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ECOLOGICAL STUDY ON THE EDAPHIC MITES POPULATIONS (ACARI: MESOSTIGMATA-GAMASINA; ORIBATIDA) IN URBAN AREAS FROM ROMANIA

VIORICA HONCIUC, MINODORA MANU

The research was made in three central parks Cişmigiu, Unirea and Izvor, in Bucharest city (in 2006 and 2007). The research of the edaphic mites (Ord. Gamasina; Ord Oribatida) was made by the transects method (T): the long side (T_1), situated parallel with the boulevard; the short side (T_2), situated parallel with street and the middle side (T_3), situated in the center. From the total number of 106 identified mites species, 83 species were decomposers and 23 predators. Common species for all urban areas were 10 saprophagous-decomposers: *Zygoribatulla terricola, Tectocepheus sarekensis, Epilohmannia cylindrical, Medioppia obsoleta, Dissorhina ornata, Ceratozetes mediocris, Oribatulla tibialis, Hermanniella dolosa, Eulohmannia ribagai, Trichoribates novus* and 2 predators: *Rhodacarellus silesiacus* and *Hypoaspis aculeifer*. Differences appeared between the saprophagous-decomposers and predators mites populations from the urban areas on the taxonomical spectrum, on the structural and dynamics of parameters. The statistical analysis of the ecological parameters (x/m^2; A_r%; C%, D%) of mites populations showed an important representation of oribatids, as well as of gamasids. The saprophagous-decomposers recorded were: 53,200 individuals/sq.m., in Unirea park; 54,800 individuals/sq.m., in Izvor park, and 33,600 individuals/sq.m., in Cişmigiu park. The predators recorded were: 8,200 individuals/sq.m., in Unirea park, 7,200 individuals/sq.m., in Izvor park, and 5,600 individuals/sq.m., in Cişmigiu park. All these aspects were due to the bioedaphic conditions, which were not so favorable to the development of the both trophical categories in urban areas as parks. The differential researches of the mites populations from the natural ecosystems and parks can be a database in parks planning strategies.

Key words: urban areas, Gamasina, Oribatida, transect, structure, dynamics.

INTRODUCTION

Among the soil group of arthropods, the mites are widely distributed, with high abundance and species diversity (Schatz, 1983; Marshall *et al.*, 1987; Honciuc, 1992; Karppinen & Krivolutsky, 1983; Vasiliu *et al.*, 1993; Evans, 1992; Walter & Proctor, 1999; Koehler, 1999; Gwiazdowicz, 2007). These live in different habitats: soil, litter, in nests of birds, on plants, on animals and even in storehouses. In soil they found the most favorable bioedaphical conditions for the development of all populations. Their presence depends on: soil structure, presence of organic matter, type of humus and on some abiotical factors such as temperature, humidity and pH.

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In different types of man affected ecosystems from temperate area (*i.e.* urban areas, mining areas; agro-ecosystems, etc.), environmental factors do not allow a proper development of the population of saprophagous oribatids and predator gamasids, in comparison with natural ecosystems, where due to the favorable bioedaphic conditions and to many habitats and microhabitats, these invertebrates had good conditions for their development (Niedbala, 1990; Ruf, 1998; Koehler, 1999; Salmane, 1999, 2000, 2001; Honciuc & Stanescu, 2000; Ruf *et al*., 2003). The ecological importance of these microarthropods from the soil of different ecosystems, highlighted in many researches done by the acarologist specialist, could be shown by many methods, one of these being the analysis of the structural and dynamic differences of the mite populations.

MATERIAL AND METHODS

The studies were made in three parks from Bucharest: Cişmigiu (N = 44°25'56.6"; E = 26°05'27.5"), Unirea (N = 44°25.5'56.6"; E = 26°08'09.9") and Izvor (N = 44°25'56.4"; E = 26°05'27.8"), in 2006 and 2007. The natural vegetation had mainly disappeared, being replaced by planted species (especially trees brought from Europe, China, Japan, America, etc.). Autochthonous and European planted species are: trees- *Acer pseudoplatanus* L., *Betula pendula* Roth., *Fraxinus excelsior* L., *Pinus sylvestris* L., *Quercus cerris* L., *Quercus robur* L., *Robinia pseudoacacia* L., *Tilia cordata* Miller., *Tilia tomentosa* Moench; shrubs- *Buxus sempervirens* L, *Cornus sanguinea* L., *Crataegus monogyna* Jacq., *Rosa canina* L., *Forsythia europaea* Degen. et Bald., *Hedera helix* L., *Ligustrum vulgare* L., *i.e*., and many herbaceous species from which we mention a few: *Achillea millefolium* L., *Agrostis stolonifera* L., *Alliaria petiolata* (Bieb.) Cavara and Grande, *Brachypodium sylvaticum* (Hudson) Beauv., *Calendula officinalis* L., *Capsella bursa-pastoris* (L.) Medik, *Dactylis glomerata* L., *Festuca arundinacea* Schreber, *Geum urbanum* L., *Lamium album* L., *Medicago sativa* L., *Poa pratensis* L., *Taraxacum officinale* Weber ex. Wiggers, *Trifolium pratense* L., *Trifolium repens* L., *Urtica dioica* L. Some elements of the natural vegetation remained and were adapted to the modified conditions of the city (either fertilisation or lack of nutrients, changes in soil pH, increased temperature, etc.).

For the soil mites (ord. Gamasina and ord. Oribatida) sampling, in each ecosystem, a certain surface was established. From each surface were collected 15 samples with MacFadyen corer, on 10 cm soil depth. Each sample was shared in two layers: litter-fermentation and soil. The samples were taken from three transects of each area (T_1 – near to the main road, T_2 – the lateral side of the studied area and T_3 – in the middle of the studied area).

The extraction was performed with a modified Berlese-Tullgren extractor, in ethyl alcohol and the mites samples were clarified in lactic acid. After taxonomical identification, the numerical abundance was the base for the quantification of others statistical parameters, such as: numerical density (x/sq.m.); relative abundance (Ar%) and constance (C%).

The relative abundance was calculated using the formula:

$$\mathbf{A = 100*n/N},$$

where:

N – number of individuals of one species from one sample;

N – total number of individuals of all species from one sample.

After this parameter the mites could be classified in five classes: eudominant (over 10%), dominant (5.1 – 10%), subdominant (2.1 – 5%), recedently (1.1 – 5%) and subrecedently (under 1.1%) (Lehmann & D'Abrera, 1998).

The constancy was obtained using the formula:

$$\mathbf{C=100*pA/P},$$

where:

pA – number of samples with species A,

P – total number of samples.

The mites were classified in four constancy classes: euconstant (75.1-100%), constant (50.1-75%), accessory (25.1-50%) and accidental (1-25%) (Lehmann & D'Abrera, 1998). The mites fauna was identified on the species level.

To evidence the structural and dynamical differences between mites population from the studied areas, the main abiotical factors were measured: humidity and pH of soil (Table 1).

Table 1

Average of humidity and pH of soil, in urban areas studied

Factors	**Parks**		
	Cişmigiu	Unirea	Izvor
Humidity	10.23	12.37	9.89
pH	7.15	7.45	6.73

RESULTS

The results were presented taking account of the natural ratio between mites from saprophagous-decomposer group (Ord. Oribatida), most represented and the predators group (Ord. Gamasina) scarcely represented.

We can observe that in all investigated ecosystems, the taxonomical structure showed the presence of 83 species of Oribatida, according to taxonomical classification of Balogh (1972), Ghiliarov & Krivolutsky (1975), Marshall *et al.* (1987), Niedbała (1992), Subias & Balogh (1989) and 23 species of Gamasina, according to Ghiliarov & Bregetova (1977) and Karg (1993) (Tables 2, 3).

From the structural point of view, the mites fauna from the investigated ecosystems was characterized by the presence of the common species: oribatids such as *Zygoribatulla terricola, Tectocepheus sarekensis, Epilohmannia cylindrica, Medioppia obsolete, Dissorhina ornata, Ceratozetes mediocris, Oribatulla tibialis, Hermanniella dolosa, Eulohmannia ribagai, Trichoribates novus* and gamasids such as: *Rhodacarellus silesiacus* and *Hypoaspis aculeifer*.

Table 2

The relative abundance (Ar%) and constance (C%) of the Oribatid mite species in central parks of Bucharest city of Romania

Species	**Cişmigiu park**		**Unirea park**		**Izvor park**	
	A %	C%	A%	C%	A%	C%
Punctoribates punctum (C.L. Koch, 1839)	2.04	60	6.96	100	52.1	100
Zygoribatulla terricola V.der. Hammen, 1952	16.33	60	12.26	100	2.09	100
Tectocepheus sarekensis Trägårdh I., 1910	4.08	100			4.88	100
Trichoribates trimaculatus (C.L. Koch, 1836)	13.47	100	3.34	100	0.7	40
Tectocepheus velatus (Michael A. D., 1880)	8.16	80	12.26	100	1.16	60
Minunthozetes semirufus C.L. Koch, 1841			11.42	80	2.79	60
Protoribates lophotrichus (Berlese, 1904)	6.53	100	0.56	20		
Ramusella insculpta (Paoli G., 1908)	6.53	60	0.84	40	4.19	100
Galumna obvia (Berlese A., 1915)	1.63	60			6.28	80
Ceratozetes minutissimus Willmann, 1951	5.71	100				
Scheloribates laevigatus (C.L. Koch, 1836)	1.63	60	5.01	80	0.93	20
Epilohmannia cylindrica (Berlese), 1904	2.04	60	4.46	100		
Ophidiotrichus vindobonensis Piffl E., 1960			3.34	40		
Medioppia obsoleta (Paoli G., 1908)	1.63	60	1.39	60	3.02	100
Protoribates monodactylus (Haller, 1804)			2.23	40		
Dissorhina ornata (Oudemans A. C., 1900)	0.82	20	2.79	40	0.7	40
Carabodes femoralis (Nicolet H., 1855)					2.09	20
Pergalumna minor (Willmann, 1938)	2.04	40	0.56	20		
Pergalumna longior (Willmann, 1928)			1.67	40		
Ceratozetes contiguus Jeleva, 1962					1.63	60
Protoribates capucinus Berlese, 1908			0.56	40	1.16	40
Peloptulus phaenotus C.L. Koch, 1844	1.22	40				
Damaeobelba minutissima Sellnick, 1928	1.22	20				
Trichoribates oxypterus Berlese, 1910	1.22	20				
Achypteria oudemansi Hammen, 1952			1.11	40		
Lohmannia lanceolata turcmenica Bul.-Zachv., 1960	0.41	20	1.11	40		
Adoristes ovatus (C.L. Koch, 1840)			1.11	20		
Ceratoppia bipilis (Hermann J. F., 1804)			1.11	20		
Galumna sp.			1.11	20	0.23	20
Stachyoppia muscicola Balogh, 1961			1.11	20		
Oribotritia berlesei (Michael, 1898)					0.7	60
Galumna elimata (C.L. Koch, 1841)	0.41	20			0.7	60
Scheloribates distinctus Mihelčič, 1964					0.7	40

Oppia chitinophincta Kulijev, 1962					0.7	20
Euphthiracarus sp.					0.93	40
Nanhermannia elegantula Berlese, 1913					0.93	40
Microppia minus (Paoli G., 1908)			0.84	20		
Oppiella nova (Oudemans), 1902	0.82	40				
Suctobelbella baloghi (Forsslund K.-H., 1958)	0.82	40				
Phthiracarus dubinini Feider et Suciu, 1957	0.82	40				
Fosseremus laciniatus (Berlese), 1836	0.82	20				
Multioppia laniseta Moritz, 1966	0.82	20				
Oppia serratirostris Golosova, 1970	0.82	20				
Perlohmannia dissimilis (Hewitt), 1908	0.82	20				
Pseudachypteria magnus Sellnick, 1928			0.56	40		
Cerachipteria sp.			0.56	20		
Ceratozetes mediocris berlese, 1908	0.41	20	0.56	20		
Ceratozetella minimus (Sellnick, 1928)			0.56	20		
Cultroribula biculltrata Berlese A., 1908			0.56	20		
Haplophthiracarus pavidus Berlese, 1913			0.56	20		
Oribatulla tibialis Nicolet H., 1855	0.41	20	0.56	20		
Hermanniella dolosa Grandjean, 1931			0.56	20	0.23	20
Tropacarus carrinatus (C.L. Koch, 1841)			0.56	20		
Tropacarus sp.			0.56	20		
Oribotritia sp.			0.56	20		
Phthiracarus sp.			0.56	20		
Pergalumna altera (Oudemans, 1915)					0.47	40
Ceratozetes sp.					0.47	20
Phthiracarus pallidus Feider et Suciu, 1957					0.47	20
Amerobelba decedens Berlese, 1908	0.41	20				
Chamobates spinosus Sellnick M., 1928	0.41	20				
Eulohmannia ribagai Berlese, 1910	0.41	20			0.23	20
Eupelops acromios (Hermann L. van der1804)	0.41	20				
Euphthiracarus cribrarius (Berlese A., 1904)	0.41	20				
Nanhermannia nannus (Nicolet H., 1855)	0.41	20				
Oppia fallax Paoli, 1908	0.41	20				
Steganacarus magnus (Nicolet H., 1855	0.41	20				
Suctobelba trigona (Michael A. D., 1888)	0.41	20				
Trichoribates novus (Sellnick M., 1928)	0.41	20			0.23	20
Tropacarus pulcherrimus (Berlese, 1887)	0.41	20				
Anachypteria sp.	0.41	20				
Globozetes tricuspidatus (Willmann, 1953)			0.28	20		
Lauroppia falcata (Paoli G., 1908)			0.28	20		
Ctenobelba pectinigera (Berlese), 1908			0.28	20		
Scutovertex minutus (C.L. Koch, 1836)			0.28	20		
Zygoribatula frisie (Oudemans), 1900			0.28	20		
Belba corynopus (Herm.), 1804					0.23	20
Berninniella bicarinata Paoli, 1908					0.23	20
Lauroppia neerlandica Oudemans A. C., 1900					0.23	20
Lohmannia sp.					0.23	20

Oribotritia sp.					0.23	20
Phthyracarus anonymum Grandjean F., 1933					0.23	20
Rhyzotritia ardua (Koch, 1841)					0.23	20
Immatures	11.42857	80			4.65	60

Table 3

The relative abundance (Ar%) and constance (C%) of the Gamasina mite species in central parks of Bucharest city of Romania

Species	**Cişmigiu park**		**Unirea park**		**Izvor park**	
	A%	C%	A%	C%	A%	C%
Asca bicornis Caneastrini and Fanzago, 1887			26.83	100		
Rhodacarellus perspicuus Halaskova, 1958	32.14	80			8.57	40
Rhodacarellus silesiacus Willmann, 1936	14.29	60	2.44	20	5.71	40
Amblyseius obtusus C.L. Koch, 1839			4.88	40	8.57	40
Veigaia nemorensis C. L. Koch,1938	7.14	40			2.86	20
Hypoaspis aculeifer Caneastrini, 1883	7.14	40	7.32	40	17.14	80
Hypoaspis praesternalis Willmann, 1949			7.32	40	11.43	20
Crassicheles concentricus Oudemans, 1904			4.88	40		
Lysigamasus neoruncatellus Schweizer 1961			2.44	20		
Lysigamasus truncus Oudemans, 1901	3.57	20				
Leptogamasus sp.	3.57	20				
Parasitus beta Oudemans and Voigts, 1904	7.14	20			2.86	20
Ameroseius fimentorum Karg, 1971	3.57	20			17.14	60
Macrocheles sp.	3.57	20				
Pachyseius humeralis Berlese, 1910	14.29	20				
Pachylaelaps furcifer Oudemans, 1903	3.57	20			2.86	20
Amblyseius meridionalis Berlese, 1914			2.44	20	5.71	40
Dendrolaelaps sp.			2.44	20		
Rhodacarus denticulatus Berlese, 1921			2.44	20		
Olopachys vysotskajae Koroleva, 1976			2.44	20		
Pseudolaelaps doderoi Berlese, 1916			21.95	20	5.71	40
Alliphis siculus Berlese, 1921			2.44	20		
Pergamasus sp.					2.86	20
Veigaia exigua Berlese, 1917					2.86	20
Prozercon fimbriatus C.L. Koch, 1839					2.86	20
Immatures			2.44	20	5.71	40

Analysing the relative abundance (Ar%) and constance (C%) of the identified species from the studied ecosystems, based on the classification criterion, after statistical researches (Lehmann & D'Abrera, 1998), the mite populations can be divided into dominance and constance classes. In the structure and functions of the terrestrial ecosystems, the mite species from the highest classes of dominance and constance are the most important in populations structure and in decomposer process, that is why the discussion was focused on these mites.

In parks, the oribatid fauna was grouped after the values of the relative abundace and constance, showing a better image on their role in structure and functions of these ecosystems. In urban areas the specific spectrum of oribatid fauna was defined by the presence of the same dominance and constance categories.

In Cişmigiu, species *Trichoribates trimaculatus* was classified as eudominant-euconstant and *Zygoribatulla terricola*, as eudominant and constant. The following category of dominant species was made by 4 oribatids: *Tectocepheus velatus*, *Protoribates lophotrichus*, *Ramusella insculpta*, *Ceratozetes minutissimus*, at the same time constant. As subdominant but constant, there were identified 4 species and from these only *Tectocepheus sarekensis* is better represented from the structural point of view.

In Unirea park, in the first category, three species were included: *Zygoribatulla terricola*, *Tectocepheus velatus*, *Minunthozetes semirufus*, as eudominant-euconstant. From the dominant-euconstant class were signalled two species: *Punctoribates punctum*, *Scheloribates laevigatus*, and as subdominant 6 species, from which *Epilohmannia cylindrica* and *Trichoribates trimaculatus* were euconstant.

In Izvor park only species *Punctoribates punctum* appears, as eudominant-euconstant, recording the highest structural values in all studied ecosystems. Only one species was dominant-constant in this park: *Galumna obvia*. From the subdominant category 6 species were identified. Only 4 species: *Tectocepheus sarekensis*, *Ramusella insculpta*, *Medioppia obsoleta*, *Zygoribatulla terricola* were more important, being at the same time euconstant (Table 2).

The identified gamasids from the three parks were common species for the temperate forest ecosystems, fact demonstrated by many researchers (Karg, 1993; Salmane, 2001; Skorupski, 2001; Gwiazdowicz & Maciej, 2004) (Table 3).

In Cişmigiu park the following species were identified: *Rhodacarellus perspicuus* as eudominant–euconstant; *Rhodacarellus silesiacus* as eudominant–constant; *Hypoaspis aculeifer* and *Veigaia nemorensis* as dominant-accessories species; *Pachyseius humeralis* eudominant-accidental.

In Unirea park the situation was presented as follows: species *Asca bicornis* was eudominant-euconstant; species *Pseudolaelaps doderoi* eudominant–accidental; and species *Hypoaspis aculeifer* and *Hypospis praesternalis* dominant-accessory. The Izvor park presents the following classification of gamasids: *Hypoapsis aculeifer* was eudominant–euconstant; *Ameroseius fimentorum* was eudominant-constant; *Hypospis praesternalis* eudominant-accidental; *Amblyseius meridionalis* and *Pseudolaelaps doderoi* were species dominant-accessory, and *Rhodacarellus perspicuus*; *Rhodacarellus silesiacus* and *Amblyseius obtusus* dominant-accidental species. In all ecosystems the unmentioned species were classified as subdominant and accidental, due to the decreased recorded values of the abundance and constance.

In parks, there were identified 83 species of Oribatida (40 species in Cişmigiu, 33 species in Izvor and 41 species in Unirea) and 23 species of Gamasina (14 species in Unirea, 14 species in Izvor and 11 species in Cişmigiu). The dynamics of the total numerical density showed that the Oribatida mites had 54,800 ind./sq.m. in Izvor, 53,200 ind./sq.m. in Unirea and 33,600 ind./sq.m. in Cişmigiu and the Gamasina mites had 8,200 ind./sq.m. in Unirea and 7,200 ind./sq.m. in Izvor and 5,600 ind./sq.m. in Cişmigiu (Figs. 1, 2).

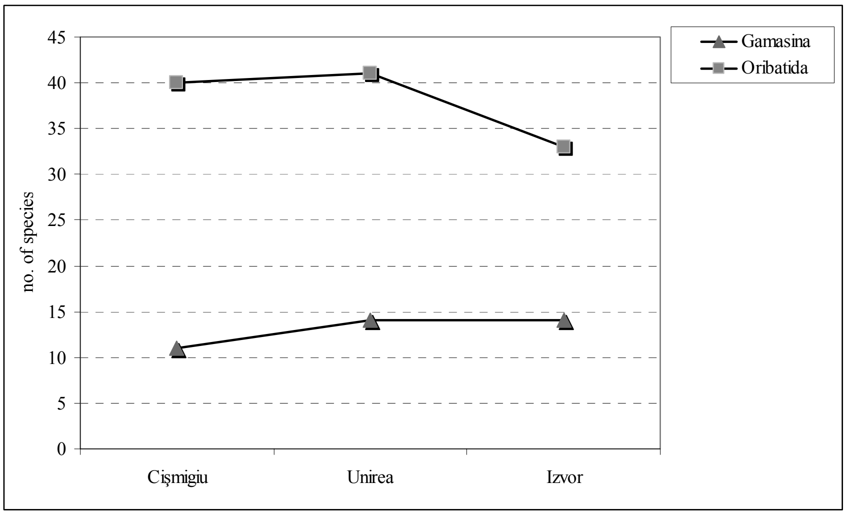

Fig. 1 – Fluctuations of the number of Gamasid and Oribatid mite species in the soil of central parks of city Bucharest/Romania, in 2006-2007.

From the structural point of view, on transect level, Oribatida populations had a dynamics in concordance with the number of species in both years. The highest density was registered in T_3 in all parks in the first year, in T_1 in Cişmigiu park, T_2 in Izvor park and in T_3 in Unirea park, in the second year. These density values on transects of the first year had the eudominant-dominant and constant species: *Trichoribates trimaculatus* (6200 ind./sq.m) in Cişmigiu park, *Zygoribatulla terricola* (3400ind./sq.m) in Unirea park, and *Punctoribates punctum* (4400 ind/sq.m) in Izvor park. In the second year, in mentioned transects, the recorded highest values were obtained by the species: *Zygoribatulla terricola* (2400 ind./sq.m; 2200 ind./sq.m) in Cişmigiu and Unirea parks, and *Punctoribates punctum* (3400 ind./sq.m.) in Izvor park (Figs. 3, 4).

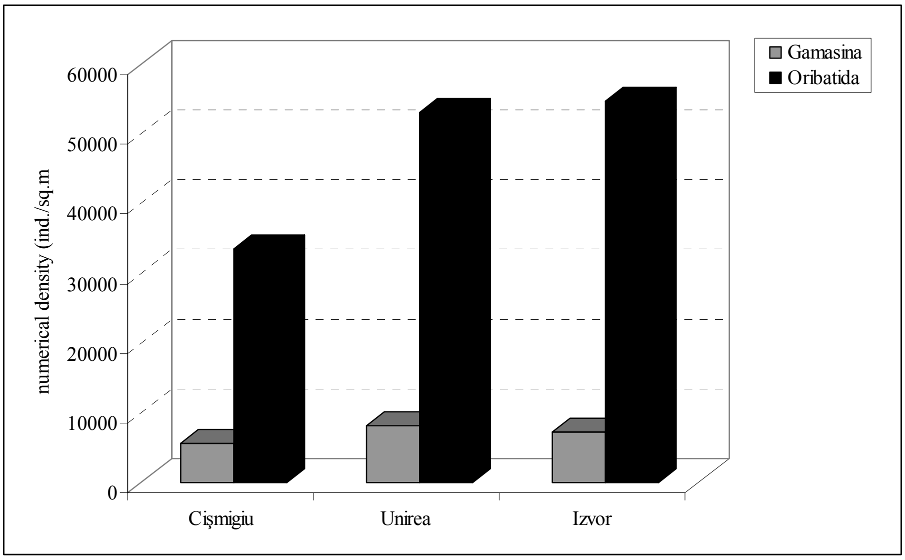

Fig. 2 – Numerical density (ind./sq.m.) of Gamasid and Oribatid mite species in soil of central parks of city Bucharest/Romania, from 2006-2007.

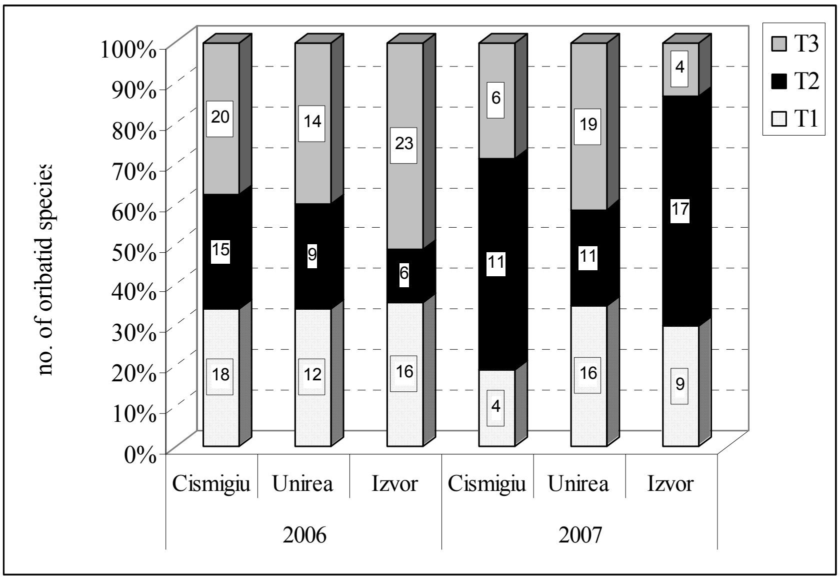

Fig. 3 – Fluctuations of the number of Oribatid mite species in soil transects of central parks, in city Bucharest/Romania, from 2006 and 2007.

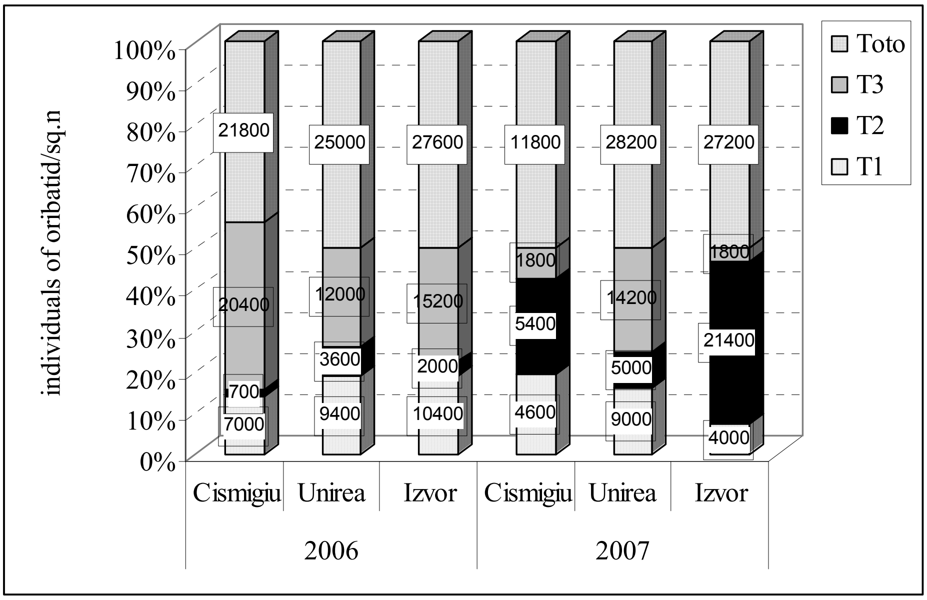

Fig. 4 – Numerical density (ind./sq.m.) of Oribatid mite species in soil transects of central parks, in city Bucharest/Romania, from 2006 and 2007.

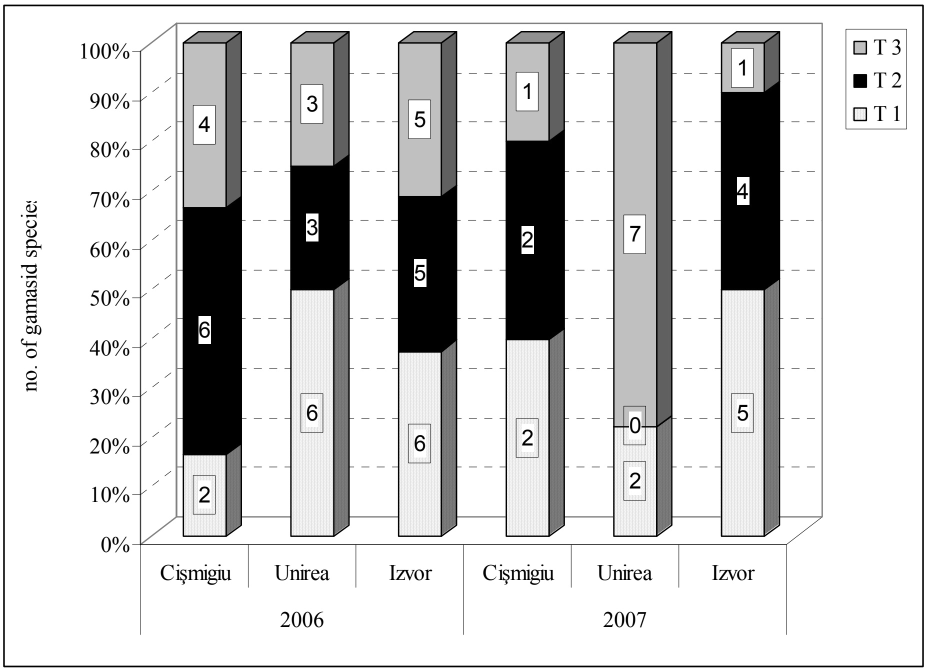

Fig. 5 – Fluctuations of the number of Gamasina mite species in soil of transect in central parks, in city Bucharest/Romania, from 2006 and 2007.

