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Seasonal Dynamics of Bacterial Indicators in the Albanian Part of Prespa Lake

E. HAMZARAJ[1,*], P. LAZO[2], O. KOJA[3], A. PAPARISTO[1], O. LAKNORI[1] and S. DUKA[2]

[1]Department of Biology, Faculty of Natural Sciences, University of Tirana, Tirana, Albania
[2]Department of General Chemistry, Faculty of Natural Sciences, University of Tirana, Tirana, Albania
[3]Department of Physics, Faculty of Natural Sciences, University of Tirana, Tirana, Albania

*Corresponding author: Tel: +355692178808, E-mail: etbur@yahoo.com

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The aim of present study is to assess the water quality of Prespa Lake according to microbiological indicators, such as total coliform bacteria and heterotrophic bacteria. Prespa Lake is part of Prespa National Park which is one of the protected areas in Albania and, apart from this, is an international focal point mentioned for its high biodiversity. MPN index was used for evaluation of total coliform bacteria in water. The number of heterotrophic bacteria was determined by counting colonies on plates with plate count agar. Samples were collected from May 2008 to May 2009 in two stations. The highest value of total coliform bacteria in water is registered in May, while the number of heterotrophic bacteria has not changed too much during the year. According to the received results for heterotrophic bacteria, the Lake Prespa water quality was in the frames of I-II class.

Key Words: Coliform bacteria, MPN index, Heterotrofic bacteria.

INTRODUCTION

Organic matter is constantly transformed in biological way throughout the biosphere and in aquatic ecosystems microorganisms play the most significant role[1]. Based on the bacteriological characteristics of water, associated with abiotic factors and above all with its chemical features, we can evaluate the water state of purity or pollution[2].

The objective of this study was to monitor the water quality of Prespa Lake based on microbiological indicators and chemico-physical parameters to evaluate the role of seasonal changes of environmental parameters on these indicators[1-12]. Prespa Lake is part of Prespa National Park, which is one of the protected areas in Albania and, apart from this, is an international focal point mentioned for its high biodiversity. Prespa National Park is situated in Balkan at 40º43'- 40º51' north and 20º00'-21º10' east, at the intersection of the frontiers of Albania, FYR of Macedonia and Greece. The Big and Small Prespa Lakes, with an altitude of 845 m above sea level and a surface area of 307 km^2, are a very important part of this park.

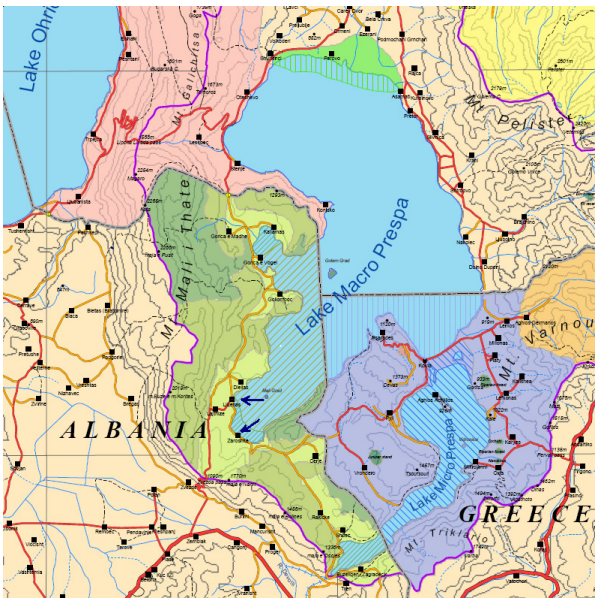
Commonly used microbial indicators of fecal contamination are total coliforms, fecal coliforms and fecal *Streptococci*. The total coliform group belongs to the family Enterobacteriaceae and includes the aerobic and facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas production within 48 h at 35 ºC[1]. This group includes *Escherichia coli*, *Enterobacter*, *Klebsiella* and *Citrobacter*. Fecal coliforms or thermotolerant coliforms include all coliforms that can ferment lactose at 44.5 ºC. The fecal coliform group comprises bacteria such as *Escherichia coli* or *Klebsiella pneumonae*. The presence of fecal coliforms indicates the presence of fecal material from warm-blooded animals.

