BABEŞ-BOLYAI UNIVERSITY

FACULTY OF BIOLOGY AND GEOLOGY

ANTHROPOGENIC INFLUENCES ON MICROBIAL POPULATIONS IN SECU AND VĂLIUG DAM RESERVOIRS, CARAŞ-SEVERIN COUNTY

ABSTRACT OF PhD THESIS

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SUMMARY

KEY WORDS: potable water, Secu and Văliug dam reservoirs, sanitary bacterial indicators, bacterial groups, enzymatic activities.

INTRODUCTION

Rivers and lakes represent sources of drinking water for many cities and human communities. Water pollution is one of the acute global problems and it is increasingly studied in our country. According to the EU Directives (Water Framework Directive 60/2000/EC), the biotic component of rivers becomes the main target for environmental quality assessment and for water quality management plans. The use of biological methods to determine water quality is motivated by the fact that they provide water quality marks reflecting the evolution of water quality in time and space, while traditional, physico-chemical methods provide only an instant image of water quality and are more expensive.

This PhD thesis analyses for the first time the microbiology and enzymology of Secu and Văliug reservoirs in the Caraș-Severin county. The thesis aims to analyze the abundance, distribution, diversity and ecological significance of some groups of bacteria in the water and sediments of the two lakes, their hygienic and sanitary condition, as well as the analysis of enzymatic activities in the sediment of the two lakes.

This PhD thesis adds valuable scientific contributions to literature on the drinking water of the town of Reșița. In this regard, the following research papers have been presented at national and international conferences and published in CNCS journals: 2 oral presentations at 2 national conferences and 1 poster presentation at the international conference in Paris; 1 abstract published in the ISI volume of an international conference; 3 scientific papers published in category B+ journals and 1 paper in a national CNCS journal.

The analysis of these findings showed that the proposed objectives of this thesis were achieved and the results are genuine and have a high novelty degree at national level, arguing the use of microbiology and enzymology techniques to determine water quality and thus quantify micro-organisms in the water structure of the two reservoirs, Secu and Văliug, in Caraș-Severin county.

AIM AND OBJECTIVES OF THE THESIS

The conceptual understanding of the role of bacteria in aquatic ecosystems and in biogeochemical circuits is closely connected to the knowledge of how their growth, abundance and diversity are controlled.

I would like to draw the attention of the authorities on the development of comprehensive control and intervention strategies in the two reservoirs, Secu and Văliug, for the development of ecological reconstruction works. Stopping the logging in the protection perimeter of the lakes. Forest felling has dramatic implications for the changing of the aquatic ecosystem, especially as these lakes are the water supply source for 70,000 people downstream. The rehabilitation of the links in the food chain, the assessment and monitoring of the impact of tree felling activities and riverbed destruction of watercourses that feed these lakes. Log exploitation with heavy machinery and trucks belonging to forest robbers affect the entire river upstream. The greening and upgrading of sawdust piles, multiple wastes and human and animal manure, implementation of economic development and environmental programs directed towards agritourism by exploiting the existing natural resources according to the European standards and compliance with environmental protection regulations.

I. AQUATIC MICROBIOLOGY

Aquatic microbiology research represents a relatively new field, initiated by a series of strictly sanitary approaches. Over the last decades, scientists have been increasingly directed their attention towards the complex, ecological study of large water reservoirs, knowing that the present industrial civilization, by its side effects, has caused the acceleration of highly complex ecological processes in the aquatic environments, with consequences often opposite to the concept of sustainable development. The approach of aquatic microbiology researches in terms of eco-physiology reveals yet unsuspected possibilities for the assessment of the general state of water.

"Water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such" Art 1 Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Lakes provide very different conditions for the growth of microorganisms according to: flow rate, water flow, depth, mineral content dependent on the geological conditions. Due to the intensified contact with the lithosphere along the banks and to their washing by the water course, erosion phenomena occur together with enrichment with mineral and organic substances from soil and with allochthonous microorganisms. These particularities of rivers confer on them a wide variety of conditions, which corresponds to great variability in the structure of microorganisms communities.

Depending on the pollution degree, there are three major types of waters:

- *oligosaprobic*, with a large amount of oxygen, low content of dissolved organic matter and low rate of organic decomposition;
- *mesosaprobic*, with a medium pollution degree, smaller amount of oxygen and moderate rate of organic matter degradation;
- *polisaprobic*, with high organic matter levels and low or absent oxygen concentration.

The main bacteriological indicators of water and sediment pollution are: the total and fecal coliform group (considered as the primary indicator of fecal water contamination) and fecal enterococci. Depending on the isolated or associated presence of these bacteria, as well as on their seasonal and annual quantitative variation, an assessment of the hygiene and sanitary state of waters can be conducted. A minor quantitative or qualitative change in case of microbial populations enables the assessment of the environmental status of the aquatic environment and the possible identification of the pollution source, where appropriate. Baums et al., (2007) employ the method for detecting fecal indicators in river water which uses luminex. Luminex® 100™ is a suspension which enables the rapid analysis on a microtitration plate. For most of the waterborne diseases, the etiologic agents are represented by the microorganisms removed from the digestive tract. As a result, the bacteriological monitoring is mandatory for surface waters, enabling the identification of the possible fecal pollution sources.

I chose as subject two reservoirs, Secu and Văliug, from the Caraş-Severin County in order to detect anthropogenic influences on the microorganism populations living in their waters.

The aim of this research is to detect the presence of microorganisms used as pollution indicators, of the pathogens, and of several groups of bacteria involved in the biogeochemical cycles in the water and sediments of the two reservoirs, Secu and Văliug, as well as to determine the bacterial and enzymatic potential of the sediments, to conduct the analysis of bacterial activity in the water sediments and of enzyme activity, namely, the sanitary quality assessment of these waters. The enzymatic potential of the sediments directly or indirectly reflects the microbiota activity, the influence of various physical, chemical, and anthropogenic factors and even that of the intensity of enzymatic activities. Therefore, the functioning of an ecosystem cannot be understood without the active anticipation of the enzymatic processes.

In this context, the objectives of this study are the following:

- Determination of the physico-chemical parameters of the waters in the two reservoirs, Secu and Văliug (determination of temperature, pH, Eh, organic matter, dissolved oxygen, biochemical oxygen demand (BOD_5), electrical conductivity, ammonium content, nitrates, nitrites, lead, iron, cadmium, etc.);
- Study of the dynamics and ecological significance of certain groups of bacteria (bacteria involved in the biogeochemical cycle of the nitrogen, sulphur, iron and carbon) in the water and sediments of the two lakes in relation to biotic factors - environmental and abiotic - determinants;
- Determination of the enzymatic activity in the sediments of the two reservoirs both quantitatively (dehydrogenase, catalase, phosphatase activity) and qualitatively (by highlighting some oligases and polyases);
- Determination of the enzymatic indicator of sediment quality (EISQ) and of the two bacterial indicators of water (BIWQ) and sediment (BISQ) quality;
- Determination of water sanitation level by determining the sanitary indicators (total coliform germs, fecal coliforms, fecal enterococci) due to the repercussions that these indicators may have on human health;
- Determination of the water pollution degree of the two reservoirs, Secu and Văliug, by the ecotoxicological testing based on *Vibrio fischeri* bioluminescent bacteria;
- Monitoring and assessment of the water quality of the two lakes - Secu and Văliug - under the influence of the anthropogenic factors in order to develop strategies for the rehabilitation and conservation of affected aquatic ecosystems, applicable to other similar hydrosystems.

