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Original paper

The assessment of biotic composition related to environmental extremes conditions in natural salted Lake Letea

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Abstract Current work describes novel data recorded for biological composition correlated to environmental extremes in salted Lake Letea, located in the Danube Delta. The environmental extremes of the investigated site recorded were assessed in terms of their cultivable microbiological communities and relationships among plankton communities. The data show the absence of a cultivable archaeal community in the saline lake Letea. The bacterial communities are most probably represented by moderately halophilic or halotolerant strains. The extracellular enzymatic activities involved in the degradation of organic compounds revealed high aminopeptidase activity and occasionally showed β-glucosidase in supernatant, which appears over the sapropelic mud from the same lake. The phytoplankton of Salt Lake Letea consists of four groups of taxa: *Cyanobacteria* (7 sp.), *Bacillariophyceae* (12 sp.) *Chlorophyceae* (6 sp.) and *Pyrophyceae* (1 sp.). The richness of the zooplankton species is highest in spring and decreases progressively to autumn. The relevance of our study of consists in the novelty of the research in the areas; no other records can be found in the literature. Also, the systemic approach of our research, including both physical–chemical parameters and different biotic levels, can represent a very attractive way to study the salt lakes in Romania.

Keywords Salt lake, halophilic, Danube Delta, plankton communities.

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Introduction

Halophilic microorganisms represent a well-adapted microbial community in saline and hypersaline environments, either in salt lakes, or rock salt. In spite of limited life conditions due to high ionic strength and low water activity for enzyme functioning, saline environments host a relatively wide variety of organisms, mainly phyto- and zooplankton (ENACHE & al [11]; PĂCEȘILĂ & al [24]). In the last decade a huge research effort was made to explore life at high salinity in various saline and hypersaline environments. According to previous works, salted environments can be regarded as saline (containing 3-35 gL^{-1} chloride) and hypersaline (over 36 gL^{-1} 58 chloride content) (ENACHE & al [10]; JAVOR [15]). Due to restricted range of physical–chemical parameters in salted environments, the various life forms present are mainly represented by archaea and bacteria communities as the predominant organisms along with some photosynthetic flagellates from the genera *Dunaliella*, *Asteromonas*, and *Synechococcus*, the brine shrimp *Artemia salina*, and the brine fly *Ephydra*. In order to cope with these challenging conditions, organisms which can grow in salted areas developed various strategies for balancing external ionic strength. In this way, halophilic microorganisms either accumulate huge concentrations of salt inside the cell (archaea) or synthesize osmoprotective agents, namely compatible solutes (bacteria) (OREN [23]).

“Biological complexity” is still a misunderstood term, used to express *sensu lato*, various relationships between biology and the other sciences, and in *sensu stricto*, the relation between nuclei and cytoplasm, for example. In many areas of science, the term “complexity” is associated with mathematics, computers, and information theory or, in various cases, with social sciences. Taking into account the functional dynamics of the components in biological organisms, whether microbial life forms or microorganisms, and their interactions with external environments, biological complexity in saline areas can also be used to express the level of interaction for optimal functioning despite boundaries imposed by natural levels between biological structures present in a given area (ADAMI & al [1]). Salted environments are well represented in Romania, and they can be found as salt mines (i.e. Slanic Prahova, Ocnele Mari, Praid, Cacica etc.) or salt lakes (Bride Cave, Bride Hillock, Techirghiol etc.) (COJOC & al [4]; ENACHE [9]; NEAGU & al [21]). In the present work we summarize data regarding the biological composition of salt lake Letea (inner Danube Delta) and the ecological connections inside this saline ecosystem. Although the origin of the salted lakes located in the Romanian Plain is generally accepted as resulting from ancient origin or the intense evaporation processes in the area (GÂȘTESCU [12]), little is known about the specific origin of salt lake Letea. As is generally known, the Danube Delta harbors fresh water accumulated by the River Danube and represents the point where this water is mixed with salt water from the Black Sea. Consequently, the sand soils in the connection area probably define the origin of the salt lake, since these represent a source of the salt for the saline water from the lake. The relevance of this study consists in the novelty of the research in the area; no other records can be found in the literature. Also, the systemic approach of our research, including both the lake’s physical–chemical parameters and different biotic levels, represents a very attractive way to study the salt lakes in Romania.

Material and Methods

Investigated ecosystem – the Letea Salt Lake (surface around 1920 square meters) is located in the proximity of the village with a similar name in the inner Danube Delta (Fig. 1), between the Chilia and Sulina – branches of the Danube River, close to the Letea Forest of Danube Delta Biosphere Reserve, Romania. The predominant soils in that area (Letea–Cardon) are psamosoils, either gleized or salted (POPESCU & al [25]).

During winter and spring, the area experiences precipitation and several 99 temporary lakes appear until summer time, when these lakes are completely evaporated due to intense solar radiation and wide surface areas; afterward their surface is covered by vegetation (*Salicornia* sp.). The Letea Salt Lake is the only lake that keeps water in all seasons and doesn’t have a connection with other superficial water resources. This lake is not covered by aquatic vegetation and is known by the local population as a therapeutic and recreational place, especially in the warm season. The samples were taken in different seasons: spring (May), summer (August) and autumn (November), 2015 from four sampling points as represented in (Fig. 1).

