ROMANIAN SALT LAKES: SOME PHYSICAL-CHEMICAL FEATURES AND COMPOSITION OF BIOLOGICAL COMMUNITIES

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Abstract. The present paper deals with biological studies of natural salt lakes located in the Romanian plain. There were investigated for first time the halophilic microorganisms (bacteria and archaea) and also phyto- and zooplankton species in relation with physical-chemical parameters as control factors. The chemical composition of all tested lakes is marked by the abundant presence of chlorides (sodium, magnesium, potassium) and also some relatively important concentrations of aluminium and strontium detected in the particular case of Ocnele Mari. The biological diversity was represented by several types of archaea and halophilic bacterial species and some species of cyanobacteria and diatoms. There were also identified ciliates, rotifers and copepods mainly in the samples taken from Amara and Balta Albă. These lakes are considered hyposaline, because total chloride concentrations do not exceed 20 g L^{-1} and sodium appears to be absent. The results revealed the presence of magnesium and calcium ions and a chloride content that influences the phytoplankton and zooplankton diversity in these saline environments.

Keywords: salt lakes, halophilic archaea, plankton communities, saline ecosystems, salt biodiversity.

Rezumat. Lacuri sărate din România: caracteristici fizico-chimice și compoziția comunităților biologice. Lucrarea de față abordează studii biologice în lacuri naturale sărate din Câmpia Română. Au fost investigate pentru prima dată microorganismele halofile (bacterii și arhee), precum și speciile fitoplanctonice și zooplanctonice, în relație cu principalii factori de control fizici și chimici. Compoziția chimică a lacurilor testate este caracterizată de prezența abundantă a clorurilor (sodiu, magneziu, potasiu). Au fost detectate, de asemenea, concentrații importante de aluminiu și stronțiu (în special în cazul Ocnele Mari). Diversitatea biologică a fost reprezentată de câteva tipuri de arhee și specii bacteriene halofile, precum și specii de cianobacterii și alge diatomee. În probele prelevate din ecosistemele Amara și Balta Albă au fost identificate ciliate, rotifere și copepode. Aceste lacuri sunt considerate hiposaline, concentrația totală de cloruri nu depășește 20 g L^{-1} iar sodiul este absent. Rezultatele au relevat prezența ionilor de magneziu, calciu și a clorurilor, factori de control care influențează diversitatea fitoplanctonului și a zooplanctonului în mediile saline studiate.

Cuvinte cheie: lacuri sărate, arhee halofile, comunități planctonice, ecosisteme saline, biodiversitate halofilă.

INTRODUCTION

Salt lakes attracted researchers in the last period as a spring of novel microorganisms capable to grow and develop in extreme conditions, namely high ionic strength due to high salt content of water body of such environments. Following this approach, more than 150 species of halophilic archaea distributed in 40 valid published genera were found to populate saline and hypersaline environments (MINEGISHI, 2013; OREN, 2013). Regarded initially as low diversity areas, saline lakes appear to harbour a rich endemic biological diversity represented by brine shrimp *Artemia salina* (Linnaeus 1758), brine fly *Ephydra*, photosynthetic flagellates belonging to the genera *Dunaliella, Asteromonas, Synechococcus* and a lot of prokaryotes either Bacteria or Archaea, which represent the predominant organisms (VENTOSA et. al., 1998). The biodiversity of saline habitats, typical examples of extreme environments, is determined by several physical-chemical parameters like the chloride content, salinity and iron content, temperature and oxygen solubility, pH value (HAMMER, 1986; JAVOR, 1983; WILLIAMS, 1998). These parameters act as factors that can control the diversity towards physiological mechanisms (WILLIAMS, 1998; OPREAN, 2008).

It is difficult to attribute a spectral characteristic of salinity for a salt lake in the absence of generally agreed classification. On the base of their taste, the lakes are divided in fresh water lakes and salt lakes. The salinity of salt lakes may be different; for example, in the case of Dead Sea the salinity is an average of 28%, while in Asal Lake from Africa is 35% (OREN, 1993; 2002a). On the other hand, our previous work (ENACHE et al., 2008) revealed that some salt lakes from Romania are characterized by salt concentrations varying from 6% (Techirghiol Lake) to 25% (Movila Miresei; Ocnele Mari). In this frame, a classification would assign salt lakes with salt content over 3g L^{-1} as hyposaline, over 20 g L^{-1}as mezosaline and over 50 g L^{-1} as hypersaline (HAMMER, 1986).

