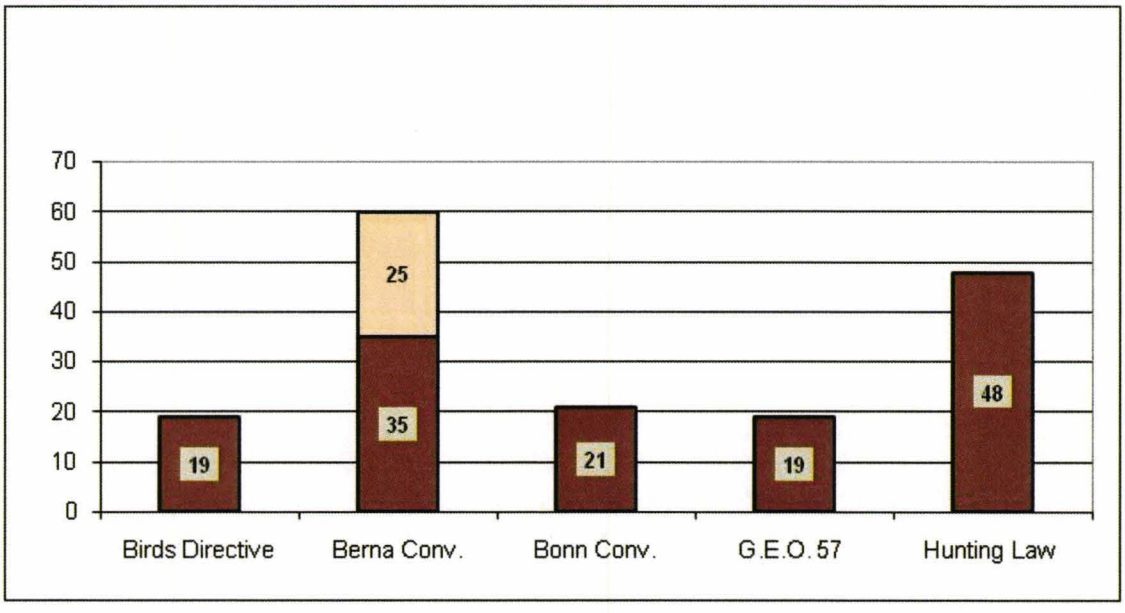

Figure 2. The avifaunistical graphic distribution of ROSPA0137 Pădurea Radomir, in terms of protection status.

The graphic shown in Fig. 2 reflects a significant proportion of preserved species. There should be taken management measures, which are required in order to ensure their preservation and perpetuation in the studied site.

We specify that the species *Ixobrychus minutus, Nycticorax nycticorax, Ardeola ralloides, Ardea purpurea, Himantopus himantopus, Dendrocopos syriacus* identified by us in the area of the site, during the research from this year (2014) are not included in the standard sheet of the site. Because these species are of European conservation interest, as cited in the annexes of the laws mentioned above, we consider necessary to include them in standard sheet of the site for the benefit of management.

CONCLUSIONS

The general avifauna analysis of the ROSPA0137 Pădurea Radomir site reveals that although the site area is part of the Oltenia Plain with little vegetation, less shelter and nesting places for birds, existing biotopes here (arable land (76%), pasture (14.6%), forest cover (1.6%), ponds and reed (5.89%), it attract birds and fauna of an important value of conservation interest such as: *Ixobrychus minutus, Nycticorax nycticorax, Egretta garzetta, Ardea purpurea, Aythya nyroca, Falco vespertinus, Himantopus himantopus, Chlidonias hybridus, Lanius collurio, L. minor, Emberiza hortulana* etc. The observations made during breeding have shown that, in order to benefit from the trophic resources available on the site, a close relationship cohabitation was created between ardeide species (*Egretta garzetta, Nycticorax nycticorax* and *Ardea cinerea*) that joined a numerous colony of *C. frugilegus* installed in Grozăveşti Oak Forest (south-eastern half of the site), near the Eleşteului Valley. The vacant nests in the mixed colony were occupied by Red-Footed Falcon (*Falco vespertinus*), which started nesting at the end of May and the first half of June, when the abovementioned species already had baby birds in various stages of development.

Prenuptial events and the juvenile specimens, which were observed in adults around other various terrestrial species (*Falco tinnunculus, Columba palumbus*, Passeriformes such as *Alauda arvensis, Lanius collurio, L. minor, Emberiza hortulana* etc.) and aquatic (*Podiceps cristatus, Ixobrychus minutus, Ardea purpurea, Anas* sp., *Himantopus himantopus, Chlidonias hybridus, Vanellus vanellus* etc.) were indications that they were nesting also in the area.

Most bird species analysed by us are protected by the National and International Laws and Conventions and Birds Directive, which requires the development of an appropriate management programme of the studied area.

The new regarding the avifaunistic diversity of the site and estimate the number of populations of species that nest and / or station in the site during the spring-autumn migrations can be used as a scientific support for updating or improving safety standard of the site and database required to draw up an area plan management.

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We thank the Local Councils of Drăghiceni and Dioşti cities, and the Olt Forest Department and A.P.I.A - DOLJ, for the information provided.

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Photo 1 and 2. Grăzăveşti Forest (*Quercus* sp.), the habitat for mixed colony of: *Egretta garzetta, Nycticorax nycticorax, Ardea cinerea, Falco vespertinus, Corvus frugilegus* (originals).

Photo 3. Adults of Night-Heron (*Nycticorax nycticorax*) (original).

Photo 4. Chickens of Night-Heron (*Nycticorax nycticorax*) (original).

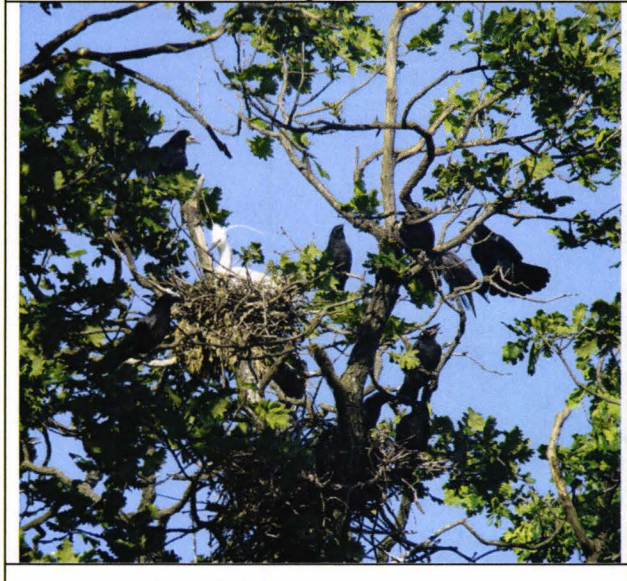


Photo 5. Adults of Little Egret (*Egretta garzetta*) on nest and Rook (Corvus frugilegus) (original).

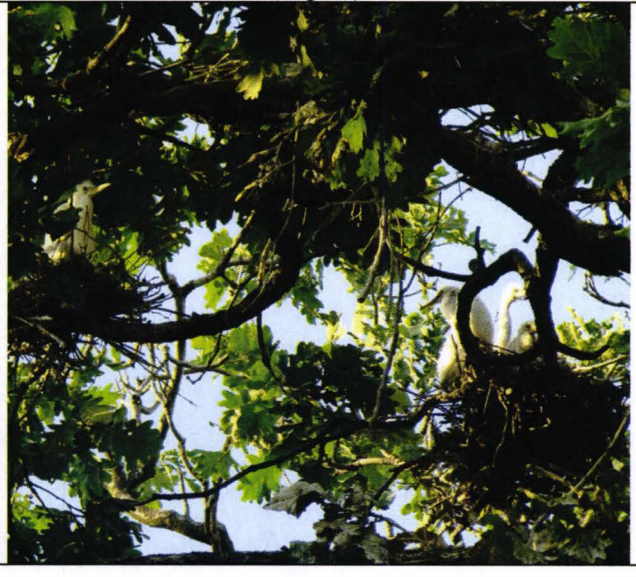


Photo 6. Chickens of Little Egret (*Egretta garzetta*) (original).

Photo 7. Juveniles of Grey Heron (*Ardea cinerea*) (original).

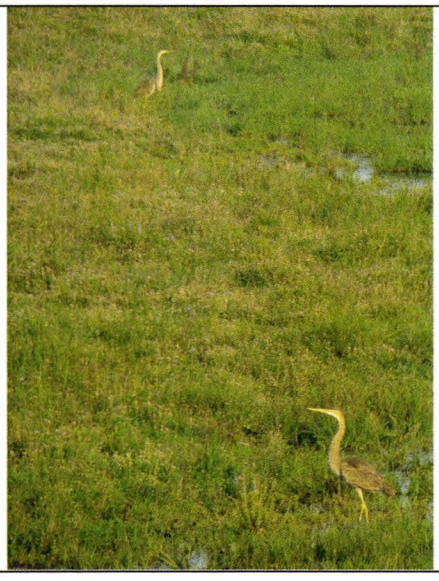
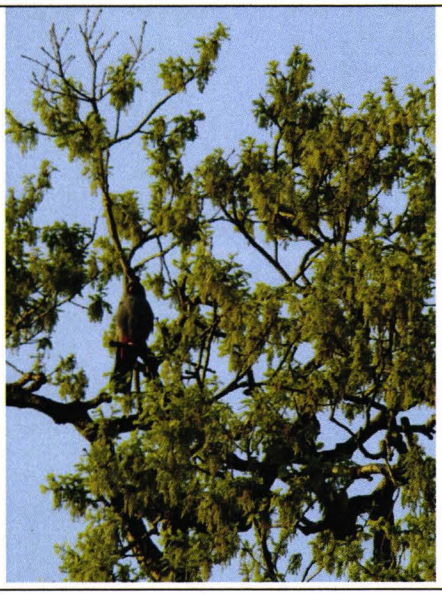


Photo 8. Juveniles of Purple Heron (*Ardea purpurea*) (original).



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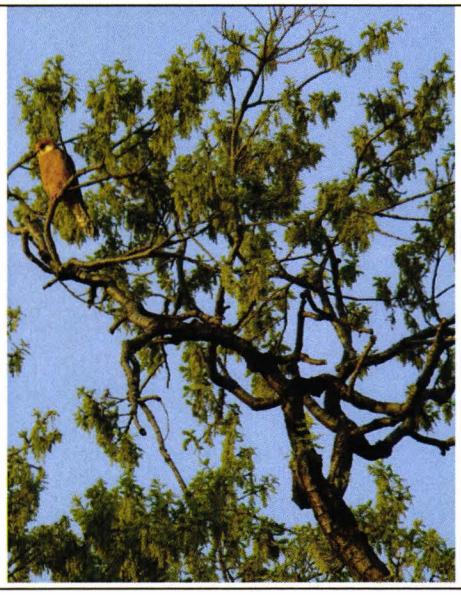

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Photo 9 and 10. Adults of Red-footed (*Falco vespertinus*), male (left) and female (right) (originals).

Photo 11. Chickens of Red-footed (*Falco vespertinus*) (original).

ECOSYSTEM ANTHROPIZATION – NEW NESTING SITES FOR BIRDS

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Abstract. The research conducted on some anthropized environment, especially on the urbanized one, creates preconditions for the assessing of the rhythm of the process of urbanization of bird species. The adaptive behavioural changes in some bird species as regarding the nesting in urban areas are manifested by changes in nest location on trees, shrubs and buildings, the composition of building material for nests compared to that used in the natural environment, the sites of food collection, etc. High buildings have attracted to cities other species such as the Black Redstart (*Phoenicurus ochruros*), the Common Swift (*Apus apus*), the Northern House-Martin (*Delichon urbica*), the Barn Swallow (*Hirundo rustica*), etc. which prefer to build their nests on tall buildings. These species built their nests in the cracks between the slabs of blocks of flats and under the eaves of buildings. In recent decades, the species Common Raven (*Corvus corax*), Eurasian Jackdaw (*C. monedula*), Carrion Crow (*C. corone*) and Rook (*C. frugilegus*) have changed the support for their nests.

Keywords: birds, anthropized ecosystems, modified biotypes, ecological plasticity, adaptation.

Rezumat. Antropizarea ecosistemelor - noi locuri de cuibărit pentru păsări. Cercetările realizate în mediul antropizat şi, în special, în cel urbanizat crează premize de apreciere a tempoului procesului de urbanizare a speciilor de păsări. Modificările adaptive comportamentale ale unor specii de păsări la cuibărit în mediul urban se manifestă prin modificarea amplasării cuiburilor pe arbori, arbuşti, clădiri, componenţa materialului de construcţie a cuiburilor, comparativ cu ceea ce sunt înregistrate în mediul natural, locului de colectare a hranei etc. Prezenţa clădirilor înalte au atras în teritoriul oraşului specii, precum, codroşul de munte (*Phoenicurus ochruros*), dreapneua neagră (*Apus apus*), lăstunul de casă (*Delichon urbica*), rândunica (*Hirundo rustica*) etc. Aceste specii îşi construiesc cuiburile în fisurile dintre plăcile blocurilor de locuit, sub streşina clădirilor. În ultimele decenii sunt înregistrate modificări ale suportului de amplasare a cuiburilor şi anume la speciile: corb (*Corvus corax*), stăncuţă (*C. monedula*), cioara grivă (*C. corone*) şi cioară de semănătură (*C. frugilegus*).

Cuvinte cheie: păsări, ecosisteme antropizate, biotopuri modificate, plasticitate ecologică, adaptare.

INTRODUCTION

Anthropized ecosystems have a substantial importance in biodiversity conservation. They are also an essential component in the ambiance creation in the territories occupied by modified biotopes (buildings, squares, gardens, vineyards, forest strips, etc.). The emergence of anthropized ecosystems creates favourable conditions only for a limited number of species with a higher adaptive potential. The research conducted on some anthropized environments, especially on the urbanized one, creates preconditions for the assessing of the rhythm of the process of urbanization of bird species. Urban areas occupy a large and increasing portion of the globe. So far, anthropization produced a disastrous effect on native species of birds. However, some regions are better for wild species of birds, suggesting that people do not destroy nature by their mere presence. Urban green spaces are important both ecologically and socially. Gardens and parks are often the only places where the urban population gets in contact with the natural fauna and it seems that this contact is very beneficial. For example, in Great Britain about 75 % of the people who possess a household provide food for birds in their gardens (CHAMBERLAIN et al., 2003).

MATERIALS AND METHODS

Quarterly observations were made from February to August in ecosystems with a different level and nature of anthropogenic influence. The following research methods of investigations were used:

a) transect method (BIBBY et al., 1997);
b) method of point estimation (BIBBY et al., 1997);
c) mapping method (BIBBY et al., 1997);
d) method of photographic recording;
e) method of video recording.

RESULTS

The investigations were carried out in different regions of the Republic of Moldova. Territories-samples were selected in different types of ecosystems with different levels of anthropogenic influence and heterogeneity. Stationary research was carried in the following study locations: the Scientific Reserve "Plaiul Fagului", forests - "Străşeni", "Chetrosu", the forest strip "Ghidighici", and the reed strips and town parks "La Izvor", "Valea Morilor" and the Botanical Garden.

Each type of biotope was studied every two weeks during the spring and breeding periods, and the composition and the number of species, the spatial structure of the communities and the functionality of this structure were recorded.

The basic parameters that influence the ecological ability of ecosystems in terms of birds, namely specific diversity, density and organic structure, were also studied.

The urbanized landscape is a specific technogenic environment. Some elements of this landscape may be comparable with the natural habitat. During the urbanization process new biotopes (habitats) are created, which create favourable conditions for some species, and less favourable conditions for those that previously inhabited them. Quite many researches have been conducted regarding the composition and the tendencies of avifauna formation in the urbanized habitat. This process occurs continuously depending on the degree of adaptability and the anthropogenic pressing on avifauna, and is manifested by the phenomenon of bird species sinantropization. Ecological, demographic and behavioural plasticity are among the general requirements that must be met by urbanization. The particular conditions which must be met are the spectrum of food, which has to be as wide as possible, and the preferred habitats.

Depending on the trends of the urbanization of the natural landscape, bird species develop a range of adaptive features to facilitate the colonization of urban (anthropized) environment. In addition, the process of urbanization of some species of birds is influenced by their wide ecological plasticity towards various changes of the anthropized environment, reflected by an increased density of certain species, changes in the terms of the breeding period and other seasonal features.

In this context, we can speak about the adaptability recorded in some species of passeriformes in the conditions created by the urban ecosystem. The adaptive behavioural changes in some bird species as regarding the nesting in urban areas are manifested by changes in nests location on trees, shrubs and buildings, the composition of building material for nests compared to that used in the natural environment, the sites of food collection, etc.

For example, a few decades ago, the Black Redstart (*Phoenicurus ochruros* Gmelin 1774), a species which has a rapid adaptation to urban conditions, could be found only on the rocky banks of the river Dniester, in the north of the country (GANEA, 1978). Nowadays this species can be found on construction sites, tall buildings, in city parks, etc. The nests of the Black Redstart birds were found in various places. The most original nest (which has been found up to now by us) was located at a height of 1.30 m inside an old electricity pole lined with cables in the courtyard of the children's hospital V. Coţaga. Another pair of Black Redstart as smart as the previous ones built its nest at a height of about 1.70 m in the crack of a brick wall of the kindergarten No. 26 in the district of Old Post in Chişinau.

High buildings have attracted to cities other species such as the Common Swift (*Apus apus* Linnaeus 1758), the Northern House-Martin (*Delichon urbica* Linaeus 1758), the Barn Swallow (*Hirundo rustica* Linnaeus 1758), etc. which prefer to build their nests on tall buildings. These species built their nests in the cracks between the slabs of blocks of flats and under the eaves of buildings. Similar nonspecific nesting places were also recorded in Iasi (CROITORU, 2009). Until recently, the Barn Swallow has not practically been observed in city areas. Nowadays, it is seen quite often. Nests of this species are usually located on the windows of the blocks of flats, inside closed balconies, but they can also be found on the beams which support the roofs of commercial halls in downtown area, namely at the Central Commercial Market. Their nests have been also found in abandoned buildings in parks where there are aquatic biotopes.

In urban areas, depending on nest sites, differences were found in some species of birds regarding the nest appearance, the building material used and their dimensions. For example, the Great Tit (*Parus major* Linnaeus 1758), the Blue tit (*P. caeruleus* Linnaeus 1758) and the Black Redstart build their nests inside pipes used as supports for vines surrounding apartment buildings and inside the legs of benches in parks and squares.

Another peculiar case is the Eurasian Collared-Dove (*Streptopelia decaocto* Friv.1838) that built its nest in the pot of a house plant that hang from a balcony of a flat on the 3rd floor. Some other peculiarities that reveal the adaptability of some species of Passeriformes to the anthropized environment are changes in their innate behaviour regarding the choice of nest sites and their building. In recent decades, the species Common Raven (*Corvus corax* Linnaeus 1758), Eurasian Jackdaw (*C. monedula* Linnaeus 1758), Carrion Crow (*C. corone* Linnaeus 1758) and Rook (*C. frugilegus* Linnaeus 1758) have changed the support for their nests. In some cases these species form associations; for example, a daw and a raven have built their nests on a high voltage power line pillar, and a Rook, Common Raven and Eurasian Jackdaw on another pillar (Fig. 1). Such nesting associations are not random; carrion crows, hooded crow and daws benefit from a higher protection because a raven has an imposing figure and is visually larger that potential predators. It has been observed that usually the nests of these species are placed at the same level with a raven nest, nearby it. When such microcolonies are invaded by potential predators the nests of associated species are affected, thus ensuring a higher rate of ravens' reproduction.

During the 1960s and 1970s of the 20th century, a Common Raven usually nestled in woods; it placed its nest in tree canopies or at the base of high tree trunks (20-30 m). In recent decades, there has been a gradual increase of birds' pairs that build their nests on high voltage power line pillars, its number varying from year to year between 30-50 % of the whole species population.

Table 1. The abundance of the *Corvus corax* species as regard the nest sites in various habitats

Period	Average no. of pairs in Moldova	Pairs that nest, %		
		Forest	Rocks	Electric pillars
1960 -1970	350	80	20	—
1990	1000	84-90	10-15	1
2013	750	60-65	10	25-32

During the 1960s and 1970s of the last century, the raven abundance was between 1/2 pairs per 5 km in forest ecosystems of Lozova, 2 pairs per 6 km in Trebujeni, and 1 pair per 2 km in the forest of Vertujeni along the bank of the Dniester river. According to the results obtained and the analysis of data, during the 1970s, the nesting sites of most pairs of ravens (80 %) were the trees in forests (Table 1). During the 1980s and 1990s of the 20th century, 84-90 % of the Common Raven population in the Republic of Moldova built their nests in forests, and some of them on power line pillars. There were also observed a reduction in the number of pairs that build their nests in rocks.

During the last decade, the number of pairs nesting on the power line pillars has significantly increased. For example, in the sector Ghiliceni – Chișcareni on a stretch of 20 km, there were observed nine nests, in the sector Scoreni – Strășeni on the same stretch there were six nests, in Anenii Noi – 4 nests, and on the route Pîrlița – Pervomaisc there were 8 nests. The power line pillars are beside forest sectors or at a small distance from woods.

DISCUSSIONS

This adaptive feature of nesting behaviour of the species, i.e. its nesting in anthropogenic sectors instead of forests, ensures the perpetuation of the species and its evolution over time.

One of the causes ravens change their nesting sites are the changes occurring in the forest ecosystems of the republic, i.e. the irrational forest exploitation that causes a significant reduction of forest habitats.

A key priority is the trophic basis. During the 1980s and the 1990s of the last century, in the Republic of Moldova, there were large cattle farms, which had special pits for carrions. They served as a source of food for ravens. In the recent transitional period, there has been a significant reduction in the number of these farms, thus the trophic basis for ravens has decreased.

The anthropogenic disturbance and the predators, for example mustelids (stone marten, pine marten), squirrels etc., are other reasons for nesting on electric pillars. Consequently, the destruction of eggs and chicks by predators has decreased significantly. All this has helped to increase overall the species density by increasing their reproduction. At the same time, the location of nests on pillars offers to the adult birds a better visibility both of the vital space and of the feeding territory. It also ensures a maximum protection of the eggs laid by a female, since it reduces the access of natural predators to the nests.

It has been observed that the number of raven nests on the electric pillars that are near forests or woodland is greater than the nests on electric pillars that are situated in open areas. Nevertheless, the number of nests in the region of the town of Bălți has increased, as on the outskirts of this town there are unauthorized dumps that serve as a trophic source for ravens.

Saker Falcon (*Falco cherug* Gray 1834) - one of the rare species of birds of fauna of Europe. In Moldova, the number and distribution of the Saker Falcon have strongly changed in the last years. The results of the inspection of the territory of Moldova in search of nesting places of the Saker Falcon have shown that the behaviour of a kind during the nested period has strongly changed. In 2005, on electric pillars, there were registered nesting sectors of Saker Falcon (10-12 nesting pairs). Routing accounts have revealed only one compact settlement in the south of the country (MUNTEANU et al., 2010).

The species Eurasian Jay (*Garrulus glandarius* Linnaeus 1758) shows a tendency of nesting in anthropogenic areas of parks and public gardens. Thus, during the 1980s, this species visited sporadically urbanized areas during nesting period and very often during post reproductive and hiemal periods. This was explained by bad conditions of cold seasons because of which they were forced to search for food in concealed places. In recent years, the percentage of the species Eurasian Jay has ranged from 0.7 to 2.9 % in forests, and 0.4% to 1.0 % in anthropogenic areas (the Botanical Garden, urban parks, public gardens) in the reproduction period. This year, their number in anthropogenic areas is 2-3 times greater during post reproduction period: in the Rose Valley – 0.9% to 2%, in the park in spring – 1.2% to 2.1%, and in the Botanical Garden – 3.0 to 3.5 %.

A similar situation has been observed in the Eurasian Jackdaw, which until the 1980s, nestled mostly in rural areas, placing their nest in unfinished buildings, churches bell towers, chimneys and in empty electric pillars (GANEA, 1978). After the 1980s, this species has shown a phenomenal adaptability to cavity nesting in hollow pillars. This has happened because the number of rural settlements with chimneys has significantly decreased, and the abandoned buildings have been destroyed. The comparative results of the studies on the Eurasian Jackdaw species during 1984-1986 and 2011-2012 on the same route, namely the forest strips Chetrosu on a stretch of 800 m, showed a growing trend of nesting in empty cavity of electric pillars: in 1984 – 3 pairs, in 1986 – 4 pairs, in 2011 – 8 pairs, and in 2012 – 6 pairs.

CONCLUSIONS

- In urban biotopes significant changes in some Passeriformes species (Black Redstrat, Common Swift, Northern House-Martin, Barn Swallow and Eurasian Collared-Dove) have been recorded regarding the nest-site selection, which differs essentially from the nest-sites selection in natural environments.
- The adaptive behaviour of bird species with a solitary lifestyle – the Common Raven and a colonial lifestyle – the Eurasian Jackdaw and Rook in poor nesting conditions is manifested by joint nesting (microcolonies) on power line pillars that provides an advanced protection against natural predators and the anthropogenic disturbance factor.

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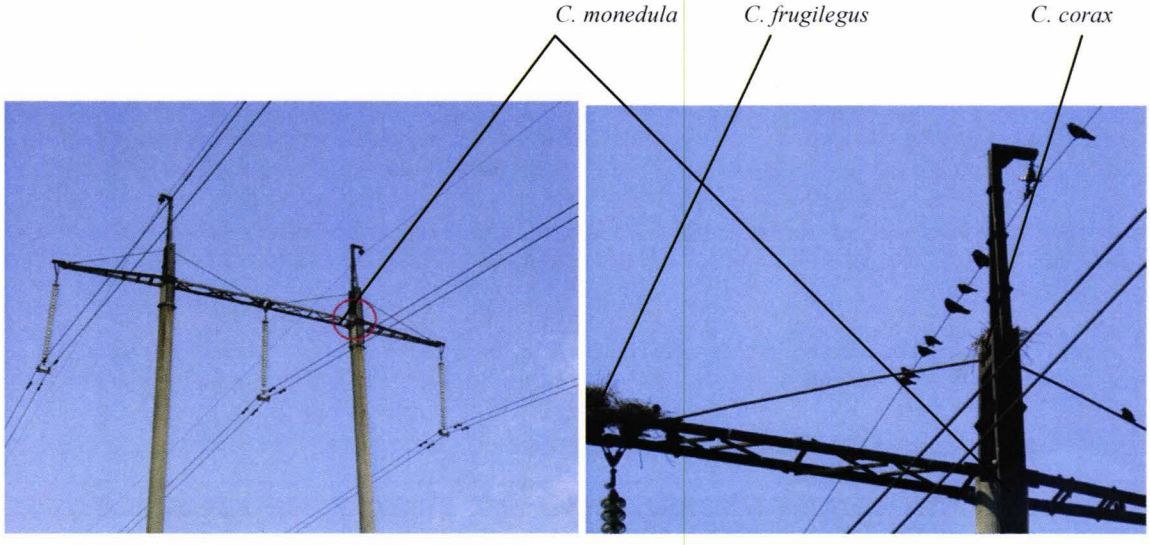

Figure 1. The sites of location of nests of *Corvus monedula*, *C. frugilegus* şi *C. corax* on the electric pillars (original photo).

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ETHOLOGICAL PECULIARITIES OF *Apodemus* GENUS SPECIES IN THE ECOSYSTEMS FROM THE REPUBLIC OF MOLDOVA

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Abstract. The species of the genus *Apodemus* are the main species in rodent fauna. The open field behaviour of the genus *Apodemus* representatives is complex, having sex, intra- and inter-specific features. According to the degree of emotional response to the new situation the most fearless (curious) were *A. flavicollis* individuals and the most precocious were *A. sylvaticus* representatives. The females of *A. sylvaticus*, *A. flavicollis* and *A. uralensis* were more curious than the males, while in *A. agrarius* the males were more curious than the females. The sex differences in the dynamics of horizontal activity are connected with greater emotionality of females compared with males, with the presence of species-specific behaviour and sexual dimorphism. The lowest values of vertical activity were detected in *A. agrarius* females and the highest in *A. sylvaticus* males. The grooming parameter had low values in all *Apodemus* genus species: the individuals need more time to fully adapt to new conditions for manifest comfort behaviour. With increasing of time spent in open field chamber and adaption to the situation the duration of freezing periods also increased. The identified interspecific and intraspecific differences in the horizontal and vertical activity showed that the motivation of exploring new environment in the studied species is stronger than the emotional response to the new situation.

Keywords: *Apodemus* genus species, open field, latent period, exploratory behaviour, horizontal activity, vertical activity, grooming, freezing.

Rezumat. Particularităţile etologice ale speciilor genului *Apodemus* în ecosistemele agricole ale Republicii Moldova. Comportamentul speciilor genului *Apodemus* în „câmp deschis" este complex, având particularităţi de sex, intra şi interspecifice. În funcţie de gradul răspunsului emoţional la noua situaţie reprezentanţii *A. flavicollis* au fost mai curioşi, iar cei mai precauţi au fost indivizii *A. sylvaticus*. Femele de *A. sylvaticus*, *A. flavicollis* şi *A. uralensis* au fost mai curioase decât masculii, în timp ce la *A. agrarius* masculii au fost mai curioşi decât femelele. Diferenţele între sexe în dinamica activităţii orizontale sunt legate de emotivitatea mai mare a femelelor în comparaţie cu cea a masculilor, cu prezenţa comportamentului specio-specific şi cu dimorfismul sexual. Cele mai scăzute valori ale activităţii verticale au fost înregistrate la femelele *A. agrarius*, iar cele mai ridicate la masculii de *A. sylvaticus*. Groomingul are valori scăzute la toate speciile studiate, indivizii necesită mai mult timp pentru adaptarea completă la noile condiţii şi manifestarea comportamentului de confort. Odată cu creşterea timpului petrecut în câmpul deschis şi adaptarea la situaţia nouă, durata perioadelor de freezing, de asemenea, a crescut. Diferenţele interspecifice şi intraspecifice în activitatea pe orizontală şi verticală la speciile studiate, demonstrează că motivaţia de explorare a noilor condiţii de mediu este mai puternică decât răspunsul emoţional faţă de mediul nou.

Cuvinte cheie: Speciile genului *Apodemus*, câmp deschis, comportament de explorare, perioada de latenţă, activitate orizontală, activitate verticală, grooming, freezing.

INTRODUCTION

The species of genus *Apodemus* are the main species in rodent fauna, having an important role in the community food chains and a special importance as consumers of tree seeds and of agricultural crops. On the territory of Moldova the genus is represented by four species: *Apodemus uralensis, Apodemus sylvaticus, Apodemus flavicollis, Apodemus agrarius*. In the conditions of anthropogenic landscape each of these species inhabit a certain habitat, which is optimal for their existence. Thus, in *A. flavicollis* such habitats are the forests, in *A. sylvaticus* island forests, forest plantations and shelter belts, as well as, gardens and orchards, *A. uralensis* is confined to fallow grounds grown with shrub and grass vegetation, also often found in forest shelter belts, orchards and gardens, while *A. agrarius* is less frequent, occurring mostly in acacia stands and other tree or shrub biotopes (SAVIN, 2004, 2005; SAVIN et al., 2011).

The open-field test is widely used in ethological studies and allows the quantification of behavioural elements in standardized new environment in a fixed time period. The open-field method is an analytical tool for assessing species-typical behavioural tendencies in a relatively unstructured situation (WILSON et al., 1976). The *Apodemus* genus species are suitable for ethological study; it includes closely related species representing diverse ecological strategies. There are several studies on *Apodemus* genus species open field behaviour in their inhabiting range (DOJDEV 1983; DOJDEV & MOLLE 1983, 1985; DOJDEV et al., 1983; FRYNTA, 1992; ATANASOV, 1983; LODEWIJCKX, 1984; STOPKA & GRACIASOVA, 2001 etc.). Although such studies in R. Moldova have been conducted for many years, only several data were published in literature (MUNTEANU & CEMIRTAN, 2007; MUNTEANU et al., 2009; CEMIRTAN et al., 2012).

This research was conducted in order to collect comparative data on open-field behaviour in wild *Apodemus* species occurring on the territory of the Republic of Moldova. The exploratory behaviour of *Apodemus* genus species in new environment will allow to emphasize some adaptation peculiarities of the species under the influence of external factors and to explain the difference between their adaptability and ethological strategies of the species.

MATERIAL AND METHODS

Studies were performed on adult specimens of both sexes of four species of forest mice: *A. uralensis* – 69 individuals, *A. sylvaticus* – 31 individuals, *A. flavicollis* – 30 individuals and *A. agrarius* – 26 individuals. The individuals were caught with live traps during spring-autumn period in various types of agricultural ecosystems. The open field orientation-exploratory behaviour (HUGHES, 1978) was studied by placing the animals for 15 min in the experimental chamber and registering each 3 minutes and in total for 15 minutes the following parameters: horizontal activity (number of crossed squares), vertical activity (jumps and vertical stands), duration of grooming (comfort behaviour) and freezing (resting periods), latent period – time of entering from the portable cage into open field chamber. Before starting the experiment the animals were weighed.

RESULTS AND SICUSSIONS

In small rodent communities the most numerous was *A. uralensis,* followed by *A. sylvaticus, A. flavicollis* and *A. agrarius*. Sex ratio (males: females) in *A. uralensis* population was 59%: 41%, in *A. sylvaticus* – 62% : 38%, in *A. flavicollis* – 62% : 38% and in *A. Agrarius* – 50% : 50%. The mean body weight was higher in females of three species: at *A. sylvaticus* it constituted 24.38±5.22 g in females and 19.98±2.92 g in males, at *A. agrarius* 23.3±4.76 g in females and 22.4±3.94 g in males, at *A. uralensis* 17.21±2.07 g and 16.89±2.44 g, respectively, and only in *A. flavicollis* the female weight (31.12±4.23 g) was lower than of males (34.87±4.68 g), although the differences between sexes were insignificant.

The latent period parameter indicates the time amount necessary for the animal to overcome the fear of the new open environment. Among rodents of all species there were recorded very suspicious individuals, which were not able to overcome the fear and did not leave the portable cage within 10 minutes and were transferred in open field by the experimenter. More curious and fearless individuals left the portable cage and entered open field by themselves within 10 minutes period.

The ratio of suspicious and curious individuals in the populations varied. Thus, the least suspicious proved to be *A. flavicollis*: 37.5% of males and 30% of females, with a latent period of 104.6±13.12 seconds in males and 171.43±21.14 s in females. In *A. agrarius* 30% of males and 60% females were suspicious, while the latent period of curious individuals was 102.57±10.25 s in males and 74.25±8.39 s in females. In *A. uralensis* 65% of males and 30% of females were suspicious and the latent period of curious animals was 173.36±15.73 s and 178.63±16.36 s respectively. The most suspicious was *A. sylvaticus* (61% males and 45% females), but in curious individuals the lowest latent period was recorded: 15.53±2.38 s and 90.67±8.13 s. When comparing these indicators between males and females, it was found that three species: *A. uralensis* (70% vs. 35%), *A. sylvaticus* (55% vs. 39%) and *A. flavicollis* (70% vs. 62.5%) the females were more curious and fearless than the males (Fig. 1).

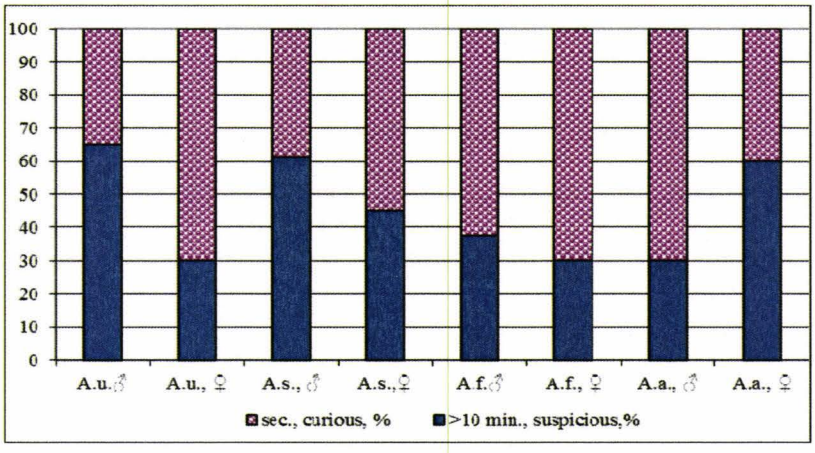

Figure 1. Intra and interspecific ratios of curious and suspicious individuals of *Apodemus* sp. genus species.

Horizontal activity. The number of crossed squares was counted every consecutive 3 minutes and on the whole for 15 minutes. This parameter reflects the locomotor activity of the animal, its capacity to explore the environment and to adapt to new conditions.

The total horizontal activity was the highest in *A. sylvaticus*, followed by *A. uralensis*, *A. flavicollis* and the lowest was registered in *A. agrarius* (Fig. 2). Only in *A. uralensis* the females recorded higher values of horizontal activity, while in other three species the males were more active, but the sex differences in total horizontal activity were insignificant.

The behaviour strategy of males in studied species is similar: the highest index values were in the first 3 minutes in open field, toward the 6th minute a significant decrease in motor activity occurred, and then, in 3 species

gradual decreasing to minimum values at the end of the experiment was observed, and only *A. sylvaticus,* remained at the 6th minute level (Fig. 2). In *A. flavicollis* males the values of horizontal activity are much lower than in females and in other species, starting at values below 50 crossed squares in the first minutes, then insignificantly decreasing below 40 crossed squares in the next 3 minutes and remained practically at the same level till the end of experiment. The highest values of horizontal activity in the first minutes were registered in *A. uralensis* males. In general, the parameter values in the first minutes of open field indicated a high level of emotional reaction to new conditions, while their decline indicated the ability to overcome fear and to adapt to the environment. Thus, all males successfully overcome fear and quickly adapted to the environment, except for *A. sylvaticus* males, which need longer time period for adaptation than other species.

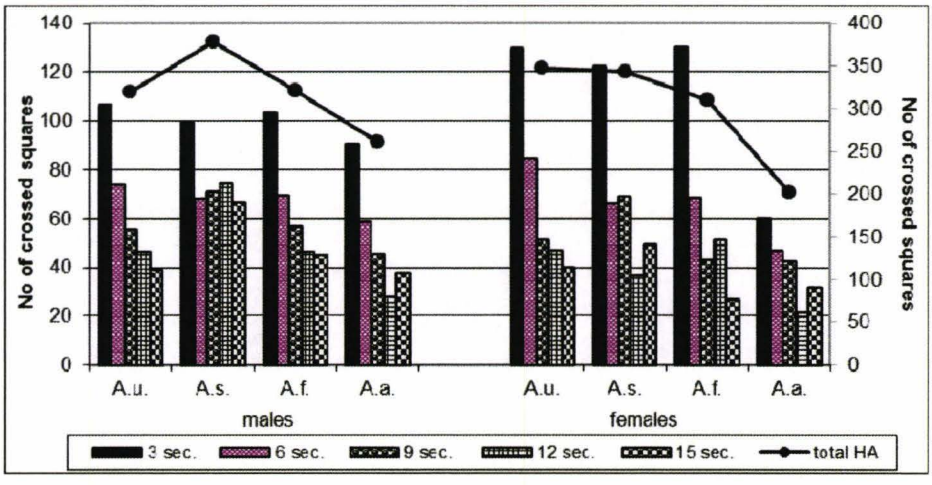

Figure 2. Horizontal activity of males and females in *Apodemus* species during 15 seconds and total horizontal.

In females, at the beginning of the experiment, almost all species had higher values of activity than males (Fig. 2), except for *A. agrarius*, which values were situated around 60 crossed squares in the first minutes. In the rest of the species the maximum values reached over 120 crossed squares in the first minutes. The reduction of horizontal activity, first sharply, then gradually down to minimum values at the end of experiment, occurred in *A. uralensis* and *A. flavicollis* females. At the last species on the 12th minute there was a slight and transient increase of the parameter. *A. agrarius* and *A. sylvaticus* females showed significant reduction in motor activity only in the 12th minute and in the 15th minute – it increased again. In general, in *A. sylvaticus* females, sudden change in motor activity was registered. The observed differences in the dynamics of horizontal activity (Fig. 2) are, probably, connected with greater emotionality of females compared with males, with the presence of species-specific behaviour and sexual dimorphism. Some researchers (DOICHEV, 1983) reported significantly higher number of squares crossed during 15 min. of open-field tests in males than in females of *A. sylvaticus* and no significant differences between the sexes were found in *A. flavicollis* (DOICHEV et al., 1983).

Vertical activity in all *Apodemus* species consisted in two indicators: the vertical jumps and vertical standings. The vertical standings were considered as proper research activity and vertical jumps – as emotional reaction to the new situation. There was also analysed the total vertical stand and jump activity.

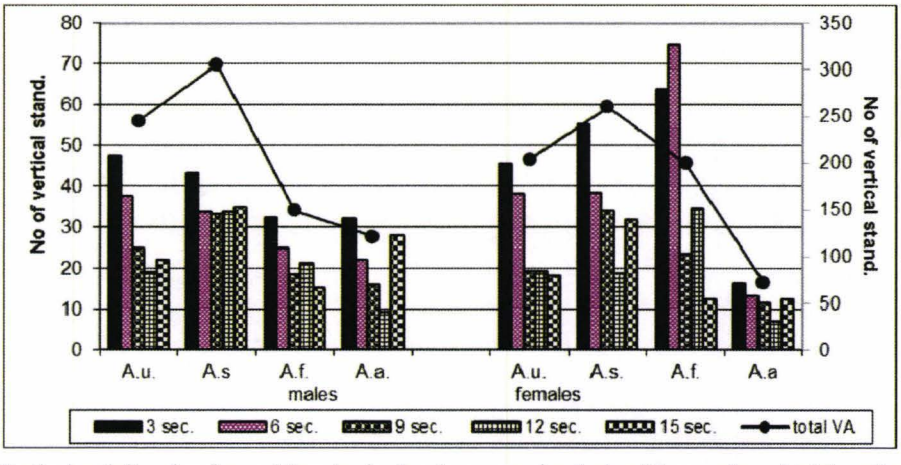

Figure 3. Vertical activity of males and females in *Apodemus* species during 15 seconds and total vertical activity.

In general, the total vertical activity was the highest in *A. sylvaticus* species, followed by *A. uralensis*, then *A. flavicollis*, and the lowest – in *A. agrarius*, the males being more active than the females in all studied species except *A. flavicollis*, where the females registered higher values of vertical activity (Fig. 3).

The lowest values of vertical activity were detected in *A. agrarius* females; this also applies to the number of jumps (lowest number of all species) and to the number of vertical stands (Fig. 3). The males of this species were more emotional and more curious than the females. This was reflected in higher values of jumps and vertical stands. The vertical activity of both males and females increased in the last 3 minutes of the experiment (Fig. 3).

Low levels of jump activity were registered in *A. flavicollis* individuals (Fig. 3), which practically disappeared toward the end of 15th minute. At the same time, they have different values of exploration activity dynamics between both sexes. In males, compared to females, the exploration activity was lower in the first minutes of the experiment, then in males this parameter is gradually declining to the lowest value at 6th minute and remained practically the same by the end, while in females the parameter remained at a high level until the 6th minute, sharply decreased to 9th min., slightly increased at the 12th min. and reached a minimum value at the end of the experiment. In general, the exploring activity of this species males was the lowest of all studied species.

In *A. uralensis* the values of jump activity was higher than in the above-mentioned species, their value remained practically unchanged for the duration of the experiment, being higher in males (Fig. 3). The dynamics and the values of exploring activity of males and females of this species are similar and, in general, higher than that of *A. agrarius* and males of *A. flavicollis*, starting with maximum values at the beginning of experiment, drastically decreased at 6th minutes, then gradually decreased toward the end of the experiment. The vertical activity is the highest in the first minutes then decreases and remains at the same level in the last minutes of the experiment, fact registered in previous studies (MUNTEANU et al., 2009).

A. sylvaticus males occupied the first place after the number of jumps and in females this parameter values were close to those of *A. uralensis* females (Fig. 3). At the same time, their jump activity was the highest of all the species, reaching maximum values in the middle of the experiment. The dynamics of exploring activity in *A. sylvaticus* male and female was also different. It was established that the values and dynamics of the "jump" parameter practically has no effect on the dynamics of the total vertical activity (jumps + stands), i.e., in general, it did not influence upon the dynamics of proper exploring activity. Thus, we can assume that the motivation for exploration in studied rodent species is stronger than the emotional response to a new situation.

Grooming is another element that characterizes animal behaviour. According to its value amount (Fig. 4) this parameter is not very particular for *Apodemus* genus species. Apparently, within 15 minutes of being in the open field chamber the animals did not have enough time to fully adapt to the environment, therefore the elements of comfort manifestations of behaviour, which include grooming, are not so significant in duration and stability. The highest time amount spent in grooming activity was registered in *A. agrarius* females, while the lowest – in *A. uralensis* females. It should be noted that intra-specific dynamics of the parameter, as well as the interspecific ones of males and females are different. Thus, between the males and females of *A. uralensis* and *A. flavicollis* the differences are significant, the males being almost two times longer involved in grooming activity than the females. In *A. sylvaticus* and *A. agrarius* the sex differences are insignificant with slight prevailing of the males in *A. sylvaticus* and of the females in *A. agrarius*. In other studies there was no evidence that the distributions of self-grooming bouts are different between sexes and that the total time spent self-grooming per time unit does not differ between sexes (STOPKA & GRACIASOVA, 2000).

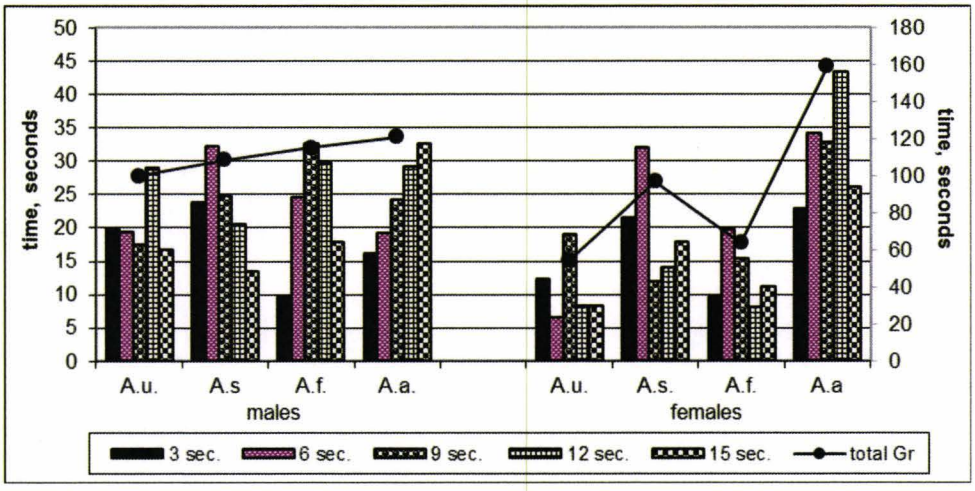

Figure 4. Grooming duration of males and females in *Apodemus* species during 15 seconds and total grooming activity.

Freezing in comparison with grooming lasts longer in time in all the species, being the highest in *A. uralensis* and the lowest in *A. flavicollis* males (Fig. 5). In females and males of *A. agrarius* species were emphasized the following features: with increasing of time spent in the open field chamber and adaption to the situation the duration of

inactivity periods also increased. In *A. flavicollis* and *A. uralensis* males the relatively high values of freezing in the first 3 min of the experiment significantly decreased in the 6th min. and then began to increase, reaching a maximum on the 9th min. without further changings (in *A. uralensis*), or reaching a maximum at the 15th min. (*A. flavicollis*). In *A. sylvaticus* males sharp changes of the parameter values were revealed during the whole period of the experiment.

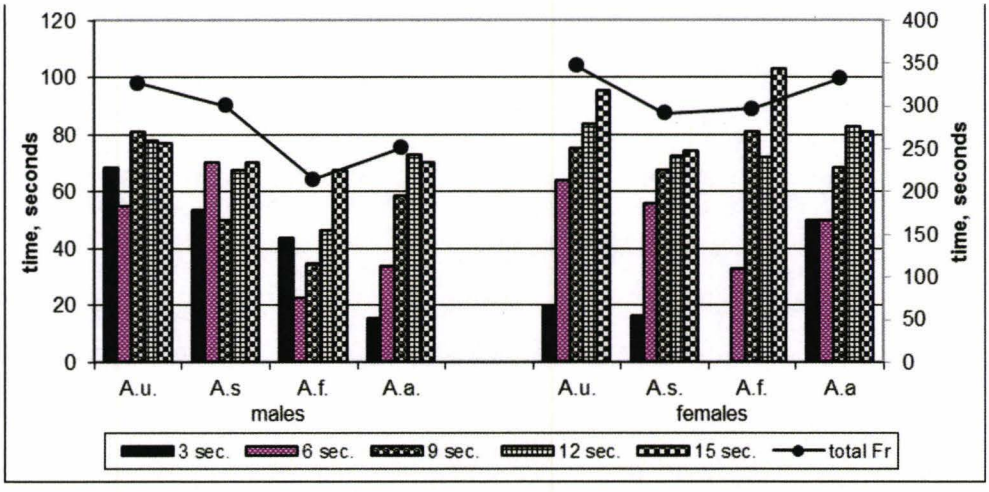

Figure 5. Freezing duration of males and females in *Apodemus* species during 15 seconds and total freezing activity.

In spite of apparently significant interspecific variation, there were not found considerable sex differences in the majority of behavioural elements registered during the open field tests. Similar results were obtained in several studies conducted in *Apodemus sylvaticus* and in *A. flavicollis* from other areas of its range (LODEWIJCX, 1984; FRYNTA, 1999; ATANASSOV, 1983).

As to the interspecific differences, our results showed marked variation. It was emphasized that in *Apodemus agrarius* the majority of behavioural elements for every 3 minutes of the tests and the total values are differ from other species under study. Its horizontal and vertical activities are the lowest, while the grooming activity is the highest. These differences can be explained by its ecological peculiarities: *A. agrarius* inhabit mostly humid biotopes covered with dense herb vegetation, while other *Apodemus* species are less specialized.

CONCLUSIONS

The open field behaviour of the genus *Apodemus* representatives is complex, having sex, intra-and inter-specific features. According to the degree of emotional response to the new situation the most fearless (curious) were *A. flavicollis* individuals and most precocious were *A. sylvaticus* representatives. The females of *A. sylvaticus, A. flavicollis* and *A. uralensis* were more curious than the males, while in *A. agrarius* the males were more curious than the females.

The sex differences in the dynamics of horizontal activity are connected with greater emotionality of females compared with males, with the presence of species-specific behaviour and sexual dimorphism. The lowest values of vertical activity were detected in *A. agrarius* females and the highest in *A. sylvaticus* males. The identified interspecific and intraspecific differences in the horizontal and vertical activity showed that the motivation of exploring new environment in the studied species is stronger than the emotional response to the new situation.

The grooming parameter had low values in all *Apodemus* genus species; the individuals need more time to fully adapt to new conditions for manifest comfort behaviour. With increasing of time spent in open field chamber and adaption to the situation the duration of freezing periods also increased.

The time period of 15 minutes is not enough for complete adaptation of studied rodent species toward new environment and manifestation of comfort behaviour elements.

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ASPECTS REGARDING THE PARASITIC INFESTATION DEGREE AND BODY RESISTANCE TO PHYSICAL TRAUMA AT MOUFLON *(Ovis ammon mussimon)* FROM DOGROGEA

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Abstract. Taking into account that, in Romania, the mouflon is a gregarious species and it lives only into fenced areas, a disease of viral, bacterial or parasitic origin could be deadly for the entire group. The study presents the diseases that affect the mouflon from Dobrogea, which have been observed in the last 5 years, and identifies those diseases that require prophylactic treatment from the diseases that might affect the mouflon (presented by NESTEROV in 1984, 2009 and 2010). This study also aims at presenting the disorders caused by environmental conditions (soil substrate, nutriment, limited space).

Keywords: mouflon, wild fauna, disease, injury, Dobrogea.

Rezumat. Aspecte privind gradul de parazitare şi rezistenţa organismului la traume fizice la muflonul (*Ovis ammon mussimon*) din Dobrogea. Luând în considerare faptul că muflonul este o specie gregară şi care, în România, trăieşte numai în spaţii împrejmuite, o boală de origine virală, bacteriană sau parazitată poate fi letală pentru un întreg grup. Lucrarea prezintă un tablou al bolilor observate la muflonii din Dobrogea în ultimii 5 ani şi identifică dintre bolile ce pot fi întâlnite la muflon (prezentate de NESTEROV în 1984, 2009 şi 2010) pe cele pentru care este necesar tratament profilactic. Prezentul studiu şi-a propus să prezinte şi afecţiuni care apar datorită condiţiilor de mediu (substrat, hrană, spaţiu limitat).

Cuvinte cheie: muflon, faună salbatică, boală, traumă, Dobrogea.

