

## VARIATIONS OF THE STRUCTURE OF BIOLOGICAL COMMUNITIES RELATED TO SALINITY IN SOME SALINE LAKES FROM ROMANIA

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**Abstract.** The study was conducted in several natural saline lakes from the Buzău and Brăila counties (Romania). The Lakes Căineni, Balta Albă and Amara are hyposaline, only the Lake Movila Miresei can be considered as hypersaline according to mineral content. The aim of the study was to have a quantitative approach to the biological structure of the lakes in relationship with abiotic factors, mainly salinity. Using the Principal Component Analysis (PCA), the Lake Movila Miresei was associated with salinity and conductivity, the Lake Căineni with temperature, while Balta Albă and Amara were influenced mainly by DO, pH, TDS, ORP, turbidity. The level of primary producers was represented by four groups of phytoplankton and the highest species richness was registered in Lake Amara (38 species), with low salinity. The zooplankton community showed a species richness significantly smaller than phytoplankton, even if it presented species belonging to several taxonomic groups (9). The high zooplankton diversity of the Lake Amara was correlated with low salinity. The Shannon index decreased with the reduction of salinity in the other three lakes. It is noteworthy the presence of *Artemia salina* Linnaeus 1758, both in the Lakes Amara and Movila Miresei, known as indicator species of therapeutic mud. The additional technique applied for investigating the diversity of the phytoplankton confirmed the values of Shannon's diversity index and confirmed the values of the species richness trend. Thus, the Lake Căineni (with high salinity, second after Movila Miresei) held the first place in terms of diversity, the second was Balta Albă, and the less diverse were Amara and Movila Miresei. The Amara Lake experienced a cyanobacterial bloom event, with *Oscillatoria tenuis* Agardh 1813. We conclude that salinity not always is the main factor shaping the diversity of saline lakes. Other factors, such as temperature, DO, pH, TDS, ORP, turbidity can influence the biological communities of these lakes.

**Keywords:** salt lakes, phytoplankton, zooplankton, bacterioplankton, saline ecosystems, salinity.

### **Rezumat. Variații în structura comunităților biologice în relație cu salinitatea în câteva lacuri saline din România.**

Studiul a fost realizat în câteva lacuri saline naturale din județele Buzău și Brăila (România). Lacurile Căineni, Balta Albă și Amara sunt hiposaline, numai lacul Movila Miresei poate fi considerat hipersalin în funcție de conținutul de minerale. Scopul studiului a fost de a avea o abordare cantitativă a structurii biologice a lacurilor, în relație cu factorii abiotici, în principal salinitatea. Folosind Analiza Componentelor Principale (PCA), lacul Movila Miresei a fost asociat cu salinitatea și conductivitatea, lacul Căineni cu temperatura, în timp ce Balta Albă și Amara au fost influențate, în principal, de turbiditate DO, pH, TDS, ORP. Nivelul producătorilor primari a fost reprezentat de patru grupe de fitoplancton, iar cea mai mare bogăție de specii a fost înregistrată în lacul Amara (38 specii), cu salinitate scăzută. Comunitatea zooplanctonică a prezentat o bogăție de specii semnificativ mai mică decât fitoplanctonul, chiar dacă speciile prezente aparțin mai multor grupe taxonomice (9). Diversitatea ridicată a zooplanctonului din lacul Amara a fost corelată cu o salinitate scăzută. Indicele Shannon scade cu reducerea salinității în celelalte trei lacuri. Este de remarcat prezența speciei *Artemia salina* Linnaeus 1758, cunoscută ca specie indicator de nămol terapeutic, în lacurile Amara și Movila Miresei. Tehnica suplimentară aplicată pentru investigarea diversității fitoplanctonului a confirmat valorile indicelui de diversitate Shannon și a infirmat valorile bogăției de specii. Astfel, lacul Căineni (cu salinitate ridicată, după Movila Miresei) se află pe primul loc în ceea ce privește diversitatea, al doilea fiind Balta Albă, lacurile Amara și Movila Miresei fiind cele mai puțin bogate din punct de vedere al diversității biologice. Lacul Amara a cunoscut un eveniment de înflorire cianobacteriană, cu *Oscillatoria tenuis* Agardh 1813. Se observă astfel că salinitatea nu este întotdeauna principalul factor care modelează diversitatea lacurilor saline. Alți factori, ca temperatura, DO, pH, TDS, ORP, turbiditatea pot influența comunitățile biologice ale acestor lacuri.

**Cuvinte cheie:** lacuri sărate, fitoplancton, zooplancton, bacterioplancton, ecosisteme saline, salinitate.

## INTRODUCTION

Saline lakes have a more simple biological structure than freshwater ecosystems (HAMMER, 1986; COMÍN et al., 1992). Salt lakes are geographically widespread, being mostly inland aquatic ecosystems. These natural systems have considerable aesthetic, cultural, economic, recreational, scientific, conservation and ecological values (WILLIAMS, 2002). In such systems, diversity, ecological factors driving biological components, biogeography and evolution in the microbial world can be investigated much more easily than in complex freshwater and marine systems (MA et al., 2010; ENACHE et al., 2017).