II. MATERIALS AND METHODS

Secu lake: reservoir in the Bârzava basin, elevation 350 m, area 105.67 ha, water volume 15,132,000 m^3, maximum depth 27 m, located in the Semenic Mountains. Built in 1963, it supplies water to the municipality of Reşiţa and it has also a recreational role in the Secu resort.

Văliug lake: reservoir in the Bârzava basin, elevation 500 m, area 66.2 ha, water volume 11,732,000 m^3, depth 40 m, located in the Semenic Mountains. Built in 1953, it supplies water to the Secu lake located downstream and implicitly to the municipality of Reşiţa and it has also a recreational role in the Crivaia resort.

The sampling, storing, transportation, keeping and identification of samples were conducted according to SR 2852/1994.

Determination of physico-chemical parameters. In order to determine the influence of the abiotic environmental factors on the density and composition of the microbial communities subjected to study, the following physico-chemical parameters are determined: temperature (T), pH, dissolved oxygen (O_2), conductivity, biochemical oxygen demand (BOD_5), organic matter determined by CCO-MN, total mineral nitrogen (total N), nitrates, nitrites, ammonia, sulfates, total phosphorus (total P) and total iron (total Fe), the presence of heavy metals (Cu, Pb, Zn, Cd).

The determination of the ecophysiological groups of bacteria was conducted to assess the functional diversity of the microbiota and biochemical processes in the aquatic habitats of the two reservoirs. *The aerobic heterotrophic bacteria* were determined by the heterotrophic plate counts (HPC) method (Drăgan-Bularda, 2000). The multiple tube method MTM is employed to determine the *ammonifying bacteria* (Cuşa 1996). For the *nitrifying bacteria* and *iron-reducing bacteria*, the basic principle is that of the multiple tube method MTM (Carpa et al., 2014). Based on the number of bacteria falling into several ecophysiological groups, the *bacterial indicator of water and sediment quality* is determined (Muntean, 1995-1996).

Enzymological methods. Quantitative and qualitative enzymatic activities were determined. Among the *quantitative enzymatic activities* studied, we mention the determination of the: *dehydrogenase activity* - Casida method, (Carpa et al., 2014; Drăgan – Bularda, 2000), *catalase activity* - Kappen method, (Muntean et al., 1996; Drăgan – Bularda, 2000), *phosphatase activity* - Kramer and Erdei method (Muntean et al., 1996; Drăgan – Bularda, 2000) from the sediment of the lakes subjected to study. Determination of the *qualitative enzymatic activities (oligases and polyases):* sucrose, lactose, cellobiose, lactose, starch, dextran, levan, inulin.

Determination of sanitary bacteria. According to the ISO and STAS standards, the following were determined: *the probable number of mezophilic bacteria at 37 ºC* (STAS 3001-91), *the probable number of total coliform bacteria by the membrane filter method* in the AQUACARAŞ laboratory, *the probable number of fecal coliform bacteria – thermotolerant* bacteria by the multiple tube method (Cuşa 1996, Drăgan-Bularda, 2000) or the membrane filter method, *fecal enterococci*.

Ecotoxicological methods for pollution testing in the waters of the two reservoirs – *the bioluminescence testing with Vibrio fischeri* (SR ISO 11348-3: 2003; SR ISO 11348-1:1998).

Statistical methods. When calculating and correlating the analyses results, statistical methods are employed. The arithmetic mean, standard deviation σ or S, standard error of the mean, tests for statistical significance, analysis of variance - ANOVA, correlation coefficient.

III. THE PHYSICO-CHEMICAL ANALYSIS OF WATER

In order to determine the influence of the abiotic environmental factors on the density and composition of the microbial communities subjected to study, the following physico-chemical parameters were determined: temperature (T), pH, dissolved oxygen (O_2), conductivity, biochemical oxygen demand (BOD_5), organic matter determined by CCO-MN, total mineral nitrogen (Nmin.t), nitrates, nitrites, ammonia, sulfates, total phosphorus (total P) and total iron (total Fe), the presence of heavy metals (Cu, Pb, Zn, Cd).

IV. THE MICROBIOLOGICAL ANALYSIS OF WATER

To determine the relationship between microbial populations in the aquatic ecosystems and the influences that the bacteria in the sediments may have on water quality, in addition to the enzymological activity and the hygienic and sanitary aspect, I also analyzed the composition of the bacterial ecophysiological groups involved in the decomposition and mineralization of the substrate in water and sediments, and I studied the aerobic heterotrophic bacteria and the bacteria involved in the biogeochemical cycle of nitrogen and iron.

IV.1. Quantitative determination of the aerobic heterotrophic bacteria

This thesis analyses for the first time the dynamics and ecological diversity of the groups of bacteria involved in the biogeochemical cycle of nitrogen and iron. In the waters of the Secu reservoir, in 2009 and 2010, the aerobic heterotrophic bacteria were of the order of 10^6-10^7. Large oscilations were recorded between the sampling points, but maximum values were recorded in the middle section of Secu lake (Fig. 1).

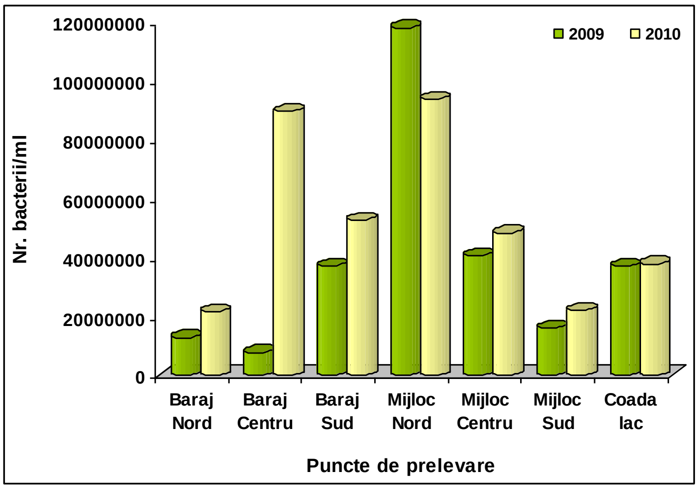


Fig. 1. The numerical distribution of the aerobic heterotrophic bacteria in Secu lake water, in the years 2009 and 2010.

The aerobic heterotrophic bacteria in Văliug lake water indicated no significant fluctuations between the sampling points in 2009 and 2010 (Fig. 2).

