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RESEARCH OF THE MICROBIAL CONTAMINATION OF A LAKE IN THE CITY OF SOFIA WITH REGARD TO ASSESSMENT OF ITS ENVIRONMENTAL SAFETY

Dorothea Y. Iosifova, Mina–Maria T. Marinova, Teodora P. Popova[*]

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: tpopova@ltu.bg

ABSTRACT

Microbiological studies were performed on ten samples from a water body in a metropolitan district with a view to evaluating its epidemiological safety. The amounts of microorganisms from the main groups that include pathogenic species, were determined using microscopical, cultural and biochemical methods. The quantities of Gram–negative aerobic and facultative–anaerobic bacteria ($5.50x10^2±6.43$ – $5.74x10^2±8.87$ CFU/ml) and staphylococci ($0.42x10^2±0.84$ – $5.62x10^2±1.07$ CFU/ml) prevailed. The sanitary indicative species *Escherichia coli* was abundant, especially in the surface water layers ($2.92x10^2±5.54$ CFU/ml). *Pseudomonas* species were less ($0.30x10^2±0.51$ CFU/ml) and found only in the surface layer. Clostridia were isolated from 40% of surface water samples and 80% of bottom samples, but the sanitary indicative species *C. perfringens* was not detected. The isolated microorganisms were in greater quantities in the surface water layer, but the differences were statistically significant only for staphylococci. Fungi and clostridia were established in greater quantity at the bottom of the lake.

Key words*:* pathogenic microorganisms, lake, epidemiological safety.

Introduction

Water bodies in or near populated areas are attractive for walking people, pets and are part of the range of wild animals. This is a prerequisite for their contamination with biological materials. Thus, there are dangers of contamination with pathogenic microorganisms and the risk of infecting people and animals with the causative agents of various infections. Microbial pollution not only of water bodies used for drinking water supply, but also for recreational activities and catching fish, crayfish and other aquatic gifts poses a risk to the health of animals and people in the respective regions. It is the basis of numerous outbreaks of gastrointestinal diseases. It is important to bear in mind that a link has been established between heavy rainfall, droughts and floods, which are increasingly common today, and outbreaks of water–borne diseases. Jung *et al.* (2014) pointed out that the monitoring of microbial contamination of water bodies is important from an epidemiological point of view. For this purpose, it is recommended to use simple and quick indicators, mainly faecal bacteria (*E. coli* or enterococci).

The large amount of microorganisms and toxic chemicals present in non–potable water that is used for swimming, fishing, boating, irrigation and other activities can be a serious health hazard (Some *et al.*, 2021). A wide range of anthropogenic pollutants are concentrated in the water and sediments of lakes, including organic matter and toxic substances that accumulate over time. Besides the direct danger to human and animal health, this pollution can also affect microbial communities, which play a crucial role in biogeochemical cycling and food chains. In a study of Lake Geneva, Lyautey *et al.* (2021) reported that anthropogenic pollution of a lake, including organic waste, nutrients and toxic substances, can adversely affect the diversity, structure and functions of surface sediment–associated microbial communities, as well as their resistance to metals and antibiotics.

Gunnarsdottir *et al.* (2013) found that a septic system located 80 m upstream of a catchment in a village in Iceland next to Lake Mývatn was a source of contamination of the drinking water they studied, even in a cold climate. Fong *et al.* (2007) also identified surface–groundwater interactions in Ohio and found that groundwater contamination with total coliforms, *E. coli*, and enterococci may also be a source of human infection. A role in this regard is played by extreme rainfall, which caused a sudden rise in water levels, a rapid exchange of surface and underground water and a significant influx of organic material. On the other hand, microbial communities play a crucial role in the functioning of ecosystems, especially in organic matter degradation, nutrient cycling and pollutant removal (Bourhane *et al.*, 2022), and the structure and composition of the microbial community can be used to assess environmental health.

The aim of the present work was to carry out microbiological studies for the isolation, identification and quantification of microorganisms from major groups, including pathogenic species, with a view to assessing the epidemiological safety of water of a lake in a large metropolitan district.