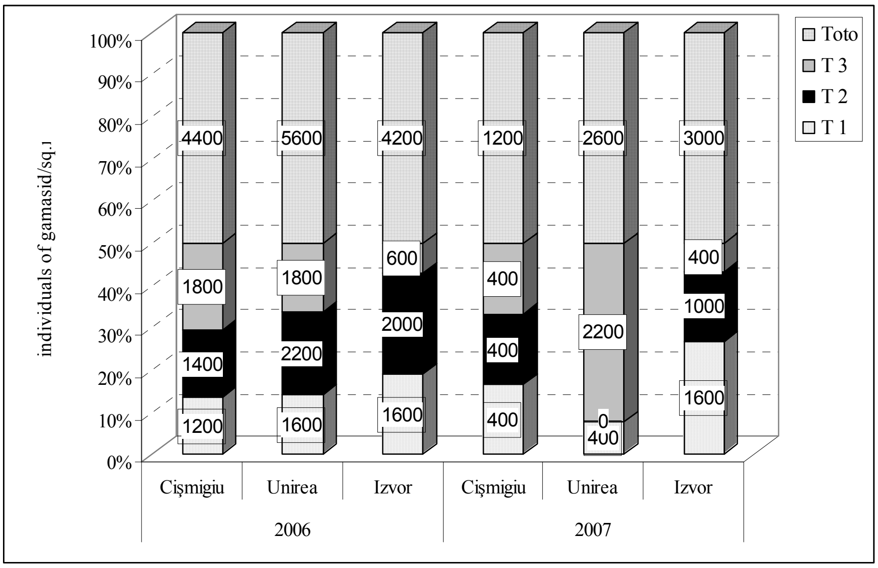

Fig. 6 – Numerical density (ind./sq.m.) of Gamasina mite species in soil transects of central parks, in city Bucharest/Romania, from 2006 and 2007.

Analysing the specific structure, the predator mites recorded the highest values at T_1 in Unirea and Izvor and at T_3 in Cişmigiu in the first year. The highest values of numerical density were recorded in T_2 at Unirea and Izvor and in T_3 at Cişmigiu. These values were obtained in 2006, in mentioned transects at *Pseudolaelaps doderoi* (1800 ind./sq.m.) in Unirea, at *Asca bicornis* (1000 ind./sq.m.) in Izvor and at *Rhodacarellus perspicuus* (1000 ind./sq.m.) in Cişmigiu. In second year, the species richness as well as numerical density had the highest values in T_1 at Izvor, in T_2 at Cişmigiu and in T_3 at Unirea. Dominant species in Izvor were: *Amblyseius meridionalis*, *Hypospis aculeifer* and *Hypoaspis praesternalis* (each recording 400 ind./sq.m.); in Cişmigiu there were *Rhodacarellus perspicuus* and *Hypospis aculeifer* (each recording 200 ind./sq.m.) and in Unirea *Asca bicornis* (800 ind./sq.m.) and *Hypoaspis praesternalis* (600 ind./sq.m.) (Figs. 5, 6).

DISCUSSION

The research methods followed the taxonomical and ecological structure and the dynamics of mites groups, in correlation with their importance in the structure and dynamics of the ecosystems studied. The structural differences (as the species richness) and their classification in classes of dominance and constance, as well as the dynamical ones (as numerical abundances expressed by ind./sq.m), between the mites populations from the investigated ecosystems, were determined by the various composition of the primary producers, by the different values of the soil humidity and pH, by the different ecological preferences of the identified species.

The structural and dynamical differences of the oribatid populations identified in parks were: the highest species richness in Cişmigiu and Unirea and the highest numerical abundance in Unirea and Izvor. These were due to the specificity of the vegetation structure, especially in Cişmigiu where the higher diversity of trees, bushes and plants is possible to determine a soil more rich in organic matter, with a higher humidity, providing a favorable habitat for oribatids, in comparison with Izvor where the sandy soil and the dryness could be perturbative factors for the mites dynamics. These variations affected the populations dynamics even on the transects level, by increasing or decreasing the species richness and the numerical densities.

The higher abundances of species in the transects from the middle of parks were due to the decreased influence of the perturbative factors (as the anthropical interventions or the pollution) and to the higher diversity of the plants which assured a proper habitat for the mites. The proximity of the other two transects to the main roads or boulevards determined a decrease of investigated parameters, possibly due to the air and soil pollution.

Species eudominant and dominant, characterized as euconstant and constant, too, were ubiquitous. The identification of species *Zygoribatulla terricola* and *Trichoribates trimaculatus* as eudominant-euconstant only in Cişmigiu and Unirea can be explained by the diversity and the provenience of the primary producers from these parks. These species are ubiquitous, having preferences strictly on the organical level of woody origin. Besides this species *Punctoribates punctum, Tectocepheus velatus, Minunthozetes semirufus* are dominant and ubiquitous too. The identification of *Eulohmania cylindrica* as subdominant-constant species in Izvor park shows the xerophilous nature of this urban area and the presence of sandy soil, the preferred habitat for this species as is mentioned even in the other researches (Paucă-Comănescu *et al*., 1996). Taking account of the species richness and of the numerical densities, a similarity between Unirea and Izvor parks was observed. The fact that almost half of the identified species are common to these ecosystems was due to the ubiquitous character of these microarthropods as well as to the soil characteristics and vegetation in Câmpia Română, where the investigated ecosystems are situated.

The particularities of the species classifications from parks were due to the environmental characteristics of each urban area. In Cişmigiu park, eudominant-euconstant identified species were found on the soil surface, being very mobile species, of small dimensions, which can adapt easily to the unfavorable environmental conditions (as low soil humidity, sandy soil, high temperature, missing of organic matter, air and soil pollution) (species from *Rhodacarellus* genera). The dominant species *Hypoaspis aculeifer* and *Veigaia nemorensis* are ubiquitous, predators, common for the terrestrial ecosystems. They prefer different habitats from the deciduous and coniferous forests, to agroecosystems, inland and coastal meadows, washed ashore material, dunes. In Unirea park *Asca bicornis* signaled is predator, being identified in similar xerophytic habitats such as those from this urban area (Skorupski & Luxton, 1998; Koehler, 1999; Salmane, 1999, 2000, 2001, 2003; Gwiazdowicz, 2007). It was also reported in industrially polluted areas. Other dominant species *Hypoaspis aculeifer* and *Hypospis praesternalis* were identified in this park, are polyphagous, their food source being the other soil invertebrates fact demonstrated by Ruf (1995 a, b; 1998). Having a wide trophical spectrum, these could adapt easily to the environmental conditions in the urban areas. These species were recorded in xerophytic habitats such as: coastal and inland meadows, dunes, spruce stands, pine forests, dung, bogs, by Skorupski & Luxton (1998) and Salmane (2001). In Izvor park, the poor vegetation and the dry soil can determine the appearance of some insects, their larva representing the food source for these predators, generally, and for the *Phytoseidae* species, especially: *Amblyseius meridionalis* and *Amblyseius obtusus*.

CONCLUSIONS

In urban areas, the dryness, the missing or the poor litter layer were unfavorable conditions for the development of these groups especially for gamasid populations. That is why there were recorded lower values of the gamasid species richness and of the numerical densities (almost half the values in forests) in all parks and in Cişmigiu park for oribatid species.

The presence of a higher number of accessory and accidental species reflects the modifications on the trophic source of mites, due to the biotical and abiotical factors.

Besides these above mentioned species, classified in dominance and constance classes, the higher number of accessories and accidental species from all three parks were due to the anthropical interventions. These species could be brought once of the plantation of the ornamental plants or with modifications of soil structure.

The lower number of common species between the three parks showed the different influence of the diversity of primary producers (which determined a specificity of the mites habitats), of the soil humidity and pH on the structure of oribatid and gamasid populations, as well as the variabilty of the soil structure.

As results from the saprophagous-detritophagous oribatid mites researches of the three central parks in Bucharest there appeared significant structural differences (species richness) and not so important dynamical differences regarding the recorded densities, the Cişmigiu park being a particular case from these points of view.

Regarding the predator mites population, the differences were obviuos on the structural and dynamical levels, due to the to the lower diversity of the primary producers, lower soil humidity and to the sandy soil poor in organic matter, which affected indirecly the trophical spectrum of the gamasid mites.

Environmental conditions (vegetation, humidity, pH of soil, characteristics of each transect) determined in parks similar species richness and numerical density for oribatid mites populations (except for the Cişmigiu park) and lower values of these two parameters for gamasid populations.

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ECOLOGICAL RESEARCH ON THE SOIL MITES POPULATIONS (ACARI: MESOSTIGMATA-GAMASINA, ORIBATIDA) FROM FOREST ECOSYSTEMS NEAR BUCHAREST CITY

MINODORA MANU, VIORICA HONCIUC

The researches were made in two forestry ecosystems Baloteşti and Băneasa situated near Bucharest, in 2007. The ecological study on the edaphic mites (Ord. Mesostigmata-Gamasina; Ord Oribatida) was made by the transects method (T): the long side (T_1), situated parallel with forestry road; the short side (T_2), situated parallel with forestry path and the middle side (T_3), situated in the center of the forest. In forestry ecosystems, from the total number of 80 identified mites species, 55 were saprophagous-decomposers (ord. Oribatida) and 25 predators (ord. Mesostigmata-Gamasina). Common species for studied ecosystems were 7 saprophagous-decomposers species: *Berniniella bicarinata, Ramusella insculpta, Oribatella berlesei, Phthiracarus globosus*, *Belba pseudocorynopus*, *Carabodes femoralis*, *Ctenobellba pectinigera* and 13 gamasids: *Lysigamasus truncus, Leptogamasus* sp., *Pergamasus* sp., *Veigaia nemorensis*, *Asca aphidoides, Asca bicornis, Rhodacarellus silesiacus, Pseudolaelaps doderoi, Olopachys suecicus, Hypospis miles, Zercon hungaricus, Prozercon traegardhi, Prozercon fimbriatus.* Differences were recorded between the saprophagous-decomposers and predators mites populations from the ecosystems on the taxonomical spectrum, on the structural and dynamics of parameters. The statistical analysis (x; s^2; s) of the ecological structural parameters (x/m^2; CV; A_r %; C%) of the mites populations showed an important representation of the oribatids as well as of mesostigmatids. The saprophagous-decomposers recorded were by 67,800 individuals/sq.m., in Baloteşti forest and 63,200 individuals/sq.m. in Băneasa forest. The predators recorded were: 18,200 individuals/sq.m., in Băneasa forest and 10,400 individuals/sq.m., in Baloteşti forest.

Key words: forest ecosystem, Mesostigmata, Oribatida, transect, structure, dynamics.

INTRODUCTION

The ecological studies regarding the mites fauna from temperate forestry ecosystems were made in Romania (Călugăr & Vasiliu, 1980; Georgescu 1981; Solomon, 1985; Honciuc, 1992, 1993; Vasiliu *et al*., 1993; Honciuc & Stănescu 2000, 2003; Stănescu & Honciuc 2005, 2006), as well as in the world, in all types of forests, by different researches (Balogh, 1972; Balogh & Mahunka, 1983; Schatz, 1983; Marshall *et al*., 1987; Schafer & Schauermann, 1990; Niedbala, 1992; Ruf, 1998; Ruf *et al*., 2003; Skorupski & Luxton, 1998; Koehler, 1999; Salmane, 2000; Honciuc & Lundqvist, 2004; Masan & Fenda, 2004; Gwiazdowicz & Maciej, 2004; Gwiazdowicz, 2007; Masan, 2007).

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The systemic approach (Botnariuc, 1999) in ecological researches in these types of ecosystems shows the implication of each biotic or abiotic factor into their structure and dynamics. Regarding the forests, the most complex ecosystems, the ratio between these components determines the homeostasis. From the multitude of biological systems of a forest ecosystem, the soil mites fauna (Arachnida: Acari: Mesostigmata-Gamasina and Oribatida) could be considered one of the main links in sustaining the ecological equilibrium. Each group of mites acts in different ways, on the soil level. Oribatids are known by their number of individuals and by the role of their trophical categories (macrophytophagous, microphytophagous and panphytophagous) as saprophagous and second consumers, decomposing the organic matter, involving in soil structure and in turnover process. The gamasids are the second and the third consumers, with an important role in transformation and recycling of the secondary organic matter (other soil invertebrates) (Koehler, 1999).

MATERIAL AND METHODS

The studies were made in two forestry ecosystems Baloteşti (N = 44°42' 45.8''; E = 26°08'49.9'') and Băneasa (N = 44°29'31.6''; E = 26°04'46.6'') near Bucharest, in 2007. The both forests were characterized by native trees, xero-mesophillous to mesophillous, meso-eutrophic to eutrophic, heliosciaphillous, frequent from sylvosteppe to beech forests and from oak to durmast forests zones *(Carpinus betulus* L., *Quercus cerris* L., *Quercus robur* L., *Robinia pseudoacacia* L., *Tilia cordata* Miller, *Ulmus minor* Miller) and by trees cultivated for forestry and ornamental purposes, thermo-subthermophillous, frequent from sylvosteppe to durmast forests zones, in forests and edge of the forests, shrubs (*Acer tataricum* L., *Ulmus minor* Miller). At the altitude 100-200 m a.s.l. of the sylvosteppe and forestry areas, the mean temperature was high (10-9,5 °C) with mean precipitations quite low (700-800 mm even 450-750 mm) typical for these forests. The herbaceous layer was dominated by xeromesophillous to mesophillous perennials, rarely biennials and annuals, frequent from steppic to beech forests and boreal zones, shadowy areas, forests, edges of the forests, shrub-lands, ruderal places and sometimes segetal weeds: *Alliaria petiolata* (Bieb.) Cavara et Grande, *Bromus arvensis* L., *Chenopodium album* L., *Dactylis glomerata* L., *Daucus carota* L., *Lamium amplexicaule* L., *Plantago media* L., *Poa angustifolia* L., *Prunella vulgaris* L., *Rumex crispus* L., *Stellaria media* (L.) Vill., *Taraxacum officinale* Weber ex. Wiggers, *Trifolium repens* L., *Urtica dioica* L. There were also mesohygro-hygrophillous perennials, frequent from steppic to beech forests zones, meadows (*Agrostis stolonifera* L.). The forests were represented by small patches of diverse phytoassociations, the distribution of the herbaceous and shrubs layers was discontinuous because of anthropic impact, increased especially in Băneasa forests.

For the soil mites (ord. Mesostigmata-Gamasina and ord. Oribatida) sampling, in each ecosystem was established a certain surface. From each surface were collected 15 samples with MacFadyen corer, on 10 cm deep. Each sample was shared in two layers: litter-fermentation and soil. The samples were taken from three transects of each area (T_1 – near to the main road, T_2 – the lateral side of the area and T_3 – in the middle of the area).

The extraction was performed with a modified Berlese-Tullgren extractor, in ethyl alcohol and the mites samples were clarified in lactic acid. After taxonomical identification, the numerical abundance was the base for the quantification of other statistical parameters, such as: average (x), variance (s^2), standard error of the average (s), standard deviation of the average (s'), variation coefficient (CV %); numerical density (x/sq.m.); relative abundance (Ar%); constance (C%). The mites fauna was identified on the species level.

To evidence the structural and dynamical differences between mites population from the studied areas, the main abiotical factors were measured: humidity and pH of soil (Table 1).

Table 1

Averages of humidity and pH of soil
in investigated areas

Factors	Forests	
	Băneasa	Baloteşti
Humidity	22	15.27
pH	5.87	5.7

RESULTS AND DISCUSSION

In the forest ecosystems studied oribatids were represented by 55 species (43 in Băneasa and 36 in Baloteşti), and gamasids by 25 species (21 in Băneasa and 16 in Baloteşti) (Tables 2-3). From the structural point of view, the mites fauna was characterized by the presence of common oribatids species for both forests: *Berniniella bicarinata, Ramusella insculpta*, *Oribatella berlesei, Phthiracarus globosus, Belba pseudocorynopus, Carabodes femoralis, Ctenobellba pectinigera* and common gamasids: *Lysigamasus truncus, Leptogamasus* sp., *Pergamasus* sp., *Veigaia nemorensis, Asca aphidoides, Asca bicornis, Rhodacarellus silesiacus*, *Pseudolaelaps doderoi, Olopachys suecicus*, *Hypospis miles, Zercon hungaricus*, *Prozercon traegardhi, Prozercon fimbriatus*.

Analyzing the relative abundance (Ar%) and constance (C%) of the identified species from the studied ecosystems, the mite populations could be divided in dominance and constance classes. At Baloteşti, the identified oribatid species were classified in certain classes as follows: *Medioppia obsoleta* as eudominant-euconstant and *Tectocepheus velatus* as euconstant-accessory. In the dominant species category there were identified: *Oribatulla tibialis* as dominant-euconstant and *Tropacarus pulcherimus* as dominant-constant. In subdominant, but constant category 7 species were included, from which *Ramusela insculpta, Metabelba pulverulenta, Punctoribates punctum, Ceratoppia bipilis, Tectocepheus sarekensis* were defined by more relevant values of the constancy index.

At Băneasa, the specific spectrum presented the species *Ceratoppia bippilis* as eudominant-constant, *Hypochthonilella pallidula*, *Damaeollus ornatissimus*, *Metabelba pulverulenta, Oribatulla pannonicus* as dominant euconstant. From subdominant-constant category 9 species were identified, from which: *Tectocepheus veletus, Berniniella bicarinata, Oribatella berlesei, Tropacarus pulcherimus* were the most constant. The biggest category belongs to the recedent-subconstant and accidental species, in both ecosystems (Table 2).

Table 2

The relative abundance (Ar%) and constance (C%) of the Oribatida species in forest ecosystems near Bucharest

Species	Baloteşti forest		Băneasa forest	
	A %	C%	A%	C%
Medioppia obsoleta (Paoli G., 1908)	16.38	100	0.95	20
Ceratoppia bippilis (Hermann J. F., 1804)	2.26	60	17.41	100
Tectocepheus velatus (Michael A. D., 1880)	11.86	20	3.80	80
Oribatulla tibialis Nicolet H., 1855	9.60	100	1.27	40
Hypochthonilella pallidula (C. L. Koch sensu Willmann C., 1931)	1.13	40	7.28	100
Damaeollus ornatissimus (Berlese), 1904	1.58	40	7.59	100
Metabellba pulverulenta (C. L. Koch, 1840)	2.82	60	6.33	80
Atropacarus pulcherimus (Berlese, 1887)	5.65	80	2.22	80
Oribatulla pannonicus Willmann C., 1949	0.56	40	5.38	80
Scheloribates laevigatus (C. L. Koch, 1836)	4.80	100	3.16	40
Berniniella bicarinata Paoli, 1908	3.11	40	3.16	80
Ramusela insculpta (Paoli G., 1908)	3.11	80	1.90	40
Oribatella berlesei (Michael A.D., 1898)	2.54	40	2.85	60
Achypteria coleoptrata (Linné, 1758)			2.85	40
Eporibatulla rauschenenis Sellnick M., 1928			2.85	40
Punctoribates punctum (C. L. Koch, 1839)	2.54	60		
Tectocepheus sarekensis Trägårdh I., 1910	2.26	60	0.95	40
Oribotritia serrata Feider et Suciu, 1958	0.85	40	2.22	40
Tropacarus carinatus (C. L. Koch, 1841)				
Ceratoppia sexpilosa Willmann, 1938			1.27	60

Nothrus biciliatus C. L. Koch, 1841	0.28	20	1.27	60
Protoribates monodactylus (Haller, 1804)			1.27	20
Zetorchestes mychronicus (Berlese, 1883)	1.13	60		
Suctobelbela baloghi (Forsslund K.-H., 1958)	1.41	20	1.90	60
Globozetes tricuspidatus (Willmann, 1953)			0.95	60
Phtriracarus sp.			0.95	60
Steganacarus magnus (Nicolet H., 1855)			0.95	40
Ceratozetes fusifer Mihelčič F., 1956	0.85	60		
Ophidiotrichus vindobonensis Piffl E., 1960	0.85	40	0.32	20
Oribatella tenuis Csiszar, 1962			0.63	20
Suctobelba aliena Moritz, 1970	0.56	40		
Ceratozetes minutissimus Willmann, 1951	0.56	40		
Chamobates cuspidatus (Michael A. D., 1884)	0.56	40		
Oribotritia sp	0.56	40		
Phthiracarus globosus (C. L. Koch, 1841)	0.56	20	0.32	20
Belba pseudocorynopus Märkel, 1960	0.28	20	0.32	20
Brachychthonius berlesei Willmann, 1928			0.32	20
Carabodes femoralis (Nicolet H., 1855)	0.28	20	0.32	20
Ctenobelba pectinigera (Berlese), 1908	0.28	20	0.32	20
Damaeollus asperatums Berlese, 1904			0.32	20
Minunthozetes semirufus C. L. Koch, 1841			0.32	20
Nanhermannia elegantula Berlese, 1913			0.32	20
Nothrus parvus Sitnikova, 1975			0.32	20
Phtiracarus piger (Scopoli, 1973)			0.32	20
Protoribates pannonicus Willmann, 1931			0.32	20
Suctobelbella acutidens (Forsslund K.-H., 1941)			0.32	20
Atropacarus pulcherimus (Berlese, 1887)			0.32	20
Achypteria sp.			0.32	20
Melanozetes sp.			0.32	20
Bellba sp.			0.32	20
Epilohmania cylindrica (Berlese), 1904	0.28	20		
Eremaeus oblongus Koch, 1836	0.28	20		
Liacarus vombi Dalenius P., 1950	0.28	20		
Peloptulus phaenotus C. L. Koch, 1844	0.28	20		
Protoribates lophotrichus (Berlese, 1904)	0.28	20		
Immatures	13.84	100	11.71	100

Regarding the Mesostigmata-Gamasina order, at Baloteşti, the gamasids were classified as eudominant-euconstant species: *Prozercon fimbriatus* and *Zercon hungaricus*; eudominant-constant species: *Asca aphidoides* and *Pseudolaelaps* *doderoi*; dominant-accessories species: *Prozercon traegardhi*; subdominant-accessory species: *Veigaia exigua* and *Olopachys suecicus*.

At Băneasa, the eudominant–euconstant species were: *Prozercon fimbriatus* and *Veigaia nemorensis*, followed by the dominant-euconstant species *Pergamasus laetus* and by *Pseudolaelaps doderoi,* as dominant-constant. Species *Lysigamasus cornutus* and *Prozercon traegardhi* are subdominant-constant (Table 3).

Table 3

The relative abundance (Ar%) and constance (C%) of the Gamasina species in forest ecosystems near Bucharest

Species	Baloteşti forest		Băneasa forest	
	A%	C %	A%	C %
Prozercon fimbriatus C. L. Koch, 1839	26.53	80	33.70	100
Zercon hungaricus Sellnick, 1958	18.37	80	2.17	40
Pergamasus laetus Ilinca Juvara Bals, 1970			4.35	80
Asca aphidoides Linne, 1758	8.16	60	1.09	20
Pseudolaelaps doderoi Berlese, 1916	8.16	60	5.43	60
Veigaia nemorensis C. L. Koch,1938	4.08	40	16.30	80
Lysigamasus cornutus Schweizer 1961			4.35	60
Veigaia exigua Berlese, 1917	4.08	40		
Olopachys suecicus Sellnick, 1950	4.08	40	2.17	40
Prozercon traegardhi Halbert, 1923	6.12	40	4.35	60
Lysigamasus truncus Schweizer 1961	2.04	20	2.17	40
Leptogamasus sp.	2.04	20	2.17	40
Pergamasus quisquiliarum G. and R. Canestrini, 1882			2.17	40
Pachyseius humeralis Berlese, 1910			2.17	40
Pachylaelaps pectinifer C. and R. Canestrini, 1882			2.17	40
Prozercon sellnicki Halaskova, 1963			2.17	40
Pergamasus sp.	2.04	20	1.09	20
Asca bicornis Caneastrini and Fanzago, 1887	4.08	20	1.09	20
Rhodacarellus silesiacus Willmann, 1936	2.04	20	1.09	20
Protogamasellus singularis Karg, 1962	2.04	20		
Hypospis miles Berlese, 1892	4.08	20	2.17	20
Zercon fageticola Halaskova, 1970	2.04	20		
Lysigamasus sp.			1.09	20
Holoparasitus calcaratus C. L. Koch, 1839			1.09	20
Immatures			5.43	40

In dynamics, the 55 oribatid species population recorded similar density values with very small differences. From this aspects we could observe that in

Baloteşti forest was recorded 67,800 ind./sq.m., and in Băneasa forest 63,200 ind./sq.m. Recording to Gamasina, the values of population density were twice more in Băneasa (18,200 ind./sq.m.), in comparison with Baloteşti (10,400 ind./sq.m.) (Fig. 1).

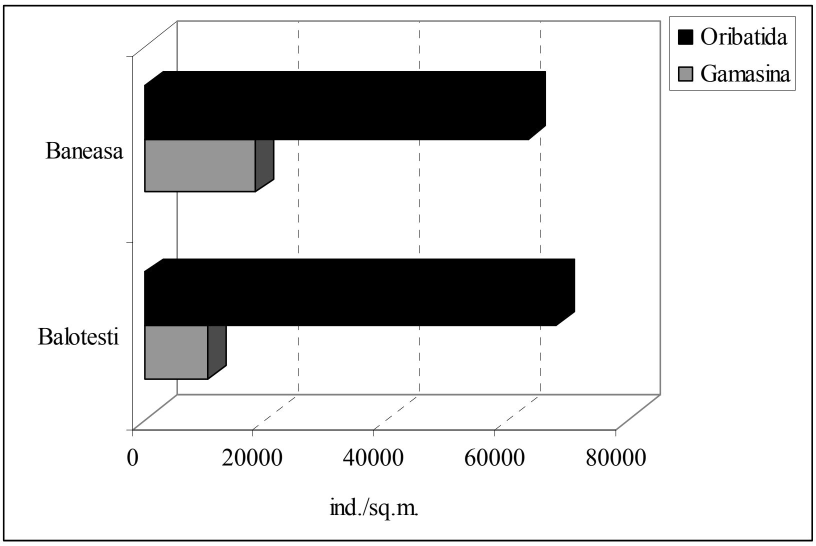


Fig. 1 – Comparison between Gamasina and Oribatida number of individuals in soil transects of two forest ecosystems near Bucharest.

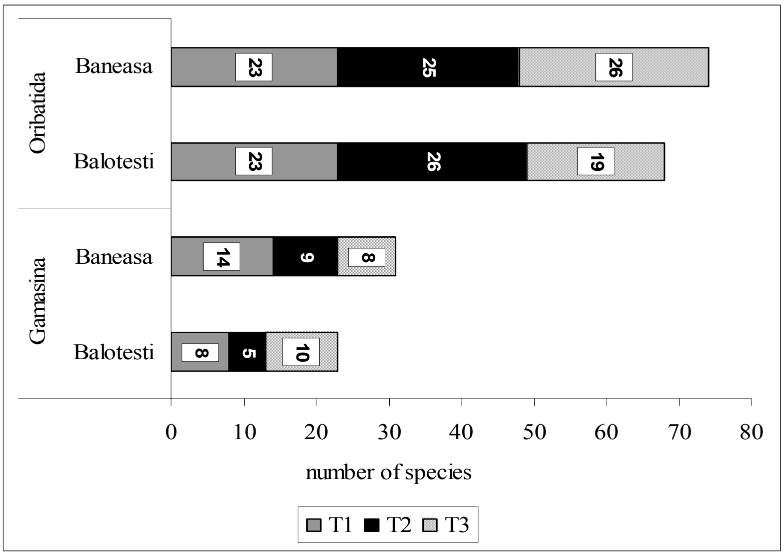
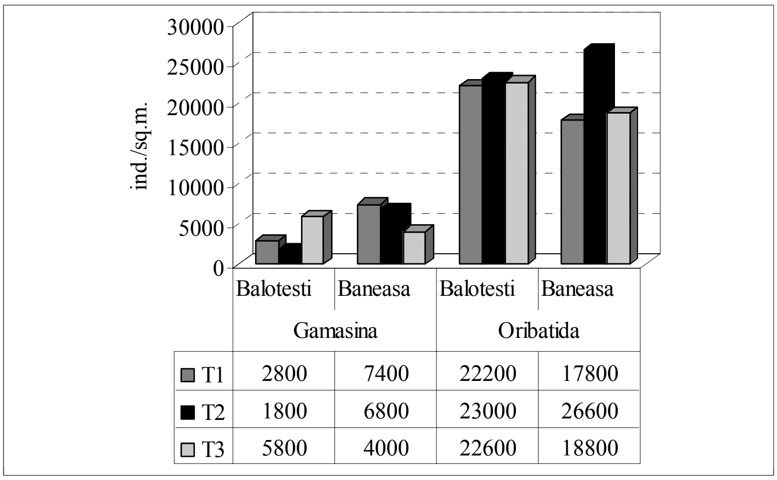


Fig. 2 – Comparison between Gamasina and Oribatida species richness in three soil transects of two forest ecosystems near Bucharest.