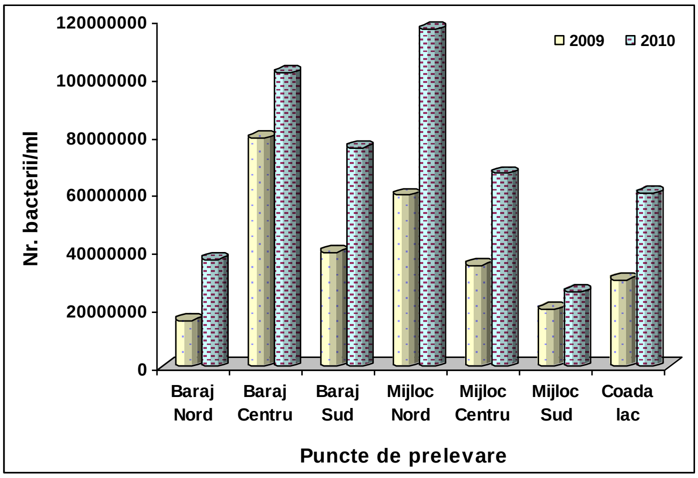


Fig. 2. The numerical distribution of the aerobic heterotrophic bacteria in Văliug lake water, in the years 2009 and 2010.

These findings indicated that the group of aerobic heterotrophic bacteria was the largest in both lakes (Secu and Văliug) throughout both years of study (2009 and 2010).

IV.2. The quantitative determination of the ammonifying bacteria

The ammonifying bacteria (BAM) are found in large numbers in the waters of the two lakes, which demonstrates the presence of increased amounts of ammonia in these waters (Fig. 3).

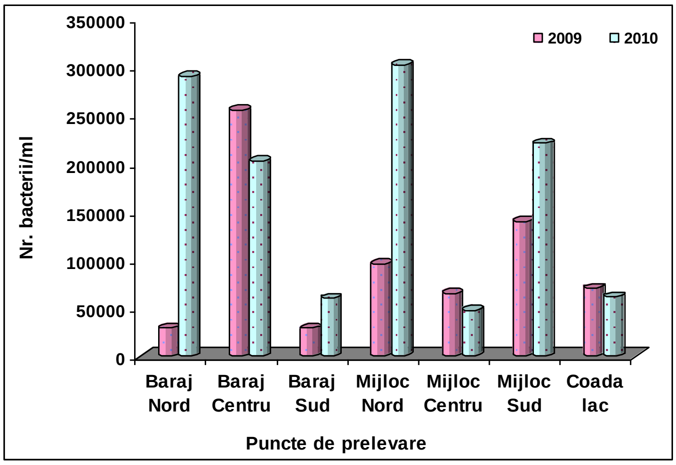


Fig. 3. The numerical distribution of the ammonifying bacteria in Secu lake water in 2009 and 2010.

Compared to Secu lake, the ammonifying bacteria were detected in a higher number in Văliug lake water (Fig. 4).

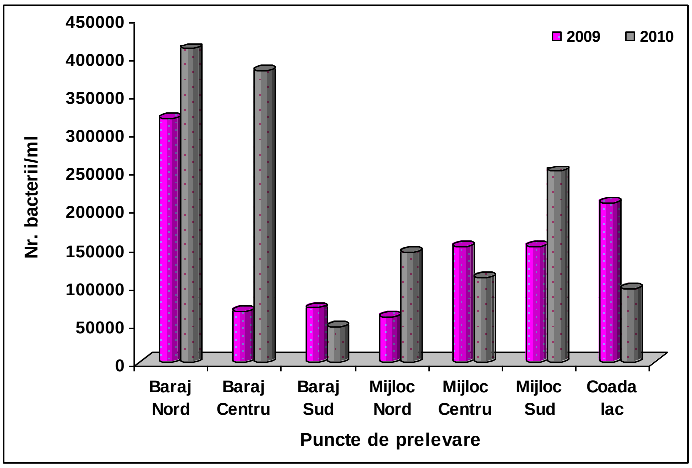
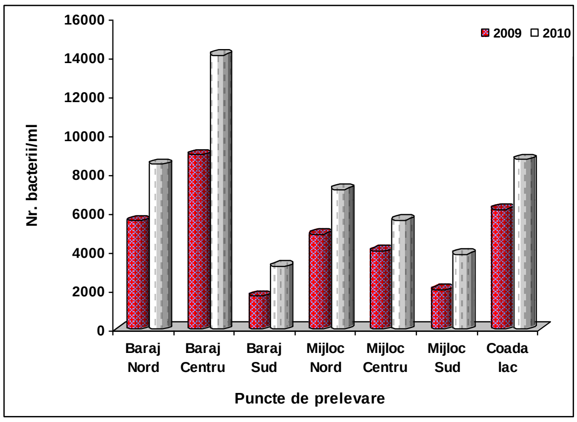


Fig. 4. The numerical distribution of the ammonifying bacteria in Văliug lake water in 2009 and 2010.

The ammonifying bacteria were found in large number in the waters of the two reservoirs, Secu and Văliug, in the two years. The maximum values are reached in the dam part (end). These high values can be explained by the accumulation of organic matter of plant or animal origin and higher water temperatures that stimulate the development and activity of microorganisms in these habitats.

IV. 3. The quantitative determination of the nitrifying bacteria

The number of nitrite bacteria in the water samples from the Secu lake revealed fluctuations between the analyzed sections, while the highest values were recorded in the dam part (Fig. 5). The lowest values were recorded in the southern part of Secu lake.



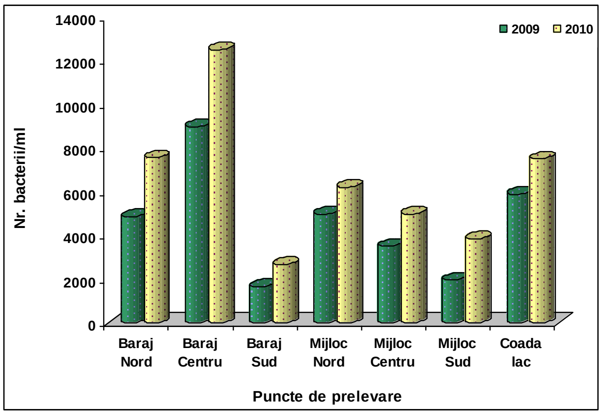
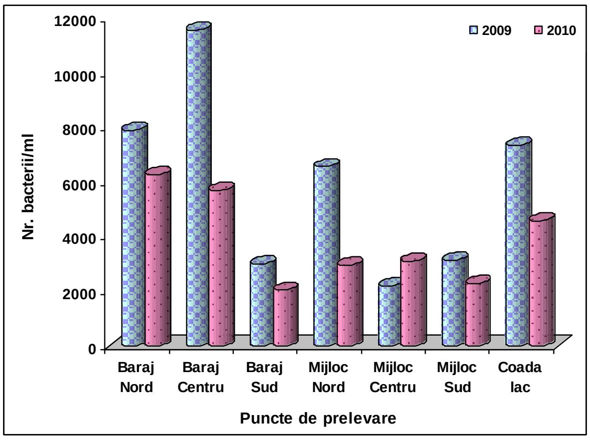


Fig. 5, 6. The numerical distribution of the nitrite bacteria (left) and of the nitrate bacteria (right) in Secu lake water in the years 2009 and 2010.

The number of nitrite bacteria in the water samples from the Secu lake revealed fluctuations between the analyzed sections. The values of these bacteria were somewhat lower compared to those of the nitrite bacteria. The highest values were recorded in the central part of the dam (Fig. 6).

The number of nitrite bacteria in the water samples from the Văliug lake revealed fluctuations between the analyzed sections, while the highest values were recorded in the central part of the dam (Fig. 7).