Materials and methods

Samples

Tests were carried out on 10 water samples from the Druzhba lake in the Druzhba district in the city of Sofia, obtained from five different places, on 05.12.2022. They were taken in sterile containers from the surface and from the bottom (at about 0.5 meters deep) from each investigated place.

Site description

Researched object. Lake Druzhba is located within the city of Sofia in the Druzhba district. It was formed in 1970 by underground water after excavation works to expand the residential area. The lake consists of two parts separated by a pedestrian bridge. The surrounding area is landscaped and a park has been formed. Fountains are installed in the larger part of the lake, and there is a chapel on the shore of the smaller part. The lake and its shore are home to many animal species, represented by fish and birds to small mammals.

Nutrient media

For the *isolation* of microorganisms, cultures were made from the water samples on elective and selective nutrient media. Mueller Hinton agar, Columbia blood agar (Biolab Zrt. H–1141, Budapest Ov. utra 43), as well as selective and differentiation media were used: Colorex Chromogenic Orientation agar (Ridacom–Sofia), Chapman Stone agar with mannitol for staphylococci, Endo agar (Antisel – Sharlau Chemie S. A., Spain) for Gram–negative bacteria, Cetrimide agar (Biolab Zrt. H–1141, Budapest Ov. utr.) for *Pseudomonas aeruginosa* and Sabouraud dextrose agar with chloramphenicol (Antisel – Sharlau Chemie S. A., Spain) for *Candida* spp. and filamentous fungi. For the isolation and cultivation of *Clostridium perfringens*, a thioglycollate broth in a high column (Antisel – Sharlau Chemie S. A., Spain) was used, and for this purpose the water samples were previously thermally treated in a boiling water bath for 2 min to remove non–spore–forming microorganisms.

The microbiological studies

The microbiological studies were carried out by inoculating the investigated water samples on the elective and selective media with 0.1 ml each. The results were reported after incubation under aerobic conditions at 37°C for 48–72 hours, as well as anaerobically (thioglycollate broth). To create anaerobic conditions, the Anaerob Pack with palladium catalyst – H_2 + CO_2 system (Bul Bio NCZPB

– Sofia) in Jar was used. Formed colonies were counted and the results calculated and presented in colony forming units/ml (CFU/ml) in the source water samples.

Microscopic investigations

Microscopic investigations of the microorganisms were performed under immersion at a magnification of 1000 x in a covered drop slide and after staining of the studied materials according to the classical methods of Gram, Pfeiffer and Klett (for capsules). A digital microscope with a camera, model B–190TV, Optika, Italy, was used.

Taxonomic identification

Taxonomic identification of the isolated microorganisms was performed by conventional methods according to the 9th edition of Vergey's Identifier (Holt *et al.*, 1994). It was carried out by means of microscopic examination of preparations stained by different methods and reporting the cultural, hemolytic and some biochemical properties on solid and in liquid media.

Quantification

Quantification of microorganisms was performed by counting the developed colonies, determining their arithmetic mean number and calculating the amount of colony forming units (CFU) in 1 ml of the starting material.

Statistical analysis

The results were processed mathematically by finding the arithmetic mean and standard deviation. A Student's t–test analysis for independent samples was applied to test the statistical dependence and reliability of the results.

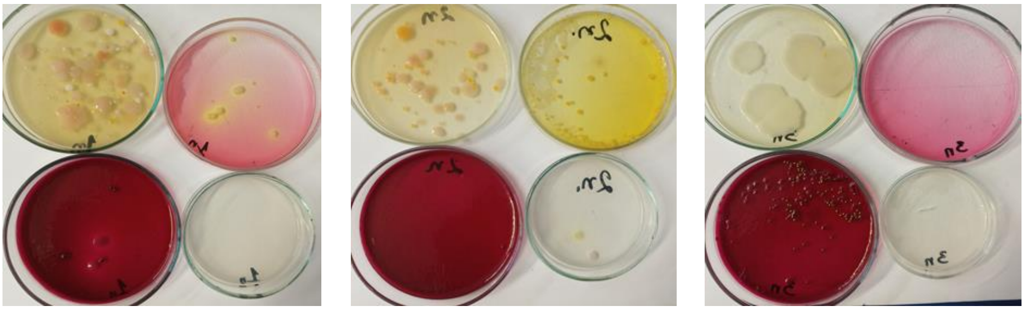
Results

The data from the microbiological tests performed on the water samples are presented in Table 1. Some of the results of the microbiological tests can also be seen in Figures 1 – 4.