INTRODUCTION

The mouflon is an allochthonous species. In the last years, the mouflon regained its importance and interest for hunting in Romania. Being a game that can be consumed, it is characterized by the lack of adipose tissue and percentages of proteins content is almost identical with that in lambs and sheep. As in Romania there are no published studies concerning the characteristics and issues of mouflon, in order to develop a suitable management it is necessary to make a study of the (specific) anatomopathological and ecological features.

Diseases never occur without a reason. They are triggered by an imbalance of the population or of the environment (PFLIEGER, 1982). Illness and mortality are significantly influenced by the climatic conditions, the quantity and the quality of the fodder, the dissemination of salt. Raptors, health agents of nature, play an important role in checking the number of the small rodents, which are very prolific and have a role in maintaining the diseases that can be transmitted from game to man (NESTEROV, 1984; 2010).

MATERIAL AND METHODS

The personal study (January, 2008 - January, 2013) covers 5 years and is focused upon 2 effectives of mouflons: those living in semi-freedom conditions (Negureni hunting complex - 45 specimens, in 2009) and those living in captivity from the patrimony of the Museum Complex of Natural Sciences of Constanţa (C. M. S. N.) 22 specimens, in 2009, which represented, at that time, the biggest nucleus of mouflons from the zoological gardens in Romania (ANTONE & UNICI, 2011; ANTONE, 2013).

In order to better understand the pathology of the mouflon, 21 dead specimens and 45 alive specimens were analysed. To highlight the possible states of parasitic infestation, faecal samples were collected from 20 specimens for laboratory tests. The samples were taken from the rectum-anus of 10 alive specimens and from different parts of the large intestine (colon, cecum, rectum) of 10 dead specimens.

Regarding the necropsy research, it should be noted that the corpses were examined in terms of the external conformation of the trunk, head, limbs and tail, in order to observe possible injuries. Those corpses were opened to study the topography and layout of the internal organs, in order to highlight some specific features of the species. Attention was mainly focused on the digestive, excretory and respiratory systems. Each internal organ was examined in detail, to identify any macroscopic anatomopathological changes, which could be attributed to the evolution of a disease. Concerning the healthiness of meat, microbiological examinations were made (total number of germs, *Escherichia coli*, *Salmonella*) on samples of muscular tissue. The samples were collected from 15 specimens, from different body parts with developed muscles.

The coproparasitological examination was based on the method LCSVD Olteanu identification of eggs and cysts of parasites by flotation and sedimentation.

RESULTS AND DISCUSSIONS

In 12 analysed cases of of dead specimens (specimens from C. M. S. N), it resulted that pulmonary congestion prevails, followed by hypothermia.

Following coproparasitological examination of 20 faecal samples from live and dead specimens, there were identified infestations with *Eimeria* sp. and *Trichostrongylius* sp. (Table 1).

Coccidiosis is a parasitic enteritis caused by 11 species of coccidia of the genus *Eimeria* that affects all categories of sheep breeds, especially the youth (DULCEANU & TERINTE, 1994). Each species that parasitizes is localized in a certain intestinal segment. At sheep breeds, coccidiosis is clinically characterized by diarrheal stools mixed with blood, dehydration and weakness.

Table 1. Parasites identified in samples.

No.	SAMPLE IDENTIFICATION	IDENTIFIED PARASITES	
		Trichocephalus ovis	*Eimeria spp.*
1	Sample 1	-	+
2	Sample 2	-	+
3	Sample 3	+	++
4	Sample 4	-	+
5	Sample 5	+	++
6	Sample 6	-	-
7	Sample 7	-	+
8	Sample 8	+	++
9	Sample 9	-	-
10	Sample 10	-	+
11	Sample 11	-	+
12	Sample 12	-	+
13	Sample 13	+	++
14	Sample 14	-	+
15	Sample 15	+	++
16	Sample 16	-	+
17	Sample 17	-	+
18	Sample 18	+	++
19	Sample 19	+	++
20	Sample 20	-	+

Legend: + - weak infection
++ - medium infection
+++ - strong infection

Synergism should be taken into consideration if 2 or 3 species of coccidia occur simultaneously, which causes severe disorders compared to those produce by each species. Mixed infections last longer.

In case of the infections with *Eimeria* sp. and *Trichostrongylius* sp., the nematode presence has no influence on the growth of lambs or the elimination of oocysts.

Of 20 faecal samples examined, 2 samples have been found free of parasitic infestation. It is worth mentioning that the 2 samples came from females aged 2-3 years, fact which is contrary to the literature. Medium infection with *Eimeria* sp. has always been associated with *Trichocephalus ovis* weak infection.

Infections clinically manifested with dysentery forms were observed in spring, after the rainy season and were due to the consumption of water from puddles. A single specimen, a male, required medication.

From 21 lung samples analysed to evaluate the sanitation of specimens, only 1 sample showed weak injuries due to the infection with *Muelleris capillaris* (nematoda) (MUELLER, 1889). The mouflon lung injuries caused by *M. capillaris* were identified by different types of grey stains with varying dimensions – extended, diffuse and/or nodular (Fig. 1.). In our case, the size of stains was less than 5 mm on the lung surface and, in addition, there were observed small hard nodules with a diameter of 1-5 mm under the lung pleura and within the left lobe of the mouflon lung (Fig.1. b). Those nodules were quite similar to the granulomas described by Beresford-Jones (1967), found in sheep infected with *M. capillaris*. McFadyean (1894) (cit. by BERESFORD-JONES, 1967) describes those nodules as pseudo-tuberculi.

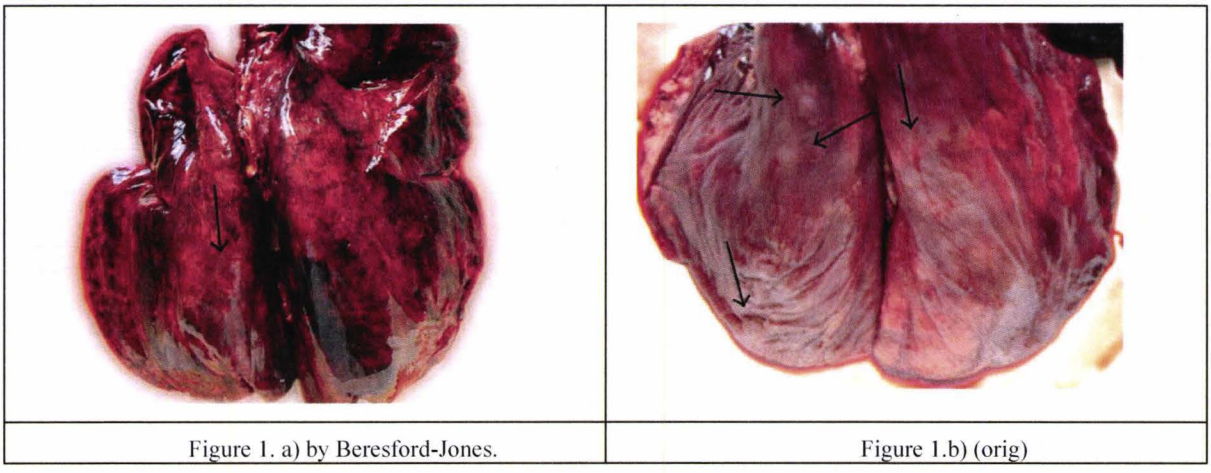

Figure 1. a) by Beresford-Jones. Figure 1.b) (orig)

Figure 1. Mouflon lungs infected with *Protostrongylids* (a) by Beresford-Jones. (b) Mouflon lungs infected with *Protostrongylids.*

In accordance with the subjective adopted criteria, the abnormalities of the mouflon lung were classified.

From possible diet-related diseases, only one case was found during the study - avitaminosis, male lamb, in 2010 (Fig. 2.) (The mother was 2 years old, at first birth). Avitaminosis is a disease caused by insufficiency or lack of one or more vitamins. It is manifested by excessive hair fall, leaving bare patches of dry skin. The most affected areas are the head and the joints of the limbs. It was associated with dysfunctions of growth and dry skin.

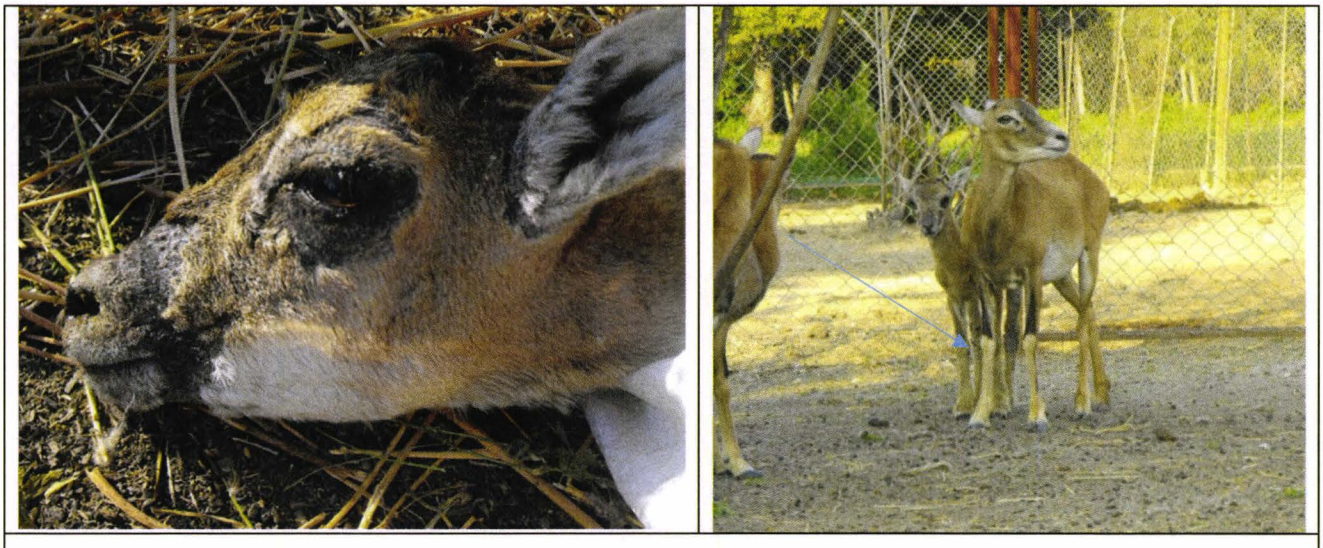

Figure 2. a) Mouflon lamb with signs of avitaminosis - head area. Figure 2. b) Mouflon lamb with signs of avitaminosis - limb joints.

Figure 2. Infections to a mouflon lamb (orig.).

The treatment consisted in disinfecting the affected areas by dabbing with methylene blue and administration of vitamins. Vitamins were injected (subcutaneously), in five consecutive days. The dose of vitamins has been: 5ml of calcium gluconate, 1 ampoule of B12, 5ml of B1-B6 and 5ml of vitamin C.

Mechanical injuries which may occur:

- Crush wound or contusion; it is caused by a hit with a hard object, which has a smooth or irregular surface; this type of wound is relatively common during the rut, at males, as a result of fights, when the horns collide with the rump or the abdomen of the opponent;
- Dislocation and torn tissue caused by certain types of predators; this type of wound has not been observed because there are no predators;
- Cut wound: it is caused by sharp instruments; blood loss following a cut wound is variable and is directly influenced by the calibre of blood vessels (from light to severe bleeding). Just 2 cases of cut wounds were registered, caused by wire threads protruding from fence - some mouflon have been put into a small enclosure (1ha) from Negureni. The wounds were produced at the head; both specimens were males, 3-4 years old. No medical intervention was necessary, because the wounds were superficial, without significant loss of blood;

- Closed fractures are bone lesions characterized by interrupting the continuity of a bone upon mechanical factors interference. The fracture was accompanied by soft tissue injuries of the musculoskeletal system. Such closed fracture was observed in the case of an aged ram (12 years), who was pushed into the shelter wall by the side, kicked by another young male (6-7 years). The impact effect was the fracture of 2 ribs on the right flank with lung perforation, diaphragm rupture and internal bleeding - death occurred in 10-12 minutes after impact;
- Comminuted fracture; it is a fracture with displacement of bone ends, which pierces and injures the surrounding soft tissues, including the skin. This type of open fracture has been studied in case of a ram (1.5 years). The ram was brought to C. M. S. N. from the hunting complex Mereni / Covasna. According to the rules, he was kept in a quarantine area for a period of 21 days. Being a wild animal and accustomed to open spaces, in an attempt to escape, went through a window with grille and fractured its right leg. He was found near the quarantine area, in a state of lethargy and was transported to a specialized veterinarian (Fig. 3.a). He had a rupture of the femur in two places, with bone chips that have pierced the muscles and skin. By operation, the bone was fixed with a rod attached to the round ends (lateral condyle) of the femoral bone.

Figure 3. a) - Mouflon with open fracture (orig.).

Figure 3. b) - Mouflon with operated fracture (orig.).

Figure 3. Mouflon with right hind fractured.

After the surgery, when anaesthesia went off, it was introduced into the enclosure, with other specimens (Fig. 3.b) and it adapted fast to the group. The affected foot was held off the ground about a month, after that it started to support on it. After six months, the foot was effectively used only in emergency or when it was forced to run with the group. Locomotor differences are hardly noticeable after one year, even for those who know the case.

Table 2: Types of injuries.

Wound type	Description	No. of specimens	Age (years)	Sex	Cause	Effect
Crush wound	Concussion by hitting	6	3-7	M	Fight/rut	Healing in 1-2 weeks
Torn wound	No such case	-	-	-	-	-
Cutting wound	Cutting caused by protruding wire	2	3-4	M	Escaping	Healing in 2-4 days
Closed fracture	Fracture of ribs, lung perforation, diaphragm rupture, internal bleeding	1	12	m	Fight	Death in 10-12 min.
Comminuted fracture	Femoral bone, open fracture	1	2	m	Escaping	Healing, after surgery 1 year

Analyzing all cases of mechanical trauma, it may be observed that all cases were due to the struggle for reproduction or fight for survival / defense. All 10 cases of mechanical trauma were registered at males (Table 2.). Those 10 specimens fall into three age classes: juvenile (1 specimen), adult (8 specimens), senescence (1 specimen).

During the study, 3 problems due to environmental conditions have been highlighted.

One problem mentioned by many works (COTTA et al., 2001; UNICI et al., 2009) is the excessive growth of the hoof and hoof deformation at the mouflon that live on other type of soil than the rocky one or with rock at surface.

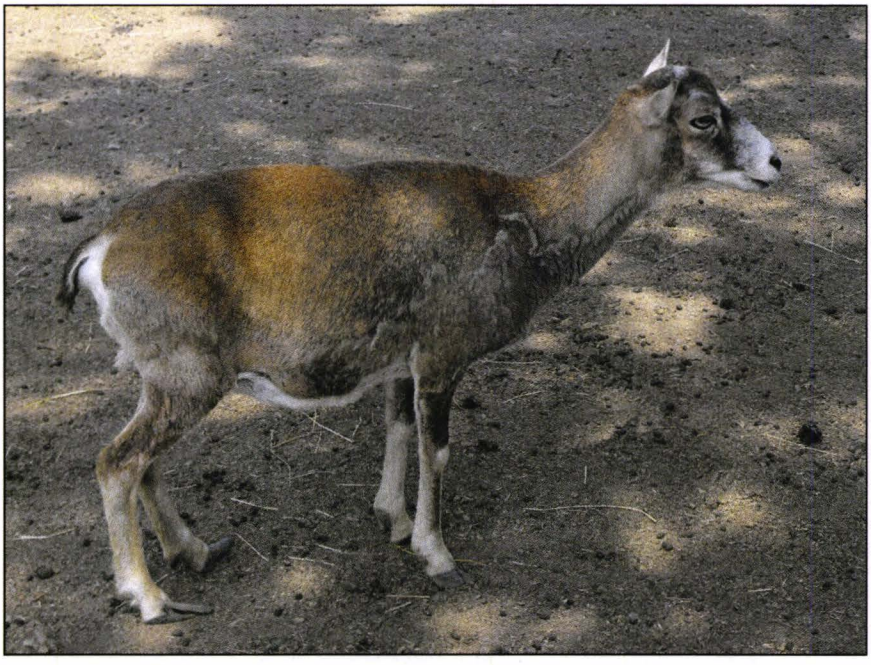

Figure 4. Female with grown and deformed hoovesh. (orig.).

At Negureni, because the substrate of the soil is rough in many areas, it was not registered an excessive growth of the hoof (Fig. 4.) At the specimens from C. M. S. N., it was registered a higher growing trend in case of the old females. There were no cases of excessive nail growth for the specimens of up to 3 years. In hunting complexes, the mouflon with an excessive nail growth are captured, the hooves are trimmed and they are proposed for selection.

The mouflon has a low resistance to moisture. The rainfalls, during spring or summer, when lambs are small, cause most deaths. The lung congestion is a pulmonary alveoli inflammation and is often associated with pleura contribution. It resembles pneumonia, because it is caused by the same microbial agent - pneumococcus. The mouflon does not seek shelter in case of rain, but only in case of downpour, storms or heavy snowfalls.

Hypothermia may occur, because the mouflon does not make shelters. They seek shelter under branches of trees or shrubs or at the base of the cliffs. The risk of hypothermia is very high when the temperatures decrease below -10°C for a longer time and the organism is weak. In this case lambs may die.

CONCLUSIONS

The mouflon analysed effectives have had as the starting core a small number of specimens and thus the actual specimens are characterized by genetic abnormalities due to the inbreeding.

In springtime, non-specific enteritis may occur, due to the change of feed and, in some cases, stress. The assessment of health and hygiene is mandatory for the mouflon specimens living in semi-freedom conditions.

The mouflon has remarkable capacity of recovery/healing in case of physical and mechanical trauma. Resilience of specimens that suffered serious injuries (fractures of limbs) is remarkable.

A summary of lesions/injuries of different biological units and systems reveals some macroscopic changes that can be presented in future studies/works of wild ruminant species pathology.

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WARM WINTER OF 2013-2014 IN OLTENIA

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Abstract. In Oltenia as well as in most part of Romania, the winter of 2013-2014 was warm, marked by long intervals of warm weather. After the warm and rainy autumn, in the end of November, a short episode of very early winter was registered, followed by an interval of 55 days of warm weather. In the interval January 25 - February 8, phenomena of severe winter were registered, with snowfalls, blizzards and snow layer, and then the warm weather returned and air temperature increased to 20°C in the middle of February. From a pluviometric point of view, winter was droughty overall. December was excessively droughty, January was very rainy and February was very droughty. At the ground surface, the maximum thermal values registered values specific to the spring months. The ground maintained unfrozen during long intervals of time. Consequently, significant effects affected the autumn crops and the ecosystems in general. The paper is a continuation of some extended studies on climatic variability in the South-West of Romania. It is useful to master graduates and specialists interested in climate evolution and climate changes.

Keywords: temperature monthly means, Hellmann criterion, phenomena of severe winter, vegetative processes.

Rezumat. Iarna caldă 2013-2014 în Oltenia. În Oltenia ca şi în cea mai mare parte a României, iarna 2013-2014 a fost caldă, marcată de intervale lungi de vreme caldă. După toamna caldă şi ploioasă, la sfârşitul lunii noiembrie s-a înregistrat un scurt episod de iarnă foarte timpurie, a urmat apoi un interval de 55 de zile de vreme caldă. În intervalul 25 ianuarie – 8 februarie s-au înregistrat fenomene de iarnă severă cu ninsori, viscole şi strat de zăpadă, apoi vremea caldă a revenit şi temperatura aerului a urcat la mijlocul lunii februarie la 20°C. Din punct de vedere pluviometric iarna a fost secetoasă în ansamblul ei, cu decembrie excesiv de secetoasă, ianuarie foarte ploioasă şi februarie foarte secetoasă. La suprafaţa solului maximele termice au înregistrat valori specifice lunilor de primăvară. Solul s-a menţinut dezgheţat pe intervale lungi de timp. Ca urmare a vremii calde, importante efecte s-au produs asupra culturilor de toamnă şi ecosistemelor în general. Lucrarea este o continuare a unor studii extinse privind variabilitatea climatului în sud-vestul României. Este utilă masteranzilor şi specialiştilor interesaţi de evoluţia climatului şi schimbările climatice.

Cuvinte cheie: medii lunare de temperatură, criteriul Hellmann, fenomene de iarnă severă, procese vegetative.

INTRODUCTION

In the 21st century, so far there were registered the first 13 warmest years in the history. The warmest years were 2005 and 2010, characterized by global average temperature values, which exceeded with 0.55°C the normal mean, followed by 1998, marked by an extremely strong phenomenon called El Nino (OMM documents).

During the entire 2013, weather evolutions in Oltenia, Romania and in the whole European continent were atypical. In Oltenia, we note the following:

- *Weather intense cooling in the interval March 25-28, 2013*, which brought the winter again all over the continent, and in the south-west of Romania (Oltenia) after 84 days of warm weather, more than normal, the snow layer thickness was comprised between 4 and 10 cm on March 28, 2013 at 08 o'clock, being the largest snow layer in Romania on that date.

– *Intense drought in the interval April 10-May 21, 2013*, which lasted for 40 days and damaged spring and autumn crops, causing *gradual enforcements in the South-East of the region at barley crop* in the area of sands from Olt County, which ripened prematurely and damaged the production badly.

– This drought appeared immediately after the *period with pouring rains in the interval April 4-6, 2013*, which caused *floods in some areas in Oltenia* (on the Jiu, the Olt and their tributaries).

– During summer *three important heat waves* were registered, which led to maximum thermal values closed to 40°C (the maximum thermal value of the summer of 2013 was 39.8°C registered on July 29, 2013 at Bechet).

– *In the end of every summer month, intense weather cooling occurred,* which caused the fall of temperature below the normal average values reaching the autumn thermal regime.

– *In the end of September, two days with pouring rains were registered*, in which weather cooled significantly. These led to quantities of precipitation, which exceeded 100 l/m^2, and *September 2013 has been the rainiest month in the entire year*, although, usually, this is the second droughty month after February.

– *Weather cooling in the end of September continued and became more severe in the first days of October 2013 reaching the climax on October 5*, when the minimum air thermal values were comprised between -4.5°C at Voineasa and 0°C in Drobeta Turnu Severin, and at the ground surface between 0°C at Caracal and -4.7°C at Polovragi. This intense cooling destroyed unprotected vegetable crops significantly.

– Afterwards, there has been *a long interval of gradual weather warming* from October 6 to November 25, which made from November a warm month in general, thus prolonging the optimum period of setting up the autumn crops up to November 25 (one month more than its normal).

– *Weather massive cooling, which occurred in the rainy interval November 23-27 and especially in the night of 26/27 November, led to the appearance of the thermal regime specific to December beginning with November 27.*

The snow layers with thicknesses comprised between 3 cm in Târgu Jiu and 22 cm in Craiova and Caracal were the largest in the country on November 28 at 08 o'clock.

– *The atypical weather* continued in December 2013, January, February and March 2014.

The paper is a continuation of some extended studies on the growing climatic oscillations and risks as a consequence of the climatic variability increase in the south-west of Romania, as well of the effects on environment, society and bioclimate in general (BOGDAN et al., 2008, 2010; BOGDAN & MARINICĂ, 2009; MARINICĂ & CHIMIŞLIU, 2008; MARINICĂ et al., 2010, 2011, 2012, 2013; MARINICĂ & MARINICĂ, 2012).

MATERIAL AND METHODS

For this paper we analysed the results of the daily processing with special software from the weather forecast, the data from Oltenia MRC Archive, the current maps from the operative activity, and those on the internet provided by the analysis and forecast international centres and NAM Bucharest (National Administration of Meteorology). We used the facilities provided by Office for drawing the tables and charts.

The paper analyses the climatic conditions during the winter of 2013-2014, on the basis of the thermal and pluviometric regime of December 2013, January and February 2014 and the thermal and pluviometric regime on the whole of the winter of 2013-2014.

RESULTS

1a. The thermal regime of December 2013

In December 2013, *air temperature means* were comprised between -2.6°C at Voineasa in the mountainous area and 0.7°C, and their deviations from the multiannual means[1] were comprised between -1.6°C at Tg. Logreşti and 0.2°C at Plovragi and Bâcleş, and in the mountainous area there were 2.9°C in Parâng.

According to Hellmann criterion, the thermal time type at the meteorological stations in Oltenia was comprised between cool (CO) in Getic Piedmont at Slatina, Tg. Logreşti, Apa Neagră and Tg. Jiu and normal (N) in most part of the region (Table 1).

The air temperature mean for the entire region was -0.4°C, and its deviation from the normal was -0.3°C which according to Hellmann criterion classifies December 2013 as a normal pluviometric month, overall for the entire region.

The air temperature minimum values were comprised between -12.1°C registered at Târgu Logreşti on December 20, 2013 and -6.3°C in Dr. Tr. Severin on December 11, 2013, and the monthly mean for the entire region was -8.7°C. Most of the minimum thermal values were registered on December 11. Consequently, in December 2013, agrometeorological[2] frost was not registered.

The maximum air temperature values were comprised between 7.8°C at Voineasa registered on December 27 and 28 and 14.4°C in Râmnicu Vâlcea, registered on December 27, the monthly mean for the entire region was 11.5°C (Table 1). Most of the maximum thermal values were registered on 27 and 28 December, which constitutes a climatic anomaly.

Frost units[3] in December 2013 were comprised between 19.5°C in Drobeta Turnu Severin and 91.3°C at Voineasa, and their mean for the entire region was 41.7°C, which means a month of mild winter.

The sums of the daily active temperature means[4] were comprised between 9.3°C at Voineasa and 43.7°C at Calafat, their mean for the entire region was 27.8°C, and in the mountainous area was 38.3°C in Parâng. The value in Parâng, comparable to those in the west of the region is due to the high frequency of the phenomenon of thermal inversion in December 2013.

The chart of the air temperature variation in December 2013 presents a slight decreasing tendency for the daily average and minimum temperature values (Fig. 1).

The minimum temperatures at ground surface were comprised between -12.6°C registered at Polovragi on 20 December and -3.7°C at Calafat on December 25, and their monthly mean for the entire region was -8.7°C.

The maximum temperatures at ground surface were comprised between 14.1°C registered in Slatina on December 13 and 19.5°C registered in Drobeta Turnu Severin on the same date, as well as all the other maximum temperature values (excepting Drăgăşani meteorological station).

The monthly mean for the entire region of the maximum temperature values at ground surface was 17.1°C.

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[1] The comparisons are made reported to the multiannual means calculated for the interval 1901-1990, which we consider normal values.

[2] ***The degree of winter bitterness*** in agrometeorology (winter type) classifies according to the sum of frost units (∑ differences between the daily minimum temperature values <-15°C and the agroclimatic critical threshold of -15.0°C, in the interval December - February). Therefore, ***a frost unit*** **is the difference of 1°C between the critical threshold of -15.0°C and an air minimum thermal value ≤ -15°C** (for example for T min = -16.0°C then the difference -15.0°C - (-16.0°C) = 1, namely a frost unit, (SANDU et al., 2010)

[3] Frost units for all the cold season is calculated as Σ of daily average temperatures <0°C, in November-March; A day of frost is the day in which the average temperature is ≤ 0°C;

[4] The active temperatures are those ≥0°C, and the temperature of the biological minimum is 0°C.

Table 1. The regime of air temperature in Oltenia and the minimum and maximum temperature values at ground surface in December 2013.

Meteorological station	Hm	NXII	M	ΔT=M-N	CH	air min T		air maxT		soil minT		soil maxT	
						(°C)	Data	(°C)	Data	(°C)	Data	(°C)	Data
Dr. Tr. Severin	77	1.4	**0.7**	-0.7	**N**	**-6.3**	11	11.4	21	-8.0	4; 6	19.4	13
Calafat	66	1.0	0.6	-0.4	**N**	-8.6	11	11.3	9	**-3.7**	25	15.9	13
Bechet	65	0.4	-0.3	-0.7	**N**	-7.0	21	12.5	21	-8.7	11	16.6	13
Băileşti	56	0.4	-0.3	-0.7	**N**	-7.4	11	11.2	21	-9.5	1	17.7	13
Caracal	112	-0.1	-0.5	-0.4	**N**	-8.1	11	8.3	9	-7.2	1	16.6	13
Craiova	190	0.1	-0.7	-0.8	**N**	-7.7	21	8.8	9	-9.2	1	16.8	13
Slatina	165	0.3	-0.7	-1.0	**CO**	-7.5	11	10.9	28	-4.6	11	***14.1***	13
Bâcleş	309	-0.4	-0.2	**0.2**	**N**	-7.0	11	13.0	21	-	-	-	-
Tg. Logreşti	262	0.1	-1.5	***-1.6***	**CO**	***-12.1***	20	13.9	28	-10.8	20	17.2	13
Drăgăşani	280	0.6	0.5	-0.1	**N**	-8.2	21	13.3	28	-9.7	5	16.4	28
Apa Neagră	250	0.1	-0.9	-1.0	**CO**	-11.8	20	13.4	28	-11.0	20	**19.5**	13
Tg. Jiu	210	0.1	-1.2	-1.3	**CO**	-8.0	4	11.2	27	-8.5	11	17.2	13
Polovragi	546	0.1	0.3	**0.2**	**N**	-9.4	11	12.3	27	***-12.6***	20	16.1	13
Rm.Vâlcea	243	0.5	0.5	0.0	**N**	-8.4	21	**14.4**	27	-9.5	21	19.1	13
Voineasa	587	-1.9	***-2.6***	-0.7	**N**	-9.9	11	***7.8***	27;28	-	-	-	-
Parâng	1585	-3.7	-0.8	**2.9**	**W**	-11.8	11	9.5	18;20	-	-	-	-
Mean Oltenia		-0.1	-0.4	-0.3	**N**	-8.7		11.5		-8.7	-	17.1	-

(Source: Data processed)

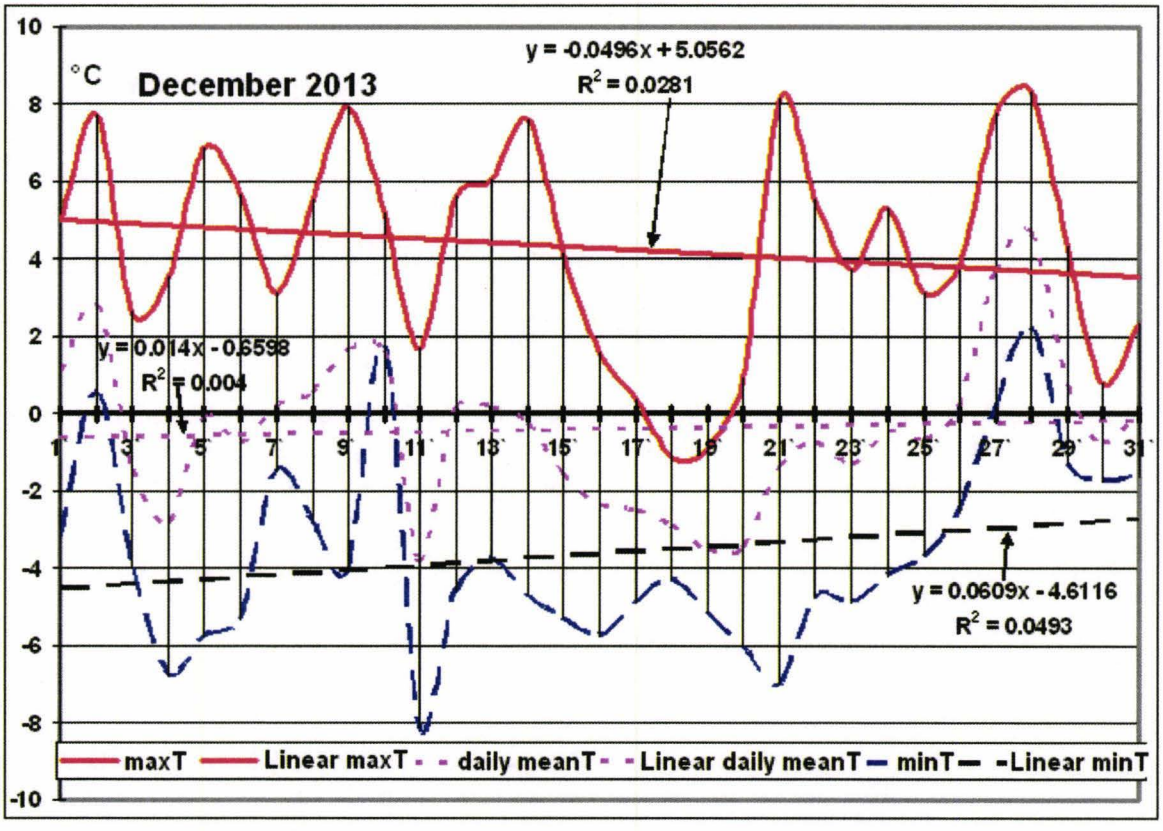

Figure 1. Air temperature variation (daily minimum temperature values, daily average temperature values and daily maximum temperature values) in December 2013. (Source: Data processed).

1.b. The pluviometric regime of December 2013

In December 2013, *the monthly quantities of precipitation* registered were comprised between 0.0 l/m^2 at Târgu Logreşti and Târgu Jiu and 2.9 l/m^2 at Polovragi, and their percentage deviations from the multiannual monthly means were comprised between -100.0% and -94.8%, which according to Hellmann criterion leads to classifications of the pluviometric time type of excessively droughty (ED) in the entire region. This situation is also confirmed by the *monthly mean* for the entire region of 2.8 l/m^2 and the percentage deviation from the normal of -94.5%.

During December, there was not any snow layer excepting the area of Polovragi Subcarpathian Depression, where in the first decade, the snow layer was insignificant or patched in some days.

On December 31, 2013, the water reserve in the ground layer of 0-100 cm in the autumn wheat crop had satisfactory and closed to optimum values, as a consequence of the abundant rains in the autumn of 2013.

Table 2. Quantities of precipitations registered during the winter of 2013-2014 (Σ), in comparison with the normal values.

Meteorological station	Hm	December 2013				January 2014				February 2014				Winter 2013 – 2014			
		ΣXII	N	Δ%	CH	ΣI	N	Δ%	CH	ΣII	N	Δ%	CH	ΣW	N	Δ%	CH
Dr. Tr. Severin	77	1.2	61.2	-98.0	**ED**	70.4	51.4	37.0	**VR**	26.8	47.9	-44.1	**VD**	98.4	160.5	-38.7	**VD**
Calafat	66	0.4	45.5	-99.1	**ED**	***52.2***	40.4	***29.2***	**R**	***4.4***	38.0	**-88.4**	**ED**	***57.0***	123.9	***-54.0***	**ED**
Bechet	65	0.2	36.3	-99.4	**ED**	54.8	33.5	63.6	**ER**	9.2	34.8	-73.6	**ED**	64.2	104.6	-38.6	**VD**
Băileşti	56	0.3	46.8	-99.4	**ED**	63.4	38.5	64.7	**ER**	12.7	36.1	-64.8	**ED**	76.4	121.4	-37.1	**VD**
Caracal	112	0.3	39.5	-99.2	**ED**	63.5	34.7	83.0	**ER**	5.5	34.5	-84.1	**ED**	69.3	108.7	-36.2	**VD**
Craiova	190	0.6	41.8	-98.6	**ED**	81.7	37.5	117.9	**ER**	8.2	30.4	-73.0	**ED**	90.5	109.7	-17.5	**LD**
Slatina	165	0.3	42.8	-99.3	**ED**	80.8	36.0	124.4	**ER**	8.1	38.4	-78.9	**ED**	89.2	117.2	-23.9	**D**
Bâcleş	309	1.1	54.7	-98.0	**ED**	74.1	50.5	46.7	**VR**	30.5	44.1	***-30.8***	**VD**	105.7	149.3	-29.2	**VD**
Tg. Logreşti	262	***0.0***	44.8	***-100***	**ED**	73.0	35.9	103.3	**ER**	17.7	41.0	-56.8	**ED**	90.7	121.7	-25.5	**D**
Drăgăşani	280	0.2	44.6	-99.6	**ED**	64.0	34.1	87.7	**ER**	9.9	35.4	-72.0	**ED**	74.1	114.1	-35.1	**VD**
Apa Neagră	250	1.6	82.3	-98.1	**ED**	**101.5**	70.9	43.2	**VR**	**32.6**	66.4	-50.9	**ED**	**135.7**	219.6	-38.2	**VD**
Tg. Jiu	210	***0.0***	64.0	***-100***	**ED**	86.6	53.9	60.7	**ER**	29.8	52.0	-42.7	**VD**	116.4	169.9	-31.5	**VD**
Polovragi	546	**2.9**	56.1	**-94.8**	**ED**	74.8	48.9	53.0	**ER**	32.0	48.4	-33.9	**VD**	109.7	153.4	-28.5	**VD**
Rm. Vâlcea	243	0.2	46.2	-99.6	**ED**	81.8	35.5	**130.4**	**ER**	18.5	38.4	-51.8	**ED**	100.5	120.1	**-16.3**	**LD**
Parâng	1585	32.3	54.6	-40.8	**ED**	22.4	57.7	-61.2	**ER**	17.1	47.7	-64.2	**ED**	71.8	160.0	-55.1	**ED**
Mean Oltenia		2.8	50.7	-94.5	**ED**	69.7	44	58.5	**ER**	17.5	42.2	-58.5	**ED**	90.0	136.9	-34.3	**VD**

(Source: Data processed).

2a. The thermal regime of January 2014

The monthly thermal means were comprised between -0.1°C at Voineasa and 1.7°C in Drobeta Turnu Severin, and their deviations from the multiannual means were comprised between 2.4°C in Slatina and 4.6°C at Voineasa, leading to the classification of warm month (W) for the entire region, according to Hellmann criterion, excepting the mountainous area where the deviation was of 5.6°C in Parâng, which designates a very warm month (VW) (Table 3). In the intervals January 1-14 and January 16-23, there were registered 22 days in which the daily average temperature for the entire region was positive.

The monthly average temperature calculated for the entire region was 0.4°C, and its deviation from the normal was of 3.2°C, which confirms the classification of warm month (W).

The monthly minimum temperatures were registered in the last day of the month and were comprised between -9.9°C at Voineasa and -15.3°C at Drăgăşani, which shows that the agrometeorological frost was registered only in the last day of the month on restricted and insignificant areas. The air temperature monthly mean was of -12.8°C.

The monthly maximum temperatures were comprised between 11.7°C at Voineasa on January 10, 2014 and 17.1°C in Râmnicu Vâlcea on January 9, 2014, and the monthly maximum temperature mean was of 14.6°C. The monthly maximum air temperature values were registered in the interval January 9-13. Beginning with January 24, weather cooled gradually, and in the last day of the month a moderate cold wave was registered, which continued in the first days of February.

At the ground surface, *the minimum temperature values* were registered in the last day of the month and fell within -6.2°C at Bechet on the Danube Floodplain and -15.0°C at Calafat, and the monthly mean for the entire region was -11.8°C.

The maximum temperature values at ground surface were comprised between 10.3°C in Slatina and 21.4°C at Drăgăşani, and their deviation for the entire region was 16.4°C. *Frost units in January* were comprised between 33.2°C in Drobeta Turnu Severin and 55.6°C at Bâcleş, and their mean for the entire region was 47.5°C, which means a mild winter from an agrometeorological point of view.

Active temperatures[5] were comprised between 35.5°C at Voineasa and 87.1°C in Drobeta Turnu Severin, and their deviation for the entire region was 60.3°C, being higher than that of the frost units, which confirms the positive thermal balance of January.

The chart of the air temperature variation in January indicates linear decreasing tendencies for the three parameters of temperature analysed (daily minimum temperature values, daily average temperature values and daily maximum temperature values), of which the most decreasing one was the maximum temperature with a variation difference of 28.7°C (the lowest maximum thermal values was of -11.6°C registered at Bechet on January 31, and the highest was of 17.1°C registered in Râmnicu Vâlcea on January 9) (Fig. 2).

[5] The temperature values exceeding 0°C, in agrometeorology are active temperatures, and their persistence during some consecutive days establishes the start over of the vegetative processes. The decrease of the air and ground surface temperature below 0°C induces the vegetative sleep.

Table 3. The thermal air regime in Oltenia and the minimum and maximum temperature values at ground surface in January 2014.

Meteorological station	Hm	NI	M	ΔT=M-N	CH	air minT		air maxT		soil minT		soil maxT	
						(°C)	Data	(°C)	Data	(°C)	Data	(°C)	Data
Dr.Tr. Severin	77	-1.1	**1.7**	2.8	**W**	-11.5	31	14.3	11	-10.2	31	19.6	13
Calafat	66	-1.8	0.7	2.5	**W**	-14.0	31	14.9	11	-7.9	31	13.5	13
Bechet	65	-2.2	0.4	2.6	**W**	-13.6	31	15.7	13	**-6.2**	31	18.1	11
Băileşti	56	-2.3	0.6	2.9	**W**	-13.8	31	14.6	11	-13.2	31	15.5	23
Caracal	112	-2.9	0.1	3.0	**W**	-14.4	31	13.9	12	***-15.0***	31	17.8	13
Craiova	190	-2.6	0.2	2.8	**W**	-14.5	31	14.9	12	-13.2	31	17.3	13
Slatina	165	-2.4	0.0	***2.4***	**W**	-14.8	31	14.2	12	-14.4	31	***10.3***	25
Bâcleş	309	-3.0	0.5	3.5	**W**	-11.3	31	14.9	12				
Tg. Logreşti	262	-2.7	0.1	2.8	**W**	-12.8	31	15.9	9	-13.2	31	16.5	9
Drăgăşani	280	-2.2	0.4	2.6	**W**	**-15.3**	31	14.2	12	-14.4	31	**21.4**	13
Apa Neagră	250	-2.6	0.6	3.2	**W**	-11.4	31	15.4	10	-11.0	31	13.8	6
Tg. Jiu	210	-2.6	0.3	2.9	**W**	-10.9	31	14.8	12	-10.4	31	15.2	12
Polovragi	546	-3.2	0.6	3.8	**W**	-13.3	31	15.1	9	-11.9	31	14.4	9
Rm.Vâlcea	243	-2.2	0.9	3.1	**W**	-12.3	31	**17.1**	9	-12.6	31	19.7	12
Voineasa	587	-4.7	***-0.1***	**4.6**	**W**	***-9.9***	31	***11.7***	10				
Parâng	1585	-5.9	-0.3	5.6	**VW**	-10.8	27	12.4	9				
Media Oltenia		-2.8	0.4	3.2	**W**	-12.8		14.6		-11.8		16.4	

(Source: Data processed)

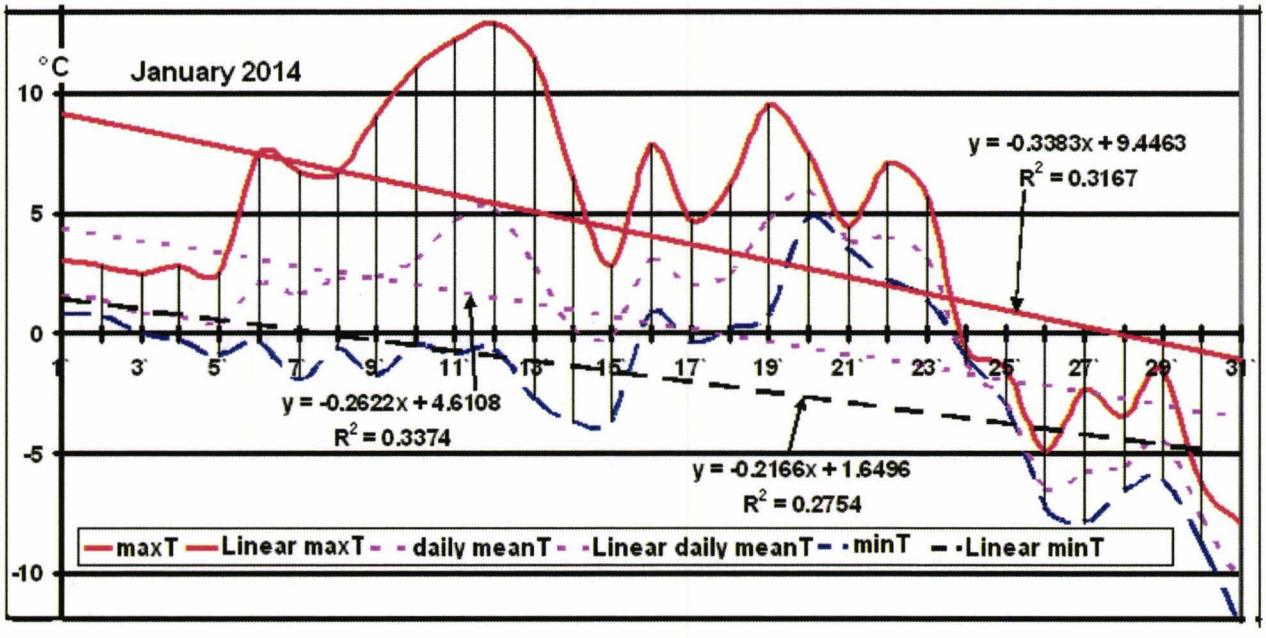

Figure 2. Air temperature variation (daily minimum temperature values, daily average temperature values and daily maximum temperature values) in January 2014. (Source: Data processed).

2b. The pluviometric regime of January 2014

The monthly quantities of precipitations were comprised between 52.5 l/m^2 at Calafat in the South-West of the region and 101.5 l/m^2 at Apa Neagră in Gorj Subcarpathians, and their percentage deviations from the multiannual means were comprised between 29.2% at Calafat and 130.4% in Râmnicu Vâlcea, which according to Hellmann criterion, leads to classifications of pluviometric time type comprised between rainy (R) at Calafat and exceedingly rainy (ER) in most part of the region.

The quantities of precipitation mean calculated for the entire region was 69.7 l/m^2, and the percentage deviation from the normal mean was 58.5% confirming the general characterization of excessively rainy month (ER) (Table. 2). In the interval November 28, 2013 - January 18, 2014 there was registered a number of *52 days of excessive atmospheric drought.*

The precipitation of January occurred after January 19, and in the night of January 24/25, 2014 precipitation turned into snowfall, and thus a snow layer formed.

As *climatic risk phenomena two blizzards were registered* in the intervals January 24, 2014, at 18 o'clock – January 26, 2014, at 22 o'clock and January 29, 2014, at 06 o'clock- January 30, 2014, at 10 o'clock for which a *yellow and orange code warnings* were remitted, and for Muntenia a red code was remitted. In the interval January 29 at 23 o'clock–January 30, at 10 o'clock, for some areas in Muntenia, *a red code warning* was remitted, *for the first time from the entering into force of code warnings.*

The interval with the most significant precipitation was January 24, 2014, at 18 o'clock- January 26, 2014, at 22 o'clock, in which the quantities of precipitation amounted values comprised between 28.9 l/m^2 at Băileşti and 50.7 l/m^2 in Slatina, which compared to the multiannual monthly means represents percentage values comprised between 64.6% at Apa Neagră and 140.8% in Slatina.

The most significant quantities of precipitation were registered on January 25, 2014, and their mean for the entire region was 19.3 l/m^2. *The snow layer* was formed beginning with January 25, and the maximum thickness was registered on January 29, at 20 o'clock when at the meteorological station from Oltenia it was comprised between 29 cm at Băileşti, Bechet and Râmnicu Vâlcea and 66 cm at Olăneşti-Băi (Vâlcea County), and in the mountainous area 85 cm at Obârşia Lotrului (Fig. 3).

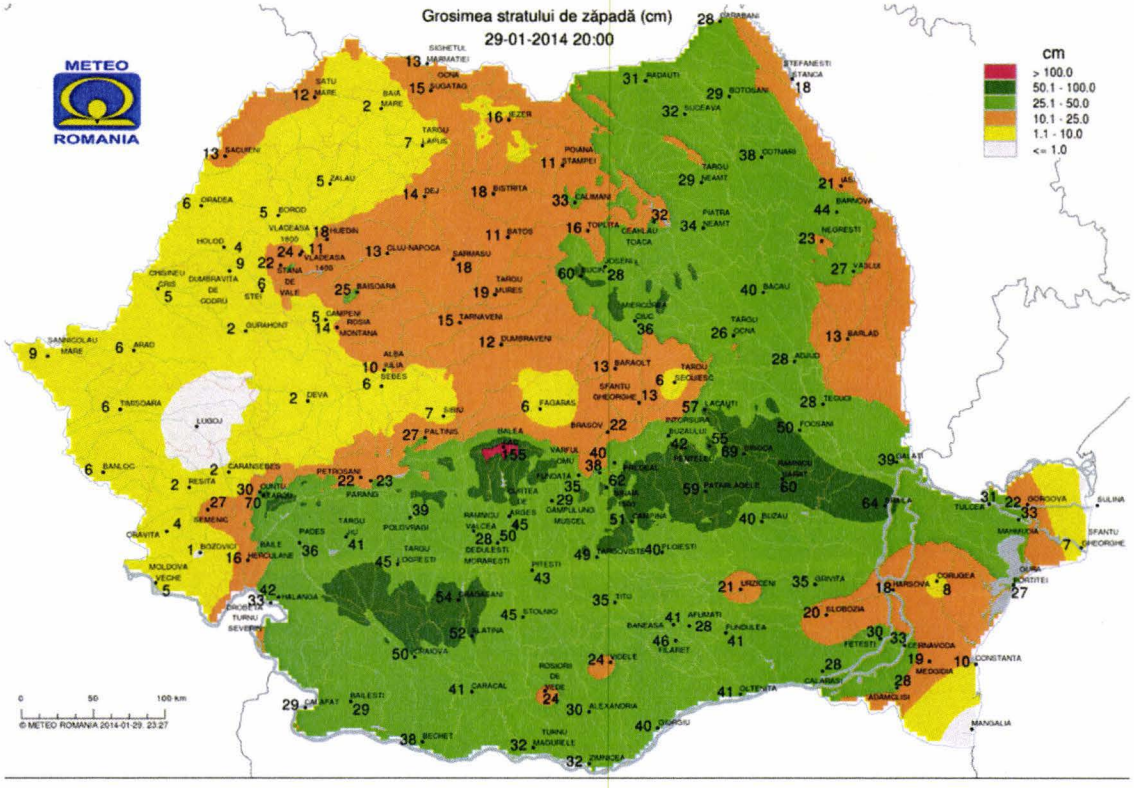

Figure 3. The maximum snow layer in January 2014 (according to NAM Bucharest).

We also note that *Oltenia was the second area in the country (at the southern limit of hills), after the area from Carpathians Curvature, in which the snow layer exceeded the thickness of 50 cm* (excepting the mountainous area). On January 25, 2014, *the maximum quantity of precipitation registered was of 46.8 l/mp in Băbeni and Berislăveşti* (Vâlcea County), which constitutes the maximum quantity of precipitation registered in 24 hours for the whole winter of 2013-2014.

As a consequence of this abundant precipitation in the end of January, in the autumn wheat crop, the water supply in the ground depth of 0-100 cm was optimum. In the interval January 31- February 1, 2014, a moderate frost wave was registered, then the air temperature has increased gradually and in the interval February 1-13, the snow layer melted.

There were no floods due to the snow layer melting, and the water reserve in the ground maintained at an optimum level in the entire region until the end of February.

3a. The thermal regime of February 2014

The monthly average air temperature values were comprised between 0.9°C at Voineasa and 3.2°C in Drobeta Turnu Severin, and the deviations from the multiannual means were comprised between 1.2°C at Calafat and 3.7°C at Polovragi, which according to Hellmann criterion leads to the classifications of the thermal time type comprised between warmish (WS) in most part of Oltenia Plain and warm in the hilly area and Subcarpathians, and in the mountain, in Parâng, very warm (VW) (Table 4).

The phenomenon of thermal inversion, which is frequent in Oltenia, contributed to the registration of low deviations in Oltenia Plain.

Air temperature mean calculated for the entire region was 1.9°C, higher with 2.3°C than the mean of January, and its deviation from the normal was 2.7°C confirming that February was a warm month (W).

The monthly minimum air temperatures were comprised between -18.1°C at Târgu Logreşti registered on February 5 2014 and -10.4°C at Bâcleş registered on February 1, and their monthly mean for the entire region was -12.8°C, equal with the mean in January and with 4.1°C lower than that of December.

The monthly maximum air temperatures were registered on February 17 and were comprised between 16.4°C at Polovragi and 20.3°C at Calafat, and their mean for the entire region was 17.6°C, being the highest value during the whole winter.

The chart of the air temperature variation presents highly linear increasing tendencies (Fig. 4).

Table 4. Air temperature regime in Oltenia and the minimum and maximum temperature values at ground surface in February 2014.

