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CHEMICAL AND MICROBIOLOGICAL STUDY FOR THE WATERS OF LAKE BRĂTENI

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Abstract

The present paper is a study of the water quality sampled from Brăteni Lake and the streams near the lake. The analysis targets the microbiology via DNA extraction, PCR and sequencing of DNA fragments and the chemistry through nitrate and ammonium determination. For the quantification of bacteria present in water samples, 3 culture media were used: R2A for oligotrophic microorganisms, Standard I as a complete medium and Burks for nitrogen fixing bacteria enrichment. The identified bacteria included, Pedobacter, Streptomyces, Bacillus, Flavobacterium and Sphingobacterium. Nitrate and ammonium had higher concentrations in one of the streams near the lake. The results highlighted a close link between land use and lake water quality, agricultural activity having a strong influence on water quality.

Key words**:** *Brăteni Lake, water quality, microbiology, DNA extraction, nitrate*

INTRODUCTION

The available freshwater resources of the planet are starting to become scarce. The European Union makes considerable efforts in protecting them [11], e.g. with the Water Framework Directive that is an important document in this direction [3]. Water is one of the most important and protected resources in the EU and worldwide, inducing studies on physiochemical water quality [9], [1]. The environmental system can be defined and understood by knowing the microorganisms present in the study area [4]. Some more chemically distinct media (alkaline, acidic) have a specific diversity of microorganisms that can create an endemic system [14]. Other studies placed a rather large emphasis on the microbiological composition, the bacteria being an important consumer of organic matter and implicitly a regulating factor of water quality [6]. The microbiological water quality can influence the fauna in the studied water body [5]. An important influence on water quality is the human activities around the water body [8]. In particular, the land use is an important factor for the chemical and microbiological components controlling the surrounding water quality [16]. A preliminary study conducted during November 2015 and April 2016 pointed to intermediate nitrate pollution in the studied water body [10].

MATERIALS AND METHODS

Study area

The studied lake is in the northern central part of Romania. It is located in the Transylvanian Depression and covers approximately 25 ha. The studied area is under a moderate continental climate. The rainfall regime varies between 600 - 650 mm/year and the average annual temperature is just above 8°C [2]. The land surrounding the lake is used for agriculture with some of the streams that supply water to the lake (Fig.1) receiving inflow from agricultural fields. The fauna and flora of the lake is rich and representative for

the studied area. The riparian vegetation surrounding the lake provides excellent shelter for the lake fauna consisting of many fish and amphibian species. The most common fish species found in this lake are: *Carassius gibelio, Abramis brama, Cyprinus carpio, Aristichtys nobilis, Silurus glanis, Perca fluviatilis, Lepomis gibbosus, Hipophtalmychtis mollitrix and Scardinius erythrophthalamus.*

Methodology

Water samples were collected from Brăteni Lake and the streams near the lake (Fig.1). Points P1, P2 and P3 were sampled from the lake surface and points P4 - P7 were located in the creeks near the lake, while point P8 was downstream from the lake. The water samples were collected in February 2017 in sterile polyetylene containers of 500 mL. For all water samples, nitrate and ammonium concentrations were measured (Sera Kit, Hamburg, Germany; readout with Genesys Spectrophotometer Thermo Fisher, Waltham, USA). To perform microbiological analyses, three media including Standard I (Roth, Karlsruhe, Germany), R2A [12] and Burks [13] for plating dilutions of up to 10^{-3} in triplicates. Pure cultures were obtained after taking the number of colonies per mL. After DNA extraction with Chelex [7], PCR (primers 27f:5´AGAGTTTGATCCTGGCT CAG3´and1492r:5´ACGGCTACCTTGTTAC ACTT-3´) was performed [15]. Sequencing (GATC, Cologne, Germany) yielded phylogenetic determination performed by comparison to known sequences (NCBI Genbank, USA).