Table 1: Microbial content of the investigated water samples

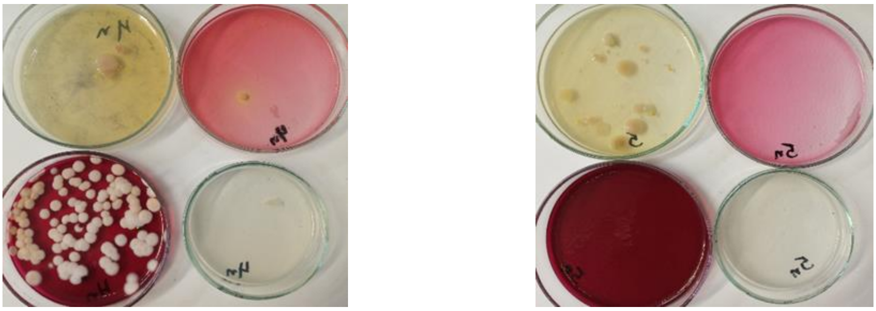
Microorganisms	**Quantities of isolated microorganisms – CFU/ml**	
	From the surface (n=5)	**From depth (n=5)**
Total number	$1.62 \times 10^3 \pm 1.08$	$1.13 \times 10^3 \pm 0.81$
Gram–negative bacteria	$5.50 \times 10^2 \pm 6.43$	$5.74 \times 10^2 \pm 8.87$
Escherichia coli	$2.92 \times 10^2 \pm 5.54$	$0.02 \times 10^2 \pm 0.04$
***Pseudomonas* spp.**	$0.30 \times 10^2 \pm 0.51$	0
***Staphylococcus* spp.**	$5.62 \times 10^2 \pm 1.07$	$0.42 \times 10^2 \pm 0.84$*
Clostridium perfringens	0	0
Fungi	$0.66 \times 10^2 \pm 0.73$	$1.60 \times 10^2 \pm 3.15$

*(*p<0,001)*



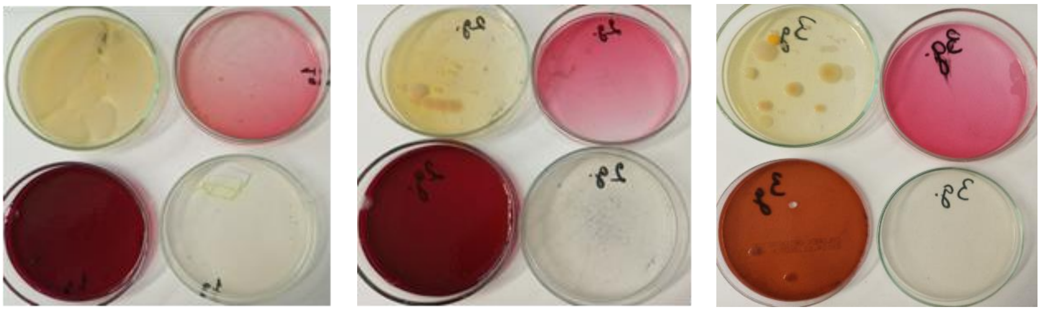
a **b** **c**

Figure 1: Results of some of the cultural studies of samples from the water surface in the lake: a - sample 1, above - on Sabouraud's agar and with mannitol, below - EMB and Cetrimide; b - sample 2, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimid; c - sample 3, above – on Sabouraud’s agar and with mannitol, below – on EMB and Cetrimide.