Meteorological station	Hm	NII	M	ΔT=M-N	CH	air Tmin		air Tmax		Tmin soil		Tmax soil	
						(°C)	Data	(°C)	Data	(°C)	Data	(°C)	Data
Dr. Tr. Severin	77	0.9	**3.2**	2.3	**W**	-10.5	1	17.5	17	-11.0	1	23.8	19
Calafat	66	0.4	1.6	***1.2***	**WS**	-12.1	1	**20.3**	17	-8.1	1	16.6	17
Bechet	65	-0.1	1.2	1.3	**WS**	-12.5	1	17.0	17	**-6.1**	1	24.7	17
Băileşti	56	-0.1	1.5	1.6	**WS**	-12.9	1	18.0	17	-13.2	8	**25.0**	17
Caracal	112	-0.7	1.1	1.8	**WS**	-12.1	1;8	18.5	17	-14.7	1	18.6	19
Craiova	190	-0.4	2.0	2.4	**W**	-11.8	1	19.1	17	-13.0	1	19.0	19
Slatina	165	-0.2	1.5	1.7	**WS**	-12.2	7	17.9	17	-15.5	7	***12.9***	19
Bâcleş	309	-0.9	2.4	3.3	**W**	**-10.4**	1	17.0	17	-	-	-	-
Tg. Logreşti	262	-0.7	1.9	2.6	**W**	***-18.1***	5	18.3	17	***-19.8***	5	23.0	19
Drăgăşani	280	-0.2	2.7	2.9	**W**	-11.8	1	18.5	17	-13.6	7	21.0	19
Apa Neagră	250	-0.6	2.7	3.3	**W**	-15.0	5	20.2	17	-15.6	5	21.0	19
Tg. Jiu	210	-0.4	2.6	3.0	**W**	-15.1	5	18.1	17	-16.5	5	23.0	17
Polovragi	546	-1.4	2.3	**3.7**	**W**	-13.2	5	***16.4***	17	-18.0	6	21.8	18
Rm. Vâlcea	243	0	2.9	2.9	**W**	-13.3	5	18.2	17	-16.8	6	**25.0**	17
Voineasa	587	-2.5	***0.9***	3.4	**W**	-13.9	4	17.4	17	-	-	-	-
Parâng	1585	-5.6	0.6	6.2	**VW**	-10.3	2	9.8	17	-	-	-	-
Media Oltenia	-	-0.8	1.9	2.7	**W**	-12.8		17.6	-	-14.0	-	21.2	-

(Source: Data processed)

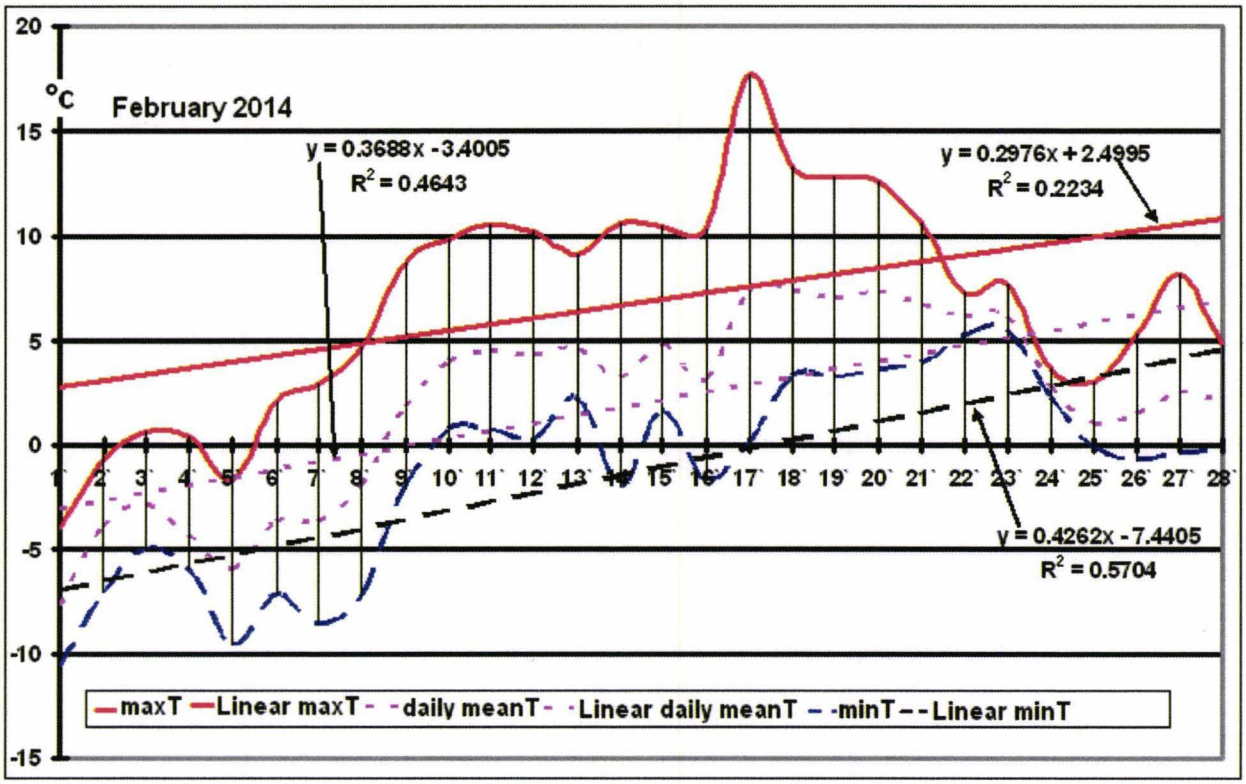

Figure 4. Air temperature variation (daily minimum temperature values, daily average temperature values and daily maximum temperature values) in February 2014. (Source: Data processed).

The most significant interval of warm weather was registered in the interval February 9-21 when the maximum thermal values often exceeded 10.0°C.

The agrometeorological frost was insignificant.

The frost units in February were registered in the interval February 1-9 and were comprised between 25.3°C at Apa Neagră and 46.7°C at Caracal, and their monthly mean was 48.7°C.

Active temperatures were comprised between 67.0°C at Voineasa and 117.7°C in Drobeta Turnu Severin, and their deviation for the entire region was 90.0°C, meaning a warm winter month.

The monthly means of the daily minimum temperatures at ground surface were comprised between -4.8°C at Târgu Logreşti and -0.7°C at Calafat, and the mean calculated for the entire region was -2.9°C.

The monthly minimum temperatures at ground surface were comprised between -19.8°C registered at Tg. Logreşti on February 5 and -6.1°C registered at Bechet on February 5, and their mean was -14.0°C.

The monthly means of the daily maximum temperatures at ground surface were comprised between 5.5°C at Slatina and 10.5°C in Drobeta Turnu Severin, and their mean calculated for the entire region was 8.2°C.

The monthly maximum temperatures at ground surface were comprised between 12.9°C registered on February 19 and 25.0°C registered at Băileşti and Râmnicu Vâlcea on February 17, and their monthly mean calculated for the entire region was 21.2°C.

3.b. The pluviometric regime of February 2014

The monthly quantities of precipitation were comprised between 4.4 l/m^2 at Calafat in the extreme South-East and 42.6 l/m^2 at Apa Neagră in the Subcarpathian area, and their percentage deviation from the multiannual means were comprised between -30.8% in Bâcleş in Mehedinţi Hills and -88.4% in Calafat, which according to Hellmann criterion leads to the classifications of the pluviometric time type in Oltenia meteorological stations comprised between very droughty (VD) and exceedingly droughty (ED) (Table 2).

The general precipitation mean for the entire region was 17.5 l/m^2, and its percentage deviation from the multiannual mean was -58.5%, which confirms that February was exceedingly droughty for the entire region.

There was a *snow layer* in the first decade of the month and has gradually melted, thus February 1 had thicknesses comprised between 25 cm at Bechet and 54 cm at Drăgăşani, on February 11 it became patched, and on 14 February it disappeared completely. The snow layer from Oltenia, as well as in most part of Europe and South-Eastern Europe, contributed to the persistence of low temperatures especially at night in the first 8 days of the month, moderated the spring arrival and contributed essentially to the registration of some spring arrival indexes with moderate values.

The ground water reserve in the end of February (that is in the end of winter) in the autumn wheat crop, the water supply available for plants on the ground depth of 0-100 cm, had satisfactory, closed to optimum and locally optimum values, in all the agricultural areas, due to the slow melting of the snow layer, the low consumption of the vegetal carpet and the low thermal regime compared to the warm season.

The overall thermal regime of the winter of 2013-2014

The seasonal temperature means for the winter of 2013-2014 were comprised between -0.6°C at Voineasa Intracarpathian Depression (the only area with negative values) and 1.9°C in Drobeta Turnu Severin in the extreme West of the region, and their deviations from the multiannual seasonal means were comprised between 1.0°C at Bechet and 2.6°C at Polovragi, which according to Hellmann criterion leads to the classification of warm winter (W) in most of Oltenia meteorological stations, and within Polovragi Subcarpathian Depression and in Parâng the winter was very warm (VW) (Table 5).

Table 5. Overall average thermal characteristics of the winter of 2013-2014.

Meteorological station	Hm	N-meanTwinter	MeanTwinter2013-2014	Δ=meanT-N	CH
Drobeta Turnu Severin	77	0.4	**1.9**	1.5	**W**
Calafat	66	-0.1	1.0	1.1	**W**
Bechet	65	-0.6	0.4	***1.0***	**W**
Băileşti	56	-0.7	0.6	1.3	**W**
Caracal	112	-1.2	0.2	1.4	**W**
Craiova	190	-1	0.5	1.5	**W**
Slatina	165	-0.8	0.3	1.1	**W**
Bâcleş	309	-1.4	0.9	2.3	**W**
Târgu Logreşti	262	-1.1	0.2	1.3	**W**
Drăgăşani	280	-0.6	1.2	1.8	**W**
Apa Neagră	250	-1	0.8	1.8	**W**
Târgu Jiu	210	-1	0.6	1.6	**W**
Polovragi	546	-1.5	1.1	**2.6**	**VW**
Râmnicu Vâlcea	243	-0.6	1.4	2.0	**W**
Voineasa	587	-3	***-0.6***	2.4	**W**
Parâng	1585	-5.1	-0.2	4.9	**VW**
Mean Oltenia		-1.2	0.6	1.8	**W**

(Source: Data processed)

Winter general mean for the entire region was 0.6°C, and its deviation from the normal was 1.8°C, which confirms the fact that the winter of 2013-2014 was warm (W) for the entire region. This general aspect is due to the increase of the general mean because of the high values in January and very high in February.

The interval comprised between January 25 and February 8 is *a severe winter* in which air temperature dropped to extremely low values and there were risk climatic phenomena: snowfalls, blizzards, snow layer, which show that winter lasted only 13 days. However, the risk climatic phenomena have occurred early, since the end of November, when in the interval November 27-30 a snow layer with a thickness of 24 cm was registered at Pieleşti (Dolj county) on 28 November, which rendered some crops vulnerable, unprepared for wintering (especially rape crops) after the warm November in most part of it. This early snow layer melted until December 1, 2013. *The number of frost unit for the entire winter* was comprised between 80.2°C in Drobeta Turnu Severin and 172.0°C at Voineasa with a general seasonal mean of 124.7°C, which from an agrometeorological point of view means a mild winter.

Active temperatures for the entire winter were comprised between 111.8°C in Voineasa Intramountainous Depression and 247.2°C in Drobeta Turnu Severin in the extreme west of the region, and the mean for the entire region was 178.1°C, which confirms the positive thermal balance of the whole winter of 2013-2014, announcing an early spring arrival, and in the mountainous area 131.7°C in Parâng.

4b. Overall pluviometric regime of the winter of 2013-2014

The seasonal quantities of precipitation were comprised between 57.0 l/m^2 at Calafat in the South-West of Oltenia and 135.7 l/m^2 in Apa Neagră Subcarpathian Depression, and their percentage deviations from the multiannual seasonal means were comprised between -54.0% at Calafat and -16.3% in Rm. Vâlcea, which according to Hellmann criterion leads to classification of the pluviometric time type in this winter comprised between little droughty (LD) on restricted areas in Râmnicu Vâlcea and Craiova and exceedingly droughty (ED) at Calafat. The very droughty weather (VD) predominated with a spatial-temporal extension of 60.0%. The mean of the seasonal quantities of precipitation calculated for the entire region was 90.0 l/m^2, and its percentage deviation from the multiannual mean was -34.3% which means a very droughty winter overall for the whole region (Table 2).

DISCUSSIONS

Although *winter phenomena* started early with snowfalls in the night of November 26-27 2013, and the snow layer was dense in November, after a passing weather cooling in the interval November 28-30 2013, weather has gradually warmed. The snow layer disappeared beginning with December 2.

The installation of the positive phase of the North-Atlantic Oscillation led during all December and most part of January (until January 24) a pluviometric regime poor in precipitation (atmospheric drought), and thermally warmer than normal.

Ground water reserve maintained closed to optimum and optimum during almost the entire winter as a consequence of autumn abundant rains, which led in some periods to periods of humidity excess in the ground.

Vernalization[6] occurred especially in the cold season in the interval January 25- February 8, 2014 and in the intervals in which the minimum thermal values dropped below 0°C. With this regard, the temperature increase during winter, the long warm periods and the climate warming, in general, represents a major bioclimatic risk for the vegetal species from our country's latitude.

Consequently, the high temperatures and long warm intervals in the winter of 2013-2014 represented a bioclimatic risk for autumn crops, especially for fruit trees. In the west of the country, especially in Banat temperatures were much higher than in all the winter months and there wasn't any snow layer.

During winter the fruit trees and roses blossomed, and in the end of autumn the strawberries ripened for the second time. In Oltenia, in the end of autumn wheat, barley and rape crops were very developed, and in case of rape the floral stems and bottoms were formed at some plants. Snowdrops blossomed in December.

During winter, in warm periods the phases of plants' development started over, which led to the increase of the vulnerability degree to frost. Most of the parts have been surprised by the snowfalls in the end of November, since they were unprepared for wintering, and weather warming in December improved the climatic conditions by removing the danger of degradations until January 25 when the intense cooling, blizzard and snowfalls represented a new danger.

[6] In most species of plants, positive low temperatures have a significant influence on the initiation and development of reproduction organs. For this purpose, the annual plants need low temperatures in the first growing phases, and the biennial plants remain vegetative in the first year and blossom in the second year, if they are exposed to a treatment with low temperatures. *Vernalization is the acquisition or acceleration of plant's ability to flower by exposure to low temperatures.* According to the requirements for low temperatures plants fall within 3 groups: 1. Autumn annual plants-which begin their vegetation in the end of autumn, resist during winter under the form of seedling and the germinated seeds are vernalized. 2. Biennial plants- which enter in the winter under the form of quite big plants, blossom in the next year and are vernalized in a more advanced growing phase. 3. Perennial plants- which produce sprouts or new ramifications every season and need low temperatures in order to form flowers. This Vernalization process can be explained due to the presence of a hypothetical (vernalin) hormone, which is still unidentified and is formed during Vernalization. This hormone can be transmitted to plants through graft. The Vernalization effect is transmitted through cellular division from one cell to another, and the light has an influence only on sprout meristems with cellular division and Vernalization. This Vernalization process, which has an autoreproduction character of *plants.* (https://sites.google.com/site/biokorinna/botanica/transformarea-circulatia-si-depunerea-substantelor-la-plante, accessed February, 2014).

The thick snow layer protected crops well, and after the snow melting weather fast warming in the interval February 11-28 led to the start over of the vegetation phases. Thus, on February 17, 2014 the buds of stone fruit trees bulbed. The intervals with periods in which there is a passing through the isotherm of 0°C constitute *periods in which plants are prepared for wintering.*

Taking into account the increase of the global average temperature, the winter is expected to become warmer and warmer, plants preparation for wintering may lack in some winters, and the appearance of short periods of intense cooling (as for example, the interval in this winter January 25-February 8, 2014) can cause significant damages for crops, and can seriously affect ecosystems.

The synoptic causes of blizzards in the interval January 25-29, 2014.

The blizzard and abundant snowfalls in the interval 24-26 January were caused by the coupling of a Mediterranean Anticyclone with the Scandinavian Anticyclone. The Mediterranean Anticyclone was formed very quickly, in the thalweg of the Icelandic Cyclone, during 6 hours in the interval January 24, 2014 at 00 o'clock UTC-January 24, 2014 at 06 o'clock UTC, in Genoa Gulf. Its movement above Romania and Balkan Peninsula was performed on Vc classic trajectory. On January 25, 2014 at 00 o'clock UTC the blizzard in the Southern half of Romania was on-going.

The position of baric centres above the European continent, *at ground level,* was the following: the Mediterranean Cyclone was cantered at the ground surface above the south of Italy where the cyclonic nucleus has pressure values below 1000 hPa (Fig. 5). An anticyclone girdle joined the Azores High, Scandinavian Anticyclone and East-European Anticyclone. In the inferior troposphere the circulation of air masses for Oltenia was North-Eastern, causing the advection of a mass of polar continental cold air (cP).

In altitude at the level of 500 hPa there was the geopotential ridge with the value of 548 damgp of the high geopotential field located above the Atlantic Ocean, which extended over the Scandinavian Peninsula sustaining the anticyclone girdle from the ground. In these conditions, for the North-West of Europe, there was a circulation of atmospheric blockage. Downwards this blockage, above Eastern and Central Europe, there was the geopotential thalweg in which a low geopotential nucleus with the isohypse of 536 damgp was visible over the South of Italy that was exactly above the cyclonic nucleus on the ground.

Thus, the vertical axle of the Mediterranean Cyclone in that moment was perpendicular on earth, which indicates the fact that the cyclone was on-going, in its maximum phase. Now, in altitude, the circulation of air masses was Southern, advecting a mass of warm and moist air above the Mediterranean Sea, a maritime tropical mass (mT). On the posterior side of the altitude geopotential thalweg, a mass of cold and moist air was advected, which was recirculated from the Northern seas and was maritime polar (mP).

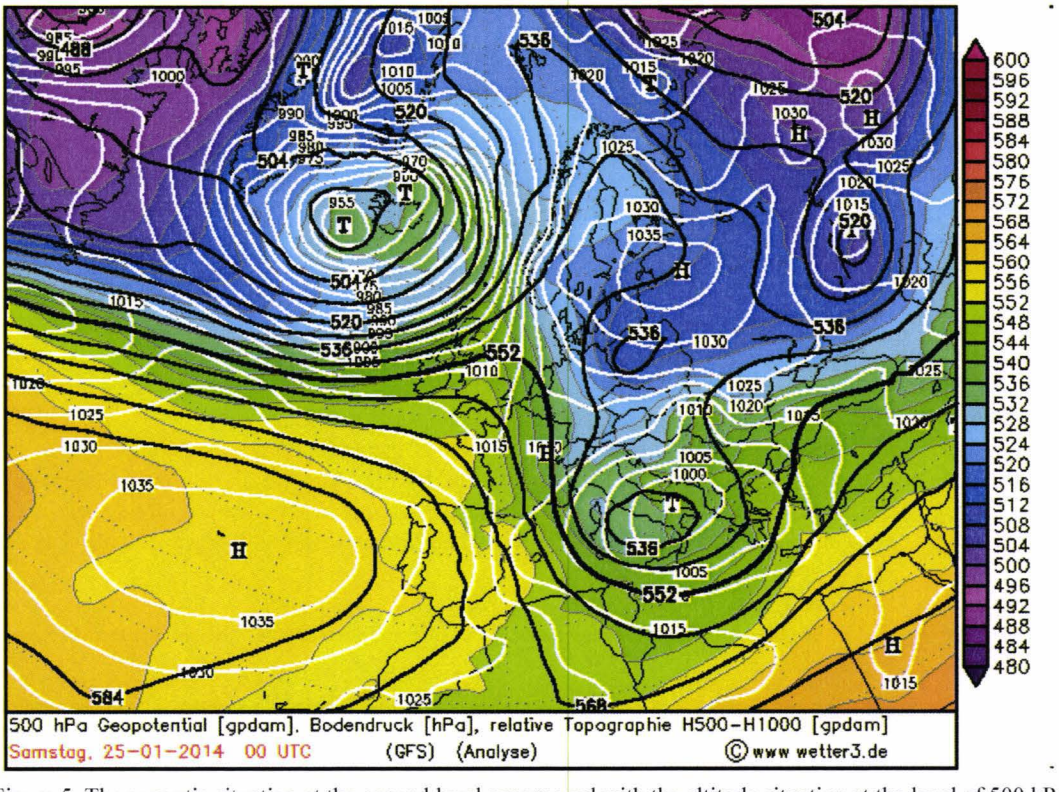

Figure 5. The synoptic situation at the ground level superposed with the altitude situation at the level of 500 hPa and the relative baric topography TR500/1000 on January 25, 2014 at 00 UTC (according to wetter3.de/).

Consequently, the strong Mediterranean Cyclone had a triple supply with air rich in moisture and strong and vast cloudy systems associated to this cyclone caused abundant snowfalls and the blizzard drifted the snow on extended areas in the south of the country. In the counties from the Carpathian Curvature, entire localities were buried in snow, and the drifts of snow reached thicknesses of over 3m in some places, thus the situation in the interval January 26-28, 2012 was repeated, but with a low intensity.

This kind of synoptic situation occurs quite often in the interval January 24-27 and is followed by intense frost waves. According to the old calendar the Epiphany was celebrated around 25 January, and the frost which followed was called in the popular tradition "bitter cold/Epiphany frost". In time, in the last century, for example, these frosts were much more intense.

We mention as example the frost in the interval February 24-29 1942[7] when in the morning of January 25, 1942 the lowest minimum temperatures of the European continent and even on the Northern hemisphere were registered, many of which have remained records up to the present.

These kind of excessively cold intervals occurred after long warm periods, in which plants start the vegetation phase, can cause massive destructions in autumn crops.

CONCLUSIONS

After a very early and cold winter beginning in the interval November 26-December 1, 2014, warm weather in the interval December 2- January 24, 2014 led to the start over of vegetation phases of autumn crops, which even from the end of November were in advanced stages of vegetation, exposing them to the danger of freezing.

The winter of 2013-2014 was warm overall, and in the interval January 25- February 8, winter phenomena were registered, consequently this winter lasted only 13 days.

February has been the warmest month of this winter with thermal maximum values comprised between 16.4°C at Polovragi and 20.3°C at Calafat, and their mean for the entire region was 17.6°C, being the highest in all winter.

Active temperatures in February were comprised between 67.0°C at Voineasa and 117.7°C in Drobeta Turnu Severin, and their mean for the entire region was 90.0°C, meaning a warm winter month.

During the entire winter *active temperatures* were comprised between 111.8°C in Voineasa intramountainous depression and 247.2°C in Drobeta Turnu Severin in the extreme west of the region, and the mean for the entire region was 178.1°C, which confirms the positive thermal balance of the winter of 2013-2014, announcing an early spring arrival, and in the mountainous area it was 131.7°C in Parâng.

From a pluviometric point of view, the winter of 2013-2014 was very droughty, and the *seasonal quantities of precipitation* were comprised between 57.0 l/m^2 at Calafat in the South-West of Oltenia and 135.7 l/m^2 at Apa Neagră Subcarpathian Depression, their percentage deviations from the multiannual seasonal means were comprised between -54.0% at Calafat and -16.3% in Râmnicu Vâlcea, which according to Hellmann criterion leads to classifications of the pluviometric time type in this winter comprised between little droughty (LD) on restricted areas in Râmnicu Vâlcea and Craiova and excessively droughty (ED) at Calafat.

The very droughty weather (VD) predominated with a spatial-temporal extension of 60.0%.

The agrometeorological frost was insignificant, registering only in the Subcarpathian area for short periods of time and with low values of the frost units.

In the last 20 years, the frequency of warm winters has increased and high values of active temperatures and exceptional maximum values were marked, confirming climate warming at a regional level and the extension of Mediterranean climate towards North.

These changes are reflected in ecosystems through their effects and the gradual extension of some species of plants and animals specific to the Mediterranean climate northwards (intense development of fig trees, some Mediterranean plants from meadow vegetation, the extension of the habitat area of golden jackal, etc.). Oltenia climate is in perfect harmony with the European climate.

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THE EFFECT OF SALTS COMPOSITIONS ON THE EXTRACELLULAR AMYLASES ACTIVITY FROM *ACIDIPHILIUM* POPULATIONS ISOLATED FROM MINING EFFLUENTS

CISMAŞIU Carmen Mădălina

Abstract. Increased environmental pollution as a result of mining activities generated an increase of the interest for understanding the mechanisms of the adaptation of microorganisms to these environments and especially for their potential biotechnological application. We isolated two populations of acidophilic heterotrophic bacteria of the *Acidiphilium* genus found in water and sediment samples from the mining sites at Roşia Poieni (Alba county) and Baia (Tulcea county) with increased resistance to metallic ion (Fe^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}). These bacteria were tested for the influence of chloride and sulphate salts on their growth profile and for the extracellular starch degradation activity in the presence of environmental cations (Ca^{2+}, Na^{+}) and anions (Cl^{-}, SO_4^{2-}). Our results indicate significant differences on the adaptation of the two bacterial populations to anions and cations in medium culture, differences correlated with specific conditions in the original sites. The population isolated from Baia has an increased efficiency of starch degradation in the presence of the above-mentioned salts, indicated by a decrease of pH in the culture medium in a relatively short period of time (7-14 days of incubation at 28°C) as a result of organic acids production, efficiency correlated with an increased bacterial growth. Our study illustrates the efficiency of degradation of organic substances under the influence of acidophilic heterotrophic bacteria in the presence of the above mentioned salts.

Keywords: mining effluents, anions, organic substances, amylases.

Rezumat. Efectul compoziţiei sărurilor asupra activităţii amilolitice extracelulare a populaţiilor de *Acidiphilium* izolate din efluenţi minieri. Accentuarea gradului de poluare a mediului în urma exploatării miniere a determinat creşterea interesului pentru mecanismele de adaptare a microorganismelor şi mai ales pentru extinderea potenţialului de aplicaţii biotehnologice a acestora. Probele de ape şi sedimente miniere de la Roşia Poieni (jud. Alba) şi Baia (jud. Tulcea) au fost folosite pentru izolarea de populaţii de bacterii heterotrofe acidofile din genul *Acidiphilium* cu toleranţa crescută la ioni metalici (Fe^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}). Acestea au fost testate cu privire la influenţa clorurilor şi sulfaţilor asupra profilului de creştere şi a activităţii de degradare extracelulară a amidonului în prezenţa cationilor (Ca^{2+}, Na^{+}) şi anionilor (Cl^{-}, SO_4^{2-}) existenţi în situsurile de prelevare a acestora. Rezultatele obţinute au evidenţiat diferenţe semnificative privind adaptarea celor două populaţii bacteriene la prezenţa în mediul de cultură a anionilor şi cationilor, fapt corelat cu condiţiile specifice situsului de origine al probelor. Populaţia izolată de la Baia prezintă o eficienţă sporită a degradării amidonului în prezenţa sărurilor menţionate anterior, observată prin scăderea valorii pH-ului în mediul de cultură într-un timp relativ scurt (7-14 zile de incubare la temperatura de 28°C) ca urmare a producerii de acizi organici, eficienţă corelată cu creşterea celulară a bacteriilor heterotrofe acidofile. Studiul de faţă a evidenţiat creşterea eficienţei procesului de degradare a substanţelor organice sub acţiunea bacteriilor heterotrofe acidofile, în prezenţa sărurilor existente în situsurile menţionate anterior.

Cuvinte cheie: efluenţi minieri, anioni, substanţe organice, amilaze.

INTRODUCTION

Research on ecology of extreme acidic environments revealed significant / considerable diversity among acidophilic microorganisms. These encompass acidophilic bacteria and archaea that perform the oxidation of sulphide ore as well as other microorganisms having synergistic or antagonistic effects on the process. The study of the interaction between acidophilic microorganisms is crucial for the development of mining technologies. Also, ecological studies have opened new horizons for understanding the biochemical processes that occur in the minerals leaching environments (AL-AZKI, 2006; 2010-2011; GONZALES TORIL et al., 2003).

Acidophilic bacteria are present as mixed populations both in natural and man-made environments. In many cases, their presence is indicated by their metabolic products or by biomass accumulation. These bacteria are widespread in the mining areas: ore and acid mine drainages, as well as the neighbouring soil. Acidophilic bacteria from the above-mentioned habitats are either heterotrophic or chemolithotrophic, being involved in the bioleaching, bioaccumulation, bioprecipitation and biofixation of metals. Their presence indicates negative changes in ecosystems, resulting from the activity of pollutants or other disturbing factors before these changes affect more evolved, higher organisms (CISMAŞIU, 2004; GIANFREDA & RAO, 2004; JOHNSON, 2003; SHAFAAT et al., 2011).

During the last decade, the discovery of acidophilic heterotrophic bacteria ability to degrade a variety of organic compounds from the inorganic synthesis products contributed to the development of bioremediation technologies. In order to estimate the contaminants biodegradability and to specify the result of a bioremediation strategy, as well as the potential risks to human health associated with the transport of contaminants in soil, it is crucial to understand the various processes affecting organic substances (CARLSON, 1998; DAS et al., 2010; JOHNSON, 2012).

It is also very important to understand the mechanisms through which acidophilic microorganisms are involved in metal accumulation, especially for the development of these microbial processes of concentration, removal or recovery of metals from aqueous solutions. Therefore, understanding the chemical and physiological reactions that take

place during the accumulation of metal ions may lead to a more effective efficient control of the process parameters in order to increase the retention rate and specificity of metals accumulation (BOND et al., 2000; SINGH et al., 2011).

It is widely accepted that the chemical and physiological reactions during retention of metal ions depend on the physiological conditions of microbial cells, the metals chemical state during interaction with cells and these are strongly influenced by the environment (JOHNSON, 2012; RAJVINDER et al., 2012).

Understanding the metabolic activity of the acidophilic heterotrophic bacteria represents a landmark in establishing the influence of physico-chemical conditions on the processes of metals sorption through their action on the degradation of organic substances in the environments polluted with metallic ions. Depending on the variation of essential parameters in their natural habitat (temperature, pH, degree of humidity concentration of O_2 and CO_2, Eh and metallic ions), morphological and physiological changes of indigenous acidophilic bacteria occur (CISMAŞIU, 2004; GIANFREDA & RAO, 2004).

In this context, due to the influence of physico-chemical factors on the development of acidophilic heterotrophic bacteria from the *Acidiphilium* genus, our study presents the effects of cations and anions on the starch extracellular degradation activity in the presence of these microorganisms.

MATERIAL AND METHODS

1. Microbial population and media

The investigated bacterial populations in this study (noted P_4 and P_7) were isolated from waters and mining sediment samples resulted from industrial activity in Baia (P_4) and Rosia Poieni (P_7) areas. Baia village is located in Tulcea county, situated approximately 300 km east of Bucharest and Rosia Poieni village is located in Alba county, approximately 280 km north-west of Bucharest. The investigated sites are characterized by high concentrations of metallic ions and isolated populations containing strains belonging to the *Acidiphilium* genus. The populations were isolated on GYE solidified culture media having pH value 3.0 before autoclaving. The medium has been solidified by using 20 g/l agar. When the liquid form of culture medium was used in experiments the growth of heterotrophic population of the *Acidiphilium* genus was recorded after three weeks of incubation at 28^0C. The growth of population has been demonstrated also by decreasing of pH value of GYE medium if compare with the initial value of 3.0 (HIRAISHI & IMHOFF, 2005).

2. Physical parameters and experimental conditions

The experiments have been conducted in 100ml volume Erlenmeyer flask, containing 50ml of culture medium related to each experimental variants (described in Table 1) and five ml of inoculums.

Table 1. Experimental variants.

Exp. no.	Medium composition
1.	GYE medium + 3g/l starch + 0.1% $CuCl_2$ + 0.1% $MgCl_2$
2.	GYE medium + 3g/l starch + 0.1% $CuCl_2$ + 0.1% $MgCl_2$ + 0.1% $CaCl_2$
3.	GYE medium + 3g/l starch + 0.1% $CuCl_2$ + 0.1% $MgCl_2$ + 0.1% NaCl
4.	GYE medium + 3g/l starch + 0.1% $CuSO_4$ + 0.1% $MgSO_4$
5.	GYE medium + 3g/l starch + 0.1% $CuSO_4$ + 0.1% $MgSO_4$ + 0.1% Ca SO_4
6.	GYE medium + 3g/l starch + 0.1% $CuSO_4$ + 0.1% $MgSO_4$ + 0.1% Na_2SO_4

The culture growth has been conducted for seven, 14 and 21 day. The temperature conditions during all experiments were 28^0C and the microbial culture were shacked at moderately value of 150rpm. In order to estimate the microbial growth, the optical density at 660 nm has been recorded spectrophotometrically.

3. Amylases activity assay

The starch degrading activity has been evaluated following the Wohlgemuth method (GUPTA et al., 2003). Regarding the study of raising the efficiency of the starch degradation process, the experiments were accompanied by chemical controls and biological controls (the P_4 population with low resistance to metallic ions).

The starch degradation by the extracellular enzymatic activity is influenced by the physiological characteristics of the acidophilic heterotrophic bacteria and was measured spectrophotometrically at 580nm using an Analytic Jena equipment.

RESULTS

To better understand the degradation mechanisms of organic materials from environments subject to metal ions contamination, we performed a comparative analysis of the influence that salts composition in mining effluents have on the amylolitic activity of heterotrophic acidophilic bacteria belonging to genus *Acidiphilium*. To this end, we isolated two bacterial populations from waste waters and mining sediments from Baia and Rosia Poieni, two highly polluted industrial regions where the metal ions concentration is much higher than the current accepted international standards.

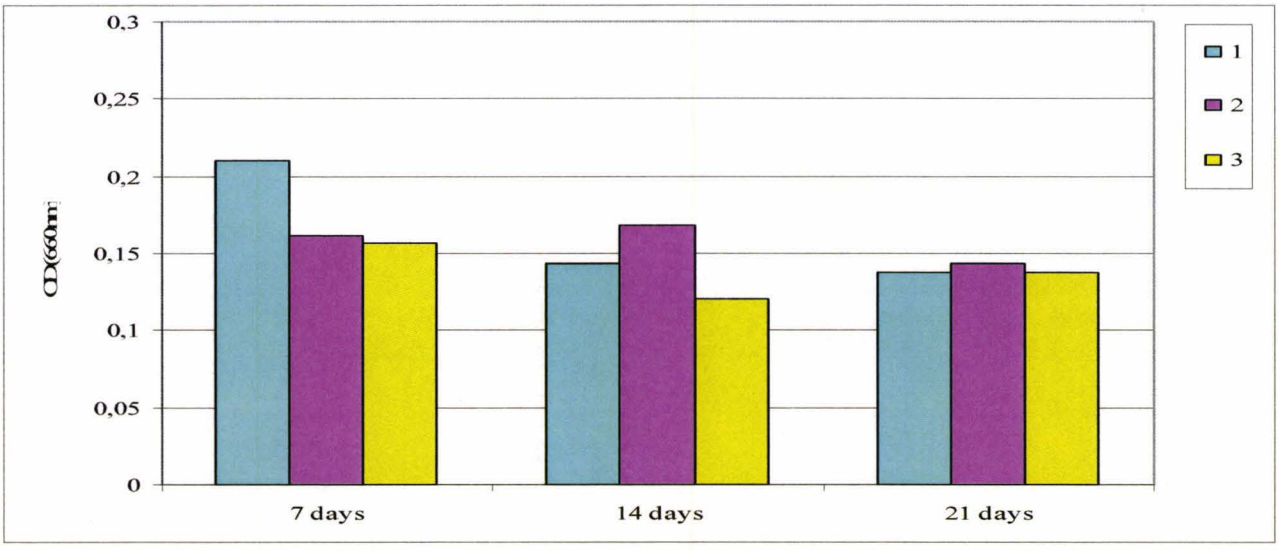

Figure 1. The influence of chloride from salts compositions on the growth profile of P_4 population.
The experimental conditions 1, 2, 3 are described in Table 1.

Data shown in figure 1 indicated that the growth of the microbial population P_4 from Baia site was less sensitive to the cations from salts (chloride) in culture medium. Thus, after seven days of incubation, in condition 1 (presence of Cu^{2+} and Mg) we recorded an optical density around 0.2 at 660 nm. A relatively similar value was observed in conditions 2 (containing calcium ion additionally to condition 1) and 3 (containing sodium), namely around 0.15 at 660 nm.

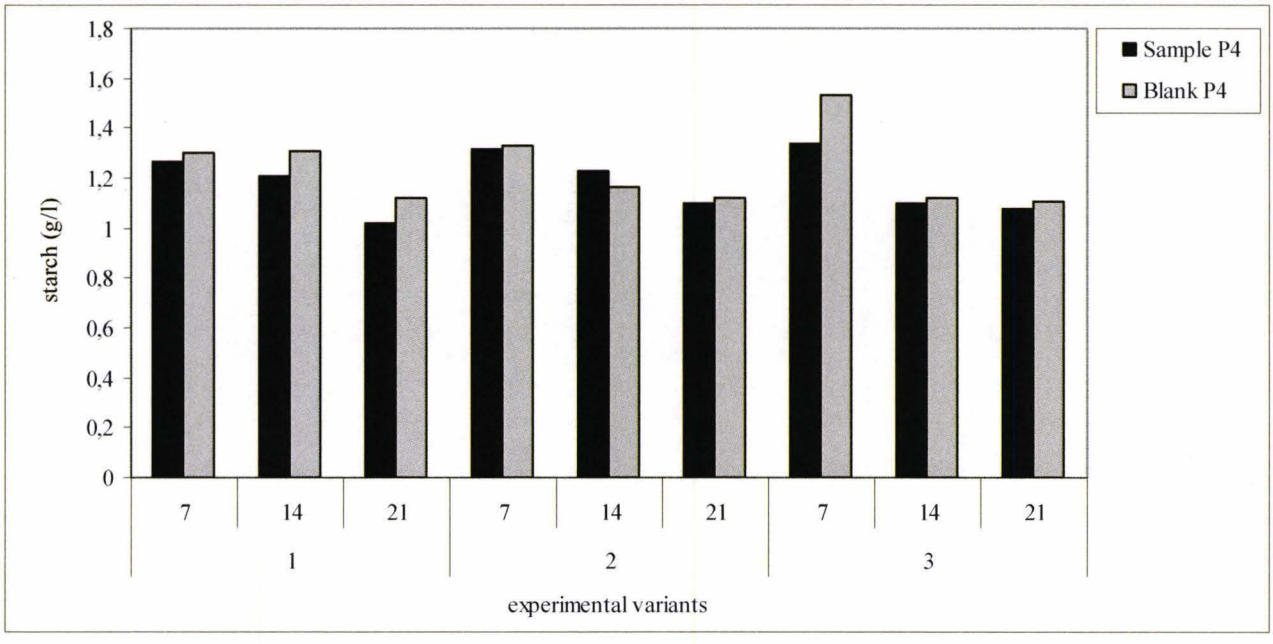

Figure 2. The influence of chloride from salts compositions on the extracellular amylases activity of the P_4 population.
The experimental conditions 1, 2, 3 are described in Table 1.

We further observed slightly differential effects induced by the presence of sodium and calcium on the growth kinetics of the microbial population P4: while calcium stimulated bacterial growth during the first 14 days, sodium had an adverse effect. On the contrary, in condition 1 and 3 the growth was diminished. After 21 days of growth, similar profiles were recorded under all conditions, with values close to the data obtained at 14 days. One exception was condition 3 where the presence of sodium appears to slightly stimulate the microbial population growth (Fig. 1).

Data showed in figure 2 revealed that extracellular amylase activity of the microbial population P_4 has a similar behaviour at seven, 14 and 21 days of cultivation. In condition 1 (absence of sodium and calcium chlorides), the activity values were higher and the presence of calcium (condition 2) and sodium (condition 3) induced a slight decrease in activity (Fig. 2).

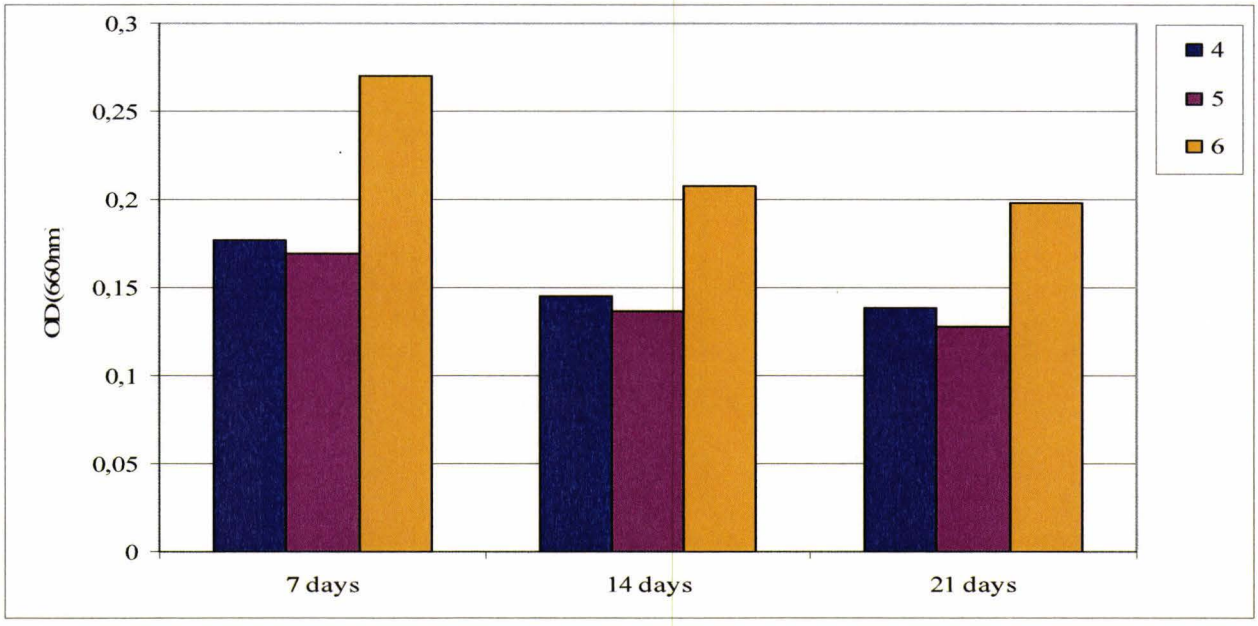

Figure 3. The influence of sulphates from salts compositions on the profile growth of the P_4 population. The experimental conditions 4, 5, 6 are described in Table 1.

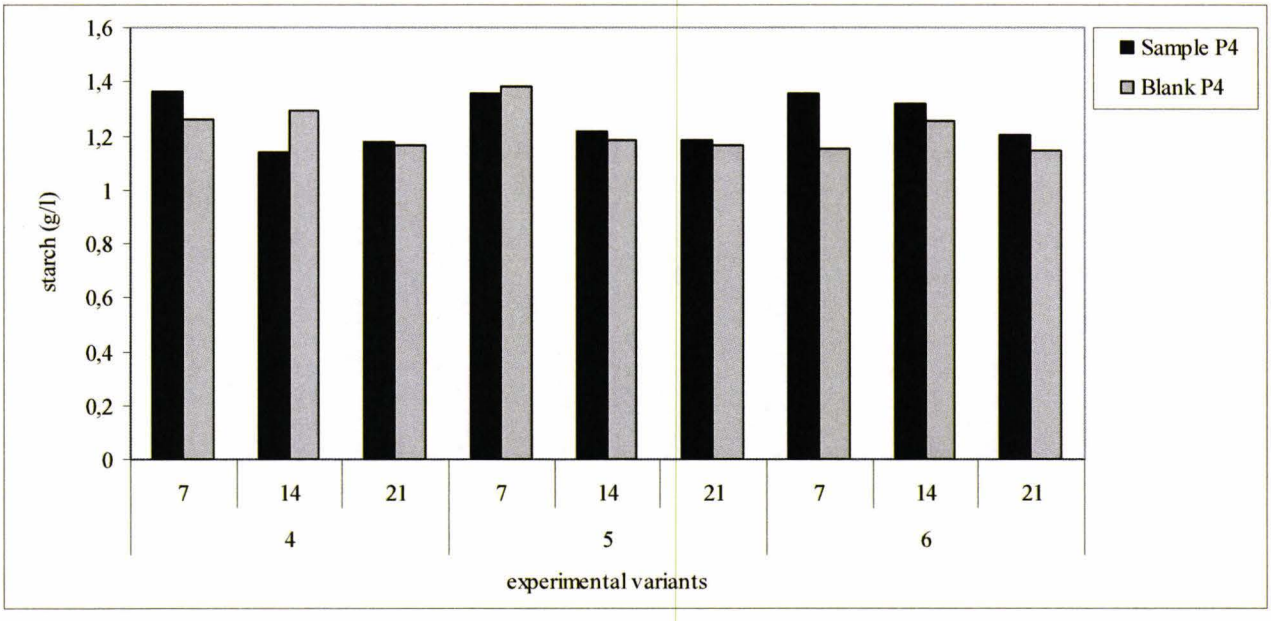

Figure 4. The influence of sulphates from salts compositions on the extracellular amylases activity of the P_4 population. The experimental conditions 4, 5, 6 are described in Table 1.

Figure 3 shows how the presence of cations as sulphates salt in culture medium influences the growth of the microbial population P_4. Thus, the best growth was obtained in the presence of sodium sulphate (condition 6) after seven days of incubation.

When culture medium contained sodium sulphate, after 14 and 21 days of cultivation the growth of population P_4 decreased. Similar behaviours were observed in other experimental conditions, namely the presence of calcium sulphates (5) and absence of sodium and calcium sulphates (4).

The extracellular amylases activity measured in the presence of sulphates in culture medium (Fig. 4) of the microbial population P_4 revealed a similar profile compared to the activity in the presence of chlorides (Fig. 2). Thus, the maximum values are recorded in the absence of sodium and calcium at seven days of growth.

The data from figure 5 reveals that growth of microbial population P_7 (isolated from Roşia Poieni) is more strongly affected by the chlorides in culture medium. Similarly to the population P4, the most effective growth was observed in the absence of calcium and sodium chloride at seven days of cultivation (Fig. 5). After this period, the growth is diminished excepting the growth condition 3 (presence of sodium chloride) at 14 days of cultivation (Fig. 5).

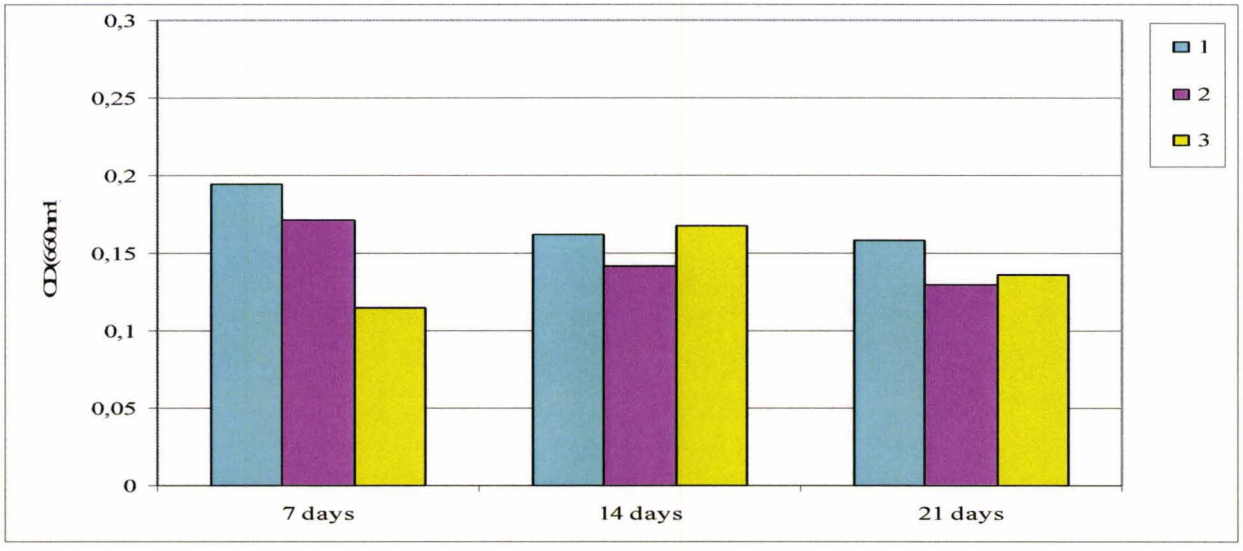

Figure 5. The influence of chloride from salts compositions on the profile growth of the P_7 population. The experimental conditions 1, 2, 3 are described in Table 1.

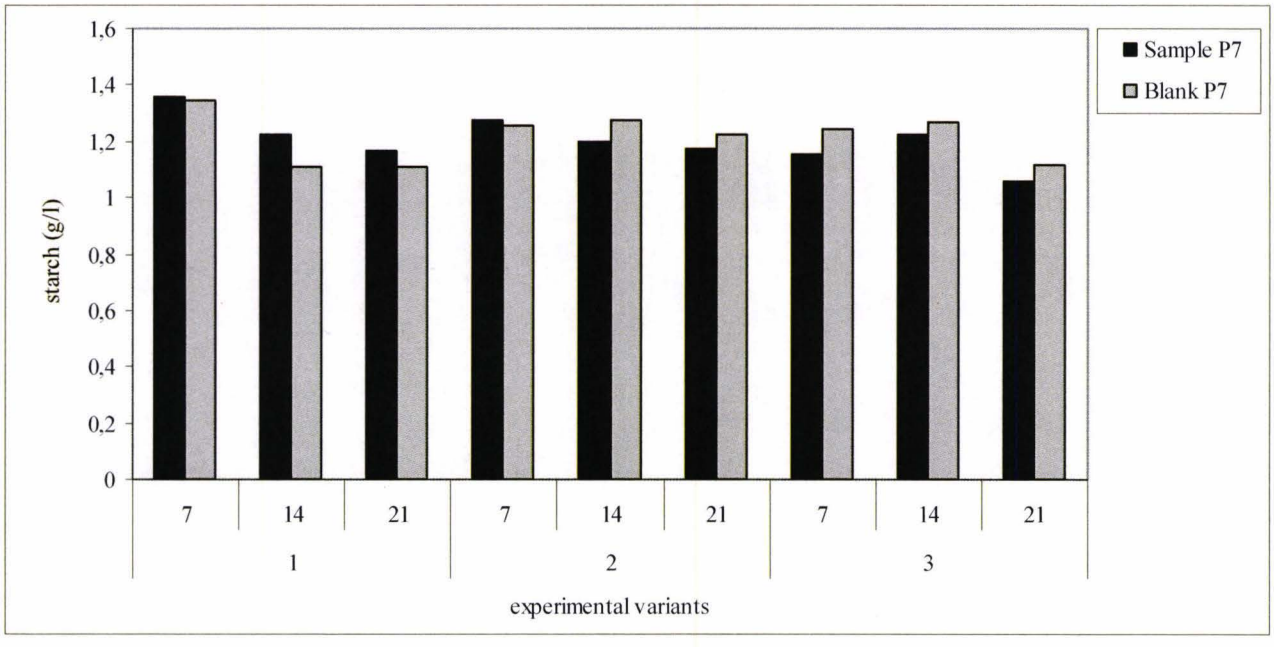

Figure 6. The influence of chloride from salts compositions on the extracellular amylases activity of the P_7 population. The experimental conditions 1, 2, 3 are described in Table 1.

Similar results were observed for the amylase activity in the presence of chlorides in culture medium for the microbial population P_7 if compare with data from figures 2 and 4. The high values are noted in the absence of sodium and calcium and the activity decreases with incubation time (Fig. 6). One exception could be observed at 14 days of incubation in the presence of sodium chlorides.

The results showed in figure 7 reveal that sulphates have a different effect on the growth of the microbial population P_7 if compared to the microbial population P_4 (Fig. 3). Thus, comparative studies about the profile growth of the microbial population P_7 is good in the absence of sodium taking into account that no influence was observed when comparing with data recorded for the experimental conditions 4, 5 and 6 in figure 7. A slight influence of sodium sulphates on the growth of the microbial population P_7 could be regarded as an exception (Fig. 7).

The extracellular amylase activities of the microbial population P_7 follow the behaviour recorded in the case of the presence of chlorides in culture medium (Fig. 6) or similar with the population P_4 (Figs. 2 and 4). Similar behaviours of amylase activity in the presence of various ratios between sodium and magnesium have been previously reported from halophytic archaea isolated from various natural salt lakes (ENACHE et al., 2009).

The literature data revealed that such kind of microorganisms survive in harsh environments where they exist developing a variety of strategies (ENACHE et al., 2001; ENACHE & FAGHI, 1999; GIANFREDA & RAO, 2004).