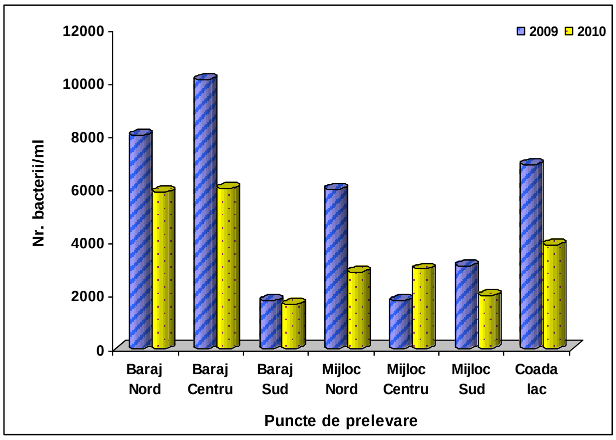


Fig. 7, 8. The numerical distribution of the nitrite bacteria (left) and of the nitrate bacteria (right) in the Văliug lake water in the years 2009 and 2010.

The number of nitrite bacteria in the water samples from the Văliug lake revealed fluctuations between the analyzed sections. In this lake too, as in the case of the Secu lake, the values of the nitrate bacteria were somewhat lower compared to those of the nitrite bacteria. The highest values were recorded in the north and central part of the dam (Fig. 8). In the middle section and in the end (tail) part of the lake, the fluctuations in nitrate bacteria numbers were not very high. In general, the number of the nitrate bacteria was lower in the year 2010 compared to the year 2009.

The nitrifying bacteria are present both in water and in sediments; their maximum density is reached in the upper layers of the aquatic basins, at the interface of water and sediment. As they are sensitive to the degree of water oxygenation, the number of bacteria in the sediments can provide us with important data about the water circulation status in these basins.

IV.4. The quantitative determination of the denitrifying bacteria

The number of denitrifying bacteria in the water samples from the Secu reservoir revealed fluctuations between the analyzed sections. The highest values were recorded in the middle central part of the Secu reservoir (Fig. 9). The values recorded by the denitrifying bacteria in other sections of the dam were much lower compared to the middle central part of the reservoir.

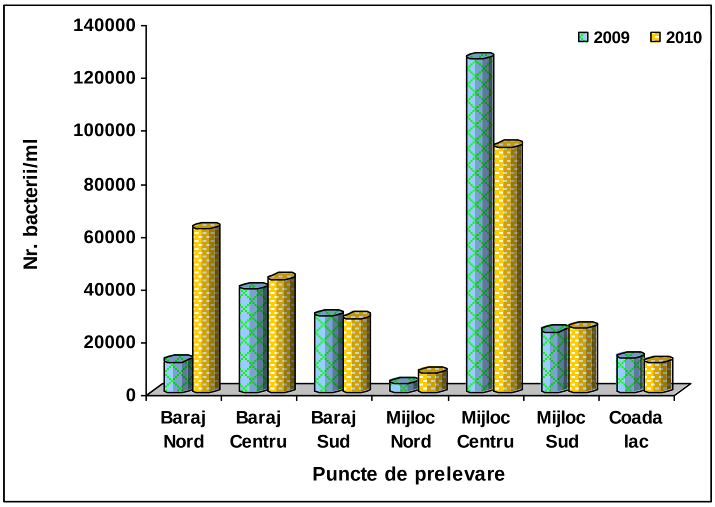


Fig. 9. The numerical distribution of the denitrifying bacteria in Secu lake water in the years 2009 and 2010.

There are certain similarities between the two lakes as the highest density is recorded in the middle section of both reservoirs (fig. 10).

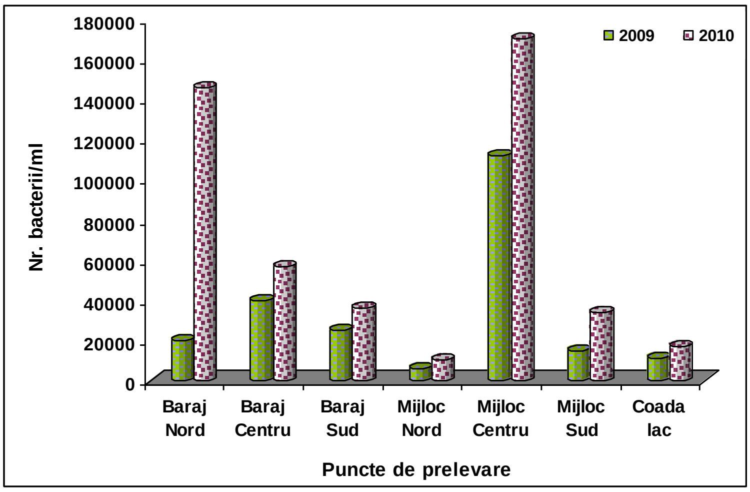
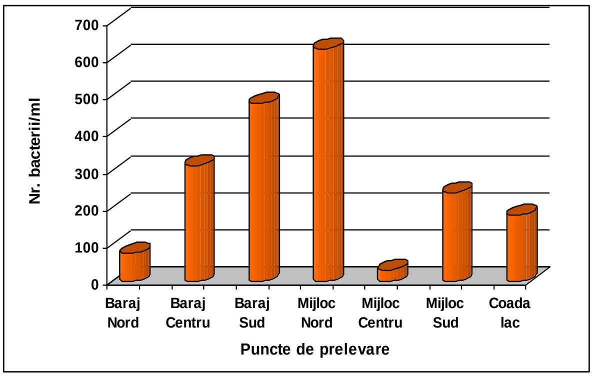


Fig. 10. The numerical distribution of the denitrifying bacteria in Văliug lake water in the years 2009 and 2010.

The number of the denitrifying bacteria in the analyzed water samples from the two reservoirs ranged between $14x10^4$ and $18x10^4$.

IV.5. Determination of the iron-reducing bacteria

The values of the iron-reducing bacteria in Secu reservoir in 2009 were in the region of hundreds. It is noteworthy that the maximum load was recorded in the middle section of the Secu reservoir (Fig. 11).



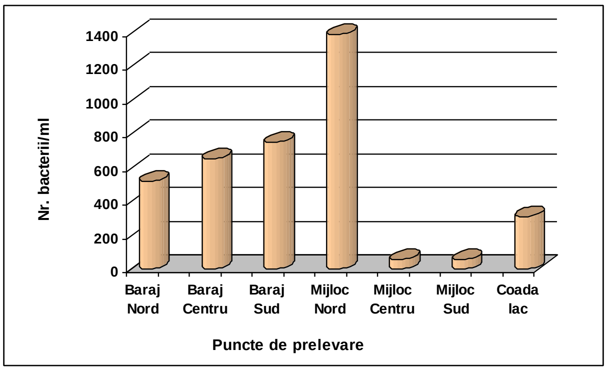
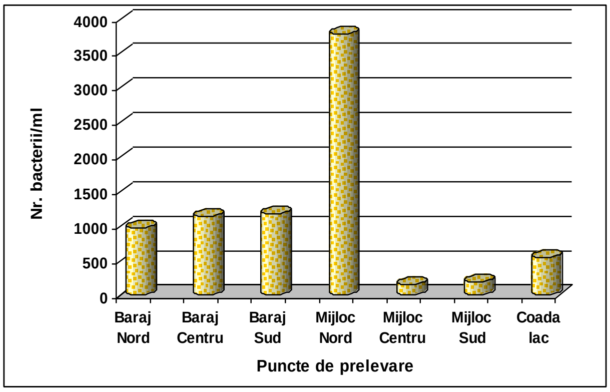


Fig. 11, 12. The numerical distribution of the iron-reducing bacteria in Secu lake water in the years 2009 (left) and 2010 (right).