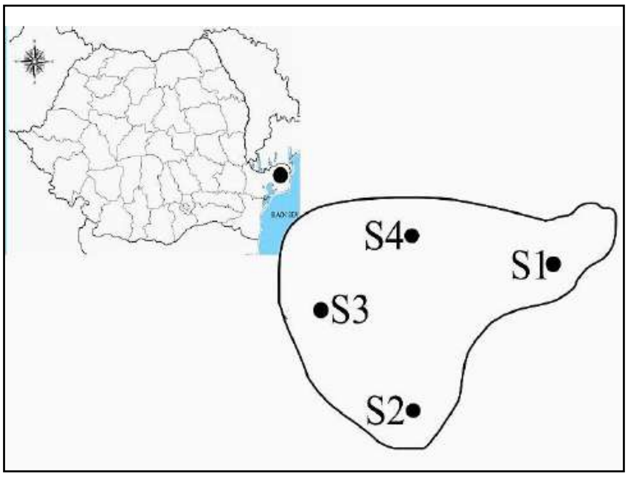

Figure 1. The location of Salt Lake Letea inner Danube Delta. The detail marked S1, S2, S3 and S4 represent the sampling points related for this study.

The waters' physical–chemical parameters – pH, conductivity, total dissolved solids salinity, oxidation-reduction potential (ORP), and temperature – were assessed in the field using a multiparameter HI 9828 from Hanna Instruments.

Phytoplankton and zooplankton analysis. The plankton samples were collected from water column by filtering 20 L of water through a 30 µm Ø mesh network using a Patalas Schindler plankton trap (4 L volume) and preserved with a 4% formaldehyde solution. Species identification and abundance were determined using a Zeiss inverted microscope according to the method described by (EDMONSON [6]) and specific taxonomic keys. The counting and taxonomical identification for each organism was performed simultaneously, a measured volume of subsample into a counting Kolkwitz chamber. Their abundance (ind L^{-1}) was assessed by the following equation (EDMONSON [6]):

$$\text{Species density} = \frac{\text{individual numbers in 1 mL X sample volume (mL)}}{\text{volume of water filtered (L)}}$$

The activity of four extracellular enzymes, L-alanine-aminopeptidase (EC 3.2.1), alkalin phosphatase (EC 3.1.3.1), α-glucosidase (EC 3.2.1.20) and β-glucosidase (EC 3.2.1.21), were assessed using fluorogenic substrates as described previously (PĂCEȘILĂ & al [24]). Soil, water and sediment samples were collected from the shore areas of the investigated lake. Water samples were processed after settling suspensions occurred. The results were reported per water liter or per gram of wet sediment.

The chloride content in the samples was determined following previously described protocols, namely by the Mohr method (ENACHE & al [8]).

The cultivable halobacterial strains were counted from water samples in a MH and JCM 168 medium as detailed in our previous works (COJOC & al [4]; NEAGU & al [21]). Thus, the viable microorganisms were quantified by the plate count method. To determine the of colony forming units (c.f.u.), serial dilutions of samples were prepared into sterile saline solution. One milliliter from each water sample and dilution was inoculated in drops onto Petri dishes and then molten agar medium was poured over it. The samples were inoculated in duplicate on different culture media. The composition of the medium used for the cultivation of moderate halophiles (MH) was as follows (gL^{-1}139): NaCl = 100, MgCl2x6H2O 7, MgSO4x7H2O = 9.6, CaCl2x2H2O = 0.36, KCl = 2, NaHCO3 = 0.06, NaBr = 0.026, glucose = 1, protease peptone = 5, yeast extract = 10 (VENTOSA & al [31]). The culture medium for extreme halophiles (JCM 168) contained (gL^{-1}): bacto casamino acids = 5; yeast extract = 5; sodium glutamate = 1; trisodium citrate = 3; MgSO4x7H2O = 29.5; KCl = NaCl = 175.5; FeCl2x4H20 = 0.036; MnCl2x4H2O = 0.36 mg; agar = 20. The pH of the culture medium was adjusted to 7.0-7.2 before autoclaving. Cultures were incubated at 28 for seven days.

The statistical analyses were performed using the XLSTAT pro, 2013, Addinsoft for Microsoft Excel, NY, USA.

Results and Discussions

The pH values of the investigated samples were around 8.7 in August and 9.2, in May and the temperatures between 7.8 and 25.5°C. The salinity varied between 8.43 gL^{-1} in spring to 45.08 gL^{-1}, in summer (Table 1). The conductivity and total dissolved solids (TDS) had a similar tendency.

Table 1. Physical and chemical parameters of Letea Salt Lake

Parameters	**May**	**August**	**November**
pH	9.23	8.78	8.91
Conductivity (mS cm^{-1})	14.51	66.20	65.05
TDS/PPM (Total Dissolved Solids)(mgL^{-1})	7.26	33.13	32.54
Salinity (total ions) (gL^{-1})	8.43	45.08	43.57
ORP (Oxidation-Reduction Potential) (mV)	59.33	-17.00	11.03
T (°C)	23.77	25.58	7.82

The bacterial community registered the highest values in November (365 C.F.U. mL^{-1}) and the minimum in spring (6.7 C.F.U. mL^{-1}) according to minimum salinity (8.43 gL^{-1}) (Table 1, Table 2).