The species richness in salt lakes decreases with the increasing of salinity (WILLIAMS, 1998). Thus, in the range of salinity bellow 50 g L^{-1} there could be found vertebrates, invertebrates, angiosperms, macrophytes, phytoplankton and prokaryotes. Over the salinity of 50 g/l, the vertebrates and angiosperms are not generally able to survive. On the other hand, at a salt content over 120 g L^{-1}, saline waters harbour only some invertebrates like *Dunaliella* sp., sulphate reducing bacteria, cyanobacteria and archaea. At a salinity level between 170-220 g L^{-1}, sulphate reducing bacteria and cyanobacteria cannot not be found, and over 220 g L^{-1} the biodiversity is limited only to *Dunaliella* sp. and archaea (ENACHE, 2011). In order to cope with high ionic strength from hypersaline and saline environments, prokaryotes developed two strategies, namely “salt-in” and “compatible solutes” (OREN, 1999; 2002b). In the first strategy the salts are accumulated in high concentrations inside cells to equivalate the osmolarity with external environment. The enzymes and proteins present special adaptations i.e. the increasing of acidic amino-acid residues to their surface (LANYI, 1974; GRAZIANO & MERLINO, 2014). The second strategy is based on the synthesis or accumulation in the cell of some organic molecules named compatible solutes in order to face external osmolarity (OREN, 1999).

The salt lakes are widely distributed in Romania, some of them being formed in the operating holes of former salt exploitations or having a natural origin (GÂŞTESCU, 1971; ENACHE et al., 2012). Some salt lakes are very well known from microbiological point of view (ŢUCULESCU, 1965) or, in the case of pelogenous lakes, by their use in the therapy with sapropelic mud (BULGĂREANU, 1996). The study of IONESCU et al. (1998) revealed that in the case of 23 analysed karst and man-made salt lakes "high densities and occurrence frequencies characterize the most representative species". In their study, the authors noted the ecological conditions only for three species, namely *Amphora veneta* (Kützing 1844), *Artemia* sp. and *Stuckenia pectinata* (*Potamogeton pectinatus*) (Börner 1912) (IONESCU et al., 1998).

The present paper presents the biological studies of several salt lakes with natural genesis located in the Romanian plain, being investigated for the first time the presence of halophilic microorganisms both bacteria and archaea, phytoplankton and zooplankton species in relation with physical-chemical parameters as control factors.

MATERIALS AND METHODS

Sampling sites. The water samples have been taken from several Romanian salt lakes as it follows: Amara, located in Ialomiţa county, approximately 120 km south-east of Bucharest, Balta Albă (White Pool) located at the border between Buzău and Brăila counties, approximately 150 km south-east of Bucharest, Movila Miresei (Bride's Hill) located in Brăila county, approximately 200 km south-east of Bucharest and Ocnele Mari area (High Salt Mines) in the area with the same name located in the proximity of Râmnicu Vâlcea city, 180 km north-west of Bucharest. The samples have been taken in summer and autumn period as it follows: the end of June 2013 (Amara sample 1) and the end of August 2013 (Amara sample 2); beginning of August 2013 – Ocnele Mari; beginning of September 2013 – samples from Balta Albă and Movila Miresei. The physical aspect of the lakes was recorded using a digital camera Canon Power Shot model Pro 1.

Physical-chemical analysis of the water samples. The estimation of pH values, density and chloride content has been performed following previously described protocols (ENACHE et al., 2000). The content in mono and divalent cations or anions was determined using a Supermini X-Ray Fluorescence Spectrometer (Rigaku Corporation, Japan), following the semi quantitative method for light elements analysis in helium atmosphere. 10 ml of the surface water sample were weighted and placed in the spectrometer. The percent of elements was transformed into mg/ml dates, considering the weight of the sample (COJOC et al., 2013; NEAGU et al., 2014; PĂCEŞILĂ et al., 2014). The UV-VIS investigations were performed using a Nano drop spectrophotometer conducted to record the absorbance and protein content as mg/ml at 280 nm.

Isolation of halobacterial strains. The halobacterial strains were isolated from samples in a MH medium as described in our previous works (COJOC et al., 2013; NEAGU et al., 2014; PĂCEŞILĂ et al., 2014). Briefly, 1 ml of the sample was placed in a Petri dish and mixed with 30 ml of the autoclaved molten agar culture medium (cooled to 55-60^{0}C). After solidification, the plates were incubated at 37^{0}C and 28^{0}C for 7-10 days and after this period the bacterial colonies were counted.