Differences in the composition and biological structure of freshwater and saltwater ecosystems are very visible and easy to study. Instead, these differences are harder to investigate between waters with different salinity values. Salty waters are classified according to salinity in several categories: (over 3 g L<sup>-1</sup>) as hyposaline, (over 20 g L<sup>-1</sup>) as mesosaline and over 50 g L<sup>-1</sup> as hypersaline (HAMMER, 1986; ENACHE et al., 2017).

In lakes a certain degree of salinity, most authors consider that this factor is the one that structures the entire biological composition of the ecosystem (KIPRIYANOVA et al., 2007). However, some authors have shown that the species richness and diversity can be significantly influenced by other factors such as oxygen, ionic composition, pH, hydrological patterns, geographic position, palaeoclimatic events, anthropogenic influences, and biological interactions (WILLIAMS, 1998).

The present work represents the continuation of the pilot research conducted in 2013 in some saline lakes from România published by MOLDOVEANU et al., in 2015. This time, we intend to have a quantitative approach to the biological structure of lakes in relationship with abiotic factors, mainly salinity. We keep sampling in our study the Lakes Movila Miresei and Balta Albă, from Brăila county, and in addition, we sampled the Lakes Amara from Buzău county (another lake with the same name is located in the Ialomița county) and Căineni from Buzău county.

Multivariate analysis is less used in literature to highlight the relationships in the saline lakes, between habitat and communities, habitat and physicochemical factors that characterize it, or the relationship between biological communities and measured physicochemical factors. We have used multivariate statistics techniques as Principal Component Analysis, Diversity Indices and Diversity Profiles to investigate these relationships.

The novelty degree of the present study is revealed by the biological complexity approach for communities' evaluation in relation with other physico-chemical parameters than salinity, seen in terms of content in sodium chloride considering the chemical composition in some investigated sites where sodium was not detected.

## MATERIAL AND METHODS

**Sampling sites.** The investigated saline lakes have a natural origin and are located in the Romanian plain (Fig. 1).



Figure 1. The map of sampling points (modified after Google Maps, accessed March 5, 2018).

The water samples have been taken from several salt lakes namely: Amara, located in Buzău county, Balta Albă (White Pool) located at the border between Buzău and Brăila counties, Movila Miresei (Bride's Hill) and Căineni, situated in the Brăila county. The Căineni Lake is the fourth important lake of the site, is surrounded by agricultural land and is located in the village of Căineni, a former spa resort. It has a high salinity, and one of the best sapropelic sludge is extracted from here.

The samples were collected in summer 2016, in July, as following: 2 sampling points from Movila Miresei (45°13'14.62"N, 27°38'31.58"E and 45°13'15.46"N, 27°38'19.6"E) and Amara (45°14'43.32"N, 27°18'7.29"E and 45°13'44.70"N, 27°16'52.25"E) and 1 sampling point from Balta Albă (45°17'38,99"N, 27°20'54,56"E) and Căineni (45°10'56.39"N, 27°19'29.41"E). The warmest season was selected for sampling in order to reveal the high values of salinity in relation with biological composition of lakes. In summer, due to an aride climate of the region, the evaporation processes are intense and the salinity of the water increase.

**In situ physicochemical measurements.** Using a portable water multiparameter system HI 9828 from Hanna Instruments, the dissolved oxygen (DO), the oxygen saturation (%), conductivity, pH, redox potential ORP, total dissolved solids TDS, salinity were measured in the field for each lake.

Also, the geographical coordinates for all lakes were established using a GPS device. Water turbidity was measured with a Hanna Instruments turbidimeter in the field, avoiding sample alteration.

The light intensity was determined with a lightmeter, at the moment of water sampling.

**Laboratory analyses.** The chemical composition of the water samples (10 mL) was determined using a Supermini X-Ray Fluorescence Spectrometer (Rigaku Corporation, Japan), following the semi quantitative method for light elements analysis in helium atmosphere.

**Bacterioplankton density.** The total colony forming units (c.f.u.) were estimated by incubation of the samples on MH culture medium at 30°C for 7 days as previously described (COJOC et al., 2009; MOLDOVEANU et al., 2015).

**Extracellular enzymatic activities** were estimated as previously described (COJOC et al., 2009) in terms of the presence of enzymes which degrading starch, cellulose, inulin, Tween 80, pectin, xilan, casein and olive oil.