In the year 2010, the values of the iron-reducing bacteria in the Secu reservoir almost doubled. The maximum values were recorded also in the middle-north section of the Secu lake (Fig. 12).

The numerical distribution of the iron-reducing bacteria in the waters of the Văliug reservoir in 2009 and 2010 was better represented, in the region of thousands (Fig. 13).



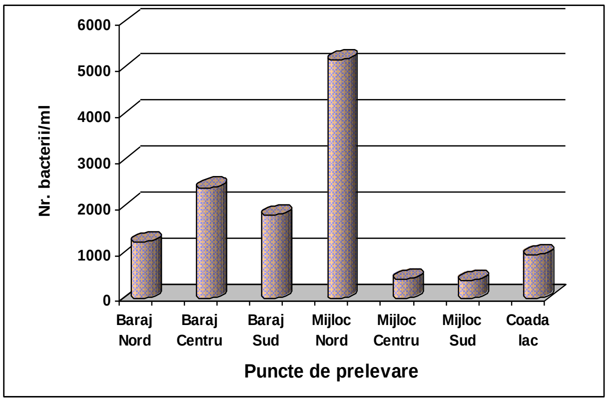


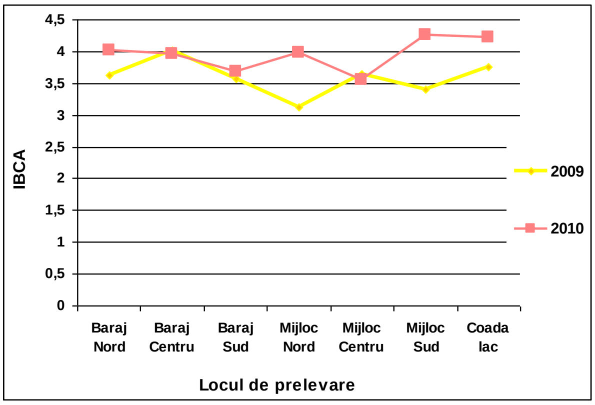
Fig. 13, 14. The numerical distribution of the iron-reducing bacteria density in the Văliug lake water in the year 2009 (left) and 2010 (right).

In the year 2010, the values of the iron-reducing bacteria in the Văliug reservoir slightly increased. The maximum values were recorded in the middle-north section of the Văliug lake (Fig. 14).

From the biological and microbiological point of view, the study of this water has a crucial role in determining the impact pathways and application of effective methods for maintaining the water of the two reservoirs, Secu and Văliug, at high-quality values.

IV.6. Determination of the bacterial indicator for water quality testing

The values of the bacterial indicator of water quality (BIWQ) were calculated using the individual values for each ecophysiological group from the Secu and Văliug lakes.



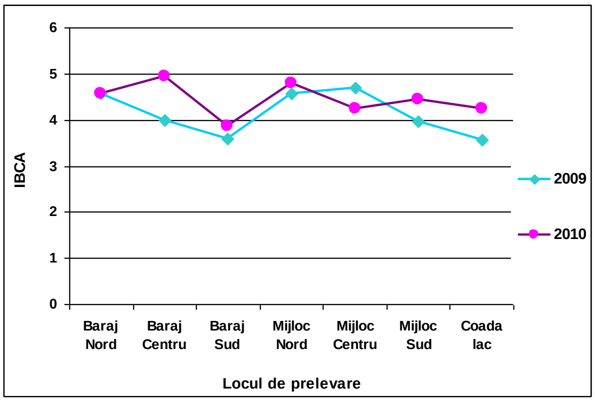


Fig. 15, 16. The values of the bacterial indicator of water quality (BIWQ) throughout the years subjected to study in the Secu reservoir (left) and in the Văliug reservoir (right).

In Secu reservoir, the values of the bacterial indicator were much higher in the year 2010 as compared to the year 2009 (Fig. 15). The BIWQ values underwent changes depending on the season when the analyses were performed.

It is the same situation in Văliug reservoir, with higher values of the indicator recorded in the year 2010 (Fig. 16).

V. THE ENZYMOLOGICAL ANALYSIS OF WATER AND SEDIMENT

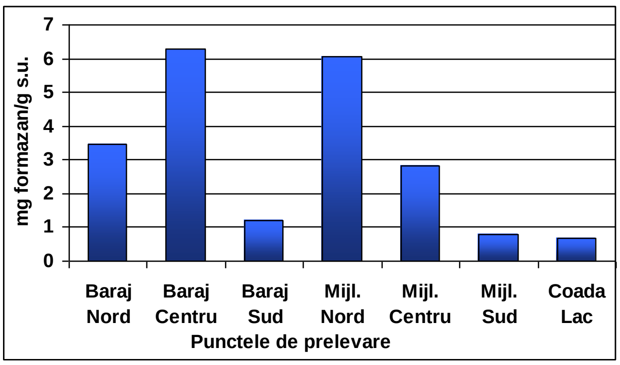
V.1. Determination of the quantitative enzymatic activity

In all the 7 sediment samples, the following enzymatic activities were determined in quantitative terms: the actual dehydrogenase activity (ADA) (reduction of TTC- the 2,3,5-triphenyl tetrazolium chloride, in samples without any glucose addition) and the potential dehydrogenase activity (PDA) (with glucose addition), the phosphatase activity (PA) and catalase activity (CA).

V.1.1. The actual and potential dehydrogenase activity

In 2009, the values of actual and potential dehydrogenase activity in Secu reservoir were average, which indicates the existence of an average microbial potential in this lake (Fig. 17 and Fig. 18).

The glucose addition has a constant, yet not very high incentive effect on the dehydrogenase activity in the sediment of the analyzed lake. The values of the potential dehydrogenase activity in Secu reservoir in 2009 were increased by at least an order of magnitude due to glucose addition (Fig. 17).