Table 2. Variation of colony-forming units in 1 ml of sample water related to chloride content and seasons. The culture media used to estimate bacterial and archaeal communities were MH and JCM 168. The data of C.F.U. represent the results on MH media. On JCM 168 media no colony growth was recorded

Month/Sampling point		**S 1**	**S 2**	**S 3**	**S 4**
May	Chloride (g L^{-1})	6.7	7.1	7.1	7.0
	x10^2 C.F.U. mL^{-1}	5	3	3	4
August	Chloride (g L^{-1})	33	30	32	30
	x10^2 C.F.U. mL^{-1}	88	13	96	96
November	Chloride (g L^{-1})	28	28	31	32
	x10^2 C.F.U. mL^{-1}	356	334	365	93

Extracellular enzymatic activities revealed that aminopeptidase showed the highest value in all seasons. The activities of alkaline phosphatase were also recorded in all samples, with values between 25 nmol substrate L^{-1} h^{-1} in May and 150 in August (Fig. 2).

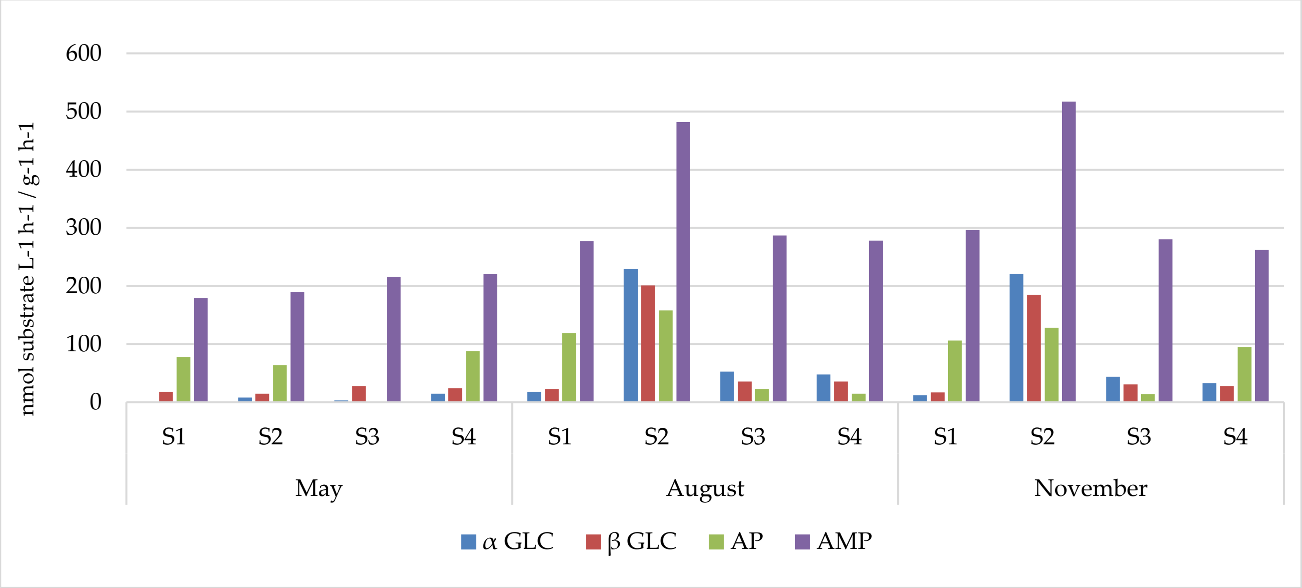

Figure 2. Extracellular enzymatic activities measured in Letea Salt Lake. α GLC = α-glucosidase; β GLC = β-glucosidase; AP = alkaline phosphatase; AMP = aminopeptidase; Sampling points (S) are described at (Fig. 1)

Phytoplankton and zooplankton communities

In terms of phytoplankton taxonomic composition, 34 species were registered, belonging to the phytoplankton of the lake consists of four taxonomic groups: (Table 3), of which the most predominant were *Cyanobacteria* (7 sp.), *Bacillariophyceae* (12 sp.) and *Chlorophyceae* (6 sp.) and *Pyrophyceae* (1 sp.) (Table 3).

Table 3. Species composition of phytoplankton from salt lake Letea ("+" = species is present; values (1, 2, 3, 4, 9) = the total species numbers attributed to one group)

	May	**August**	**November**
CYANOBACTERIA	**2**	**4**	**3**
Anabaena variabilis Kützing ex Bornet & Flahault, 1886		+	+
Anabaena sp.	+		
Microcystis flos-aquae (Wittrock) Kirchner, 1898		+	
Oscillatoria limosa C.Agardh ex Gomont, 1892			+
Oscillatoria sp.			+
Pleurocapsa sp.		+	
Spirulina sp.	+	+	
PYRROPHYCEAE			**1**
Ceratium hirundinella (O.F. Müller) Dujardin, 1841			+
BACILLARIOPHYCEAE	**9**	**3**	**4**
Amphora ovalis (Kützing) Kützing, 1844			+
Amphora sp.	+	+	
Cyclotella sp.	+		
Diatoma sp.	+	+	+
Gyrosigma acuminatum (Kützing) Rabenhorst, 1853.	+		
Melosira granulata (Ehrenberg) Ralfs in Pritchard 1861			+
Navicula sp.	+		
Nitzschia sigmoidea (Nitzsch) W. Smith, 1853	+		
Pinnularia gibba Ehrenberg, 1843			+
Pinnularia sp.	+		
Surirella sp.	+	+	
Synedra sp.	+		
CHLOROPHYCEAE	**2**	**4**	**2**
Cladophora sp.		+	
Mougeotia sp.		+	
Oedogonium sp.	+		+
Spirogyra sp.	+		
Ulothrix zonata (F.Weber & Mohr) Kützing, 1843		+	+
Ulothrix sp.		+	

The last one group was only occasionally found at one sample. One species belonging to Pyrophyceae has an accidental appearance in November, with a frequency below 25%. In total, the phytoplankton community registered 34 species during the 2015 year. Most phytoplankton species were constant, occurring in over 50% of the sampling time points (Fig. 3).