**Phyto and zooplankton samples.** The phytoplankton and zooplankton samples were taken on the water column with a Patalas Schindler plankton device at the same moment with the *in situ* measurements. Immediately, all samples

were filtered through a plankton net mesh with 50  $\mu\text{m}$   $\varnothing$  and preserved in 4% formalin solution. The species identification was made using a Zeiss inverted microscope according to the method described by UTERMÖHL (1958) and specific taxonomic keys.

**Statistical analyses.** Data processing was performed using PAST software (HAMMER et al., 2001). The Principal Component Analysis (PCA) was used in order to reveal the relationship between lakes, biological components and environmental factors. As PCA is a non-parametric method of classification, there is no need to transform the data for a normal distribution (VEGA et al., 1998; HELENA et al., 2000).

## RESULTS AND DISCUSSION

Though the site ROSCI0005 Balta Albă-Amara-Lacul Sărat Căineni-Jirlău has a Community importance (Habitats Directive), it is subjected to riverine activities: household waste discharges into the lakes, significant water volume fluctuations due to weather conditions, natural and artificial water composition changes, intensive agriculture, uncontrolled tourism (<http://ananp.gov.ro>). The Movila Miresei Lake does not have a conservational status and the occasional use of water for bathing by local population may be a disturbance factor for the saline system.

The waters of the lakes are different in terms of salinity, turbidity, total dissolved solids (TDS), oxidation-reduction potential (ORP), dissolved oxygen and saturation. The order of classification of the lakes according to salinity and conductivity is: Movila Miresii > Căineni > Balta Albă > Amara (Table 1). According to HAMMER, 1986, the Lakes Căineni, Balta Albă and Amara are hyposaline, only the Movila Miresei Lake can be considered as hypersaline.

The Principal Component Analysis showed which environmental factors describe significantly (PC 1 = 51.34 %, PC 2 = 28.35 %, PC1 and PC2 reached 79.69 %) the studied lakes (Fig. 2). Thus, the Movila Miresei Lake was associated with salinity and conductivity, the Căineni Lake with temperature, while Balta Albă and Amara were influenced mainly by DO, pH, TDS, ORP, turbidity. Our findings were in agreement with the results of WILLIAMS, 1998. In the past (early 70s), the Amara Lake has been flooded with fresh water and salinity never increased at a high level, affecting the therapeutic quality of water (<https://www.agerpres.ro>).

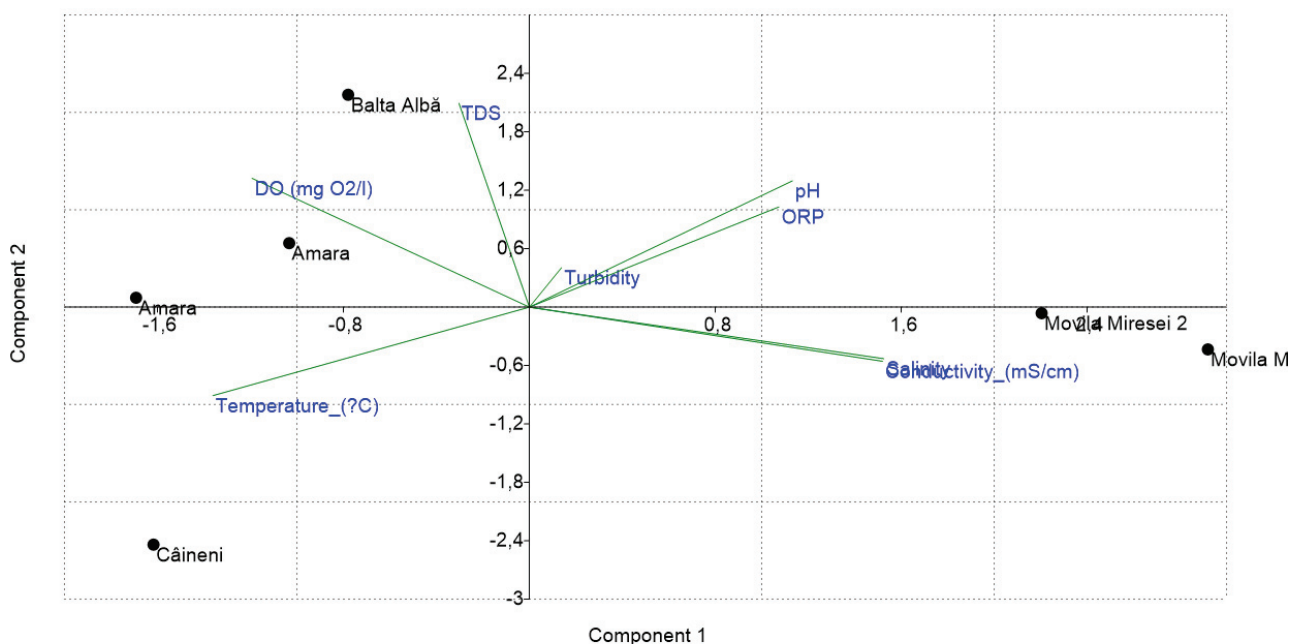


Figure 2. The PCA biplot of the environmental factors characterising sampled lakes.