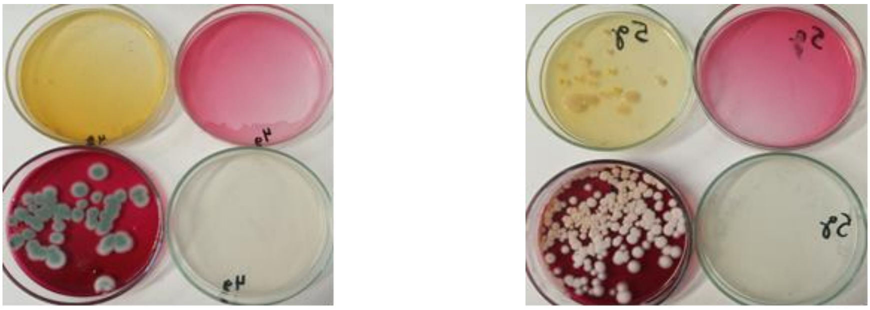
a **b**

Figure 2: Results of some of the cultural studies of samples from the water surface in the lake: a - sample 4, above - on Sabouraud's agar and with mannitol, below - on EMB and Cetrimide; b - sample 5, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimide.



a **b** **c**

Figure 3: Results of some of the cultural studies of water samples from the bottom of the lake: a - sample 1, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimide; b - sample 2, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimide; c - sample 3, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimide.



a b

Figure 4: Results of some of the cultural studies of water samples from the bottom of the lake: a - sample 4, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimide; b - sample 5, above – on Sabouraud's agar and with mannitol, below – on EMB and Cetrimide.

As can be seen from the data summarized in the table, the isolated microorganisms were in larger quantities in the surface layers of the water. This applied both to their total number and to the individual groups of bacteria studied. However, the differences were statistically significant only for staphylococci. Only the fungi had been settled in greater quantity at the bottom.

Gram–negative aerobic and facultative–anaerobic bacteria, as well as staphylococci, were isolated in the largest quantities. All isolated staphylococci belonged to the group of pathogenic mannitol–positive species. The significant amount of *E. coli*, especially in the surface layers of the water ($2.92 \times 10^2 \pm 5.54$ CFU/ml) was also impressive. The amount of *Pseudomonas* species, which were found only in the surface layer, was the least ($0.30 \times 10^2 \pm 0.51$ CFU/ml). Clostridia were isolated from only 40% of the surface water samples and from 80% of the bottom samples. The sanitary indicator species *C. perfringens* has not been isolated.

Discussion

Not only drinking water sources, but also recreational water bodies can be a source of contamination for humans and animals. Their contamination with pathogenic microorganisms is a serious problem, which necessitates the need for monitoring, with *E. coli* and fecal coliforms (bacteria from the *Enterobacteriaceae* family) being accepted as the main indicator. The climate disturbances we have witnessed in recent years can significantly affect pathogen levels in water resources. This negative effect is compounded by the dumping of urban, agricultural and industrial waste into them (Pandey *et al.*, 2014; Custodio *et al.*, 2022). Faecal streptococci, *C. perfringens* and *Klebsiella* spp. are widely used as an alternative to coliform bacteria as indicators of faecal contamination. *Salmonella* spp., *Shigella* spp. and *Proteus* spp. also are predominant bacterial flora in wastewater and indicate contamination of water bodies with such waters. Some *et al.* (2021) pointed out that the acceptable limits of coliforms and *E. coli* are 0/100 ml for drinking water and up to 126 CFU/100 ml for that for domestic and recreational purposes. Total coliform count should be 0/100 ml in drinking water as per Bureau of Indian Standards specification recommendation IS: 10500–2012 (Arghyam, 2020).

Our isolation of *E. coli* in the large quantity was indicative of fecal contamination and the possibility of the presence of other pathogenic microorganisms in the tested waters. This danger was also confirmed by the establishment of pathogenic mannitol–positive staphylococci species, also in significant quantities. Species of the genus *Pseudomonas* are normal aquatic inhabitants and their isolation was not surprising, but among them there may also be those with pathogenic potential, for example *P. aeruginosa*. The fact that no *C. perfringens* was isolated is favorable. In a similar study of microbial and heavy metal contamination of the water, sediments and fish of Lake Manzala in Egypt, Hamed *et al.* (2013) investigated the total number of microorganisms and that of faecal coliform bacteria. They reported a high degree of contamination of the water, as well as the fish in it, with these microorganisms, confirming that lake fish are dangerous to human health. These authors recommended regular assessment of pollutants in the lake. Prasanna *et al.* (2012) also conducted a study to assess microbial contamination and metal concentrations in lake waters in and around the city of Miri, East Malaysia. The higher *E. coli* values they found indicate microbial contamination in the lake waters.