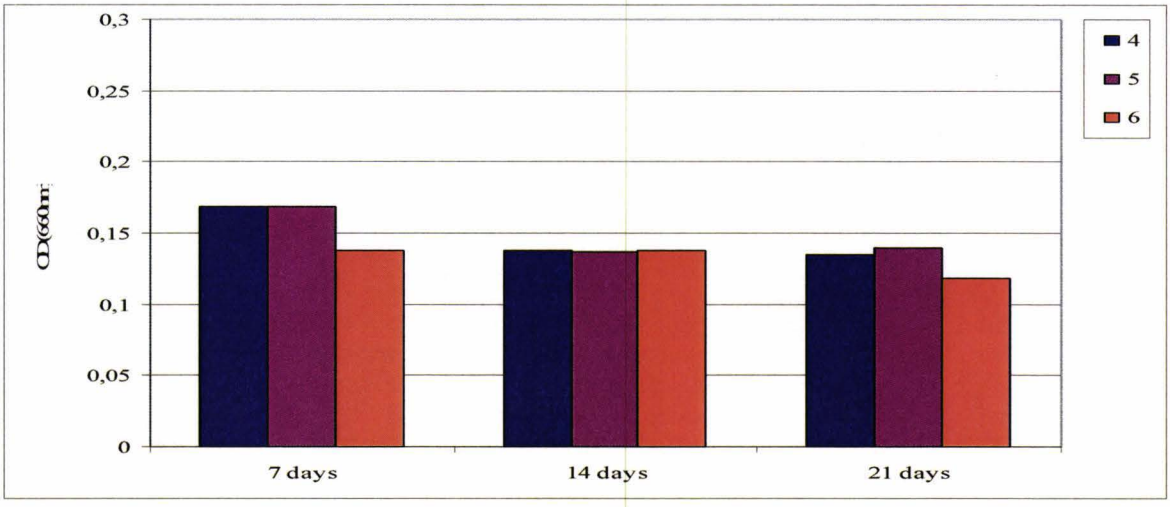

Figure 7. The influence of sulphates from salts compositions on the profile growth of the P_7 population. The experimental conditions 4, 5, 6 are described in Table 1.

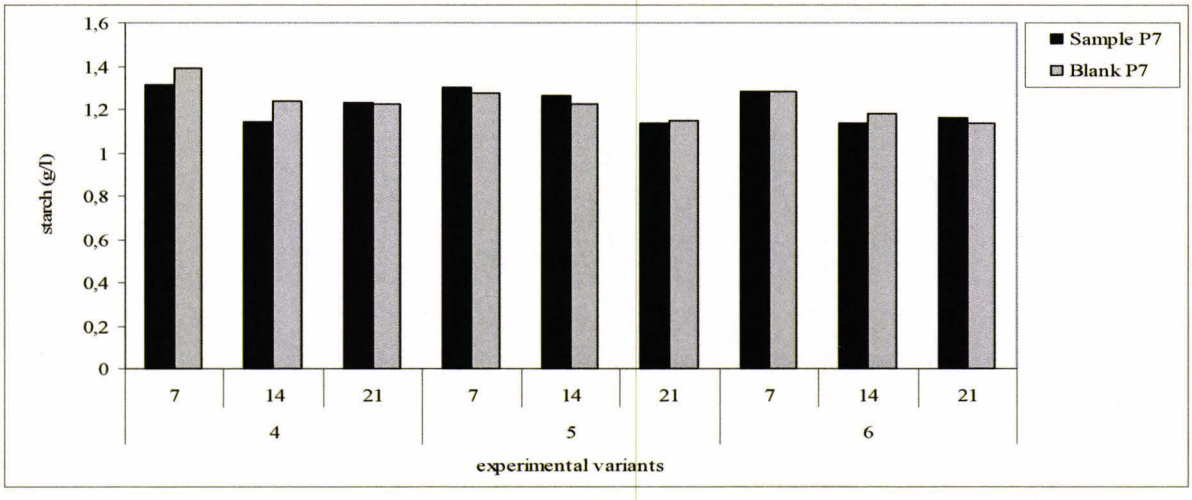

Figure 8. The influence of sulphates from salts compositions on the extracellular amylases activity of the P_7 population. The experimental conditions 4, 5, 6 are described in Table 1.

The enzyme of these types of extremophilic microorganisms are able to cope with high ionic strength in the presence of salt mixtures, and their stability in harsh pH conditions could constitute an advantage for exploitation in several biotechnologies destined to environmental protection and historically polluted area decontamination (AL-AZKI, 2010-2011; DAS et al., 2010; ENACHE et al., 2001; GIANFREDA & RAO, 2004).

CONCLUSIONS

Our results bring an important contribution to understanding the correlation between specific environmental conditions and corresponding bacterial strains.

The two bacterial strains have different growth profiles and different sensitivity to chloride and sulphate from salts. Sulphate salts stimulates growth of the microbial population from Baia site (P_4) from an initial 70% increase to 50% after longer periods of time (21 days).

On the contrary, chloride has a moderate influence on bacterial growth in the case of P_4 population (25-30% after 7 days) and the effect is not maintained after 21 days. Also, our results show a different growth pattern of P_4 population in environments with different cations: while $CaCl_2$ slightly stimulates growth after 14 days, NaCl has an inhibitory effect.

On the other hand, P_7 population from Roşia Poieni site has an improved growth in the presence of chloride compared to sulphates although the effect is lost after longer times (21 days). Comparative analysis regarding the influence of cations on the P_7 population growth profile shows a stimulative effect of Na^+, especially after 14 days (30%).

Continuous agitation conditions in the presence of 3g/l starch induce an increased efficiency of extracellular amylases from P_4 population when chloride is present in the environment, while sulphate salts have a moderately

inhibitory effect. In case of chloride, extracellular amylolitic activity is stimulated by the presence of calcium ions in the culture medium, when compared to sodium ions.

When studying the influence of salts (sulphates and chlorides) on starch degradation, we observed a stimulation of enzymatic activity in the presence of P_7 population, which reflects the adaptation of these bacteria to the culture conditions.

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TAXONOMY OF A NOVEL EXTREMELY HALOPHILIC ARCHAEON BELONGING TO GENUS *HALOARCULA* ISOLATED FROM A LOW SALINE ENVIRONMENT, TECHIRGHIOL LAKE, ROMANIA

ENACHE Mădălin, COJOC Roxana, ECHIGO Akinobu, MINEGISHI Hiroaki, ITOH Takashi, KAMEKURA Masahiro

Abstract. This paper deals with the polyphasic taxonomy of an extremely halophilic strain, T1/2S95 isolated from Techirghiol Lake, Romania, a low saline environment. The strain T1/2S95 was yellow to orange pigmented, stained Gram-negative, and was able to hydrolyse Tween80, growing between 1 and 5.2 M sodium chloride. The membrane polar lipids were PGS, PGP-Me, PG, and two glycolipids, DGD-1 and S-DGD-1. Genomic DNA G+C content was 65.1 mol%. According to the analysis of 16S rRNA gene sequence (AB469844) in BLAST, the strain showed the highest degree of similarity with *Haloarcula japonica*, a haloarchaeon in the family *Halobacteriaceae*. The strain has been deposited to Japan Collection of Microoganisms (RIKEN, BioResource Center) as JCM 19933. The strain could be considered to represent a novel species of the genus *Haloarcula*.

Keywords: halophilic archaea, salt lakes, hypersaline environments, Techirghiol lake.

Rezumat. Taxonomia unui arheon halofil aparţinând genului *Haloarcula*, izolat din Lacul Techirghiol, România. Lucrarea abordează caracterizarea taxonomică polifazică a unui haloarheon, respectiv tulpina T1/2S95 izolată din Lacul Techirghiol, România, un mediu caracterizat printr-o salinitate scăzută. In baza comparaţiilor în baza de date BLAST, tulpina arată un grad mare de înrudire cu *Haloarcula japonica*. Secvenţa genică aferentă 16S ARNr provenind de la tulpina haloarheană investigată a fost depozitată la banca de gene DDBJ având numărul de acces AB469844 iar exemplarul de tulpină tip s-a depozitat la Colecţia de Microorganisme a Japoniei (RIKEN, Centrul de Bioresurse) unde are numărul de acces JCM 19933. Rezultatele obţinute în cadrul acestui studiu conduc către concluzia că tulpina T1/2S95, Gram-negativă, pigmentată, capabilă să hidrolizeze Tween80 şi să crească pe medii de cultură conţinând clorură de sodiu de la 1M până la 5.2M, având profilul lipidic membranar alcătuit din PGS, PGP-Me, PG, DGD-1 şi S-DGD-1, precum şi un conţinut în baze azotate G+C al ADN de 65.1 mol%, izolată din Lacul Techirghiol, România, poate fi considerată ca specie nouă pentru ştiinţă aparţinând genului *Haloarcula*.

Cuvinte cheie: arhee halofile, lacuri sărate, medii hipersaline, Lacul Techirghiol.

INTRODUCTION

Halophilic microorganisms belonging to the *Archaea* domain are spread in all investigated saline and hypersaline environments distributed in the entire world such as salt waters, saline soils, salt lakes, salterns and salt mines (OREN, 2013). Physiological and biochemical investigations revealed that such kind of microorganisms cope with the high ionic strength of the salty environments by either compatible solutes or salt-in strategies (OREN, 1999). The particular features of the halophilic microorganisms made them model for investigations in astrobiology (DasSARMA, 2006), taking into account that some halophiles were identified in a primary salt crystal brine dated around 250 million years old (VREELAND et al., 2000) and sodium chloride (salt) has been detected in Martian origin meteorites (GOODING, 1992). The abundance of microorganisms either bacteria or archaea in saline environments with low pH or high pH values (MINEGISHI, 2013), revealed by increasing numbers of publications in recent years, supported the high interest in the characterization and description of novel microbial strains inhabiting niches considered hostiles to normal live conditions.

In Romania, a lot of salty areas could be found as salt lakes, salt mines or saline soils harbouring halophilic archaea able to hydrolyse several polysaccharides or proteins (NEAGU et al., 2014). Among the saline and hypersaline environments in Romania, Techirghiol Lake located near the Black Sea coast is characterized by variable concentrations of salt depending on site, ranging from negligible salinity up to 60 g/L (AONOFRIESEI, 2007).

Techirghiol lake is relatively well known in terms of biological communities, biodiversity and of salinity regime, being quite well investigated for many years either from relatively high importance of therapeutic properties of the medicinal (sapropelic) mud present in the lake (ŢUCULESCU, 1965) or towards understanding of the dynamics of putative moderately halophilic microbial community (DUMITRU et al., 1996). The unique biodiversity of this area led to conclusions to include Techirghiol Lake in the European network Natura2000 in order to guarantee the protection and support for conservation status of the area (IOJĂ et al., 2010). The intensive use of medicinal mud extracted from this lake for the treatment of several diseases, mainly in rheumatology (DEMIRGIAN et al., 2012; IONESCU et al., 2012; PROFIR et al., 2012; SURDU et al., 2012) supported the significance and importance of the investigations in the area to characterize archaeal and bacterial populations. Previous investigations revealed that Techirghiol Lake harbour either moderately halophilic bacteria able to decontaminate some pesticides (ONCESCU et al., 2007) or some extremely halophilic archaea (ENACHE et al., 2009).

This paper deals with the polyphasic taxonomy of a haloarchaeal strain, T1/2S95 isolated from Techirghiol Lake. According to the analysis of 16S rRNA gene sequence in BLAST, the strain showed a high degree of similarity

with *Haloarcula japonica*. Torreblanca et al. described the genus *Haloarcula* in 1986, with *Haloarcula vallismortis* as the type species. From that time many new species were described, namely *Har. amylolytica* (YANG et al., 2007), *Har. argentinensis* (IHARA et al., 1997), *Har. hispanica* (JUEZ et al., 1986), *Har. japonica* (TAKASHINA et al., 1990; 1991), *Har. marismortui* (OREN et al., 1990), *Har. salaria* (NAMWONG et al., 2011), *Har. tradensis* (NAMWONG et al., 2011), *Har. vallismortis* (GONZALEZ et al., 1979; TORREBLANCA et al., 1986), *Har. quadrata* (OREN et al., 1999). The 16S rDNA sequence of the investigated strain has been deposited in DDBJ with accession number AB469844 and the type strain has been deposited in the Japan Collection of Microorganisms (RIKEN, BioResource Center) having access number JCM 19933.

MATERIALS AND METHODS

The strain T1/2S95 has been isolated from a water sample from Techirghiol Lake in a medium containing per litre 125 g NaCl, 160 g $MgCl_2 \cdot 6H_2O$, 5 g K_2SO_4, 0.1 g $CaCl_2 \cdot 2H_2O$, 1 g peptone, 2 g soluble starch, and 1 g yeast extract. The pH of the medium was 7.0 – 7.2 before autoclaving. Further experiments for the characterization of the isolate were performed using the strain cultivated on JCM medium no. 168 which contained per litre: Bacto casamino acids 5 g, Bacto yeast extract 5 g, sodium glutamate 1 g, trisodium citrate 3 g, $MgSO_4 \cdot 7H_2O$ 29.5 g, KCl 2 g, NaCl 175.5 g, $FeCl_2 \cdot 4H_2O$ 0.036 g and $MnCl_2 \cdot 4H_2O$ 0.36 mg. The medium pH was also 7.0-7.2 before autoclaving. When necessary, the media were solidified by adding 20 g/l agar. Generally, the strain was cultivated at 32^0C – 37^0C, in the absence of light, with moderate agitation at 250 rpm, in 100 ml culture volume containing 10 ml inoculum and 90 ml culture medium.

The characterization of the strain T1/2S95 followed the proposed minimal standards in order to describe a new haloarchaeal strain in the order Halobacteriales (OREN et al., 1997). The classification of the strain T1/2S95 to the archaea has been evaluated in the above-described media containing 0.25 g/l taurocholic acid sodium salt or 20 mg/l chloramphenicol, taking into account that haloarchaeal cells lyse or do not grow in the presence of bile acids (KAMEKURA et al., 1998; KAMEKURA & SENO, 1991). The membrane lipids extraction and identification were performed using thin layer chromatography method (KAMEKURA, 1993). Haloarchaeal cells obtained from 100 ml cultures were mixed with distilled water and chloroform/methanol (1/2 v/v). The resulted mixture were centrifuged at 10 000 rpm and the supernatant was separated. The supernatant was treated with a mixture of chloroform/methanol. The lower chloroform phase resulted in separator funnel was isolated, dried and dissolved in the chloroform-methanol solvent mixture. The evaluation for halocin production (bacteriocin produced by halophilic archaea) was performed according to the procedure described by Meseguer and Rodriguez-Valera (1985). Two ml of culture tested as halocin target were mixed with 20 ml molten agar medium. After the solidification of this medium, 100 µl of culture tested as halocin producer were deposited into wells and incubated at 37^0C for 7-10 days. In the presence of a clear inhibition zone surrounding the well, the activity was recorded positive. The other biochemical tests were performed following the previously described protocols (ENACHE et al., 2007).

Genomic DNA of the strain T1/2S95 was isolated and purified following the method of Tamaoka (1994) adapted for halophilic archaea and the G+C content was determined by the HPLC method. The 16S rRNA genes were amplified by PCR, using the archaeal specific forward and reverse primers, 5'-TCCGGTTGATCCTGCCG (position 8 – 24 in *E. coli*) and 5'-GGAGGTGATCCAGCCG (position 1540 – 1525), respectively. The resulted DNA fragment was sequenced using BigDye Terminator Cycle Sequencing Kit (Pharmacia Biotech) and ABI Prism DNA genetic analyzer (Applied Biosystems). The orthologous 16S rRNA gene (MINEGISHI et al., 2012) was amplified with the *pyrD* primer set2: forward primer pyrD2, 5'-TCGTTGTTNARNCCCATNCGGTT-3' (corresponding to nt 346–368 of *pyrD* of *Haloarcula marismortui*); reverse primer 23SRev, 5'-GCTTWTCGCAGCTTGG-3' (corresponding to nt 56–71 of the 23S rRNA gene of *Halobacterium salinarum*). The 16S rRNA gene sequences obtained was analysed using BLAST and aligned with other reported haloarchaeal 16S rRNA gene sequences using CLUSTAL W 1.7 software. A phylogenetic tree was reconstructed by the neighbour-joining method (SAITOU & NEI, 1987).

RESULTS AND DISCUSSION

The strain T1/2S95 investigated in this work has been isolated from Techirghiol Lake, a saline lake having sodium chloride content around 60 g/l, located nearby the Black Sea coast, in Constanta county, Romania, in the proximity of Eforie city. The Gram-negative strain lysed in distilled water and was not able to grow on media supplemented with bile salts thus being assumed as haloarchaeal strain. On the solid medium colonies were elevated, circular and having whole margins and transparent. When cultivated on liquid JCM 168 medium containing various concentrations of sodium chloride (absence of sodium chloride to saturation) for 48 hours, the strain showed no pigmentation, but after 72 hours the pigmentation became weak orange in culture media with 4 – 5.2 M NaCl and yellow in media having 1 – 4 M NaCl (Fig. 1b). The strain was unable to grow bellow 1 M NaCl and grew optimally at 2.0 – 2.5 M. The strain was not able to synthetize halocins but its growth was inhibited by a halocin produced by other haloarchaeal strain, GR1 isolated from Bride Cave, a man-made salt lake located in Slănic Prahova and assigned to *Haloferax* genus as previously described (ENACHE et al., 2008). The biochemical investigations revealed that the strain was catalase and oxidase positive. sulphide was produced from sodium thiosulphate but indole was not produced

from tryptone. The strain hydrolyzed Tween80 but was negative for starch and casein hydrolysis. Gelatin liquefactions were also negative (Table 1).

The sequence of 16S rRNA gene of the strain T1/2S95, amplified with the archaeal specific forward and reverse primers, was most closely related to those of the strains of the genus *Haloarcula.*

The chemotaxonomic features, namely lipid profile and DNA G+C content complied with the already characterized members of the genus *Haloarcula.* The genomic DNA G+C content for the strain T1/2S95 was 65.1 mol% whereas members of the genus *Haloarcula* showed values from 60.1 mol% in *Har. quadrata* to 64.7 mol% in *Har. vallismortis*. Membrane lipid investigation by TLC revealed the presence of $C_{20}C_{20}$ glycerol diether derivatives of phosphatidyl glycerol sulphate (PGS), methyl ester of phosphatidyl glycerol phosphate (PGP-Me) and phosphatidyl glycerol (PG) as phospholipids (Fig. 1a). The data shown in Fig. 1a related to glycolipid analysis revealed the presence of diglycosyl archaeol-1 and their sulfated derivative (DGD-1 and S-DGD-1).

Table 1. Characteristics features of the members of *Haloarcula* genus.
1 = Formation of sulphide from sodium thiosulphate; 2 = catalase; 3 = oxidase; 4 = indole from tryptone; 5 = starch hydrolysis; 6 = Tween80 hydrolysis; 7 = Gelatin liquefactions; 8 = casein hydrolysis; 9 = range of NaCl for growth (M); 10 = optimum NaCl for growth (M); 11 = isolation site; 12 = origin of isolation site; 13 = DNA G+C content (mol%)

	1	2	3	4	5	6	7	8	9	10	11	12	13
amylolytica	+	+	+	+	+	+	+	-	2.0 -5.1	3.1	Salt lake	N	62.4
argentinensis	nd	+	+	nd	+	+	+	nd	2.0 -4.5	2.5 - 3.0	Soil of saltern	A	62
hispanica	nd	nd	nd	+/-	+	+	+	+/-	2.5 -5.2	3.5 - 4.2	Saltern	A	62.7
japonica	+	nd	nd	+	-	-	-	-	2.5 -5.0	3.5	Salted soil	N	63.3[&]
marismortui	nd	nd	nd	-	-*	-	-	nd	1.7 -5.1	3.4 - 3.9	Dead Sea	N	62
quadrata	nd	+	+	-	+**	-***	-	-	2.7- 4.3	nd	Salted pool	N	60.1
salaria	nd	+	+	-	+	+	-	-	2.5 -5.1	3.4- 4.2	Salt from fish sauce	N	61.6
tradensis	nd	+	+	-	+	+	-	-	2.5 -5.1	3.4- 4.2	Salt from fish sauce	N	62.2
vallismortis	nd	nd	nd	+	+****	-	-	-	<=2.5	4.3	Salted pool	N	64.7
T1/2S95	+	+	+	-	-	+	-	-	1.0 -5.2	2.0 - 2.5	Saline lake	N	65.1

Legend:
*= weak positive in Bergey's Manual
**= negative in paper of Namwong et al., 2011; positive in Bergey's Manual and paper of Yang et al., 2007
***= positive in paper of Namwong et al., 2011; negative in Bergey's Manual and paper of Yang et al., 2007
****= negative in paper of Yang et al., 2007
& = value corresponding to strain TR-1^T (D28872)
+/- = variable results
nd = no data available
N = natural environment; A = man-made environment

The data summarized in Table 1 suggested that the members of *Haloarcula* genus have been isolated from natural saline and hypersaline environments except two members isolated from man-made hypersaline environments, namely saltern (*Har. hispanica*) and soil from saltern (*Har. argentinensis*).

Since *Haloarcula* spp. possess multiple, heterogeneous 16S rRNA genes, the *pyrD* primer set was used to amplify the DNA fragment encompassing the orthologous 16S rRNA & tRNA-Ala with *pyrD* and 23S rRNA genes. According to the data of MINEGISHI et al. (2012) related to gene orders in the upstream of 16S rRNA genes (one or two in species of some genera, and three in *Haloarcula* spp.), orthologous 16S rRNA gene is preceded by a gene coding for dihydroorotate dehydrogenase, an enzyme involved in enzymatic synthesis process of nucleic acid base pyrimidine. Sequence of the orthologous 16S rRNA gene of the strain T1/2S95 showed high similarities with those of *Haloarcula* spp. Thus, the phylogenetic tree (Fig. 2) was reconstructed based on sequences of orthologous 16S rRNA gene. Sequence used as out-group was adopted from KEGG (Kyoto Encyclopedia of Genes and Genomes).

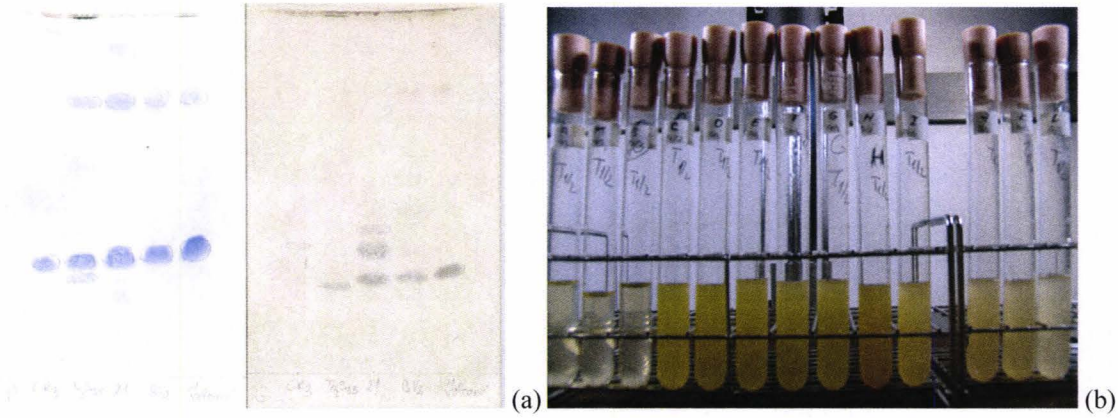

Figure 1. (a) Phospholipid (left) and glycolipid (right) pattern of the strains T1/2S95 revealing the presence of PGS, PGP-Me, PG and DGD-1 and their sulphate derivate; starting from left of either pattern the lane 1 = strain GR3, lane 2 = strain T1/2S95, lane 3 = strain 21, lane 4 = strain BV2 and the lane 5 = *Haloferax volcanii*; (b) growth and pigmentation of the strain T1/2S95 in the presence of various NaCl concentrations from nil up to saturation (from left to right) (original).

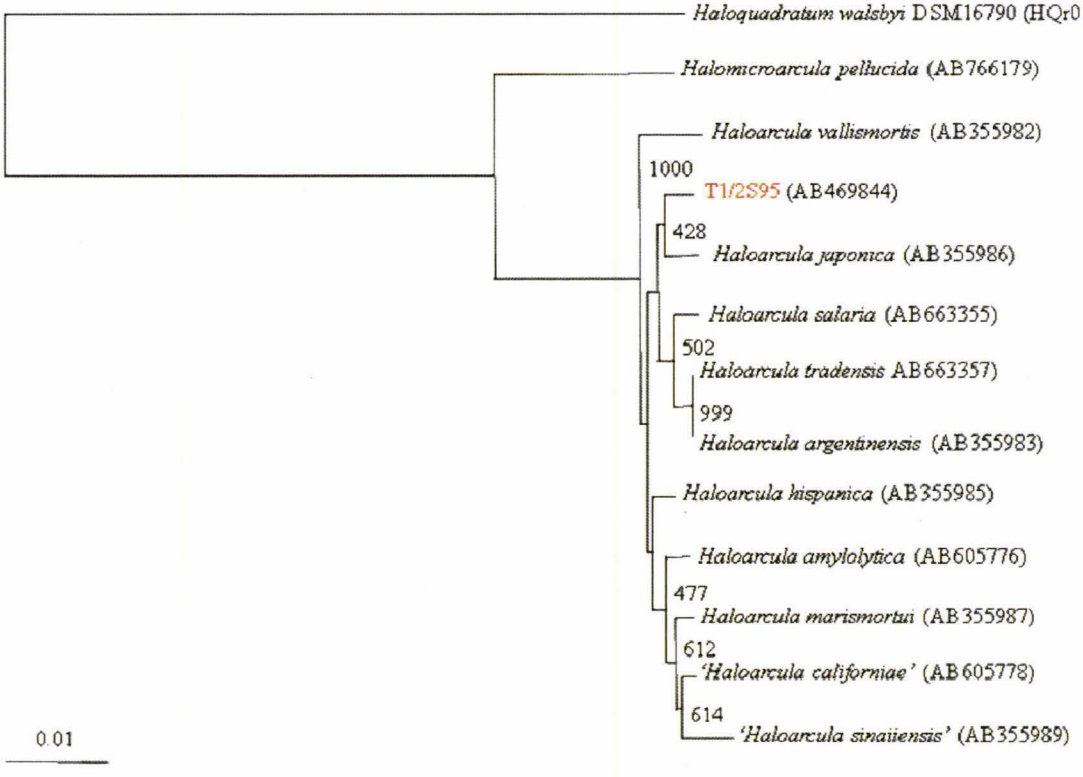

Figure 2. Phylogenetic tree revealing the position of strain T1/2S95 amongst the members of the genus *Haloarcula*. The tree was reconstructed by the neighbour-joining method. Bootstrap values over 400 are represented. Bar representing one substitution per 100 nucleotide position.

The strain T1/2S95 formed a clade together with *Har. japonica, Har. salaria, Har. tradensis* and *Har. argentinensis*. The maximum parsimony supported the group (strain T1/2S95 and *Har. japonica*) well, with a bootstrap value of 42%. The clade is also well supported by a value of probability over 30%. Another clade in Fig. 2 constituted of remaining five species, *Har. hispanica*, *Har. californiae*, *Har. sinaiiensis*, *Har. marismortui* and *Har. amylolytica*, and grouping of this clade with *Har. vallismortis* is supported very well by a probability of 100%. When compared in BLAST, the similarities of strain T1/2S95 with strains of genus *Haloarcula* forming the clade together were 99%, and 98% with remaining five species and *Har. vallismortis*.

As a general conclusion, it should be noted that pigmented Gram-negative strain T1/2S95 isolated from Techirghiol Lake in Romania, growing between 1 and 5.2 M sodium chloride concentrations, able to hydrolyze Tween80, with lipid profile constituted of PGS, PGP-Me, PG, DGD-1 and S-DGD-1, and with genomic DNA G+C content of 65.1mol%, could be considered as a novel species of the genus *Haloarcula*.

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NEW STRATEGIES FOR BIOREMEDIATION OF SOIL CONTAMINATED BY OBSOLETE PESTICIDES IN THE REPUBLIC OF MOLDOVA

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Abstract. This paper is focused on the elaboration of the new approaches to activate indigenous soil microflora for the remediation of long-term pesticides contaminated soil collected nearby the former storehouse of persistent organic pesticides: DDTs and trifluralin. The bioremediation of the contaminated complex was carried out in two main directions: strictly oxic conditions and alternating anoxic and oxic conditions. Bioremediation procedures have had a beneficial effect, resulting in the reduction of the pesticides content and mineralization of DDT.

Keywords: bioremediation, soil, pesticides, DDT, trifluralin.

Rezumat. Noi strategii de bioremediere a solului îndelung contaminat cu pesticide în Republica Moldova. Articolul este axat pe elaborarea unor procedee noi de activizare a microflorei indigene pentru remedierea solului, îndelung contaminat cu pesticide, din apropierea fostului depozit de poluanţi organici persistenţi: DDTs şi trifluralina. Bioremedierea complexului contaminant a fost realizată pe două căi principale: crearea condiţiilor strict oxice şi alternarea condiţiilor anoxice cu cele oxice. Procedurile de bioremediere au avut efect benefic, soldat prin reducerea conţinutului de pesticide si mineralizarea DDT-ului.

Cuvinte cheie: bioremediere, sol, pesticide, DDT, trifluralina.

INTRODUCTION

Pesticides are the only toxic substances released intentionally into our environment to kill living things. This includes substances that kill weeds (herbicides), insects (insecticides), fungi (fungicides), rodents (rodenticides), and others. The use of toxic pesticides to manage pest problems has become a common practice around the world (http://www.toxicsaction.org/problems-and-solutions/pesticides).

The Republic of Moldova has never produce pesticides, but the country still has frequent pollution accidents in the small spaces of the natural environment (water, soil). Despite the restrictions, considerably reducing the volume of pesticide in use, the problem related to environmental pollution in Republic of Moldova, including accidental pollution, remains acute. Currently over a thousand locations in the country - the former territories of pesticide deposits, reached the deplorable state - is a continuing source of pollution and threat to the environment and to human health in adjacent areas.

The management of domestic and hazardous wastes is considered as one of the most urgent environmental problems in Moldova. Currently, in Moldova, approximately 3,000 tons of obsolete pesticides are stored in various former collective agricultural warehouses or disposed in uncontrolled dumps (NATIONAL IMPLEMENTATION PLAN, 2004). The spatial analysis showed a strong pollution impact to surrounding agricultural territory near old pesticide storage (BOGDEVICH & CADOCINICOV, 2007; 2009). Huge areas of contaminated soil around old storehouses are a continuous danger for the environment and public health (The Eliminators in Moldova, 2011). However, the information about the actual condition of soil after the repacking on former storages is not sufficient at present. This investigation is important also for the assessment of the remediation technologies that can be used for future soil detoxification.

There are many methods available for soil remediation like excavation, In-situ vitrification, soil dressing, washing and bioremediation. Bioremediation methods have drawn the attention of the researchers as chemical detoxification methods failed to handle the issue of soil remediation economically. Bioremediation, including stimulation of native microflora, bioaugmentation, phytoremediation, and rhizoremediation are methods or procedures for cleaning soil contaminated with persistent organic pollutants that have already become common. The activation of native microorganisms destructive abilities by introducing additional sources of carbon, nitrogen, phosphorus, hydrogen peroxide, in aeration, maintaining high humidity, is one of the technologies using microorganisms to degrade pesticides, petroleum products, detergents, etc., in soil and water (PHILLIPS et al., 2004; DENYS et al., 2007; AYOTAMUNO et al., 2009).

Our goal was to identify more efficient approaches to activate soil microorganisms' biodestructive capacity for remediation of long-term pesticides contaminated soil.

MATERIAL AND METHODS

Soil samples were collected from the site nearby the former storehouse of persistent organic pesticides (POPs) located in the central part of the Republic of Moldova, Chişinău municipality, Sangera village. Collected soil was cleared of roots and other impurities, sieved (mesh number 2) to 2-3 mm, air-dried at 22-23°C and analysed. Soil pH, moisture content (SMC), water-holding capacity (WHC) and soil organic matter content were determined using standard methods (ARINUSHKINA, 1970; KOZLOVA, 2009).

The extraction of DDTs and trifluralin from soil has been performed in four repetitions per option according (KLISENKO & ALEXANDROVA, 1983). The determination of pesticide residues in soil was confirmed by gas chromatography with mass spectrometry GC/MS multiresidue method, at the gas chromatograph "Agilent Technologies" 6890N coupled with MSD mass selective detector "Agilent Technologies" 5973.

The bioremediation was established in experimental plastic jars, each containing 1,000 g of contaminated soil (Table 1). The duration of the experiment was 135 days. The experiment was carried out in two main directions: (1) strictly oxic conditions, the constant humidity were maintained at 60% of WHC, and (2) alternating anoxic and oxic conditions, each cycle consisting of two phases – anaerobic and aerobic. There were two variants that served as a control: initial air-dried soil and variant 1 – without stirring and amendments, the soil moisture maintained at 60% of WHC.

Table 1. Treatment protocol for bioremediation of soil in laboratory conditions.

Variant	Bioremediation factors						
	Biostimulation					Bio-augmentation	Phyto-remediation
	Soil moistening, % of WHC	Periodic tilling	Amendments			*Rh. meliloti*	*M. sativa* L.
			Peptone	K_2HPO_4 + $(NH_4)_2HPO_4$	Ammophoska NPK 15:15:15		
Oxic conditions							
Initial	–	–	–	–	–	–	–
1	60	–	–	–	–	–	–
2	60	+	–	–	+	–	–
3	60	+	–	–	–	–	+
4	60	+	–	+	–	+	+
Anoxic/oxic conditions							
5	80/60	– / +	+	+	–	–	–
6	80/60	– / +	+	+	–	–	+

Section 1: strictly aerobic conditions. The constant humidity was maintained at 60% of WHC. There were variants:

1. Without stirring, without amendments, soil moistened to maintain 60% of WHC.

2. At the beginning of experiment this variant was amended with ammophoska, 0.49 g/kg of soil, aerated by tilling twice a week.

3. Without amendments, aeration by tilling weekly. For phytoremediation purpose, it was chosen alfalfa (*Medicago sativa* L.). Periodically plants were cut, mixed with soil and new seeds were planted. Aeration by tilling weekly.

4. At the beginning of the experiment, this variant was amended with potassium and ammonium phosphates (K_2HPO_4 and $(NH_4)_2HPO_4$) at a concentration of 1.0% by weight of soil each. Phytoremediation – alfalfa (*M. sativa* L.). Periodically plants were cut, mixed with soil and new seeds were planted. Soil bacterium *Rhizobium meliloti* was introduced to soil once at the same time when the seeds were planted. Aeration by tilling weekly.

Section 2: alternating anoxic and oxic conditions, each cycle consisting of two phases – anaerobic (for 14 days) and aerobic (for 7 days). Anaerobic conditions were created by saturating the contaminated soil with water (up to 80% of WHC) in the dark plastic jars sealed with Parafilm, and stored in the dark at 20-22°C. At the beginning of the aerobic phase Parafilm was removed, soil mixed with a metal spatula and gradually brought soil moisture up to 60% of WHC. At the beginning of each anaerobic phase peptone and phosphates salts were added at a concentration of 0.5% and 0.2% respectively, soil humidity was maintained at 80% of WHC.

5. At the beginning of experiment, there were added potassium and ammonium phosphates (K_2HPO_4 and $(NH_4)_2HPO_4$) in concentration of 1.0% by weight each, and peptone in concentration of 0.5%.

6. Alternating aerobic-anaerobic conditions as in variant 5 for 63 days. Then, during the last aerobic phase, the soil humidity was brought up to 60% of WHC, sawdust was added to soil in amount of 1/3 of the volume of soil. Alfalfa was planted as for variant 3 for 72 days.

RESULTS AND DISCUSSIONS

At the start of the experiment, the soil pH was 8.0 and the air-dry soil moisture content was 1.84%. Water holding capacity was 33.60% and soil organic matter content was 2.06%. Soil type was determined as carbonated chernozem (Table 2).

Table 2. Characterization of experimental soil samples (Sangera storehouse, mun. Chişinău).

Soil type	pH	SMG,%	VHC, %	Organic Matter, %
Carbonated chernozem	8.0	1.84	33.6	2.06

The total content of pesticides exceeded 21.00 mg/kg soil, the rate of POPs – DDTs content (total amount of DDT and its metabolites DDE and DDD) was estimated to 1.48 mg/kg. The major component of pollution was halogenated herbicide trifluralin; its concentration was 19.52 mg/kg (Table 3). According to the Hygienic norms, established for the Republic of Moldova, DDTs and trifluralin Maximum Residue Limits (MRL) in soil is 0.1 mg/kg (NORMATIVELE IGIENICE, 2003). Thus, soil pollution by DDTs and trifluralin exceeded the MRL about 15 and 200 times respectively. Pesticides contaminated soil nearby the storehouse area for a long time and pollution was complex, so soil from that site was selected for modelling bioremediation project.

Table 3. The level of soil contamination with POPs.

Contamination, mg/kg						
Trifluralin	∑DDTs	DDT		o,p'-DDD	DDE	
		o,p'-DDT	p,p'-DDT		o,p'-DDE	p,p'-DDE
19.52 ± 0.22	1.48	0.21± 0.01	0.11± 0.005	0.27± 0.027	0.29 ± 0.020	0.60 ± 0.012

Chromatographic analyses of soil extracts at the end of the experiment demonstrated the effectiveness of bioremediation processes in decreasing concentrations of organochlorine pesticides and trifluralin in the contaminated soil. The results of bioremediation and phytoremediation procedures are displayed in figure 1.

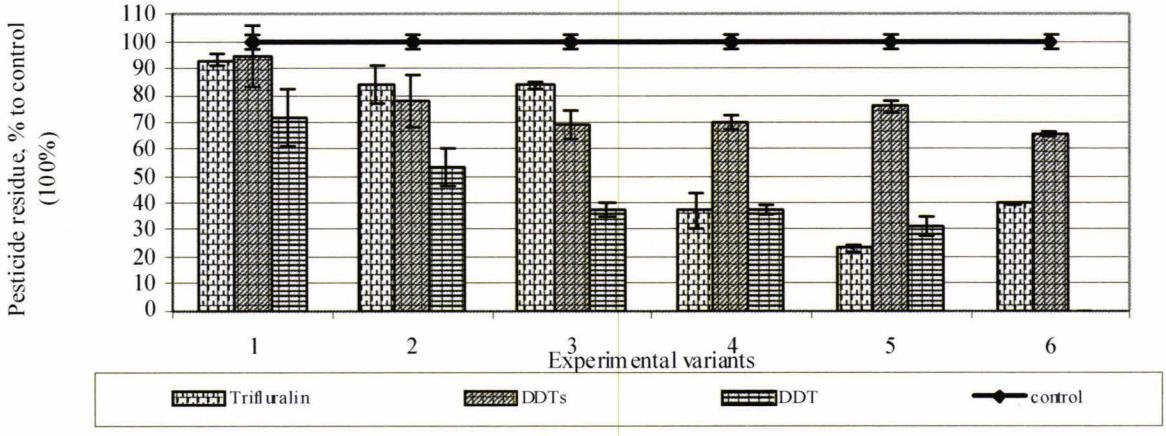

Figure 1. DDTs, DDT and trifluralin degradation.

Regular soil moistening without stirring and amendments contributed to the mineralization of 28% of DDT and slightly influenced trifluralin degradation. Bioremediation treatments setting in oxic conditions reduced the total DDTs' content up to 31% and the content of trifluralin up to 63% in 135 days. The most significant results were obtained using bioaugmentation as a factor of remediation (Fig. 1). While DDT and total DDTs removal was at the same rate as in variant 3 (phytoremediation without amendments), trifluralin concentration decreased more than 2 times. Recently, there are also reports that microorganisms associated with plants, especially rhizobacteria, induced the degradation of xenobiotics, such as phenanthrene and simazine herbicide (TAKASHI & RYOTA, 2004; TURKOVSKAYA et al., 2007).

The amendments of the soil with phosphates and organic compounds in anaerobic/aerobic conditions favoured the reductive cleavage of organochlorine pesticide DDT and intensive accumulation of its metabolites. The alternation of anoxic and oxic treatment combined with the stimulation of indigenous microflora by phosphates and peptone amended to soil reduced the DDT concentration by 3-3.5 times, and the total concentration of DDTs by 35%. The prolongation of the experiment in aerobic conditions enhanced DDT metabolization by soil microflora and resulted in complete mineralization of DDT.

Cycled anaerobic and aerobic treatment provided the degradation of 60-77% of the initial rate of trifluralin. As a result of phosphates and peptone application in anaerobic/aerobic conditions maintained for 135 days (6 full cycles), trifluralin content decreased by more than 4 times. Trifluralin removal in variant 6 was not so significant, and, thus, it can be suggested to increase the duration of anaerobic/aerobic phase (from 3 cycles to 6 or more).

Additionally, two consortia of microorganisms adapted to high concentrations of organochlorine toxicants were isolated from the contaminated soil. By analyzing soil microbiota long-term adapted to toxic presence of pollutants, there were selected 4 strains with ability to degrade the organochlorine compounds.

CONCLUSIONS

Thus, by using different bioremediation factors and treatments, we elaborated two strategies for the remediation of a long time and complex contamination with persistent organic pesticides DDT and trifluralin. Bioaugmentation of rhizobacteria associated with alfalfa in oxic conditions enhanced the trifluralin degradation.

Alternation of anoxic and oxic treatment combined with stimulation of indigenous microflora by phosphates and peptone resulted in complete mineralization of DDT.

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THE TOXICITY LEVEL OF THE LOWER JIU WATER

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Abstract. In accordance with Article 8 (1) of the Water Framework Directive (2000/60/EC), European Union Member States have established monitoring programs for surface waters, groundwater and protected areas in order to know and classify the „status" within each basin (HATTON-ELLIS, 2008). In Romania, the established monitoring programs became operational on December 22, 2006 and are applied on surface water bodies, groundwater bodies and protected areas. The lower Jiu is part of this category, respectively Răcari and Podari sections. There have been monitored biological (macrobenthos, phytoplankton) and physico-chemical parameters (oxygen regime, biotic substances, toxic substances, general ions). Following the analysis, Răcari section belongs to the II quality class from the biological point of view and to the IV quality class regarding the physico-chemical parameters. Podari section was framed, from the biological point of view, in the II and III quality class, in the II quality class in terms of chemical indicators and in the IV quality class regarding the toxic substances.

Keywords: lower Jiu, biological and physico-chemical indicators, saprobic index, quality class.

Rezumat. Nivelul de toxicitate al apei Jiului inferior. În conformitate cu Articolul 8 (1) al Directivei Cadru din domeniul apelor (2000/60/EC), Statele Membre ale Uniunii Europene au stabilit programele de monitorizare pentru apele de suprafaţă, apele subterane şi zonele protejate în scopul cunoaşterii şi clasificării „stării" acestora în cadrul fiecărui bazin hidrografic (HATTON-ELLIS, 2008). În România programele de monitorizare stabilite au devenit operaţionale la 22 decembrie 2006, aplicându-se corpurilor de apă de suprafaţă, corpurilor de apă subterane şi zonelor protejate. Din această categorie face parte şi râul Jiul inferior respectiv, secţiunile Răcari şi Podari. Au fost monitorizaţi parametrii biologici (macrobentosul, fitoplanctonul), şi fizico-chimici (regimul de oxigen, substanţele biotice, substanţele toxice, ionii generali). În urma analizelor, secţiunea Răcari se încadrează, din punct de vedere biologic, în clasa a II-a de calitate şi, din punct de vedere fizico - chimic, în clasa a IV-a de calitate. Secţiunea Podari a fost încadrată, din punct de vedere biologic, în clasa a II şi a III-a de calitate, iar în ceea ce priveşte indicatorii chimici în categoria a II -a de calitate şi substanţele toxice, în categoria a IV-a de calitate.

Cuvinte cheie: Jiu inferior, indicatori biologici şi fizico-chimici, index saprobic, clasă de calitate.

INTRODUCTION

Integrated management of water resources is an activity that promotes the development and sustainable management of water, land and related resources, in order to obtain a maximum growth of the economic resultant and social status in a balanced manner, without affecting the sustainability of vital ecosystems (MEYBECK & VÖRÖSMARTY, 2004).

Water quality does not remain constant over time, but may vary due to natural or artificial sources of contamination (CÎRŢÎNĂ, 2010), which requires permanent control of the parameter which defines the quality of surface waters and their ability to be the power source of human settlements or of use in industrial processes and agricultural activities.

From a legal perspective, preventing degradation of the aquatic environment is a concern in Europe dating since the 70s, when it was drafted the first Water Directive (75/440/EEC) and culminated in the drafting of the Directive 2000/60/EC which establishes a framework for Community action in the field of water policy (MOSS, 2008). Entered into force in 2000, the Water Framework Directive is a bold and far-reaching instrument in the sustainable use of water resources in Europe and has as a main objective achieving and maintaining good status of water by 2015.

Initially, in our country, water quality assessment for their management was based mainly or exclusively on physico-chemical analysis, biological assessment methods become fully accepted in the 70s of last century (BALABAN & CONSTANTINESCU, 2006). Currently, in Romania, surface waters are assessed in accordance with the Normative 161/2006 by which the classification is done in terms of ecological and chemical parameters or all surface waters.

MATERIAL AND METHODS

Surface waters quality from the lower basin of the river Jiu was evaluated during 2013, using monitoring data from two distinct areas. Water quality assessment was performed using biological, general physico-chemical and chemical indicators (heavy metals and organic micropollutants) analysed in Răcari and Podari sections.

Monitoring sections were chosen from the National Network for Monitoring and aim to highlight critical points in terms of water quality, which are located both upstream and downstream of the main pollution sources, providing information on their impact on water surface.

The characterization from the biological perspective of watercourses quality in the lower basin of the river Jiu was based on monitoring macrozoobenthos and phytoplankton. Saprobic index was calculated by the Puntle-Buck method for each section monitored and was correlated with the quality class from Normative 161/2006.

Physico-chemical quality elements used in the development of ecological status for all water bodies (natural and heavily modified) are: acidification status (pH), the oxygen regime (dissolved oxygen), nutrients ($N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$, P_{total}).

Specific pollutants are represented by synthetic and non-synthetic substances (metals: chromium, nickel, lead, mercury). For the synthetic substances were used, as evaluation limits of the ecological status, the environmental standards of Directive 105/2008.

Due to the natural existence of metals in surface waters, the natural background value was calculated first, in order to know the contribution coming from anthropogenic sources, only in situations in which the concentration obtained exceeded the quality standards of the Directive. For non-synthetic specific pollutants, high ecological status is defined by concentrations which remain within the range normally associated with background values.

Good environmental status for both specific synthetic pollutants, and for the non-synthetic, is defined by concentrations which do not exceed the environmental quality standards. The determinations were performed with the following instruments: water harvesting kit, equipped with vacuum pump, Hanna ph-meter, incubation vials for determining biochemical oxygen demand, Hach digital titrator, system Kjeldahl for determination of total nitrogen, flamephotometer model FLM-KRS, DR 2010 spectrophotometer, RQ-flex19+. Toxic substances, namely cadmium, nickel, lead and mercury were analysed using atomic absorption spectrophotometer AAS-ML-1200.

RESULTS AND DISCUSSIONS

Although biological elements are considered integrator of all types of pressure, to assess the ecological status, analysis needed certain support elements represented by: general physico-chemical indicators, hydromorphological elements and specific pollutants (Fig. 1).

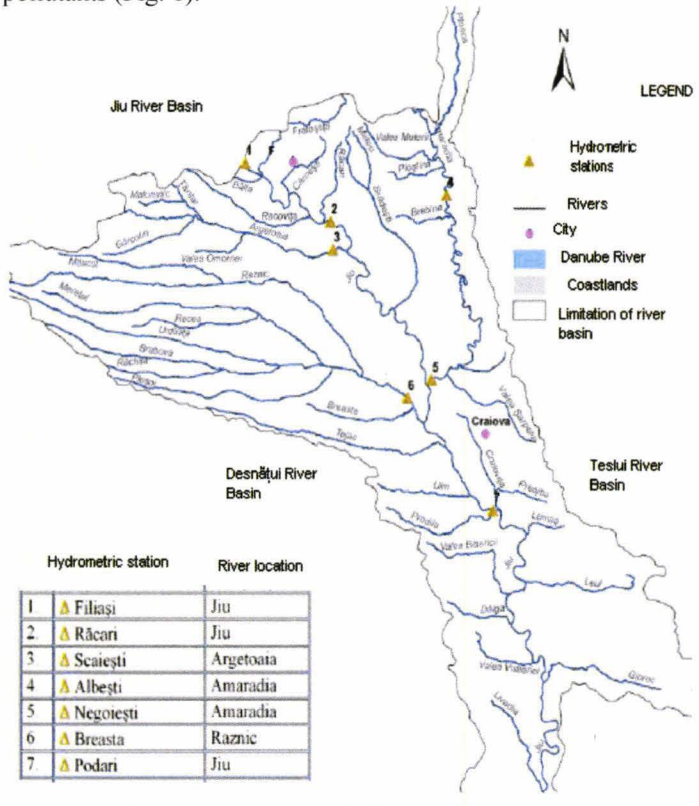

Figure 1. Delimitation of Jiu river course (Source: WBA Jiu).

On the river Jiu, in the first section monitored in terms of biological indicators (Răcari), zoobenthos samples were collected in June, August and September and phytoplankton in April, July and September 2013.

With an annual average density of 1,000 specimens/sq. m and a saprobic index of 2.135, macrozoobenthos was represented by species belonging to: Gastropoda (*Physella acuta, Sphaerium rivicola*), Amphipoda (*Gammarus fossarum*), Ephemeroptera (*Caenis macrura, Cloeon dipterum*), Odonata (*Calopteryx virgo, Coenagrion pulchelum, Gomphus vulgatissimus*), Diptera (*Corynoneura scutellata, Cricotopus bicinctus, Simulium balcanicum*), so the water body is in the II quality class according to normative 161/2006.

Phytoplankton, with an annual average density of 275,567 specimens/l and a saprobic index of 1.84, has placed this water body still in the second quality class. In this section there were identified species belonging to Bacillariophyta (*Synedra ulna, S. acus, Melosira granulata, Gomphonema constrictum, Pinnularia viridis, Diatoma vulgar, Ceratoneis arcus*) and Chlorophyta (*Closterium Navicula, Ulothrix zoned*), Cyanophyta (*Merismopedia tenuissima*).

The main indicator of quality in terms of physico-chemical characteristics is represented by the hydrogen ion concentration (pH), which may vary due to discharges of industrial and domestic wastewater. In the lower basin analysed, the variation of pH is relatively low 7.25-7.45 upH, its value being within in the permissible limits (6.5-8.5).

In river waters, soluble gases, oxygen and carbon dioxide vary within limits which depend on the solubility of the compounds in accordance with the environmental conditions. The highest content of dissolved oxygen in the river waters is not more than 15 mg/l, and the maximum amount of carbon dioxide does not exceed 20-30 mg/l. Daily and annual regime of these gases dissolved in river waters is conditioned by water temperature, intensity of photosynthesis, power sources of the rivers, as well as pollution sources. Within this class of water quality assessment (rivers category), there were analysed three indicators:

- dissolved oxygen (mg O_2/l) is in close relation to water temperature (inverse variation), the amount of microorganisms and oxidable substances;
- biochemical oxygen demand (BOD_5), expressed in mg O_2/l, depending on the amount and capacity of biochemical decomposition of organic substances existing in water;
- chemical oxygen demand (COD), expressed in mg O_2/l, represents the quantity of oxidable substances in water and can be determined using potassium dichromate (COD) or potassium permanganate (CCOMn).

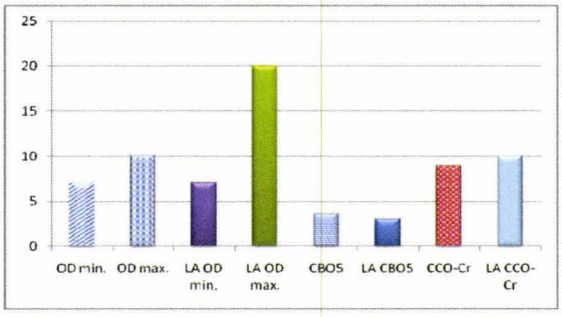

Figure 2. Variation of oxygen regime on river Jiu, section Răcari.

From figure 2, we can notice that the dissolved oxygen minimum value of 7.21 mg/l is above the allowable minimum and the maximum value of 10.13 mg/l is under the maximum limit of 20 mg O_2/l.

The second indicator is the biochemical oxygen demand (BOD_5) at which is observed a non-significant difference of 0.59 mg O_2/l, compared to the allowed limit. Chemical oxygen demand determined by potassium dichromate is below the permissible value of 10 mg O_2/l. We can conclude that water from Răcari section is part of the II quality class.

The main biogenic elements (Nutrient regime) consist of nitrogen compounds (ammonia, nitrates, nitrites, total nitrogen), phosphorus ions (orthophosphate, total phosphorus).

Ammonium ion (ammonia: $N\text{-}NH_4^+$) can occur in natural waters as a result of decomposition of organic matter under anaerobic conditions in the presence of bacteria or following the reduction of nitrites ions. In the majority of natural waters, ammonium ion predominates, because they have a pH around 7, but in alkaline waters free ammonia may reach levels that exceed the maximum allowable concentration.