The turbidity ranged widely, from 9.21 FNU in Lake Căineni to 216 FNU in Lake Amara. All lakes can be considered alkaline having a pH over 8.45. The lower ORP value was registered in the Balta Albă and Amara Lakes, while the highest value was measured in the Movila Miresei Lake (152) (Table 1).

Salinity has a significant impact on biological communities, like phyto and zooplankton, because it requires them to adjust the saline concentrations in their bodies to the surrounding environment. (PATUJ et al., 2017). Thus, any changes in salinity can lead to the disappearance of some species and their replacement with others, more well adapted (OJAVEER et al., 2010).

The cultivable colony forming units (C.F.U) numbers varying from  $9 \times 10^2$  (Lake Căineni) until to  $64 \times 10^2$  (in Lake Movila Miresei) (Table 2). The numbers are relatively close to previous reports from other saline lakes from România (MOLDOVEANU et al., 2015) but is relatively low if compared with data for the Dead Sea or the Great Salt

Lake inhabited by halophiles until to  $10^6$  cells/g with seasonal fluctuation (OREN, 1993; NEAGU et al., 2014). On the other hand, the C.F.U. numbers appear to be related to the salinity and increase with its content (Table 2). In terms of the presence of NaCl, the recorded data (Tables 1 and 2) revealed the absence of a correlation between C.F.U. numbers and this compound.

Table 1. The environmental factors of the salt lakes.

Lake	St.	Light intensity	Depth (m)	Turbidity FNU	Temp. (°C)	pH	Cond. (mS/cm)	DO (mgO <sub>2</sub> /l)	DO %	OR P	Salinity	TDS
Movila Miresei	st1	1056X100	1.00	90.90	27	9.38	78.82	1.17	9.17	152	54.9	31.41
Movila Miresei	st2	1056X100	1.00	86.40	27	9.48	78.74	4.58	80	50.6	55	39.31
Balta Albă	st1	1135X100	1.00	11.10	28	9.56	15.42	10.96	156	28	8.97	75.71
Amara	st1	1151X100	0.50	84.40	30.76	8.65	10.08	8.03	114.9	55	5.64	50.52
Amara	st2	1180X100	1.00	216.00	28.55	8.77	10.64	7.82	106.5	32	5.59	50
Căineni	st1	1108X100	1.00	9.21	30.68	8.45	27.47	5.48	80	124	16.82	13.78

Table 2. The bacterioplankton density and XRF analysis results in salt lakes.

Lake	Sampling	Bacterioplankton	Na <sub>2</sub> O	SO <sub>3</sub>	K <sub>2</sub> O	Cl	MgO	
	point							density
								CFU ml <sup>-1</sup>
Movila Miresei	st1	64x10 <sup>2</sup>	17.10	15.55	3.27	62.39	0	
Movila Miresei	st2	64x10 <sup>2</sup>	24.59	15.17	2.88	56.37	0	
Balta Albă	st1	39 x10 <sup>2</sup>	20.40	11.19	12.88	50.08	4.12	
Amara	st1	-	0	14.17	11.33	28.53	3.04	
Amara	st2	-	0	12.07	10.10	24.45	1.96	
Căineni	st1	9 x10 <sup>2</sup>	17.56	22.37	4.78	39.64	7.37	

The species number of phytoplankton ranged from 3 species in Movila Miresei, to 38 species in the Amara Lake. The dominating taxonomic groups were Bacillariophyceae (19 species in Amara) and Cyanobacteria (8 species in Lake Amara and Căineni) (Table 3).

The zooplankton community showed a species richness significantly smaller than phytoplankton, even if it presented species belonging to more taxonomic groups (9). The highest number of zooplanktonic species is recorded in the Amara Lake (13), of which five are rotifers (Table 3). It is noteworthy the presence of *A. salina*, belonging to the Artemiidae family, Branchiopoda class, both in the Amara and Movila Miresei lakes. The species is known to tolerate salinity values between 3-50 g L<sup>-1</sup> (BANISTER, 1985). Also, this species is considered an indicator of the therapeutic quality of mud in salty lakes in which it is present, especially if the abundance is high, as is the case of the Movila Miresei Lake (509 ind L<sup>-1</sup>). In the meantime, it contributes, by its microbial degradation and sediment deposition, to the enrichment of the organic matter of the sludge, becoming a sapropelic mud (MUNTEANU & DUMITRAȘCU, 2011).

 Table 3. The species richness of phytoplankton (cell L<sup>-1</sup>) and zooplankton (ind.L<sup>-1</sup>) in salt lakes.