The detection of *Pseudomonas* spp. and *Staphylococcus aureus* in large quantities in water bodies is also an indicator of fecal pollution and the possible presence of pathogenic microorganisms. Contamination of lake water with coliforms, including *E. coli*, can pose a major sanitary risk to the catch of fish and other hydrobiological products. The transferring of pollutants by rain from other anthropogenic activities and the presence of animals in the surrounding areas can be the cause of surface water pollution (Rondón–Espinoza *et al.*, 2022). Faecal streptococci have certain advantages over coliform bacteria as indicators because they are more resistant in the environment, as well as to chlorination, compared to coliforms. Fecal streptococci include species of the genera *Enterococcus* and *Streptococcus*. *Enterococcus faecalis* and *Enterococcus faecium* are a part of the normal microflora of the large intestines of humans and animals and their detection in the tested samples is an indicator of faecal contamination (Some *et al.*, 2021).

It is interesting that the content of microorganisms in the surface layers of the water in the reservoir studied by us was greater than in depth. The opposite is common, since the sun's rays have a strong bactericidal effect on the surface (Popova, 2016). We suppose that this may be a result of the season and weather conditions during which we took our samples. In winter with cloudy weather, there was little sunshine and too low an UV index, which would otherwise cause a bactericidal effect in the surface layers of the lake. Without the active action of this factor, microorganisms can not only survive, but also multiply. Apparently, however, fungal and clostridia spores settle to the bottom, where they can remain viable for a very long time. Contamination of surface water with bacterial pathogens is common. Fishing, bathing and swimming in such bodies of water are frequent ways of transmitting pathogens to humans, but also to animals. The results of a study of Lake Ma Vallé (Mwanamoki *et al.*, 2014) also showed low concentrations of microorganisms in the water but very high in the sediment – *E. coli* $2.65\text{x}10^3$ CFU/g^{-1} and Pseudomonas ssp. $3.27\text{x}10^3$ CFU/g^{-1}. In Lake Nubagabo in Uganda in 2015 *E. coli* has been found to be as high as 0–2.63 CFU/100 ml depending on the time of sampling (Doreen *et al.*, 2015). The researchers explained these results with the strong solar radiation in this geographical location and the high UV index, which has a bactericidal effect.

The present results show that water bodies in populated areas contain significant amounts of microorganisms from groups with pathogenic species. Therefore, the direct contacts of animals and people with them pose risks of infection, in order to prevent which it is not desirable to enter them and especially to drink water or use it for other purposes.

Lyautey *et al.* (2021) pointed out that benthic microbial communities from the most polluted sites were characterized by the lowest bacterial diversity and the highest abundance of antibiotic resistance genes. A high frequency of antibiotic resistance among coliform groups has been found in various water bodies, including marine and wastewater. Antibiotic–resistant strains reach the environment mainly through human and animal feces (Some *et al.*, 2021).

The sanitary quality of recreational water and especially its microbial contamination is of great epidemiological importance. The presence of pathogenic bacteria, protozoa and viruses in such waters is a serious threat to human health (Some *et al.*, 2021). The free access of animals and people to the waters of the lake that we studied hides the danger of infection with pathogenic microorganisms and, accordingly, the risk of spreading infectious diseases among people and animals. In addition to *E. coli*, coliforms and staphylococci, another finding in the waters are fungal microorganisms, including *Candida* spp. The results obtained by us, as well as those of other authors, show the need for sanitary supervision of the quality of water in reservoirs in and around populated areas and instructions for the prohibition of fishing, bathing and swimming of people and animals in polluted waters in order to prevent the appearance and spread of infections.

Conclusion

The water body we studied is contaminated with microorganisms with pathogenic potential, especially mannitol–positive staphylococci, Gram–negative bacteria, *E. coli, Pseudomonas* spp. and fungi, therefore they can be a source of infection for animals and humans.

The results give us reason to recommend avoiding direct contact with the lake waters (bathing, swimming or drinking) by people or pets.

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