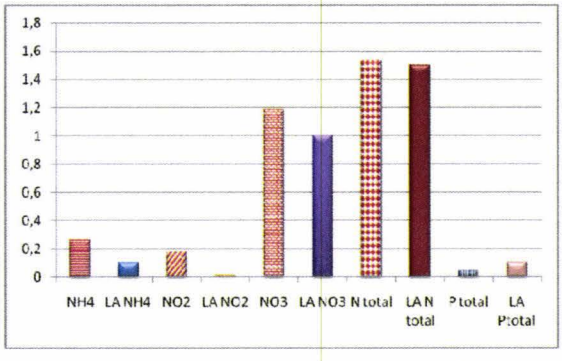

Figure 3. The content of biogenic substances in Răcari section.

In figure 3, there are represented biogenic substances from the lower basin of the Jiu river, Răcari section. There were recorded high values of the concentrations of ammonium ion 0.256 mgN/l (0.172 mg/l compared to the allowed limit 0.01 mg/l), nitrate (1.18 mg/l, at 1 mg/l), total nitrogen 1.53mg/l - 1.5 mg/l limit allowed), total phosphorus 0.043 mg/l does not exceed the limit of 0.1 mg/l.

The nitrite ion (NO_2^-) is the result of the nitrification process by which bacteria convert the ammonium ion into nitrite ion. In addition, nitrite ions may also occur due to the reduction in anaerobic conditions. Ions "nitrite" indicates faecal contamination of water. A possible cause could be due to partially treated sewage disposal of Filiaşi. The nitrites are found in much smaller amounts than nitrates. Nitrites occur during the normal cycle of decomposition of organic substances, typically in the late summer and fall. In case of the discharge of polluted waters, the nitrate content increases up to tenths of mg/l.

The nitrate ion (NO_3^-) is found in almost all water categories. Under natural conditions, surface waters are characterized by minimum variable nitrate content in summer when it is consumed by aquatic plants; in case of intense processes of photosynthesis nitrates disappear completely.

Regarding the regime "general ions – salinity", there were analysed the following chemical parameters: chloride (Cl^-), sulfate (SO_4^{2+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+). The elements of chemical quality of this "regime" are substances of natural origin and do not indicate pollution; therefore Răcari section is part of the I quality class.

From Table 1 it is observed that the values determined for the aforementioned chemical indicators in section Răcari do not exceed the allowed limit.

Table 1. Regime "general ions - Salinity".

Indicators	Determined Value	Allowed limit
Chlorides (mg/l)	21.8	50
Sulfates (mg/l)	70.3	80
Calcium (mg/l)	59.6	75
Magnesium (mg/l)	21.2	25
Sodium (mg/l)	13.4	25

The last reviewed regime is the "specific toxic pollutants of natural origin". In this group, there were analysed total concentrations (µg/l) of the following ions: total chromium (Cr^{3+}, Cr^{6+}), copper (Cu^{2+}), zinc (Zn^{2+}), total iron (Fe^{2+}, Fe^{3+}), total manganese (Mn^{2+}, Mn^{7+}), as well as non-synthetic substances Cd – 0.1, Ni – 1.431, Pb – 0.482, Hg – 0.124 (µg/l).

Special toxicity of these heavy metals (cadmium, nickel, lead and mercury) manifested on the aquatic environment has led to the need for their separate assessment. The presence of cadmium in surface waters is due both to the natural and anthropogenic sources of pollution (chemical, mining). The main natural source is the ore containing 0.5% cadmium, but is found in the form of CdS and $CdCO_3$.

Widely spread in nature, mercury is in the form of mercurous chloride $(Hg_2Cl_2)_3$, iodide mercury (Hg_2I_2) and mercuric sulfide, cinnabar (HgS). It may derive from anthropogenic sources as: mining, chemical industry and agriculture (GAVRILESCU & BUZATU, 2014).

The levels of these two toxic metals recorded in the lower basin of the Jiu river were below the detection limit of the device mass spectrometry with inductively coupled plasma (for cadmium) and for mercury variation interval was between 0.085 µg/l - 0.185 µg/l, with values well below the limit values set by normative 161-2006.

Nickel is a metal relatively non-toxic to humans and is rare in nature in elemental form; it is usually combined with sulfur, arsenic or antimony. In contrast, aquatic ecosystems are influenced by the presence of nickel, the concentrations lethal to fish (CALABRESE et. al., 1973) varying within very wide limits (4 mg/l - 43 mg/l). From this point of view the water falls in the IV quality class.

In Podari section, located 81 km from the confluence of the Jiu river with the Danube, zoobenthos and phytoplankton samples were collected in the months of May, July and September 2013.

The macrozoobenthos was represented by species belonging to the orders: Oligochaeta (*Chaetogaster limnaei, Dero obtusa, Nais communis, Pristina longiseta, Tubifex tubifex, Eiseniella tetraedra, Lumbriculus variegatus*), Gastropoda *(Physella acuta, Bithynia tentaculata*), Bivalvia (*Sphaerium corneum, Unio pictorum*), Amphipoda (*Gammarus fossarum*), Ephemeroptera (*Caenis macrura, Baetis vernus, Cloeon dipterum*), Odonata (*Calopteryx virgo, Gomphus flavipes, Platycnemiss pennipes*), Coleoptera (*Hydraena riparia, Platambus maculatus*), Diptera (*Brillia monilis, Orthocladius thienemmani, Chironomus thummi, Cricotopus bicinctus*). Saprobic index of 2.43 calculated in the monitoring section, as well as the annual average density of 1,835 specimens/sq. m, led to the conclusion that the water body belongs to the III quality class.

The phytoplankton, with an annual average density of 636,875 specimens/l and a saprobic index of 2.14, framed the water body in the II quality class. The representative species identified belong to the orders: Bacillariophyta (*Synedra ulna, S. acus, Melosira granulata, Gomphonema constrictum, Pinnularia viridis, Diatoma vulgare, and Ceratoneis arcus*) and Chlorophyta *(Closterium navicula, Ulothrix zonata*), Cyanophyta (*Merismopedia tenuissima*).

The value of pH determined in section Podari (average) is 7.54 upH, which can vary due to discharge of industrial and domestic wastewater. The variation interval for the pH is relatively small, the only situation in which its value is not within the allowed limits (6.5-8.5) was registered in May 2013, when it was measured a value of 6.31. The acidic nature is justified by the fact that in this body of water untreated wastewaters of Craiova are discharged.

Regarding the oxygen regime, respectively the minimum dissolved oxygen was 7.18 mg O/l and chemical oxygen demand caused by potassium dichromate exceeded the allowed limit (10 mg O/l). In Podari section, oxygen regime, represented by dissolved oxygen, BOD_5 and COD, exceeds the allowed maximum.

The second indicator of this analysed regime is the biochemical oxygen demand (BOD_5). On the lower Jiu, the range of the quality indicator BOD_5 is between 3.76 mg O_2/l (section Răcari) and 5.74 mg O_2/l in Podari section.

The last indicator of quality from this group is the chemical oxygen demand (COD) determined using the potassium dichromate (CCOCr) and expressed in mg O_2/l. On the Jiu river, the chemical oxygen demand, an indicator

of pollution, varies from 9.79 mg/l recorded in Răcari section to 17.1 mg/l, Podari section (Figs. 2, 4). Higher values are due both to seasonal variation (summer is characterized by maximum values) and pollution sources.

In the plain area, oxidative processes are carried out with a low efficiency due to high temperatures and various sources of pollution - agriculture, urban wastewater, leading to an increasing amount of organic matter in surface waters.

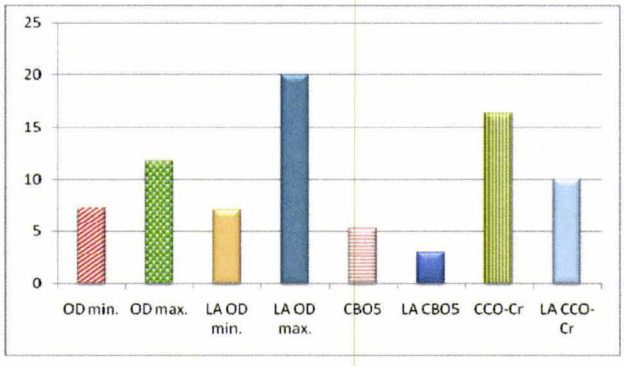

Figure 4. Variation of oxygen regime on river Jiu, section Podari.

The biogenic substances accumulated in this section exceed the maximum limit for all indicators (Fig. 5), except for the content of total phosphorus (0.038 mg/l compared to 0.1 mg/l limit allowed).

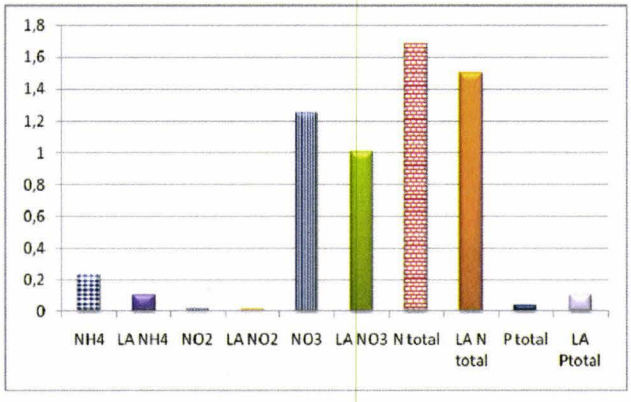

Figure 5. The content of biogenic substances in Podari section.

Ammonium ion (ammonia: N-NH_4^+) can occur in natural waters as a result of the decomposition of organic matter under anaerobic conditions in the presence of bacteria or following the reduction of nitrites ions. Depending on the pH of the water, ammonia in water is in the form of the ammonium ion NH_4^+ (low toxicity) or as free ammonia NH_3, which is highly toxic. Ionic form (NH_4^+) is 50 times less toxic than the unionized form.

Within the regime "general ions - salinity" were analysed the following chemical indicators: chloride (Cl^-), sulfate (SO_4^{2+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+). The determined values for sodium and chlorides indicators exceed the allowed limit (Table 2); the source of these elements is of anthropogenic nature, being represented by the discharges of wastewater from Podari industrial platform.

Table 2. Regime "general ions – salinity".

Indicators	Determined Value	Allowed limit
Chlorides (mg/l)	58.3	50
Sulfates (mg/l)	70.3	80
Calcium (mg/l)	62.4	75
Magnesium (mg/l)	22.1	25
Sodium (mg/l)	27.1	25

The last reviewed regime is the "specific toxic pollutants of natural origin". In this group, there were analysed the total concentrations (µg/l) of the following ions: total chromium (Cr^{3+},Cr^{6+}), copper (Cu^{2+}), zinc (Zn^{2+}), total iron (Fe^{2+}, Fe^{3+}), total manganese (Mn^{2+}, Mn^{7+}). The range of variation of these metal ions was relatively small and did not adversely affect the status of water bodies. According to the regime "toxic pollutants of natural origin", in 2013, Podari section belonged to the I quality class.

Table 3. Average concentrations of non-synthetic substances recorded in Podari section.

Monitoring section	Average concentrations recorded in 2013 (µg/l)			
	Cadmium	Nickel	Lead	Mercury
Podari	0.1	**1.567**	0.337	0.097
	Exceedances compared to the limit value			

From table 3, one can observe that the values recorded for the average concentrations exceed the limit stipulated by the Regulations 161/2006 in Podari section for nickel.

CONCLUSIONS

The Saprobic' index values calculated for the two sections of the Jiu river ranged from 2.135 to 2.43 regarding the macrozoobenthos and from 1.84 to 2.14 for phytoplankton.

On the lower Jiu, the variation of BOD_5 quality indicator is between 3.76 mg O_2/l (section Răcari) and 5.74 mg O_2/l in Podari section. The chemical oxygen demand, an indicator of pollution, varies from 9.79 mg/l recorded in Răcari section to 17.1 mg/l in Podari section.

The ammonium ion concentrations varied between 0.256 mg N/l in Răcari section and 0.231 mg N/l in section Podari. Ions "nitrite" indicates faecal contamination of water. On the Jiu river, the highest concentration was recorded in section Răcari (1.18 mg N/l) and the highest average concentration of the nitrate ion is found in section Podari (1.25 mg N/l). In the "toxic pollutants specific natural origin" group, there were analysed total concentrations (µg/l) of the following ions: total chromium (Cr^{3+}, Cr^{6+}), copper (Cu^{2+}), zinc (Zn^{2+}), total iron (Fe^{2+}, Fe^{3+}) and total manganese (Mn^{2+}, Mn^{7+}). The variation of these metal ions was relatively small and did not affect the status of water bodies in a negative way.

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THE IMPACT OF HYDROGEOMORPHOLOGICAL CHANGES ON PLANKTON EVOLUTION IN MUSURA LAGOON (THE DANUBE DELTA)

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Abstract. Musura Lagoon belongs to the avandelta, part of the Danube Delta, Biosphere Reserve. The aim of this study was to compare the historical data recorded in Musura with those more recent (2005-2007), in order to reveal the impact of hydrogeomorphological changes on structural and functional diversity of the plankton communities. In the last 30 years, the ecological researches in lagoon highlighted the relative fast dynamics at spatial - temporal scale. The resultant of the ecosystem evolution consists in a gradual transition from marine gulf stage to a half-closed lagoon, with many freshwater traits. The impact of factors as: the variation of water level (the decreasing of depth), the progressive decreasing of initial value of salinity (1942-12‰; 2005-0.18‰) and the geomorphological changes, were favourable to the proliferation of typical freshwater vegetation. The structural diversity of phytoplankton assemblages recorded increased values in the period 1987-1989, but a low taxonomic constancy. It was reported the presence of 7 halophilic species. During 2005-2007, the species richness reached a total number of 200 species, belonging to 7 taxonomical groups, mainly ubiquitous and typical for freshwater ecosystems. The average of zooplankton species richness during 1954-1974 was 32 species and, currently, has increased up to 76 species in the last decades, according with the changes of the water chemistry. Only one marine zooplanktonic species, the meroplanktonic larvae of *Balanus improvisus* Darwin 1854 (Cirripedia), was revealed between 2005 and 2007. The functional diversity of bacterioplankton was defined by the dominance of the members of the organic matter with sulphur decomposers group. This fact is a peculiarity of the marine environment. Nowadays, the physiological groups of bacterioplankton became characteristic to the freshwater ecosystem. From 2000 until present, Musura Lagoon has underwent an increased trend of isolation from the marine water of the Black Sea.

Keywords: hydrogeomorphological changes, Musura lagoon, plankton diversity, the Danube Delta.

Rezumat. Impactul modificărilor hidrogeomorfologice asupra evoluţiei planctonului din Golful Musura (Delta Dunării). Laguna Musura este localizată în avandeltă, parte a Deltei Dunării, Rezervaţie a Biosferei. Scopul acestui studiu a fost realizarea unei comparaţii a unor date istorice cu altele mai recente (2005-2007), în vederea evaluării impactului schimbărilor hidrogeomorfologice asupra diversităţii structurale şi funcţionale a comunităţilor planctonice. În ultimii 30 ani, cercetările efectuate în lagună, au evidenţiat o dinamică relativ rapidă la scara spaţio-temporală. Rezultanta evoluţiei ecosistemului a constat într-o tranziţie graduală de la stadiul de golf maritim la o lagună semi – închisă, cu multiple trăsături de apă dulce. Impactul unor factori ca: variaţia nivelului apei (descreşterea adâncimii), descreşterea progresivă a valorii salinităţii (1942-12‰; 2005-0.18‰), şi modificările geomorfologice au fost favorabile proliferării unei vegetaţii tipice de apă dulce. Diversitatea structurală a fitoplanctonului a înregistrat valori crescute în perioada 1987-1989, dar o constantă scăzută. A fost raportată prezenţa a 7 specii halofile. Între anii 2005-2007, bogaţia specifică a atins un număr de 200 specii, aparţinând la 7 grupe taxonomice, majoritatea ubicviste şi tipice pentru un lac deltaic. Media bogăţiei specifice a zooplanctonului în perioada 1954-1974 a fost de 32 specii şi a crescut la 76 specii în perioada actuală, în strânsă legătură cu schimbarea în chimismul apei. Doar o singură specie marină a fost întâlnită în perioada 2005-2007, şi anume larva meroplanctonică *Balanus improvisus* Darwin 1854 (Cirripedia). Diversitatea funcţională a bacterioplanctonului a fost dominată de grupul descompunătorilor materiei organice cu sulf. Acest aspect este o particularitate a mediului marin. În perioada actuală, bacterioplanctonul este reprezentat de grupe fiziologice caracteristice apelor dulci. Din 2000 până în prezent, Laguna Musura a avut o tendinţă de izolare de apele Mării Negre.

Cuvinte cheie: schimbări hidrogeomorfologice, laguna Musura, diversitate planctonică, Delta Dunării.

INTRODUCTION

Worldwide, the transitional waters are an important topic in ecology studies because of their status as interface between two different environments that gives them distinct features (GONENC & WOLFLIN, 2005). Musura Lagoon represents the interface of transitional waters between the Danube Delta and the northwestern part of the Black Sea. This area belongs to coastal lagoons, covering 13% of the world's coastal zone (GÜREL et al., 2005). The water of Musura channel and bay comes from two sources – the Chilia branch and the lagoon located between the Chilia and Sulina branches and the Black Sea; consequently, the geomorphological evolution of the Romanian – Ukrainian border and of the territorial waters in the immediate proximity of the Danube mouth is highly influenced by this contact (GÂŞTESCU & ŞTIUCĂ, 2006).

From the geomorphologic point of view, the channel and Musura bay are very recent: they formed through alluvial processes occurring near Cardon, between the delta of the Chilia branch (original much smaller and located north of the present location) and Sulina; this process started in the nineteenth century and the Musura bay appeared in the first half of the twentieth century, being then part of Romania, in the nineteenth century (DRIGA, 2004; FLORESCU & MOLDOVEANU, 2008). The same phenomenon that formed the Musura Bay presently transforms it in a swamp with reeds; east of it, there emerges a new bay, located between the mouth of the Old Stambul and the end of dam located along the Sulina branch. A sandy coastal belt formed and there appeared an island, still unnamed (Fig. 1).

During the last 30 years, the ecological researches in this lagoon revealed the relative fast dynamics at spatial - temporal scale. The outcome of the ecosystem evolution consists in a gradual transition from marine gulf stage to a half-enclosed lagoon, with many freshwater traits. The decrease of the water level, the progressive reduction of salinity (1942-12‰; 2005-0.18‰) and the geomorphological changes allowed the proliferation of the typical freshwater vegetation. As a result, both structural and functional diversity of the plankton community (phyto, zoo and bacterioplankton) changed (ZINEVICI et al., 2005; ZINEVICI & PARPALĂ, 2007; IONICĂ et al., 2008).

The aim of this study was to determine, by comparison between historical data and our data, the impact of the hydrogeomorphological changes on the structural and functional diversity evolution of the plankton community from Musura Lagoon.

MATERIAL AND METHODS

Study site and sampling:

Musura Lagoon, (45°10'48.12"N latitude, 29°39'21.34"E longitude), belongs to the avandelta, part of the Danube Delta, Biosphere Reserve (Fig. 1).

The samples were collected between 2005 and 2007, seasonally (May, July and October) from five stations, using a Patalas-Schindler (5 litres) device on water column.

Methods

The phytoplankton conservation was made in 500 ml plastic containers, with 4% formaldehyde solution. In the laboratory, phytoplankton samples were concentrated by sedimentation and filtration, using an Ø 65 mm network (VOLLENWEIDER, 1969; BRITTON & GREESSON, 1987). The identification of the phytoplankton species and abundance (10^3 ind L^{-1}) assessment were made using a Zeiss inverted microscope according to UTERMÖHL (1958). Phytoplankton biomass (mg wet weight L^{-1}) was established by volumetric and gravimetric measurements (OLRIX et al., 1998).

The zooplankton samples were collected by filtering 50 litres of water using a Patalas-Schindler device (5 l) on water column through a 65 µm Ø mesh network, and preserved in 4% formaldehyde solution.

The zooplankton species were identified by following keys: for Ciliata (FOISSNER et al., 1991-1995), Testacea (BARTOŠ, 1954; GROSPIETSCH, 1972), Lamellibranchia (MARSDEN, 1992), Rotifera (VOIGHT, 1956; RUDESCU, 1960), Cladocera (NEGREA, 1983; BROOKS 1959), Copepoda (DAMIAN-GEORGESCU, 1963; 1966; 1970).

The abundance (ind L^{-1}) was assessed by microscopic methods, using a Zeiss inverted microscope type, by direct counting into a Kolkwitz chamber (UTERMÖHL, 1958).

For zooplankton biomass calculations, it was used the wet weight of the organism (µg wet weight L^{-1}) according to: WINBERG (1971) for Ciliata and Testacea, STANCZYKOWSKA (1976) for Lamellibranchia, DUMONT et al. (1975) for Rotifera and ODERMATT (1970) and SEBESTYEN (1958a, b) for biomass estimation of the crustaceans. The biomass was evaluated by volumetric and gravimetric measurements, taking account the organism volume of each identified species.

To identify the structure of the bacterioplankton community and to assess the abundance of the different physiological groups, cultivation on selective media was performed.

Data analysis

Statistical analyses were performed using SPSS 15.0 Windows Evaluation Version, available for download at http://www.spss.com.

For comparison, a data base (old records from 1954, 1961, 1970, 1987-1989) was used. The long-term results belong to the database of the Institute of Biology Bucharest.

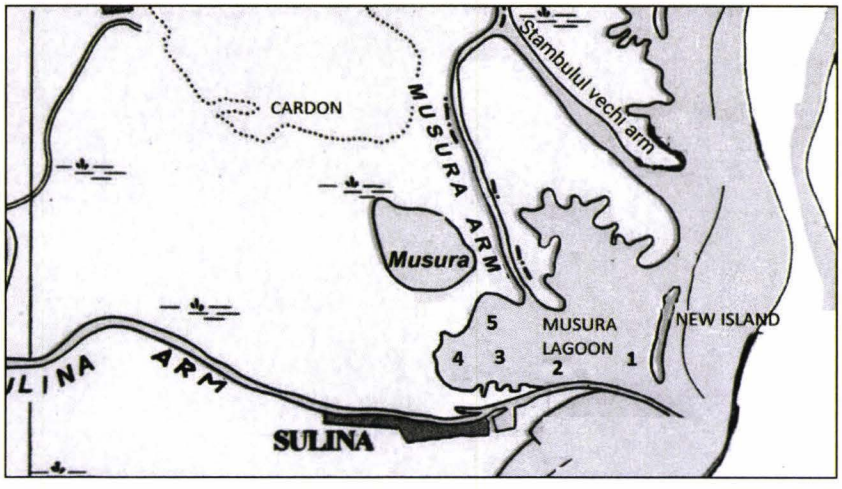

Figure 1. The map of Musura Lagoon (the Danube Delta) with sampling points (adapted after wikimedia.org).

RESULTS AND DISCUSSION

The assessment of physical and chemical parameters confirmed that Musura had specific characteristics of a freshwater ecosystem. It is possible that, certain features of brackish water appeared when the NE winds blew, but the influence of the Black Sea between 2005 and 2007 was insignificant (POSTOLACHE, 2006; COMAN & SANDU, 2009). In the 6th decade, the Musura Bay was strongly influenced by the Black Sea waters (BURGHELE, 1946). Due to process of silt deposition at the mouths of these two branches and sea currents, its connection with the sea got narrower (STANCU-STOIANOVICI, 1992; ZINEVICI et al., 2005).

In the year 1955, research conducted in Musura, described the existence of two distinct biotypes, freshwater and marine with brackish areas (ENĂCEANU, 1955). There was certain salinity, but it decreased halfway between the sea and Cardon, in this area approaching 0. In the marine area, 7 species of zooplankton were reported (BREZEANU & ZINEVICI, 1971). Phytoplankton was very monotonous and poor and the marine species were present (*Ceratium tripos* O. F. Müller 1777; Nitzsch. 1817, *Chaetoceras* sp.). In 1967, the study area was classified as brackish waters (salinity ranged from 0.11 to 7.12 ‰), having an unstable character, due to the fluctuations of marine waters depending on the hydrology and local climate (freshwater input, wind direction, direction of sea currents) (BREZEANU & ZINEVICI, 1971).

Between 1987 and 1989 a high structural diversity was recorded at phytoplankton level. 7 species were halophilic and the others ubiquitous, with a wide range of salinity tolerance (Fig. 2).

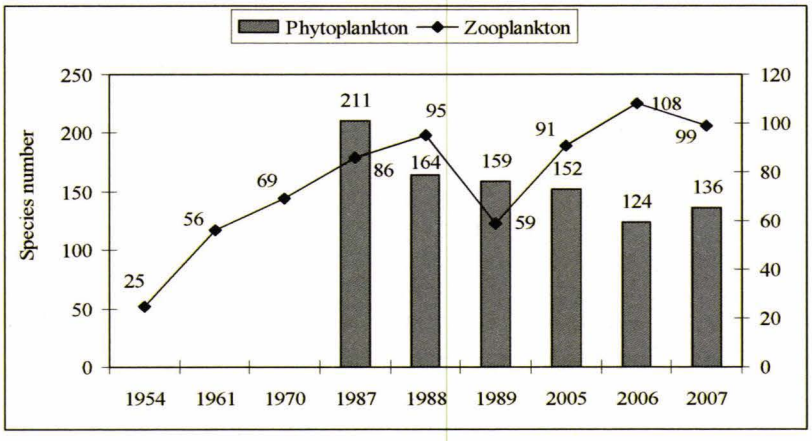

Figure 2. The evolution of species richness of phytoplankton and zooplankton.

In 1988, 8.42% of total number of zooplankton species were halophilic (*Oikopleura dioica* Fol 1872), (*Podon polyphemoides* Leuckart 1859), (*Balanus improvisus* Darwin 1854) and in 1989, the percentage of marine species was 11.32%, including species as (*Tintinnopsis meunieri* Kofoid & Campbell 1929), (*Noctiluca miliaris* Suriray 1816), Polichaeta larvae, (*Synchaeta litoralis* Rousselet 1902).

By comparison, in 2005-2007, the marine species were completely absent, only freshwater or brackish species were identified (*B. improvisus*) larvae (Cirripedia). Regarding the species richness, in Musura Bay, a total of 200 species of phytoplankton was recorded (Fig. 2). In 1988, Musura Bay showed a specific diversity of 134 species (STANCU-STOIANOVICI, 1992).

It can be appreciated that there was a high biodiversity of phytoplankton, sensitive close or even higher than in the Danube Delta lacustrine ecosystem. In 2001, Lake Roşu diversity registered 144 species, and in Puiu, in 1987, 82 species were reported (TUDORANCEA & TUDORANCEA, 2006).

In 2005-2007, in terms of species frequency, 33.5 % of phytoplankton species were constant, 25% accessory and 41% accidental (Fig. 3a).

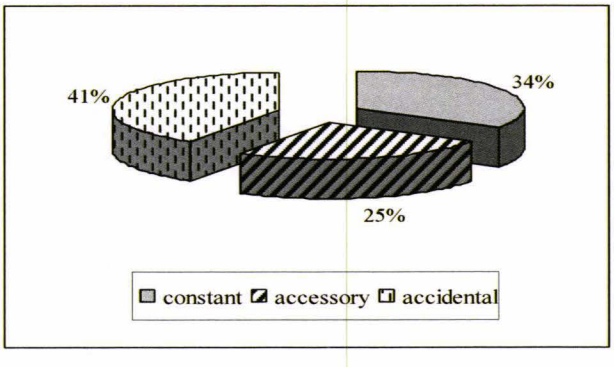

Figure 3a. The frequency of phytoplankton species in 2005-2007.

The frequency analysis of groups revealed the persistency of diatoms (38.04%), followed closely by euglenoids and chlorophytes (35%, respectively 33.33%). The Cyanobacteria had only 19.35 % contribution to the phytoplankton assemblage. The significantly higher number of constant species and high specific diversity indicated a considerable stability degree of the ecosystem found in a state of transition from brackish to lacustrine deltaic ecosystem, with 0 salinity. The typical freshwater macrophytes appeared and the general aspects of the ecosystem tend to be a deltaic lake (RADU et al., 2008). Also, the phytoplankton communities included ubiquitous species, characterized by a wide range of tolerance to salinity. In the period 1954-2005, 220 zooplankton species were recorded (Fig. 2).

Zooplankton species richness showed an ascending trend, due, probably, to the reverse relationship between the number of species and the decrease of salinity.

The evolution of specific diversity was estimated using diversity indices. Notice the minimum phytoplankton diversity in 2006, which showed the decline of species; the Shannon index decreased, indicating that it takes into account the numerical abundance (Figs. 2; 3b). The minimum zooplankton diversity was achieved in 1989, which is emphasized by the two evaluated indices (number of species and Shannon index).

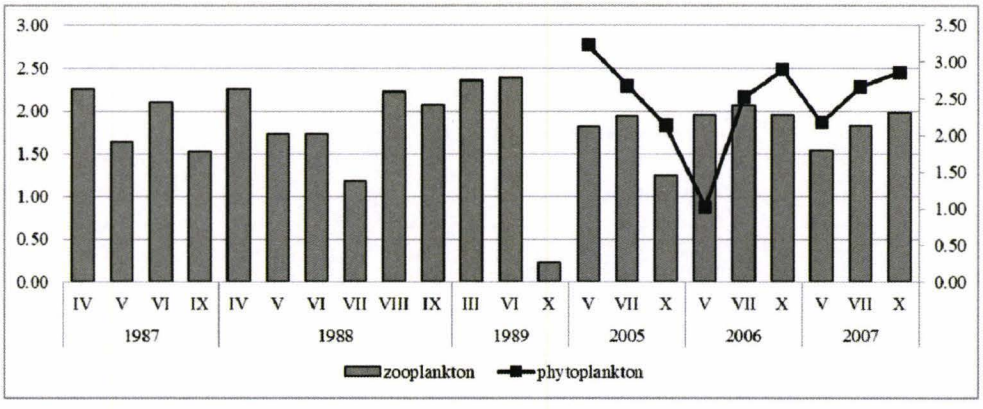

Figure 3b. The variation of Shannon-Wiener Diversity Index of phyto- and zooplankton species.

The gravimetric abundance of phytoplankton has been changed during 2005-2007 compared to the 1987-1989 period, especially regarding the number and percentage of the taxonomical groups. Thus, Bacillariophyceae decreased from 58% to 40%, Chlorophyceae from 34% to 16%, and a new group (Xanthophyceae) was observed in the last period (Fig. 4).

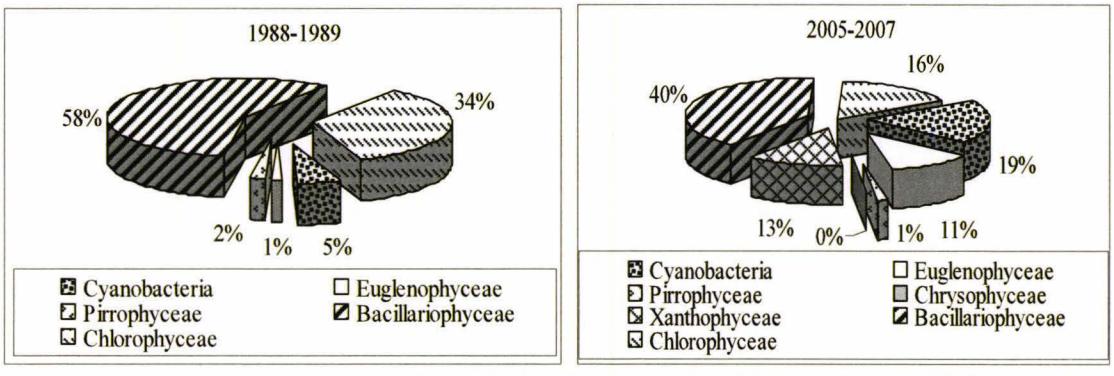

Figure 4. The gravimetric abundance of phytoplankton between 1987 and 1989 and between 2005 and 2007.

Zooplankton community showed a shift in number and dominance; the dominance of Cladocera was replaced by Rotatoria in 2005-2007 (Fig. 5).

Taxonomically, there is a structural simplification due to the gradual disappearance of marine species. These observations are proved by the values of Shannon-Wiener index (Table 1).

Table 1. Diversity indices of planktonic taxonomical and physiological groups.

Diversity indices	Phytoplankton		Zooplankton		Bacterioplankton	
	1988-1989	2005-2007	1987-1989	2005-2007	1987-1988	2006-2007
Shannon-Wiener Diversity	0.960	1.529	1.203	0.927	1.687	1.614
Evenness	0.597	0.853	0.868	0.844	0.941	0.901

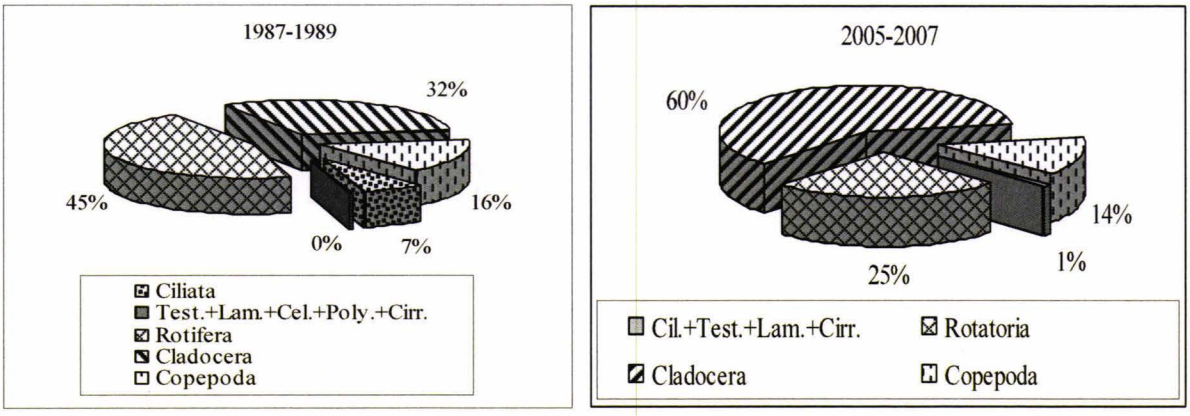

Figure 5. The gravimetric abundance of zooplankton between 1987 and 1989 and between 2005 and 2007 (Cil. = Ciliata; Test. = Testacea; Lam. = Lamellibranchia; Cel. = Coelenterata; Poly. = Polychaeta; Cirr. = Cirripedia).

The functional diversity of bacterioplankton is less or more sensitive to hydrogeomorphological changes (Fig. 6, Table 1). It is notable the presence of the sulphur decomposers of organic matter, which is a peculiarity of the marine environment.

During 1974-1975, the bacterioplankton community had not a characteristic seasonal dynamics, because the environmental factors from the impact area between the Danube water and marine water were instable (NICOLESCU & IONICĂ, 1995).

The response of plankton communities is reflected at structural and functional diversity level (the species richness of phytoplankton and zooplankton and diversity of physiological groups of bacterioplankton).

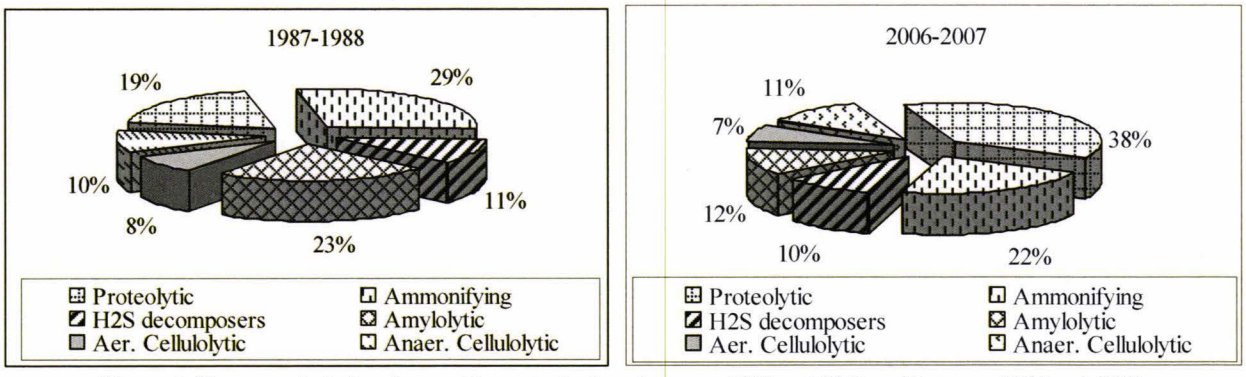

Figure 6. The numerical abundance of bacterioplankton between 1987 and 1989 and between 2005 and 2007.

The seasonal, annual and spatial distribution of biotic parameters among the sampling station was assessed by analysis of variance (ANOVA). The influence of spatial distribution of the sampling sites was assessed using the same statistical test, but no significant relationship was found with the parameters of interest ($F<Fcrit.$, $p>0.05$). This result supports our hypothesis that Musura Bay has changed to a semi-enclosed freshwater lagoon, with homogenous structural and functional characteristics.

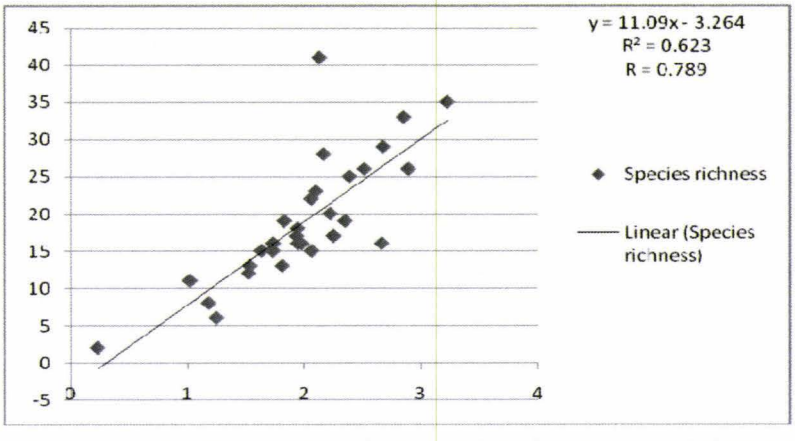

Figure 7. Pearson correlation between Shannon diversity and species richness of plankton communities.

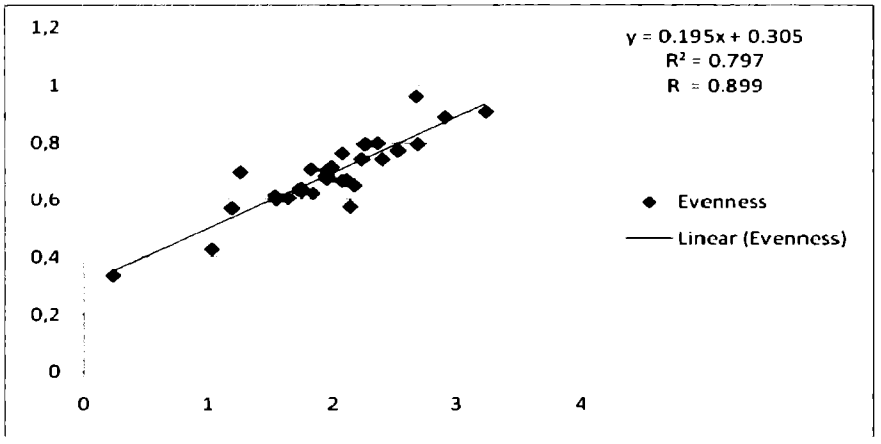

Figure 8. Pearson correlation between Shannon diversity and evenness of plankton communities.

In order to establish the relationships between diversity and other indices that characterize the populations of plankton (phytoplankton and zooplankton) simple correlations were used. By analysing Pearson correlation coefficient and the coefficient of determination R^2, it was concluded that diversity is determined mainly by evenness (81%) ($R = 0.8996$, $R^2 = 0.8094$), the number of species ($R = 0.7896$, $R^2 = 0.6235$) having a lower influence (62%) (Figs. 7, 8).

The control factors that modulate the evolution of this ecosystem were essentially the natural, climatic, hydrological, geomorphological ones, which led to the relatively rapid transition from a marine bay to a semi-enclosed lagoon with freshwater features, comparable to those of a typical deltaic lacustrine system.

CONCLUSIONS

From 2000 until present, Musura Lagoon has underwent an accelerated trend of isolation from the marine water of the Black Sea, due to an active process of remodelling of its borders between the real Delta, marine waters and the allochthonous flow of the Danube River.

Species richness and specific diversity, taxonomical composition, gravimetric abundance of taxonomical groups, marine/freshwater species ratio are the most sensitive diversity parameters of the plankton communities which responded to the hydrogeomorphological changes.

These changes have led to the formation of an ecosystem similar to that of a freshwater lake from the maritime delta. Currently, the interaction between the Danube, the Danube Delta and the Black Sea waters enhanced the alluvial and clogging processes that led to to the formation of a new island, still nameless.

Our study has a practical meaning and should be a valuable tool for the management and conservation politics of the transitional waters.

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SMALL RESERVOIRS VALEA PREAJBA (ROMANIA) – STRUCTURAL BIOCOENOTIC INDICES OF GASTROPOD POPULATIONS

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Abstract. The research made within some small reservoirs from the hydrographical basin of the Jiu emphasized the structure of the biocoenosis, an important role being played by the Gastropod populations. Thus, there have been established the structural biocoenotic indices. Their values highlight the distribution of gastropod populations within the reservoirs.

Keywords: Gastropods, the Preajba Valley, Oltenia, structural parameters.

Rezumat. Lacurile de baraj Valea Preajba (România) - indicii structurali biocenotici ai populaţiilor de gastropode. Cercetările efectuate asupra unor lacuri mici de baraj din bazinul hidrografic al Jiului au pus în evidenţă structura biocenozelor în care un rol important îl au populaţiile de gastropode. În acest scop au fost stabiliţi indicii structurali biocenotici. Valorile acestora reflectă distribuţia populaţiilor de gastropode în lacuri.

Cuvinte cheie: gastropode, Valea Preajba, Oltenia, indicii structurali.

INTRODUCTION

The small reservoirs Preajba Valley, located in the plain area from the hydrographical basin of the Jiu, are characterized by the great diversity of ecosystems (springs, streams, rivers, basins and swamps) (Fig. 1). In these ecosystems, there have been identified 18 species of Gastropods, 5 of which are new for Oltenia fauna: *Esperiana esperi* (A. Ferussac 1829), *E. (Microcolpia) daudebardii acicularis* (A. Ferussac 1829), *Aplexa hypnorum* Linnaeus 1758, *Segmentina nitida* (O. F. Muller 1774) and *Stagnicola corvus* Gmelin 1788 (CIOBOIU, 2002).

An important role in the structure of the reservoirs biocoenoses is played by the gastropods through their specific diversity, numerical and biomass density, as well as biocoenotic structural indices (BOTNARIUC et al., 1964; BREZEANU & GRUIŢĂ, 2002; BREZEANU et al., 2011; CIOBOIU, 2011). There have been calculated the following indices: frequency, relative abundance, indices of ecological significance, affinity, diversity and equitability (BOTNARIUC & VĂDINEANU, 1982; CIOBOIU, 2003; STĂNICĂ & NEACŞU, 1998).

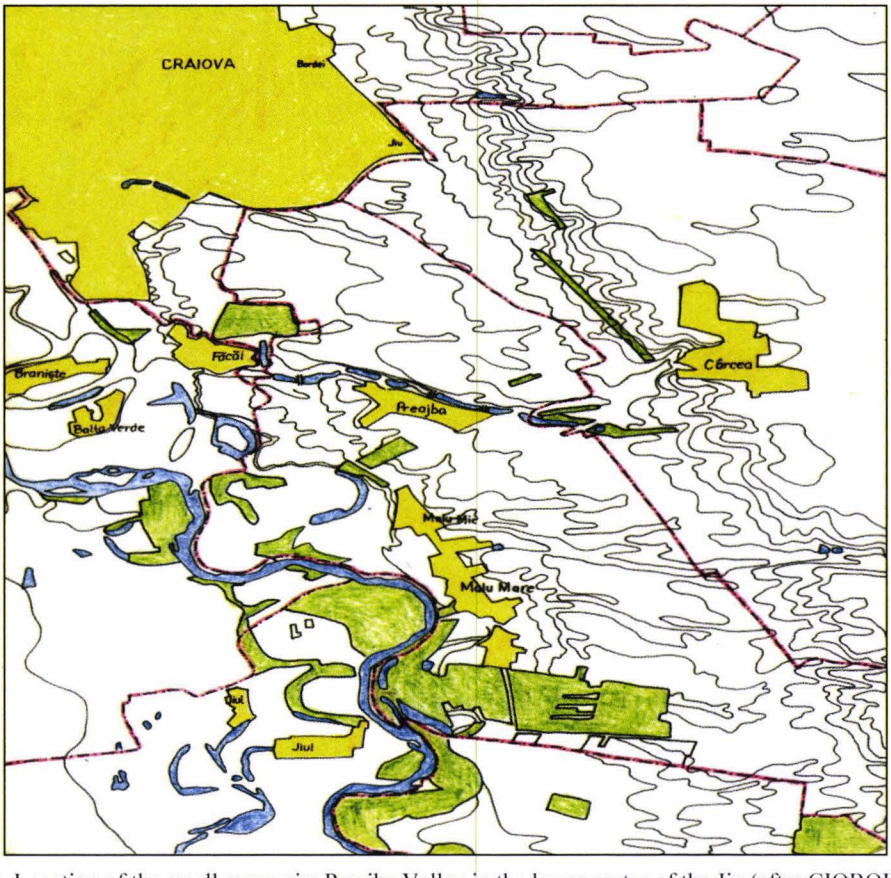

Figure 1. Location of the small reservoirs Preajba Valley in the lower sector of the Jiu (after CIOBOIU, 2011).

MATERIAL AND METHODS

In order to emphasize the qualitative and quantitative structures, there were taken samples each season. Within the framework of a vast research program, there were analysed more than 4,000 gastropods (CIOBOIU, 2002; 2011). The laboratory works included: taxonomic determinations, distribution within the habitat, structure according to age, dynamics of the populations increase and of the structural indices, determination of the production. The structural indices were established on the basis of statistical calculation.

RESULTS AND DISCUSSIONS

The structure of the gastropod populations displays certain specific features characteristic to each reservoir, features that are illustrated by the structural indices of the populations of this group. Some species were identified in only one reservoir or in two or three reservoirs at most, as it is the case of the species *Esperiana esperi* (A. Ferussac 1829), *Stagnicola corvus* Gmelin 1788 and *Oxyloma (O.) elegans* (Risso 1826). The main cause is the specificity of the microhabitat these species prefer, which are specific to the studied reservoirs - areas without decaying organic silt, with a sandy bottom washed by a slow water flow. They present a more or less uniform distribution, most of them being present in all the reservoirs; they are relatively ubiquist, adapted to the varied conditions characteristic to an eutrophic environment, such as the ones of these reservoirs, specific to the ecosystems from both stagnant waters and streams.

It was noticed that the greatest number of species populates the silty-detritic bottom of the shallow near-shore areas. These areas present the best feeding conditions. Gastropods find abundant food on the coarse detritus, on the leaves fallen into the water which are not decayed but covered by a rich periphyton, on the silt pellicle rich in organic substances. The lowest species diversity was determined in those areas characterized by a preponderantly sandy bottom or exclusively silty bottom (Table 1).

Table 1. The taxonomic composition of the Gastropod species within the studied reservoirs.

SPECIES	RESERVOIRS								
	V	VI	VII	VIII	IX	X	XI	XII	XIII
Viviparus acerosus Bourguignat 1870					+	+	+	+	
Viviparus viviparus Linnaeus 1758					+	+	+	+	
Valvata (Cincina) piscinalis O. F. Muller 1774						+	+	+	
Esperiana esperi (A. Ferussac 1829)	+			+					
E. (Microcolpia) daudebardii acicularis (A. Ferussac 1823)	+	+	+			+			
Physa fontinalis (Linnaeus 1758)				+				+	+
Physella (Costatella) acuta (Draparnaud 1805)	+	+	+	+	+	+	+	+	+
Aplexa hypnorum Linnaeus 1758	+	+	+	+	+			+	+
Stagnicola palustris (O. F. Muller 1774)	+								
Stagnicola corvus Gmelin 1788				+					
Radix auricularia (Linnaeus 1758)	+	+	+	+	+	+	+	+	
Radix ampla (Hartmann 1821)	+		+	+	+		+	+	+
Radix balthica (Linnaeus 1758)	+	+	+	+	+	+	+	+	+
Planorbis planorbis (Linnaeus 1758)			+			+	+		+
Anisus (A.) spirorbis (Linnaeus 1758)			+						
Segmentina nitida (O. F. Muller 1774)	+		+			+		+	
Oxyloma (O.) elegans (Risso 1826)	+								

The calculated indices emphasize that the reservoirs can be classified into two groups: those located in the upper and in the lower river. This grouping reflects the distribution of gastropods within the reservoirs (CIOBOIU, 2002; 2011; CIOBOIU & BREZEANU, 2009).

In the first group of reservoirs, the population *Physella (Costatella) acuta* (Draparnaud 1805), followed by *Radix balthica* (Linnaeus 1758) registered the highest frequencies: up to 72.22% in the first case, 50-55% in the second. In these reservoirs, the diversity index is the highest, as a direct consequence of the diversity of the habitat types: vast areas with paludous and aquatic macrophytes, while nearshore there is found a detritic and sandy facies (Fig. 2).

With regard to the second group of reservoirs, the highest frequency index is registered by the species *Viviparus acerosus* Bourguignat 1870 and *V. viviparus* Linnaeus 1758. Both of them are stagnant species that prefer especially the eutrophic lake ecosystems. Mostly inhabiting the nearshore areas with macrophytes, where a rich periphyton develops, as well as the submerged part of the concrete panels securing the shores, the two species, but also other accompanying species, find abundant sources of food here (Fig. 3). Characteristic to this area is the appearance of the species *Valvata (Cincina) piscinalis* O. F. Muller 1774 for the first time, the frequency index of which is 34.61 %; this species is considered to be oligotope (CIOBOIU & BREZEANU, 2002).

Taking into account their distribution and frequency in the studied reservoirs, it results that the species *Viviparus acerosus* Bourguignat 1870, *V. viviparus* Linnaeus 1758, *Physella (Costatella) acuta* (Draparnaud 1805) and *Radix balthica* (Linnaeus 1758) have a relative abundance and an important role in the functioning of the benthic biocoenosis. Besides these species, *Aplexa hypnorum* Linnaeus 1758 and *Radix auricularia* (Linnaeus 1758) are the dominant species accounting for over 74% of the total, the other species having a share of 25.9% (Fig. 4).

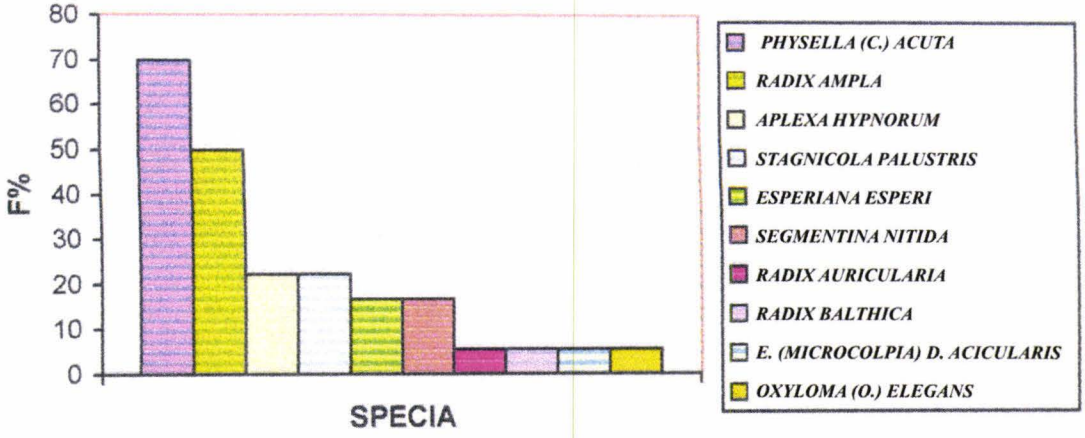

Figure 2. The frequency of the Gastropod species within the upper sector of the river (reservoir V).

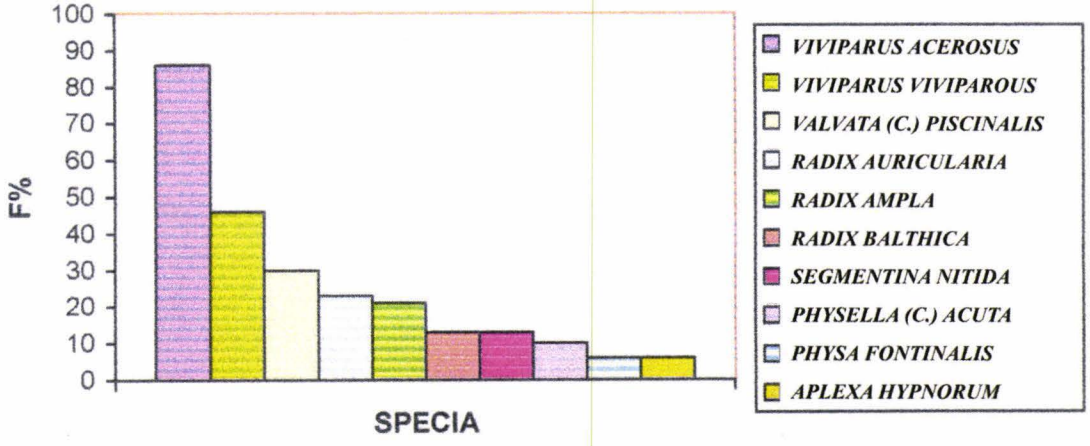

Figure 3. The frequency of the Gastropod species within the lower sector of the river (reservoir XII).