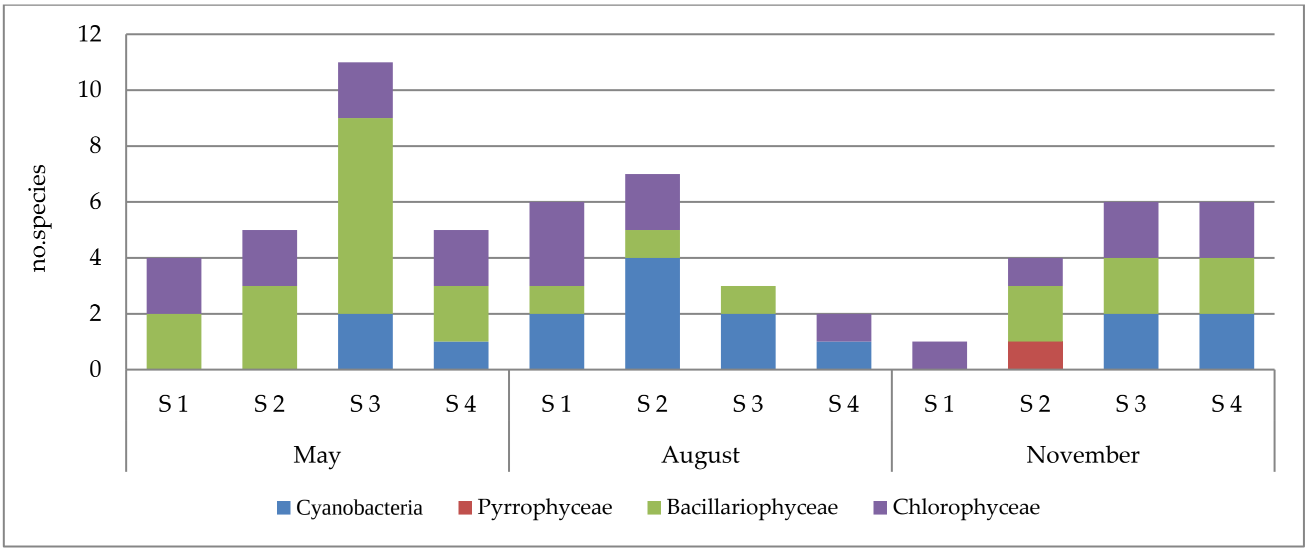

Figure 3. Seasonal species fluctuation of phytoplankton during 2015.

More rarely there are months or seasons where the taxonomic composition is represented only by one or two groups of phytoplankton; generally, all detected groups are present through all seasons and sampling points.

Chlorophyceae and *Cyanobacteria* were the dominant groups in terms of abundance, especially during the warm period. The density of Chlorophyceae varied from 2.69E+07 cell L^{-1} in May to 5.99E+02 cell L^{-1}in August, and Cyanobacteria registered the highest value in May (6.89E+06 cell L^{-1}). The density of *Bacillariophyceae* was lowest in August (1.85E+01 cell L^{-1}) (Fig. 4; Table 3).