Phytoplankton/zooplankton groups	Balta Albă	Căineni	Amara	Movila Miresei
Cyanobacteria	5	8	8	1
Euglenophyceae	2	3	3	0
Bacillariophyceae	12	11	19	2
Chlorophyceae	0	0	8	0
<b>Total phytoplankton</b>	<b>19</b>	<b>22</b>	<b>38</b>	<b>3</b>
Ciliata	1	0	1	0
Testacea	1	0	1	1
Rotifera	2	1	5	2
Ostracoda	0	0	1	0
Cladocera	0	1	1	0
Copepoda	2	2	3	0
Branchiopoda (Artemiidae)	0	0	1	1
<b>Total zooplankton</b>	<b>6</b>	<b>4</b>	<b>13</b>	<b>6</b>

Regarding the abundance of the total phytoplankton, an inverse upward trend was seen with the decreasing of the salinity gradient in the lakes. The only system with a high abundance of phytoplankton at a relatively high salinity ( $17 \text{ g L}^{-1}$ ) was the Căineni Lake (Figs. 3; 4).

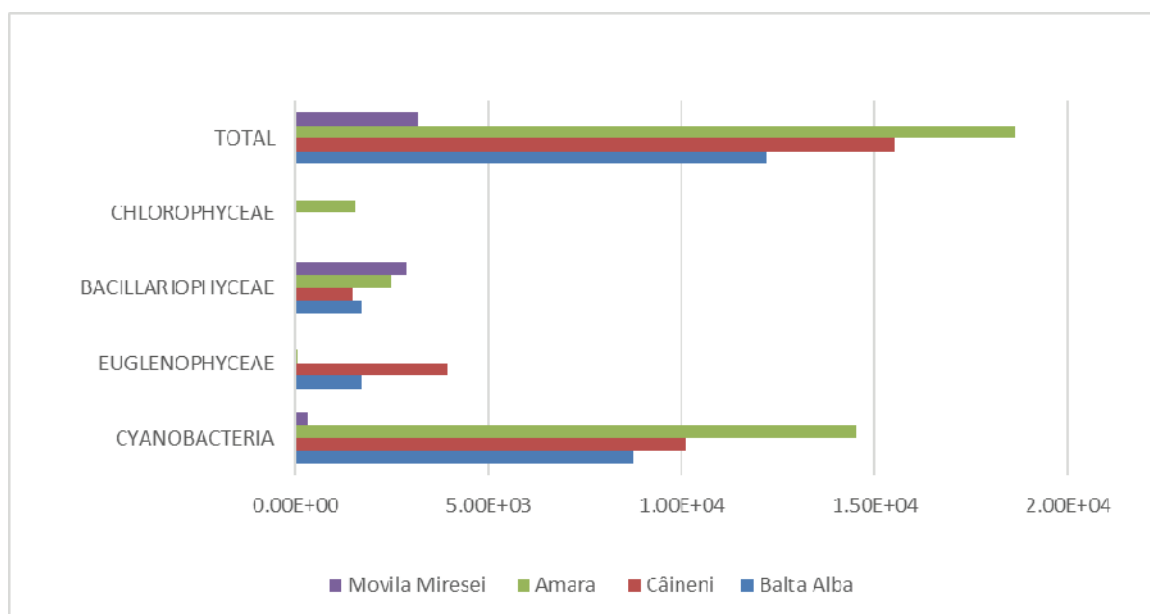


Figure 3. The variation of phytoplankton density ( $\text{cell L}^{-1}$ ) in the sampled lakes.

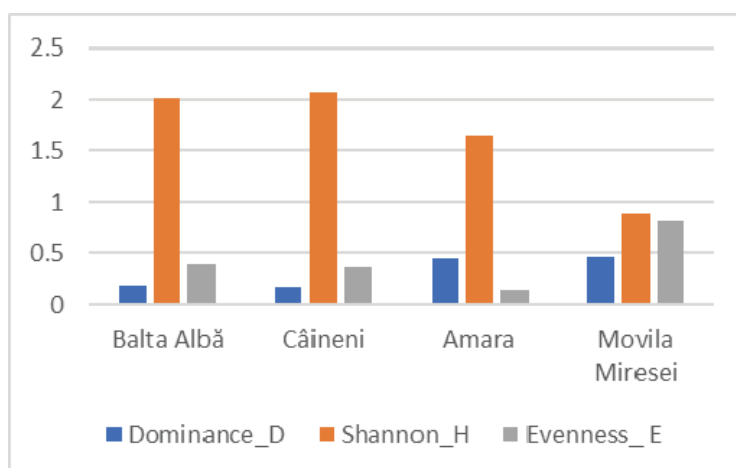


Figure 4. Diversity indices of phytoplankton in saline lakes.