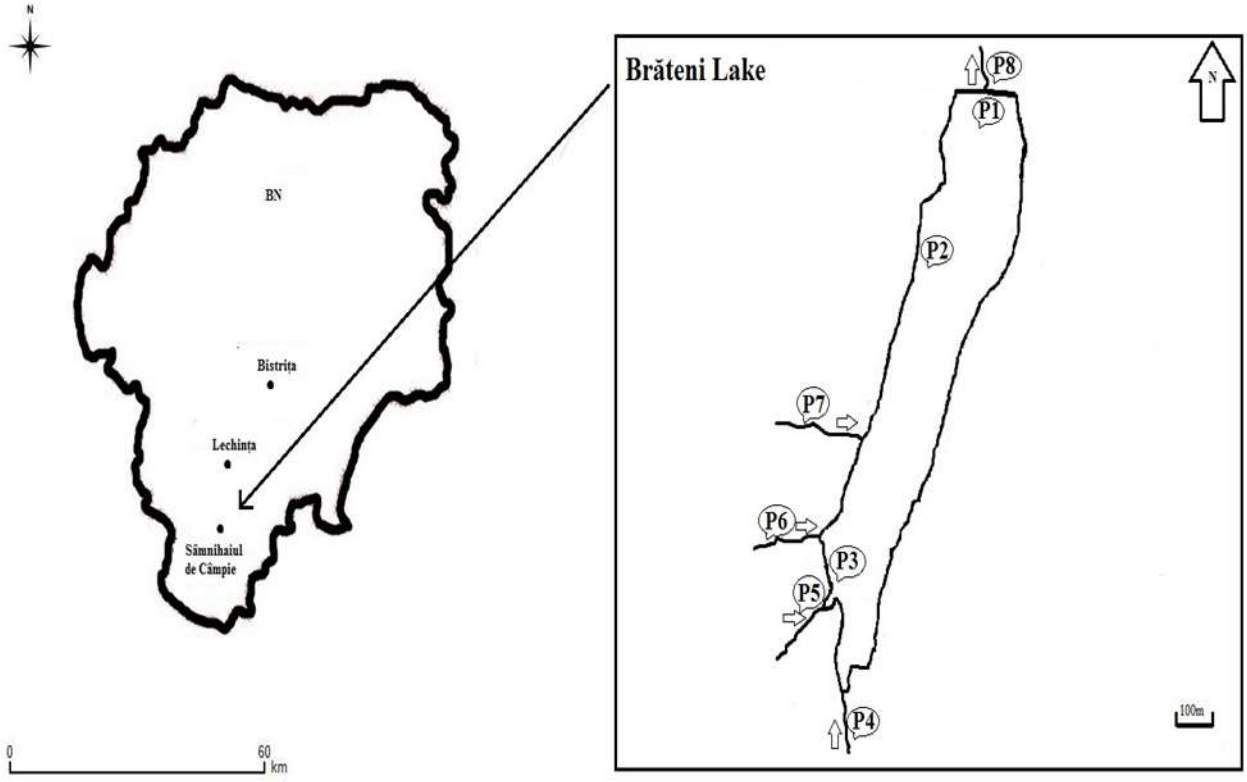

Fig. 1. The study area with sampling points (P1 – P8).
Source: Own determination

RESULTS AND DISCUSSIONS

The colonies obtained from non-selective Std I media (Fig.2) showed that the points inside the lake (P1,P2,P3) had lower colony forming units compared to the external sampling points, which at P5 exceeded 3,000 colonies/ ml.

R2A medium is more selective and facilitates the emergence of bacteria from an aquatic

environment, which was visible in higher concentrations of colonies at the sampling site P2 in the lake, with up to 8,000 colonies/ml.
Measuring point P5 yielded increased bacterial loads for the stream environment. Burks medium allows enrichment of soil bacteria, especially including nitrogen fixing species, which was seen with higher yields for the external sampling points of the lake.