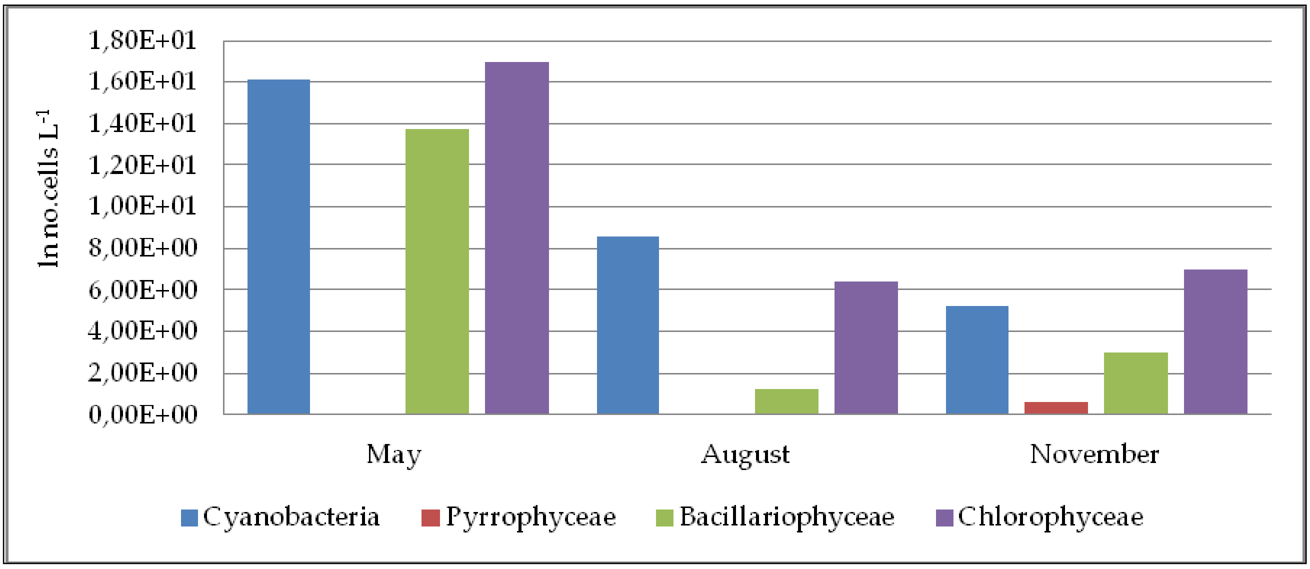

Figure 4. The abundance of phytoplankton groups

All these values are not log transformed. In terms of taxonomic composition, the zooplankton of the lake consist of six groups (*Ciliata, Testacea, Rotifera, Cladocera, Copepoda, Insecta*) (Table 4). The rotifers were the dominant group in all seasons, while cladocerans were reported only in spring (7 species). In total, the zooplankton community registered 27 species during the 2015 – year sampling period. The abundance of Ciliata and Rotifera reached the maximum values in August (294.39 ind. L^{-1}) and November (363.53 ind. L^{-1}), respectively. The Copepoda group registered the high values in spring and summer (43 ind. L^{-1}), the value dropped to 4.07 ind. L^{-1} in November (Table 4).

Table 4. Abundance of zooplankton in Salt Lake Letea (ind. L^{-1})

	May	**August**	**November**
Ciliata	0.11	294.39	184.93
Testacea	-	0.81	-
Rotifera	0.84	167.44	363.53
Cladocera	4.93	-	-
Copepoda	43.31	43.94	4.07
Insecta	0.20	7.79	-

The values of conductivity, total dissolved solids and salinity showed the same trend, depending on the season and the water temperatures. The values of pH and temperatures are reflected in the numbers of colony-forming units obtained seasonally from investigated samples taken from various points of the lake (Table 2). There has been observed that number of cultivable halobacterial strains increased with the chloride content revealing their importance for the ecosystem when salinity increased. Their number is higher in summer and autumn if compare with spring. On the other hand, should be noted that when culture media with very high sodium chloride content were used for isolation of cultivable halophilic microorganisms no colony growth were recorded. In this way, most probably predominant presence in the investigated lake is for halophilic bacteria, either moderately halophilic bacteria or halotolerant bacteria and not for extremely halophilic archaea). The pH values of the water appear to have no influence on the microbial communities and are relatively similar in the investigated samples. In terms of extracellular enzymatic activities, the values of aminopeptidase increased slowly when bacterial communities became more abundant. A similar profile was observed for alkaline phosphatase. The glucosidases showed a weak increase (Fig. 2). In most cases, extracellular enzymatic hydrolysis is the first step in the decomposition of detrital organic matter (WETZEL [32]). Thus, the assessment of enzymatic activity can provide information about the nature of the microbial decomposition processes in the analyzed ecosystems. α-glucosidase and β-glucosidase are part of hydrolytic enzymatic complexes that act on polysaccharides like starch and cellulose (REGE & CHOWDHARY [27]; TEUGJAS & VALJAMAE [29]), while alanine–aminopeptidase catalyzes the hydrolysis of various protein molecules (MIETTINEN & al [19]). Alkaline phosphatase hydrolysis of a large variety of phosphate esters (JANSSON & al [14]) releases orthophosphate, and is frequently used as an indicator for phosphorus conditions in aquatic ecosystems (JAVOR [15]). The presence of extracellular enzymatic activity suggests that an important source of carbon and energy for plankton was provided in this lake by the remineralization processes of detrital organic matter. Diatoms are microscopic algae living in both fresh and salt waters, being more numerous in cold seasons than in summer. These algae prefer colder seasons (SOMMER & al [28]). In order to test the differences in phytoplankton abundance during different seasons, an ANOVA test was applied. This method is able to show the statistical differences among two or more data sets. The results indicated significant differences in the seasonal abundance dynamics of the main phytoplankton groups, *Bacillariophyceae* ($F = 27.85$, $p = 0.0001$) and *Chlorophyceae* ($F = 18.42$, $p = 0.001$). Zooplankton species richness presented the highest values in spring and decreased progressively to autumn (Table 4). Most common species recorded in spring were found in the warm period. The decrease in species diversity is due mainly to increased salinity from spring into summer, to about five times the recorded value in spring (Table 1). In the spring, the salinity of the lake is lower due to freshwater input during winter. These conditions may favor diversity in that period. Even though the richness of species was higher in spring, the abundance recorded was low compared with other periods of sampling (Table 4). Note that salinity is the main driver of the dynamics of the observed plankton communities. Thus, in spring, the number of species is higher for both phytoplankton (13 species) and zooplankton (18 species). However, when the salinity increases in summer, there is a succession of species: salt-tolerant species appear in response to new conditions and their abundance increases. Most of phytoplankton species were susceptible to increasing conductivity, salinity and TDS. Thus, the identified species are not characteristic of these water types, which exhibit high salinity and a high degree of mineralization. Some research has reported the decrease of phytoplankton with increase of electrical conductivity and salinity (KAWABATA & al [16]). We found, however, that pH was the parameter that influenced *Cyanobacteria* ($R = 0.591$; $p = 0.04$), *Bacillariophyceae* ($R = 0.69$; $p = 0.01$) and *Chlorophyceae* ($R = 0.62$; $p = 0.02$) while ORP determined *Bacillariophyceae* ($R = 0.58$; $p = 0.04$) (Fig. 5).