	Gamasina Balotesti	Gamasina Baneasa	Oribatida Balotesti	Oribatida Baneasa
▨ T1	2800	7400	22200	17800
■ T2	1800	6800	23000	26600
□ T3	5800	4000	22600	18800

Fig. 3 – Numerical density (ind./sq.m.) of Gamasina and Oribatida in three soil transects of two forest ecosystems near Bucharest.

On the transects level, analyzing the specific and structural differences of the Oribatid populations, the high number of species were observed in T_1 and T_2 in Baloteşti forest and in T_2 and T_3 in Băneasa forest (Fig. 2). The eudominant-dominant-constant species from these transects at Baloteşti forest were: *Medioppia obsoleta* (4800 ind./sq.m.; 1200 ind./sq.m.) and *Tectocepheus velatus* (2400 ind./sq.m.; 5600 ind./sq.m.) in both transects. In Băneasa forest these species were: *Metabelba pulverulenta* (2400 ind/sq.m.) in T_2 and *Damaeollus ornatissimus* (2800 ind./sq.m) in T_3. Regarding the dynamics of density values, the results were different, the highest density values were in T_2 for both forests realized by the species: *Tectocepheus velatus, Metabelba pulverulenta* with the above mentioned densities, and by the high number of immature individuals (Fig. 3). The most increased number of gamasids species as well as the numerical density were recorded in T_3 at Baloteşti forest and in T_1 at Băneasa forest. The dominant species from these transects, at Băneasa forest, was *Prozercon fimbriatus* (1200 ind./sq.m.) and in Baloteşti forest *Veigaia nemorensis* (1000 ind./sq.m.) and *Prozercon fimbriatus* (1400 ind./sq.m.). All these species with their highest density values had a contribution to the density values in these forests (Figs. 2, 3).

Taking into account that in the environmental conditions of the forest ecosystems, Oribatida order had the most increased number of species and individuals, because of their role as decomposers, in comparison with Mesostigmata-Gamasina order, which are in general predators. As we can see in the taxonomical, structural and dynamical researches, the oribatid fauna was dominant in all ecosystems. In forest ecosystems the oribatids were more representative because of their ecological role, are presented in other researches

(Călugăr & Vasiliu, 1980; Honciuc, 1992, 1993; Honciuc & Stănescu, 2000; Honciuc & Stănescu, 2003, 2004, 2005; Stănescu & Honciuc, 2005).

The equal presence of the oribatids, having very close values of the numerical densities in forests, was due to the similar environmental conditions created by the presence of the same vegetation layer (primary producers). The small differences of the populational parameters of the oribatids from the forests: a more increased number of species at Băneasa, more increased numerical density at Baloteşti, as well as the species ratio from the dominance and constance categories, were due to the specificity of each ecosystem (especially to abiotical factors such as soil, humidity and pH), fact demonstrated by the species diversity. This specificity was obvious on the transect levels, where differences appeared on the number of species and individuals, with a small increase in the middle of Băneasa forests (T_3). Taking account of the transects level, the numerical densities evolution showed that oribatids had proper environmental conditions in T_2 in both forests, in comparison with T_1. The decrease of the number of individuals of oribatids was possible due to the cars pollution, T_1 being situated in the proximity of the main road. In T_3 there were obtained the medium values of the numerical densities in both areas, but more increased at Baloteşti. This forest has not such an anthropical pressure as Băneasa, and this fact was reflected in the values obtained of the populational parameters studied.

Besides the two forests which had the same ecological characteristics, from all identified species, only 7 were common: *Berniniella bicarinata, Ramusella insculpta, Oribatella berlesei, Phthiracarus globosus, Belba pseudocorynopus, Carabodes femoralis, Ctenobellba pectinigera*. This fact reflects the habitats similarity in these forests. The signaled species in forests were characteristic for these types of ecosystems in Romania. This group of species is ubiquitous and some eudominant and dominant species were identified in other types of ecosystems such as: coniferous forests, meadows, agroecosystems. Majority of oribatid species are panphytophagous and microphytophagous as was shown by other researchers (Luxton, 1982, 1983; Honciuc & Stănescu, 2000).

Eudominant and dominant species signaled in these forests as: *Medioppia obsoleta, Ceratoppia bippilis, Tectocepheus velatus, Oribatulla tibialis, Hypochthonilella pallidula, Damaeollus ornatissimus*, are ubiquitous, being signaled in other researches in forest ecosystems from Romania (Călugăr & Vasiliu, 1980; Honciuc, 1992, 1993; Honciuc & Stănescu 2000, 2003, 2004, 2005; Stănescu & Honciuc, 2005).

Regarding the predator mites, the situation in forests was different, due to their trophical characteristics. The most increased number of species and of numerical densities recorded in Băneasa forest were due to the better environmental condition (more increased soil humidity), compared to Baloteşti forest. These conditions determined the most increased number of the identified species, as well as the numerical density in T1 at Băneasa forest and in T_3 at

Baloteşti forest. These increased values could be correlated with the presence of a developed herbaceous layer, in comparison with the T_2 where the destroying of vegetation (due to the forest cutting) involved a decrease of the numerical densities, especially in Baloteşti. In Băneasa forest, T_2 is represented by a tourist path, where the vegetation is not completely damaged, creating a habitat favorable to gamasids development.

However, the recorded numerical densities were not so high, in comparison with other natural forest ecosystems in our country, due to: sandy soil, to the decreased acidity of the soil (5.7-5.8), to the high mean temperature (10-9.5°C) and to the quite low mean precipitations (700-800 mm even 450-750 mm) (Honciuc 1992, 1993; Honciuc & Stănescu, 2000, 2003, 2004, 2005; Stănescu & Honciuc, 2005).

The eudominant-euconstant species from Baloteşti and Băneasa were signaled by us, and by other specialists, as common species for deciduous forest ecosystems from temperate area, having as preferred habitats the litter and soil layers (Honciuc & Stănescu, 2000; Stănescu & Juvara-Balş, 2005; Stănescu & Honciuc, 2006). They are omnivorous species, having a wide trophical spectrum, which allowed a better adaptation to the variations of the environmental conditions. From eudominant species signaled in these forests, *Veigaia nemorensis* is ubiquitous, surface dweller, hemiedaphic species, predator, characteristics mentioned in different papers (Skorupski & Luxton, 1998; Koehler, 1999; Honciuc & Stănescu, 2000; Salmane, 2001; Stănescu & Juvara-Balş, 2005; Stănescu & Honciuc, 2006; Gwiazdowicz, 2007).

CONCLUSIONS

Taking into acount our results we can conclude that the oribatid species, in majority saprophages-decomposer as it knows, with an important role in the decomposition process of the detritus and organic matter in soil, were the most abundant, in comparison with gamasid species, which are predators, with an important role in regulation of other invertebrates groups in the soil. Both groups were in direct correlation with the presence and the availability of the food source, which depends on the environmental factors.

In forest ecosystems the similarity of the soil structure and vegetation determined the close values of the number of species and of numerical densities for all identified mites.

Making a comparison between specific structure and numerical densities of the oribatid and gamasid populations, we observed a dependence of these populational parameters on the specific bioedaphic conditions.

The differences between studied population parameters of the two groups of mites are due to the trophical preferences (being saprophagous-decomposer and predators). The differences of the faunistical structure between the two forests show the variation of the abiotical factors and the similarities are due to the same type of primary producers.

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NATIVE AND ALIEN ARTHROPODS IN SEVERAL GREENHOUSES (BUCHAREST AREA)

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The paper is the outcome of surveys performed on various ornamentals and vegetables in several greenhouses from Bucharest area during a few years and also of an elaborate study of literature concerning alien arthropods recorded in the Romanian geenhouses. It provides data on a number of 61 arthropod species (5 Acarina and 56 Insecta), from which 31 are native, 28 are alien and 2 are cryptogenic. Among the 61 species recorded, 19 are beneficial (predators, parasitoids, pollinators) and 42 are plant pests. Arthropod pests have damaged 142 plant species belonging to 62 botanical families and 33 orders (nine ferns, two gymnosperms and 131 angiosperms, from which 40 species are monocotyledonous and 91 dicotyledonous). To species where there were serious pests, average numerical density, attack frequency and intensity and damage degree have been assessed.

Key words: alien, neobiota, non-native, autochthonous, greenhouse.

INTRODUCTION

Species (plants, animals and microorganisms) present a natural tendency to enlarge their native area, to pervasion into the new territories. This natural process is determined by exploiting of new trophic resources, by the evasion of intra- and interspecific competition, by the orientation to areas with the optimum climate conditions, by escape of predators, parasites and pathogens pressions. To areas where they break into**,** these species are considered “aliens”.

Alien taxa are species, subspecies or lower taxa introduced outside of their past or present natural range and outside of their natural dispersal potential (inclusively any part, gamete or propagule of such species that might survive and subsequently reproduce) (Pyšek *et al*., 2009). To alien species are used different terms such as: non-indigenous, non-native, exotic, foreign, new, allochthonous, adventive, allogenous, immigrant, neobiota (neozoan, neophyta, neomicrobia). Cryptogenic are taxa of unknown origin, for which it could not be established if they are native or alien.

Invasive are alien taxa that produce significant harm to biological diversity, ecosystem functioning, socio-economic values and human health, in invaded regions (natural, semi-natural or human-made habitats) (Teodorescu, 2009).

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Greenhouses are structures wherein temperature, light, moisture and soil can be rigorously managed, being favourable and protected habitats to acclimatization, reproduction and establishment of alien species. These alien species are easily introduced in the greenhouses both intentionally as different planting or ornamental species, and unintentionally as contaminants of various materials used in culture (seed, seedling, planting, soil, substrates, etc.). Furthermore, in the absence of their natural enemies, alien species populations could develop excessively. Greenhouses can also concur to the conversion of some alien species into pest or invasive species and can be reservoirs for their propagation into the neighboring habitats and ecosystems.

MATERIAL AND METHODS

Surveys have been performed on various ornamentals and vegetables in the greenhouses from the Botanical Gardens Bucharest (ornamentals), Popeşti, Berceni (vegetables) during several years (1979-1981, 1983-1984, 1989, 1995-2000, 2007-2009, 2010).

Researches have implied the examination of aerial parts of greenhouse plants before chemical treatments application, arthropods collection, and species identification. For species with very serious attack there have also been estimated average numerical density, attack frequency and intensity, and damage degree.

The average numerical density has been assessed per surface unit (cm^2), by counting of *Trialeurodes* puparia, respectively *Lecanium hesperidum*, *Saissetia haemispherica* and *Pseudococcus citri* individuals on a surface of 12 cm^2 from each leaf. Four to eight leaves on each plant have been analyzed, total controlled surface varying between 48 and 96 cm^2.

Attack frequency (F %) and intensity (I %) and damage degree (Dd %) have been estimated using formulae: $F\ \% = n \times 100/N$; $I\ \% = \sum (i \times f)/n$; $Dd\ \% = = \sum (i \times f)/N$, where: n = number of attacked plants or organs; N = total number of investigated plants or organs; i = percent of atacked plants or organs in the analyzed plants; f = number of cases having an “i” value.

Furthermore, the working paper infered an elaborate study of literature concerning alien arthropods ever recorded in the Romanian geenhouses.

RESULTS AND DISCUSSION

In the surveied greenhouses a number of 61 arthropod species has been recorded (56 insects belonging to nine orders and five mites belonging to four families), from which 31 are autochtonous, 28 are neobiota (alien) and two are cryptogenic species.

The alien species have belonged to Homoptera (Aleyrodidae, Aphididae, Diaspididae, Lecaniidae, Pseudococcidae), Thysanoptera (Thripidae), Hymenoptera

(Aphelinidae), Diptera (Agromyzidae), Lepidoptera (Noctuidae) and Acari (Phytoseiidae) orders (Fig. 1).

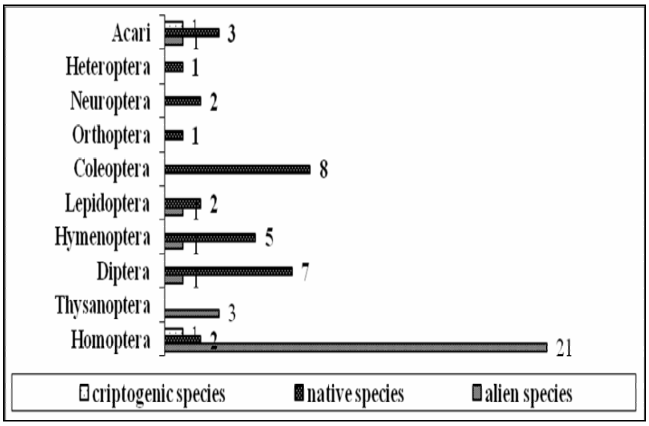

Fig. 1 – Arthropod orders with alien, native and cryptogenic species from greenhouses.

The highest species richness has been registered to Homoptera (especially Diaspididae), the other orders being represented by one or two species. Most of alien species are native to Asia and North America, the others being native to Central America, tropical, subtropical or mediterranean regions (Fig. 2).

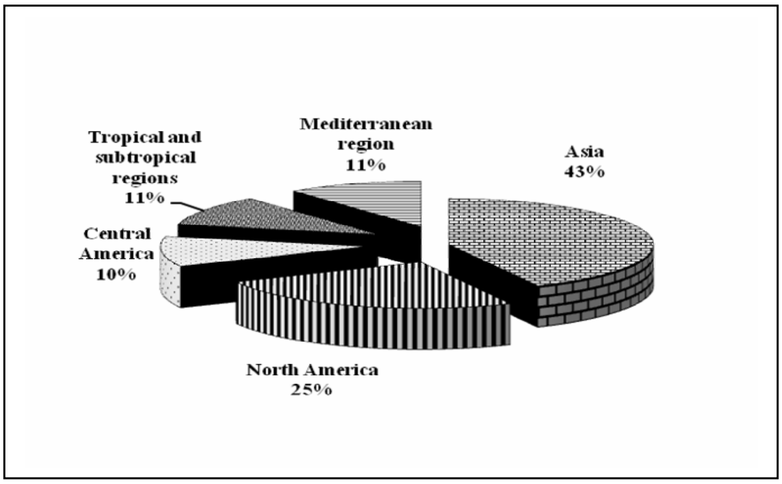

Fig. 2 – Native area of alien arthropod species from some Romanian greenhouses.

Most of neobiota from the Romanian greenhouses (26 species) have been introduced unintentionally, as contaminants of planting or ornamental materials. Two species have been introduced intentionally: *Encarsia formosa* (Hymenoptera, Aphelinidae), as parasitoid of *Trialeurodes vaporariorum*, and *Phytoseiulus perssimilis* (Acarina, Phytoseiidae), as predator of *Tetranychus*.

Among these alien species, the first detected in Romania was *Diaspis echinocacti*, in 1859, and the last detected was *Liriomyza trifolii,* in 1981 (Deheleanu & Macedon, 1981; Iacob *et al.*, 1983).

Some species were signaled in the last part of the nineteenth century: *Aphis gossypii* detected in 1877, and signaled by Horvath (1897, a), *Brevicoryne brassicae* and *Aulacaspis rosae* also by Horvath (1897 b), *Aspidiotus hederae* by Horvath (1897 b) and Arion (1912), *Diaspis echinocacti* in 1859 (Paşol, 2007).

Most of the species were signaled during the twentieth century: *Heliothrips haemorrhoidalis* in 1900 (from Knechtel, 1923), *Thrips tabaci* in 1906 (Popovici), *Trialeurodes vaporariorum* in 1929 (Anonymous and Dobreanu & Manolache, 1955), *Chrysomphalus dictyospermi*, *Aonidiella aurantii, Saissetia haemisphaerica, Lecanium hesperidum and Pseudococcus adonidum* in 1930 (Knechtel from Paşol, 2007; Teodorescu & Procheş, 1997), *Thrips dianthi* in 1938 (from Knechtel, 1951), *Macrosiphoniella sanborni* and *Neomyzus circumflexus* in 1942 (Knechtel & Manolache), *Diaspis boisduvalii* in 1949 (Teodorescu & Procheş, 1997; Teodorescu *et al*., 2006), *Pseudococcus citri* in 1960 (Teodorescu & Procheş, 1997; Teodorescu *et al*., 2006; Paşol, 2007), *Bulgarialeurodes cotesii* in 1969 (Teodorescu & Procheş, 1997; Teodorescu *et al*., 2006), *Encarsia formosa* in 1969 (Dobreanu & Manolache), *Phytoseiulus perssimilis* in 1969 (Iacob, 1972, 1975, 1978; Teodorescu & Procheş, 1997; Teodorescu *et al*., 2005; Paşol, 2007), *Liriomyza trifolii* in 1981 (Deheleanu & Macedon). For five species (*Chrysomphalus aonidum, Lepidosaphes beckii, Pulvinaria floccifera, Pseudococcus mammilariae* signaled by Teodorescu & Procheş (1997), Teodorescu *et al*. (2006) and *Heliothis armigera* (syn *Chloridea obsoleta*) (Teodorescu *et al*., 2006; Paşol, 2007) the first introduction date could not be established.

Concerning preferred habitats, 21 alien species are encountered exclusively in the greenhouses and on houseplants, four species appear both in the field and greenhouses, one species appears outside (in gardens, parks) only during the summer and two are field pests which appear accidentally in the greenhouses.

As regards trophic regime, 26 alien species are phytophagous (12 polyphagous and 14 oligophagous or monophagous) (Fig. 3), one species is predator and one is parasitoid.

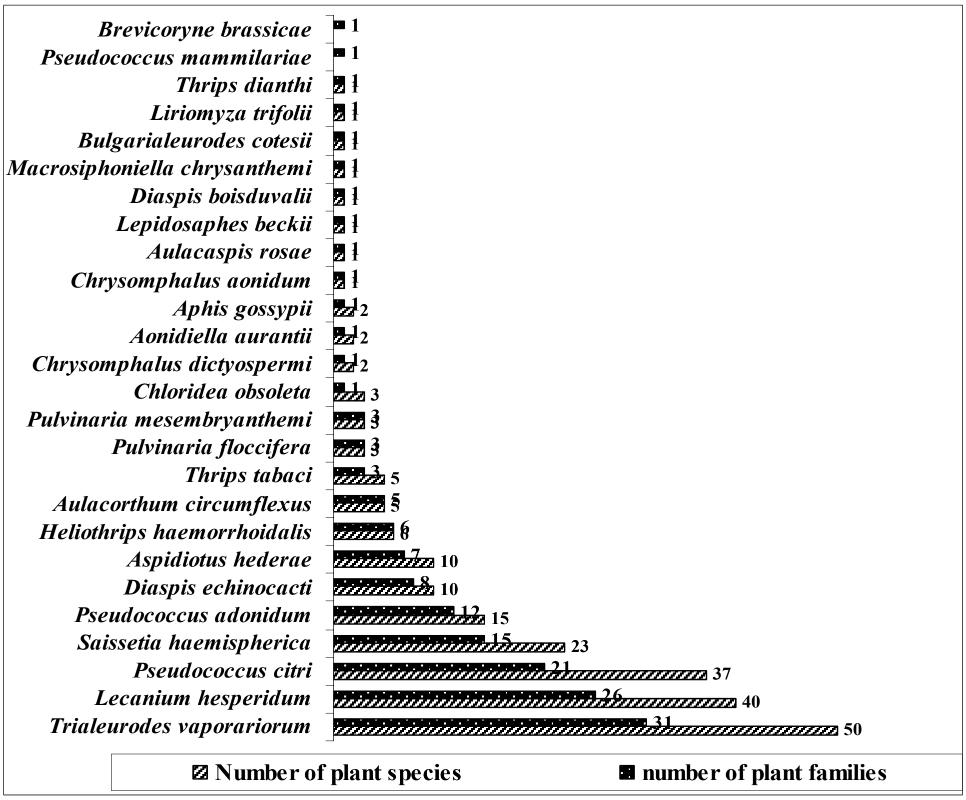

Fig. 3 – Number of plant families and species damaged by alien insect species.

The 31 autochtonous species have belonged to Coleoptera (Coccinellidae and Elateridae), Diptera (Agromyzidae, Syrphidae and Cecidomyiidae), Hymenoptera (Megachilidae, Argidae, Aphidiidae and Encyrtidae), Homoptera (Aphididae), Lepidoptera (Noctuidae and Pieridae), Neuroptera (Chrysopidae), Orthoptera (Gryllotalpidae), Heteroptera (Anthocoridae) and Acarina (Tetranychidae, Acaridae and Tarsonemidae) orders. As trophic category, 13 autochthonous species are phytophagous (seven polyphagous and five oligophagous) (Fig. 4). Other 14 species are predators and three are parasitoids. The three parasitoid species belonged to Hymenoptera order: *Aphidencyrtus aphidivorus* (Encyrtidae), *Aphidius matricariae* and *Diaeretiella rapae* (Aphidiidae). *Megachile centuncularis* (Megachilidae) is a pollinator species.

The autochthonous predator species belonged to Coleoptera Coccinellidae (*Coccinella septempunctata, Adonia variegata, Adalia bipunctata, Synharmonia conglobata, Propylaea quatuordecimpunctata* and *Chilocorus bipustulatus*), Diptera, Cecidomyidae (*Aphidoletes aphidimyza)* and Syrphidae (*Episyrphus balteatus, Syrphus ribesii, Eupeodes corollae, Syrphus vitripennis*), Neuroptera, Chrysopidae (*Chrysopa carnea, Chrysopa perla*) and Heteroptera, Anthocoridae (*Orius majusculus*).

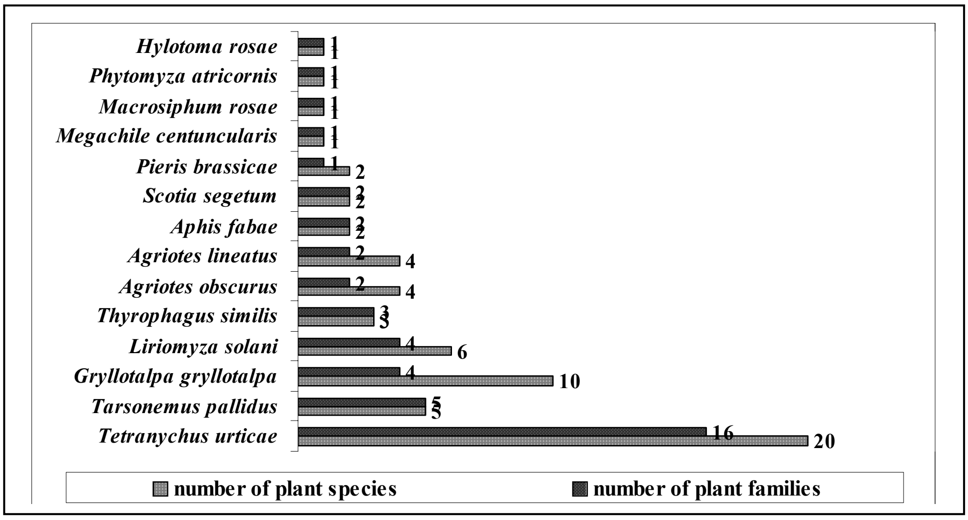

Fig. 4 – Number of plant families and species damaged by native arthropod species.

As to preferred habitats, among the 14 phytophagous autochthonous species, two appear exclusively in the greenhouses, ten are recorded both in the field and greenhouses and two are field pests encountered accidentally in the greenhouses.

The predator species are encountered in the greenhouses only during the summer. They come from outside, being attracted by abundant food.

The two cryptogenic species belonged to Tarsonemidae (Acari) and Aphididae (Homoptera) families. Both are phytophagous with a polyphagous dietary, one of them being encountered only in the greenhouses and the other appearing in the orchards too.

Attack frequency has been assessed to nine polyphagous species (seven neobiota, one autochthonous, one cryptogenic), attack intensity to five species (three neobiota, one autochthonous, one cryptogenic) and damage degree to one alien invasive species (Table 1). The highest attack frequency values have obviously been registered to *Trialeurodes vaporariorum*, especially on *Lantana camara, Lycopersicon esculentum, Gardenia thunbergia* and *Datura* species.

For *Lecanium hesperidum* attack frequency values were sometimes high on *Nerium oleander* leaves. *Pseudococcus citri* was frequent on *Nerium oleander* leaves and *Citrus* fruits, and *Polyphagotarsonemus latus* on *Capsicum annuum* leaves. To *Polyphagotarsonemus latus* on *Capsicum annuum* leaves and to *Pseudococcus citri* on *Nerium oleander* leaves, the highest attack intensity values have been registered. Damage degree has only been assessed to *Trialeurodes vaporariorum* on *Lantana camara* leaves and their values were high.

The average numerical density of *Trialeurodes* puparia has been assessed on 25 plant species, the highest value being registered on *Pachistachis* leaves. On *Lantana camara* and *Gardenia thunbergia* leaves, average numerical density of *T. vaporariorum* puparia was high and on *Nerium oleander, Musa paradisiaca, Rosa, Pelargonium zonale, Euphorbia pulcherima* leaves it was moderate.

A very low numerical density of *T. vaporariorum* puparia has been registered on the other 16 investigated plant species (under 2 puparia/cm^2) (Fig. 5). The average numerical density was low (around or under one individual/cm^2) for *Lecanium hesperidum, Pseudococcus citri* and *Saissetia haemisphaerica* (Figs. 6, 7, 8).

In the greenhouses the attack of arthropod pests has been detected on 142 plant species, belonging to 62 families, 33 orders, 4 classes and 3 phyla. Nine of these species belonged to Phylum Pteridophyta (four families, three orders), two species belonged to Phylum Gymnospermatophyta (one family, one order) and 131 species belonged to Phylum Angiospermatophyta, from which 40 to Monocotyledonae class (17 families, six orders) and 91 to Dicotyledonae class (40 families, 23 orders).

Table 1

Attack frequency and intensity and damage degree of some arthropod pests on several ornamentals and vegetables

Plant hosts	Attack frequency (F%)	Attack intensity (I%)	Damage degree (DD%)
Trialeurodes vaporariorum			
Nerium oleander	20; 90		
Hibiscus rosa sinensis	56		
Datura sp.	87.5		
Gardenia thunbergia	90		
Lantana camara	90		84; 88; 100
Lycopersicon esculentum	62; 98; 100	4.5; 7; 12.4	
Cucumis sativus	23; 26; 31	0; 1.2; 1.5	
Citrus limon	50		
Jacobinia sp.	50		
Laurus nobilis	50		
Brunfelsia calycina	8		
Lecanium hesperidum			
Aralia brauerii	50		
Kalanchoe sp.	56		
Laurus nobilis	20-25; 32		
Citrus limon	20-25; 32		
Nerium oleander	57.14; 62.5; 100; 68.9; 70; 95.5	73.21; 78.1	

Saissetia haemisphaerica			
Jacobinia carnea	38.89		
Pulvinaria floccifera			
Homalocladium sp.	50		
Pulvinaria mesembyanthemi			
Ficus sp.	30		
Homalocladium sp.	30		
Hedychium speciosum	30		
Pseudococcus citri			
Ficus religiosa	26		
Citrus sp., fruits	23-100		
Citrus limon, fruits	95-100		
Nerium oleander	63.4; 75; 100; 86.2; 90; 94	79.4; 90	
Pseudococcus adonidum			
Jasminum grandiflorum	18.75		
Tetranychus urticae			
Carica papaya	17.65		
Cucumis sativus		1.8; 2.1; 15.2	
Capsicum annuum		3.7; 6.4; 11.4	
Jacobinia sp.	5		
Hibiscus sp.	5		
Polyphagotarsonemus latus			
Capsicum annum	100	100	

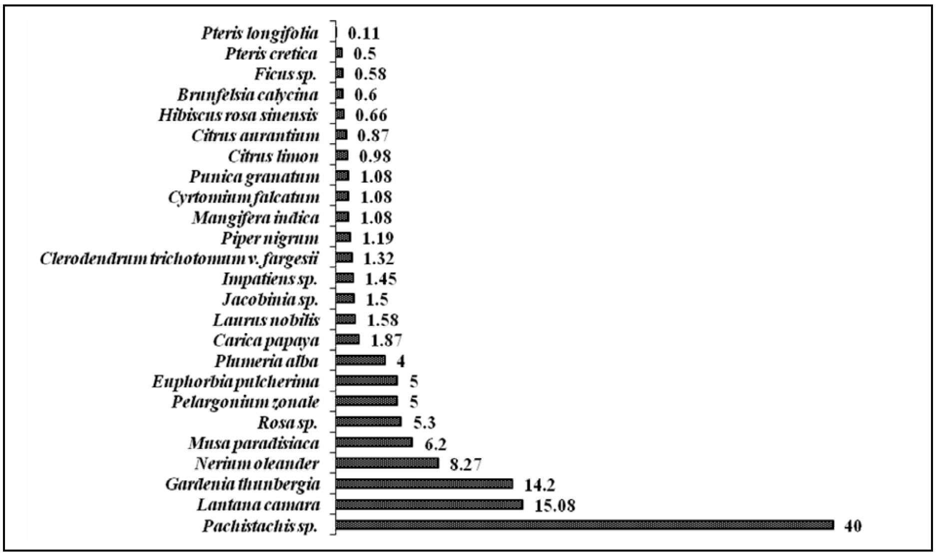

Fig. 5 – *Trialeurodes vaporariorum* average numerical density on various ornamentals.