EXPERIMENTAL

Samples were collected almost every month from May 2008 to May 2009 in two stations near two villages, Liqenas and Zaroshke, that are located nearby the Big Prespa Lake (Fig. 1).

(a)

(b)



(c)

Fig. 1. Photos of the monitoring sites, Liqenas (a) and Zaroshka (b) Map of monitoring sites (c)

Microbial water quality is traditionally monitored using culture based techniques that slectively promote the growth of bacterial indicators of fecal pollution[11,12]. Indicators of fecal pollution comprises the bacteria which are normally taken as indicators of the degree of purity of the water. The most commonly used indicators for surface waters are the fecal coliforms and *Escherichia coli*. This is a faecal bacterium which is found in the intestinal canal of man and warm-bloaded animals and is discharged with faeces and for this reason is used as an indicator of fresh faecal pollution[8].

Water samples were collected prior to the collection of sediment and using sterilized glass bottles. The closed container was submersed in water, than it was opened, rinsed two times with sample water, filled with sample and closed again in sub-surface. The water samplers were placed in cool boxes and transferred to the lab within 24 h[13].

Detection method: MPN index was used for evaluation of total coliform bacteria in water. Diluted samples were cultivated in series of five tubes with Lauryl Tryptose Broth, that were incubated at 35 ºC, for the preliminary test and at 44 ºC for the confirmation test[3].

The number of heterotrophic bacteria was determined by counting colonies on plates with PCA, cultivated with 0.1 mL sample after three series dilutions.

Environmental parameters like pH, temperature, turbidity, ammonia, phosphate, nitrite and dissolved oxygen where estimated using standard methods[12-14].

RESULTS AND DISCUSSION

Water quality bio-classification: The analyses for the most probable number of total coliform bacteria demonstrate present loading of the water with communal wastewater in the course of the whole investigation period (Fig. 2).

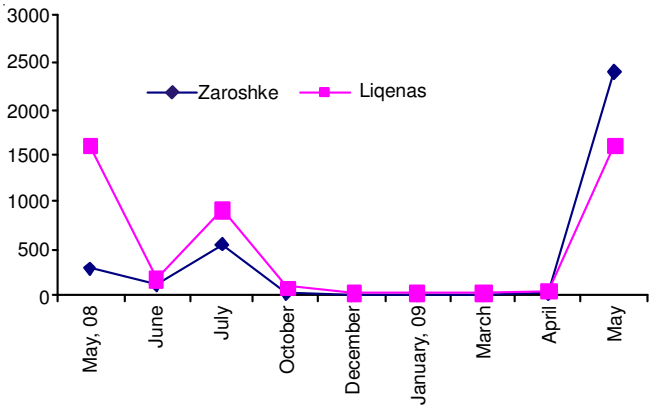


Fig. 2. MPN index for total coliform bacteria

As it was expected and revealed by previous investigations[6], the number of total coliform bacteria decreased from summer (with a maximum of 2400 bact mL^{-1} in May, 2009) to winter (with a minimum of 4 bact mL^{-1} in December, 2008), a pattern that is normally influenced by seasonal changes of temperature. The highest value of total coliform bacteria in water is registered in May. In our opinion these values could be due to heavy rains during spring months followed by high intake flows to the lake from land. Our investigation shows also that the number of total coli form bacteria in the littoral is higher than in the pelagic region, where these bacteria sometime were not even evidenced (Fig. 3).