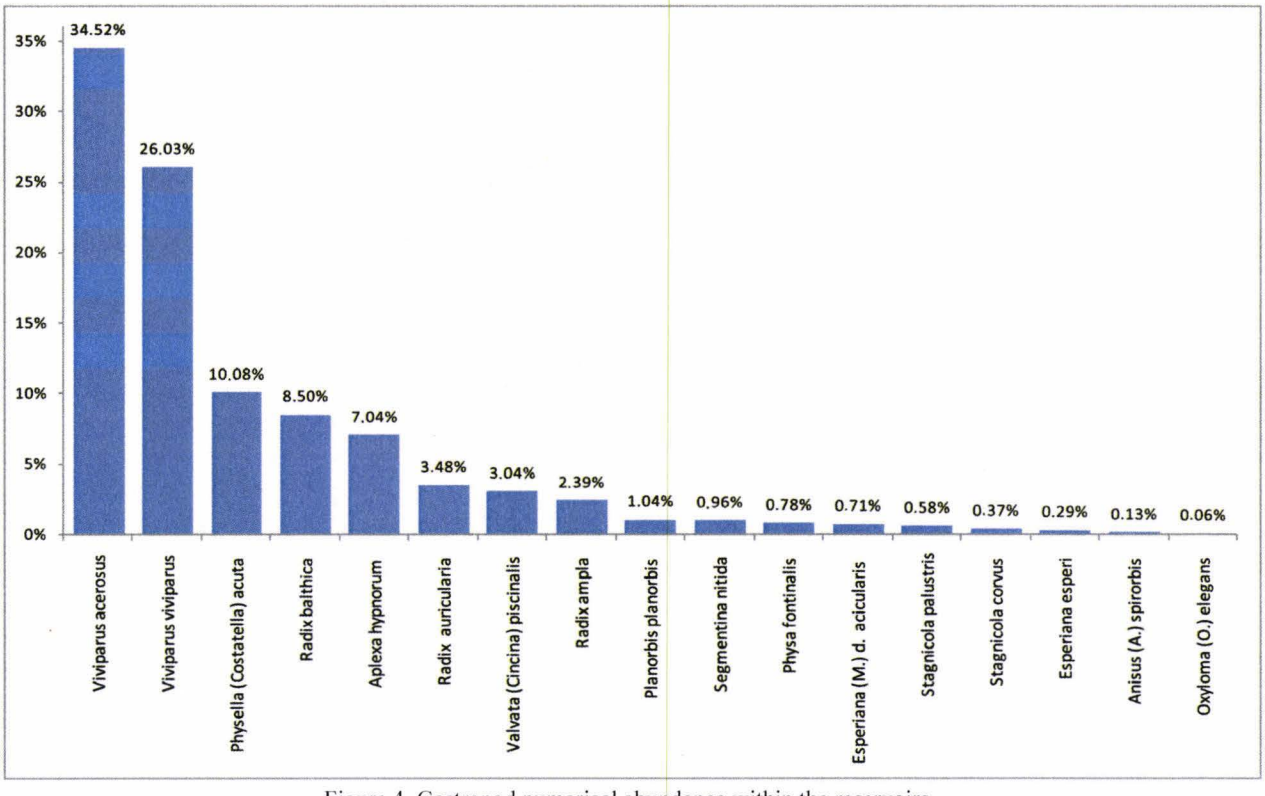

Figure 4. Gastropod numerical abundance within the reservoirs.

In order to calculate the affinity index, the species were classified according to the index of ecological significance (Table 2) (STĂNICĂ & NEACŞU, 1998). The affinity index, calculated according to Jaccard formula:

$$q = \frac{c}{a+b-c} X\ 100$$

in order to establish to what degree the different Gastropod species are characteristic for the biocoenosis they populate, emphasized that there is a certain link or association degree among the species.

Table 2. The distribution of Gastropod species according to the index of ecological significance (W).

(reservoir V)

SPECIES	W	TYPE OF SPECIES
Physella (Costatella) acuta	22.27	characteristic
Radix ampla	17.29	characteristic
Stagnicola palustris	2.28	accessory
Aplexa hypnorum	1.76	accessory
Esperiana esperi	0.78	accessory
Segmentina nitida	0.78	accessory
Radix balthica	0.34	accessory
Oxyloma (O.) elegans	0.08	accidental
Radix auricularia	0.03	accidental
E. (Microcolpia) daudebardii acicularis	0.03	accidental

(reservoir XII)

SPECIES	W	TYPE OF SPECIES
Viviparus acerosus	44.37	characteristic
Viviparus viviparus	14.52	characteristic
Valvata (Cincina) piscinalis	1.73	accessory
Radix ampla	0.85	accessory
Radix auricularia	0.79	accessory
Segmentina nitida	0.42	accessory
Radix balthica	0.15	accessory
Physella (Costatella) acuta	0.11	accidental
Aplexa hypnorum	0.04	accidental
Physa fontinalis	0.02	accidental

The maximum affinities that oscillate between 70.1 and 100 % have been registered to a reduced number of species: *Physa fontinalis* + *Radix ampla*, *Physa fontinalis* + *Physella (Costatella) acuta*, *Radix ampla* + *Physella (Costatella) acuta*; *Viviparus acerosus* + *V. viviparus*, *Viviparus acerosus* + *Radix balthica*; *Physella (Costatella) acuta* + *Stagnicola corvus*. It is known that the affinity between species does not always directly depend on each other, but also on the environment conditions (GROSSU, 1993; BREZEANU & GÂŞTESCU, 1996; VĂDINEANU, 2004). This is the case of the species *Viviparus acerosus* Bourguignat 1870 and *V. viviparus* Linnaeus 1758, that have similar conditions and thus, their affinity oscillates between 70 and 100 %. In fact, these two species, due to the high numerical and biomass density, play an important role in the functioning of the biocoenosis they populate, in transferring the matter and energy (GROSSU, 1986; 1987).

A much lower affinity can be noticed between *Physella (Costatella) acuta* (Draparnaud 1805) that populates all reservoirs as it finds optimum development conditions and *Oxyloma (O.) elegans* (Risso 1826), that rarely appeared; the affinity between them is only 7.7 %. If the first species can be considered eurytopic, as it is present within all the reservoirs, the other is an accidental species as it was identified within only one reservoir.

We can conclude that the affinity has increased values among the Gastropod species that are well established within the reservoirs and present a maximum number of individuals, as is the case of the species *Viviparus acerosus* Bourguignat 1870, *V. viviparus* Linnaeus 1758, *Physella (Costatella) acuta* (Draparnaud 1805), *Radix balthica* (Linnaeus 1758) and *Aplexa hypnorum* Linnaeus 1758 and low or null affinity values between the poorly ecologically consolidated species, characterized by a reduced number of individuals: *Anisus (A.) spirorbis* (Linnaeus 1758) and *Oxyloma (O.) elegans* (Risso 1826) (Table 3).

The calculation of the diversity and equitability indices demonstrates to what extent and which are the causes that triggered the differences of diversity at the Gastropod populations located within the reservoirs. The diversity index varied between 1.68 and 2.49 (Table 4).

According to the two groups of reservoirs, diversity indices highlight greater species diversity in the large areas with macrophytes. In the second group of reservoirs, where the bottom of the reservoirs is covered by sapropelic silt rich in organic substance, the dominant species are *Viviparus acerosus* Bourguignat 1870, *V. viviparus* Linnaeus 1758 and *Radix balthica* (Linnaeus 1758).

For the Gastropod populations, the equitability presents values between 0.59 and 0.88 (Table 5), the lowest value being registered within certain reservoirs located downstream, where the anthropogenic impact is greater.

Table 3. Affinity coefficients of the Gastropod populations.

0 – 10 %		10.1 – 30 %		30.1 – 50 %		50.1 – 70 %		70.1 – 100 %

Reservoir V

	Physella (C.) acuta	*Radix ampla*	*Stagnicola palustris*	*Aplexa hypnorum*	*Esperiana esperi*	*Segmentina nitida*	*Radix balthica*	*Oxyloma (O.) elegans*	*Radix auricularia*	*E.(Microcolpia) daudebardii acicularis*
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1		53.3	21.42	6.25	14.3	23.07	0	7.7	0	16.6
2			27.3	0	18.2	44.4	0	10	10	22.2
3				0	0	0	25	0	0	0
4					0	0	0	0	0	0
5						50	0	0	0	0
6							0	0	0	0
7								0	0	0
8									0	0
9										0
10										

Reservoir XII

	Viviparus acerosus	*Viviparus viviparus*	*Valvata (C.) piscinalis*	*Radix ampla*	*Radix auricularia*	*Segmentina nitida*	*Radix balthica*	*Physella (C.) acuta*	*Aplexa hypnorum*	*Physa fontinalis*
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1		61.5	34.2	23.1	26.9	15.4	11.5	11.1	3.7	7.7
2			33.3	16.6	29.4	11.8	5.9	11.8	6.3	0
3				15.4	33.3	18.9	0	8.3	0	22.2
4					0	0	0	25	0	14.3
5						10	25	0	12.5	0
6							0	0	0	0
7								0	25	0
8									20	0
9										0
10										

Table 4. The diversity of Gastropod species within the reservoirs.

REAL DIVERSITY								
RESERVOIRS								
V	VI	VII	VIII	IX	X	XI	XII	XIII
2 .49	2 .04	2.45	2 .02	2 .36	2 .33	1 .81	1 .98	1 .68

Table 5. The equitability of the Gastropod species within the reservoirs.

EQUITABILITY (E)								
RESERVOIRS								
V	VI	VII	VIII	IX	X	XI	XII	XIII
0 .83	0 .88	0 .82	0 .87	0 .82	0 .83	0 .65	0 .66	0 .59

CONCLUSIONS

The distribution of the Gastropod populations depends on the location of the reservoirs along the schemed Preajba Valley course and their classification into two groups: the reservoirs located on the upper sector of the river and the ones located on its lower sector. According to the analyses of the populations structural indices, there resulted that *Physella (Costatella) acuta* (Draparnaud 1805), *Radix balthica* (Linnaeus 1758) and *Aplexa hypnorum* Linnaeus 1758 species register the highest frequencies, as they are constant species; *Radix auricularia* (Linnaeus 1758), *Segmentina nitida* (O. F. Muller 1774) and *Anisus (A.) spirorbis* (Linnaeus 1758) are accidental species. In the second group of the reservoirs, *Viviparus acerosus* Bourguignat 1870 and *V. viviparus* Linnaeus 1758 displaying the highest frequency index, represent constant populations and present the most increased abundance. The calculation of the affinity index emphasizes that *Viviparus acerosus*, *V. viviparus*, *Physella (Costatella) acuta*, *Radix balthica* and *Aplexa hypnorum* are the best ecologically consolidated species.

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RESEARCH ON THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN SEVERAL FISH SPECIES UNDER THE ACTION OF THE FUNGURAN FUNGICIDE

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Abstract. Intensive agriculture increasingly depends on chemical fertilizers and pesticides. Considerable amounts of these substances persist in the soil and, then, they reach surface water bodies, favouring eutrophication phenomenon or adversely affecting aquatic life. The existence and evolution of aquatic organisms decisively depend on the environment they live in - water. This paper presents the results of the investigations regarding the changes of the physiological indices in fish under the action of the fungicide Funguran OH 50 WP (70% copper hydroxide, 50% metallic Cu). Copper hydroxide is toxic to aquatic organisms and it may cause long-term adverse effects in the aquatic environment. The toxic action of copper on fish manifests by the appearance of abundant mucus on gills and skin, cough, bleeding lips, stress, loss of balance and eventually death. Copper ions alter the ability of fish to distinguish toxic substances. In case of subacute concentrations (0.05 mg/l), the Prussian carp and other fish species are attracted to the ion source or tend to avoid it, according to the slope of the concentration gradient (low slope - attraction, steep slope - avoidance) and temperature, which sometimes, can reverse the reaction of fish. According to the research performed by us, it was found that the fungicide Funguran OH 50 WP has an inhibitory effect on the respiratory rate in all three investigated fish species (*Carassius auratus gibelio* Bloch, *Perca fluviatilis* L. and *Alburnus alburnus* L.); it also triggered the decrease of the number of erythrocytes and reduction of the glycaemic index. Perch proved to be the most sensitive fish species among the three studies species. The experiments performed show that the fungicide Funguran OH 50 WP is toxic to fish at very low concentrations.

Keywords: Funguran, pesticides, fish, temperature, respiratory rate, erythrocyte, glycaemia.

Rezumat. Cercetări asupra modificărilor unor parametri fiziologici la mai multe specii de peşti sub acţiunea fungicidului Funguran. Agricultura intensivă depinde tot mai mult de fertilizanţii chimici şi de pesticide. Cantităţi apreciabile din aceste substanţe persistă în sol, de unde ajung în apele de suprafaţă, favorizând fenomenul de eutrofizare a corpurilor de apă sau acţionând advers asupra vieţii acvatice. Existenţa şi evoluţia organismelor acvatice depind în mod hotărâtor de mediul în care evoluează - apa. Lucrarea de faţă prezintă rezultatele investigaţiilor referitoare la modificările unor indici fiziologici la peşti sub acţiunea fungicidului Funguran OH 50 WP (70% hidroxid de cupru, 50% Cu metalic). Hidroxidul de cupru este toxic pentru organismele acvatice, putând provoca efecte adverse pe termen lung asupra mediului acvatic. Acţiunea toxică a cuprului asupra peştilor se manifestă prin apariţia de mucus abundent pe branhii şi piele, tuse, buze hemoragice, stres, pierderea echilibrului şi în final moartea. Ionii de cupru alterează abilitatea unor peşti de a distinge substanţele toxice. La concentraţii subacute (0.05 mg/l), carasul şi alţi peşti sunt atraşi de sursa de ioni sau tind să o evite, în funcţie de panta gradientului de concentraţie (pantă mică-atracţie, pantă mare-evitare) şi de temperatură, care uneori poate inversa reacţia peştilor. În urma cercetărilor efectuate s-a constatat că fungicidul Funguran OH 50 WP are efect inhibitor asupra ritmului respirator la toate cele trei specii de peşti investigate (*Carassius auratus gibelio* Bloch, *Perca fluviatilis* L. şi *Alburnus alburnus* L.); de asemenea a determinat scăderea numărului de eritrocite şi reducerea nivelului glicemic. Dintre cele trei specii de peşti cel mai sensibil s-a dovedit a fi bibanul. Experienţele efectuate ilustrează faptul că fungicidul Funguran OH 50 WP este toxic pentru peşti în concentraţii foarte mici.

Cuvinte cheie: funguran, pesticid, peşte, temperatură, ritm respirator, eritrocite, glicemie.

INTRODUCTION

The contamination of freshwater with a wide range of pollutants has become a matter of concern in recent decades (VINODHINI & NARAYANAN, 2008). Increased human activities, especially the rapid development of agriculture and industry, have led to a considerable increase in the level of pollution, such as heavy metal pollution, which is the main anthropogenic pollutant that causes serious and long-term negative effects for all living organisms (SASTRY & SUKLA, 1993; MURUGAN et al., 2008).

Some metals such as copper, which is also essential for cell metabolism, become highly toxic to aquatic animals if their concentration in water increases (CARVALHO & FERNANDES, 2006).

Fish have the ability to accumulate heavy metals in tissues, gills being the main target of pollutants, and in case they are affected, there can be noticed an immediate impact on the entire body (AL-YACOOB et al., 1994). The human body can be also exposed at a high risk due to the contamination of the food chain (COSTA & HARTZ, 2009).

Many researchers have reported nocive effects of copper on aquatic life (OLAIFA et al., 2004; MUTHUKUMARVEL et al., 2007).

The negative effect of heavy metals on fish is linked to disturbances in the biochemical and physiological processes (VIELLA et al., 1999).

Biochemically, copper interferes in a series of enzymatic and redox processes, which are vital to living organisms as an activator or inhibitor. In case of the rainbow trout, *Salmo gairdneri*, the oxidation of the lactic acid in the gills has been inhibited in 53% of the survivors exposed to lethal concentrations of copper (DIUDEA et al., 1986).

The main cause of death in the fish exposed to heavy metals is hypoxia, as there occur changes in the brachial epithelium, disturbances in the osmoregulation process, decrease of the oxygen consumption and, finally, death (ALBASTER & LIOYD, 1982; PEURANEN et al., 1994; HASSAN, 2005). The gills are very sensitive to metals often

appearing various metal-induced lesions. This leads not only to osmotic imbalance, but may also cause a deterioration of the function of the respiratory system of fish, which varies depending on the type of metal and the site of action (JEZIERSKI & SARNOWSKI, 2002; DOBREV et al., 2008).

Funguran OH 50 WP (77% copper hydroxide and 50% metallic Cu) is a contact fungicide characterized by a very good capacity of penetration in plants and high protection. These features confer the product the ability to combat pathogenic fungi, especially those belonging to Phycomycete group (which produce "mana"), as well as the fire blight in the Rose family. Funguran OH 50 WP has great preventive action by inhibiting spore germination and blocking the development of the mycelium in plants. The period of protection depends on the local conditions and varies from 7 to 14 days; treated plants: potato, tomato, cucumber, beans, onion, vine, apple tree, pear tree, cherry tree, sugar beets, hop. It is classified in group III of toxicity. Copper hydroxide is toxic to aquatic organisms and it may cause long-term adverse effects in the aquatic environment.

MATERIAL AND METHOD

The research was conducted on specimens belonging to three species of fish from the Olt river: *Carassius auratus gibelio* (Bloch), *Perca fluviatilis* L. and *Alburnus alburnus* L. (Figs. 1, 2, 3). The biological material was chosen taking into consideration the physiological state, body integrity and size of the specimens, sensitivity to toxic substances, as well as the fact that they easily adapt to the 'retention' conditions in the aquarium, tolerate high temperature variations and show resistance to oxygen deficiency, displaying a low lethal hypoxic limit, especially the Prussian carp.

Figure 1. *Carassius auratus gibelio* (Bloch 1783).

Figure 2. *Perca fluviatilis* (Linné 1758).

Figure 3. *Alburnus alburnus* (Linné 1758).

The transfer of fish in aquariums was done about a month before the start of the experiments; thus, they have adapted to the new conditions.

The aquariums used during the experiments had a capacity of poisoning of 20-30 litres and were equipped with lighting, stirring and aeration systems. These containers have been cleaned and sanitized.

In case of the variants made at a temperature of 5-7°C, fish were placed in refrigerators, lighting being artificial.

The solutions of toxic substances from the aquariums were renewed at intervals of 24 hours.

The fish specimens used in different experimental variants were selected and sorted by weight categories in order to avoid or, on the contrary, to emphasize the effect of the individual weight factor. We used groups of fish of ten specimens.

The measurement of the respiratory rate was performed by successive determinations by means of a timer (their arithmetic mean representing the respiratory rate at the respective moment).

For the experiments carried out to underline the change in the number of erythrocytes and blood glucose levels under the action of the fungicide Funguran OH 50 WP, there were taken blood samples from the analysed fish. Blood was sampled from the caudal artery. Erythrocytes were determined using Thoma counting chamber (PICOŞ & NĂSTĂSESCU, 1988). Glucose determination was performed using Accutrend GCT device that allows the measurement of its value in a drop of blood using strips in a short time.

All the performed determinations were carried out under strict control.

The experiments that required series conducted at different dates were always made within a nyctemer as narrow as possible (same time each day) to avoid any influence of the circadian variations in the respiratory rate.

There were 12 experimental variants designed to determine the respiratory rate and 6 experimental variants to determine the number of erythrocytes and blood glucose.

The measurements were made after 14 days of treatment at two thermal levels: 5-7°C and 20-22°C.

The concentrations of Funguran OH 50 WP used in this work are 0.25 and 0.5 mg/l, concentrations corresponding to quantities of 0.2 respectively 0.4 mg copper hydroxide/l.

RESULTS AND DISCUSSIONS

1. The action of the fungicide Funguran OH 50 WP upon the respiratory rate

The variation of the respiratory rate in case of the Prussian carp specimens exposed to the fungicide Funguran OH 50 WP is rendered in figure 4.

At a temperature of 20-22°C, the fungicide has an inhibitory effect in case of both concentrations used. The concentration of 0.25 mg/l has a weak effect on the respiratory rate. The inhibitory effect increases at the concentration of 0.5 mg/l. After completing the experiment, the reductions are 6.25% and 12.3% compared to control group (according to the increase of the concentration).

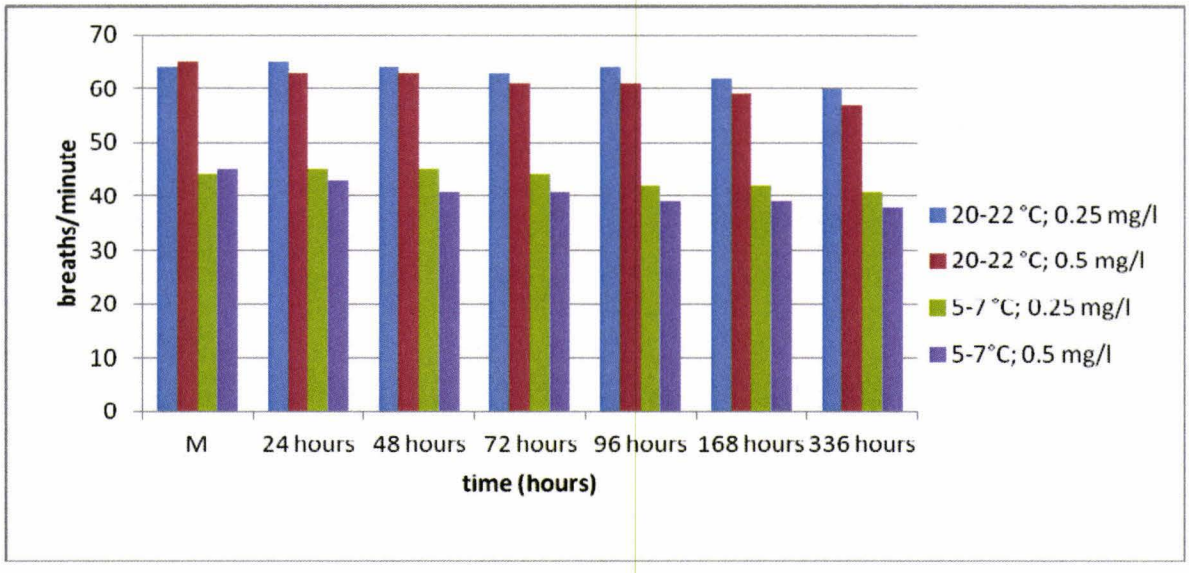

Figure 4. Variation of the average respiratory rate at the Prussian carp specimens exposed to the fungicide Funguran OH 50 WP at two thermal levels.

At a temperature of 5-7°C, the inhibitory effect of the fungicide is stronger as compared with that registered at the temperature of 20-22°C for both concentrations, but especially at the concentration of 0.5 mg/l (reduction of 7% and 15.5% respectively compared to the control group).

The action of the fungicide Funguran OH 50 WP on the respiratory rate at perch and bleak is rendered in figures 5 and 6.

The inhibitory effect of the fungicide on the respiratory rate occurs in the first 24 hours after exposure and continues to increase until the end of the experiment (14 days).

The respiratory rate decreased at the perch specimens poisoned with Funguran OH 50 WP by 10% at a temperature of 20-22°C and 12% at 5-7°C at a concentration of 0.25 mg/l and by 17% at a temperature of 20-22°C and 19% at a temperature of 5-7°C at a concentration of 0.5 mg/l as compared to the control group.

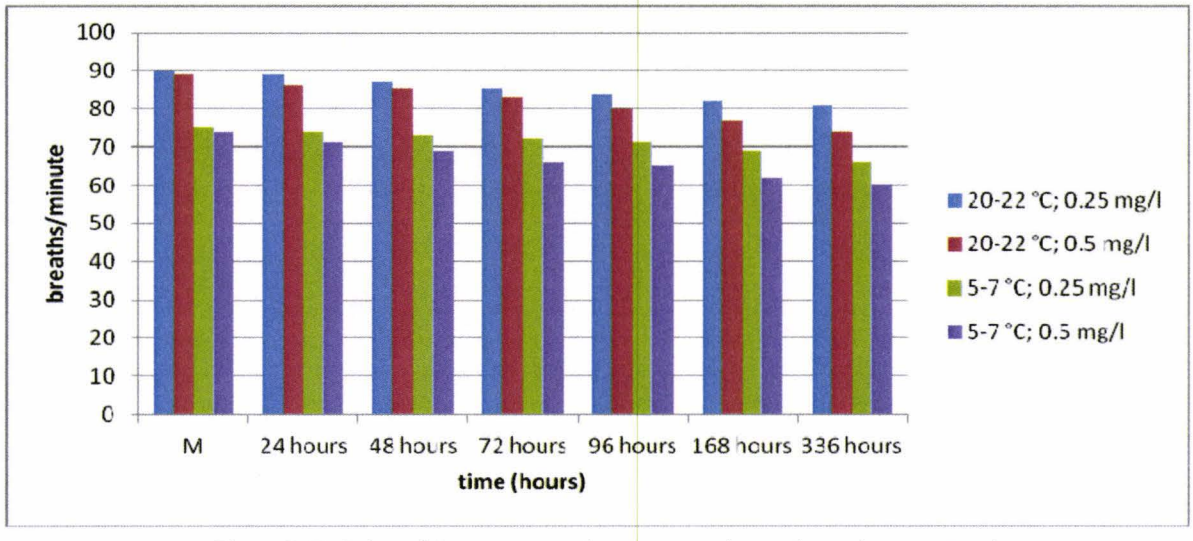

Figure 5. Variation of the average respiratory rate at the perch specimens exposed to the fungicide Funguran OH 50 WP at two thermal levels.

The respiratory rate decreased at the bleak specimens poisoned with Funguran OH 50 WP by 8% at a temperature of 20-22°C and 9% at the temperature of 5-7°C at a concentration of 0.25 mg/l and by 13.5% at a temperature of 20-22°C and 15.5% at a temperature of 5-7°C at a concentration of 0.5 mg/l as compared to the control group (Fig. 6).

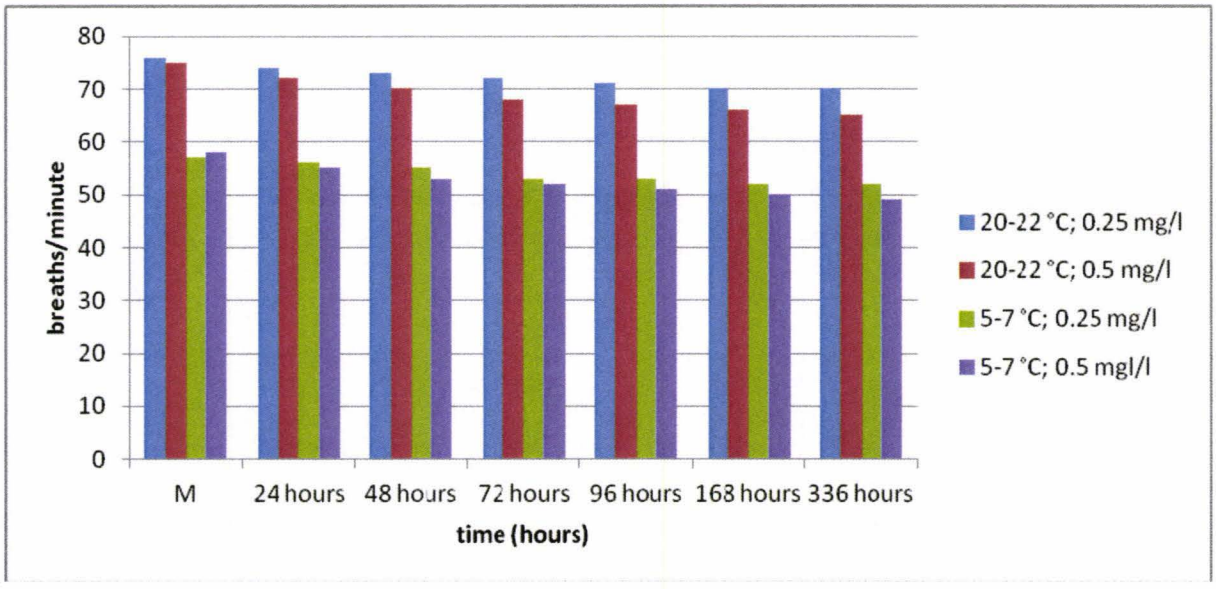

Figure 6. Variation of the average respiratory rate at the bleak specimens exposed to the fungicide Funguran OH 50 WP at two thermal levels.

The decrease of the respiratory rate is more pronounced at perch specimens than at bleak specimens. The toxic action of the fungicide should be correlated with the respiratory lesions.

Copper is concentrated in gills and liver (DURVE 1980, quoted by DIUDEA et al. 1986) at the species *Ictalurus nebulosus* after 30 days of exposure to 0.027 mg / l copper.

Metals can affect the respiratory function by reducing the respiratory surface, by atrophy and fusion of secondary lamellae, as well as by the internal action of the metal, which enhances the action of the respiratory inhibiting factors (MUTHUKUMARVEL et al., 2007; SHEREEN & LOGSWAMY, 2008).

Copper exposure causes histopathological changes leading to the separation of the epithelium from the secondary lamellae, hyperplasia, fusion of the secondary lamellae and necrosis (HASSAN, 2011).

The same effects were reported by MUHVICH et al. (1995) after exposing the fish to sub-lethal concentration of copper sulphate for 96 h, PANDEY et al. (1997) at sub-lethal concentrations of lead for 15 days, VUTUKURU (2005) and HASSAN (2005) after exposing the species *Carassius Carassius* to sub-lethal concentrations of cadmium.

2. The action of the fungicide Funguran OH 50 WP upon the number of erythrocytes

By analysing figure 7, where it is rendered the action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg / l at carp, perch and bleak at a temperature of 20-22°C, it can be noticed that the reduction in the number of red blood cells is higher in case of perch specimens (20%). At bleak, the reduction in the number of erythrocytes is 16.5% compared to the control group, while at the Prussian carp the reduction is 15%.

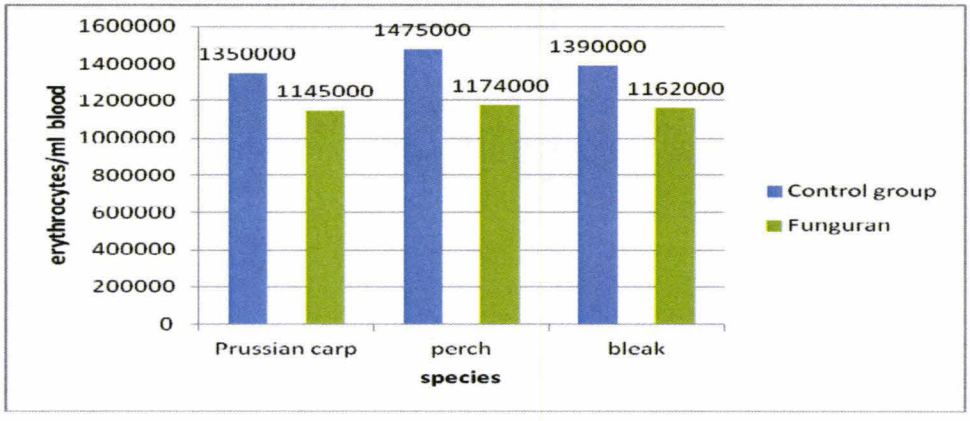

Figure 7. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon the number of erythrocytes at Prussian carp, perch and bleak at 20-22°C.

At a low temperature (5-7°C), the reduction in the number of erythrocytes is 19% for carp, 17% for perch and 14% for bleak. Low temperature influences the reduction in the number of erythrocytes only at perch and bleak (Fig. 8).

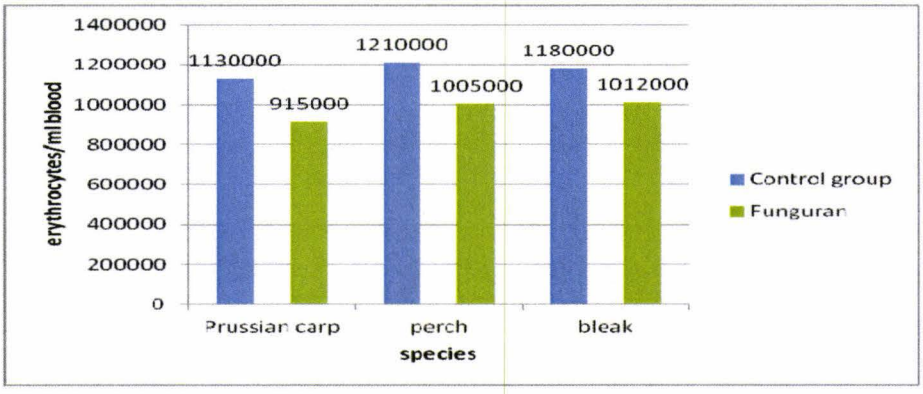

Figure 8. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon the number of erythrocytes at Prussian carp, perch and bleak at 5-7°C.

The reduction in the number of erythrocytes was reported in the species *Carassius auratus gibelio* Bloch by MIHAI (2013) under the action of the fungicides Funguran Champion, Ridomil and Curzate manox (which contain metallic copper).

At the same time, a decrease in the number of erythrocytes, haemoglobin and haematocrit levels along with a severe anaemia was reported in the species *Channa punctatus* subjected to a treatment with copper sulphate (0.36 mg/l) by SINGH et al. (2008) and copper and chromium (SINGH, 1995). The metal penetrates the fish body and is eliminated slowly (NEWMAN & MITZ, 1988; JAMES & MITZ, 1996; JAMES et al., 1996); therefore, the haematological parameters are affected by the toxicity of the metal. Oxygen transport in the blood depends on the amount of haemoglobin.

Acute exposure of the species *Colisa fasciatus*, *Oreochromis mossambicus* to sub-lethal concentrations of lead, copper and zinc showed that haemolytic anaemia occurs due to the lysis of erythrocytes with concomitant decrease in haemoglobin and haematocrit (SOIVEO & NIKINMAA, 1981; SAMPATH et al., 1998).

Anaemia under the stress induced by copper may also be due to cell injury and disruption of the blood haemoglobin synthesis (McKIM et al., 1970; GROSS et al., 1975). Similar results, rendering significant reduction of erythrocytes and haemoglobin levels in fish exposed to different heavy metals have been previously reported by GOEL et al. (1985) and GOEL & SHARMA (1987). According to DE BOECK (1995) and (SINGH et al. (2008), the reduction of the haemoglobin level in the fish exposed to toxic substances could also be a result of the inhibitory effect of the toxic substance on the enzyme system responsible for the synthesis of haemoglobin. JOSHI et al. (2002) suggested that the exposure to heavy metals also decreased the number of erythrocytes, haemoglobin and haematocrit due to the dysfunction of iron intestinal absorption. Anaemia is an early manifestation of an acute and chronic intoxication with heavy metals.

3. The action of the fungicide Funguran OH 50 WP upon glycaemia

The fungicide Funguran OH 50 WP lowers the blood glucose at 20-22°C in all three studied fish species. At the end of the experiment (Fig. 9), higher reductions in the glucose levels were recorded at perch (33%) and bleak (27.6%); in the case of the Prussian carp, the reduction was 21.2%.

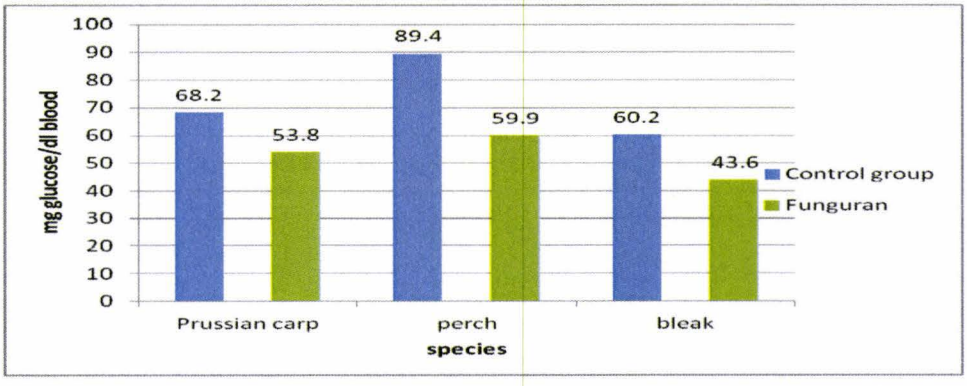

Figure 9. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon glycaemia at Prussian carp, perch and bleak at 20-22°C.

The temperature of 5-7°C determines the decrease of glycaemia by 19% in the Prussian carp, 30.9% in perch and 26.75% in bleak (Fig. 10).

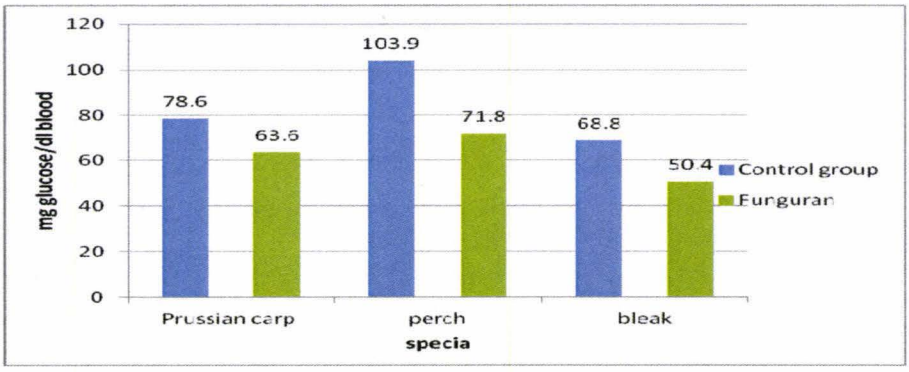

Figure 10. Action of the fungicide Funguran OH 50 WP at a concentration of 0.25 mg/l upon glycaemia at Prussian carp, perch and bleak at 5-7°C.

CONCLUSIONS

Low temperatures (5-7°C) lead to the decrease of the respiratory rate, low number of erythrocytes and to the increase of glucose levels compared to the values recorded at 20-22°C. The fungicide Funguran OH 50 WP reduces the respiratory rate in the three studied species of fish (*Carassius auratus gibelio* Bloch, *Perca fluviatilis* L. and *Alburnus alburnus* L.). The number of erythrocytes reduces after the exposure to Funguran OH 50 WP. The glucose values decrease in the fish exposed to Funguran OH 50 WP, this fungicide having hypoglycaemic effect. Of all the three studied fish species, perch registered greater reductions in all the investigated physiological indices, this species being the most sensitive, followed by bleak and the Prussian carp.

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RESEARCH ON THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP UNDER THE ACTION OF THE FASTER INSECTICIDE

MIHAI Florina

Abstract. The production and use of pesticides is a serious threat to the hydrosphere due to the discharge of residual water from factories of antiparasitic products or to the action of rainfalls, which remove these substances from the treated agricultural lands (MOHAN & ARDELEAN, 1993). The spread of pesticides has become a global issue, as they are present in all inland waters and carried by the rivers and streams in seas and oceans (BREZEANU & SIMON-GRUIŢĂ, 2002). The existence and evolution of aquatic organisms decisively depend on the environment they live in - water. In case of water bodies contaminated with pesticides, the environmental impact is mainly induced by the capacity of bioaccumulation and biomagnification of pesticides in the organisms living in water (GAVRILESCU, 2008). Of all the pesticides, the most important in terms of the danger of water contamination are particularly insecticides (MĂLĂCEA, 1969). This paper presents the results of the investigations on the changes of certain physiological indices in fish under the action of the insecticide Faster 10 CE, the active substance of which is cypermethrin at a concentration of 100 g/l. Cypermethrin is a synthetic non-systemic pyrethroid, with contact action and through ingestion. It is toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. According to our investigations, it was found that the insecticide Faster 10 CE exerts an inhibitory effect on the oxygen consumption and respiratory rate in the investigated species (*Carassius auratus gibelio* Bloch); at the same time, it increased the number of erythrocytes and blood glucose levels having a hyperglycaemic effect. The performed experiments show that the insecticide Faster 10 CE is toxic to fish at very low concentrations.

Keywords: faster, fish, energetic metabolism, erythrocytes, glycaemia.

Rezumat. Cercetări asupra modificărilor unor parametri fiziologici la caras sub acţiunea insecticidului Faster. Producerea şi folosirea pesticidelor constituie o gravă ameninţare pentru hidrosferă în care ajung datorită deversărilor de reziduuri de la fabricile de produse antiparazitare sau datorită spălării acestor substanţe de apele de ploaie de pe terenurile agricole tratate (MOHAN & ARDELEAN, 1993). Răspândirea pesticidelor a devenit globală, fiind prezente în toate apele continentale şi duse de apele râurilor şi fluviilor în mări şi oceane (BREZEANU & SIMON-GRUIŢĂ, 2002). Existenţa şi evoluţia organismelor acvatice depind în mod hotărâtor de mediul în care evoluează - apa. În cazul apelor contaminate cu pesticide, impactul ecologic se datorează în principal capacităţii de bioconcentrare şi biomagnificare a pesticidelor din apă în organisme (GAVRILESCU, 2008). Dintre toate pesticidele, cele mai importante din punctul de vedere al pericolului de contaminare al apelor sunt îndeosebi insecticidele (MĂLĂCEA, 1969). Lucrarea prezintă rezultatele investigaţiilor referitoare la modificările unor indici fiziologici la peşti sub acţiunea insecticidului Faster 10 CE, ce are ca substanţă activă cipermetrinul în concentraţie de 100 g/l. Cipermetrinul este un piretroid de sinteză nesistemic, cu acţiune de contact şi prin ingestie. Este toxic pentru organismele acvatice, putând provoca efecte adverse pe termen lung asupra mediului acvatic. În urma cercetărilor efectuate s-a constatat că insecticidul Faster 10 CE are efect inhibitor asupra consumului de oxigen şi ritmului respirator la specia de peşti investigată (*Carassius auratus gibelio* Bloch); de asemenea a determinat creşterea numărului de eritrocite şi a nivelului glicemic, având efect hiperglicemiant. Experienţele efectuate ilustrează faptul că insecticidul Faster 10 CE este toxic pentru peşti în concentraţii foarte mici.

Cuvinte cheie: faster, peşte, metabolism energetic, eritrocite, glicemie.

INTRODUCTION

Numerous research studies on the biological effects of environmental pollution are geared toward the detection of the functional changes of the animal organism resulting from the action of certain chemical agents used in the industrial technological processes or agriculture, which are then collected by inland waters (DRĂGHICI, 1976; 1979).

The contamination of freshwater bodies with a wide range of pollutants has become a matter of concern in recent decades (VINODHINI & NARAYANAN, 2008).

As they reach the aquatic environment, pesticides affect a wide range of non-target organisms, such as invertebrates and fish (OTLUDIL et al., 2004). Because of the accumulation of pesticides in the tissues and organs of fish and other hydrobionts, they intoxicate and, in their turn, become sources of pollution.

ZAMFIR (1974), synthesizing a rich specialized literature, shows that pesticides detected in some food products today are incriminated to cause reproductive impairment of the body and induce mutagenic, teratogenic and carcinogenic effects.

Faster 10 CE is an insecticide in the form of an emulsifiable concentrate, having cypermethrin as the active ingredient in a concentration of 100 g/l. Cypermethrin is a synthesis non-systemic pyrethroid of Type II - alpha-cyano pyrethroid, which acts at contact and through ingestion.

Pyrethroid insecticides are highly toxic for fish. They damage the nervous system of invertebrates, inducing paralysis (MILLER & SALGADO, 1985). It has a broad spectrum of action, particularly on the species of Lepidoptera, Coleoptera and Diptera; it blocks the transmission of nerve impulses (BRADBURY & COATS, 1989).

Even low levels of cypermethrin in water can have significant effects on the reproduction and development in carp (AYDIN et al., 2005).

It inhibits the production of ATP-ase enzyme (CLARK & MATSUMURA, 1982). This is of great importance to understand why aquatic organisms are more sensitive to pyrethroid insecticides than terrestrial organisms.

It has a good residual action on treated plants, with inhibitory effect on the feeding process of phytophagous insects. It is very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment.

Cypermethrin has relatively low toxicity to mammals and birds, although it is extremely toxic to fish and aquatic invertebrates (BRADBURY et al., 1989). It is classified in group III of toxicity.

MATERIAL AND METHOD

The research was conducted on the Prussian carp (*Carassius auratus gibelio* Bloch), which we sampled from the Olt River (Fig. 1). When choosing the biological material we have considered the physiological state, body integrity and size of the specimens, the sensitivity to toxic substances, as well as the fact that the species easily adapts to the conditions of retention in the aquarium, tolerates high temperatures and shows good resistance to oxygen deficiency having a low lethal hypoxic limit.

Figure 1. *Carassius auratus gibelio* (Bloch, 1783).

The introduction of fish in the aquariums was done about a month before the start of the experiments, to live them enough time to adapt to the new conditions. The aquariums used during the experiments had a capacity of poisoning of 20-30 litres and were equipped with lighting, stirring and aeration systems. These tanks have been cleaned and sanitized. In case of the variants made at 5-7°C, fish were placed in refrigerators, lighting being artificial. The solutions of toxic substances from the aquariums have been renewed at intervals of 24 hours.

The specimens of Prussian carp used in different experimental variants were selected and sorted according to the weight category in order to avoid or, on the contrary, enhance, the effect of the individual factor represented by body weight. We used fish lots consisting in ten specimens each.

Of the three methods for determining the amount of dissolved oxygen (iodometric, gasometric and polarographic method), we used Winkler iodometric method in the achieved experiments, as described by the author at the end of the last century (1888). This method is considered the safest way to quantitatively assess the content of dissolved oxygen in water, under various temperature conditions. To assess the oxygen consumption it was used the technique of confined space described by Stroganov (1962), which allows to easily determine the exact quantity of the oxygen consumed by fish within a known time, based on the difference between the amount of oxygen measured in a control tank and the amount determined at the end of the period (quoted by PICOŞ & NĂSTĂSESCU, 1988).

The measurement of the respiratory rate was performed by successive determinations by means of a timer (their arithmetic mean representing the respiratory rate at the time).

The experiments that required series performed in different days were always made within a nyctemeral as narrow as possible (same time each day), in order to avoid any possible influence of the circadian variations of the oxygen consumption and respiratory rate.

As we aimed at achieving experimental conditions as close as possible to natural conditions, there have been kept and used in the experiment only the fish specimens found in the conditions of natural photoperiodism. Although it was not demonstrated a direct relationship between energy metabolism and light, it was taken into account the importance of the light factor in triggering certain functional manifestations.

Much attention was paid to the effect of fish handling during the pre-experimental stage, which may alter the results of the determinations (FRY, 1957; MARINESCU, 1972). For the experiments carried out by the technique based on the principle of confined space, where it is not possible to entirely avoid perturbing fish due to manipulation, we tried to limit its action. Consequently, for the experiment, we chose fish species characterized by low mobility (gibel carp) and we always performed control samples before the actual experiment.

For the experiments carried out to determine the change in the number of erythrocytes and blood glucose levels under the action of the insecticide Faster 10 CE, there were taken blood samples from the fish specimens. Blood samples were taken from the caudal artery. The number of erythrocytes was determined using Thoma counting chamber (PICOŞ & NĂSTĂSESCU, 1988).

The determination of the blood glucose level was performed using Accutrend GCT device that allows the measurement of its value in a drop of blood on a testing strip in a very short time. All the determinations performed on fish were carried out under strict control. There were eight experimental variants designed to determine the four physiological indices.

Measurements were made after 14 days of treatment at two thermal levels: 5-7°C and 20-22°C.

The concentrations of Faster 10 CE used in this work are 0.000005, 0.00001, 0.00002 and 0.00004 ml Faster 10 CE/l, respectively 0.5, 1, 2 and 4 µg cypermethrin/l.

RESULTS AND DISCUSSIONS

1. The action of the insecticide Faster 10 CE upon the oxygen consumption

The insecticide Faster 10 CE caused the intensification of the oxygen consumption in fish, within the first 72 hours after placing the fish in the toxic solution at lower concentrations (0.000005 and 0.00001 ml Faster 10 CE/l), the most pronounced increase in the energy metabolism occurring 24 hours after the exposure to the insecticide (114%, respectively 117% compared to the control group) (Fig. 2).

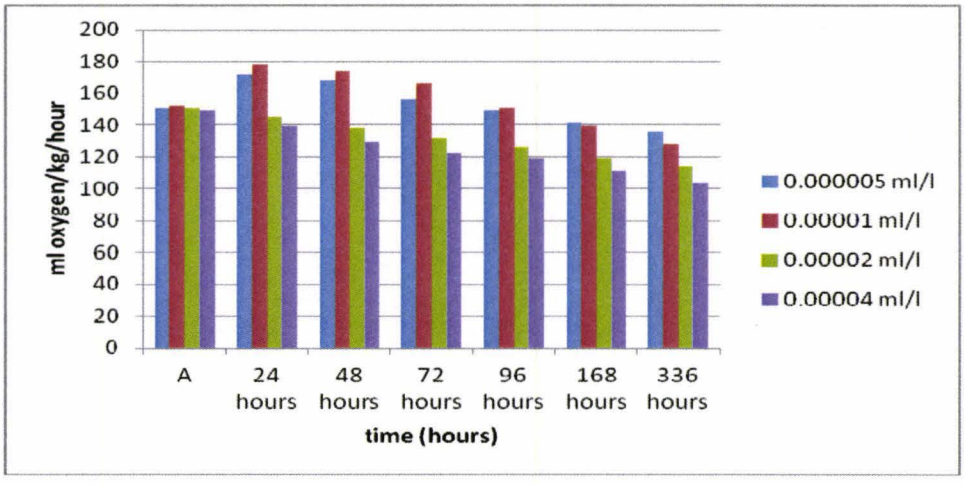

Figure 2. Variation of the average oxygen consumption at the Prussian carp specimens exposed to the action of the insecticide Faster 10 CE at different concentrations at 20-22°C.

The insecticide Faster 10 CE determined the decrease of the oxygen consumption at the end of the experiment (14 days), at all concentrations. The highest decrease was registered at the concentration of 0.00004 ml/l of Faster CE (30% less compared to the value recorded before the start of the experiment). In case of the first three concentrations, the reduction of the oxygen consumption was 10, 16 and 24% (in order of increasing concentration) compared to the control sample.

The temperature of 5-7°C determined the increase of the oxygen consumption at the Prussian carp specimens, during the first 72 hours after the exposure at lower concentrations (0.000005 ml and 0.00001 Faster 10 CE/l), as it was registered in case of the temperature of 20-22°C. The most pronounced increase in the energy metabolism was registered 48 hours after the exposure to the insecticide (110.3%, respectively 115.3% compared to the control group) (Fig. 3).

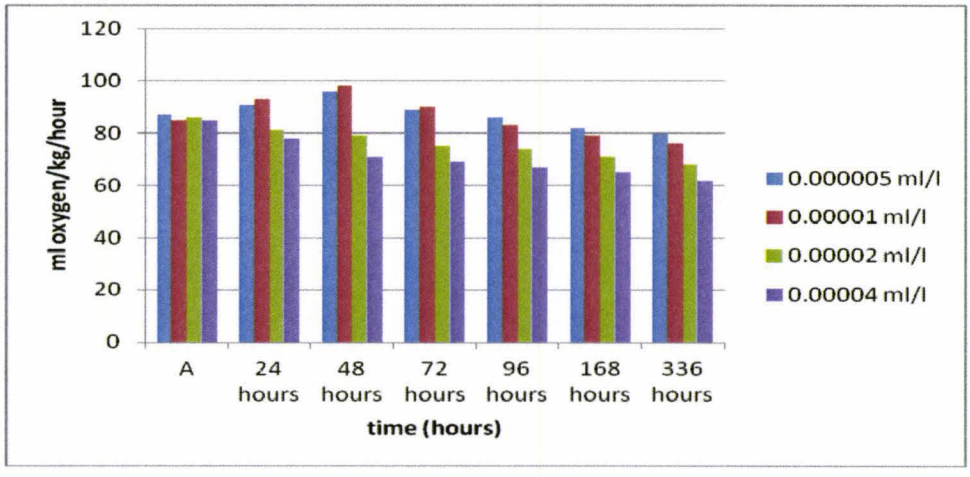

Figure 3. Variation of the average oxygen consumption at the Prussian carp specimens exposed to the action of the insecticide Faster 10 CE at different concentrations at 5-7°C.