Phyto and zooplankton samples. The phytoplankton and zooplankton samples were taken on water column with a Patalas Schindler plankton trap, filtered through plankton net mesh with 65μm Ø and preserved in 4% formaldehyde. Species identification was made using a Zeiss inverted microscope according to the method described by UTERMÖHL (1958) and specific taxonomic keys.

RESULTS AND DISCUSSIONS

Amara and Balta Albă salt lakes are included in the European ecological network Natura 2000 for nature conservation as part in ROSPA 0004 and 0065 and ROSCI005 sites. The recorded data revealed the salinity in investigated lakes is due to the presence of chloride and sulphur salts with ions from alkaline and alkaline-earth groups. Table 1 shows that potassium quantities varying from 0.5 mg mL^{-1} in Ocnele Mari to 12 mg mL^{-1} in Amara Lake were present in all tested samples. Similarly, silica was present in a different content in all lakes. In Amara and Balta Albă lakes the sodium was not identified. In opposition, concentrations of 25 mg mL^{-1} in Ocnele Mari and 18 mg mL^{-1} in Movila Miresei lakes were recorded.

Various chloride concentrations were determined as showed in table 1 arguing to consider the investigated lakes as hyposaline (Amara and Balta Albă) or hypersaline (Movila Miresei and Ocnele Mari). The water in Amara Lake contained 21 mg mL^{-1} sulphur, Balta Albă 7 mg mL^{-1} and the remaining lakes (Movila Miresei and Ocnele Mari) contained only traces. The samples taken from Amara and Ocnele Mari presented some magnesium content. The absence of calcium ions was recorded only in Balta Albă. In the other lakes, the concentration of these ions was recorded as 2 mg mL^{-1} in Amara, 0.1 mg mL^{-1} in Movila Miresei and 4 mg mL^{-1} in Ocnele Mari. Aluminium was detected as trace element in all tested samples. Movila Miresei sample harboured phosphorus and bromide content. Based on these results presented in table 1, salt lakes Amara and Balta Albă could be considered as hyposaline lakes and Movila Miresei and Ocnele Marias hypersaline.

Table 1. The ionic composition and physical-chemical characteristics of the investigated surface water samples from hyposaline and hypersaline lakes. The presence of fungi is marked by "+".

	Amara – sample 1	**Amara – sample 2**	**Balta Albă**	**Movila Miresei**	**Ocnele Mari**
K (mg mL^{-1})	12	13	11	2	0.5
Si (mg mL^{-1})	0.04	0.3	0.4	0.3	0.6
S (mg mL^{-1})	21	21	7	7	0.4
Absorbance at 280 nm	0.18	0.18	0.17	2.6	0.05
Protein content (mg mL^{-1})	0.2	0.2	0.16	3	0.05
pH	8.6	8.7	9.2	8.9	6.7
Chloride content (g L)	17	15	13	80	252
Density (g mL^{-1})	1.01	1.01	1.02	1.1	1.2
Presence of fungi	+	+	+	+	+

(a) (b) (c)

Figure 1. The physical aspect of the investigated lakes recorded in photo images using a digital camera Canon Power Shot model Pro 1: Lake Movila Miresei (a); Lake Balta Albă (b); Lake Amara – Ialomiţa (c) (original).

The results recorded in table 1 showed a protein content of 0.05 mg mL^{-1} in Ocnele Mari, 0.16 mg mL^{-1} in Balta Albă, 0.2 mg mL^{-1} in Amara salt lake, and 3 mg mL^{-1} in the hypersaline lake Movila Miresei. The values observed for protein content are related with the absence of sodium ions (Amara and Balta Albă salt lakes – hyposaline lakes) and a low level of chloride content around 15 g L^{-1}. In the case of Movila Miresei and Ocnele Mari (hypersaline lakes) the protein content is influenced by the presence of sodium ions and high values of chloride content - 80 and 252 gL^{-1}, respectively. Movila Miresei lake is characterized by a green (yellow-green) colour (Fig. 1) influenced by the intensity of sun light. The presence of algae from Bacillariophyceae and Chlorophyceae contributed to this colour. The water colour of the other lakes showed no particular aspect being transparent (Balta Albă and Ocnele Mari) or turbid, in case of Amara, due to human impact by sapropelic mud exploitation and extensive touristic use.