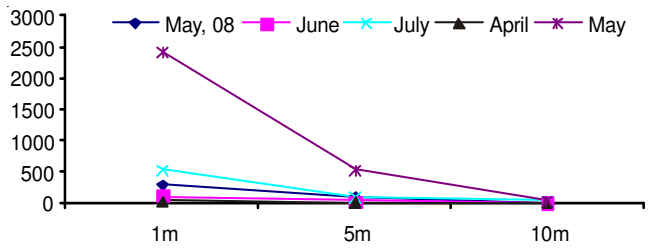


Fig. 3. Distribution of total coliform bacteria in the pellagic zone

On the other hand, the number of heterotrophic bacteria has not changed too much during the year, except some very high values registered in June and in January (11670 bact mL^{-1}) (Fig. 4). In first case, it is suggested that it is due to high temperatures in that month, while in the second case the high concentration of heterotrophic bacteria can be due to high intake flows to the lake after heavy rainfalls. According to the received results for heterotrophic bacteria, the Lake Prespa water quality was in the frames of I-II class[5-9].

TABLE-1
CONTENT OF NUTRIENTS AT PRESPA LAKE

Index	NO_2-N	(µg/L)	NO_3-N	(mg/L)	NH_4-N	(mg/L)	PO_4-P	(mg/L)	TN/TP	(Atoms ratio)
	Zaroshke	Liqenas	Zaroshke	Liqenas	Zaroshke	Liqenas	Zaroshke	Liqenas	Zaroshke	Liqenas
Min.	1.65	1.78	0.044	0.058	0.058	0.044	0.046	0.040	5.04	8.54
Mean	2.21	1.95	0.071	0.087	0.083	0.061	0.066	0.056	6.42	9.12
Max.	2.57	2.12	0.090	0.012	0.102	0.081	0.099	0.084	5.33	7.93

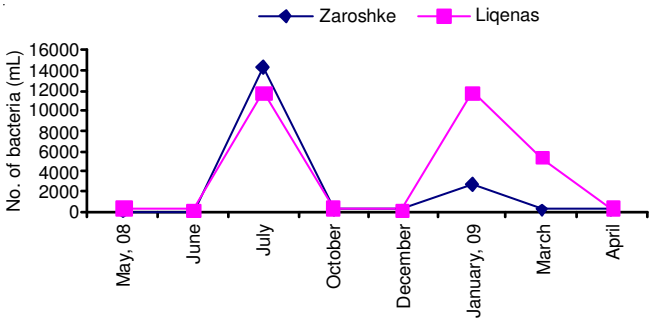


Fig. 4. Number of heterotrofic bacteria in the water of Prespa Lake

The quantity of bacterial populations in an aquatic community is determined in part by the physical and chemical properties of the body of water[5]. Measurement of total phosphorus and nitrogen show that there are no significant differences on nutrients content tested in different times for each station. According to these data and based on OECD classiffication, 1982, Prespa Lake can be classified in the group of eutrophic lakes (Table-1).

Water quality classification according to chemical parameters: Four water quality parameters dissolved inorganic nitrogen and dissolved inorganic phosphorus (NO_2-N, NO_3-N, NH_4-N and PO_4-P) are presented below. Chemical parameters (the content of nutrients) in water was determined by chemical methods through SFUV-VIS[13-16]. The obtained results are given in Table-1 and Fig. 5.

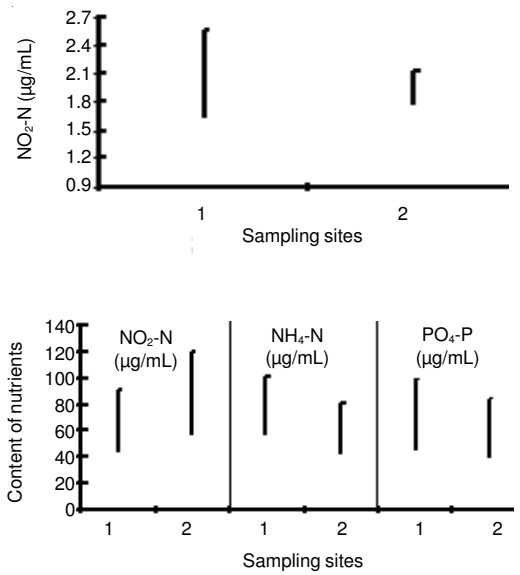


Fig. 5. Mean content of nutrients for each sampling period in Zaroshke (1) and Liqenas (2) sites