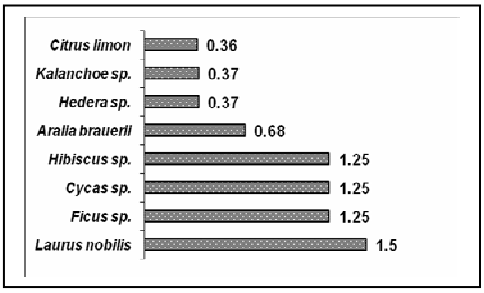

Fig. 6 – *Lecanium hesperidum* average numerical density on various ornamentals.

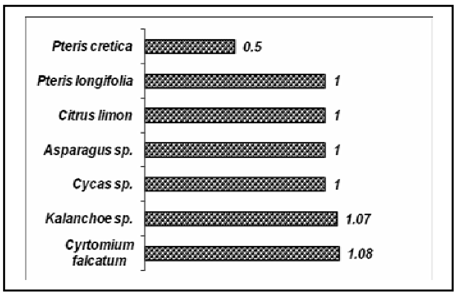

Fig. 7 – *Saissetia haemisphaerica* average numerical density on various ornamentals.

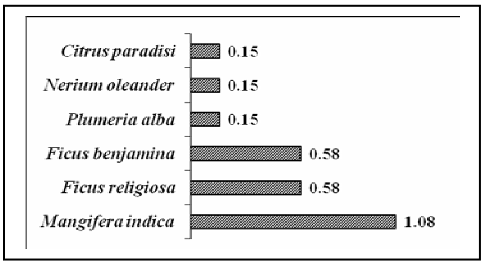

Fig. 8 – *Pseudococcus citri* average numerical density on various ornamentals.

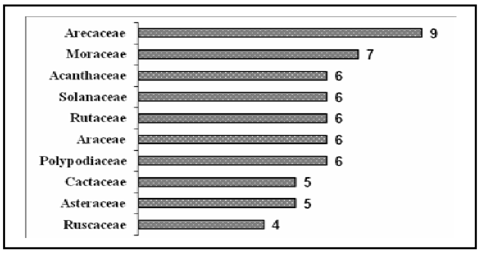

Fig. 9 – Plant families with the highest number of attacked species.

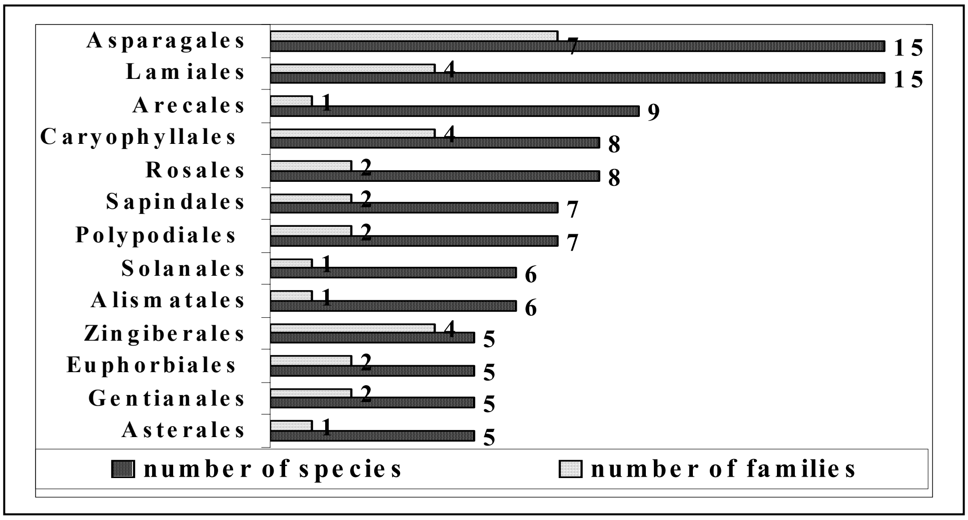

Fig. 10 – Plant orders with the highest number of attacked species and families.

The highest number of damaged plants belonged to 10 families (one Pteridophyta, three Monocotyledonae and six Dicotyledonae) and 13 orders (one Pteridophyta, four Monocotyledonae and eight Dicotyledonae) (Figs. 9, 10).

On houseplants have been detected: *Pseudococcus citri* on *Cycas* sp.; *Lecanium hesperidum* on *Nerium oleander*, *Citrus limon, Murraya* sp., *Asparagus* sp.; *Saissetia haemisphaerica* on *Asparagus* sp. and *Aspidiotus hederae* on *Nerium oleander*. Outdoor, *Trialeurodes vaporariorum* was detected during the summer on many Leguminosae, Cruciferae, Cucurbitaceae, Solanaceae, Geraniaceae Malvaceae, Theaceae and other plant species, from gardens and parks.

CONCLUSIONS

Surveys performed during several years in a few greenhouses in Bucharest area have recorded 61 arthropod species (5 Acarina and 56 Insecta), from which 28 are neobiota, 31 are autochthonous and two are cryptogenic. Among the recorded species, 20 are beneficial and 41 are plant pests. Most of the plant pests have been alien and cryptogenic species (28), only 13 of them being native species.

Trialeurodes vaporariorum, *Lecanium hesperidum*, *Pseudococcus citri*, *Saissetia haemisphaerica*, *Aspidiotus hederae, Pseudococcus adonidum*, *Diaspis echinocacti*, and *Heliothrips haemorrhoidalis*, among alien species, *Tetranychus urticae*, *Tarsonemus pallidus*, *Gryllotalpa gryllotalpa* and *Liriomyza solani* among native species, and the two cryptogenic species (*Polyphagotarsonemus latus* and *Myzus persicae*), were the most noxious species. *Megachile centuncularis* damaged *Rosa* leaves, but at the same time it is a beneficial species, as pollinator.

Among useful species two are neobiota (predator and parasitoid) and 18 are autochthonous (14 predators, three parasitoids and one pollinator).

The attack of arthropod pests has been recorded on 142 plant species, belonging to 62 families, 33 orders, four classes and three phyla. Among plant species damaged, nine are ferns, two are gymnosperms and 131 are angiosperms, from which 40 species are monocotyledonous and 91 are dicotyledonous.

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CONFIRMATION OF THE PRESENCE OF *OCHLEROTATUS ZAMMITII* (DIPTERA: CULICIDAE) IN ROMANIA

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IRINA TEODORESCU**, PAUL REITER***

Ochlerotatus (*Acartomyia*) *zammitii* (Theobald, 1903) was considered as uncertain species until now in Romania. The presence of this species in our country has been confirmed by morphological features of 12 females captured in Grindul Lupilor, a marine sandbank located in the Southern part of the Danube Delta Biosphere Reserve. It seems that this species has a limited area in Romania, along the Black Sea shore, perhaps because of its larvae preference for high water salinity. We consider that it is reasonable to add *Ochlerotatus* (*Acartomyia*) *zammitii* to the list of the Romanian mosquitoes.

Key words: Ochlerotatus zammitii, mosquito, halophylous species, Danube Delta, Romania.

INTRODUCTION

Zotta (1932) listed *Ochlerotatus* (*Acartomyia*) *mariae* (as *Aedes mariae*) among 29 species in Romania, but Giurcă (1982) considered it doubtful, because according to "Limnofauna Europaea" (Dahl & White, 1978), it was not present in the Eastern Mediterranean. Nicolescu (1995), however, believed that Zotta had actually found *Ochlerotatus zammitii* and this is compatible both with the "Limnofauna Europaea" and with the geographical distribution of sibling species of the *mariae* complex (Coluzzi *et al.*, 1974, 1976).

The *mariae* complex is classified in the *Acartomyia* Theobald (1903) subgenus of *Ochlerotatus* genus (Reinert *et al.*, 2008) and comprised three coastal Mediterranean species: *Ochlerotatus mariae* s. str. (Sergent and Sergent, 1903) – described from Algeria, *Ochlerotatus zammitii* (Theobald, 1903) – described from Malta) and *Ochlerotatus phoeniciae* (Coluzzi and Sabatini, 1968) – described from Cyprus (Coluzzi *et al.*, 1970). *O. zammitii* is considered as restricted to the eastern Mediterranean, whereas *O. mariae* s. str. is a coastal species found in the western Mediterranean only. Both species have been reported from Greece, although there are doubts about the record of *O. mariae* (Becker *et al.*, 2003) and *O. zammitii* as being recorded from Bulgaria (Minár, 1990). *Ochlerotatus phoeniciae* has been recorded from the coasts of Cyprus, southeastern Turkey, Lebanon and Israel (Coluzzi *et al.*, 1974; Becker *et al.*, 2003).

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Ochlerotatus zammitii was catalogued as "uncertain" in a list of Romanian mosquitoes (Nicolescu, 1995, 2000) and in maps of European mosquitoes (Snow & Ramsdale, 1999; Schaffner *et al.*, 2001; Nicolescu *et al.*, 2003 a, b), but it has yet to be included in keys of the Romanian mosquitoes.

MATERIAL AND METHODS

Outdoor collections were made by CDC-light traps and pigeon-baited traps, human bait, and sweep net in vegetation. Resting mosquitoes were captured by battery-powered aspirator in houses and animal shelters. All were made at 2-4 week intervals from spring to autumn of 2006-2008 at three sites in ecologically distinct areas of the Danube Delta Biosphere Reserve: (i) Grindul Lupilor, in the Razim-Sinoe lagoon area; (ii) Sălcioara, at the edge of the Dobrogea tableland on the eastern bank of Lake Razim and (iii) Mila 26, in the Delta proper.

Grindul Lupilor is a low relief (0.5-1.5 m) marine sandbank in the southern part of the Danube Delta Biosphere Reserve, between the Dobrogea tableland and the Black Sea. It is bounded by three brackish lakes, all former maritime lagoons: Sinoe to the south-west, south and south-east, Zmeica to the north-west and Goloviţa to the north and north-east. The soils are calcareous gleyed psammosols and gleyic solonchaks of high salinity (>15 dSm^{-1}) (Munteanu, 1996). They are associated with thin deposits of fine marine sands, arenaceous and halophilic plants, and small areas of *Elaeagnus angustifolia*. There are also wetland areas with hygrophilic thickets, reed and sedge beds, and depressions frequently flooded.

Sălcioara is a village surrounded by reeded ponds and channels on the banks of Lake Razim. Soil salinity is lower than in the Grindul Lupilor.

Mila 26 is an area on the left bank of Sulina Branch of the Danube where there are semi-feral horses, cattle and pigs. It includes a network of natural and artificial channels where water circulation is low.

In June and October 2008 other ten sites situated in the proper Delta were investigated: (i) an area with *Phragmites australis* and *Salix* sp. formations, on the bank of Ghermandi Channel; (ii) a young *Salix* sp. forest, between Ileana Channel and Rotundu Lake; (iii) an area with *Phragmites australis* nearby Dovnica Channel; (iv) puddles surrounded by reeds, on the edge of the Răducu Lake; (v) an isle of *Populus* sp. inside of a mixed forest, on a Caraorman marine sandbank; (vi) an area with *Tamarix ramossissima* on the bank of Crişan-Caraorman Channel; (vii) a *Populus alba* plantation on the bank of Crişan-Caraorman Channel; (viii) a riparian area with *Salix* formations and *Amorpha fruticosa*; (ix) a dry meadow area with *Eleagnus angustifolia* formations, on the Stipoc river sandbank; (x) a wet meadow area, on the Sireasa agricultural polder.

RESULTS AND DISCUSSION

In all sites there were collected 25.683 adult mosquitoes but the twelve specimens, all female, identified as *O. zammitii* were captured only in Grindul Lupilor (44°41'45.00" N, 28°56'15.06" E) in June 2006 and July 2008, alongside brackish marine lagoons (Table 1).

Table 1

Collections of *Ochlerotatus zammitii* in Grindul Lupilor

Habitat	**Date**	**Collecting method**	**No. of specimens**
Area of frequently flooded depressions with reeds and some willow formations	June 01, 2006	Human bait	1
Elaeagnus angustifolia forest on sandy soil	June 02, 2006	Human bait	2
Idem	July 06, 2008	Light trap	6
Idem	July 06, 2008	Human bait	2
Idem	July 23, 2008	Human bait	1

Water and soil salinity at the site is high compared to other investigated areas of the Reserve; as on the Mediterranean coast, the larvae of *O. zammitii* favour high water salinity (Hassaïne *et al.*, 2001).

These species can be separated from other mosquito species using morphological features. All specimens had pale basal and apical rings on the hind tarsomeres (Fig. 1 a). Pale basal bands on the abdominal terga with no other markings differentiated them from *Ochlerotatus* (*Ochlerotatus*) *caspius* (Pallas, 1771) and *O.* (*Ochlerotatus*) *dorsalis* (Meigen, 1830), which both have additional pale median stripes. The wings had pale scales scattered among the dark ones, unlike *O.* (*Ochlerotatus*) *pulcritarsis* (Rondani, 1872) in which all are dark, except for a small white patch at base of the costa. The scutum had submedian longitudinal stripes of white scales and a bare metameron (Fig. 2 a, b), distinct from *O. mariae* in which the scutum has no longitudinal stripes and the metameron has a patch of scales (Nicolescu, 2000; Samanidou-Voyadjoglou & Harbach, 2001). The pale scales at the apex of maxillary palpi described by Schaffner *et al.* (2001) were also present (Fig. 2 c).

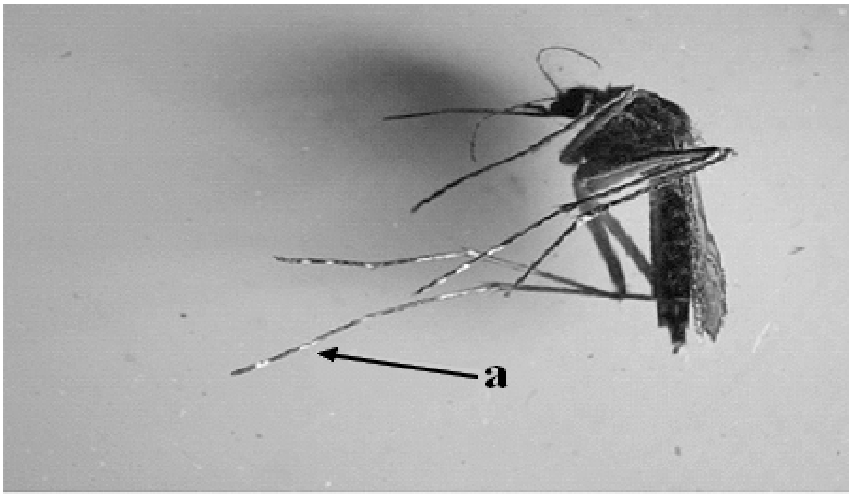

Fig. 1 – *Ochlerotatus zammitti*. Note the legs with basal and apical pale rings (a) (orig.).

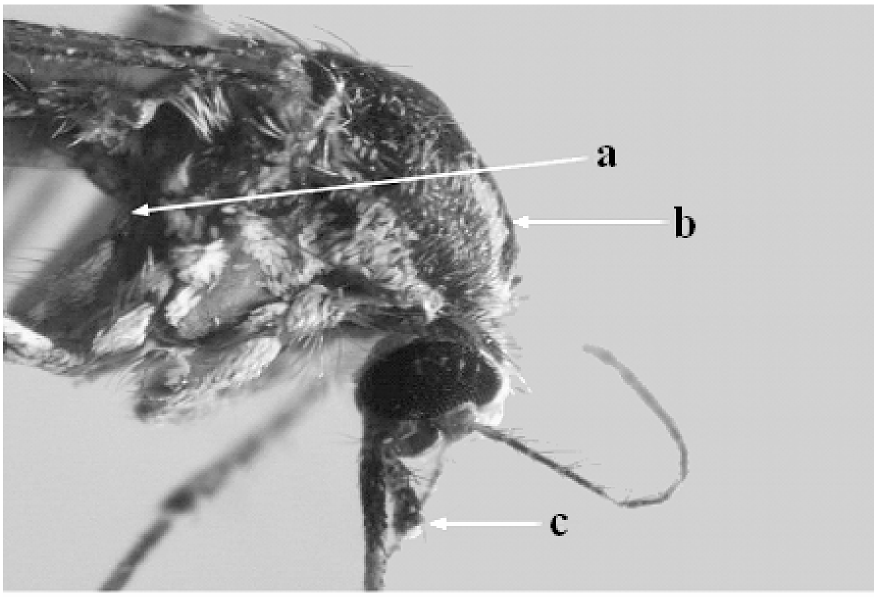

Fig. 2 – *Ochlerotatus zammitti.* Note the bare metameron area (a) the submedian longitudinal stripes (b) on the scutum and the pale scales (c) on the tip of the maxillary palpus (orig.).

CONCLUSIONS

We report the capture of 12 female of *Ochlerotatus* (*Acartomyia*) *zammitii*, during some investigations of the mosquitoes fauna from the Danube Delta

Biosphere Reserve. We confirm that *Ochlerotatus zammitii* is present in Romania but with low abundance (0.05 % and 0.1% of the total catch from Grindul Lupilor in 2006 and 2008 respectively). It seems that this species has a limited zone in Romania situated on a narrow strip along the Black Sea shore-perhaps because of its larvae preference for high water salinity. Based on our result it is reasonable to add *Ochlerotatus* (*Acartomyia*) *zammitii* to the list of the Romanian mosquitoes.

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BLOOD-MEAL PREFERENCES FOR *ANOPHELES MACULIPENNIS* (DIPTERA: CULICIDAE) COMPLEX SPECIES IN COMANA, GIURGIU COUNTY (ROMANIA)

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The present study has been performed in order to establish the feeding preferences of the species belonging to the *Anopheles maculipennis* complex in Comana, Giurgiu County. A total of 150 *Anopheles maculipennis* s.l. females have been tested by direct enzyme-linked immunosorbent assay (ELISA) for blood-meal identification. *Anopheles maculipennis* complex species are very closely related and almost indistinguishable by morphological characters. The anopheline mosquitoes have been identified to species level by Polymerase Chain Reaction (PCR) method on the ribosomal DNA sequences of the second internal transcribed spacer (ITS2) region. Three species have been distinguished in Comana: *Anopheles atroparvus*, *Anopheles messeae* and *Anopheles maculipennis* s. str. A proportion of 85.5 % out of 150 tested females were fed on bovines, 2.1% on horses, 5.5% on pigs and 6.9% on multiple hosts.

Key words: Comana, *Anopheles maculipennis* complex, feeding preferences, ELISA blood-meal identification, PCR.

INTRODUCTION

Anopheles maculipennis complex includes the historical vectors of malaria in Europe. *A. maculipennis* was recognised in 1920-1930 as the first example of a sibling species complex among mosquitoes. The mosquito complexes include morphologically similar species with ecological, behavioural, physiological and genetic differences, as well as different vector competence and geographic distributions.

Different authors contributed to the establishment of species composition of the complex taking into consideration the methods used for species identification at a moment (White, 1978; de Zulueta *et al*., 1983; Cianchi *et al*., 1987; Ribeiro *et al*., 1988; Romi *et al*., 2000; Linton *et al*., 2002, Sedaghat *et al*., 2003; Nicolescu *et al*., 2004). The new classification of *Anopheles* genus includes the European vectors in *A. maculipennis* subgroup with the species *A. atroparvus* van Thiel, *A. daciae* Linton, Nicolescu & Harbach, *A. labranchiae* Falleroni, *A. maculipennis* Meigen, *A. martinius* Shinagarev, *A. melanoon* Hackett, *A. messeae* Falleroni, *A. persiensis* Linton, Sedaghat & Harbach, *A. sacharovi* Favre (Harbach, 2004).

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In Europe, malaria was eradicated in 1975 but its recent re-emergence in some countries of the former USSR (Romi *et al.*, 2002) and countries along the Black Sea shore (Baldari *et al.*, 1998; Alten *et al.*, 2000; Kurdova *et al.*, 2001; Sabatinelli *et al.*, 2001; Kampen *et al.*, 2003) have increased the interest regarding *Anopheles maculipennis* complex.

Romania is a malaria-free country since 1962. Before the eradication campaign there were about 270,000 new clinical cases of malaria reported yearly. Historical transmission of malaria in Romania is attributed to four members of the *Anopheles maculipennis* complex: *A. maculipennis*, *A. messeae*, *A. atroparvus* and *A. sacharovi* (Martini & Zotta, 1934; Zotta, 1938; Zotta *et al.*, 1940). *Anopheles sacharovi* disappeared after malaria eradication from its spreading area on the Black Sea coast in Romania (Bîlbîe *et al.*, 1978).

The feeding preference with blood is of paramount importance in malaria epidemiological studies because the anopheline anthropophily or zoophily are elements influencing the level of malaria risk emergence. The blood-meal analysis and the molecular identification of the *Anopheles maculipennis* complex species have been performed on samples collected in Comana, Giurgiu County.

MATERIAL AND METHODS

Study area. The investigation have been performed on anophelines collected in Budeni village, located in touristic Comana area (26°00' – 26°10' E and 44°08' – 44°14' N) in the South of the Romanian Plain, at the confluence of Neajlov and Argeş rivers (Fig. 1). This area was a part of the flooded zones of "malaria stratification" in Romania, with *Anopheles messeae* as the dominant species and *A. atroparvus* as the main vector of malaria (Zotta, 1938).

Fig. 1 – Location of the Giurgiu County including the study area.

Mosquito samples. The fresh blood-fed anopheline females were collected with hand battery aspirator in June and August 2005 from their resting places represented by cattle shelters in Budeni village. The abdomens of 150 anopheline females have been separated from the rest of the body with a scalpel, squashed individually on confetti filter papers and placed in numbered vials. The head-thorax parts of the females were placed individually in other vials numbered in concordance to the respective abdomens and kept for molecular identification of the species. The samples were preserved at –20°C.

Blood-meal identification. The direct ELISA for blood-meal identification of *Anopheles* (Beier *et al*., 1988) was used. The confetti filter papers containing the mosquito blood meal were let individually in 800 µl of phosphate-buffered saline (PBS) for 1 h. Fifty µl of each mosquito blood meal sample (in PBS) were added to 96-well microtiter plates. The plates were covered and incubated at + 4ºC during the night. After incubation, the plates were washed 2 times with PBS/Tween 20. Fifty µl of host-specific conjugate, diluted in 450 ml PBS containing 2.5 g boiled casein in 50 ml NaOH 0.1 N, 0.025 % Tween 20, 0.05 g Thimerosal, and 0.01 g Phenol red were then added. Each sample was tested against the following antibody conjugates: human, bovine, sheep, pig, horse, dog and chicken. After a 1 hour incubation at room temperature, the plates were washed 4 times with PBS/Tween 20, and 100 µl of peroxidase substrate (5 mg ortho-toluidine, 0.25 ml N,N-dimethyl formamide, 30 ml citrate buffer and 4 µl H_2O_2 30%) was applied. After a final incubation for 30 minutes at room temperature, in each well was added 50 µl of H_2SO_4 and then the plates were read by ELISA reader at 620 and 450 nm. Positive control (10 µl host-specific conjugate in 990 µl PBS) and negative control (PBS) were on every plate. A sample was considered positive if its absorbance value exceeded the mean plus 3 times the standard deviation of the negative controls.

Molecular identification. Species identification has been made by PCR diagnostic assay that differentiates between six species from those of *Anopheles maculipennis* complex occurring in Europe (Proft *et al*., 1999).

DNA was extracted from a single leg/specimen following a slightly modified version of the protocol of Edwards (1998). Each sample was homogenized in 200 µl of 2.0% CTAB (Cetyl trimethyl ammonium bromide). The tubes were incubated at 65° C for 5 min, and 200 µl chloroform was added before spinning at 12000 rpm for 5 min. The supernatant was transferred to a clean tube and then was added 200 µl isopropanol before the next spinning at 12,000 rpm for 15 min. The liquid phase was thrown, 200 µl 70 % ethanol was added and then the tubes were spun at 12000 rpm for 5 min. The pellet was air-dried and suspended in sterile water to give 50 µl of DNA solution. Four microliters of re-suspended DNA were used in each PCR reaction.

PCR procedure (Proft *et al*., 1999) was used with minor modification. The rDNA-ITS2 region was amplified using two specific primers for the conserved regions of the 5.8S and 28S genes. The PCR mixture had a total volume of 25 µl and contained 10 pmoles of each of the specific primers of 5.8S and 28S, 0.5 U of Taq polymerase, 0.2 mM of each of dNTPs, 2.5 µl of 10 × reaction buffer and 1 mM $MgCl_2$. The PCR cycling conditions were as follows: initial denaturation at 94°C for 3 min followed by 40 cycles of: denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s. Final extension was carried out at 72°C for 10 min. The target amplified DNA was loaded onto 2.0 % agarose gel. Gels were stained with ethidium bromide and run at 140 V for 1 h. DNA bands were visualized by UV transillumination (Fig. 2).

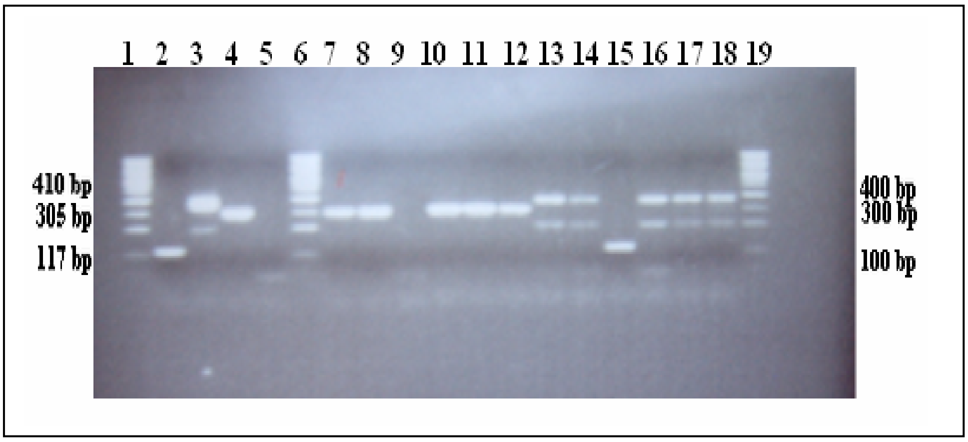

Fig. 2 – PCR products from amplification of the rDNA-ITS2 region of anopheline mosquitoes from Comana area.

Legend: 1, 6, 19 – migration marker; 2-4 – positive control; 5 – negative control; 7, 8, 10 – 12 – *Anopheles messeae*, 13, 14, 16-18 – *Anopheles maculipennis* s. str.; 15 – *Anopheles atroparvus.*

RESULTS AND DISCUSSION

This study has been performed in order to obtain data regarding the feeding preferences of the species from *Anopheles maculipennis* complex existing in Comana area. These data are important because they show the level of feeding on humans of the anopheline females and their possibility to transmit or to become infected with *Plasmodium*. The rDNA-ITS2 region of *A. maculipennis s.l.* specimens has been amplified using 5.8S and 28S conserved primers. Three species were identified: *Anopheles atroparvus* (19.3 %), *A. maculipennis* s.str. (17.3 %) and *A. messeae* (63.3 %). The length of amplified sequences was 410 bp for *A. maculipennis* s.s., 305 bp for *A. messeae* and 117 bp for *A. atroparvus* (Fig. 2).

The ELISA test identified 145 (96.7%) out of the 150 tested samples, 5 samples (3.3%) remaining unidentified. The main observation is that none of the females of *Anopheles maculipennis* complex has fed on humans. Taking into account the identified samples, it appears that females of *A. maculipennis* complex were fed in a proportion of 85.5% on bovines and 14.5% on other vertebrate hosts (7.6% on a single host – pig or horse; 6.9% on two hosts, one host being bovine and the second one being horse, pig or chicken) (Table 1).

Our results show that the species of *Anopheles maculipennis* complex have fed predominantly on bovines. *A. messeae* and *A. maculipennis* s. str. have also had preferences for pigs, horses and just one multiple hosts, while *A. atroparvus* had preferences for pigs and three different multiple hosts (bovine/horse, bovine/pig, bovine/chicken). The fact that none of the mosquitoes has fed on humans, and the limitation of human-mosquito contact in the last decades show that the risk of malaria re-emergence in the area is very low.