The registered pH values varied from 6.7 in Ocnele Mari to 9.2 in Balta Albă salt lake. The physical-chemical data presented in table 1 showed that Balta Albă salt lake harbours high salinity and alkalinity, which argue for considering this lake to have polyextremophilic conditions. The values recorded for density are correlated with the chloride content (Table 1). There were not observed any significant differences between hyposaline and hypersaline lakes.

The microbiological research revealed that all investigated lakes were inhabited by populations of halophilic microorganisms, both bacteria and archaea. The sodium ions content in the culture medium influences the number of total colony forming units (c. f. u.). By increasing sodium content, the number of c. f. u. decreased (Table 2).

Table 2. Distribution of colony forming units number (c. f. u.) in investigated salt lakes.

Lakes	Total c. f. u. number	Selected strains number	Strains growing in the presence of bile salt	Strains growing in the presence of chloramphenicol	Strains producing H_2S
Amara – sample 2	$33x10^2$	18	10	4	1
Balta Albă	$43x10^2$	7	4	1	3
Movila Miresei	$70x10^2$	10	8	0	7
Ocnele Mari	69	7	5	1	2

Amara hyposaline lake was characterized by a high number of c. f. u. and the absence of magnesium ions. The hypersaline lakes Movila Miresei and Ocnele Mari are inhabited by a low number of microbial strains and characterized by the presence of magnesium ions. A similar behaviour is observed in case of Balta Albă hyposaline lake in which magnesium ions were not present. In spite of having different physical-chemical conditions, the obtained c. f. u. number is close to previously reported data for several man-made salt lakes from Romania (ENACHE et al., 2008). Fungal species belonging to *Basipetospora* or *Walemia* genera were observed and isolated from all lakes. Several numbers of microbial strains were selected for further investigations, as showed in table 2. Data from table 2 revealed that halophilic bacteria are abundant in the investigated lakes dominating the archaeal strains, both in hyposaline or hypersaline lakes. Four strains isolated from Amara salt lake showed the capacity to grow both in the presence of sodium deoxycholate and chloramphenicol.

The same behaviour was noted for one strain from Balta Albă and one from Ocnele Mari salt lakes. The number of strains capable to produce hydrogen sulphide was significant in Movila Miresei. There is noted that such kind of microbial strains is closely related to the mechanism of sapropelic mud formation (ŢUCULESCU, 1965). The use of mud for treatment of various diseases constitutes the main economic value of salt lakes in Romania (BULGĂREANU, 1996; ENACHE et al., 2012).

The investigation of phytoplankton taxonomical structure (Fig. 2) revealed a wide spectrum of cyanobacterial species in Amara salt lake, characterized by the presence of magnesium and calcium ions, in Balta Albă being detected only *Merismopedia tennuissima* (Lemmermann 1898) (Table 3a).

Table 3a. Phytoplankton diversity in investigated hyposaline and hypersaline environments.

	Amara	Balta Albă	Movila Miresei
CYANOBACTERIA			
Merismopedia tennuissima (Lemmermann 1898)	-	+	-
Merismopedia sp.	+	-	-
Oscillatoria chalybea F. K. Mertens in G. H. B. Jürgens, 1822 ex M. A. Gomont, 1892	+	-	-
Gloeocapsa sp.	+	-	-
Chroococcus sp.	+	-	-
Crucigenia sp.	+	-	-
Microcystis sp.	+	-	-
BACILLARIOPHYCEAE			
Asterionella formosa (Hassall 1850)	-	+	+
Melosira granulata var. angustissima (Otto Müller 1899)	+	+	+
Navicula sp.	+	+	-
Navicula cryptocephala (Kützing 1844)	-	+	+
Nitzschia acicularis (Kützing) Kuntze 1898	+	+	-
Nitzschia vermicularis (Kützing) Hantzsch 1860	+	-	-
Nitzschia spectabilis (Ehrenberg) Ralfs 1861	+	-	-
Synedra ulna (Nitzsch) Ehrenberg 1832	+	+	-
Synedra acus Kützing 1844	-	+	-
Cymbella sp.	-	+	+
Cocconeis placentula Ehrenberg 1838	-	+	+
Fragillaria sp.	-	-	+
Cyclotella sp.	+	-	-
CHLOROPHYCEAE			
Pediastrumduplex Meyen 1829	-	+	+