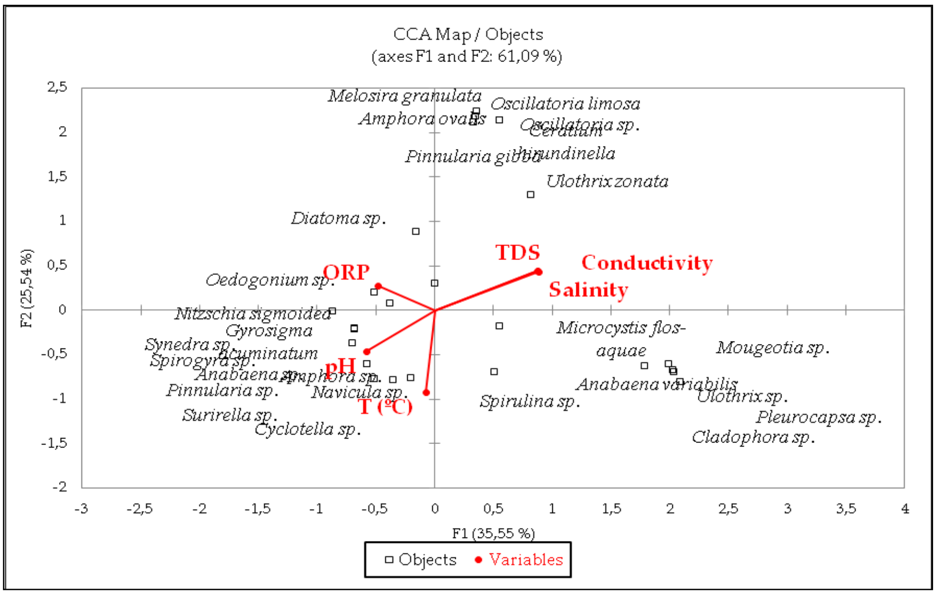

Figure 5. The canonical correspondence analysis (CCA) of relationships between phytoplankton species and environmental factors. ORP and TDS were defined in materials and methods.

At the zooplankton level, during the study two of the four recorded ciliate species were represented in abundance. In spring, *Epistylis* sp. was present in a very large number as an epibiont organism and in the last half of the year we observed *Paradileptus elephantinus* (SVEC, 1897; KAHL, 1931) (Table 4). *Epistylis* sp. was found exclusively on *Cladocera* species, even if in the same period the lake also contained copepods (Fig. 6).

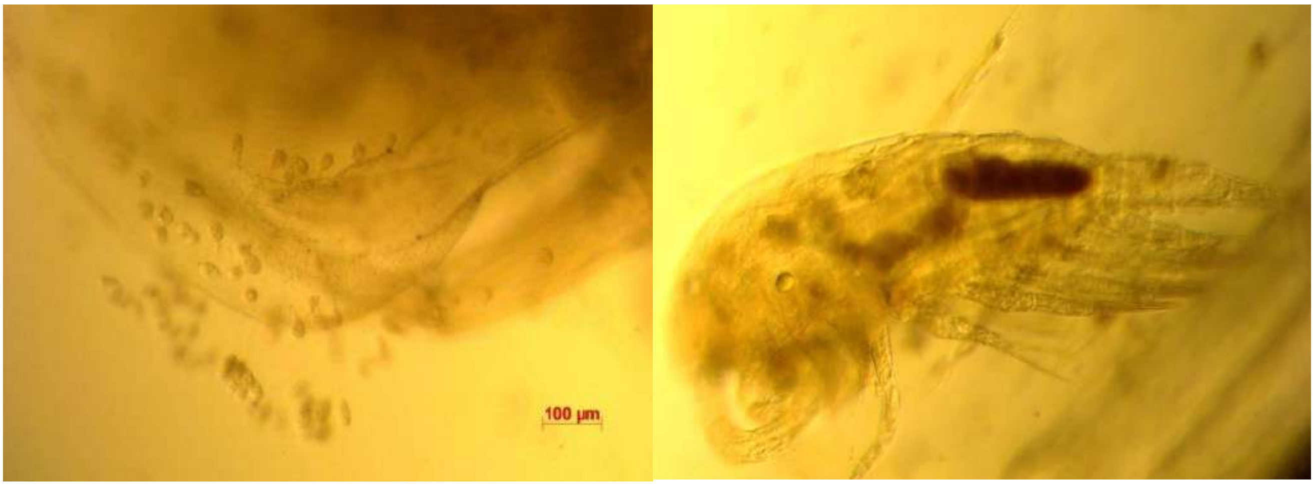

Figure 6. *Epistylis colony* (arrows) on the body of *Daphnia* sp. (left) and the absence on the body of copepod juvenile (right). The scale corresponds to 100 µm on optical microscopy.

The species belonging to the genus *Epistylis* can have epibiont–host relationships with various groups of aquatic organisms, but the most common are crustaceans, especially copepods.