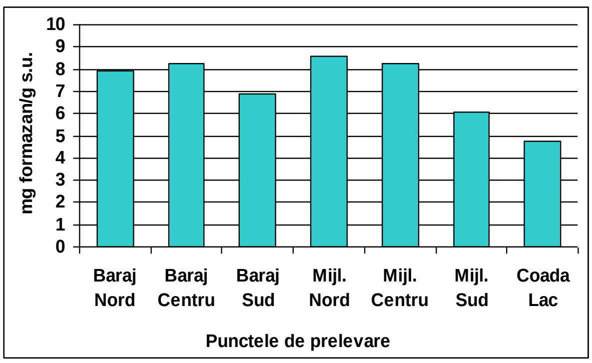
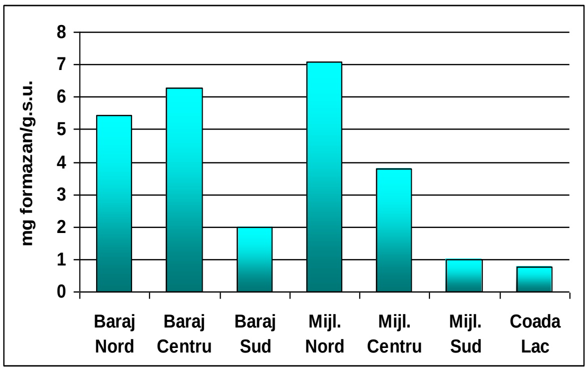


Fig. 17, 18. The intensity of the actual dehydrogenase activity (ADA) (left) and that of the potential dehydrogenase activity (PDA) (right) in the sediments of Secu reservoir (2009).

In 2010, the values of actual and potential dehydrogenase activity recorded in Secu lake were somewhat higher than the values obtained in 2009. This shows an improvement of the microbial potential in this reservoir. Compared to the actual dehydrogenase activity, the potential dehydrogenase activity in Secu lake in 2010 revealed increased values due to additional carbon source, as the intensity is almost homogeneous across all sections subjected to study (Fig. 20).



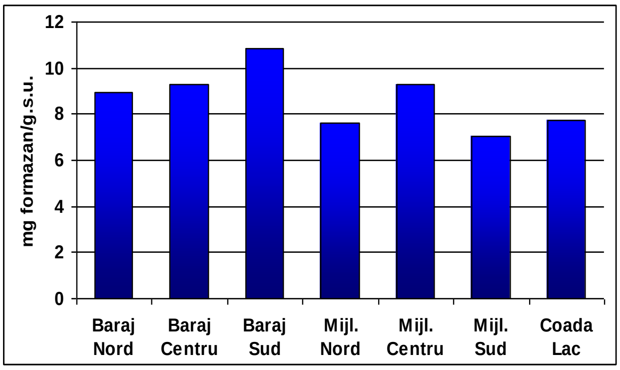


Fig. 19, 20. The intensity of the actual dehydrogenase activity (ADA) (left) and that of the potential dehydrogenase activity (PDA) (right) in the sediments of Secu reservoir (2010).

The dehydrogenase activity in the sediments of Secu lake showed oscillations in the two years of study, measuring higher values compared to Văliug lake, probably due to the presence in these sediments of a sufficient amount of organic matter, which determines a good development of microorganisms, as their activity is characterized by the level of the actual dehydrogenase activity.

V.1.2. The phosphatase activity

In general, the values of the phosphatase activity in the two reservoirs were higher in the year 2009 compared to the year 2010. A fairly large numerical fluctuation of the phosphatase activity was noticed in all sections examined in 2009 in the Secu reservoir (Fig. 21). In 2010 the phosphatase activity recorded lower values in all the examined sections compared to those recorded in 2009 (Fig. 22).

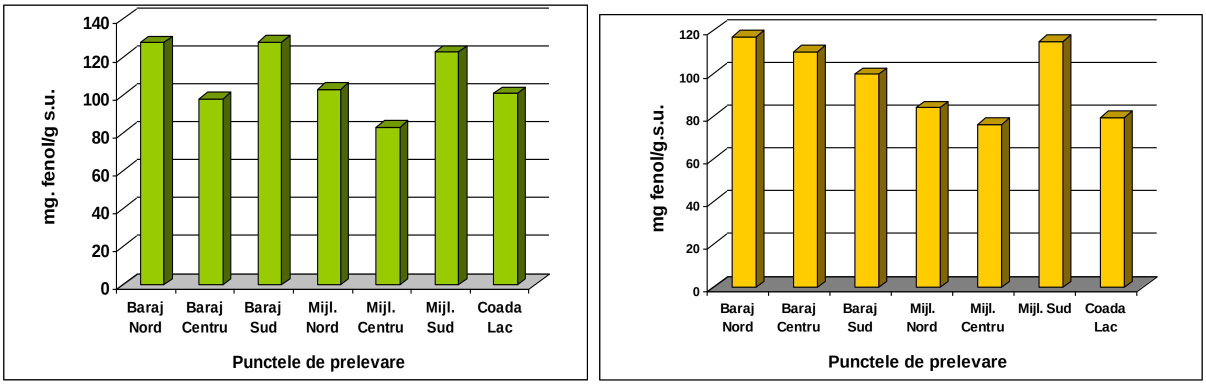


Fig. 21, 22. The intensity of the phosphatase activity (PA) in the sediments of the Secu reservoir in the year 2009 (left) and in 2010 (right).

The phosphatase activity in the sediments of Văliug lake was detected in all examined sections, showing a higher intensity towards the end (tail) of the lake (Fig. 23).

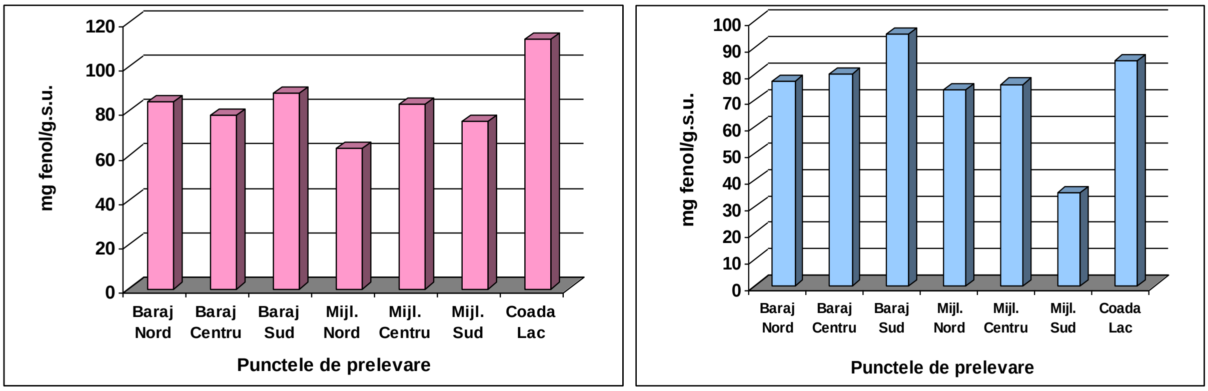


Fig. 23, 24. The intensity of the phosphatase activity (PA) in the sediments of the Văliug reservoir in 2009 (left) and in 2010 (right).

Lower values of the phosphatase activity in the sediments of the Văliug reservoir were recorded in the year 2010 (Fig. 24). Lower values were recorded in the south-middle section.

The phosphatase activity in the sediments of the Secu and Văliug reservoirs indicated fairly high values both in the year 2009 and in 2010. This intense phosphatase activity recorded in the two lakes in almost all examined sections indicates the fact that sediments represent the main phosphorus reservoir in the water bodies (Muntean et al., 1996-1997).