Pediastrum simplex Meyen 1829	-	+	-
Pediastrum tetras (Ehrenberg) Ralfs 1845	-	-	+
Pediastrum sp.	+	-	-
Scenedesmus sp.	+	+	+
Scenedesmus ecornis (C.G. Ehrenberg ex J. Ralfs 1845) R.H. Chodat 1926	-	+	-
Scenedesmus quadricauda (Turp.) Brébisson	-	+	-
Coelastrum microporum Nägeli in A.Braun 1855	-	+	-
Keratococcus raphidioides (Hansg.) Pascher 1915	-	+	-
Crucigenia sp.	-	+	-
Tetraedron muticum (A.Braun) Hansgirg 1888	-	+	-
EUGLENOPHYCEAE			
Phacus sp.	+	-	-

The high salinity of Movila Miresei is reflected in the absence of cyanobacterial species. Diatoms were present in all lakes with the higher richness in Balta Albă hyposaline lake where aluminium was detected in higher concentrations than in the other lakes. A similar result was recorded in the case of green algal species (Table 3a). *Phacus* sp. was observed only in Amara Lake. Due to the high chloride content in Ocnele Mari, no phytoplanktonic and zooplanktonic species were recorded.

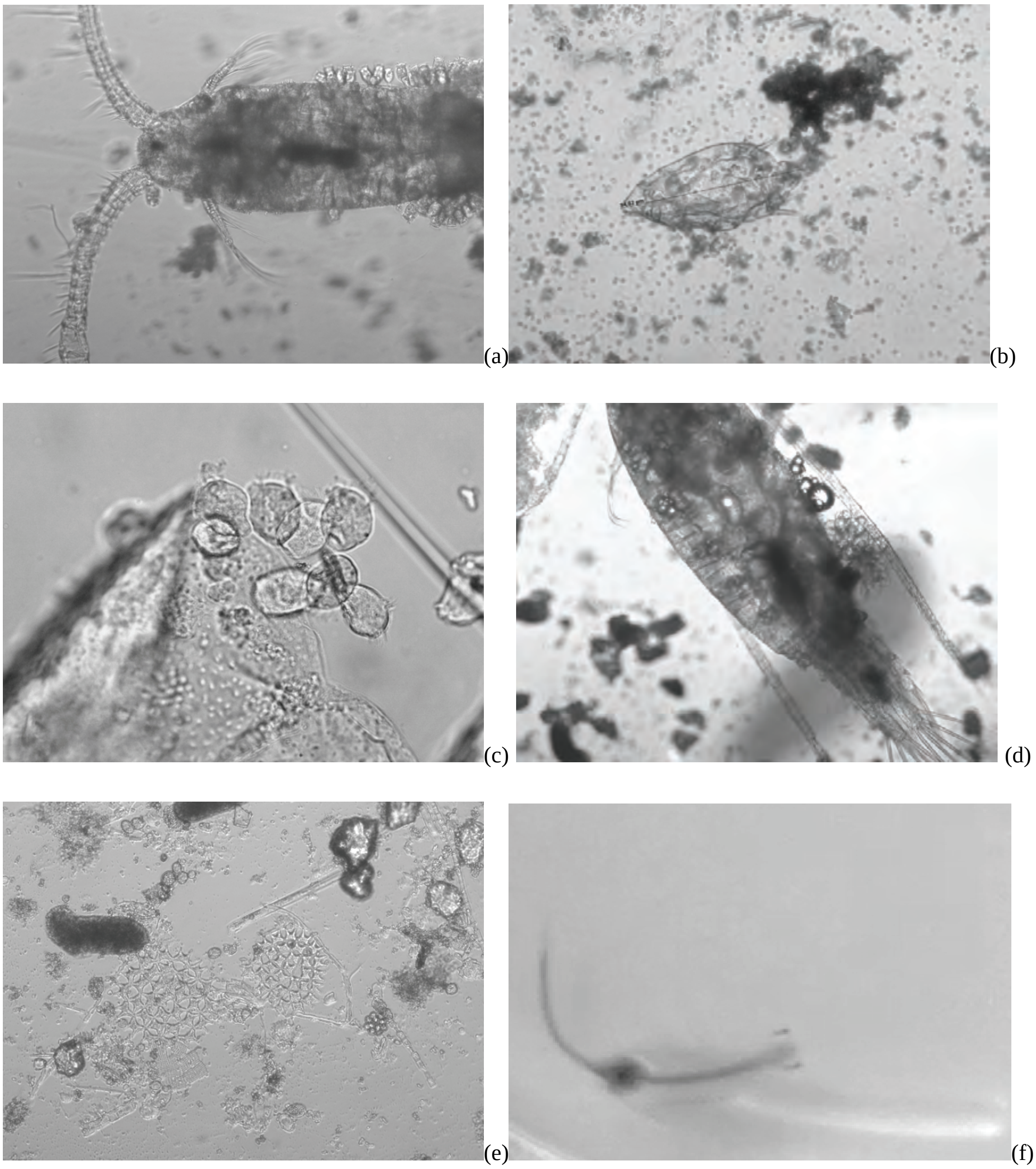

Figure 2. Phyto- and zooplankton representative species detected in salt lakes: (a) Diaptomidae (Copepoda) – Amara; (b) *Keratella* sp. (Rotifera) – Movila Miresei; (c) Epibiont ciliate on *Moina salina* – Balta Albă; (d) Diaptomidae (Copepoda) – Balta Albă; (e) *Pediastrum duplex* and *Merismopedia tennuissima* – Balta Albă; (f) *Artemia salina* in Movila Miresei (original).

Among the investigated lakes, Amara presented the higher zooplanktonic richness (Table 3b), being detected protist, rotifer and crustacean species. The distribution of zooplankton species was related to the chemical composition of lakes, at high chloride content (Movila Miresei) being detected predominantly crustaceans and cladocerans.

Table 3b. Zooplankton diversity in investigated saline and hypersaline environments.

	Amara	**Balta Albă**	**Movila Miresei**
PROTOZOA			
Protozoa g. sp.	+	+	-
Flagelata	+	-	-
Testacea	+	-	-
Difflugia sp.	+	-	-
CILIATA			
Vorticella sp.	+	-	-
ROTIFERA			
Keratella sp.	-	-	+
Cephalodella sp.	+	-	-
Brachionus sp.	+	-	-
Keratella cochlearis Gosse 1851	+	-	-
Keratella quadrata Mülle, 1786	+	-	-
Rotifera g. sp.	+	-	-
CRUSTACEA			
COPEPODA			
Diaptomida			
Arctodiaptomus salinus Dada, 1885 nauplia	+	+	+
Arctodiaptomus salinus - copepodits	+	+	-
Arctodiaptomus salinus adults	+	+	+