Meybeck *et al.*[10] and Chapman[12] suggested that, in fresh waters where the TN/TP mass ratio is greater than 7, phosphorus will be the limiting nutrient, whereas for TN/TP ratios below 7, nitrogen will be the limiting nutrient for algal growth (in practice, TN/TP less than 10 would indicate a nitrogen deficiency and TN/TP greater than 20 would indicate a phosphorus deficiency). Often TN/TP ratios are low in eutrophic lakes and high in mesotrophic and oligotrophic lakes. Both stations of this study showed TN/TP ratios between 5 and 9 value, indicating that in Prespa Lake, the limiting nutrient is azot. According to these data and based on OECD classiffication, 1982, Prespa Lake can be classified in the group of eutrophic lakes (Tables 2 and 3).

TABLE-2
ENVIRONMENTAL CLASSIFFICATION OF WATER OF TWO STATIONS POSITIONED IN THE LAKE

Zaroshke	Parameters	Liqenas
0.163	TN_{mean} (mg/L)	0.173
0.068	TP_{mean} (mg/L)	0.005
Eutrophic	Environmental situation	Eutrophic

TABLE-3
CLASSIFICATION OF NATURAL WATER QUALITY (UNECE, 1994)

	Oligotrophic	Mesotrophic	Eutrophic	Hypertrophic
TP (mg/L)	<0.013	<0.040	<0.100	>0.100
TN (mg/L)	<0.300	<0.400	<1.000	>1.00

It was seen the bacterial production was likely (NH_4-N and PO_4-P)-limited. The high direct correlation between (NH_4-N and PO_4-P) and bacterias content (Table-4) provides support for this conclusion.

TABLE-4
CORRELATION BETWEEN MEAN CONTENT OF NUTRIENTS AND BACTERIA (INVESTIGATED DURING THE SPRING)

Index	NO_2-N	NO_3-N	NH_4-N	PO_4-P	TCB	Heterotrof
NO_2-N	1	–	–	–	–	–
NO_3-N	-1	1	–	–	–	–
NH_4-N	1	-1	1	–	–	–
PO_4-P	1	-1	1	1	–	–
TCB	-1	1	-1	-1	1	–
Heterotrof	-1	1	-1	-1	1	1

The strong positive correlation between TCP's and Hetrotophes indicating both are going in the same sense; the same factors control their content in water. It is clearly demonstrated TCP's and hetrotophes have strong positive correlation only with NO_3-N and strong negative correlation with NO_2-N, NH_4-N and PO_4-P. Generally assumed that nitrogen is not a growth-limiting factor due to the low concentrations needed for growth and maintenance and high turnover of existing cellular nitrogen. It was found that nitrogen levels did not decrease as water traveled in the distribution system, indicating that it was not consumed by the microorganisms to

support growth[15]. Negative correlation of TCP's and hetrotophes with NO_2-N, NH_4-N, can lead to low levels of dissolved oxygen and negatively alter various plant life and organisms. A few studies have determined that phosphorus levels could limit microbial growth in the distribution system[15] and it is the limiting nutrient for algal growth in lakes, especially in oligotrophic-mesotrophic conditions[14], being the factor for the interpretation of negative correlation between TCP's/and or Hetrotophes with PO_4-P content in surface water of Prespa lake.

Conclusion

The obtained results for the investigated groups of bacteria show, in general, that values in the littoral water of Prespa Lake are not with a great variability during the year, except two or three months when environmental factors have a distinct impact. The total coliform bacteria were usually present in the summer season, or in other seasons after heavy rains. According to the results for heterotrophic bacteria, the Lake Prespa water quality was in the frames of I-II class (eutrophic). The same results were obtained through the investigation of the content of nutrients in the same conditions with bacteria.

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