Table 1

Blood-meal sources for the *Anopheles maculipennis* s.l. and the number that fed on each vertebrate host

Species	Blood-meal sources; number of positive samples (%)										
	No. tested	Hm	B	P	Hr	S	C	D	BHr	BP	BC	UI
Anopheles atroparvus	29	0	22 (81.5)	1 (3.7)	0	0	0	0	1 (3.7)	2 (7.4)	1 (3.7)	2
Anopheles maculipennis s.s.	26	0	19 (79.2)	3 (12.5)	1 (4.2)	0	0	0	0	1 (4.2)	0	2
Anopheles messeae	95	0	83 (88.3)	4 (4.3)	2 (2.1)	0	0	0	0	5 (5.3)	0	1
Total	150	0	124 (85.5)	8 (5.5)	3 (2.1)	0	0	0	1 (0.7)	8 (5.5)	1 (0.7)	5

Abbreviations: Hm = human; B = bovine; P = pig; Hr = horse; S = sheep; C = chicken; D = dog; BHr = = bovine/horse; BP = bovine/pig; BC = bovine/chicken; UI = unidentified.

CONCLUSIONS

In Comana, a former malaria-endemic area, the same members of *Anopheles maculipennis* complex reported during malaria-endemic period (*A. atroparvus*, *A. messeae* and *A. maculipennis* s. str.) were identified.

Our study has shown that anopheline mosquitoes had fed predominantly on bovines. None of the mosquitoes had fed on human blood whatever the mosquito species. The ELISA blood-meal results relieve that members of the *Anopheles maculipennis* complex in Comana area are highly zoophilic and the risk of malaria re-emergence in the area is very low.

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NUMERICAL DYNAMICS STUDY OF PREY (DEER) AND PREDATOR (WOLF) FROM RÂUL TÂRGULUI AREA (FĂGĂRAŞ MOUNTAINS, ARGEŞ DISTRICT, ROMANIA)

ROMICĂ MANDU

From 1996 till 2005, populations of wolf and deer were monitored in the area of Râul Târgului (the Făgăraş Mountains – Argeş County), in order to establish their numerical dynamics. Obtained data were used to draw their numerical diagrams. Using modified Lotka-Volterra model and the data collected, we configured 17 diagrams, meaning temporal representations of the relationships between wolf and deer populations. Analysing all figures, they showed the same thing: the numerical dynamics of prey (H) population and C (predator) population tended to steady-state values over time. Either changing the values of k, H0 (prey population at the beginning of simulation) and C0 (predator population at the beginning of simulation), or remaining unchanged, the two populations of prey and predator, after a number of years, reached or were close to reach a dynamic equilibrium, at the established final values of He (prey population at the end of simulation) and Ce (predator population at the end of simulation). Our results, using modified Lotka-Volterra model, confirmed the results cited elsewhere (Mitteldorf *et al.*, 2002), populations of prey and predator tending to steady-state values over time. Where the equilibrium point was not reached, the figures of He and Ce did not touch the asymptotic level: that is, the time required for reaching it had to be longer than the time the program was built up for.

Key words: wolf, deer, prey population, predator population, trophic interactions.

INTRODUCTION

Wolf and deer populations were described in time in different ways: as the influence of snow cover on both populations (Nelson & Mech, 1986; Gula, 2004); as habitat-population density relationships (Roseberry & Woolf, 1998), as wolf control on deer (Atkinson & Janz, 1994); as trophic interactions using Lotka – Volterra mathematical models (Gardini *et al.*, 1989; Abrams, 2000; Ackleh *et al.*, 2000; Chung, 2000; Saito *et al.*, 2001; Jensen & Miller, 2001; Levin, 2002; Li *et al.*, 2005; Hoppensteadt, 2006; Takeuchi *et al.*, 2006; Kazanci, 2007; Krivan, 2007, 2008; Fang & Chen, 2008; Wang, 2008; Krivan & Cressman, 2009). The interaction dynamics between a prey population H (deer) and a predator population C (wolf) is described in time by Lotka-Volterra differential equations, where *a* represents multiplying exponent

$$\frac{dH}{dt} = (a - bc)H \; ; \; \frac{dC}{dt} = (\lambda bH - m)C$$

on a prey unit in the absence of predators; *b* represents consumption index on a predator unit; λ represents prey conversion index into predator progeny; *m* is the natality of predators in the absence of prey. Equation terms have the following significance: *aH* – growth rate of herbivore population in the absence of consumers; *bHc* – diminution rate of herbivore population because of predators consumption; λb*Hc* – growth rate of predator population; *mc* – diminution rate of predator population in the absence of food. Recent researches showed that modified Lotka-Volterra model (Jensen & Miller, 2001) introduced *k* factor, interpreted as one to inhibit prey reproduction, that is, crowding factor, inhibiting prey reproduction which, in turn, put the predator in a condition of starvation. Without food, predator mortality will increase, that is, less pressure on prey population. In these conditions, keeping the notations of Lotka-Volterra equation, there were obtained modified Lotka-Volterra equations:

$$\frac{dH}{dt} = \left[a\left(1 - \frac{H}{k}\right) - bC\right]H \; ; \; \frac{dC}{dt} = (\lambda bH - m)C$$

In order to realize mathematical models of numerical variation of different populations, first of all parameters *a* and *m* have to be known, which are different from one to another species. It is possible to work either with conventional units and then to establish conversion coefficients, for instance 1 bear corresponds to 1.5 wolves, either with a and m coefficients with ponderate averages values by the form $a = \frac{a_{C_e} n_{C_e} + a_C n_C + a_i n_i}{n_{C_e} + n_C + n_i}$; $m = \frac{m_l n_l + m_u n_u + m_r n_r + m_{ps} n_{ps}}{n_e + n_u + n_r + n_{ps}}$, where a_{ce}, a_c, a_e represent "a" coefficient for deer; n_{ce}, n_c, n_i represent the number of deer from the analyzed territory; m_l, $m_{u,}$, m_r, m_{ps} represent the "m" coefficient for deer and bears; n_l, n_u, n_r, n_{ps} represent the number of wolves, bears from the analyzed territory. The coefficients *b*, λ depend on the prey-predator interaction and are in relation with "m" and "a" coefficients. The diagrams constructed, using Lotka-Volterra modified model, are different, depending on the values of λ, b, k.

In the hunting districts of South part of the the Făgăraş Mountains, namely Râuşor, Râul Târgului şi Stoeneşti, trophic relationships between deer and wolf were interpreted using modified Lotka-Volterra model, which pointed out nearly an asymptotic level of their numerical dynamics after a number of years.

MATERIAL AND METHODS

The observed area was located on the south part of the Făgăraş Mountains (South of Romania) in the district of Râul Târgului. Observations started in 1996 and were made till 2006. The places from where observations were made were located on the moving ways of deer herds, followed by packs of wolves, both from fix and from changing places.

Using mathematical Lotka-Volterra model and the data obtained during the 10 years study, we realized a number of different diagrams which showed the dynamics of numerical structure of prey and predator populations. The conversion index of 1.5 wolves to 1 bear was obtained taking into account the inventory of animals related to the area of hunting districts. The values of all coefficients used in the paper were obtained by mathematical calculation.

RESULTS

In the area of Râul Târgului, but not only, besides wolf, there are other predator species, as is bear, for which we used a conversion coefficient of 1 bear for 1.5 wolves (Table 1).

Table 1

Numerical dynamics of wolf and bear populations in the area of Râul Târgului

Year	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
Number of wolves	4	4	8	10	10	13	15	10	10	10
Number of bears	20	20	20	30	25	25	25	25	25	25
Number of deer	70	70	60	60	60	50	50	50	50	50

Considering a conversion coefficient of 1 bear for 1.5 wolves, the results are as follows:

Table 2

Numerical dynamics of prey and predator populations in the area of Râul Târgului

Time (years)	1	2	3	4	5	6	7	8	9	10
H	70	70	60	60	60	50	50	50	50	50
C	34	34	38	55	47	50	52	47	47	47

On the basis of the data in Table 2, we configured the diagrams of numerical dynamics of H and C populations (Fig. 1). Figure 1 shows that the model is asymptotic toward H, C values. Accepting the formula

$$T = \frac{2\pi}{\sqrt{ma}}$$

for the determination of constant *b* and considering the relations

$$H_e = \frac{m}{\lambda b};\ C_e = \frac{a}{b};$$

the expression is deduced

$$b = \frac{2\pi}{T\sqrt{\lambda H_e C_e}}.$$

On the basis of values for λ = 0.75 and T = 7 years, the value of b = 0.02 is obtained. At this time, modified Lotka-Volterra model, transcribed as

$$\frac{dH}{dt} = b\left[C_e \frac{1-\frac{H}{k}}{1-\frac{H_e}{k}} - C\right]H\ ;\ \frac{dC}{dt} = \lambda b(H - H_e)$$

is applied, using the data from Table 3. Numerical results are transcribed in the diagrams $C(H), H(t), C(t)$.

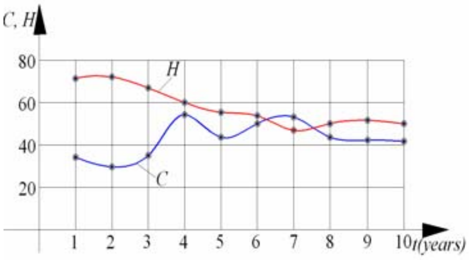


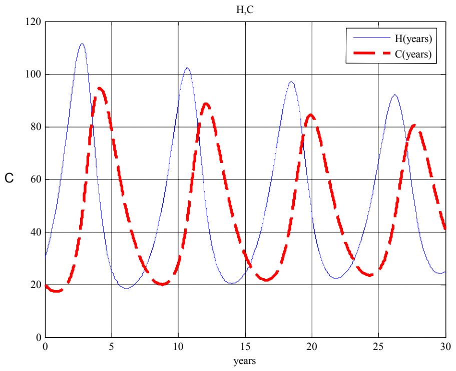
Fig. 1 – Numerical dynamics of C (prey) and H (predator) populations from the area of Râul Târgului.

x = time (years); y = individual numbers of C (predator) and H (prey) populations. Fig. 1 shows that both populations, starting approximately from the fourth year, are tending to steady-state values over time, reaching or being close to reach a dynamic equilibrium.

Table 3

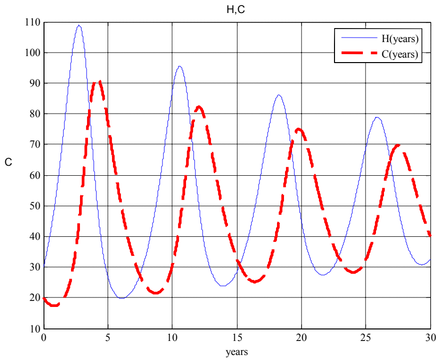
Numerical values corresponding to registered ones from Table 1

No.	H_e	C_e	λ	b	k	H_0	C_0
1	50	47	0,75	0,02	2000	30	20
2	50	47	0,75	0,02	1000	30	20
3	50	47	0,75	0,02	100	30	20
4	50	47	0,75	0,02	50	30	20
5	50	47	0,75	0,02	1000	40	30
6	50	47	0,75	0,02	200	40	30
7	50	47	0,75	0,02	100	40	30
8	50	47	0,75	0,02	50	40	30



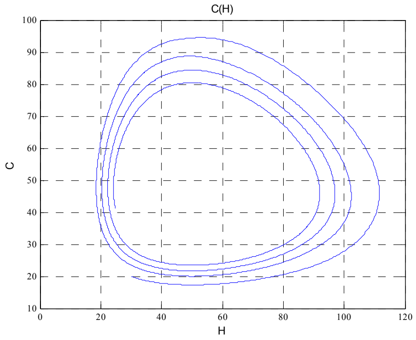
a)

Fig. 2 – Numerical dynamics of C (predator) and H (prey) populations (k = 2000; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = time (years); y = individual numbers of C (predator) and H (prey) populations.



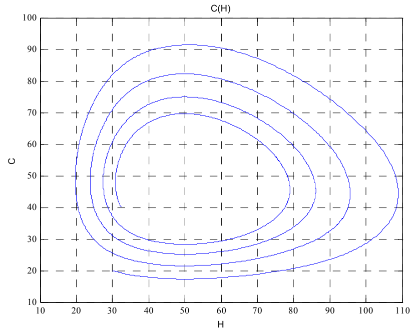
a)

Fig. 3 – Numerical dynamics of C (predator) and H (prey) populations (k = 1000; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = time (years); y = individual numbers of C (predator) and H (prey) populations.



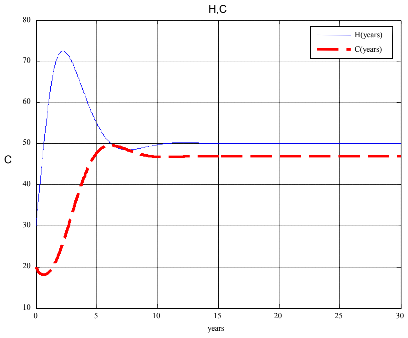
b)

Fig. 4 – Numerical dynamics of C (predator) and H (prey) populations (k = 2000; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



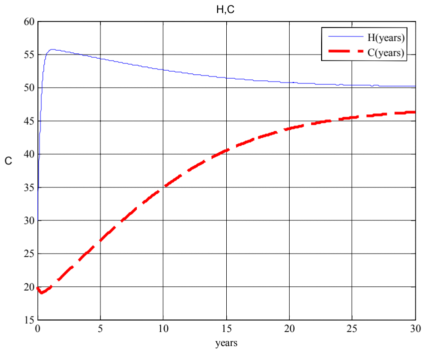
b)

Fig. 5 – Numerical dynamics of C (predator) and H (prey) populations (k = 1000; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; Y = individual numbers of C (predator) population.



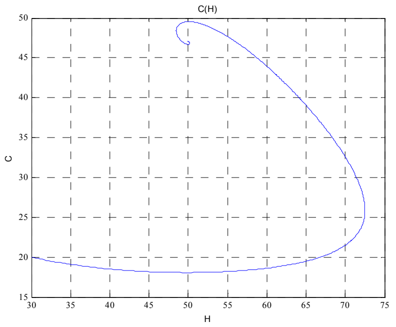
a)

Fig. 6 – Numerical dynamics of C and H populations (k = 100; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = time (years); y = individual numbers of C (predator) and H (prey) (populations).



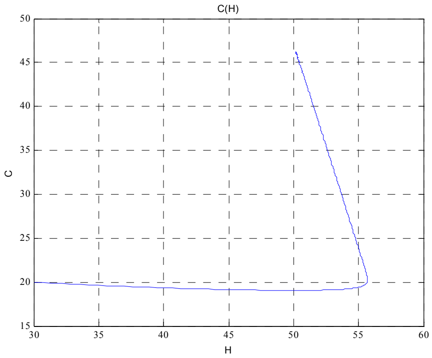
a)

Fig. 7 – Numerical dynamics of C and H populations (k = 60; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = time (years); y = individual numbers of C (predator) and H (prey) populations.



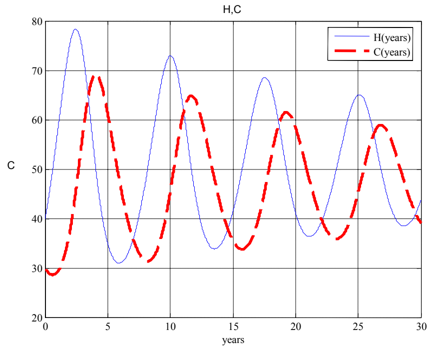
b)

Fig. 8 – Numerical dynamics of C and H populations (k = 100; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



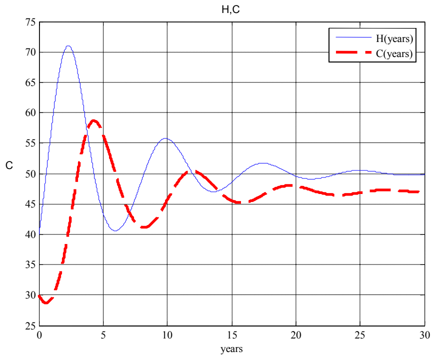
b)

Fig. 9 – Numerical dynamics of C and H populations (k = 60; H0 = 30; C0 = 20; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



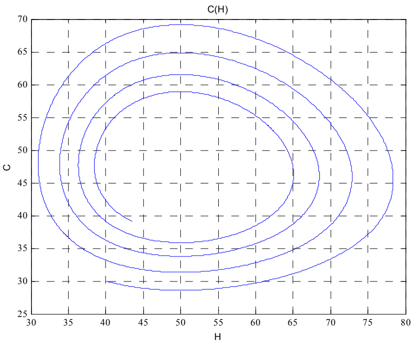
a)

Fig. 10 – Numerical dynamics of C (predator) and H (prey) populations (k = 1000; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = time (years); y = individual numbers of C (predator) and H (prey) (populations).



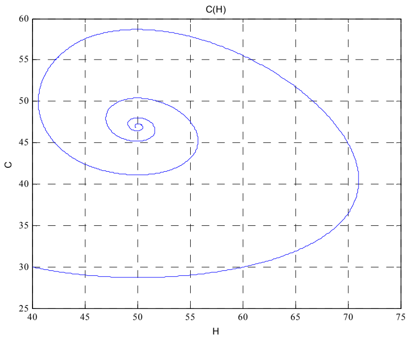
a)

Fig. 11 – Numerical dynamics of C (predator) and H (prey) populations (k = 200; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



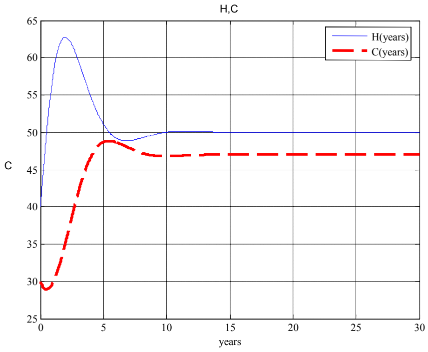
b)

Fig. 12 – Numerical dynamics of C (predator) and H (prey) populations (k = 1000; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



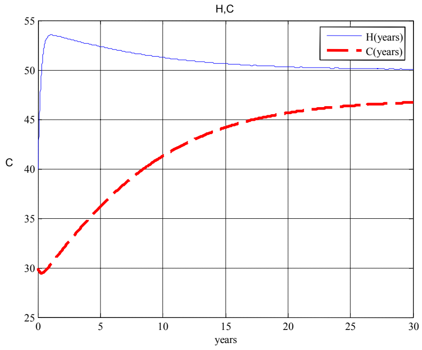
b)

Fig. 13 – Numerical dynamics of C (predator) and H (prey) populations (k = 200; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



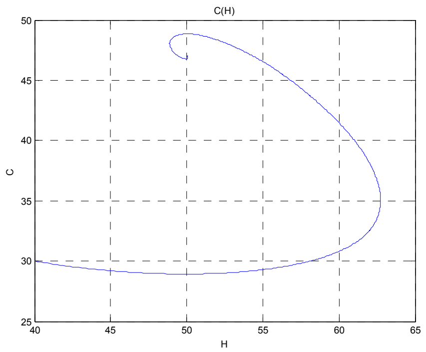
a)

Fig. 14 – Numerical dynamics of C (predator) and H (prey) populations (k = 100; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = time (years); y = individual numbers of C (predator) and H (prey) (populations).



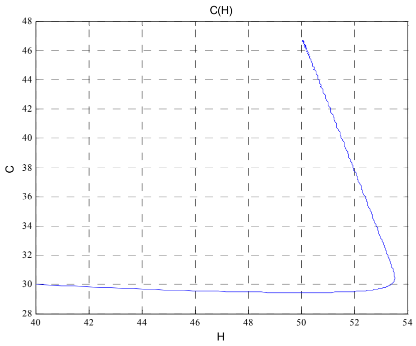
a)

Fig. 15 – Numerical dynamics of C (predator) and H (prey) populations (k = 50; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



b)

Fig. 16 – Numerical dynamics of C (predator) and H (prey) populations (k = 100; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.



b)

Fig. 17 – Numerical dynamics of C (predator) and H (prey) populations (k = 50; H0 = 40; C0 = 30; He = 50; Ce = 47; λ = 0.75; b = 0.02) from Râul Târgului; C0, H0, populations at the time = 0; x = individual numbers of H (prey) population; y = individual numbers of C (predator) population.

DISCUSSION

All those 17 figures analysed the numerical dynamic relationships between H (pray) and C (predator) populations, in different conditions. Figures 2a & 4b show the numerical dynamic relationships between H and C populations with the following values: He = 50; Ce = 47; λ = 0.75; b = 0.02; k = 2,000; H0 = 30; C0 = = 20. The difference between them consists in the change of significance of coordinated axes: in Figure 2a, x = time (years); y = individual numbers of C (predator) and H (prey) (populations); in Figure 4b, x = individual numbers of H (prey) population; y = individual numbers of C (predator) population. Both figures showed, in different ways, the same thing: the initial populations (C0 and H0) started from 20 individuals (C0) and from 30 individuals (H0). After 30 years, both Ce and He had to reach 47 and respectively 50. But they did not, being on the way to reach it, after more than 30 years. In other words, 30 years, for which the model was built up, were not enough, needing more time. Figures 3a & 5b show the same relationships between H and C populations, all the other values remaining the same, except for k, whose value was changed, being now 1000. For all figures, on “a” figures, the time was set on x axis; the individual numbers of H and C populations were set on y axis. For all figures, on “b” figures, individual numbers of H population were set on x axis; the individual numbers of C population were set on y axis. For all figures, the values of He, Ce, λ, b remained the same; the values of k, H0 and C0 were changed. Analysing all figures, they showed the same thing: the numerical dynamics of prey (H) population and C (predator) population tended to steady-state values over time. Either changing the values of k, H0 and C0, as we have done for the other remaining figures, or remaining unchanged, the two populations of prey and predator, after a number of years, reached or were close to reach a dynamic equilibrium, at the established final values of He and Ce. Our results, using modified Lotka-Volterra model, confirmed the results cited elsewhere (Mitteldorf *et al.*, 2002), populations of prey and predator tending to steady-state values over time.

CONCLUSIONS

After studying the numerical dynamics of prey (deer) and predator (wolf) from Râul Târgului area (Făgăraş Mountains – Argeş District, Romania), the following conclusions have been drawn:

1. Trophic relationships between a predator (wolf) and a prey (deer) are of type (+ –), which means that the relation is obligatory and positive for predator species and negative-inhibitive for prey species.
2. Relationships between wolf and deer in the area of high altitude of the Făgăraş Mountains were represented using Lotka-Volterra modified mathematical

model. The obtained results were illustrated in 17 diagrams, each of them keeping constant some parameter values and modifying some others. From all figures, the values of He and Ce did not coincide with the final values of H and C. That was because the final values of H and C were in fact those reached by the populations after a number of years, 30 years, for which the model was constructed, whereas He and Ce were the values to which the populations were tending to stabilize in time. If the time were not long enough, the populations did not reach the values of He and Ce but only came near them.

3. Using the Lotka-Volterra modified model, there were constructed two kinds of diagrams: on half of them, all "a" figures, the time was represented on Ox axes and individual numbers of H and C populations on Oy axes; on the other half of figures, all "b" figures, individual numbers of H population were represented on Ox axes and individual numbers of C population on Oy axes. Both types of figures went toward the same conclusion, populations of prey and predator tended to steady-state values over time.

4. *k* parameter, as a crowding factor, inhibiting prey reproduction which, in turn, put the predator in a condition of starvation, proved to be one of the most important factors which influenced the curve of numerical dynamics of predator-prey relationships: smaller the k value, almost lack of oscillations of the curves of the numerical dynamics of predator-prey diagrams. In other words, it is possible to conclude that the tendency toward steady-state values over time of prey and predator populations starts earlier, without, or with fewer numerical oscillations of their numerical dynamics.

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HEALTH CONDITION ASSESSMENT IN LAKE XOCHIMILCO (MÉXICO)

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Lake Xochimilco located in Mexico City was one of the main water supplies for Mexico City. Nowadays the lake receives wastewater from a treatment facility. The health of the lake was studied using early warning biomarkers in: *Ankistrodesmus falcatus*; *Hyallela azteca* and *Ambystoma mexicanum*; trophic state and water quality index (WQI) was assessed, and histological damages were analyzed in *A. mexicanum*. Monitoring periods were in rainy, cold and drought season, in urban (UZ), tourist (TZ) and agricultural (CZ) zones. The trophic state is eutrophic-hypereutrophic. WQI fluctuates from 47.7 in UZ to 61.8 in CZ. Sublethal exposure assays provoked oxidative stress in all species studied. UZ was the site that exerted major oxidative damage. CZ exerted inhibition in the acetylcholinesterase activity in *A. mexicanum*. Exposure to elutriates provoked histological damages in the liver and gills in *A. mexicanum*.

Key words: Early warning biomarkers, aquatic ecosystem health, Mexican urban lake.

INTRODUCTION

Lake Xochimilco, an urban water body in Mexico City, has a long historical and cultural wealth that includes its high biodiversity, and the floating gardens or "Chinampas" that have given reputation to this locality at a world-wide level (Ezcurra, 1988). Since the beginning of 1900, this lake has been one of the main water supplies for Mexico City. Nevertheless, since 1971, the inputs of waste water in the lake from a treatment facility have provoked ecosystem deterioration by water quality depletion (Guerrero *et al*., 1982). Municipal effluents are recognized as the major sources of aquatic environmental pollution, such as diverse pathogenic microorganisms, heavy metals, pesticides and estrogens, are in these effluents, all of them confirming very complex mixtures, potentially harmful to the aquatic biota (Gagné *et al*., 2002). Water extraction with provision aims and inputs of water treated into the channels of Xochimilco are probably the main causes of extinction of two species of endemic fish of the basin of Mexico, *Evarra tlahuacensis* and *E. bustamantei* (World Conservation Monitoring Centre, 1996). It has also provoked the restriction in the distribution range of other endemic species of the basin, as is the case of the viviparous fish *Girardinichthys viviparus* and of the neotenic axolotl, *Ambystoma mexicanum* both considered species critically threatened by the IUCN (2009) that are subject to special protection.

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In 1987 Xochimilco was designated as an area "of world-wide historical and cultural patrimony" by the UNESCO (Wirth, 1997), emphasizing the necessity to protect this relict of civilization. Furthermore, in 2004, the Lake of Xochimilco was included in the list of wetlands RAMSAR (2004) (site 4MX050). The RAMSAR convention on wetlands is an international treaty for the conservation and sustainable utilization of wetlands. Although since 1991 some measures have been taken for the restoration of the Xochimilco Lake, it is well-known that deterioration conditions still prevail in the ecosystem. Due to the fact that Lake Xochimilco has a wide agricultural-chinampera, urban and tourist zones inside the metropolis of the city of Mexico, this wetland can also be considered an urban wetland, which confers a high ecological value to the city besides the potential as a biotic reserve. *Ambystoma mexicanum* is a Urodele neotenic amphibian (reaches its sexual maturity without changing its larval morphology), that belongs to the Ambistomatide family. *A. mexicanum* is endemic of the basin of Mexico its unique natural habitat being the remaining lakes in Mexico City especially those of Xochimilco. The axolotl has great importance for research, since it serves as model amphibious in many physiological and morphologic studies. *A. mexicanum* has diverse hormonal processes that have been studied to analyze their regenerative capacity. Nevertheless, the information generated on Mexican species *A. mexicanum* is still scarce.

The need to detect and to determine the pollution impact of chemical compounds in the environment has led to studying indicators of biological effects, biomarkers, considered as stressors-induced variations in cellular or biochemical components or processes, structures, or functions that are measurable in a biological system or sample, such as variation in cells body fluids, tissues, or organs within an organism (Livingstone, 1993). Enzymatic responses and production of metabolites are examples of biomarkers, which can be related to exposure to toxics (Sherry, 2003). These are indicators that polluting agents have entered the organism, are distributed in tissues and are exerting effects in target organs (Van der Oost *et al*., 2003). From the point of view of conservation and environmental protection, damages assessment generated by the mixtures of xenobiotics on the aquatic biota is of greater relevance than the quantification of chemical compounds in water.

The risk that represents the water quality deterioration of Lake Xochimilco by the waste water entrance to the different endemic, native and migratory species living in this aquatic ecosystem will be explained through the study of early warning biomarkers. The aim of the present contribution is to analyze the environmental conditions and the response of some early warning biomarkers in three native species of different trophic level from the lacustrine zone of Xochimilco: the microalgae *Ankistrodesmus falcatus*, the amphipod *Hyalella azteca* and the amphibian *Ambystoma mexicanum*.

MATERIAL AND METHODS

The lacustrine zone of Xochimilco, with an extension of 2,657 ha, is located in the south of Mexico city and belongs to the basin of Mexico. Three study sites were examined in Lake Xochimilco considering the main land uses that have impact on water quality and generate contrasting conditions: 1) urban zone (UZ), 2) tourist zone (TZ) and 3) the agricultural zone (AZ) (Fig. 1). Monitoring was conducted in three periods of the year: rainy season (October 2008), the cold drought season (January 2009) and the warm drought season (May 2009). Water quality and trophic state assessments are presented for each study site and period studied and data of early biomarkers are from the rainy season.