In order to assess the degree of the plankton diversity in the four studied lakes, indices of diversity (Shannon, index of dominance, evenness) were calculated. Although these indices are often used in ecology to analyse and compare the spatial and temporal diversity of a lake or several lakes, they do not always have the necessary precision (HELLMANN & FOWLER, 1999). Therefore, a good statistical technique was chosen for shaping diversity profiles (Figs. 5; 7) to compare the four lakes from this point of view. The test is based on the calculation of the alpha index, an index that encompasses in point 0, the species richness, in point 1, the Shannon diversity and in point 2, the dominance index. The curve that is above the other lines, in the range 0-2, is interpreted to have the greatest diversity.

The diversity of plankton in these types of ecosystems, with a simpler organization than those of fresh water, is very important (GHEORGHEVICI et al., 2015). Most times, the phytoplankton community provides the primary production of the lake, because the aquatic plant community is poor or even absent, especially at high salinity. The phytoplankton community is the only one to support the entire trophic network of salty lakes, which is limited to primary consumers (zooplankton) and microbial communities that are mainly the organic matter decomposers.

The diversity curves of the phytoplankton confirmed the values of Shannon's diversity index, with the Căineni Lake holding the first place, Balta Albă the second, and the less diverse were Amara and Movila Miresei (Fig. 5).

As it can be seen both in Figure 4 and Figure 6, the Shannon index varies inversely with the dominance index, suggesting that, for example, although the Movila Miresei Lake Amara recorded the highest abundance, it is accumulated only by one species, *O. tenuis*. For this reason, in the Amara Lake, evenness showed the lowest value (0.136) (Fig. 4).

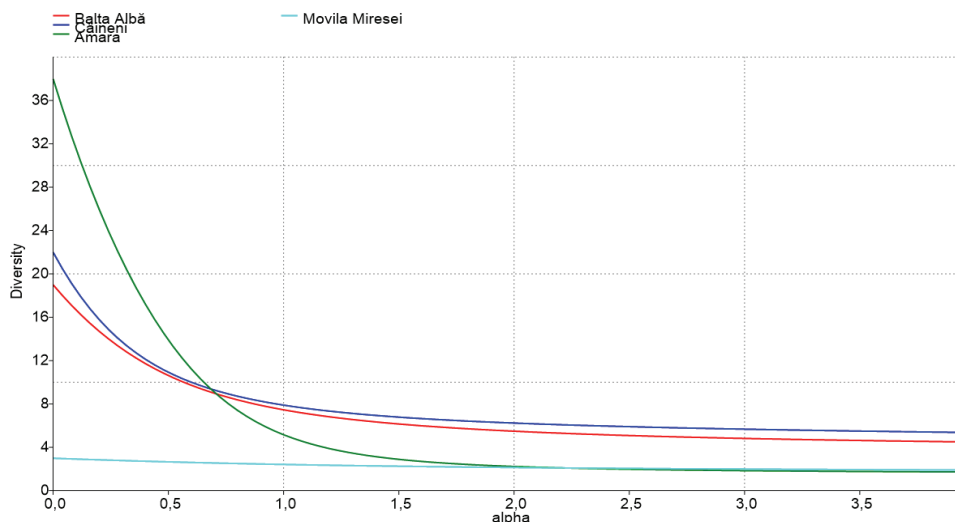


Figure 5. The diversity profile of saline lakes based on phytoplankton abundance.

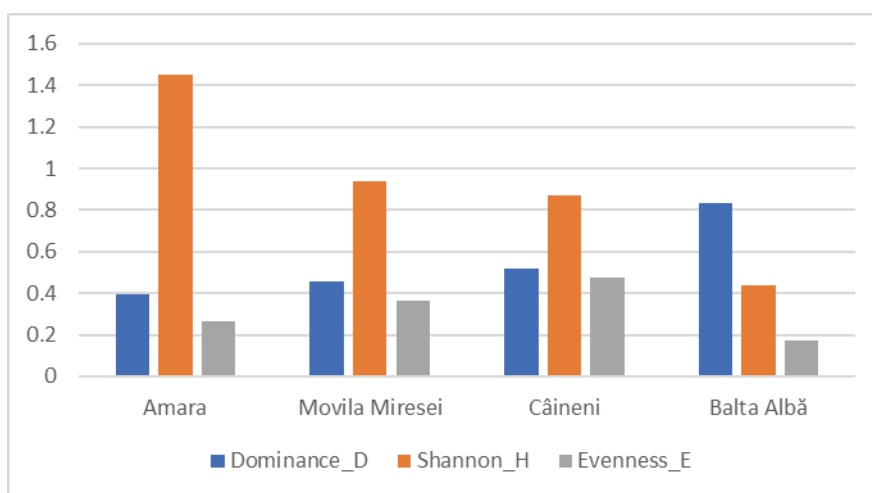


Figure 6. Diversity indices of zooplankton in saline lakes.

The high zooplankton diversity of the Lake Amara was correlated with a low salinity. The Shannon index decreased with the reduction of salinity in the other three lakes (Fig. 6).