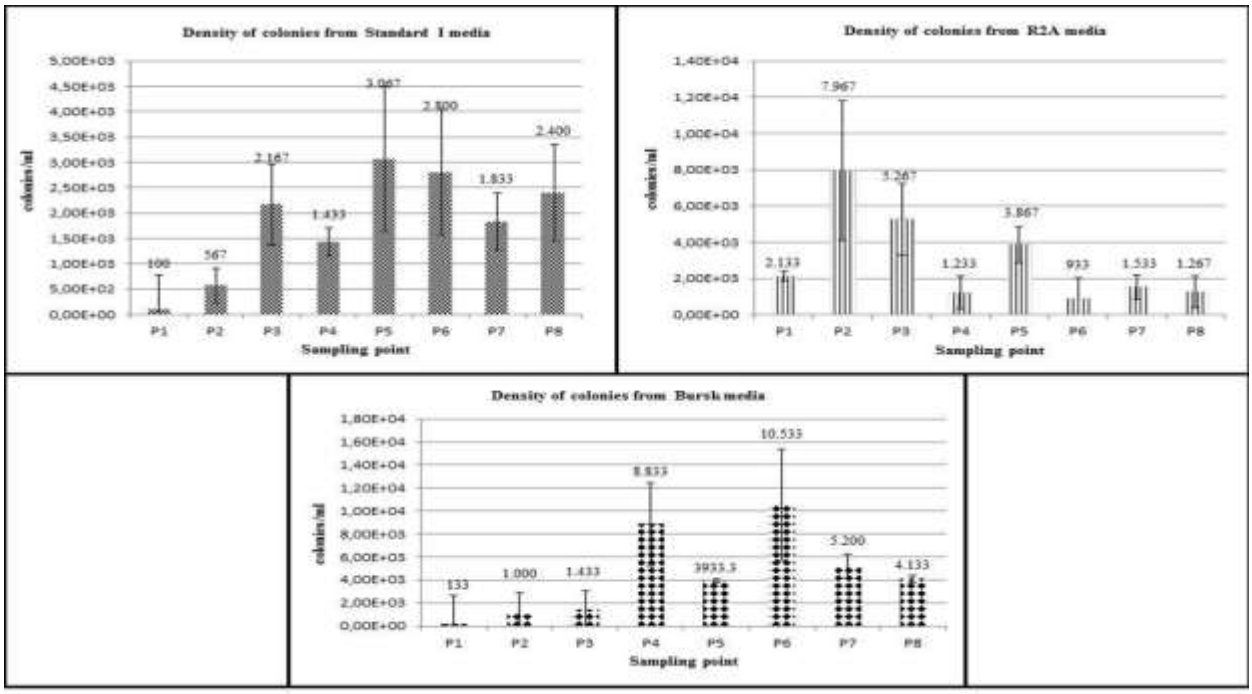

Fig. 2. Colony forming units at 8 sampling points using three different media (n=3).
Source: Own determination

Point P6 exceeded the concentration of 10,000 colonies/mL, followed by point P4 with a concentration of approximately 9,000 colonies /ml. The points inside the lake (P1, P2, P3) did not have values above 1,500 colonies/ml. At the points outside the lake there is a much larger bacterial presence (especially in the Standard I and Burks medium).
The nitrogen fixation correlated weakly with nitrate concentration (Fig.3) with a maximum at point P4, over 3 mg/L, exceeding Romanian legislation (Order 161/2006), which is ranked in the 3rd quality class. A low ammonium concentration in lake water was observed with a peak at point P6, above 1.4 mg/L (Fig.4). If we relate to the waters outside the lake (points P4 – P8), they fall into the 3rd and 4th quality class.

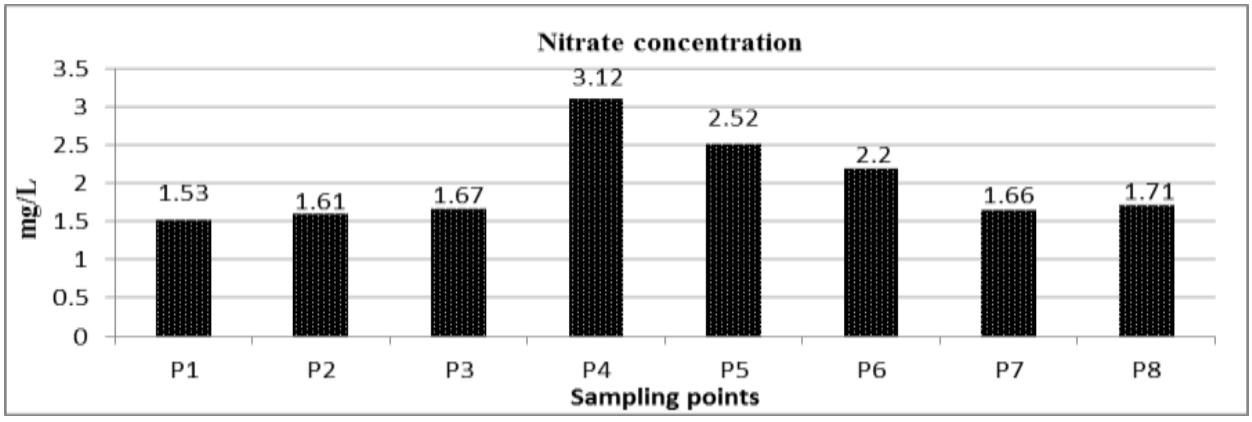

Fig. 3. Nitrate concentration (mg/L) from water samples (P1 – P8).
Source: Own determination