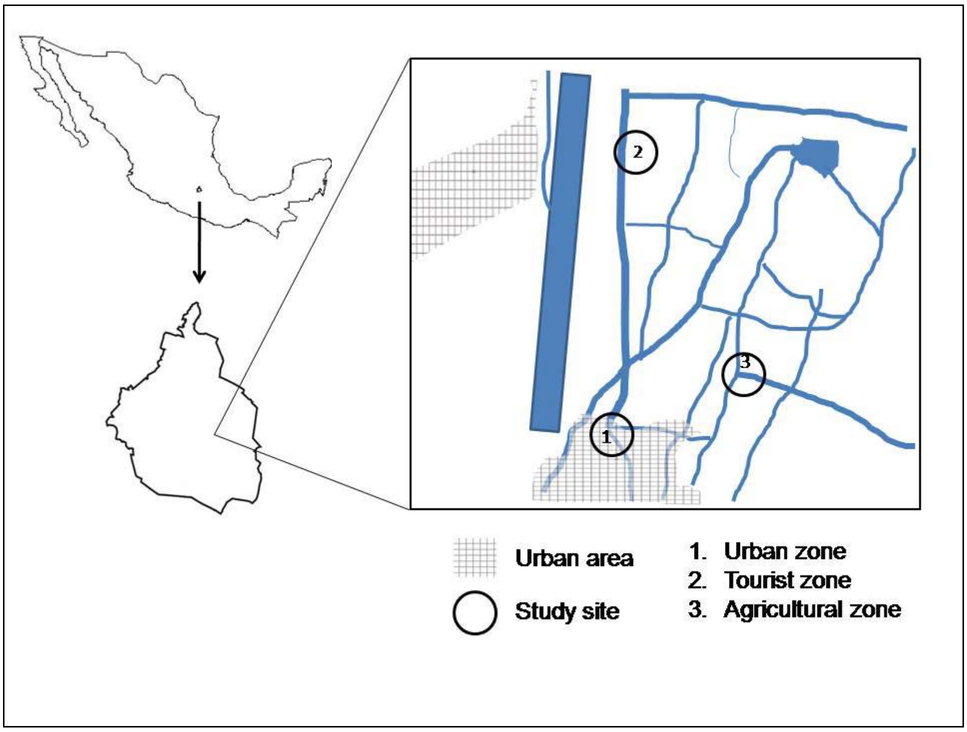

Fig. 1 – Location of study sites.

Different environmental factors were recorded at each study site using a Quanta multiparametric sonde: water temperature (°C), dissolved oxygen (mg/L), pH, conductivity (mS/cm). Transparency was recorded with a Secchi disk, and chlorophyll *a* was recorded with a Fluorometer Turner design. To assess water quality, water samples were taken in duplicate in order to quantify hardness ($CaCO_3$ mg/L), orthophosphate (mg/L), total phosphorus (P_T mg/L), ammonium (mg/L), nitrate (mg/L) and nitrite (mg/L) and N_T (mg/L) using a Hach 2500 spectrophotometer. Alkalinity (mg/L), BOD_5 (mg/L), and total and fecal coliforms (MPN) were measured according to APHA (2005) procedures. Additionally, samples of sediments were taken for each study site to obtain elutriates to be used in exposure bioassays with the three species selected in this study.

The test organisms for bioassays were the microalgae *Ankistrodesmus falcatus*, the amphipod *Hyalella azteca* and the amphibian *Ambystoma mexicanum* all of them native of the lacustrine zone of Xochimilco. *A. falcatus* and *H. azteca* were obtained from laboratory stocks and the amphibian from aquariums of the civil association "*Conservation and exploitation of the chinampera zone*".

Elutriation procedure

Sediment elutriates from samples of each study site were obtained, following the procedure approved by USEPA (2008) that consists of adding water to the dried sediments in a proportion 1:4 of sediment:water, were shaken during 30 min and after decantation the water was centrifugated at 3000 rpm.

Exposure bioassays

Static bioassays with the sediment elutriates from October of the three study sites of Xochimilco were performed to assess their toxic effect on the health of three native species. For the case of the microalgae (*A. falcatus)* bioassays were performed following the standardized protocol of the USAEPA (1978). Filtered water samples through a membrane of 0.45 µm and sterilized were used in test tubes of 10 ml of capacity with spiral cover, tubes that contained air and water in a proportion 40: 60. Each tube was spiked in aliquots of 50 µL containing *A. falcatus* in a concentration of 2.5×10^3 cells of algae mL^{-1}. Absorbance was read each 24 hrs by a period of 10 days. The algal growth potential (AGP) was obtained following Couture *et al.* (1985) as the maximum concentration of the algae when the population reaches the stationary phase. Each bioassay was done by triplicate. Results of the absorbance were transferred to the number of cells mL^{-1}.

For the assays of the second trophic level the amphipod *H. azteca* was used. Lots of 100 adult organisms were exposed in static bioassays to sediment elutriates of the three study sites for a period of 96 hrs of exposure with constant aeration and at a temperature of 22±1°C. At the end of the exposure the organisms were frozen in liquid N until their processing. The biomarkers assessed were the lipidperoxidation level with the method of Buege & Aust (1978) and acetyl-cholinesterase with the method of Hestrin (1949).

For the bioassays of the third trophic level *Ambystoma mexicanum* was used. Juveniles of *A. mexicanum* maintained in laboratory free of toxic exposure were used. Elutriates of the three study sites were placed in aquariums of 20 L with constant aeration and with 22±1°C of temperature. Lots of four organisms were exposed to elutriates of each study site for a period of 96 hours. At the end of the exposure the organisms were sacrificed and dissected for the extraction of the liver, gills and muscle, which were preserved in liquid N until their processing. Biomarkers used were in the muscle: acetylcholinesterase (AChE) (Hestrin, 1949) and the lipidperoxidation level (LPO) (Buege & Aust, 1978). In liver and gills biomarkers used were the lipidperoxidation level (Buege & Aust, 1978), the superoxide dismutase (SOD) (Sun *et al.*, 1988), the glutathione peroxidase (GPx) (Lawrence & Burk, 1976) and the catalase activities (CAT) (Cohen *et al.*, 1970).

Data Analysis

Water quality data (Table 1) was used to assess the Water Quality Index (WQI) of Dinius (1987). This geometric index clusters the values of the variables of water quality to obtain an absolute value whose magnitude is related to the water pollution state in a numeric scale associated with the potential water uses. The algorithm on which this index is based is:

$$\mathrm{WQI} = \prod_{i=1}^{n} I_i^{w_i}$$

where

WQI = Water quality index

I_i = Subindex of quality for the ith parameter and $0 \le i \le 100$.

w_i = Importance weight of the ith parameter and $\Sigma w_i = 1$

n = Number of parameters.

Trophic state was assessed with the Trophic State Index (TSI) of Carlson (1977). The interval of trophic state index of Carlson by its logarithmic nature does not display an upper limit, and it is obtained in agreement with the following expressions:

$$\mathrm{TSI\ (SD)} = 60 - 14.41 \ln (\mathrm{SD}), \text{ where SD = Secchi depth,}$$

$$\mathrm{TSI\ (CHL)} = 9.81 \ln (\mathrm{CHL}) + 30.6, \text{ where CHL= chlorophyll } a,$$

$$\mathrm{TSI\ (TP)} = 14.42 \ln (\mathrm{TP}) + 4.15, \text{ where TP = Total Phosphorus concentration.}$$

Data of biomarkers are presented as mean ± SE. Significant differences among groups were analyzed with ANOVA with Tukey`s multiple comparison. The significance of the results was ascertained at $p < 0.05$.

In order to incorporate the response of a battery of early warning biomarkers, the Integrated Biomarker Response (IBR) was performed following the procedure of Beliaeff & Burgeot (2002). It is an easy and useful multivariate method through the use of star plots that takes the variation contained in the data set into account.

Table 1

Water quality index values

Urban zone	47.73	53.85	45.78	49.12
Tourist zone	55.16	58.59	51.68	55.14
Agricultural zone	60.51	61.88	55.20	59.20
	54.47	58.11	50.89	54.48

Trophic state assessment

Trophic state index values (TSI) fluctuate from 57.21 to 73.9 (Table 2) all are within the Lake range typified as eutrophic to hypereutrophic according with TSI of Carlson (1977) (Table 3). The lowest values appear in October during the rainy season and in the cold drought in January.

Table 2

TSI of the study sites WQI values

	October	January	May	Average
Urban zone	65.02	70.25	70.96	68.75
Tourist zone	57.21	72.42	71.24	66.96
Agricultural zone	65.34	73.90	72.13	70.45
	62.52	72.19	71.44	68.72

Table 3

Trophic state according to TSI Carlson (1977)

Range of TSI	Trophic state	Water condition
0-40	Oligotrophic	Transparent water
41-50	Mesotrophic	Higher turbidity, higher algae abundance and macrophytes
51-70	Eutrophic	Usually blue-green algae blooms
> 70	Hypereutrophic	Extreme blue-green algae blooms

TSI by factor in each study site (Fig. 2) showed that TSI, by chlorophyll *a*, displayed lower values in contrast to the TSI by P as well as TSI by the Secchi transparency. These differences suggest that in the lacustrine zone an intensive grazing occurs on the phytoplankton by the zooplankton, which would give as result the low values of chlorophyll *a*, also those TSI values can be resulted by the presence of toxic compound that provokes inhibition or depletion of algal growth in spite of the high nutrient concentrations (N and P) detected in Lake Xochimilco. On the other hand, values of the TSI by Secchi transparency were also high, nevertheless, the chlorophyll *a* is not the cause of the low transparency, in turn some abiogenic materials could be producing turbidity in the water column and could be the cause of low transparency and also could be a limiting factor for the photosynthetic activity.

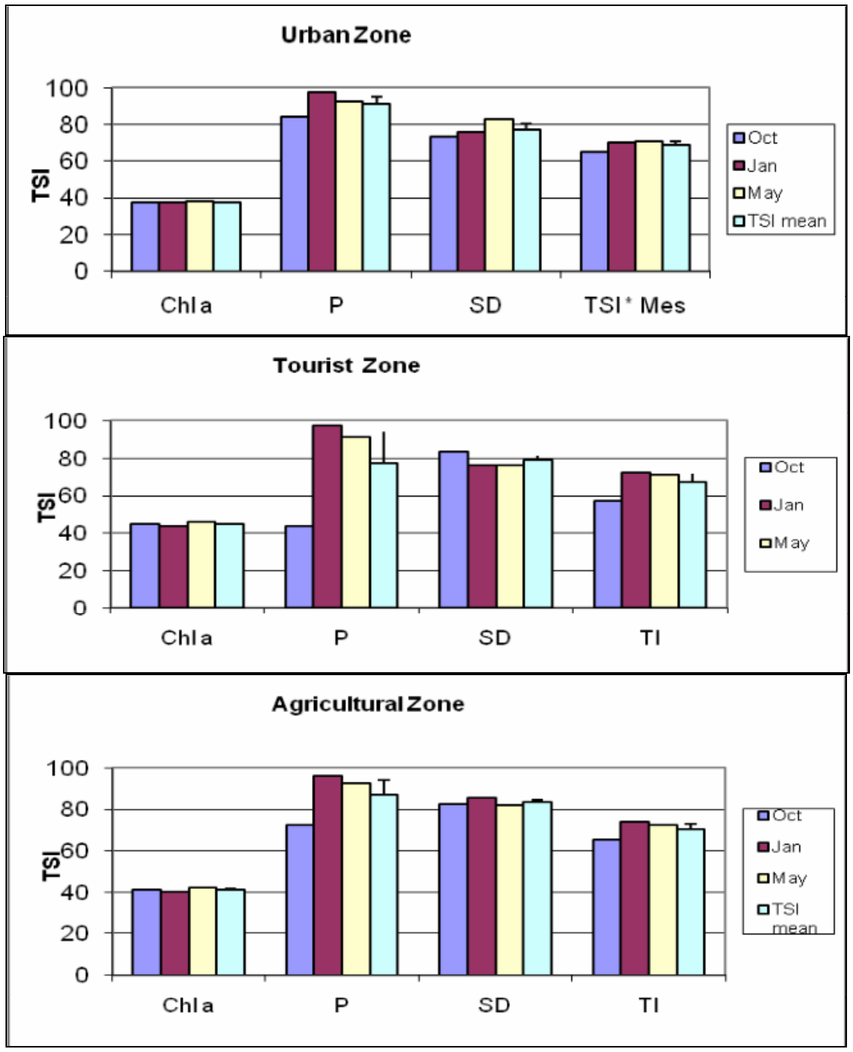

Fig. 2 – Trophic state index of the study sites of Lake Xochimilco by factor, study site and study period.

Biomarkers

Algal growth potential with *Ankistrodesmus falcatus*

The results of the AGP with sediment elutriates of the three study sites show that elutriates that generate higher AGP are those of the agricultural zone, followed by the urban zone and finally the tourist zone. The response of the AGP bioassay is strongly related to nutrients availability that stimulates the algal growth (Fig. 3).

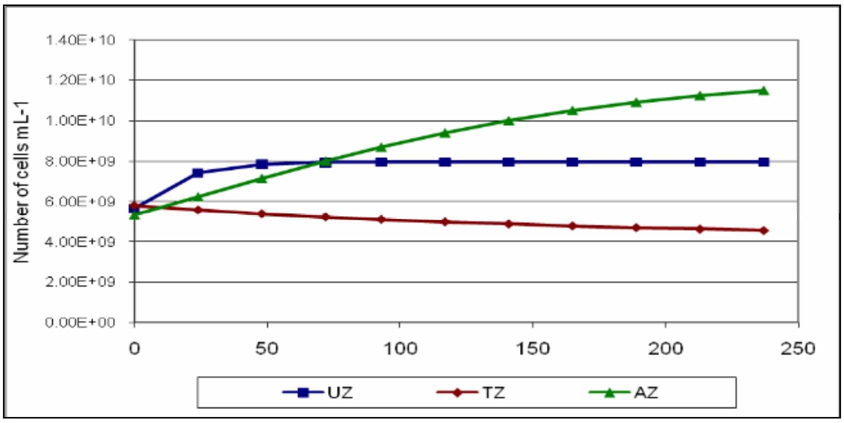

Fig. 3 – AGP bioassay of *Ankistrodesmus falcatus* during 236 hrs of exposure.

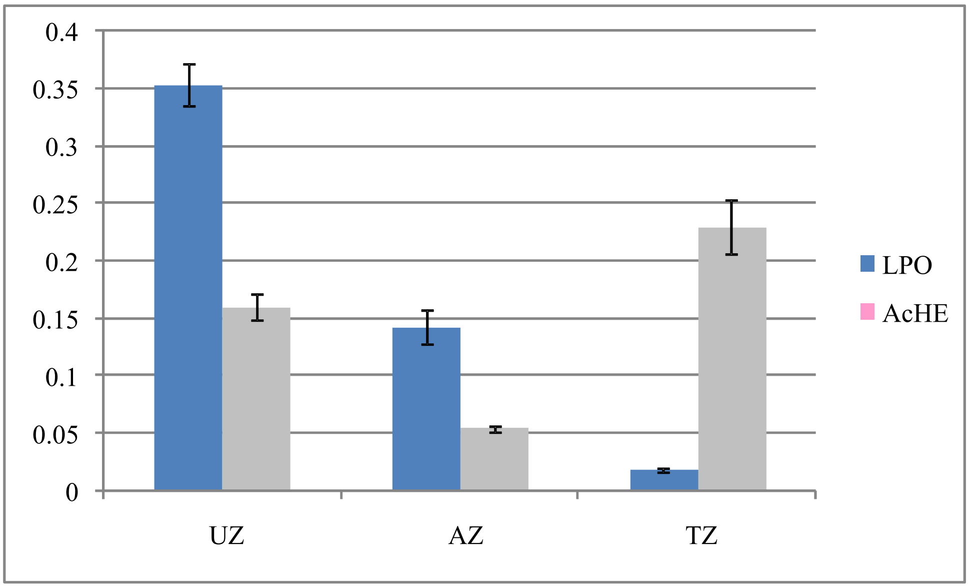

Fig. 4 – Lipidperoxidation level (nMol MDA mg/prot) and AchE (nmol AcH mg/prot) activity in *H. azteca*.

Biomarkers in *Hyallela azteca*

The lipidperoxidation level showed that sediment elutriates of the urban zone exert a higher oxidative damage, followed by those of the agricultural zone and finally of the tourist zone. In the acetylcholinesterase activity, it was detected that the agricultural zone is able to exert inhibition, followed by the urban zone and the tourist zone (Fig. 4).

DISCUSSION AND CONCLUSIONS

The mean value of the WQI was 54.48, very close to the mean value of the scale of the WQI. Results of WQI of Lake Xochimilco are in the range of those recorded in the Lerma River, 26 to 67 (Sedeño-Diaz & López-López, 2007). The Lerma River is considered as the most contaminated river basin of Mexico. Values of the WQI indicate that for water exploitation there is required to give treatment. For the case of the biota, the protection criteria are even stricter, which implies that the resource does not reach the minimum for aquatic biota protection. The trophic state index of Carlson (1977) showed that the lacustrine zone of Xochimilco is in an advanced state of nutrients enrichment, however, during the period of study massive blooms of cianobacteria did not appear. The trophic state assessed by the three indicators (chlorophyll a, total phosphorus and transparency of Secchi) give values of eutrophic to hypereutrophic. In the lake Pamvotis (Greece) a drastic change in the trophic state was recorded in the last 50 years, associated to several modifications in the catchment area of the lake basin by anthropogenic activities. The changes provoked by the man include modifications in the land use, in water level, as well as the point and nonpoint sources of pollutants (Kagalou *et al*., 2003). The advanced eutrophic state of the Lake Xochimilco can also be attributed to anthropogenic activities, since, this water body has been under severe anthropogenic pressure since pre-hispanic times (Wirth, 1997), despite the most drastic impacts in land use (increase of the urban zone), water extraction as well as the input of polluting agents of point sources and non-point have occurred in the past 60 years (Ezcurra, 1988). These conditions have led the lake to show eutrophic-hypereutrophic symptoms that in addition have been associated to the loss of biodiversity. The response of biomarkers in axolotls and in the amphipod *H. azteca*, exposed to the sediment elutriates of the different study sites (ZC, ZU and ZT), shows that besides the depleted water quality and the advanced eutrophic state, Xochimilco presents xenobiotics, among them heavy metals, that alter the physiology of the organisms. For the case of *H. azteca* the effects lead to increases in the level of lipidperoxidation. In the case of the axolotl the response included increase in the level of lipoperoxidation as well as in some enzymatic activities (SOD and CAT) and in other cases inhibition (GPx and AChE). Previous studies in Lake Xochimilco have recorded the presence of several heavy metals (As, Cd, Cr, Cu, Zn, Hg) (Bojórquez & Amaro, 2003). Damages exerted by heavy metals include neurological affectations resulted in disruption of AChe activity (Najimi *et al.*, 1997), as well as oxidative stress (Ercal *et al.*, 2001). The alterations at biochemical level that were detected in the exposure organisms affect at other physiological levels increasing the susceptibility of the organisms to diseases and parasitism, due to the chronic stress in which they live (Lafferty & Holt, 2003), reason why its survival is in risk. Furthermore, exposure to heavy metals may cause cytological, cytochemical and ultrastructural alterations to the hepatopancreas of several aquatic organisms, which has been identified as a target organ of the xenobiotics (Frías-Espericueta *et al.*, 2008). Histological effects in *A. mexicanum* exposure to elutriates demonstrated severe sub lethal damages in the liver and gill, however, these damages could affect the successful reproductivity of this species.

Finally, for the case of microalgae, it was detected that the nutrient enrichment conditions can lead to the occurrence of algae blooms. The cascade effect could exert different affectation at different trophic levels from the organisms of the Lake of Xochimilco, putting at risk the integrity of the whole lacustrine system.

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STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF ZOOBENTHIC COMMUNITY FROM MUSURA BAY (THE DANUBE DELTA) BETWEEN 2005–2007

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The paper presents some dynamics aspects of the benthic invertebrates from Musura Bay, Danube Delta, in the conditions of 2005-2007 interval. The eutrophication of the Danube Delta, occurred after 1980s as a consequence of the increased nutrient loads carried by the Danube River, affected the aquatic communities reducing drastically the biodiversity. Due to bay's shallowness and the frequent occurrence of winds, the sediment resuspension impedes the organic matter deposition, limiting the food resources for the zoobenthic organisms; therefore, the zoobenthic community exhibited only low values of structural and functional parameters. During the investigated period, only 7 taxonomic groups were recorded. Similar with the other lakes of the Danube Delta, the chironomids and oligochaetes were dominant in abundance (520 ind/m^2 and 326 ind/m^2) and biomass (15.96 g/m^2 and 12.2 g/m^2). However, due to the former salinity of the bay, polychaetes were identified especially in the area located near the Black Sea. The productivity recorded also low values: the multiannual average was 12.48 kcal/m²/year for chironomids and 11.04 kcal/m²/year for olygochaetes, providing a low trophic basis for the higher links of the food web. The food up-take by the benthic fishes is limited also by abiotic factors such as the oxygen content at the sediment-water interface or the amount of organic matter in sediment; consequently, in this ecosystem a low fish yield is expected, but this aspect needs further investigations.

Key words: the Danube Delta, zoobenthic community, productivity, benthic fish.

INTRODUCTION

Musura Bay, located at the confluence of Sulina and Chilia arms with the Black Sea, represents an important morpho-hydrographic unit of the Danube-Danube Delta-Black Sea system (Fig. 1).

The bay is characterized by a very dynamic ecosystem evolution process that reflects the effect of gradual changes of water influx from the river, delta and sea in the bay perimeter during the last 50 years (Gâştescu, 1993; Zinevici *et al*., 2006 a). The rapid progression of Chilia secondary delta in the Northern part of the bay and the construction of a new canal at the mouth of Sulina branch determined the narrowing of its mouth with more than 5 km from the middle of 19th century, when it reached over 13 km, while the depths decreased below 2 m; the basin colmation process was intensified in the last twenty years by the formation of a marine sandbank at the mouth, that contributed to the transformation of the bay into a lagoon (Zinevici *et al*., 2006 a).

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Nowadays, the main water inflows occur through surface channels such as Musura, Stambulul Vechi, Cardon (Fig. 2) and breaks along the dike located on the left side of the Sulina arm (Fig. 3).

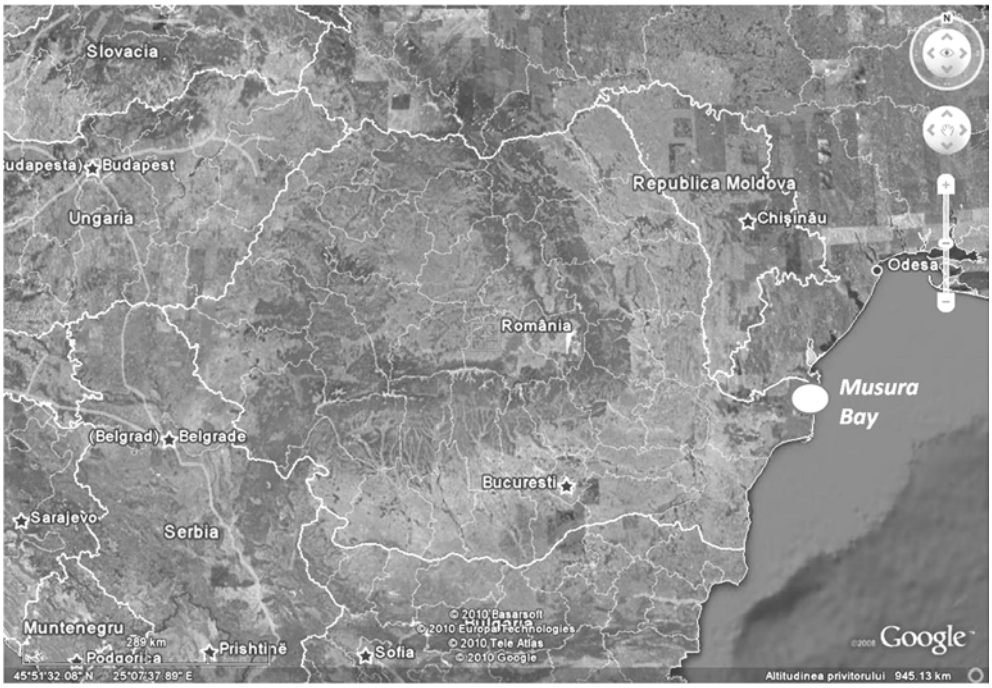

Fig. 1 – Location of Musura Bay (www.earthgoogle.com).

Fig. 2 – Side channel.

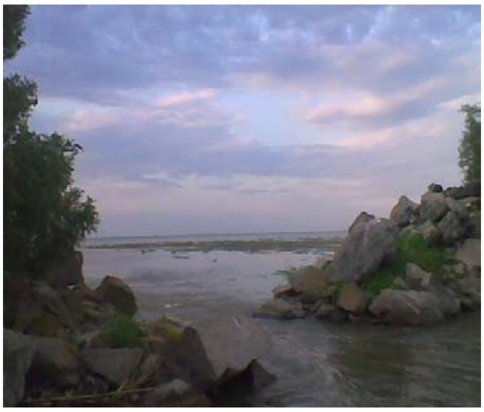

Fig. 3 – Breaks in the dike along Sulina arm.

Fig. 4 – Channel in the southern part of the bay.

Fig. 5 – Aquatic vegetation in the channel.

The partial closure of Musura Bay and the consequent diminished inflow of marine water, together with the increased amounts of freshwater brought by the Danube River, determined a progressive decrease of the salinity and a shift of maritime biocenoses to the freshwater type (Zinevici *et al*., 2006 a). The frequent floods occurred in the last decades as a consequence of the climatic changes (IPCC, 2008) determined an increased deposition of alluvia brought by the Danube River. Consequently, in the southern part of the bay the delta advances fast; new channels are formed and pioneer species are conquering the new territory: *Phragmites australis, Typha angustifolia, Salix trianda*, *Salix fragilis*, *Salix alba*, *Salix cinerea, Scirpet* associations, etc. (Fig. 4). The aquatic vegetation comprises among others *Nymphea* sp*., Nuphar lutea, Trapa natans*, *Potamogeton* sp., *Myriophyllum* sp., *Utricularia* sp. *Salvinia natans*, *Lemna* sp*., Wolffia arrhiza, Utricularia vulgaris, Spirodela polyrrhiza*, etc. (Fig. 5).

The shallowness of this ecosystem and the strong winds occurred especially from the N-E favor the sediment resuspension; consequently, the amount of organic matter in sediment is very low and the zoobenthic community is rather poorly developed in comparison with other aquatic ecosystems of the delta (Dobre, 2000, 2006; Radu *et al*., 2008).

This paper presents the results of the researches carried out on zoobenthic community from Musura Bay between 2005-2007 and represents a part of a larger project which investigated the structural and functional characteristics of the bay's biological communities during this interval.

MATERIAL AND METHODS

The sediment samples have been taken from five stations (Fig. 6), in May, July and October 2005-2007, with a Corer-type device; the surface of the sampling unit was 86.54 cm^2. According to different authors (Green, 1969; Elliot, 1977), the Corer has several advantages: a relatively low disturbance of the sediment-water interface, high efficiency in sampling different substrates, analysis of a higher number of replicates due to the reduced volume of sample units and increased statistical relevance.

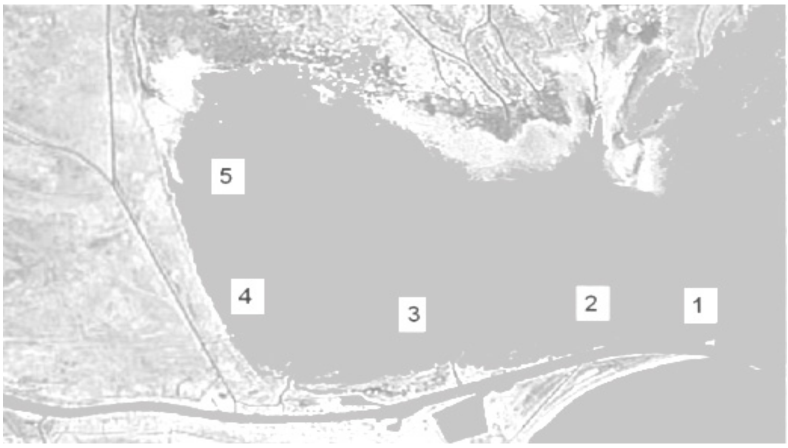

Fig. 6 – Location of sampling sites in Musura Bay (www.earthgoogle.com).

The samples were washed on site through nets with 230 µm mesh size, the material being placed in plastic bags and preserved with formaldehyde 4%. They were analyzed using a stereomicroscope Zeiss. The wet biomass was determined by weighing the organisms on analytical balance Feinwaage, while for the determination of dry biomass the organisms were dried at 60^o for 24 h.