The reason why *Epistylis* preferred only cladocerans in our study could be that copepods dominated in their juvenile stages, which have several moltings in their development (AZEVEDO & al [2]; BOZKURT & GENC [3]). Rotifer abundance increased from spring to autumn, especially that of *Brachionus plicatilis* (MULLER, 1786), otherwise a dominant species in total zooplankton. *Brachionus plicatilis* is among freshwater species which are known as salt tolerant (LEBEDEVA & ORLENKO [17]; TORUAN [30]) reaching high abundance in these conditions (ECHANIZ & al [5]). In contrast, crustaceans presented a different dynamic in the course of the year. Cladocerans were present only in spring and disappeared in the other two seasons. Salinity was the main parameter responsible for their disappearance ($R = -0.98$; $p < 0.0001$). It is known that this group is less common in salty waters, preferring a salinity of less than 1g L^{-1} (EL-DEEB GHAZY & al [7]). An increase in salinity may exert negative effects on the richness and abundance of total zooplankton (IVANOVA & KAZANTSEVA [13]). If some zooplankton species were disadvantaged by increasing salinity, *Paradileptus elephantinus* was favored by these conditions ($R = 0.97$; $p < 0.0001$). On the other hand, copepods showed sensitivity to temperature ($R = 0.91$; $p < 0.0001$), in particular calanoid copepods ($R = 0.986$; $p < 0.0001$). Due to the lake's shallow depth, its temperature decreases very much. The highest correspondence in terms of seasonal dynamics, based on AHC dendrogram (Fig. 7), was between ciliates and rotifers.

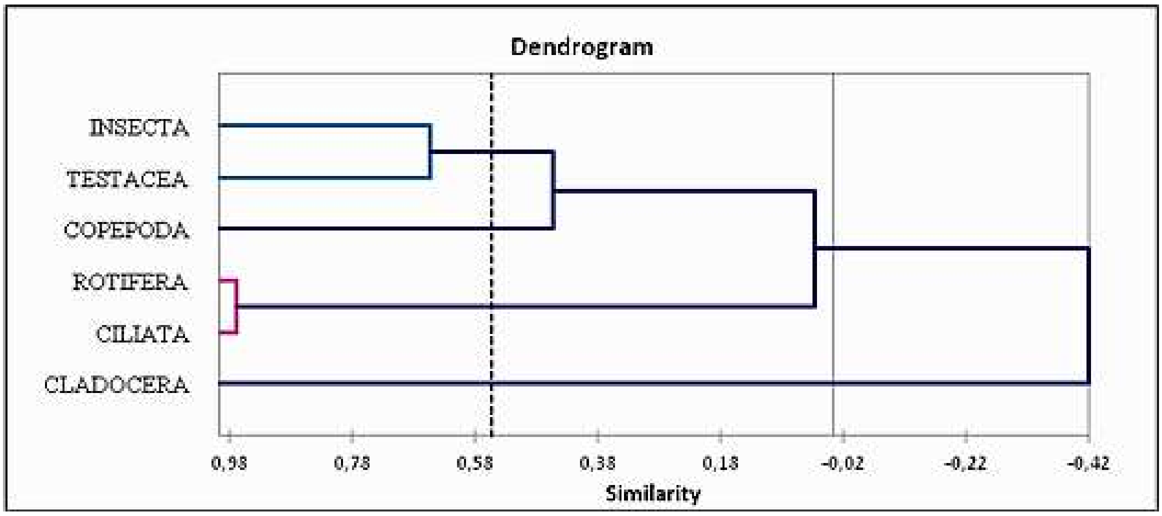

Figure 7. The similarity of zooplankton groups based on Agglomerative hierarchical clustering (AHC).

Our analysis also highlights a slight similarity in the presence of copepods, Testacea and insects. The relationship between the environmental parameters and dynamics of the zooplankton present can be highlighted by canonical correspondence analysis (CCA). Thus, we note that the environmental parameters divide zooplankton into two categories: species influenced by T, ORP, and pH, and species influenced by salinity, conductivity, and TDS/PPM (Fig. 8).

The results of CCA show the importance of some phytoplankton groups (68.83%) in the trophic spectrum of zooplankton (Fig. 9).

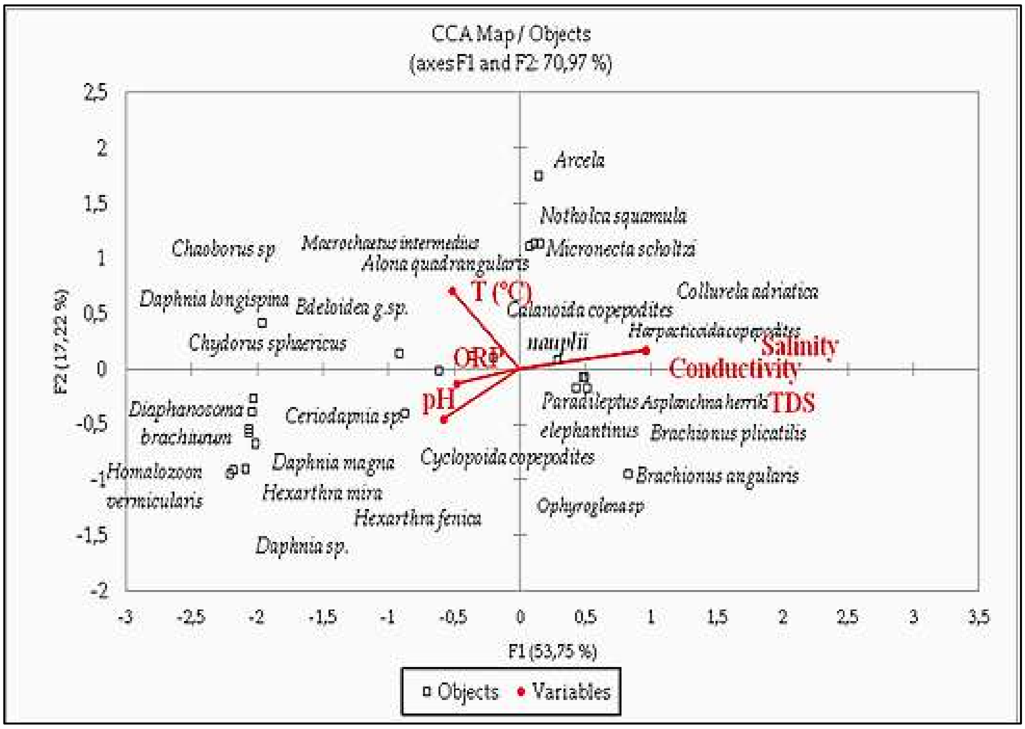

Figure 8. CCA analysis of relations between zooplankton species and registered chemical and physical parameters of the sample. ORP and TDS were defined in section Materials and Methods.