The zooplankton diversity curves confirmed this analysis, the lake with the most zooplanktonic species being Amara (Fig. 7). The high diversity of species is related to the low salinity level, the species found here being species found in freshwater and tolerant to oligohaline waters.

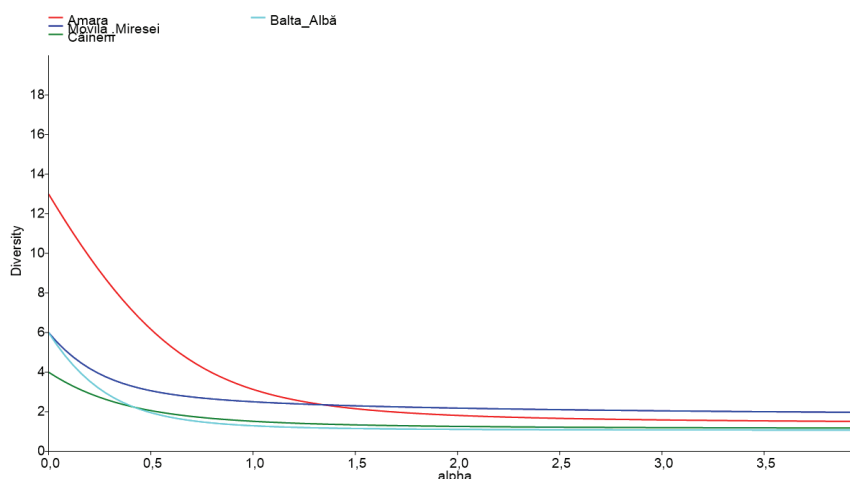


Figure 7. Diversity profiles of the saline lakes based on zooplankton abundance.

In order to find out the preference of the plankton groups for a particular lake, a multivariate statistical technique, Principal Component Analysis (PCA) was applied.

Figure 8 shows that *A. salina* belonging to the Artemiidae family preferred higher salinity conditions, such as those of Movila Miresei, while in the Căineni Lake, which ranked second in salinity, species of the Copepoda group are found. The lakes with lower salinity, such as Amara and Balta Albă, are preferred by the Rotifera group, where most of the species in this group were found. The degree of association of these groups with the studied lakes is 85.39%, combined percentage of the two PC1 and PC2 component axes of the analysis.

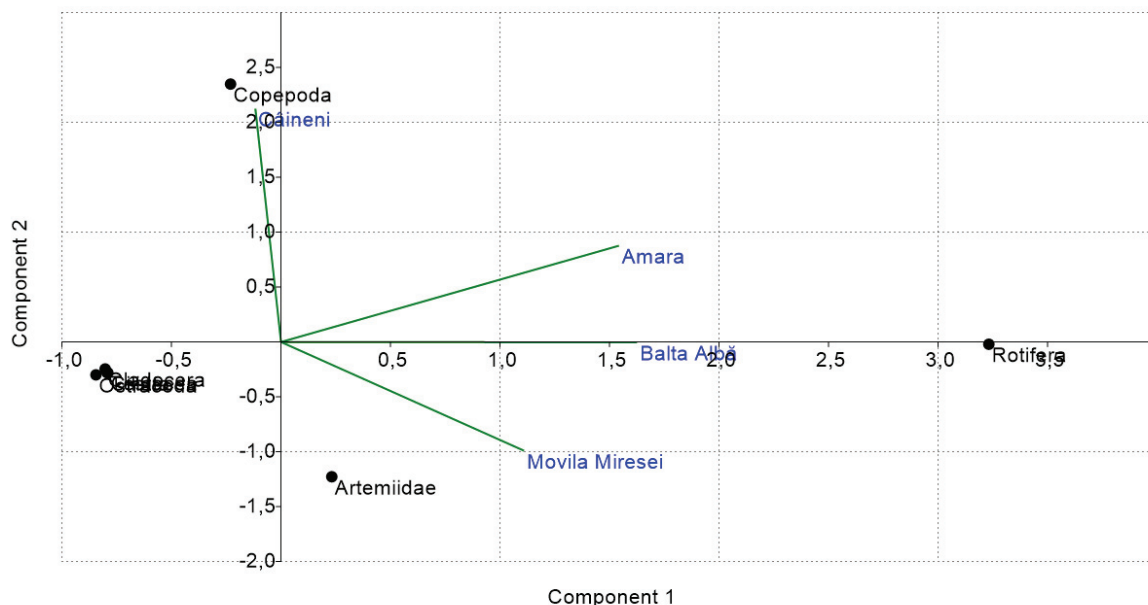


Figure 8. PCA biplot of the correlation between zooplankton groups and saline lakes.

Figure 9 shows the degree of preference of the phytoplankton groups for the studied lakes. Thus, the diatoms were found in the conditions of the high salinity that characterized the Movila Miresei Lake. Species belonging to the Cyanobacteria group preferred lower salinity conditions, from Căineni, Balta Albă and Amara. In the Lake Amara was registered a blooming episode of the cyanobacterial filamentous species, *O. tenuis*.