The structural and functional parameters (productivity) were determined based on the raw data. The amount of organic matter accumulated in the biomass and transferred to the next trophic level was transformed in energetic units using the caloric equivalent of dry biomass determined by different authors (Ivlev 1939; Winberg, 1972).

RESULTS AND DISCUSSION

During the investigated period, at structural level, a low number of taxonomic groups was recorded (average: 6-7 groups), encompassing a low abundance and biomass. Besides the eutrophication, which led to a marked decrease of biodiversity (Brezeanu *et al*, 1991), another possible cause which led to these low values was the lack of food resources, as the amount of organic matter in sediment was extremely low (Table 1), in comparison with the values recorded in other lakes (Dobre, 2006).

Table 1

Physico-chemical characterization of Musura Bay between 2005 and 2007

Parameter	**May 2005**	**July 2005**	**October 2005**	**May 2006**	**July 2006**	**September 2006**	**May 2007**	**July 2007**	**October 2007**
Depth (m)	1.64	1.56	1.66	1.44	1.68	1.34	0.68	1.20	1.04
Transparence (m)	0.74	0.38	0.36	0.78	0.36	0.70	0.58	0.88	0.44
T/D	0.47	0.25	0.23	0.60	0.23	0.54	0.90	0.74	0.43
pH sedim.	7.63	8.26	8.04	7.548	7.55	7.738	7.37	7.644	8.584
Temperature sedim. (C^{o})	24.18	27.32	23.70	18.42	25.32	22.28	14.68	25.80	17.76
Org.mat. sedim. (%)	4.61	9.086	8.004	5.142	9.62	6.04	5.344	1.212	2.056

The dominant groups, in abundance and biomass, were the chironomids (the annual average ratio ranging between 48.8-52.6 % for abundance and 50.7-54.3 % for biomass) and oligochaets (annual average ratio 29.6-33.1 % for abundance and 38.1-42%), followed by polychaetes, gasteropods and isopods (Tables 2, 3). The other taxonomic groups, represented by nematods and amphipods, recorded very low biomasses. The relative high frequency of the occurrence of polychaetes in Musura Bay, especially in the area located close to the bay's mouth, is a characteristic of this aquatic ecosystem due to its former salinity (Surugiu, 2002, 2005), this group occurring only rarely in most of the Danube Delta lakes. The chironomids species with constant frequence of occurrence and dominant in abundance and biomass were those belonging to *Chironomus* and *Cryptochironomus* genera, while the dominant oligochaetes species belong to Tubificidae family.

Table 2

The abundance of zoobenthic groups (ind/m^2) in Musura Bay between 2005 and 2007

Taxonomic group	**2005**				
	May	**July**	**October**	**Annual average**	**Ratio (%)**
Nematoda	39	70	39	49	4.16
Polychaeta	85	47	39	57	4.84
Oligochaeta	393	362	416	390	33.13
Isopoda	62	16	16	31	2.63
Amphipoda	93	8	8	36	3.05
Gasteropoda	23	31	16	23	1.98
Chironomidae	763	447	562	591	50.21
Total	1458	981	1096	1177	100
Taxonomic group	**2006**				
	May	**July**	**September**	**Annual average**	**Ratio (%)**
Nematoda	31	31	16	26	2.95

Polychaeta	62	31	8	34	3.86
Oligochaeta	301	278	262	280	31.85
Isopoda	8	31	23	21	2.38
Amphipoda	54	8	8	23	2.66
Gasteropoda	23	54	23	33	3.75
Chironomidae	524	416	447	462	52.55
Total	1003	849	787	879	100
Taxonomic group	**2007**				
	May	**July**	**October**	**Annual average**	**Ratio (%)**
Nematoda	47	70	47	55	5.27
Polychaeta	85	69	47	67	6.46
Oligochaeta	362	293	270	308	29.55
Isopoda	39	39	23	34	3.26
Amphipoda	70	23	16	36	3.45
Gasteropoda	39	47	16	34	3.26
Chironomidae	593	431	501	508	48.75
Total	1235	972	920	1042	100

Table 3

The biomass of zoobenthic groups (g/m^2) in Musura Bay between 2005 and 2007

Taxonomic group	**2005**				
	May	**July**	**October**	**Annual average**	**Ratio (%)**
Nematoda	0.042	0.0678	0.0374	0.049	0.13
Polychaeta	2.944	1.628	1.17	1.914	5.36
Oligochaeta	15.744	12.65	16.612	15.002	42.02
Isopoda	0.778	0.148	0.18	0.368	1.05
Amphipoda	0.1748	0.0156	0.015	0.068	0.19
Gasteropoda	0.202	0.264	0.17	0.212	0.59
Chironomidae	21.486	12.586	20.19	18.087	50.66
Total	41.37	27.359	38.374	35.7	100
Taxonomic group	**2006**				
	May	**July**	**September**	**Annual average**	**Ratio (%)**
Nematoda	0.036	0.03	0.014	0.026	0.1
Polychaeta	2.166	1.074	0.266	1.168	4.55
Oligochaeta	10.668	10.442	8.584	9.898	38.63
Isopoda	0.102	0.328	0.254	0.228	0.88
Amphipoda	0.1	0.014	0.014	0.042	0.2
Gasteropoda	0.256	0.568	0.234	0.352	1.37

Chironomidae	15.702	13.238	12.78	13.906	54.27
Total	29.03	25.694	22.146	25.62	100
Taxonomic group	**2007**				
	May	**July**	**October**	**Annual average**	**Ratio (%)**
Nematoda	0.052	0.0638	0.0514	0.055	0.2
Polychaeta	2.934	2.464	1.53	2.309	7.5
Oligochaeta	13.656	11.188	10.348	11.73	38.1
Isopoda	0.48	0.42	0.242	0.38	1.23
Amphipoda	0.1328	0.0444	0.029	0.068	0.22
Gasteropoda	0.416	0.42	0.17	0.335	1.08
Chironomidae	17.948	12.328	17.446	15.907	51.67
Total	35.618	26.928	29.816	30.784	100

In comparison with other lakes of the Danube Delta, the abundance and biomass recorded by the benthic community of Musura Bay were lower. For instance, Erenciuc Lake, a lake of riverine origin located in the southern part of the delta, is an eutrophic aquatic ecosystem, where the different colmation degree and retention time lead to a different structure of the benthic community (Zinevici *et al.*, 2006 b): *e.g.* the average abundance recorded between 2005-2007 was 1033 ind/m^2, while in Erenciuc Lake the average abundance was 1338 ind/m^2 (between 2002-2004); the average biomass was 42.83 g/m^2 in Erenciuc and 30.70 g/m^2 in Musura Bay, the dominant groups being chironomids and oligochaetes, as recorded in Musura (Dobre, 2006) (Figs. 7, 8).

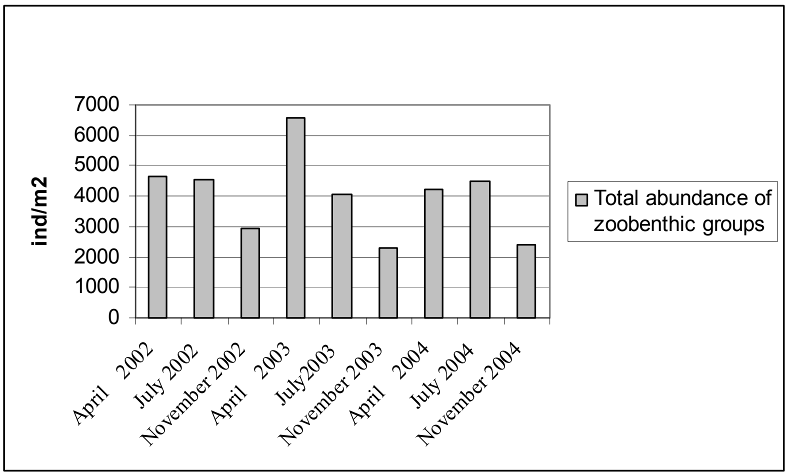

Fig. 7 – Dynamics of the abundance of zoobenthic community from Erenciuc Lake between 2002 and 2004.

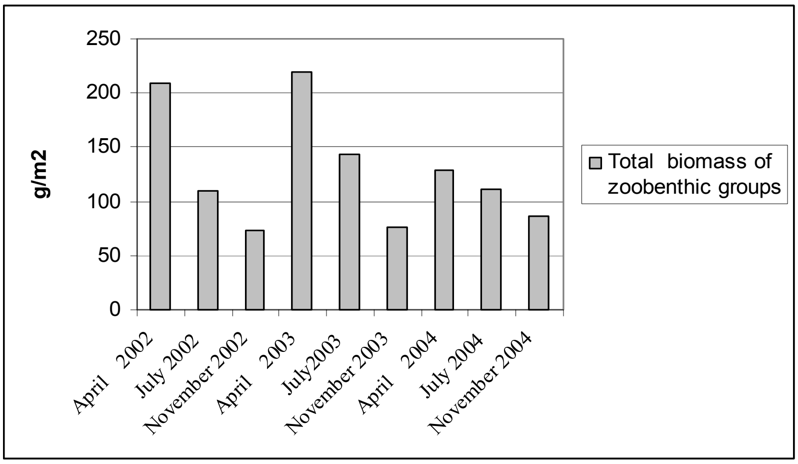

Fig. 8 – Dynamics of the biomass of zoobenthic community from Erenciuc Lake between 2002 and 2004.

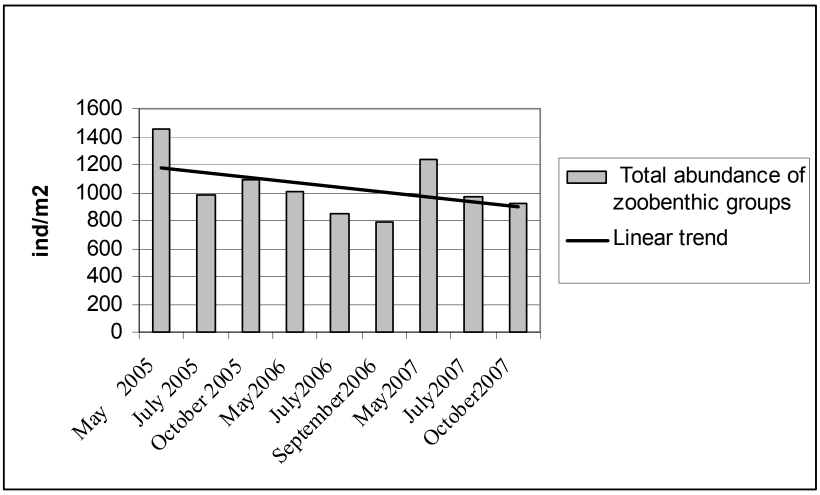

Fig. 9 – Dynamics of the abundance of zoobenthic community from Musura Bay showing the decreasing trend recorded between 2005 and 2007.

During the investigated period, in Musura Bay the dynamics of zoobenthic abundance and biomass recorded a decreasing trend (Figs. 9, 10). The maximum value of the abundance was recorded in May 2005 (1458 ind/m^2), while the minimum was reached in September 2006 (787 ind/m^2); the biomass reached the maximum in May 2006 (45.19 g/m^2) and the minimum in July 2007 (26.92 g/m^2).

The annual average productivity of the dominant groups (chironomids and oligochaetes) in Musura Bay between 2005-2007, ranged between 0.097 kcal/m²/month) in 2006 and 1.186 kcal/m²/month in 2005, respectively between 0.749 kcal/m²/month in 2006 and 1.140 kcal/m²/month in 2007 (Figs. 11, 12, 13).

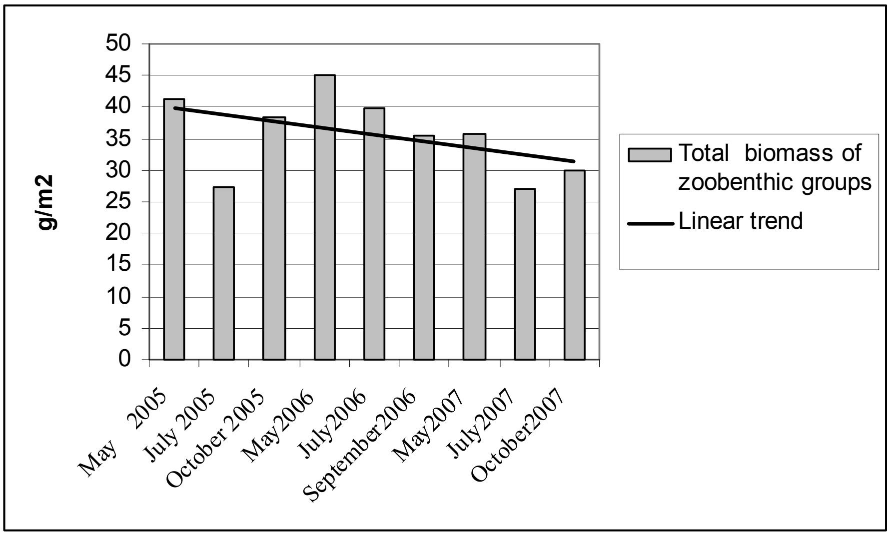

Fig. 10 – Dynamics of the biomass of zoobenthic community from Musura Bay showing the decreasing trend recorded between 2005 and 2007.

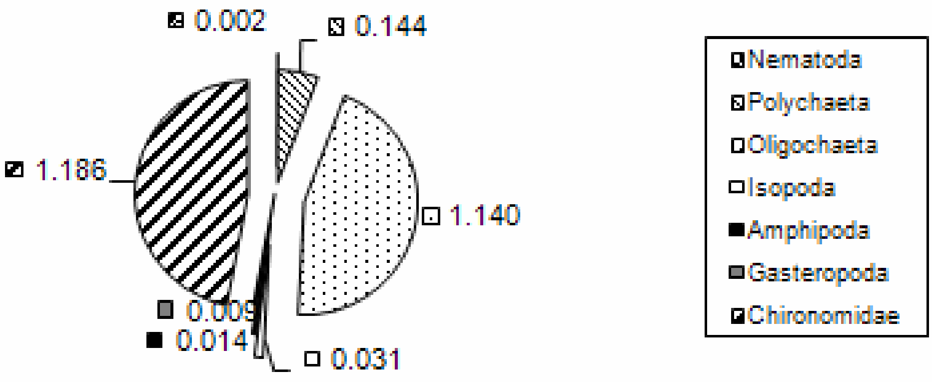

Fig. 11 – Productivity of zoobenthic groups in Musura Bay in 2005 (kcal/m²/month).

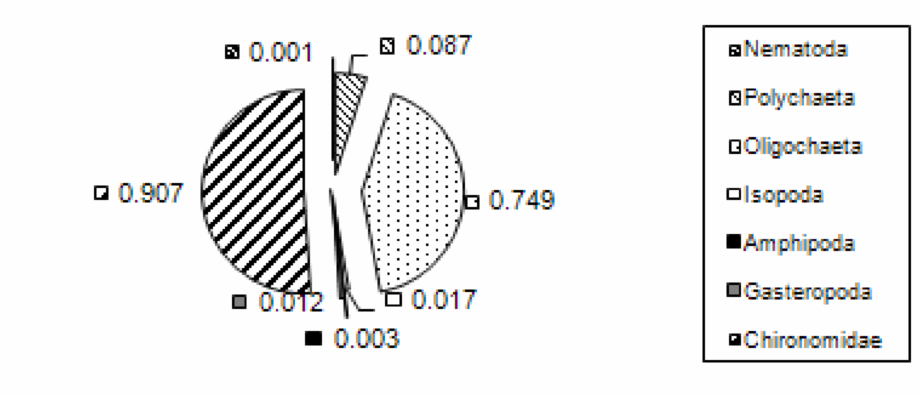

Fig. 12 – Productivity of zoobenthic groups in Musura Bay in 2006 (kcal/m²/month).

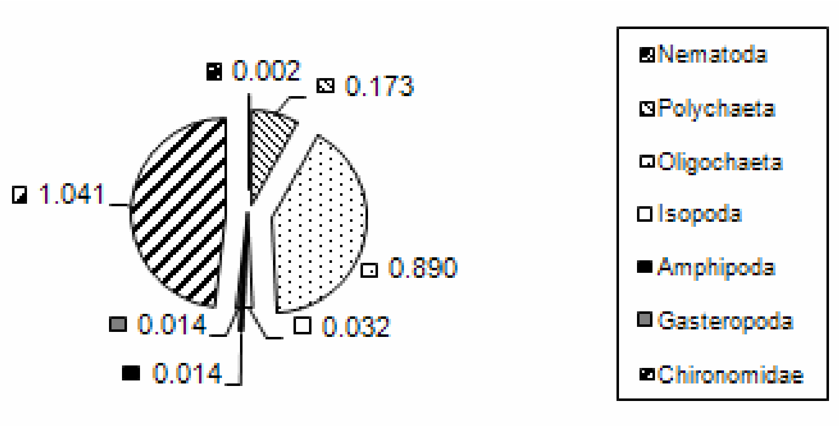

Fig. 13 – Productivity of zoobenthic groups in Musura Bay in 2007 (kcal/m²/month).

The productivity of the other groups was very low, ranging in a narrow interval: polychaetes between 0.087 kcal/m²/month (2006) – 0.144 kcal/m²/month (2005), isopods 0.017 (kcal/m²/month 2006) – 0.032 kcal/m²/month (2007), amphipods 0.003 kcal/m²/month (2006) – 0.014 kcal/m²/month (2007), gasteropods 0.009 kcal/m²/month (2005) – 0.014 kcal/m²/month (2007).

The two dominant groups, the chironomids and oligochaetes, had the highest productivity, their role in the accumulation and transfer of matter and energy to the higher trophic level (benthic fishes) being extremely important (Rîşnoveanu, 1993; Rîşnoveanu & Vădineanu, 2000; Ionică *et al.*, 2008).

Using the functional relationships of Lellak (1965), Thayer *et al.* (1973) and Hanson & Leggett (1982), we estimated indirectly their role in the transfer towards the benthic fishes. The results obtained for Musura Bay between 2005-2007 have shown that the trophic basis provided by the chironomids could support between 47.7 kg fish/ha (July 2006) and 77.4 kg fish/ha (May 2005) (Fig. 14), while the oligochaetes could support between 38.2 kg fish/ha (July 2006) and 59.8 kg fish /ha (October 2005) (Fig. 15).

However, the trend recorded during this interval indicated a reduction of the trophic basis provided by chironomids and oligochaetes, and consequently, of the potential fish biomass supported by them. This biomass represents the equivalent of the maximum biomass supported by the two dominant groups, in the absence of any other disturbance such as variation of abiotic factors (*e.g.*: the food up-take by benthic fishes is influenced by the oxygen content at the interface water-sediment; Nicolski, 1962; Oțel, 2007), ichtyophage waterfowls, anthropogenic impact, etc. which may limit significantly the fisheries production.

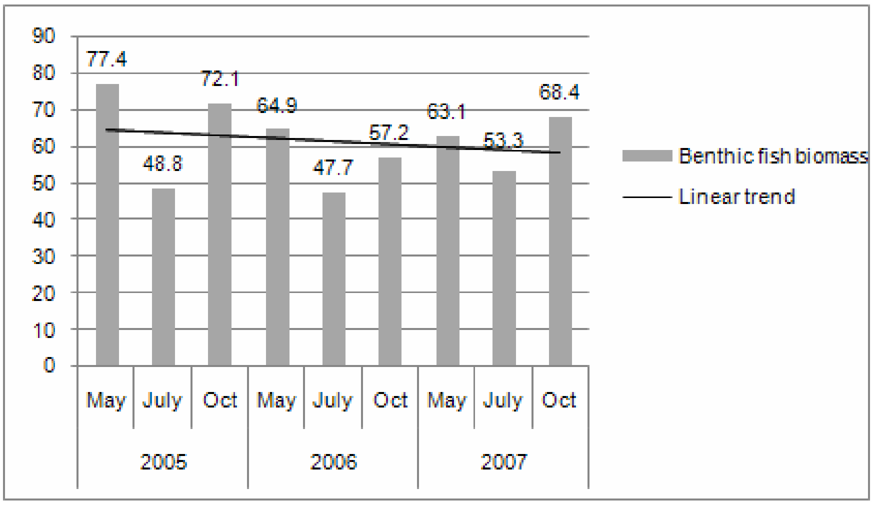

Fig. 14 – The estimated values of benthic fish biomass that could be supported by the chironomids community in Musura Bay between 2005 and 2007.

The eutrophication occurred after 1980s in the Danube Delta lakes had a dramatic impact on the aquatic food webs; among other major changes, the support of benthic fish production provided by chironomids and oligochaetes was considerably diminished (Diaconu, 1985; Ignat *et al*., 1986; Diaconu *et al*., 1994). Nowadays, although the water quality improved and the plankton communities seem to recover (Zinevici & Parpală, 2007), the structural and functional parameters of the zoobenthic community are still below those recorded before 1980s.

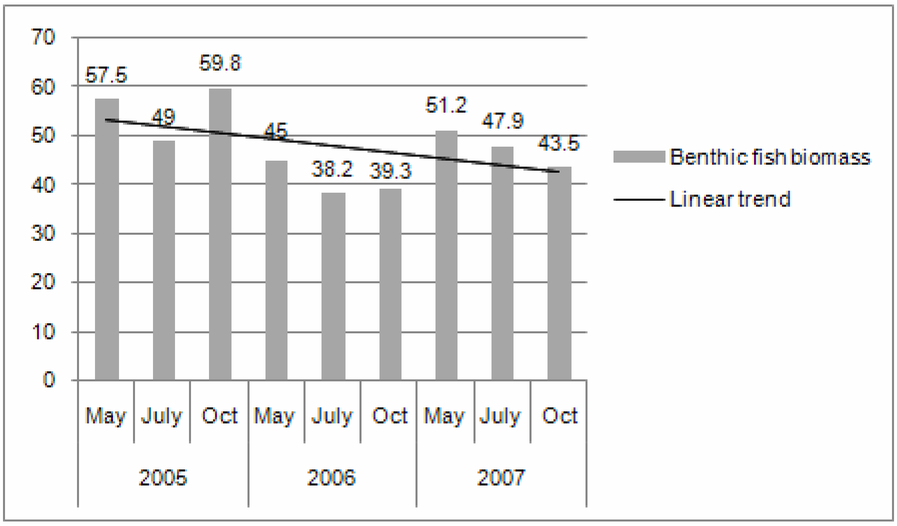

Fig. 15 – The estimated values of benthic fish biomass that could be supported by the oligochaetes community in Musura Bay between 2005 and 2007.

The eutrophication occurred after 1980s in the Danube Delta lakes had a dramatic impact on the aquatic food webs; among other major changes, the support of benthic fish production provided by chironomids and oligochaetes was considerably diminished (Diaconu, 1985; Ignat *et al*., 1986; Diaconu *et al*., 1994). Nowadays, although the water quality improved and the plankton communities seem to recover (Zinevici & Parpală, 2007), the structural and functional parameters of the zoobenthic community are still below those recorded before 1980s.

CONCLUSIONS

The high amount of alluvia brought by Kylia and Sulina branches, together with the shift of maritime currents in the Northern Black Sea, lead to the formation of a sand bank at the bay's mouth, which limited gradually the inflow of marine water, determining the decrease of salinity and the shift towards a freshwater lagoon.

During the investigated period, the zoobenthic community was characterized by low diversity, abundances and biomasses, being rather poorly developed in comparison with the communities from other lakes of the Danube Delta.

The dominant groups were chironomids and oligochaetes, these groups having the most important role in the transfer of matter and energy to the higher trophic link, the benthic fish; besides these groups, polychaetes were identified, especially in the proximity of the Black Sea.

The changes occurred in the aquatic biocenoses of the Danube Delta as a consequence of the eutrophication had significant negative effects on the food-webs, reducing the biological diversity and diminishing the support of zoobenthic community as trophic basis for the benthic ichthyofauna.

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INFRASTRUCTURAL AND MOLECULAR TUMOR-EXTRACELLULAR MATRIX INTERFACE ALTERATIONS IN THE BASOCELLULAR AND SQUAMOUS CELL CARCINOMA DEVELOPMENT

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FLORIN DANIEL JURAVLE**, DORINA MIRANCEA*

The present study deals with the infrastructural changes and some associated molecular alterations at the tumor-stroma interface. We focus our interest on the dynamics changes in the epidermal carcinomas phenotypes (basal epithelioma and squamous cell carcinoma), especially at the tumor-stroma interface, corelated with the desmosomal and hemidesmosomal junctions as well as the basement membrane aspects during degradation in the process of tumor cells invasion. Multiple invadopodia processes are expressed both by basocellular and squamous cell carcinomas. Plasma membrane recombinations between tumor and stromal cells were recorded. Extravasated inflammatory cells contribute to the activated tumor-stroma formation. Molecular investigations at the ultrastructural level for keratin filaments (panKeratin and Keratin K14) as well as for Plectin/HD1 were made. Compared to healthy epidermis, Plectin/HD1 (as connector molecules for keratins) is missing in the case of tumor cells affronted by stroma. K14 does not show a positive signal in the basal cytoplasm of the basal pole. That correlates with the defective hemidesmosomes for the inner plaque (or total absence of hemidesmosomes) at the invadopodia level.

Key words: basal carcinoma, squamous cell carcinoma, defective hemidesmosomal junction, plasma membrane recombination, invadopodia, pankeratin, Keratin 14, Plectin (HD1).

INTRODUCTION

In humans, the large majority of human cancers have an epithelial origin accounting for circa 80% of all malignancies. Malignant tumors are represented by (1) tumor cells and (2) tumoral stroma. Many solid tumors arise from sites of chronic physical-chemical irritation and inflammation. Inflammation is a very important player of the tumor progression (Coussens & Werb, 2002).

Basal cell carcinoma is the most common form of skin cancer. The high rate of the risk of developing basal cell carcinoma is increased for individuals with a family history of the disease and with a high cumulative exposure to UV light or carcinogenic chemicals. Basal cell carcinoma is a malignant epithelial tumor arising only in the skin, from the basal layer of the epidermis or of the pilosebaceous adnexa. It appears as a firm nodule, growing within the skin and below it, rather than on the surface. Although basal cell carcinoma rarely metastasizes, it grows locally without stopping; untreated can be disfiguring.

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Squamous cell carcinoma is a malignant tumor of epidermal keratinocytes and accounts for *cca.* 20% of cutanneous malignancies. High cumulative dose of UV radiation, exposure to some carcinogens, chronic immune suppression, HPV types 5, 6, 8, 11 and 16-infection and DNA repair failure are the major causes of squamous cell carcinoma.

In situ, squamous cell carcioma is histologically defined by atypia involving the full thickness of the epidermis but without invasion into the dermis. However, mention must be made that squamous cell carcinoma can grow invasively and metastasizes in ectopic places, most often to the lung.

A solid tumor is an organ-like entity comprised of neoplastic genetic altered cells and non-transformed host stromal cells embedded in an extracellular matrix (Fukumura & Jain, 2007; Mirancea *et al*., 2009).

For many years, most studies of neoplastic transformation have focused on the unit of the malignant cell. Interactions between neoplastic cells and host tissue (cells and extracellular matrix components) are determining in the carcinoma architecture and in the invasive process of malignant epithelium cells.

There are old and recent credible published studies which demonstrate the oncogenic role of the stroma. Recent work has begun to illustrate previously unappreciated or less appreciated oncogenic functions of the stromal tissue and to contribute with few considerations to the carcinogenesis knowledge. These oncogenic functions stimulate the transformation of adjacent (or in close vicinity) cells through transient signalling that mainly results in disrupting cell polarity (Mirancea *et al*., 2002, Mueller & Fusenig, 2004).

In multicellular organisms, all organs live and function thanks to the planned, harmonious disciplined cooperation of different types of cells (Mirancea & Mirancea, 1998). Unfortunately, in some circumstances, focal accidents can disrupt this harmony.

To answer the major question of what determines tumour cells remain in one place retaining their associations with their neighbours and basement membrane (desmosomal, respectively hemidesmosomal junctions) or dissociate and move elsewhere to arrest at the ectopic sites, creating secondary lesions, should arise from complex investigation at the ultrastructural and molecular level of changes in cell adhesion properties (Mirancea *et al*., 2009).

The interaction with extracellular matrix, including basement membrane, is critical for maintenance of tissue homeostasis (Glick & Yuspa, 2005).

Invasive growth of malignant tumours is often associated with loss of differentiation and cell polarization as well as decrease or absence of basement membrane and, finally, invasive growing of malignant cells into peritumoral stroma. In order to study the significance of these parameters, here we focus our investigations at the high resolution microscopy on the *in situ* basal carcinoma and squamous cell carcinoma. In order to detect some molecules of interest involved in the above mentioned events, their distribution was investigated by immune electron microscopy.

MATERIAL AND METHODS

Basal cell carcinoma tissue

Small fragments of tumor skin resulted by a surgical therapy from a patient suffering from basal cell carcinoma (the surgeon got patient consent) were processed for electron microscopic investigation.