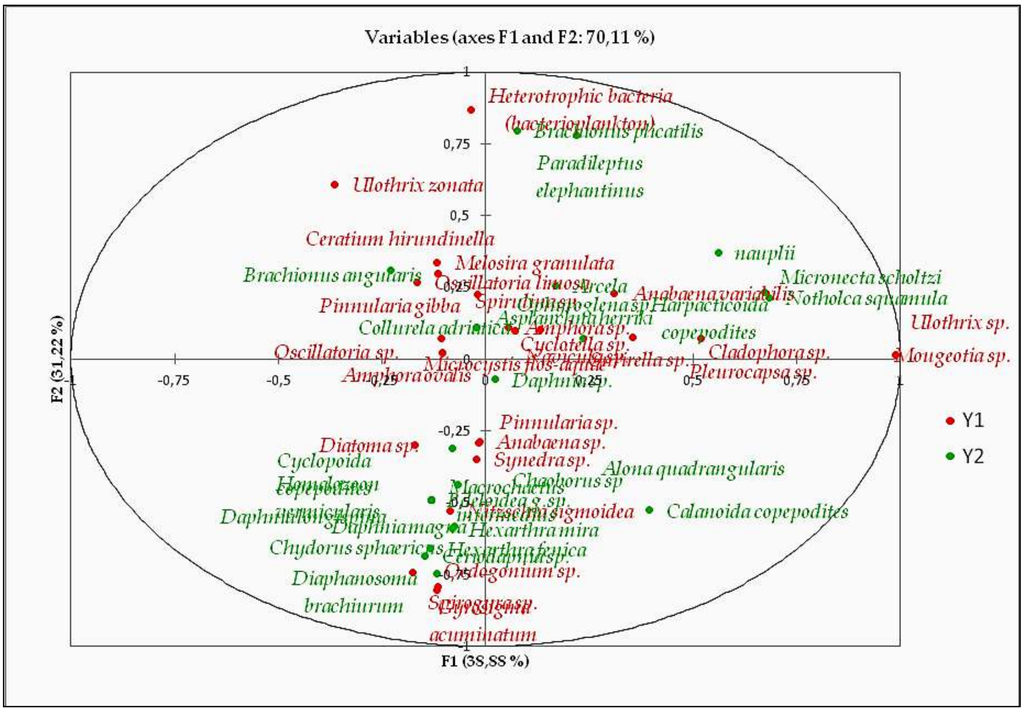

Figure 9. The CCA of the relationships among phytoplankton, zooplankton and bacterioplankton. Green color = zooplankton species. Red color = phytoplankton species.

Species belonging to *Ciliata*, *Copepoda*, *Cladocera* and *Rotifera* are able to tolerate cyanobacteria as food even if these phototrophic procariotes could be inedible or even become toxic in certain conditions (POSTOLACHE [26]). On the other hand, it can be seen that most species of cladocerans and rotifers have some preferential groups as food, primarily green algae with a rich nutrient content. The second food source was diatoms. The correlation between zooplankton and filamentous green algae (especially in the warm seasons, which registered a high rate of algae degradation) can be explained by the presence of these algae in detritobacterial aggregates. These complexes are formed by the association of algae fragments, detritus and colonized bacteria. The exposure to air of the filamentous green algae, especially near the banks where the depth of the lake is very low (<20 cm), often leads to their destruction under the influence of intense sunlight and dry conditions in the hot days of summer. Indeed, large masses of algae found in degradation were present along the lake shore. The restoring of green algae populations is ensured by the formation of akinetes, the resistance forms, induced by stressful conditions (e.g. *Ulotrix*). The green algae are resistant to temperature and salinity fluctuations and can be found in all seasons and under different salinity conditions in Lake Letea. *Brachionus plicatilis* and *Paradileptus elephantinus* were the dominant species in the structure of zooplankton community. Also notable is the correlation between these species and bacterioplankton. *Paradileptus elephantinus* is a large omnivorous ciliate, able to eat small metazoans and rotifers and also able to access aggregates (ZIMMERMANN-TIMM [33]). The availability of heterotrophic bacteria was also confirmed in our study, even considering the main food source of the ciliates ($R = 0.882$; $p < 0.0001$). *Brachionus plicatilis* is a rotifer having a low-to-medium ability to feed on bacteria (OLAV & al [22]).

Conclusions

Based on the presented results of the investigated saline lake, the phytoplankton communities are well developed and able to support an active microbial food web. The presence of green macroalgae populations in Lake Letea, in various physiological stages depending on season, argues that the microbial activity exerted on them by degradation and mineralization contributes to sediment formation.

Further investigation of the lakes' bacterioplankton and bacteriobenthos using molecular methodology will more clearly define their role in this extreme ecosystem.

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