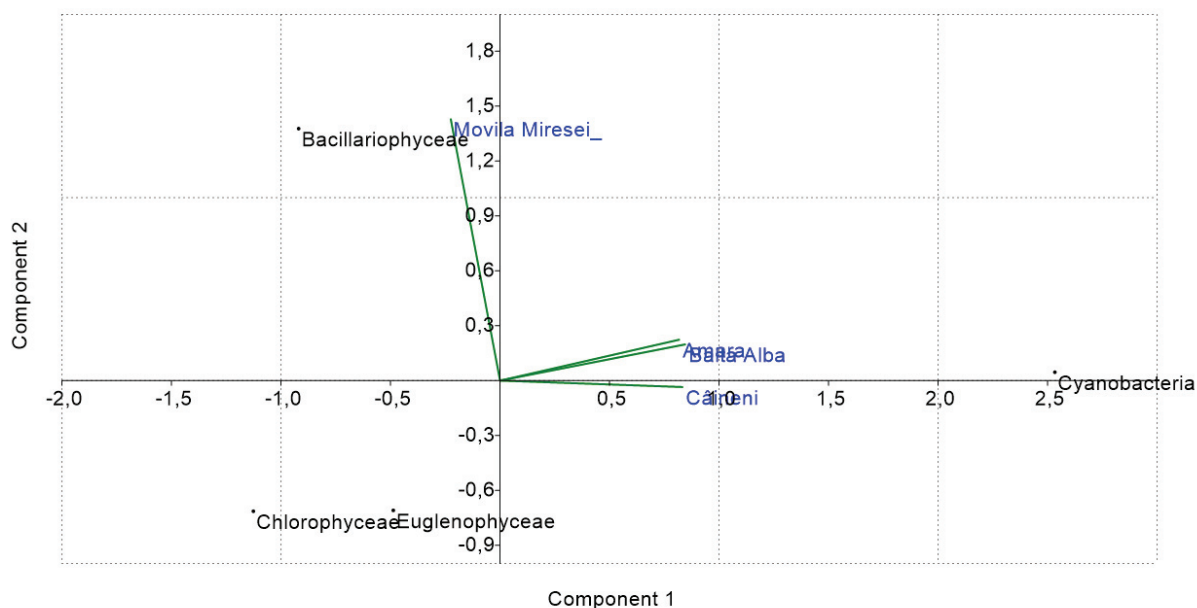


Figure 9. PCA biplot of the correlation between phytoplankton groups and saline lakes.



54 bacterial strains were randomly selected from the investigated sites, which showed halotolerant behaviour considering their ability to grow on culture media supplemented with NaCl up to 5M. From the investigated strains, 25 harbour ability to grow well on media having a NaCl concentration from 0 to 5M. A lot of them, namely 20 strains, were isolated from the sapropelic mud sample from the Movila Miresei hypersaline lake. In terms of the presence of extracellular enzymatic activities, seven strains showed capacity to degrade casein, eight strains degrading starch, four strains harbour lipolysis activities, another four hydrolyzing Tween 80, three strains used pectin as substrate, one strain degrading xylene and another one carboxy-methyl cellulose (CMC). Inulin was not degraded by the investigated strains. Two strains isolated from sapropelic mud from Movila Miresei harbour ability to degrade three tested substrates. Other seven strains degraded two tested substrates. The recorded data revealed that two extracellular activities were predominant, namely amylase and protease. The less used substrates testes in our investigations were xylene and CMC.

## CONCLUSIONS

Using the Principal Component Analysis (PCA), the Movila Miresei Lake was associated with salinity and conductivity, the Lake Căineni with temperature, while Balta Albă and Amara were influenced mainly by DO, pH, TDS, ORP, turbidity. The additional technique applied for investigating diversity of the phytoplankton confirmed the values of Shannon's diversity index and inquired the values of species richness. Thus, the Căineni Lake (with high salinity, after Movila Miresei) was on the first place in terms of diversity, the second was Balta Albă, and the less diverse were Amara and Movila Miresei. Salinity is not always the main factor shaping the diversity of saline lakes. Other factors, as temperature, DO, pH, TDS, ORP, turbidity can influence the biological communities of these lakes.

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## ACKNOWLEDGEMENTS

This study was funded by the projects no. RO1567-IBB02/2016 and RO1567-IBB05/2017 and 9PED/2017 (BioNanoTubenz) from the Institute of Biology Bucharest of the Romanian Academy and UEFISCDI. The study was conducted in the frame of the Convention of collaboration between the Oltenia Museum of Craiova and the Institute of Biology Bucharest. The authors thank Stela Sofa for the technical support.

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Received: March 31, 2018  
Accepted: July 3, 2018