Harpacticoida			
Harpacticoida g. sp.	-	+	-
CLADOCERA			
Artemia salina Linnaeus 1758	-	-	+
Moina salina	-	+	+
Bosmina sp.	+	-	-

The lake Movila Miresei is characterized by the presence of sodium, bromide and phosphorous. On the other hand, these species were observed in Balta Albă hyposaline lake characterized by the absence of sodium, magnesium and calcium ions, the salinity of this lake being a consequence of the presence of sulphur salts. These data are supported also by the relatively high numbers of halophilic microorganisms from this lake capable to produce hydrogen sulphide (Table 2). In the case of phytoplankton, the species richness appears to be double than in the case of zooplankton.

The salted environments investigated in this study are represented by salt lakes of natural origin located in the southern region of Romania. The origin of the salt lakes Balta Albă and Amara is supposed to be a consequence of the brackish sea evaporation (GÂŞTESCU, 1971). On the other hand, the salt lake from Ocnele Mari appears to have an anthropogenic origin resulted from the exploitation of the salt deposit in the area.

CONCLUSIONS

Based on the investigation from this work, the chemical composition of all tested areas resulted to be marked by abundant presence of chlorides (sodium, magnesium, potassium) and some relatively important concentrations of aluminium and strontium in the particular case of Ocnele Mari. Biological diversity was represented mostly by several types of archaeal and halophilic bacterial species. Several species of Cyanobacteria, Bacillariophyta, Ciliata, Rotifera and Copepoda were detected mainly in the samples taken from Amara and Balta Albă hyposaline lakes, where the total chloride concentrations did not exceed 20 g L^{-1} and sodium appeared to be absent. The data from this study revealed that the presence of magnesium and calcium ions and the chloride content have the role to control the phytoplankton and zooplankton populations in these saline environments.

ACKNOWLEDGEMENTS

This study was funded by the project no. RO1567-IBB05/2014 from the Institute of Biology Bucharest of the Romanian Academy. Authors thank Stela Sofa for technical support.

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Received: March 28, 2015
Accepted: June 10, 2015