Transplantation of HaCaT II-4 RT cell cultures onto athymic nude mice and subcutaneous injected HaCaT II-4 RT cells into athymic nude mice

Transfection procedure of immortal HaCaT cells to obtain HaCaT-*ras* clone II-4 (malignant) was described in detail by (Boukamp *et al*., 1990). Briefly, HaCaT II-4 RT cells were transplanted onto congenitally athymic nude mice as organotypic culture grown on type I collagen gels (Willhauck *et al*., 2007). Isolated HaCaT II-4 RT cells were subcutaneously injected in athymic nude mice (Vosseler *et al*., 2005). Transplants as well as subcutaneously developed tumors were excised at 2-3 weeks and processed for electron microscopic investigation.

Transmission Electron Microscopy

Specimens for electron microscopy were pre-fixed in 2.5-3% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.4 at 4° C for minimum 2h and post-fixed in 2.5% osmium tetroxide in 0.1 M cacodylate buffer for 2h at room temperature. The specimens were then over night stained in block with aqueous 0.5% uranyl acetate and dehydrated in a graded series ethanol, then infiltrated with propylene oxide followed by embedding in Glycidether 100 (Epon 812 equivalent). 1 µm as semithin sections were stained with toluidine blue for light microscopic examination. Ultrathin sections of 80–90 nm were obtained with an ultramicrotome equipped with a diamond knife, double counterstained with uranyl acetate and lead citrate, and investigated in an electron microscope operated at 80 kV.

Immune electron microscopy

In order to detect some molecules of interest (pankeratin, keratin K14, Plectin/HD1) at the ultrastructural level, a post-embedding immune electron microscopic method was applied to some biologic specimens following the protocol as it was described elsewhere (Mirancea *et al*., 2007). Briefly, ultrathin sections of 70-90 nm collected on nickel grids (300 mesh) covered with formvar were incubated with first antibodies overnight, followed by secondary antibodies conjugated with 5 or 10 nm gold particles (Aurion/BioTrend, Cologne, Germany) at room temperature for 2-3 hours, and counterstained as for transmission electron microscopy. In order to increase the sensitivity for immune detection, 1 nm gold conjugated antibodies were used, followed by silver enhancement according to the manufacturer's protocol (silver enhancement *Aurion* kit, The Netherlands).

RESULTS AND DISCUSSION

Basal cell carcinoma (basalioma)

Tumors developed by epidermal epithelium at the tumor-stroma interface showed an irregular front of growth. The most striking observation on the tumor epithelium refers to the very severe impairment of cell layering and loss of cell polarization for the cells affronted by stroma.

Usually, desmosomal junctions of the malignant cells of the tumor basal cell carcinoma are scarcely and impaired (Fig. 1). Inside of some tumor cells, internalized desmosomes can be detected (not shown). At the tumor-stroma interface, the basement membrane is missing (Figs. 1-3), except for some short profiles (Figs. 4-6). Hemidesmosomes are absent or, if some, then these are defective for the inner plaque and, consequently, the keratin filaments do not abut the basal aspect of the plasma membrane (Figs. 1-6). Very occasionally, scanty intermedium filaments connect some defective hemidesmosomes (Figs. 5-6). The contour of the borderline tumor-stroma interface can be relatively linear but, quite often, tumor cells in basal position extend cell projections termed invadopodia (Figs. 4-6). Mention must be made that, because of high plasticity/fragility, to some extent, plasma membrane of the invadopodia performed cell membrane recombination with adjacent stromal cell (Fig. 4) or showed short profiles of dissolution, as is depicted in Fig. 5 and detailed in Fig. 6. Interestingly, sometimes, inside of the peritumoral stroma, mummy cells can be seen (Fig. 7).

Squamous cell carcinoma

Concerning the tumor tissue phenotype resulted by experimentally reproduced squamous cell carcinoma as heterotransplanted Ha-*ras* transfected HaCaT cells first observation is that normally epithelial cell stratification is abolished.

Similar to the *in situ* basal cell carcinoma, there are evident tumor-stroma interface changes involving both epithelial tumor cells and adjacent stroma. Tumor cells showed the shedding vesicles process at their contact with stromal tissue (Fig. 8). Inside of the peritumoral stroma, microvessels are very fragile, so that, in high amount red and white blood cells can be detected in very close vicinity to the tumor mass (Figs. 8-9), even among tumor cells (not shown).

Some individual malignant cells from the growth front of the tumor mass tend to detach from the tumor (Fig. 10). The detachment and penetration inside of the stroma are facilitated by the impaired or missing desmosomal junctions between invading tumor cells and the rest of tumor mass. Moreover, some invadopodia and a pletora of shedding vesicles can be seen around the invading tumor cells (Fig. 10). Malignant cell extensions as invadopodia play a major role in cancer cell motility/invasion (Friedl & Wolf, 2003).

The tumor microenvironment differs considerably from that of normal tissue where the tumor originates from. In both basal and squamous cell carcinoma tumors we analysed, a lot of inflammatory cells are visible inside of the peritumoral stroma. The extravasated blood cells we observed can be related to the high fragility of peritumoral microvasculature. Due to a low glucose level and hypoxia as well as a low pH installed inside of the tumor epithelia, new but impaired blood vessels are developed inside or around the tumor mass (Vosseler *et al*., 2005, Miller *et al*., 2005). Hypoxia generates oxygen free radicals which may induce DNA mutations (Laconi, 2007). As a consequence, from time to time, new tumor cell phenotypes will appear and the therapeutic protocols must accordingly be changed.

Similar to the basal keratinocytes in normal epidermis, in both cases of epidermoid carcinomas, at the interface with peritumoral stroma there are tumor epithelial keratinocytes in basal position, but different from the normal epidermis, tumor cells showed remarkable infrastructural alterations. The most conspicuous above mentioned infrastructural alterations induce remarkable cell polarisation changes. As a consequence of the peritumoral stroma alteration, including basement membrane destruction leading to the enhanced release of growth factors and cytokines, cell polarity, including cell cohesions, are severely affected (Breitkreutz *et al*., 2009).

Membrane fusion is a physiological process characteristic to normal cells because it is involved in membrane biogenesis, intracellular traffic, cell secretion, (Leabu, 2006) as well as neuritogenesis, synaptic transmission, viral infection, etc. (Tareste *et al.,* 2008).

In order to grow invasive, tumor cells take advantage of the homeostatic mechanisms of the host involved in normal tissue regeneration and repair. Our electron microscopic investigations clearly showed that tumor cells exhibit a high fragility of plasma membrane as can be documented by the pictures showing plasma membrane dissolution emphasized by the tumor cells affronted with peritumoral stroma (Figs. 5-6). That may explain why tumor cells performed *in situ* recombination of their plasma membrane with plasma membrane of the adjacent normal stroma cells (Fig. 4). Bukovsky *et al*. (2001) showed that malignant cells may hybridize *in situ* with activated host stem cells, resulting malignant-normal cell hybrids.

Indeed, the high plasticity of the plasma membrane belonging to tumor cells involved in basal carcinoma development suggests the possibility of malignant-normal cell hybrids formation. If so, that may become a big advantage to overcome host mechanisms of defence against aggressive growth of tumor.

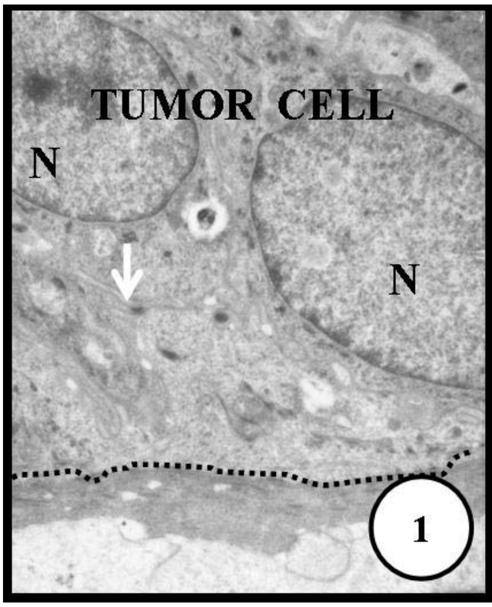
Epithelial tumor-stromal interactions are necessary for tumor growth, invasive behavior and ectopic development of microtumors. There are a lot of known cell and molecular events which took place in order to support such kind of processes involved in patho-physiology. In this context, extracellular matrix metalloproteinases contribute very much to the invasive growth of tumor cells (Fusenig *et al*., 2002; Vosseler *et al*., 2005).

The reticulo-epithelial cellular network of the most epithelial organs stromal cellular microenvironment plays a pivotal role in normal growth and in maintaining homeostatic functions. Stroma cells produce numerous cytokines with vital role in normal functioning of the epithelial components of the organs as is the case for the thymus (Bodey, 2007).

Both in basal cell carcinoma and squamous cell carcinoma tumors collected from patient *in situ* EM analysis showed numerous shedding membrane vesicles at the tumor-stroma interface, formerly described in postgrafting HaCaT II- malignant cells (Mirancea *et al*., 2002). To some extent, similar alterations are described by Hashimoto *et al*. (1973) *in situ* human squamous cell carcinoma.

According to other opinions (Dolo *et al*., 1995; Neuener *et al*., 1996), we consider the plethora of shedding membrane vesicles as a possibility of tumor cells to escape of immune surveillance. Our observations emphasized that such kind of microvesicles contain lysosomes, meaning proteolytic enzymes and, of course, other kinds of molecules. In an elegant study, Sidhu *et al*. (2004) showed that tumor shedding vesicles is a cargo infrastructure carrying a very important type I transmembrane glycoprotein termed EMMPRIN (extracellular matrix metalloproteinase inducer), a tumor derived factor (inducer) involved in the stimulation of the matrix metalloproteinases (MMPs) expression in fibroblasts (in a paracrine manner) which plays a major role in tumor-stromal interactions and hence facilitate tumor invasion and secondary mini-tumors formation at the ectopic places (metastasis), far from the primary tumor. The de-structured basement membrane in front of the invasive front of tumor cells is performed also with participation of the proteolysis enzymes including MMPs (Fusenig *et al*., 2002; Vosseler *et al*., 2005) so that we may assume that multiple shedding vesicles may be involved also in EMMPRIN deliverance (Tang *et al*., 2004).

Loss of cell-cell and cell-extracellular matrix junctions activates transition from low to high-grade human basal and squamous cell carcinoma. E-cadherin is a tumor suppressor gene. While histogenesis during embryo development is associated with specific Ca^2-dependent cell-adhesions formation, loss of E-cadherin function is involved in neoplastic lesion formation (Margulis *et al*., 2006).



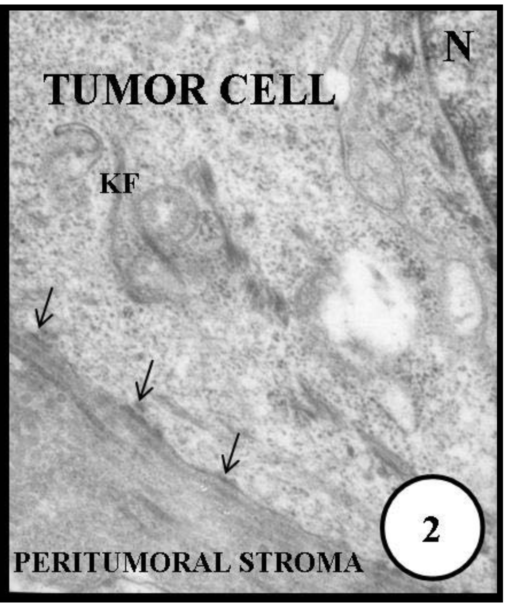


Fig. 1 – Basal pole of two tumor cells with euchromatic nuclei (N). White arrow marks an impaired desmosomal junction. Borderline between tumor cells and peritumoral stroma is delineated by black dots. (Basalioma, × 13,200).

Fig. 2 – A basal pole of a tumor cell. Keratin filaments (KF) do not abut defective hemidesmosomes (arrows). N = nucleus. (Basalioma, × 24,300).

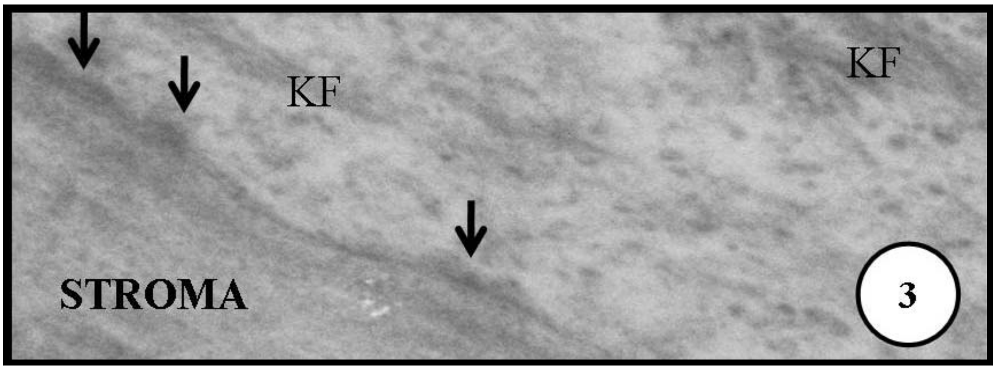


Fig. 3 – Details for defective hemidesmosomes (arrows) which do not show inner plaque and consequently keratin filaments (KF) fail to connect hemidesmosomes. (Basalioma, × 65,000).

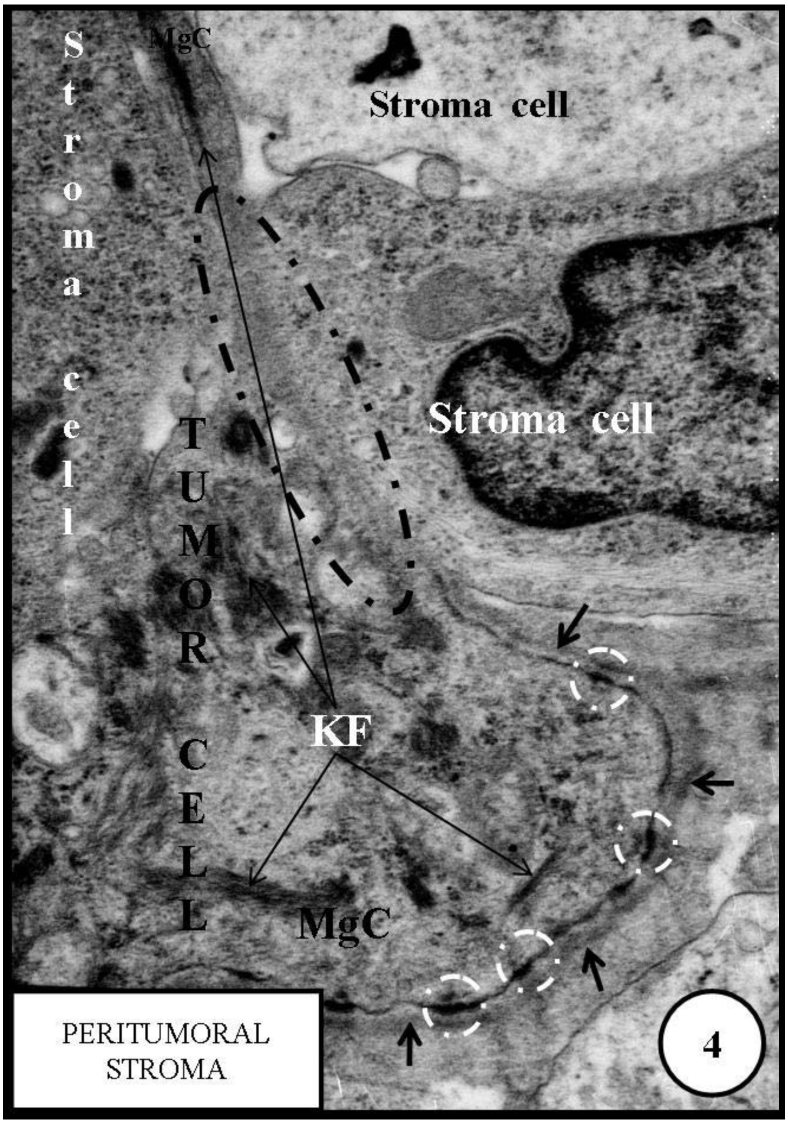


Fig. 4 – A sector of a malignant cell (MgC), showing an invadopodium which penetrates among stromal cells. To some extent, the plasma membranes of the tumor cell and an adjacent stroma cell (elliptic area delimited by black dots and lines) perform a recombinant membrane process. At the border with amorphous peritumoral stroma, invadopodium is delineated by a basement membrane (black small arrows). Keratin filaments (KF) fail to connect defective hemidesmosomes (encircled areas). (Basalioma, × 30,700).

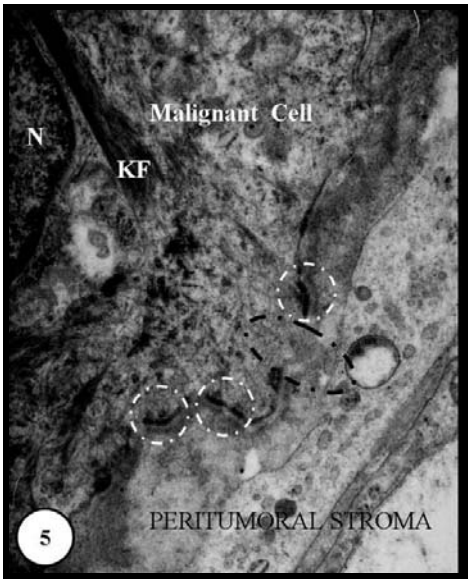


Fig. 5 – A basal pole of a malignant cell shows an illusive limit with peritumoral stroma (black elliptic area). Very scanty keratin filaments (KF) abut defective hemidesmosomes (encircled areas by white lines and dots). N = nucleus. (Basalioma, × 24,500).

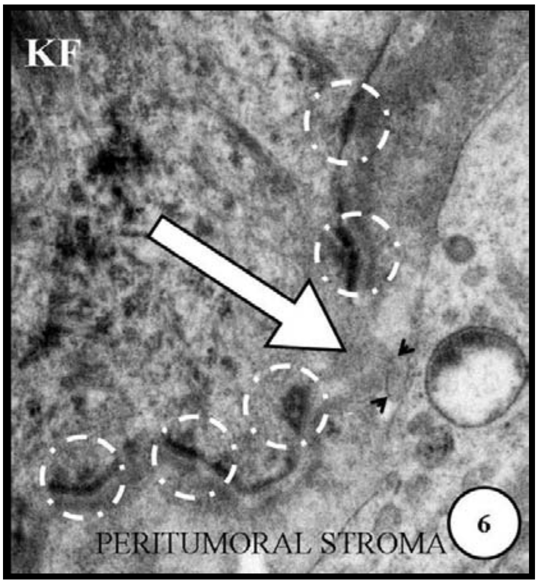
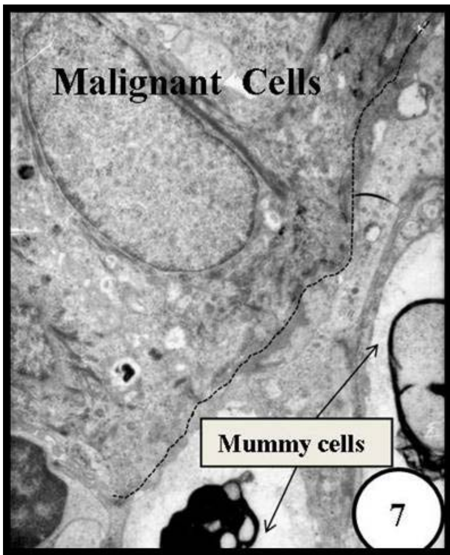
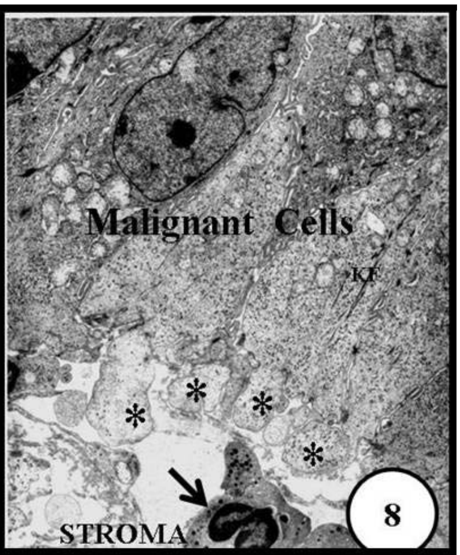


Fig. 6 – Detail for Fig. 5. White arrows mark the dissoluted plasma membrane and cytoplasm herniates inside of the adjacent stroma (head arrows). KF = keratin filaments. Encircled areas mark defective hemidesmosomes. (Basalioma, × 37,000).





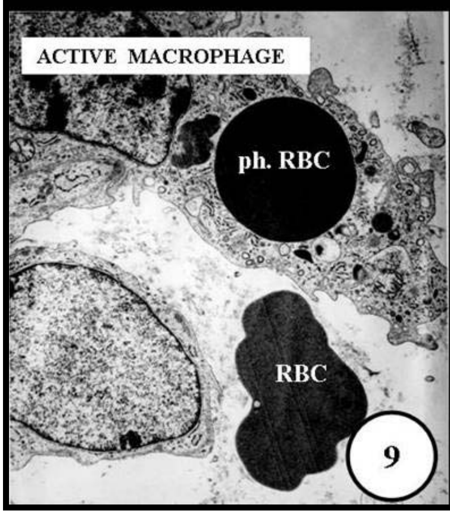


Fig. 7 – At the border of the malignant cells there are two mummy cells. (Basalioma, × 9,200).

Fig. 8 – Malignant cells show shedding vesicles (asterisks) inside of the adjacent stroma. Keratin intermedium filaments (KF) are scarce and far from the basal plasma membrane. An extravasated neutrophil granulocyte (arrow) comes in close vicinity of the malignant cells. (Squamous cell carcinoma, × 8,900).

Fig. 9 – Extravasated red blood cells (RBC), among which one (phRBC) is phagocytated by an activated macrophage. (Squamous cell carcinoma, × 9,900).

Fig. 10 – White stars mark the level of an individual malignant cell which tends to detach from the tumor mass to invade peritumoral stroma. Small arrows mark shedding vesicles in front of invasive cell. (Squamous cell carcinoma, × 8,700).

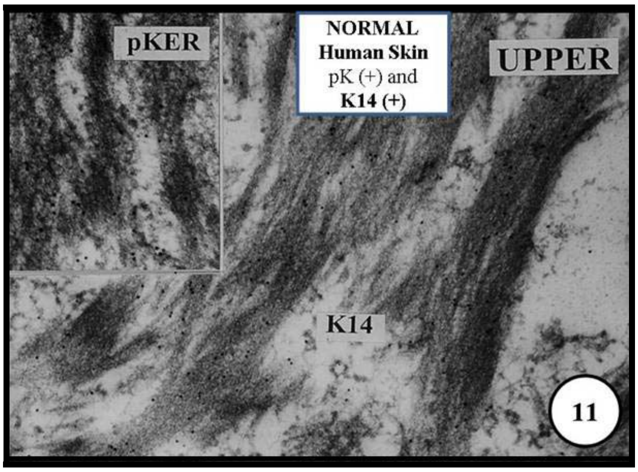


Fig. 11 – The upper part of a basal pole of a normal human epidermal keratinocyte shows positive immune reaction for both Keratin 14 (K14) as well as pankeratin (pKER, insert). (Normal human skin, × 53,000; insert × 61,000).

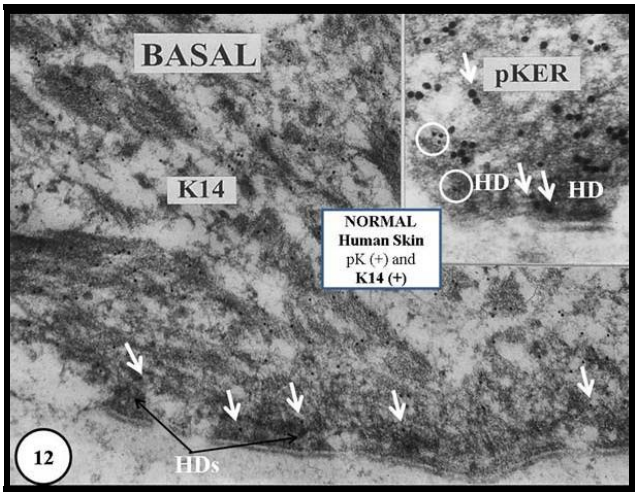


Fig. 12 – Basal pole of a normal skin basal keratinocyte shows positive immune reaction for keratin 14 (K14), including at the level of the hemidesmosomal junctions (HDs), (white arrows), (10 nm gold particles). In insert, pankeratin (pKER, white arrows) is detectable, including the hemidesmosomes (HD). Encircled areas show positive immune reaction for HD1/ plectin (1 nm gold particles + silver enhancement). (Normal human skin, × 51,000; insert × 75,000).

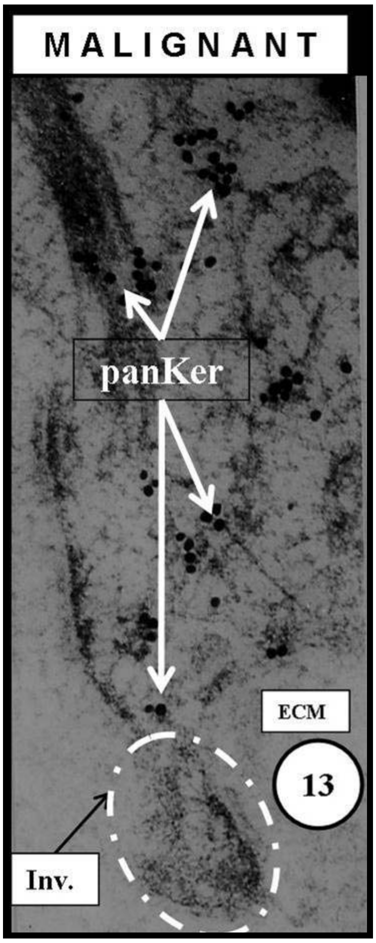


Fig. 13 – At the basal pole of a malignant cell, pankeratin (panKer) is detected by immune reaction, but is completely missing inside of the invadopodium (Inv; encircled area). ECM = extracellular matrix. (Subcutan injected HaCaT II-4 RT, × 100,000).

Indeed, impaired desmosomes or absence of desmosomal junctions as well as internalization of desmosomes (E-cadherin intercellular junction type) we have detected in both basal and squamous carcinomas sustains the theory consistent with the observation that disassembly of cadherin-cell adhesions is strongly correlated with tumorigenic epithelial-to-mesenchymal transition (Ivanov *et al*., 2005).

Immune electron microscopic examination

Taking into consideration that, similar to basal cell carcinoma, in case of tumors resulted by subcutan injected HaCaT II-4 RT cells, hemidesmosomal junctions are defective or even missing and consequently keratin filaments do not reach basal plasma membrane, we used the immune electron microscopic method to see the distribution of some molecules involved in those infrastructures. The immune electron microscopy applied to the healthy human skin showed positive reactions to pankeratin and K14 in the upper cytoplasm as well as in the basal cytoplasm of the basal keratinocytes (Figs. 11–12). When the immune electron microscopy was applied to the malignant tumor resulted by HaCaT II-4 RT cells at the ultrastructural level, K14 is missing in the basal cytoplasm of a tumor cell located at the interface with peritumoral stroma (not shown here, but see Mirancea *et al*., 2002). On the other hand, a positive signal for pankeratin was registered inside of basal cytoplasm but, mention must be made that, at the level of the invadopodia, no positive immune reaction was detectable (Fig. 13). Moreover, as the transmission electron microscopic investigation showed, the hemidesmosomes are defective for the inner plaque and, knowing that Plectin (also termed HD1) is a connector molecule for keratin filaments (Herrmann & Aebi, 2000; Mirancea *et al*., 2001), we check the immune reaction for this molecule both for the normal human skin as well as for the squamous tumor cells. While in case of the *in situ* healthy epidermis, Plectin (HD1) is clearly detectable at the hemidesmosomal junction (Fig. 12) that molecule is absent in case of the tumor cell counterpart (not shown). In such circumstances, missing Plectin molecule explains why cytoskeletal filaments of keratin do not enter the invadopodia. The consequence is the depolarization of the malignant cells and, consequently, facilitation of invasive behavior. Individual or cell masses migration is a very important process during morphogenesis, wound healing as well as during aberrant invasion and metastasis of malignant cells (Mirancea & Mirancea, 1998; Quigley & Armstrong, 1998; Vasiliev, 2004; Wang *et al*., 2005; Margulis *et al*., 2006; Breitkreutz *et al*., 2009; Mirancea *et al*., 2009).

CONCLUSIONS

Both transmission and immune electron microscopic investigations demonstrate that loss of cell-cell and cell-extracellular matrix junctions of epithelial cells affronted to the adjacent stroma lead to severe epithelial cell depolarization. High fragility of tumor cell plasma membrane (plasma membrane recombinations, invadopodia formation, shedding membrane vesicles and, even focal dissolution of plasma membrane) correlates with ectopic location or absence of some connector or cytoskeleton molecules. Altogether above mentioned alterations as well as basement membrane absence enhance the invasive ability of tumor cells inside of peritumoral stroma.

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