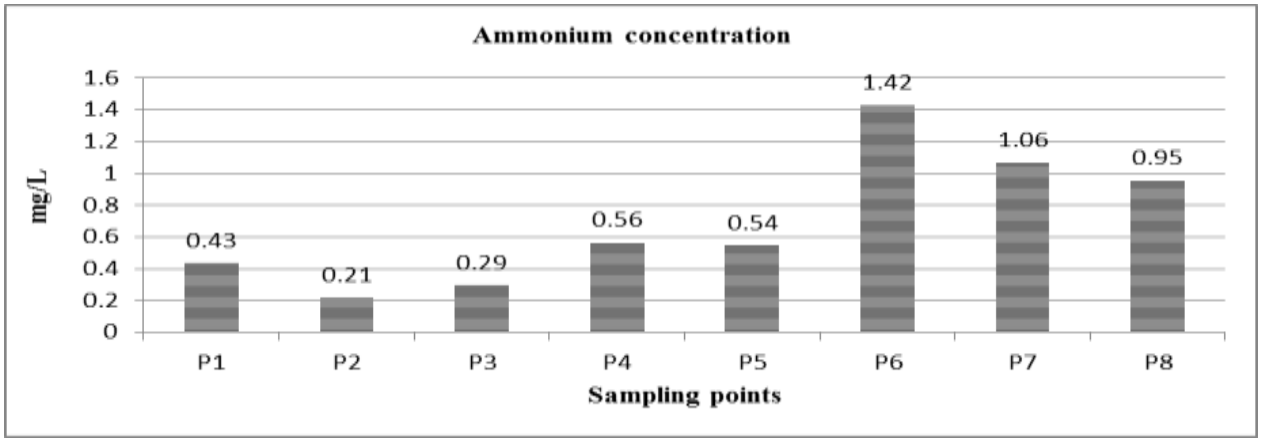

Fig. 4. Ammonium concentration (mg/L) from water samples.
Source: Own determination

The comparison with the Romanian legislation (Order 161/ 2006) confirms that the lake tributaries have a pronounced pollution, but the lake water still has acceptable quality for the type of use.

The isolates were phylogenetically analyzed and their next neighbors show very different habitats, none of them associated with eutrophic lakes (Table 1).

Table 1. Bacterial isolates from the different sampling points

Name of isolate	**Accession number for 16S rDNA sequence of nearest neighbor**	**Place of discovery**	**Sampling point**
Duganella zoogloeoides strain NBRC 102465	NR_114106.1		P1
Uncultured bacterium clone ncd2091d04c1	JF169117.1	Leather	P3
Pseudarthrobacter sp. strain MB10	KY445627.1	Soil	P3
Pseudomonas sp. FBF96 partial	HG805767.1		P4
Pseudomonas fluorescens	AB680976.1		P4
Mycobacterium sp. Ellin113	AF408955.1		P4
Bacillus sp. strain yangyueK2	KU977127.1	Soil (China)	P4
Bacillus sp. strain 70015	MF045082.1	Ocean sediment in Bohai Bay (China)	P4
Plantibacter sp. strain BAV2857	KY074037.1	Rain in Montgomery (USA: Blacksburg, VA)	P4
Flavobacterium sp. WB2.1-19	AM934633.1	Harsh water brook (Germany: Westenhoefer Bach)	P4
Variovorax sp. HP3O1	KM187456.1	Amphibian Pseudacris crucifer	P5
Pseudomonas sp. 41110	KC834307.1	Rhizosphere	P5
Streptomyces sp. WP-XU-1-2	KC555534.1		P5
Pseudomonas lini strain 48C10	KT695832.1	Soil (Wisconsin)	P5
Rahnella aquatilis strain CZ-BHG006	KT765843.1	Fecal (China: Qinghai Lake)	P5
Sphingobacterium sp. THG-CR32	KF999712.1	Artichoke tea (South Korea)	P6
Pedobacter agri strain YF28-3(1)	KT369848.1	Mount Qilian (China)	P6
Paenibacillus polymyxa strain ATCC	CP011420.1	Soil (Belgium)	P6
Variovorax sp. SAP777.1	JX067694.1	Floral nectar	P7
Pseudomonas sp. DRE-2009-B3	FM956661.1	Phenol (United Kingdom: West Midlands)	P7
Flavobacterium sp. I	KF555636.1	Soil	P7
Pedobacter terrae strain QT16	GU385862.1	Soil (China: Qinghai-Tibet)	P7

Source: Own determination.

In this points is an important variety of bacteria, but most of these bacteria are normal occurrence in the soil. A few examples from our research are: *Pseudomonas sp., Pedobacter terrae, Pseudomonas lini., Bacillus sp., Paenibacillus polymyxa., Pseudarthrobacter sp., Flavobacterium sp*.

CONCLUSIONS

The lake receives water with higher concentrations of nitrate and ammonium.

These potentially harmful substances are diluted in the lake water. At the same time, the contributaries also carried higher bacterial loads, many of which are related to soil-specific clased. Their presence in the water bodies thus might indicate agricultural input into the eutrophic lake. The highest number of isolates on R2A medium from points within the lake (P1, P2, P3), show that dilution of nitrate coincides with lower abundance of bacteria.

The creeks have a semi-permanent character and at least part of the year they do not represent a habitat for typical water body associated bacteria.

The presence of many soil bacteria as seen from isolation with Burks medium revealed that specifically sampling point P6 shows large influence of soil, possibly by erosion processes during winter. According to the results presented here, an adverse effect of the agricultural activity around the lake on water quality could be seen combining chemical and microbiological analyses. Specifically, the nitrate and ammonium concentrations necessitate further monitoring, especially for the creeks.

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