The values registered at the end of the experiment are lower than the initial values in all the studied variants (8%, 11.5%, 21% and 27%). The reduction of the oxygen consumption is more pronounced at the temperature of 20-22°C.

2. Action of the insecticide Faster 10 CE upon the respiratory rate

The variation of the respiratory rate at the Prussian carp specimens exposed to the action of the insecticide Faster 10 CE at different concentrations at 20-22°C is rendered in figure 4.

In the first 24-48 hours, it can be noticed a slight increase in the respiratory rate and then, it begins to decrease, so that at the end of the experiment, the average values of this index are 20-30% lower compared to those registered before introducing the fish specimens in the toxic solution at all tested concentrations.

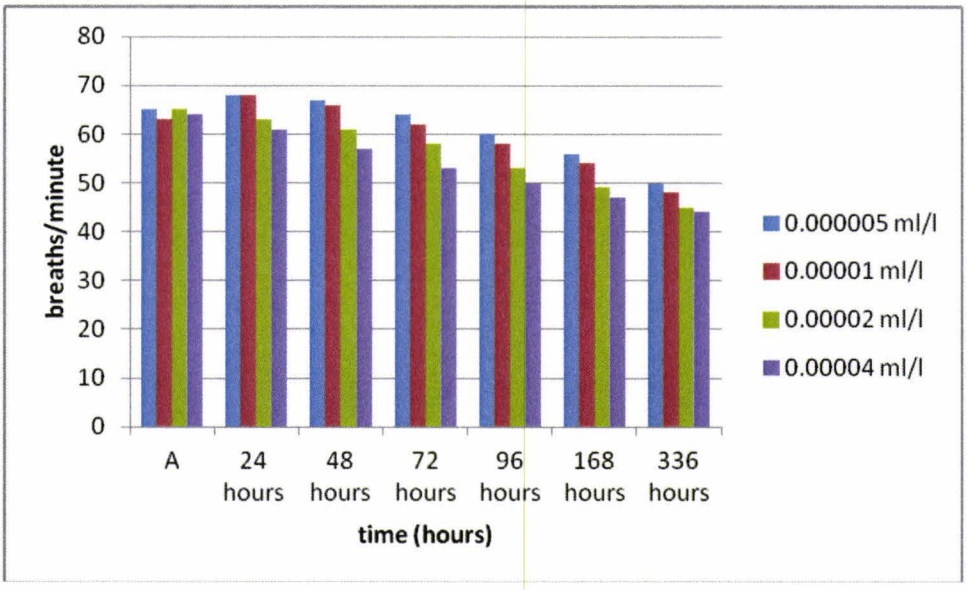

Figure 4. Variation of the respiratory rate at the Prussian carp specimens exposed to the action of the insecticide Faster 10 CE at different concentrations at 20-22°C.

Low temperature (5-7°C) reduced the respiratory rate throughout the experiment (Fig. 5), without registering an intensification of the index in the first hours after exposure, as it happened in case of the variants achieved at 20-22°C. The values determined at the end of the experiment are 17-26% lower compared to those registered before the fish exposure to the toxic substance. The reduction of the respiratory rate is more pronounced at 20-22°C.

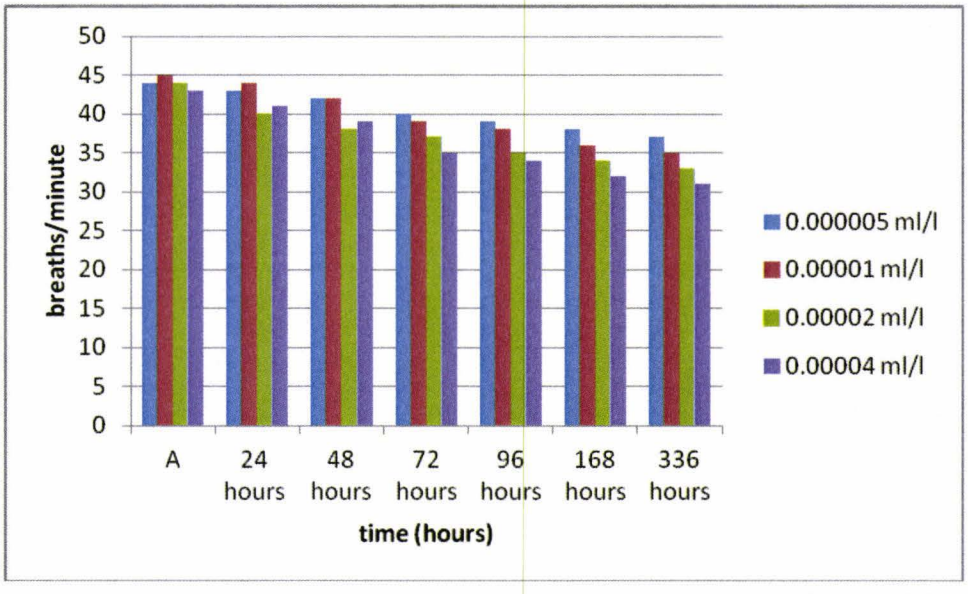

Figure 5. Variation of the respiratory rate at the Prussian carp specimens exposed to the action of the insecticide Faster 10 CE at different concentrations at 5-7°C.

The obtained results (oxygen consumption and respiratory rate) suggest a higher toxicity of the insecticide Faster 10 CE at the temperature of 20-22°C.

The decrease of the oxygen consumption can be explained by the histological changes in the gills occurred because of the toxic action of the insecticide Faster 10 CE.

COSTIN et al. (2007) and CENGHIZ (2006) reported changes at the level of the gills in the *Carassius auratus gibelio*, respectively, *Cyprinus carpio* after exposure to deltamethrin (hyperaemia, desquamation, necrosis, edema, lifting of lamellar epithelial, hyperplasia, aneurysm in the secondary lamellae, and fusion of secondary lamellae).

3. Action of the insecticide Faster 10 CE upon the number of erythrocytes

The number of erythrocytes at the Prussian carp specimens intoxicated with Faster 10 CE at a concentration of 0.000005 ml / l for 14 days, in case of both thermal levels, are rendered in figures 6 and 7.

At the temperature of 20-22°C, the insecticide Faster 10 CE at a concentration of 0.000005 ml / l determined the increase in the number of erythrocytes in exposed fish compared to the control group, where the increase was of only 4.44%.

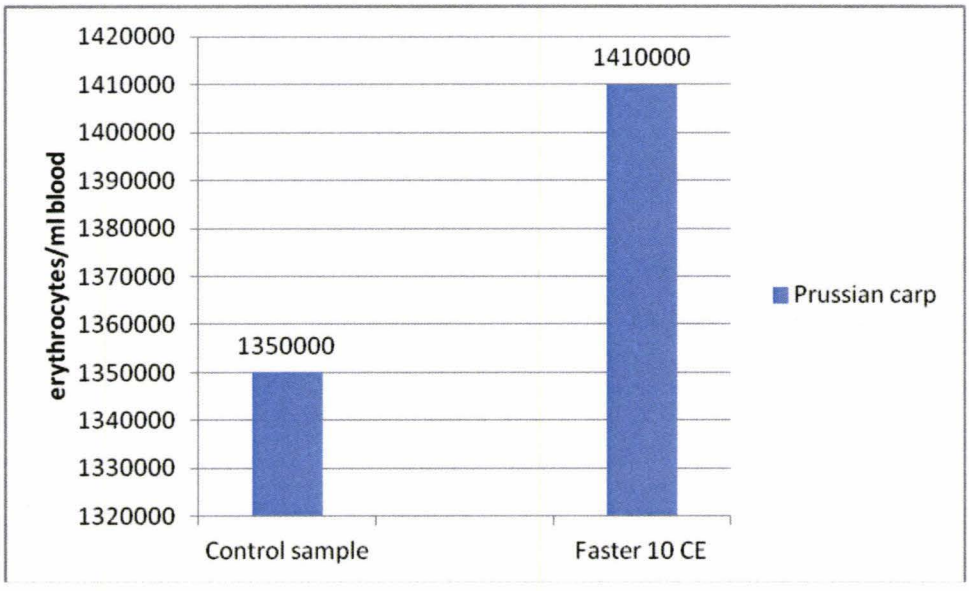

Figure 6. Action of the insecticide Faster 10 CE at a concentration of 0.000005 ml/l upon the number of erythrocytes at the Prussian carp at 20-22°C.

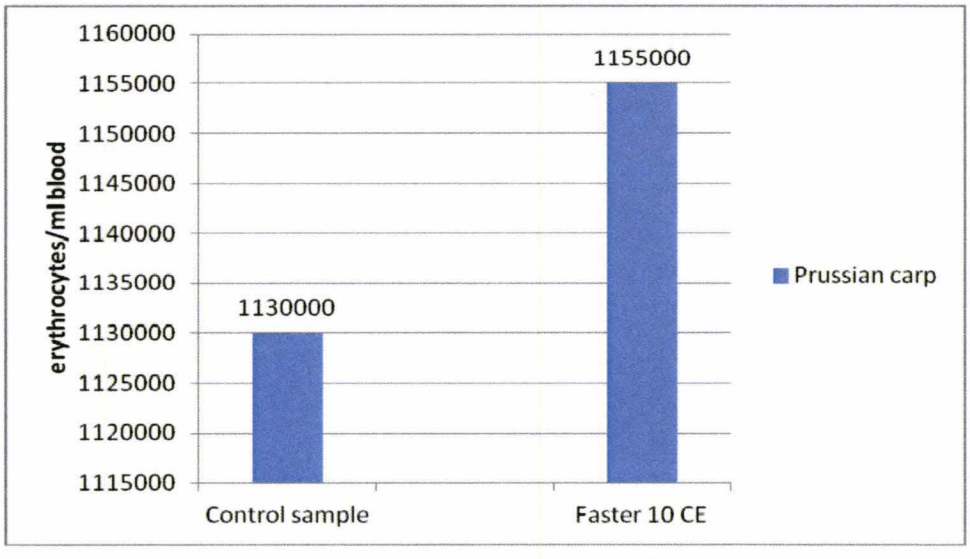

Figure 7. Action of the insecticide Faster 10 CE at a concentration of 0.000005 ml/l upon the number of erythrocytes at the Prussian carp at 5-7°C.

At the end of the experiment, at a temperature of 5-7°C, the number of erythrocytes increased by 2.21% as compared to the control group, the increase in this index being lower as compared to that registered at the temperature of 20-22°C. As the concentration of cypermethrin increased, it was also reported the increase in the number of erythrocytes, haemoglobin concentration, number of thrombocytes and sedimentation rate of erythrocyte in *Oncorhyncus mykiss* (ATAMANALP et al., 2002).

4. Action of the insecticide Faster 10 EC upon the blood glucose level

The temperature of 20-22°C increased the blood glucose level by 19.35% at the Prussian carp specimens intoxicated for 14 days with the insecticide Faster 10 CE at a concentration of 0.000005 ml / l compared to the control values (Fig. 8).

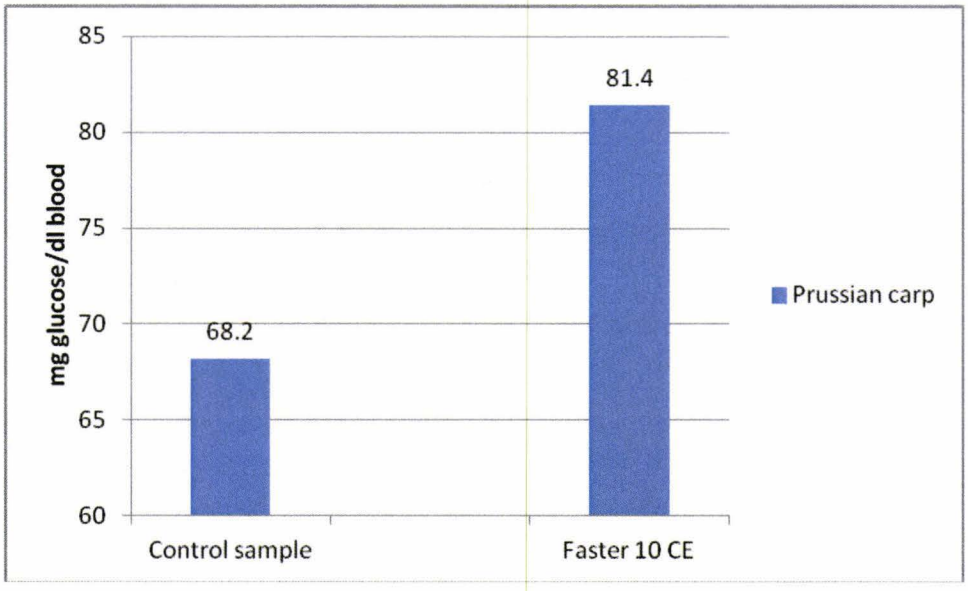

Figure 8. Action of the insecticide Faster 10 CE at a concentration of 0.000005 ml/l upon blood glucose level at the Prussian carp at 20-22°C.

The increase in the blood glucose level was also recorded at the temperature of 5-7°C (21.2% as compared to the control values) (Fig. 9).

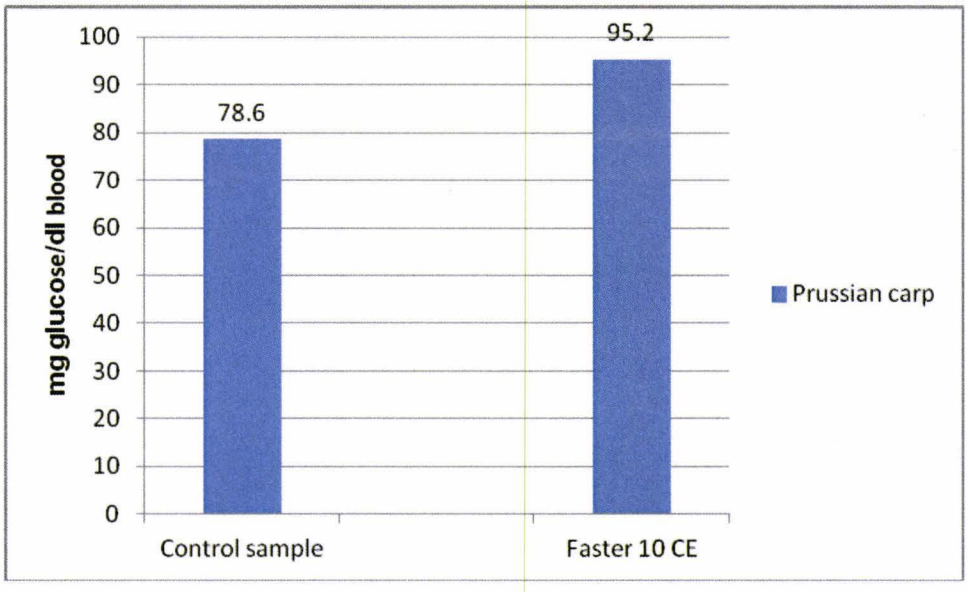

Figure 9. Action of the insecticide Faster 10 CE at a concentration of 0.000005 ml/l upon blood glucose level at the Prussian carp at 5-7°C.

A significantly higher level of blood glucose level was reported at the species *Korean rockfish* and *Sebastes schlegeli* by JEE et al. (2005) due to the long-term exposure to high concentrations of cypermethrin. This hyperglycaemia seems to determine an increase in the energy demand in fish under stress, in order to cope with the negative conditions imposed by the chronic exposure to toxic substances.

CONCLUSIONS

Low temperatures (5-7°C) determined the decrease in the oxygen consumption and respiratory rate, the reduction of the number of erythrocytes and the increase of the blood glucose level compared with the values registered at 20-22°C. The insecticide Faster 10 CE reduces the oxygen consumption and the respiratory rate in the Prussian carp (*Carassius auratus gibelio* Bloch). Initially, it caused a slight increase of these two physiological indices in the first 72 hours after the exposure to lower concentrations (0.000005 and 0.00001 ml Faster 10 CE/l); then, the values began to decrease, so that after 14 days of exposure to the insecticide, in all the used variants, the average values of these indices were lower than those registered before the exposure of fish to the toxic substance. The number of erythrocytes and

blood glucose levels increase when fish are exposed to the insecticide Faster 10 CE, as this insecticide has a hyperglycaemic effect.

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DIVERSITY: TOWARDS AN UNIFYING CONCEPT JOINING THEORETICAL AND PRACTICAL VIEWS OF ECOLOGY AND GEOGRAPHY UNDER A SPATIAL AND STATISTICAL FRAMEWORK

PETRIŞOR Alexandru-Ionuţ

Abstract. The 1992 United Nations Convention on Biodiversity brought to the attention of scientists the concept of diversity. Its definition allowed for including new elements, developing new concepts and refining the existing theories. Geographers developed their own interpretation, resulting into the concept of geodiversity. Particular concepts were defined for the human society, soils and other components of the physical realm. This paper attempts to integrate all concepts and developments into a unitary perspective, and apply this theoretical framework to analyze the diversity of an entire country in a measurable way. The results indicate the need for merging the variety of diversities into a unitary approach.

Keywords: ecodiversity, biodiversity, geodiversity, pedodiversity, territorial diversity.

Rezumat. Diversitatea: către un concept unitar ce îmbină perspectivele teoretice şi practice ale ecologiei şi geografiei în accepţie spaţială şi statistică. Convenţia Naţiunilor Unite din 1992 privind Diversitatea Biologică a adus în atenţia specialiştilor conceptul de diversitate. Definiţia sa permite includerea de noi elemente, elaborarea unor noi concepte şi rafinarea teoriilor existente. Geografii au dezvoltat propriile interpretări, conducând la apariţia conceptului de geodiversitate. Noţiuni particulare au fost definite cu referire la societatea umană, soluri şi alte componente ale lumii fizice. Lucrarea îşi propune să integreze aceste concepte şi abordări într-un cadru unitar şi să îl aplice pentru a analiza cantitativ diversitatea unei ţări întregi. Rezultatele susţin necesitatea de a integra varietatea de diversităţi într-o abordare unitară.

Cuvinte cheie: ecodiversitate, biodiversitate, geodiversitate, pedodiversitate, diversitate teritorială.

THE CONCEPT OF DIVERSITY

According to the most common understanding, diversity refers to dissimilarities between objects of the same class, making them distinguishable one from another, while preserving the common features of the class, even though there are many definitions, measurements and indices associated with it (MOR BARACK, 1999; MC DONALD & DIMMICK, 2003; WINEBERG & OPPACHER, 2003). It is also called variability or heterogeneity. Apart from this very general understanding, the diversity of physical realm has been interpreted in numerous ways by disciplines concerned with its study; components ranging from molecules to ecosystems (HUBER et al., 2005) have been joined and separated in concepts like biodiversity, ecodiversity, geodiversity, pedodiversity, territorial diversity, etc.

ECODIVERSITY, BIODIVERSITY, GEODIVERSITY, PEDODIVERSITY

For ecologists, the 1992 United Nations Convention on Biodiversity established a conceptual model which overlaps diversity (biological or ecological) to the structure and functions of ecological systems, seen as life-support systems or ecological foundation (VĂDINEANU, 1998). The simplest rationale is that the most common measurement of diversity (species richness) is also a simple descriptor of the structure of ecosystems (PIMM & RAVEN, 2000). Consequently, the conservation of diversity is identical to the protection of environment. Ecologists believe that a greater diversity gives the system better chances of adapting to the fluctuations of command factors; the range of fluctuations is diminished in terms of effects, resulting into a higher stability (WASHINGTON, 1984; VĂDINEANU, 1998; MC CANN, 2000; IVES & CARPENTER, 2007). Ecologists believed initially that diversity and stability are proportional, but later found out that there is a diversity threshold. If diversity exceeds this value, the system becomes unstable. The threshold is given by the number of species connected through stable relations (TOMESCU & SAVU, 2002; MOUGI & KONDOH, 2012).

Two concepts of diversity, *i.e.*, biodiversity and ecodiversity, are used to describe the structure – including relationships between structural elements – and functions of ecological systems (PETRIŞOR, 2008b; VĂDINEANU, 1998; 2007). Starting from this dichotomy, biodiversity and ecodiversity are defined in two perspectives. The first refers to the structure of the ecological systems and will be called "structural biodiversity", and the other to their functions, namely 'functional biodiversity' (NOSS, 1990; KAENNEL, 1998; DANOVARO et al., 2008). According to VĂDINEANU (1998, pp. 116-117), structural biodiversity includes: (1) diversity of ecological systems; embeds the diversity of supra-species biological systems integrated in the biological organizational hierarchy – biocoenosis, biome, biosphere, and the diversity of hydro-geomorphologic units, including habitats; this side is called ecodiversity in its strict sense; (2) diversity of species and levels of the taxonomic hierarchy (ω or phylogenetic diversity) – biodiversity in its strict sense; (3) genetic diversity of populations, species, genetic resources, including the human species, and (4) ethno-cultural diversity of socio-economic systems. The first three components reflect the natural capital, and the latest, the created capital. Functional biodiversity is reflected by the variety of food niches and trophic subunits of the

biocoenosis: trophic dynamic modules, guilds, trophic levels, etc. (MARTINEZ, 1996; PETCHEY & GASTON, 2006; POPESCU, 2009).

Geodiversity is disputed by three disciplines. In geography, geodiversity is the heterogeneity of "*geological features (rocks, minerals, fossils, and structures), geomorphologic features (landforms and processes) and soil features, including their assemblages, relationships, properties, interpretations and systems*" (GRAY, 2004, pp. 8). In geology, geodiversity is an expression of the "*geology of a region, including rocks, minerals, fossils and geological structures open by natural or anthropic means*" (POPA, 2007). For ecologists, geodiversity is "*a measure of environmental resource availability, which includes climate, topography, soils and geology*" (PARKS & MULLIGAN, 2010). Similar definitions or understandings are found in LESER & SCHAUB, 1995; BARTHLOTT et al., 1996; JAČKOVÁ & ROMPORTL, 2008 and PĂTRU-STUPARIU et al., 2011.

These two perspectives indicate a proprietary understanding of diversity based on discipline. In fact, ecology and geography describe the same territorial reality, and use a systemic approach to describe it, making correspondences possible based on spatial scale (PETRIŞOR, 2012), as showed in Table 1. For this reason, several authors believe that geodiversity and biodiversity overlap conceptually (MUSILA et al., 2005; SANTUCCI, 2005), while others argue that geodiversity includes biodiversity (HAKALA, 2005) and some claim the opposite (VĂDINEANU, 1998, pp. 116-117).

PETRIŞOR & SÂRBU (2010) consider that the confusion is etymological, since "biodiversity" is built upon the Greek βίος (bios) – living, as in the Rio Convention on biological diversity: "*variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems*" (United Nations, 1992). Provided that the Rio definition of biodiversity embeds the diversity of ecosystems, which include "*not only the organism-complex, but also the whole complex of physical factors*" (TANSLEY, 1935), it can be extended to include non-living (abiotic) components. The resulting diversity (of living and non-living components of ecological systems), seen as a component of biodiversity, was called ecodiversity, and constructed etymologically around the concept of ecosystem. PETRIŞOR & SÂRBU (2010) believe that the inclusion of ecodiversity in the already consecrated concept of biodiversity, as an extension, was preferred despite on their inverse logical and semantic relationship, even though erroneous, and concluded that, if understood correctly, ecodiversity overlaps with geodiversity, and represents the diversity of natural and anthropic sub-systems, including biodiversity (Fig. 1). Landscape ecologists also use "landscape ecology" as a synonym for ecodiversity (LESER et al., 1995; BARTHLOTT et al., 1996; JEDICKE, 2001; DEGÓRSKI, 2006; WALZ, 2011; PĂTRU-STUPARIU et al., 2011).

Table 1. Correspondence of the hierarchies of systems in geography, ecology and spatial planning and spatial diversity (PETRIŞOR, 2012).

Hierarchy of ecological systems	**Hierarchy of geographic systems**	**Hierarchy of territorial systems**	**Spatial diversity**
Structural and functional sub-units of ecosystems	Nano- and micro-structures, house/ block, company/ unit/ section, street/ street segment	-	α, ω
Ecosystem	Geosystem, geofacies, geotope, local system	NUTS V (LAU II)	α, ω
Regional complex of ecosystems	Natural region, geographical region, regional system	NUTS III	ß, γ, ω
Macro-regional complex of ecosystems	Domain, zone, national/ supra-national, continental system	NUTS II, NUTS I national territory, continent	γ, δ, ε, ω
Ecosphere	Geosphere, planetary system	Globe	ω

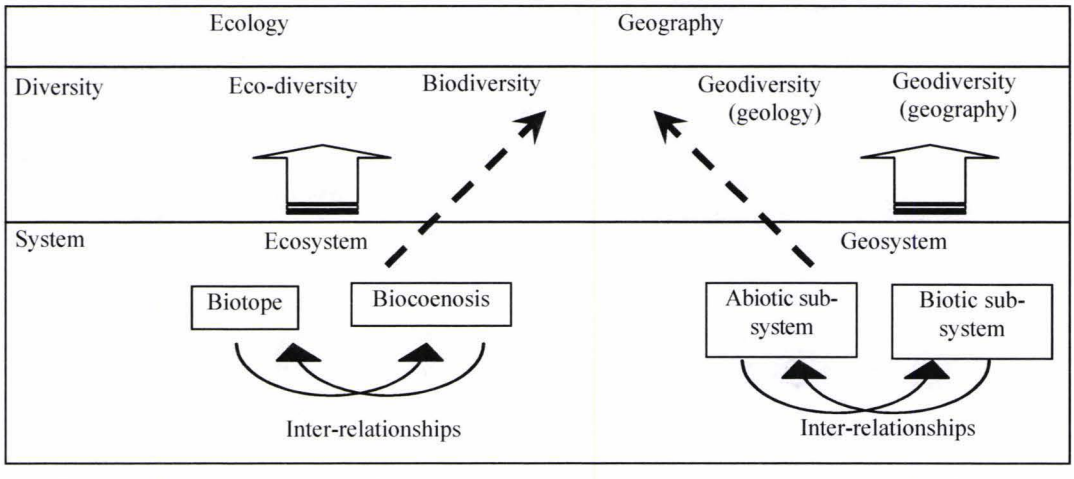

Figure 1. Relationships between biodiversity, ecodiversity and geodiversity correlated to the hierarchy of systems in ecology and geography (PETRIŞOR & SÂRBU, 2010).

The relationship between the two concepts was also discussed by PETRIŞOR & SÂRBU (2010) based on the concept of eco-energy, measuring the degree of anthropization (IANOŞ, 2000). The anthropization process is joined by impacts including pollution (BRAGHINĂ et al., 2010, 2011; PEPTENATU et al., 2010, 2011, 2012; CORNEANU et al., 2012) or the so-called "global changes", term introduce to coin land cover and use changes, climate changes and alterations of the energy flow and their connections (DALE, 1997; DALE et al., 2009, 2011; CHEVAL et al., 2009), determining the simplification and fragmentation of natural habitats and loss of biodiversity, which results in a reduced biodiversity of man-dominated systems, as it can be seen along the urbanization gradients (VĂDINEANU, 1998; SAVARD et al., 2000; HABERL et al., 2009; ŠUSTEK, 2011, 2012). Concomitantly, urbanization results into the emergence of new structures, specific to the socio-economic systems, leading to an increased complexity of territorial systems, translated into increased geodiversity (SÂRBU, 1999; IANOŞ et al., 2011). If natural resources are managed in an environmental-friendly manner (IANOŞ et al., 2009), biodiversity is "amplified" through the human contribution (VĂDINEANU, 2004), and geodiversity increases (VĂDINEANU, 1998).

Given the very particular view on soil systems (pedosystems), seen as an interface between the living and non-living realms, IBÁÑEZ et al. (2012) consider that pedodiversity, defined as "*inventory of the various discrete pedological entities (e.g. soil taxa) and the analysis of their spatial and temporal patterns*", differs from biodiversity, but can be used as its surrogate indicator. On a similar note, FLOREA et al. (2013) consider that pedodiversity is a synthetic and ingrate expression of the variety and differences between the soils of a given territory from genetic and spatial viewpoints, distinguishing between genetic pedodiversity (defined similarly to species richness, based on soil taxonomy) and spatial pedodiversity, related to the pattern and spatial distribution of soils, composing a pedo-landscape (FLOREA, 2002; PETRIŞOR, 2012).

STATISTICAL INTERPRETATION AND MEASUREMENT OF DIVERSITY

Diversity is understood in statistics **quantitatively** as scatter around a central trend (DRAGOMIRESCU, 1998, pp. 37) and **qualitatively** as different number of constituents and their different weights, *i.e.*, evenness of distribution (DRAGOMIRESCU, 1998, pp. 88; DRAGOMIRESCU & PETRIŞOR, 2009, pp. 110; MAGURRAN, 1998, pp. 7). The first view, commonly named "variability", applies to biological and ecological "metrics" – size, weight, and other measurable characteristics of individuals, and has the potential for distinguishing between species or taxonomic subdivisions of species. For example, consider a distribution of lengths of lake fishes belonging to different species; in this case, for each species lengths have a Normal distribution, as most individuals have lengths close to the average value, and only few are abnormally long or short (Fig. 2, bottom row). Each peak indicated by the average length corresponds to a species, and experts are able to distinguish, for instance, the peak of large Gibel carp *Carassius auratus gibelio* (Bloch, 1782), average bleak *Alburnus alburnus* (Linnaeus, 1758) or small bitterling *Rhodeus sericeus amarus* (Bloch, 1782). In the second view, "diversity' (*sensu stricto*) or heterogeneity produces a "diversity of diversities" (MAGURRAN, 1998) measured by many indices, such as the simple species richness or complex models (McArthur, Shannon, Motomura, etc.), including unifying indices (HILL, 1973), all based at least on the number of species, and eventually the number of individuals from each species. Even though these indices were traditionally used in ecology, different authors used them for measuring pedo-diversity (FLOREA et al., 2013) or ethno-cultural diversity (PETRIŞOR & IANOŞ, 2012).

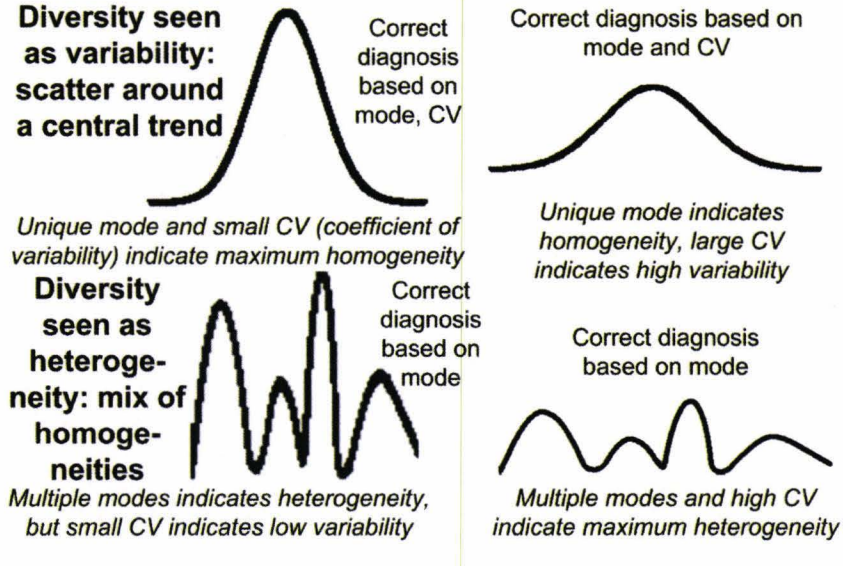

Figure 2. Statistical interpretation and indicators of diversity based on metrics (PETRIŞOR, 2012).

The distinction between variability and heterogeneity is fine-tuned; the number of modes indicates the number of homogenous distributions, as each unimodal (single-mode) distribution is homogeneous from this standpoint (see the example with the fish length distribution above; each unimodal distribution corresponds to a species). If there are several modes, they can indicate variability or heterogeneity: sexual dimorphism producing two modes when looking at the metrics is an example of variability, but the discrimination of species based on modes seen in the metrics distribution indicates heterogeneity (DRAGOMIRESCU, 1998). The coefficient of variability (CV) indicates only variability; sometimes, the two indicators might give contradictory messages, but it has to be recalled that the CV makes sense only when the mode is singular (Fig. 2).

A SPATIAL PERSPECTIVE ON DIVERSITY

The importance of temporal and spatial scales to ecology has been underlined by numerous studies (WEGENER et al., 1986; WIENS, 1989; SAVARD et al., 2000; FISHER et al., 2009). The spatial approach is particularly important to pinpointing "hotspots" defined as areas with high biodiversity or concentrated risks for the loss of biodiversity (MYERS et al., 2000). However, spatial and temporal data on species richness can hardly assess long term environmental changes due to sparseness (CIUBUC, 2004; ZINEVICI et al., 2010) resulting from financial constraints, change of priorities, or lack of taxonomists covering some groups.

Different authors (MAGURRAN, 1998, pp. 58; PETRIŞOR, 2008a, 2009a, b; PUSCEDDU, 2008, pp. 6-7) believe that diversity has different levels, corresponding to the spatial scale, namely: (1) α diversity – diversity of an ecosystem, (2) ß diversity – diversity of a micro-regional complex of ecosystems, (3) γ diversity – diversity of a regional complex of ecosystems, such as ecological regions or European biogeographical regions, (4) δ diversity – diversity of a macro-regional complex of ecosystems, such as global biogeographical regions, (5) ε diversity – diversity of life environments (oceanic, terrestrial), and (6) ω diversity – global phylogenetic diversity (included in the same categories, even though the approach refers more to structural diversity). Given the correspondence presented in Table 1, these levels can be extended to geographical systems. Furthermore, there is a correspondence with temporality, meaning that smaller systems change faster and more frequently (WIENS, 1989).

The diversity of socio-spatial systems (dominated by the human species) is analysed through the ethno-cultural component of diversity (DIETZ, 2007), consisting of the presence of more cultures belonging to different ethnic or religious groups and the linguistic diversity (PETRIŞOR, 2008b; PETRIŞOR & IANOŞ, 2012).

This spatial perspective is particularly useful in determining concrete way to assessing diversity. Since socio-ecological complexes correspond administratively and spatially to the levels of the Nomenclature of Territorial Units for Statistics (NUTS) hierarchy, a correspondence can be made between NUTS levels and geo-, bio- and ecodiversity. Table 2 lists several classifications that reflect units of different sizes with known structure and overall diversity. Some of the most important classifications are:

- Global diversity of continental systems is reflected by global biogeographical regions (PIELOU, 1979).
- Within each continent, macro-regional diversity is reflected by biogeographical regions; there are eleven European biogeographical regions: Arctic, Boreal, Continental, Atlantic, Macaronesian, Mediterranean, Alpine, Pannonian, Steppic, Black Sea (Pontic), and Anatolian (PINBORG & LARSSON, 2002).
- The diversity of regional ecological complexes of ecosystems is described at the macro-regional level by the ecological regions.
- Regional diversity is reflected by habitats. The classification of the European natural and man-dominated ecological habitats is the European Nature Information System (EUNIS) classification, developed between 1996 and 2001 by the European Environment Agency, successor to the CORINE (CoORdination INformation Environment) Biotopes Habitat Classification developed in 1991 (DAVIES et al., 2004).
- Local diversity is reflected by land cover and use. According to JENSEN (2000, pp. 413), "*land cover*" indicates what lays on the ground surface from a biophysical viewpoint, while "*land use*" indicates its use by human communities. However, the second definition is perfectly valid for man-dominated systems only; in natural systems, land use reflects only a more detailed classification (PETRIŞOR et al., 2010). The United States use Anderson's classification (ANDERSON et al., 1976), with two levels; the first shows land cover, and the second land use. Europe utilizes CORINE classification with three levels (DE LIMA, 2005); the first one shows land cover, and the last two land use, in more or less details (PETRIŞOR et al., 2010).

Table 2. Spatial approach to diversity based on the Nomenclature of Territorial Units for Statistics (PETRIŞOR, 2008a).

Diversity	NUTS levels			
	I	II	III	IV-V/ LAU I-II
Hydro-geomorphologic units (relief)	x	x	x (by case)	
Biogeographical regions	x	x		
Ecological regions	x	x	x (by case)	
Types of ecosystems and/or habitats – land cover and use	CORINE I Anderson 1	CORINE I/II Anderson 1	CORINE II Anderson 2	CORINE III Anderson 2

x – indicates that diversity can be described by a given classification at a certain spatial scale.

A UNIFYING CONCEPT OF DIVERSITY

As it has been stated in the beginning, different disciplines interpreted in particular ways the diversity of the same physical realm, producing overlapping or even identical concepts. These were a result of multiple dichotomous separations: natural vs. man-dominated, living vs. non-living, functional vs. structural, qualitative vs. quantitative. The purpose of this research was to develop a unifying concept. Fig. 3 is a graphic representation of what can be called "diversity", a multifarious concepts with sides and sub-units dictated by the dichotomous classifications and disciplines concerned with developing a particular view.

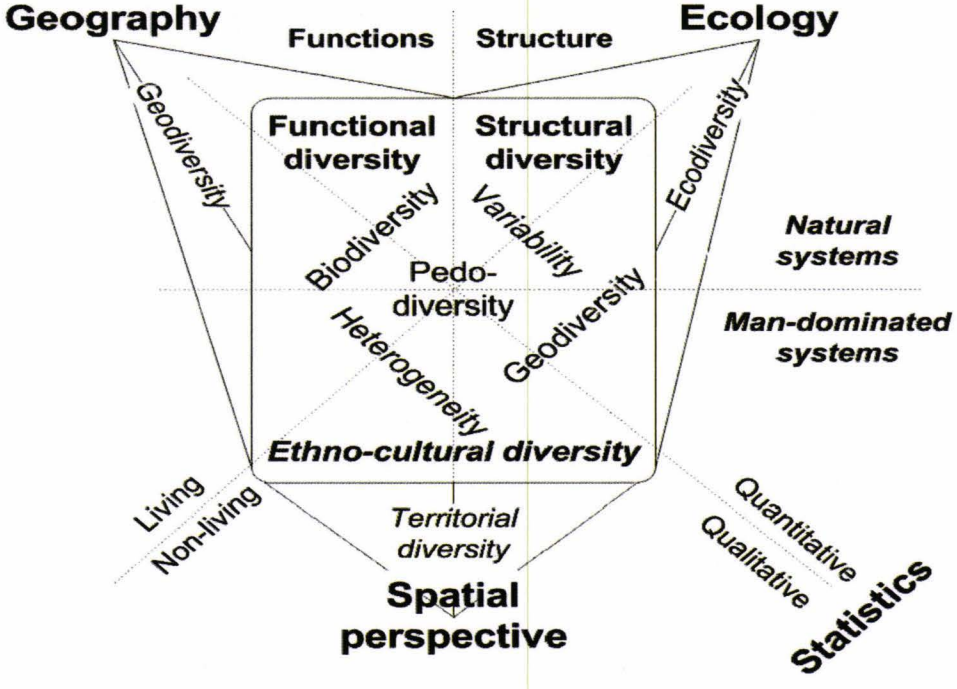

Figure 3. Towards a unifying concept of diversity (original).

ROMANIA AS A CASE STUDY

This paper proposed a unified approach for analysing the components of diversity under geographical, ecological, statistical, and spatial frameworks, addressing both natural and man-dominated systems. In order to apply this conceptual framework to describing the diversity of Romania, in all aspects (abiotic and biotic components, at several spatial scale), it is sufficient to list the constituents:

- Five types of relief – floodplain, field, hill or plateau and mountain (CAZAN et al., 2004; MÂRA, 2007)
- 900 species of Carpathian minerals (PAPP & SZAKÁLL, 1996)
- 10 classes and 39 types of soil (Ministry of the Environment and Sustainable Development, 2008)
- Five of the eleven European biogeographical regions – alpine, Pontic (Black Sea), continental, Pannonian and steppic (PETRIŞOR, 2008a)
- 22 level 1 and 57 level 2 ecological regions (COGĂLNICEANU & STANCIU, 2001)
- Over 3700 superior plant species and 33802 animal species (VĂDINEANU et al., 2003)
- 783 types of habitats identified and characterized in 261 areas spread over the national territory, analysed in the CORINE (Co-ordinated Environmental Information in the European Community) Biotopes program (Ministry of the Environment and Sustainable Development, 2007)
- Ethno-cultural diversity: 20 ethnic (National Institute of Statistics, 2008a) and 18 religious (National Institute of Statistics, 2008b) groups at the 2002 census.

CONCLUSION

The different elements of the same physical realm led to the development of a variety of diversity concepts, disputed by the disciplines concerned with their study. Even though numerous classifications are used and can be proposed, the analysis demonstrates the need for their integration in a unitary concept. Especially when the analysis is carried out from a spatial perspective, it makes little, if any sense to divide the same physical reality into different units, when each classification uses different criteria. In such situations, it seems to be more important to see the whole rather than its parts.

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"DR. FAWAZ AZKI" GEOLOGICAL MUSEUM, KISMIN – LATTAKIA, SYRIA – WORLD HERITAJE; ITS ROLE IN THE DEVELOPMENT OF SCIENCE AND ENVIRONMENTAL EDUCATION WORLDWIDE

AXINI Monica, AL-AZKI Fawaz

Abstract. "Dr. Fawaz Azki" Geological Museum is located in Kismin Village - Lattakia District, Lattakia Guvernorate - Syria. It is the only museum of its kind in the Syrian Arab Republic and the first museum of geology established in the Middle East. There is on the UNESCO list and GoogleEarth. This paper is a description of the museum. However, the work is intended as an overview of the educational and scientific activities carried out under it, from its creation until today (2014).

Keywords: geological museum, scientific research, ecological education.

Rezumat. Muzeul de geologie „Dr. Fawaz Azki", Kismin – Lattakia, Siria – patrimoniu mondial; rolul său în dezvoltarea ştiinţei şi educaţiei ecologice pe glob. Muzeul de Geologie "Dr Fawaz Azki" este situat în Satul Kismin – Judeţul Lattakia, Guvernoratul Lattakia - Siria. Este unicul muzeu de acest gen din Republica Arabă Siriană şi primul muzeu de geologie înfiinţat în Orientul Mijlociu. Se află pe lista UNESCO şi pe GoogleEarth. Lucrarea reprezintă o descriere a muzeului. Totodată, se doreşte a fi o privire de ansamblu a activităţilor ştiinţifice şi educative desfăşurate în cadrul acestuia, de la înfiinţarea sa şi până în prezent (2014).

Cuvinte cheie: muzeu de geologie, cercetare ştiinţifică, educaţie ecologică.

INTRODUCTION

The "Dr Fawaz Azki" Geological Museum was opened in July 2002. It is the only private museum in Syria, arranged in the grandparents` house of the geologist (AL-AZKI, 2011; 2014a).

The museum is located about 20 km from the Lattakia Town, in the west of the Kismin Village.

Kismin Village is located in the north-eastern part of Latakia City (Latakiyah, often locally transliterated as Lattakia), in Lattakia Mountains - coastal mountains disposed parallel to the Syrian Mediterranean coast.

Kismin Village (also named Kesmin, Kesmine, Kasmin, Qasmin, Qasmine, Qosmine, Masmin) is divided in two parts by Mashqita Lake. It shows forested limestone ribs, with altitudes between 100 and 300 m that descends gently toward the lake.

Figure 1. Earth map (from Google Earth, accessed: March 24, 2014) emphasizing the position of Syria in the Middle East – Asia.

DISCUSSIONS

The "Dr. Fawaz Azki" Geological Museum (35°38'00.62" N, 35°54'20.68" E) - Kismin Village (35°37'56.85" N, 35°54'43.09" E) (Figs. 1; 2a, b; 3), part of the scientific and cultural heritage of Syrian Arab Republic, is the rarest museum in Syria. It was opened after the completing our own research in the coastal mountains series of Syria. It

consists of two parts: the Open-Air Museum and the Closed Museum (Museum Building) (AL-AZKI, 2012c; AL-AZKI, 2014b; MGSREE, 2014).

The Open-Air Museum (Fig. 4), spread over 1.500 m^2, is located in the courtyard of the museum. Here, there are over 60 rock samples of huge size (between 50-200 cm and 75-1.500 tons) which are the dominant rocks in Syria.

Also, in the Open-Air Museum, there are samples of dinosaur species in their natural size: a herbivorous dinosaur from the Jurassic (with a height of 7 m and a length of 23 m), a carnivorous dinosaur from the Cretaceous time (with a height of 3 m and a length of 5 m) and a flying dinosaur with a length of 4 m.

Also in the Open-Air Museum, there is the reply of a volcano in Qatar. The entrance is through a gate located at its base. Inside the volcano, there are six samples of different depths volcanoes, over 30 km, and a copy of the internal structure of the Earth.

In the Open-Air Museum, there are the 3D geological map of Syria, 330 cm, and a reply of the Earth Globe with a diameter of 2 m.

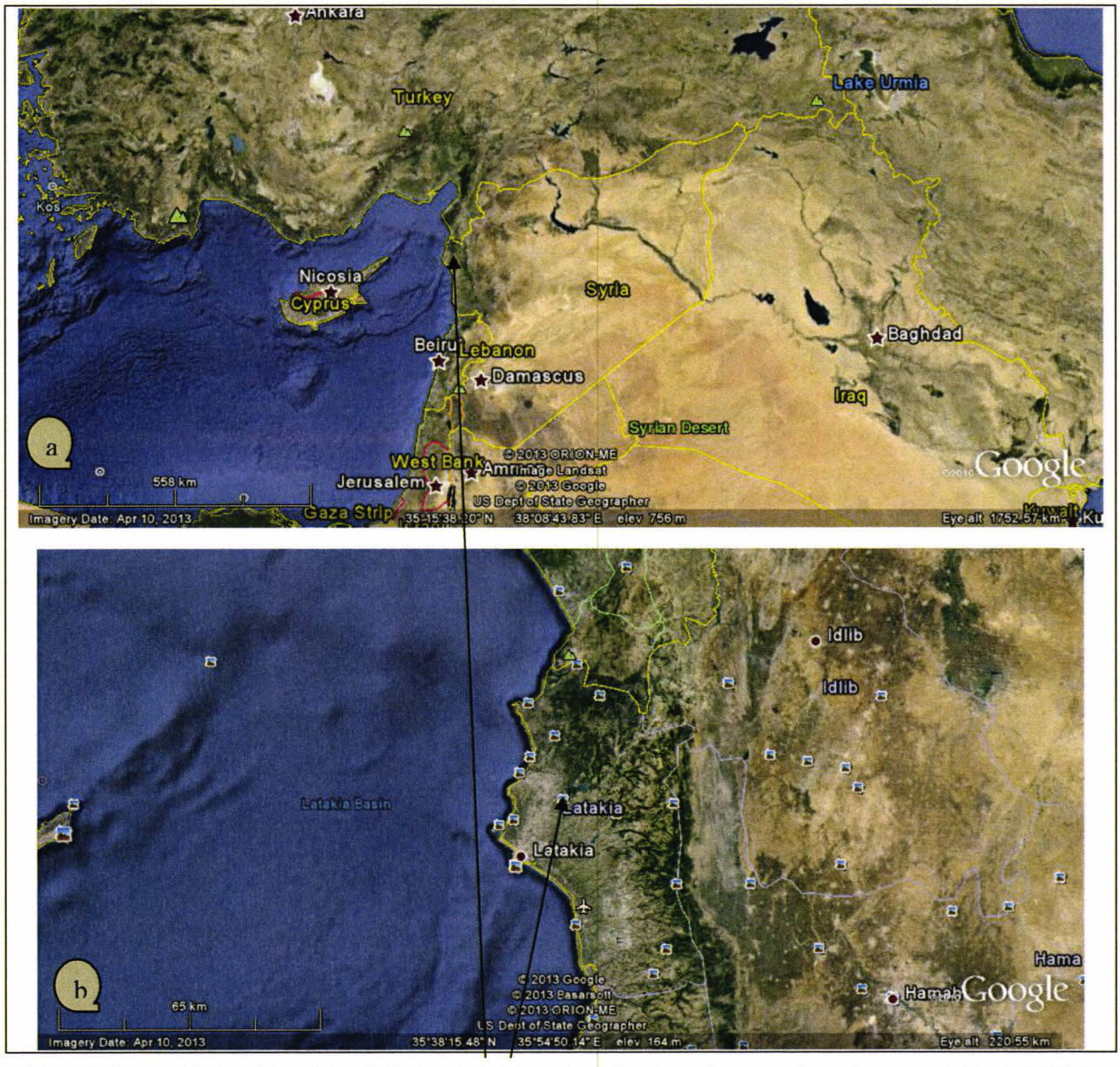

Figure 2. Geographical position of Kismin Village in Lattakia Mountains (image from Google Earth, accessed: March 4, 2014).

The Museum Building (Fig. 5), spread over 130 m^2, is built in the Syrian architectural style from boulders brought from Lattakia Mountains (35°29`21.76``N, 41°25`58.97``E), and covered only with stones. It is divided into seven sections.

1. The Fossils Section ("mosthathat" in Arabic) has a circular shape; on this roof, there is a starfish, as hologram (the symbol of the Syrian "llmsthathat"). It houses more than 120 fossils found on the whole territory of Syria and other parts of the world. The museum houses a beautiful collection of microorganisms, plants, invertebrates

(mollusks, etc.) and vertebrates (dinosaur embryos in open eggs and adult specimens of dinosaurs) fossils, collected and classified - all the fossils discovered in Syria; they are placed in wooden boxes fitted with window for viewing (AL-AZKI & AXINI 2012a, b).

2. Minerals Section has a prismatic form (quartz crystal system), on top of which it is written in stone SIO_2 (chemical formula of quartz – the symbol of the Syrian mineral). This section houses over 100 kinds of minerals and ores, representing all the minerals of Syria and in the world.

3. Innate Geology Section is built in the shape of a hemisphere. It includes a collection of some rock tools (by clay, basalt, onyx, sandstone, etc.) which are handmade by ancient Syrian peoples – Syrian cultural heritage pieces.

4. Black Hole Section is a lobby with curved roof. It is a cosmic black hole which makes the transition to the fifth section.

5. Cave Section shows the types of caves within the territory of Syria. It includes Stalagmites and Stalactites.

6. Rocks Section accommodates all existing rocks in Syria and the other regions of the worlds - sedimentary, magmatic and metamorphic rocks.

7. Seventh Section includes the library, the maps and epitomes hall, the research laboratory of the museum and administrative halls.

The Museum Library includes more than 1000 scientific papers in Arabic, Romanian, English and French languages.

The Maps and Epitomes Hall houses many geological charts and maps for Syria, the most ancient and important of which is the one drawn in 1945, and many representations of the tectonic phenomena.

The research laboratory houses samples of raw materials. Here, samples are examined and cut.

In October 2007, on the roof of the museum, it was founded the first and unique Astronomical Observatory in Syria.

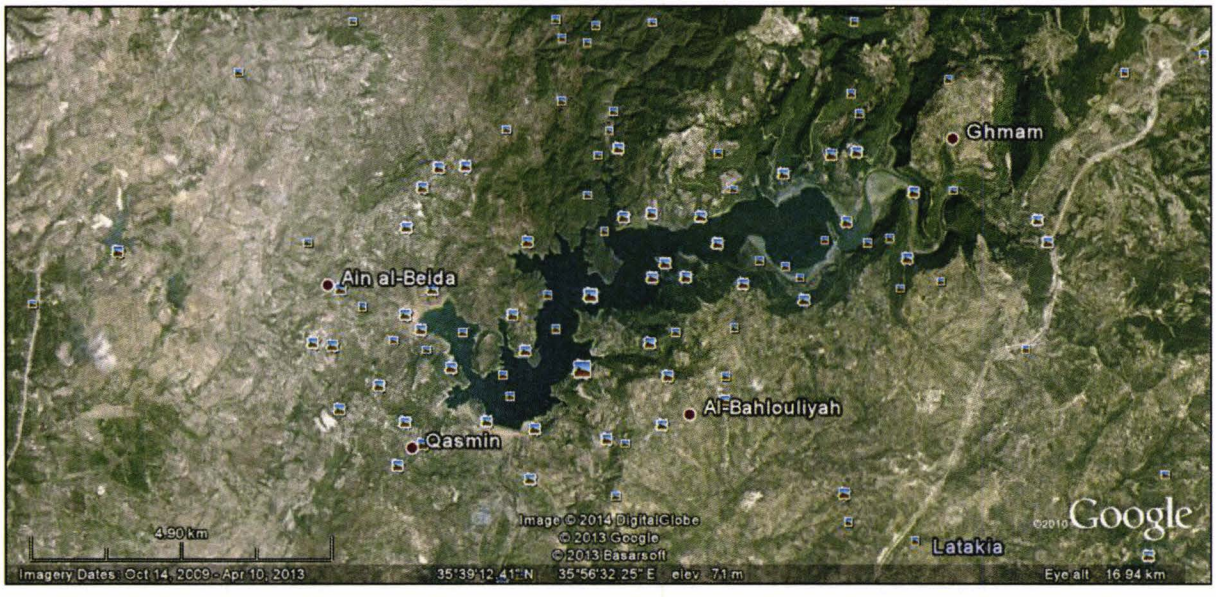

Figure 3. "Dr Fawaz Azki" Geological Museum in Kismin Village (from Google Earth, accessed: March 4, 2014).