V.1.3. The catalase activity

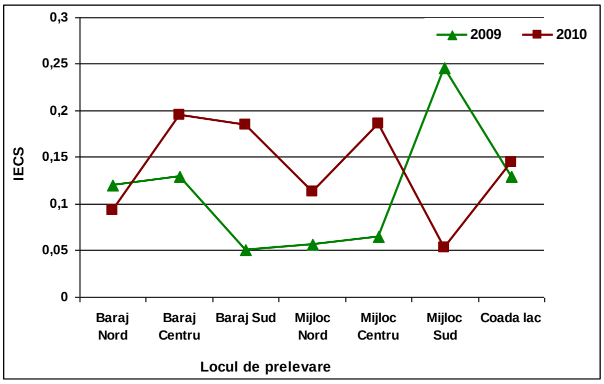
If we compare the values of the catalase activities in the two reservoirs we can notice that the levels in Secu lake were much higher than in Văliug lake. These high values of catalase activity recorded in Secu lake may be due to the presence of strong reducing substances (sulphites) and humic acids in the sediments (Muntean et al., 1999).

Table 1. Distribution of catalase activity values recorded in the sediments of Secu and Văliug reservoirs in 2009 and 2010.

The sampling point	Secu reservoir		Văliug reservoir	
	2009	2010	2009	2010
	mg H_2O_2/g.d.m.		mg H_2O_2/g.d.m.	
North of the dam	19.8	14	12	8
Center of the dam	13.6	11	6	5.3
South of the dam	7.3	8	7.5	7
Middle north	10.4	9.5	8.4	6.8
Middle center	8.5	7.6	6.5	5.2
Middle south	17	12	7	4
End of the lake	12	9.5	9	8

V.1.4. Determination of the enzymatic indicator of sediment quality

Fig. 25 illustrates the variations of the enzymatic of sediment quality in the Secu reservoir, in the years 2009 and 2010, as reflected by the values of the examined indicators.



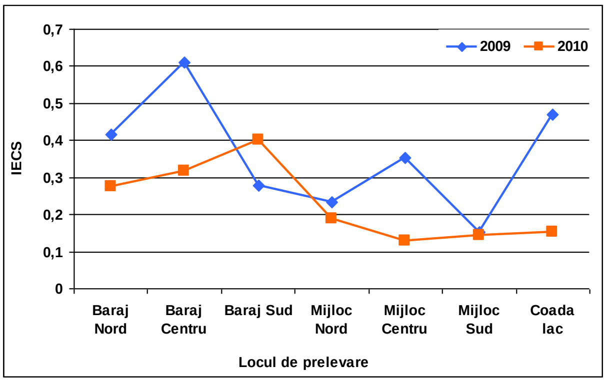


Fig. 25, 26. The values of the enzymatic indicator of sediment quality (EISQ) throughout the examined years in the Secu reservoir (left) and in the Văliug reservoir (right).

Figure 26 illustrates the enzymatic indicator of sediment quality in the Văliug reservoir. The much higher value of the indicator can be noticed here compared to that measured in Secu lake.

V.2. Determination of qualitative enzymatic activity

The qualitative enzymatic activity was determined in 3 points for each reservoir (3 samples in Secu lake and 3 samples in Văliug lake). Oligase and polyase qualitative enzymatic activities were detected. Among the oligase activities, the following were detected by paper chromatography: invertase, maltase, cellobiase and lactase. Of all these, this summary presents only the invertase and maltase activity. Among the polyase activities, the following were detected by paper chromatography: amylase, cellulase, glicogenase, dextranase and inulinase. Of all these, this summary presents only the amylase and inulinase activity.

V.2.1. Highlighting some oligase and polyase in the sediments of the two lakes

The chromatogram in fig. 27 presents the sucrose activity in the sediment samples in both reservoirs Secu and Văliug. Based on the glucose spots resulting from the hydrolysis of sucrose by the action of sucrase, it may be appreciated that this invertase activity is present in all sediment samples examined. However, there are small intensity differences marked by the intensity of spots.

Fig. 27. Sucrose activity (SA). 1-3 sediment samples Văliug; I-III sediment samples Secu + enzyme substrate (sucrose 2%), S=control – sucrose solution 2%. G=glucose.

The chromatogram in Fig. 28 presents the results of maltase activity in the sediment samples of the two reservoirs. The sediments in Secu lake were more active in terms of enzymatic activity than those in Văliug lake.

Fig. 28. Maltase activity (MA). 1-3 sediment samples Văliug; I-III sediment samples Secu + enzyme substrate (maltose 2%), M=control – maltose solution 2%. G=glucose a=maltose spot. b=glucose spot.

The chromatogram in Fig. 29 shows the amylase activity by the presence of glucose spot in all the six sediment samples examined. It can be noticed that sediment samples from Secu lake are more active in terms of amylase activity than those in the sediments from Văliug lake.

Fig. 29. Amylase activity (AA). 1-3 sediment samples Văliug; I-III sediment samples Secu + enzyme substrate (starch 2%), A=control – starch solution 2%. G=glucose a=amylase spot. b=glucose spot.

Figure 30 shows the presence of inulinase activity for all six samples, while the most intense fructose spot was in sample 1 from Văliug lake and in samples I and III in Secu lake. For sample II in Secu lake, the intensity of the fructose spot is at the detection limit.

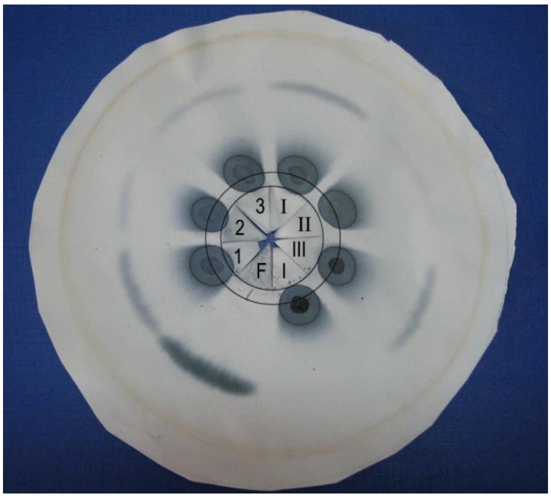


Fig. 30. Inulinase activity (IA). 1-3 sediment samples Văliug; I-III sediment samples Secu + enzyme substrate (inulin 2%), I=control – inulin solution 2%. F=fructose.

VI. ASSESSMENT OF WATER HYGIENE BY DETERMINATION OF SANITARY INDICATORS

The sanitary bacteria presented a relatively low numerical density in the Secu lake water, during the study period, but compared to the values measured in Văliug lake, the values of the examined indicators were higher in Secu lake.

A numerical fluctuation of the sanitary bacteria groups has been noticed in all analyzed points. The sanitary bacteria in Văliug lake water revealed much lower numerical densities in most of the sampling points, except the end (tail) of the lake. From the group of sanitary bacteria examined, total coliform bacteria revealed maximum densities throughout the entire survey period, while fecal enterococci were in low and very low numbers in almost all the sampling points.

Higher density of total coliform bacteria may be noticed in Secu lake in 2009 compared to 2010, with a higher number towards the end (tail) of the lake (Fig. 31).