Since 2002 until now, in the museum there were conducted several research programs and projects on education, culture, tourism, alone or in collaboration with various scientific research, educational or cultural institutions in Syria or aboard.

In the museum laboratory, there were conducted numerous research projects that focused on field and laboratory research. Here, numerous research on palaeo-diversity/palaeo-ecology and zoo-archaeology of Syria were held: research on the origin and evolution of man; methods of conservation and protection of nature (bio- and geo-diversity) of the ancestral population of the current territory of Syria; perception of primitive man on climate change; research of dinosaur fossils discovered in marine and continental sediments belonging to the Late Cretaceous and the Middle Jurassic of Lattakia Mountains, Syria (AL-AZKI, 2013; AL-AZKI & AXINI, 2013).

Also, in the museum, there were held research studies on fossils species diversity identified across Syria and the Mediterranean coast (AL-AZKI, 2012a, b). Here, there were conducted hydro-geology, geophysics, climate change and global warming, environment pollution, etc. research studies.

On the basis of this research, there were gathered the fossils, rocks and minerals species and stone tools discovered within the territory of Syria, and existing in the museum sections.

The best results of this research were presented in various scientific events, and subsequently published in scientific journals – national (Syria/Romania) and international.

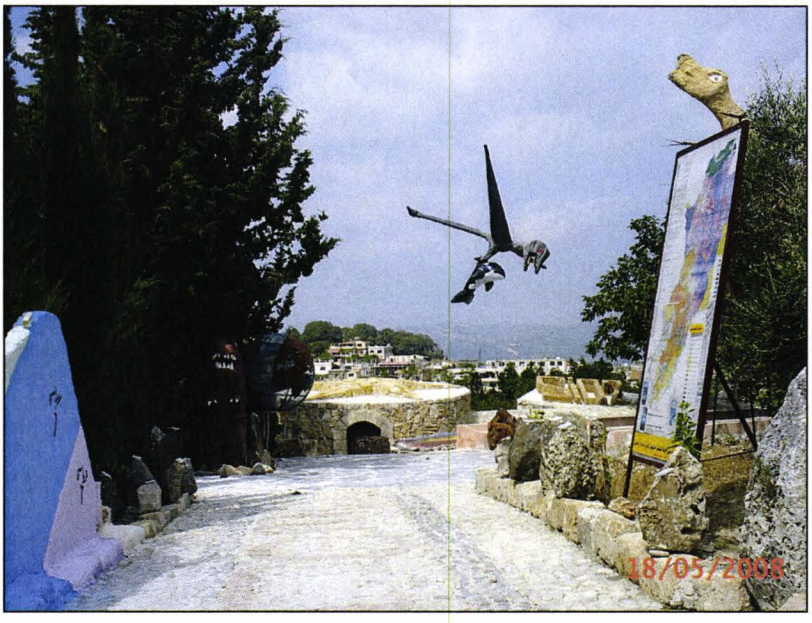

Figure 4. Overview from the Open-Air Museum (original).

Figure 5. Entrance to the Museum Building (original).

The museum organizes the International Scientific Symposium *Bio-diversity Conservation "in situ" and "ex situ"* (BCIS) / International Scientific Symposium *Geo-and Bio-diversity Conservation "in situ" and "ex situ"* (GBCIS), and it is the international partner of the educational program named DESAME (Day of Earth Sciences in Africa and Middle East).

From the beginning until now, the museum has been ~~a~~ partner of many cultural and tourist programs, and its activities represent an example to follow (DMA-UPD, 2011a, b).

In the museum (the open-air museum or the closed museum), many educational projects with pupils and students were held (Figs. 6a, b). During scientific or educational lectures, the visitors of the museum (pupils, students, etc.) can listen or take notes on specially designated banks in the museum courtyard with 80 stay places.

From the beginning until present, in the Astronomical Observatory, there have been made astronomical observations both scientific and educational – the last achieved by various visitors of the museum, eager to know the Universe (Fig. 7).

From 2002 until now, the museum and hence the observatory was visited by over 74,000 visitors, of all ages and professions, from Syria and abroad – the museum being on the list of the tourist circuits of the Ministry of Tourism of Syria (Syria-news.com, 2013).

The museum hosted several environmental education documentaries made in collaboration with the Syrian media. The documentaries themes included: knowledge of the museum and its collections, lectures on astronomy, knowledge of Syria geology (explorations in nature) – the geology TV program named "Geology of Syria" with over 66 episodes, program release in Syria.

In the museum, many scientific monographs were edited, unique in the geology field, and educational publications (brochures and flyers) (AL-AZKI 2002; 2006).

Figure 6. Educational activities in the museum – The Fossils Room (a) (original).

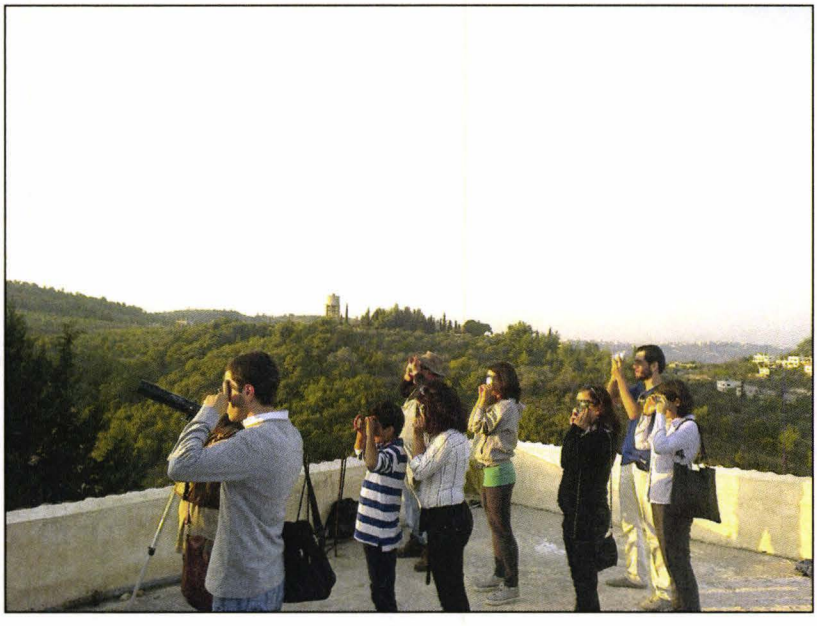

Figure 7. Educational activities in the Astronomical Observatory (original).

CONCLUSIONS

The museum is part of the cultural, educational and scientific heritage of Syria and of the whole world.

It is a scientific and cultural institution and touristic place with international fame, placed on the UNESCO list.

This museum aims at serving children, students, researchers, and other interested persons.

Entrance is free for all visitors, because AZKI opinion is: "if I don't add something to this Universe, I am just an addition in it"!!!

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GRIGORE ANTIPA (1867 – 1944) – 70 YEARS AFTER HIS DEATH. HOMAGE

NEGREA Ştefan

Abstract. Through his economic and biological work expressed in papers and monographs and the scientific institutions he founded, Gregory Antipa remains immortal. He was a contemporary and friend of another famous Romanian naturalist, Emil Racoviţă, the founder of a new science, Biospeologia (in Romanian) - Biospeleology (in English), and of the first Institute of Speleology in the world. Grigore Antipa was the initiator of the Romanian hydrobiology and ichthyology and the organizer of the state fisheries. But his fame comes from the organization, on ecological grounds, of the National Museum of Natural History in Bucharest, which bears his name. He equipped the museum with dioramas that were subsequently taken as a model by many museums in the world. There is no pupil in Romania who came on a school trip to Bucharest to see, first, the Antipa Museum. The great naturalist fought like no other to enhance the wealth of his country waters, especially of the Danube with its wonderful Delta and lakes located in the floodplain of the river. He was convinced that this complex ecosystem, partly in its natural state, is an important wetland of the planet, which had to be protected until it would not be too late (see ANTIPA, 1910; NEGREA & NEGREA, 1975; NEGREA, 1990). It could not have been otherwise, as Antipa was a brilliant disciple of the creator of Ecology, Ernst Haeckel, who awarded him *Summa cum Laude*, a distinction that the great Darwinian professor granted only three times in his career.

Keywords: Grigore Antipa: man and work, Ernst Haeckel - founder of ecology, The Antipa Museum and the ecological dioramas, Organizer of the State fisheries.

Rezumat. Grigore Antipa (1867 – 1944) – 70 de ani de la moarte. Evocare. Prin opera sa biologică şi economică, exprimată prin articole şi monografii ştiinţifice şi prin instituţii fondate de el, care funcţionează şi în prezent, Grigore Antipa rămâne nemuritor. El a fost contemporan şi bun prieten cu alt celebru naturalist român, Emil Racoviţă, fondatorul unei noi ştiinţe, Biospeologia (în română) – Biospeleologia (în engleză), şi a primului Institut de Speologie din lume. Grigore Antipa a fost iniţiatorul Hidrobiologiei şi ihtiologiei româneşti şi organizatorul, pe baze ştiinţifice, a Pescăriilor Statului. Dar faima lui vine de la organizarea, pe baze ecologice, a Muzeului Naţional de Istorie Naturală, din Bucureşti, care ii poartă numele. El şi-a dotat Muzeul cu dioramele imaginate de el, luate ulterior drept model de multe muzee din lume. Nu-i elev în România să nu fi mers cu şcoala în excursie în Bucureşti să vadă, în primul rând, Muzeul lui Antipa. Marele naturalist a luptat, ca nimeni altul, pentru punerea în valoare a bogăţiei apelor ţării sale, cu precădere ale Dunării, cu mirifica sa Deltă, şi cu lacurile din zona inundabilă a fluviului. El era convins că acest ecosistem complex, parţial încă în stare naturală, este o importantă zonă umedă a Planetei, care trebuie protejată până nu va fi prea târziu (vezi ANTIPA, 1910; NEGREA & NEGREA, 1975; NEGREA, 1990). Nici nu se putea altfel, Antipa fiind un strălucit discipol al creatorului Ecologiei, Ernst Haeckel care l-a distins cu *Summa cum Laude*, calificativ acordat de marele profesor darwinist numai de trei ori în cariera sa.

Cuvinte cheie: Grigore Antipa: omul şi opera, Ernst Haeckel - fondatorul ecologiei, Muzeul Antipa şi dioramele ecologice, Organizatorul Pescăriilor Statului.

GRIGORE ANTIPA'S ORIGIN

In my book, *In the Footsteps of Grigore Antipa* (1990) (Plate 1), I tried a first genealogy, based on the data collected from different sources (NEGREA, 1990, pp. 10-17).

From this diagram, it results that Antipa comes from Aromanian ancestors on maternal line. Regarding his paternal ancestors, I did not identify any reliable data. The descendants of the families named Antipa, in the north-eastern part of Botoşani city, could not give any information about their ancestors. In the archives of Botoşani city, I found reliable data only on his grandparents. Constantin Antipa, who lived in Săvenilor Street, no. 143, gave me the only oral and unverifiable information, namely that his father, Mihai Antipa, was a cousin of Grigore Antipa, and his grandfather, Ion Antipa, was the brother of the lawyer Vasile Antipa (father of Grigore Antipa). However, he could not show me any official document. From oral information, he knew that they were Romanians, not Armenians, Lipoveni Russians or other nations inhabiting the city.

GRIGORE ANTIPA'S PARENTS AND BROTHER

I found little reliable data about Vasile Antipa and Zoiţa Nicolau (Grigore's parents) in their marriage certificate and his father's death certificate, documents discovered in the State Archives in Botoşani and noted in the genealogical diagram (Plate 2).

From the marriage certificate with Zoiţa, it results that **Vasile Antipa** was born in 1826 in Curteşti locality, his parents being Gheorghe Antipa and Maria, born Sârbu. It is not known when and how he became a lawyer in Botoşani and married Profira Mârzescu. It is known that, at 38 years old (in 1864), he divorced, and at 41 years old, more precisely on the 19th of February, 1867 (old calendar), he married Zoiţa. Towards the end of the same year (1867), Zoiţa gave birth to a son, Grigore, the hero of the book *In the Footsteps of Grigore Antipa* (NEGREA, 1990). It is not known why the father, Vasile Antipa, died two years after the wedding, on the 18th of March, 1869.

Grigore Antipa's mother, ***Zoiţa Nicolau***, was born in 1838 in the village Băiceni, not far from Botoşani. It is not known when both her parents died. She was raised on her grandmother's mansion, Dafina Şendrea from Băiceni, along with her 14 years older brother, Panaite, born in 1824. At her grandmother's request, she got married in 1861, against her will, with Iorgu Leon. She was only 22 years old. The following year, she gave birth to Nicolai (Nicu), the stepbrother of Grigore, who was conceived with her second husband, Vasile Antipa. The two brothers, although stepchildren, were good friends, both in childhood and in the teenage and adulthood years. The unexpected death of their mother, due to typhoid fever, on the 12th of October 1873, when she just turned 35, left the two children orphan at an early age (Nicolai was 11, and Grigore 6 years old). It is touching the page of N. LEON's memories (1925) about the loss of their mother. I quote:

The most terrible misfortune I have ever felt in my life was the death of my mother. Irreparable, irremediable loss. One day, I sneaked into the room where she was lying - because I was not allowed (the doctor forbade it) - and I sat on a couch. She, seeing me, called me to her and, with her soft hand touching me gently on the forehead and cheeks, said: <You ran in all directions, you played, but nowhere is better than near your mother>. Hot tears were streaming down my fondled cheeks and her eyes were wet. A few days later, she spoke due to typhoid delirium: she made plans to go to Paris. I was barely in high school, I was 12 years old and she said: <When Nicu graduates high school I am going to stay with him in Paris to attend the University>. The next day, she was dead. In vain I touched, kissed and asked her to wake up. For me, it was all over.

CHILDHOOD IN BOTOŞANI COUNTY (1867 – 1878)

After their mother's death, the lives of the two brothers, Nicolai (Nicu) Leon and Grigore Antipa, was held under the protection of their tutor, uncle Panaite, Zoiţa's brother, oscillating between holidays and school.

Nicu Leon followed Mărgineanu's boarding school in Botosani, then, the high school, the United Institutes in Iaşi. At this school, he had famous teachers like Grigore Cobălcescu, Petru Poni and A. D. Xenopol. Finishing high school in private, he passed the baccalaureate in 1882. After high school, Nicolai Leon attended the Faculty of Sciences and Medicine at the University of Iaşi, Department of Natural Sciences (1882-1889). These studies were continued at the University of Jena brilliantly (1884-1887). Among his famous teachers, I mention Ernst Haeckel, under whose leadership he prepared his PhD thesis on the mouth parts at Hemiptera, thesis presented in 1887 and awarded with the degree *Magna cum Laude*. Returned in the country, he conducted a multiple teaching and scientific activity, dedicating himself to parasitology studies, which helped to cure certain diseases. At the University of Iaşi, he was elected Dean (1912) and, then, rector, (1918-1922). However, he refused the title of academician of the Romanian Academy, saying that he could not accept this honour as long as celebrities like Eminescu, Creangă and Caragiale were not full members of the Romanian Academy (they had to be made honorary members of the *Academy of the Socialist Republic of Romania*).

Grigore Antipa was born in Botoşani on the 25th of November, 1867, Old Calendar (the 7th of December, New Calendar). His father, Vasile, a lawyer in this city, was married to Zoiţa Nicolau from Băiceni. On the 19th of February, 1867 (Old calendar), immediately after the wedding, he was moved in a beautiful house located in M. Eminescu Boulevard, 56 (formerly Harapului), the house being part of Zoiţa's dowry. Even today (2014), the house has not become *Grigore Antipa's Memorial House* in spite of all my insistence and that of Victor Dughilă, Professor of Biology in Botoşani (now retired) and a great admirer of the scientist, as it was given to the military commissariat by the mayoralty for many years. It is in this way the authorities who lead the city understand to respect the great people of Romania, of global reputation!

In the fall of 1873, marked by the so-called Bacovian rains, after the death of their mother Zoiţa, the childhood of the two brothers was suddenly broken. Nicu was sent to *Mărgineanu's Boarding School* to attend middle school, while Grigore remained to Alecu Popovici's mansion as he was too young. He used to take him to Băiceni, where he had to do business with Sachi Pamfil, the administrator of Alecu Popovici's estate, the successor of the deceased grandmother, Dafina. In my book, *In the Footsteps of Grigore Antipa* (pp. 37-44), I render the stories about their journeys on the hills covered by endless forests, about the knell of bells from Agafton Hermitage, about the mysterious lake from Eminescu's Ipoteşti Forest, about the fishing of carps and other fish in the pond next to Băiceni, etc. One day, asked by Sachi what he wanted to become when he would grow up, Grigore replied: *Well, a lawyer like dad.* He was too young to know what to do with his life. He liked living in the country, he was attracted to the life in the woods, the groves, the pond, the animals in the yard and gardens, birds in flight and chicken nests, beehives, but he could not know that all these creatures, in their natural or anthropized environment, represent the naturalists' study subject.

The chapter *Memorable holidays* (pp. 44-58) evokes the atmosphere of the patriarchal village of Băiceni seen through the eyes of the child Grigore and renders stories heard during my trips for the book *In the footsteps of Grigore Antipa*. I am thinking even now, while writing, that the heroes of my stories went into the realm of shadows long ago, where *there is no pain, no grief, no sighing*. Although I did not know them, I feel them close. I immortalized them in my books (NEGREA, 1979; 1980; 1990; the first one printed in 30,000 copies), a century after the birth of Grigore and half a century after the publication of the *Memories* written by Nicolai (Nicu) LEON (1922-1927). He had not turned six when Moşu Panaite, the brother of the late Zoiţa, took her son, Grigore, to *Mărgineanu's Boarding School* in Botoşani, where the sons of wealthy people, learnt to read and write. You could make four primary and four secondary

classes in boarding school regime. Endowed with a remarkable memory and a brilliant intelligence, Grigore easily assimilated the taught information and was always ready to answer questions. In the autumn of 1877, when Grigore was a student in the first cycle of the school, the country was involved in the independence war. With genuine patriotism, the teacher required the students to read the poems of Vasile Alecsandri. After the final examination of the first four classes, Grigore went on vacation for the last time at Băiceni.

STUDENT AT THE UNITED INSTITUTES FROM IAŞI (1878 – 1885)

In the autumn of 1878, Moşu Panaite sent Grigore Antipa to study at the United Institutes from Iaşi, considered the best high school in Moldova. For seven years, he learnt from professors known for their erudition, graduating the middle school and high school and living as an inhabitant of this cultural city, the capital of Moldova starting from the time of Vasile Lupu. Among the teachers of Grigore Antipa, I mention Grigore Cobălcescu (1831 - 1892), reputable geologist and palaeontologist, Petru Poni and A. D. Xenopol (Plate 3). Besides the two brothers, Leon and Antipa, they had other students who, in turn, became personalities known abroad, such as Emil Racoviţă (1868 - 1947), founder of Biospeleology. However, Antipa was most influenced by Peter Missir (1896 - 1929), through his way of thinking and views. This professor of political economy, newly arrived from Germany, taught lessons in such an interesting way that Grigore, according to his own confession, remained passionate for his entire life of this science, which gave him ideas and arguments for the practical application of the results of his studies of aquatic ecology. In 1885, the student Grigore Antipa passed the baccalaureate, and, in February 1886, he received the graduation certificate of secondary education, with the highest grades at natural sciences and German language – the same subjects required for the enrolment at the University of Jena.

STUDENT AT JENA, THE FORTRESS OF EVOLUTIONISM (1886 – 1892)

In the autumn of 1886, Nicu convinced Grigore to go together to Jena, to join him and enrol at the famous University where courses were taught by famous professors, such as Goethe, Schiller, Humboldt, Hegel and Ernst Haeckel, who was also vice-rector. The Scientific Department of the Faculty of Philosophy, Grigore enrolled at, included also the Institute of Zoology, managed by Haeckel. The six years of college, of tense working under the guidance of the great Darwinian biologist, are amply mentioned in the book *In the Footsteps of Grigore Antipa* (NEGREA, 1990, pp. 79-104), in the sub-chapters: *The student Grigore Antipa and the great Haeckel, How to learn the mysteries of research, With Nicu Leon at Weimar, Leipzig and Dresden, Stops in famous biological resorts* (for his PhD thesis), *A doctorate passed at Haeckel* (he obtained *Summa cum Laude*, awarded by Haeckel only three times in his career). It is worth mentioning that the relationship between the famous professor and the Romanian student became so close that after Antipa's return in the country, they corresponded. Thus, on the 4th of May, 1917 (old calendar), three months before the battle of Mărăşeşti, deeply affected that his people was fighting against Antipa's people on the Romanian territory, he wrote:

*Dear friend Antipa [...]. I enjoyed finding out that your beautiful city escaped destruction and, thus, your splendid museum escaped destruction [...]. On the 16th of February, I was celebrated on the occasion of 83 years from the doctorate. These two communications marked the end of my scientific activity. I no longer have enough power. I welcome my last trip to nirvana (*as if he were a Dacian who enjoys going to Zamolxes – a.n.*). I have been paralyzed for six years, so there is no chance to be able to travel again. My beloved wife left me two years ago [...]. In the museum where I gathered all the documents related to the evolution, there are exhibited all the beautiful gifts from you [...]. With best wishes [...] your professor, Ernst Haeckel.*

In July 1917, Antipa received the last postcard written in shaky handwriting. I quote from Haeckel's letter: *For several months, my health has been getting worse, I cannot work methodically. My heart and nervous system no longer allow it.* It seems unbelievable, but even octogenarian and paralyzed his desire of working was still alive. Two years later, on the 16th of August, 1919, the titan of biological thinking died at 85. He will forever remain immortal through his work and life.

Grigore Antipa would not forget in his entire life the six years of strenuous work in Haeckel's lab under his direct guidance, along with his two assistants, A. Lang and W. Kukenthal. They were years when he diligently learned how to study wildlife in the field and under the microscope, how to perform scientific research culminating in the publication of the results and to prepare works for print (Plate 4).

After completing his PhD thesis, Antipa remained nearly a year in Jena, waiting for a recommendation from the Minister of Culture and Public Instruction, Dimitrie Sturdza, to Anton Dohrn, the director of the Zoological Station in Naples. This recommendation was received by post in January 1892 and on the 14th of February he was already present in Dohrn's famous Zoological Station. After six months of work under his authority, he returned in the country fully enlightened about how to study the marine environment. Bearing in mind bold plans, he returned in Bucharest. He was 25 years old, the golden age for a biologist researcher.

Given the reduced space I have for the Romanian period of the research of seawater and fresh waters, for the study of biodiversity and their bioeconomy, I shall just render the chronological presentation of the main events.

THE BEGINNING OF HIS CAREER 1892 – 1912

It is the period when Grigore Antipa studied the Black Sea, the Danube Delta and its floodplain. For further details, one may study the works: ANTIPA (1912; 1941), BOTNARIUC & BELDESCU (1961), NEGREA & NEGREA (1975), TUDORANCEA & TUDORANCEA (eds., 2006), ZINEVICI & PARPALĂ (2006), BREZEANU & CIOBOIU (2011), BREZEANU et al. (2011).

- October 1892. Grigore Antipa, through the help of the Minister D. Sturdza, got a private audience with King Carol I and he presented a memoir entitled *On the necessity of introducing a rational pisciculture in the Romanian waters*. The king recommended the resort ministers as the 25 years-old young man to be appointed General Director of the State Fisheries, to entrust him the administration of the zoological collections of the Natural History Museum in Bucharest and to facilitate his embarkation on military ships in order to start the biological research of the Black Sea. A few months later, he was appointed General Director of the State Fisheries, a position he held until 1914, and on the 4th of November, 1892, he was appointed director of the Department of Zoology, Natural History Museum, a position he held until his death in 1944.
- On the 5th of May, 1893 (old calendar), Antipa went on his first expedition to study life in the Black Sea on board of the cruiser battleship Elizabeth. It was carried out in three stages and lasted 118 days. It is considered the first Romanian oceanographic research. As he did not have a team and urgent business required his presence, the collected faunal material remained unused and unpublished. The same thing happened with the collections gathered during the 60-day expedition from 1894 with the brig Mircea on the route Sulina – Constanţa – Odessa – Mangalia – Varna and the 1895 expedition aboard the gunboat Griviţa in the Romanian waters of the Black Sea (details NEGREA, 1990, pp. 114-132). Over the years, he would publish two papers on the Biosociology and Bioeconomy of the Black Sea (ANTIPA, 1933; 1941).
- Also in 1893, as General Director of the State Fisheries, he began to make trips to the floodplain and the Danube Delta, including inland, to document directly in the field on the state of waters, fisheries and fish trade. Between two trips, although overwhelmed by administrative duties, he found time to process the data collected in the field and publish the results. In 1894, it was published the first work of Hydrobiology with economic implications about *Lake Razim and the current state of fisheries and means of recovery*. This paper shows the need to build networks of canals connecting the lakes with the Danube. This brilliant idea subsequently applied to the Danube Delta, with great practical results. Noting the damage caused by chaotic, ruthless fishing and especially by killing juveniles, Antipa published the works: *Studies on the fisheries in Romania* (1895) and the *Fisheries Law and the results that it has brought. A response to the attacks it was subject to* (1899). It is the law he devised and passed by Parliament in 1896. But his most important work from the end of the century was the synthesis published in 1895 *Studies on the fisheries in Romania*, based on the three years of research during his "numerous trips in every fishery at the mouth of the Danube and surrounding ponds", including the lakes located on the Romanian coast of the Black Sea. With brilliant insight, he wrote a modern ecologist about "the connections" that exist between creatures, "plankton standing at the basis of all". For the suppression of this serious situation, Antipa elaborated the law also mentioned in the program called PARID/FIDFR (Fisheries and Improvement of the Danube Floodplain Region) based on the cooperativization of fisheries and on the centralization of fish sale. According to the vision of the young biologist economist, fishermen freed from the intermediaries' exploitation (tenants, merchants, etc.) and organized in production cooperatives were to fish within legal limits, paying property taxes, based on which the state could execute great works (dams, canals, fisheries, etc.). One can see here the beneficial effect of studying political economy, taught in high school by Petre Missir, who, together with Ernst Haeckel, were in my opinion, role models in life, that positively influenced Grigore Antipa, making him an illustrious biologist worthy to stand in the science - cultural world pantheon with another scientist originating in Moldova, Emil Racoviţă, his high school mate from Iaşi.
- In the years 1907 – 1908, under the protection of the Fisheries Law, Grigore Antipa helped by the engineer Mihai Roco (design) and Ion Vidraşcu (execution), built the first canal in the Danube Delta, called Dunavăţ, 27 km long, linking Lake Razim (mistakenly called Razem by the geographers of the time) to the Danube. As a result, fish production in the lake increased from 38,000 to 3,600,000 kg / year. From 1907 until 1914 (when Antipa was dismissed as general inspector of the State Fisheries), the Delta has been enriched by a network of canals, which restored its initial fishery fertility.
- In 1909, there appeared Antipa's monograph entitled *Ichthyologic Fauna of Romania*, the result of 14 years of activity, which can still serve as a model in the field. In 1910, he published another synthesis entitled *The floodplain of the Danube, its current state and means of capitalization*, from which it results his care of patriotic citizen to capitalize this wetland. The hydrotechnical engineers, after 1950, drained a large part of the Delta and the large lakes located within the river floodplain (Crapina – Jijila, Insula Mare a Brăilei / Big Island of Braila, Greaca Lake almost entirely, Potelu, etc.), although NEGREA & NEGREA (1975) in their book, which is based on their PhD theses (A. Negrea, Gastropods, Şt. Negrea, Cladocerans) show that all their conclusions confirm Antipa's findings after five decades (1910) and the floodplain of the Danube must remain in its natural state and no longer be drained. However, the policy makers did not take into account our conclusions (details in NEGREA, 1990, pp. 158-161).

HIS CREATIVE UPSURGE (1913 – 1937)

- Grigore Antipa's Museum has been functioning in the current building since 1908. The history of the museum and Antipa's contribution are widely presented in the book *In the Footsteps of Grigore Antipa* (NEGREA, 1990, pp. 162-168 and 174-192), where the author reconstructs its long history of 150 years since it was founded, in 1834, under the name of the *National Museum* until 1990, when the book appeared. Because of the lack of space, I kindly require the reader to consult this guaranteed and mostly original source of information. I just underline that Dumitru Murariu, the present director, has built a new wing of the building and reorganized the visiting area, giving it a unique sense and equipping it with the latest means of viewing and information and, thus, the presence of a guide is no longer absolutely necessary. Currently, Murariu is working with his team of researchers and experts to a Memorial dedicated to the personalities who have been linked in some way to this museum. Of course, there will be represented the directors of this institution of culture that have contributed to its development: Grigore Antipa, Constantin Motaş, Mihai Băicescu and Dumitru Murariu, all reputed academicians and professors.
- In 1899, at 32 years old, Antipa got married to Alina Petrescu, the daughter of the General Zaharia Petrescu. Because of a condition of the digestive system, she was hospitalized several times in a sanatorium in Baden bei Wien, for balneary treatment, and Grigore wrote her almost daily as he had promised.
- Grigore Antipa was a true *homo viator* since the college days at Jena. *I had established a rule* – he confessed in 1934 - *from which I never wavered: to travel every year at least two months in different countries to make new purchases of collections* (see NEGREA, 1990, pp. 182-192, chapter entitled *New roads through Europe and the crossing of the Atlantic* – impossible to summarize).
- The same Romanian Academy, which rejected his work, *Studies on the Fisheries from Romania*, since Gheorghe Lazar Prize, 11 years later, recognized Grigore Antipa's theoretical and practical value of his entire work, choosing him as a corresponding member three years later, and after another three years, in 1910, electing him as an active member. On the 28th of May, 1912 (old calendar), Antipa uttered his speech entitled *Hydrobiological research in Romania and their scientific and economic importance*. The scientific department chose him for years in a row secretary during 1919-1941. It was also Antipa who, as representative of the Academy, managed the inventory of the legacy left by Jacques Menahem Elias, a Romanian banker and philanthropist. Together with Daniel Danielopol, built Elias Hospital in Bucharest, considered the most modern hospital in Europe at the time.
- Throughout his professional life, Antipa had as permanent concern the problems of the Danube, and, thus, in 1922, he published the study suggestively entitled *The Danube and its scientific, economic and political problems* (see NEGREA, 1990, pp. 197-202). Instead, the study *The issues of the evolution of the Romanian people*, published in 1919, reveals another preoccupation that the agronomist Gh. Ionescu Şişeşti appreciated as: "With this book, Dr. Antipa fulfilled his duty to the nation as an educator" being a true "Treaty of sociology, regarding the Romanian realities".
- Grigore Antipa was also founder of institutions. Besides the Natural History Museum and Elias Foundation in Bucharest, he founded the Institute of Bio-oceanography in Constanta, the Hydrobiological Station in Tulcea and the fish hatcheries from Nucet. He also initiated the founding of the Institute of Agricultural Research (ICAR) and it was also Antipa who was the initiator of the creation of the Geological Institute of Romania. Therefore, it is only about applied science institutes.
- Antipas had an active presence (different communications) at various international scientific conferences since 1927. I mention some of them: The 10th International Congress of Zoology (Budapest, 1927); CIESMM office work for the scientific exploitation of the Mediterranean Sea (Madrid, 1929); The 14th International Congress of Agriculture (Bucharest, 1930); the 11th International Congress of Zoology, Padua, 1930; the 7th International Congress of Aquaculture and Fisheries, accompanied by Alina (Paris, 1931); the General Meeting of the Mediterranean Commission (CIESMM), Paris (1933) and Naples (1934); Representative of Romania to the centenary of the National Museum of Natural History (Paris, 1935). About other trips abroad (1936, 1939), there is little and vague information.
- On the 7th of December, 1937, Grigore Antipa turned 70 and, in 1938, he was honoured in a solemn session of the Romanian Academy. On this occasion, he received the Jubilee volume *Grigore Antipa hommage a son oeuvre* (727 pages). According to C. MOTAŞ (1961), Antipa represents the *Apotheosis of a daily work of nearly half a century in the service of science, culture and common good.* It could be said that at 70 years old *he was able to work tirelessly* for 15 to 17 hours every day.
- I mention two works published by the end of his career: *The role of the Academy in combating smear campaigns of the Romanian people and state* (published in the Memoirs of the Scientific Section of the Academy, ser. 3 tom. 15, 1940) and *Oceanography, bionomy and general biology of the Sea Black* (314 pages), largely based on unpublished data. His last published work was: *Capitalization of the reed from the Danube Delta* (An. Acad. Rom. Mem. Secţ. Şt., ser. 3, tom 18) in 1942, two years before his death.

- On the 9th of November, 1940, a powerful earthquake destroyed part of the museum building and damaged its collections. With his spirit of economist, Antipa turned to Emil Prager, to repair the roof as winter approached. On the 23rd of January, 1941, another calamity: during the Legionary Rebellion, the museum building was gunned. Antipa suffered his first heart attack. On the 1st of April, 1943, when there were 50 years since he was at the helm of the museum, the country was at war and the anniversary moment passed almost unnoticed.
- On the 9th of March, 1944, when Grigore Antipa was 76 years and 3 months old, his heart failed and he died alone after midnight. The next day, Alina Antipa, who was sleeping in the next room, finding out about Grigore's death, exclaimed: *Grigore could not have done such a thing, to leave me alone!*. He took the light and went in eternity. Three days later, on the 12th of March, 1944, Alina and Grigore were together on the catafalque in the entrance hall of the museum. The urns with the ashes of the two are still together, in a niche, united by death forever, in the same room, the Museum of European fame, his dearest achievement, being their grave.

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Plate 1. Cover of the book *In the Footsteps of Grigore Antipa* by Ştefan Negrea, his face illustrating optimism and kindness (drawing by Victor Elias after a picture from Al. Marinescu's photo library).

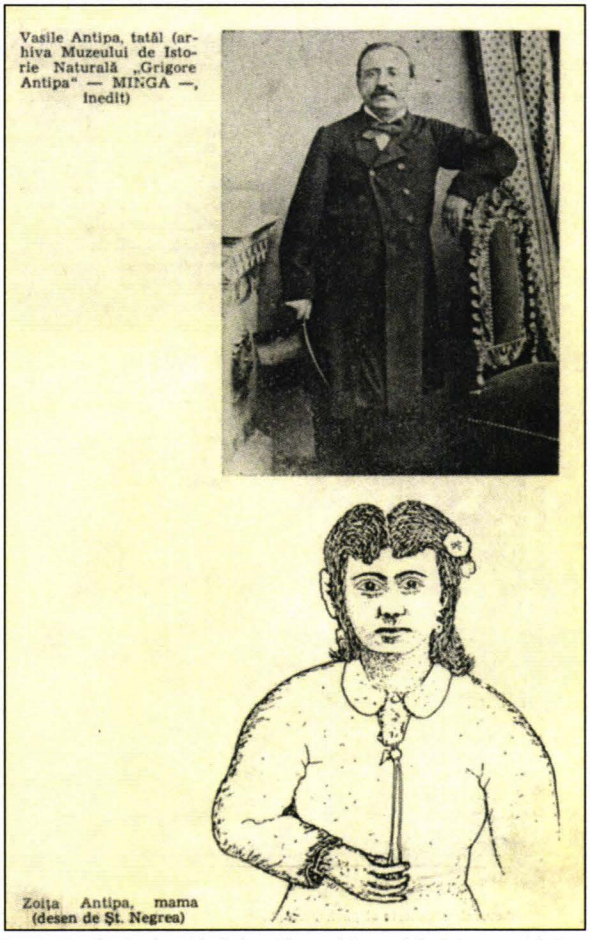

Plate 2. Vasile Antipa, his father (the archives of the Museum of Natral History "Grigore Antipa" – MINGA – inedited).
Zoiţa Antipa, his mother (drawing by Ştefan Negrea).

Plate 3. Grigore Antipa, student at the United Institutes from Iaşi (MINGA archives, inedited). The old building of "Mihai Eminescu" High School where the "Unites Institutes" functioned in Iasi (photo by Ştefan Negrea).

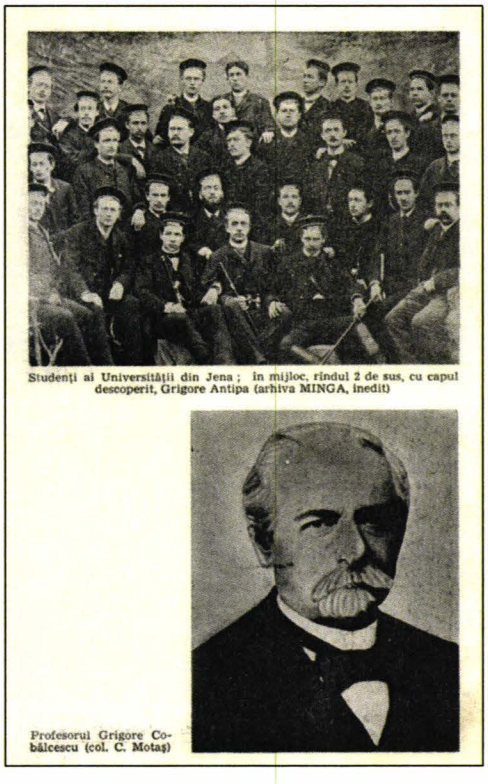

Plate 4. Students of Jena University; in the middle, in the second row from above, with the uncovered head, Grigore Antipa (MINGA archives, inedited). Professor Grigore Cobălcescu (col. C. Motaş).

QUANTITATIVE APPRECIATIONS REGARDING THE BIODIVERSITY

NEACŞU Petre, CIOBOIU Olivia

Abstract. Biodiversity represents the totality of the Earth flora and fauna species. It is considered that about 2.000.000 species have been known so far. However, it is estimated that 12.990.400 species would live on the globe.

Keywords: biodiversity, quantitative values, monitoring, the Earth.

Rezumat. Aprecieri cantitative asupra biodiversităţii. Biodiversitatea reprezintă totalitatea speciilor vegetale şi animale de pe Planeta Terra. Se consideră că în prezent se cunosc în jur de 2.000.000 de specii. Potenţial se estimează că, pe glob ar exista 12.990.400 specii.

Cuvinte cheie: biodiversitate, valori cantitative, monitorizare, Terra.

Biodiversity represents the variety of all living organisms from all terrestrial, inland waters and marine environment ecosystems. In simpler terms, biodiversity is constituted from the assemblage of living organisms, genetic material and the ecological complexes they belong to.

The knowledge of species is a long-lasting work, which is far from being finished. By the middle of the 18th century, only 9,000 species were known.

Presently, the number of vegetal and animal species was estimated to about 2 million (1,849,000 species).

However, it is considered that the real number would be 12,990,400 species (Table 1).

Table 1. Inventory of known species on large taxonomic groups and the evaluation of the potential number of species for each group (according to LEVEQUE, 1997).

Taxonomic group	The approximate number of registered species	Potential number of species
Viruses	4,000	500,000
Bacteria	4,000	1,000,000
Funguses	72,000	1 to 2 millions
Protozoa	40,000	200,000
"Alga"	40,000	400,000
Plants	270,000	320,000
Invertebrates	**1,400,000**	-
Arachnids	75,000	750,000
Crustaceans	40,000	150,000
Insects	950,000	8,000,000
Other arthropods	125,000	-
Mollusks	70,000	200,000
Nematodes	25,000	400,000
Other species	115,000	250,000
Vertebrates	**42,500 – 42,900**	-
Fish	19,000	21,000
Amphibians	4,200	4,500
Reptiles	6,300	6,500
Birds	9,000 – 9,200	9,200
Mammals	4,000 – 4,200	4,200

According to the Census of Marine Life, published in the United States of America in the scientific journal PLOS BIOLOGY, there would be 8.7 million species on the Earth (ORNATO, 2012).

A recent study of the American researchers, based on an informational model, reached the conclusion that the following groups of organisms would live on the Earth: animals (7.8 milions species), higher plants (298,000 species), funguses and molds (611,000 species), alga (21,000 species) (Table 2).

Table 2. The numerical situation of the estimated and classified groups of organisms (according to ORNATO, 2012).

Taxonomic group	Number of estimated species	Number of classified species
Animals without protozoa	7,77 millions	953,434
Plants	298,000	215,644
Funguses and molds	611,000	43,271
Protozoa	36,400	8,000
Alga, diatomeae, water molds	27,500	13,033

Of the total number of presented species, only 1.23 million (or 14%) have been discovered, described and classified so far. The rest of 86% of all the species present on the Earth live in terrestrial and continental water ecosystems and 91% live in seas and oceans and wait to be discovered and classified.

It is considered that numerous species could disappear even before being discovered and studied for their functions within the ecosystem (CALLARD & MIILIS, 2003).

Biodiversity creates the whole existence of human society providing sources of food, raw materials, health and recreation. Apart from the ethical and naturalist concerns, the diversity of the living is actually a set of *biological resources* essential for human beings.

For biotechnology, biodiversity is a factor of inspiration and use of raw materials that will be discovered in the future to the species that have not been known until now. Defending this potential is an argument to be used in the fight to protect biodiversity (CHAUVET & OLIVIER, 1992; GALLOCHAT, 1994).

Recently, the International Union for Nature Conservation finished the red list where there are registered 59,508 monitored species, 19,625 of which are threatened species.

In conclusion, the plants and animals that biodiversity refer to represented and still represents the main factors of human society development. The knowledge of species was achieved gradually once research in this field intensified.

The increase of human population and the abusive exploitation through excessive hunting, fishing, agriculture and industry led to the disappearance or reduction of the populations.

The creation of parks and natural reserves – data bases regarding biodiversity monitoring and conservation as well as of other protection means led to the rescue of numerous species.

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RECOMMENDATIONS
regarding the elaboration of the papers for the scientific journal
"Oltenia. Studii şi comunicări. Ştiinţele Naturii"

The journal is edited by the Oltenia Museum Craiova and it publishes original papers in the fields of vegetal and animal biology, ecology-environment protection, mineralogy-palaeontology, as well as scientific reports, reviews, anniversary or commemoration papers.

It appears annually, it is ISI indexed (http://science.thomsonreuters.com/cgi-bin/jrnlst/jlresults.cgi?PC=MASTER &Word=oltenia) **and accredited by CNCSIS as a B+ Journal.**

I. Structure (format) for original papers, scientific reports and reviews:

A	***Original papers***	will be structured according to the information rendered in the Table 1.
B	***Scientific reports***	will be structured according to the author's (authors') preferences, but it has to include abstract and key words, both in English and Romanian.
C	***Reviews***	there will be mentioned: author (authors) of the book (name and first name – CAPITAL LETTERS), comma, the title of the book, lowercase letters (Italic), publishing house, publishing location, year, number of pages. Use a free space and then render the text of the review with as fewer paragraphs as possible and the same characters as in the case of original papers.

Table 1.

STRUCTURE OF THE PAPER	CHARACTERISTICS	OBSERVATIONS
TITLE	capital letters, 12 pt., bold, centred	
two spare rows (12 pt.) between the title and the name of the author/s		
Author/Authors	name, capital letters, first name, noncapital, 11 pt., bold, normal, aligned right	between two or many authors, use comma
One spare row, 10 pt.		
Abstract (English)	from the beginning of the line, without tab, 9 pt., bold, normal	the abstract will be written with 9 pt., normal, maximum 300 words
One spare row, 9 pt.		
Keywords (English)	from the beginning of the line, without tab, maximum 5 words, 9 pt., normal	
One spare row, 9 pt.		
Rezumat (Romanian)	from the beginning of the line, without tab, 9 pt., bold, normal	**Complete translation of the title in Romanian** (no capital letters, except for the first letter of the title; 10 pt., bold). The content of the abstract – 9 pt., normal, maximum 300 words
One spare row, 9 pt.		
Cuvinte cheie (Romanian)	from the beginning of the line, without tab, maximum 5 words, 9 pt., normal	
One spare row, 14 pt.		
INTRODUCTION	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
MATERIAL AND METHODS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
RESULTS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
DISCUSSIONS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
CONCLUSIONS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
ACKNOWLEDGEMENTS	10 pt. (capital letters, bold)	content – 10 pt., normal
One spare row, 10 pt.		
REFERENCES	10 pt. (capital letters, bold)	content – see bibliographical references
One spare row, 10 pt.		
Bibliography enumeration	see the detailed explanations at the references heading	
Personal data	Name and surname – 8 pt., bold, normal, centred Institution and e-mail address – 8 pt., normal, centred	

Other details related to the papers:

Publishing language	English
Page format	A4 (21 x 29.7 cm), margins: top – 2.5 cm; bottom – 2.0 cm; left – 2.0 cm; right – 2.0 cm; gutter – 0 cm; header, footer – 1.27 cm. The papers will be elaborated in Microsoft Word, justified; font: Times New Roman, 10 pt., normal; single space.

- Latin names (genus, subgenus, species, subspecies) will be written with italic characters; - Suprageneric names are **not** written with italic characters. The same procedure is used when they are mentioned within figures, graphs and tables.
The first mention of a taxon in the text will be followed by the taxon author's name and the publishing year of the description, according to the zoological nomenclature code (e.g.: *Cossus cossus* (Linnaeus 1758), afterwards, it will be used abbreviated.
The name of the authors quoted in the text will be written **normal, capital letters,** while the names of the authors of the taxa will be written **normal, lowercase letters**
For the names of Romanian authors and settlements diacritics must be used.
The materials sent for publication (printed and in electronic format) has to be between 2 and 8 pages (the number of pages must be **even**).

II. References

➢ **References** in the text (quotation) includes only the author's/authors' names (CAPITAL LETTERS) and publishing year. For example:

- when it is a single author: IONESCU (1965) or (IONESCU, 1965);
- when there are two authors, it is used "&": RĂDULESCU & SAMSON (1990) or (RĂDULESCU & SAMSON, 1990);
- when there are more than two authors: IONESCU et al., (1992) or (IONESCU et al., 1992);
- in case there are many papers written by the same author/authors, published in the same year, use the letters a, b, c, etc. after the year (e.g.: IONESCU, 2000; IONESCU, 2000a, …, 2000g);
- authors are rendered alphabetically and, in case there are many papers written by the same author, they are introduced chronologically.

➢ **References** will include **only** the papers quoted in the text (10 pt.), alphabetically rendered, without numbers, as it follows:

- author (CAPITAL LETTERS), publishing year (normal), (**do not use** comma between the author and the publishing year or between the name and first name of the same author; use comma between different authors, when there are more than two), *the title of the paper* (*italic*), name of the journal, publishing house, volume number (bold), the number of fascicle (normal), number of pages (normal). When there are two authors, use "**&**"; if there are many authors, mention **all** of them. The ladies' first name is completely written; for gentlemen, use only the first letter.

The reference titles will be aligned as it follows: the first line from the beginning (no tab), the second at 1.27. For example:

EXEMPLE
Book reference:
BOŞCAIU N. 1971. *Flora şi vegetaţia Munţilor Ţarcu, Godeanu şi Cernei*. Edit. Academiei R. S. R. Bucureşti. 300 pp.
Paper published in a journal:
GULII V. & PAMUJAC M. 1994. Elemente ale protecţiei integrate a culturilor agricole de dăunători şi boli. *Protecţia integrată a plantelor*. Edit. „Ştiinţa". Chişinău: 112-118.
STAN MELANIA & BACAL SVETLANA 2006. New contributions to knowledge stafilinidelor (Coleoptera: Staphylinidae) of the landscape reserve "Codrii Tigheci" (Moldova). *Oltenia. Studii şi comunicări. Ştiinţele Naturii*. Muzeul Olteniei Craiova. **22**: 155-159.
Reference to a part of a collective paper; volume (with editors):
IFTIME Al. 2005. Amfibieni şi Reptile. In: Botnariuc & Tatole (Eds.) *Cartea Roşie a Vertebratelor din România*. Edit. Academiei Române. Bucureşti: 1-325.
Papers presented at scientific manifestations and published in a volume without editors:
CIOCHIA V. & STANCĂ-MOISE CRISTINA. 2001. Contributions to the knowledge of the Macrolepidoptera from natural complex "Dumbrava Sibiului". *Sesiunea Ştiinţifică dedicată împlinirii a 75 de ani de la înfiinţarea Staţiunii Biologice Marine „Prof. Dr. I. Borcea"*. Agigea-Constanţa. 19-20 octombrie 2001: 125-131.
Official publications (laws, decrees, official reports):
***. România. Legea nr. 13 / 1993 pentru aderarea României la Convenţia privind conservarea vieţii sălbatice şi a habitatelor naturale din Europa, adoptată la Berna la 19 septembrie 1979. *Monitorul Oficial al României*. An V, nr. 62/25 martie 1993. Bucureşti: 1-20.
PhD thesis:
COSTACHE I. 2005. *Flora and vegetation Motru River Lower Basin.* Ph. D. Thesis, University of Bucharest. Romania. 290 pp., 8 Pl.
Web pages:
Muzeul Olteniei Craiova. Secţia Ştiinţele Naturii. *Oltenia. Studii şi comunicări. Ştiinţele Naturii.* (onlline). 2011. Publisher: Museum of Oltenia Craiova, Romania. www.olteniastudii.3x.ro (accesed: May 8, 2012).

Entire electronic document or service (data base):

***. Fauna Europaea: Chironomidae. In: *Fauna Europaea: Chironomidae, Diptera, Nemathocera.* (Ed. H. de Jong) Fauna Europaea version 1.5, http://www.faunaeur.org. (accesed: June 23, 2012).

E-book:

AHMADJIAN V. 1967. *The Lichen Symbiosis.* Blaisdell Publishing Company. Massachusetts. Available from: http://books.google.ro/books?id=at7uXMn8iMC&printsec=frontcover&hl=ro&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false. 152 pp. (accesed: January 15, 2013).

Electronic publication (papers):

DANILEVSKY M. L. 2007. A check-list of Longicorn Beetles (Coleoptera, Cerambycoidea) of Europe. Available online at: http://www.coleoptera-literatura.ic.cz./literatura/checklist_cerambycidae_2007.doc. (accessed: May 20, 2009).

Note: The papers published with other characters than the Latin ones, will be re-written with Latin characters, both in the text and at references, mentioning the original language of publication between square brackets at the end:

ALEXANDROVICH O. R. 1995. Reconstruction of the ways of the ground beetles (Coleoptera, Carabidae) fauna forming at the West of the Russian plain. In: I. K. Lopatin, Pisanenko A. D., Shklyarov L. P. (Eds.). *Fauna and taxonomy: Proceed. Zool. Museum Byel.* University Minsk: Nauka Tekhnika. **1**: 52-68. [In Russian].

III. Illustration

- Images (white/black or colour), tables, graphs and maps are inserted into the manuscript, but **the original versions have to be sent also separately**: high contrast photographs, electronic images in TIFF format at a minimum resolution of 300 dpi.
- The references to the illustrations (tables, images, photographs) will be made in the text as it follows: (Fig. 1), (Figs. 1a, b), (Figs. 3; 5); (Table 1); (Photo 1).
- **Graphs must be achieved in Microsoft Excel.**

The title of a table (in English) will be placed above the table (aligned right), 9 pt., normal.	The title of a figure, photo, map (in English) will be placed below, centred, 9 pt., normal.

- For photographs and maps:
- It will be mentioned the word 'original' in case it is achieved by the author/'s of the paper;
- It will be mentioned the name of the author/'s between brackets in case it is achieved by another or other persons;
- It will be mentioned the web source (if it is the case), which has to be entirely rendered at references, also mentioning the access date.

Legend is placed below a table, graph, etc., 8 pt., normal (English)

!!!!!!**For graphs and diagrams**, use white, black, grey, different tones and textures. **They will not be published in colours.**

Illustrations references (tables, images) (in English), will be made in the text as it follows: (Fig. 1), (Fig. 1a, b), (Figs. 3; 5); (Table 1). The title of a figure (in English), will be centered below the figure; as for tables, put it above (aligned right), both 9 pt., normal. Examples: a) Table 1. List of the identified staphylinids from the Lower Dniester. b) Figure 1. Distribution of the butterfly *Papilio machaon* L.

The paper will be accepted for publishing if:

- **it respects** the aforementioned **requirements**;
- **it acquires a favourable review** from the scientific reviewers of the journal;
- **it is sustained** within the framework of the International Conference "*Museum and scientific research*";
- **the publication fee is paid**;
- **it is respected** the deadline, **March 31** of the current year.

The editorial board *has the right to reduce the number of figures and photos (if there are too many as compared to the text of the paper or if they do not correspond to the requirements) and not to accept papers sent after deadline*, **March 31, 2015**.

With all the respect for the authors, papers that do not correspond to the recommendations will be sent back.

The responsibility of the scientific content of the papers depends entirely on the authors. Authors must revise the papers reviewed by the reviewers.

The Editorial Board

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