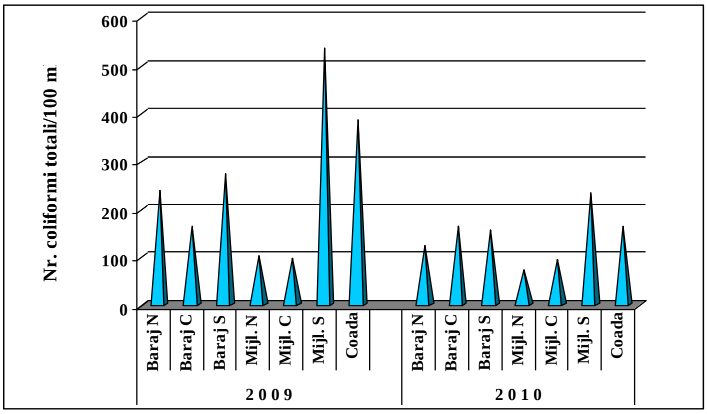


Fig. 31. The distribution of the mean annual values of total coliform bacteria densities in Secu lake in 2009 and 2010.

The number of total coliform bacteria in Văliug reservoir was higher in the year 2009 compared to the year 2010 (Fig. 32). Also, higher values of total coliform bacteria were recorded at the end of this lake, where various leisure activities are carried out.

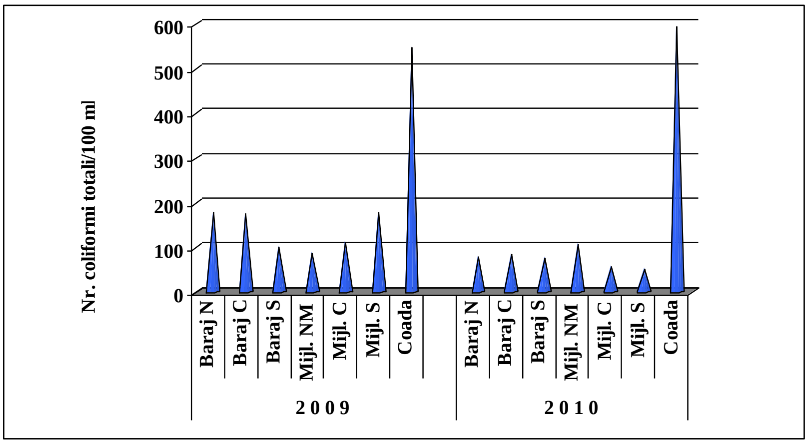


Fig. 32. The distribution of the mean annual values of total coliform bacteria densities in Văliug lake in 2009 and 2010.

VI.1. Determination of the most probable number of fecal coliform germs

A rather significant numerical fluctuation of fecal coliform bacteria has been noticed in Secu lake in 2009. The fecal coliforms values in water samples from the same lake, but in 2010, decreased to almost a half (Fig. 33).

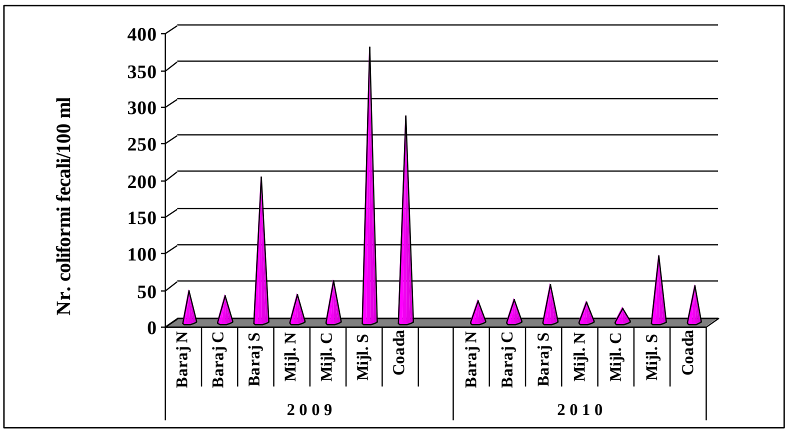


Fig. 33. The distribution of the mean annual values of fecal coliform bacteria densities in Secu lake in 2009 and 2010.

The values of fecal coliforms in water samples from Văliug lake were lower than the values measured in Secu lake (Fig. 34). The comparison between the values obtained in the two years of the survey reveals that the values of the fecal coliforms were much higher in 2009 than those measured in 2010.

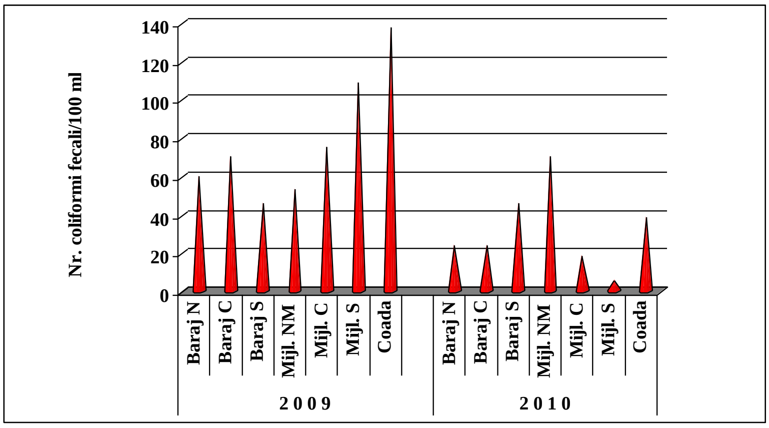


Fig. 34. The distribution of the mean annual values of fecal coliform bacteria densities in Văliug lake in 2009 and 2010.

VI. 2. Determination of the most probable number of fecal enterococci

Fig. 35 illustrates the mean annual distribution of fecal enterococci densities in Secu lake, where these bacteria were higher in 2009 compared to 2010.

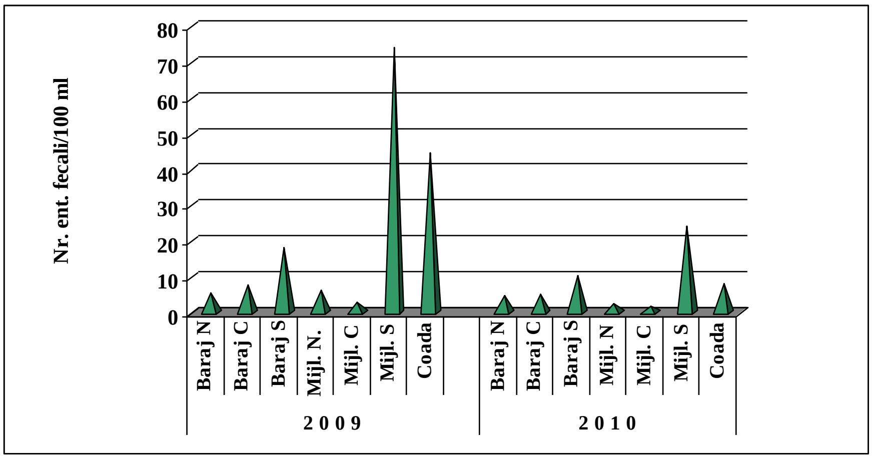


Fig. 35. The distribution of the mean annual values of fecal enterococci densities in Secu lake throughout 2009 and 2010.

Fig. 36 illustrates the mean annual distribution of fecal enterococci densities in Văliug lake. In this lake, the fecal enterococci values were much lower than those of Secu lake.

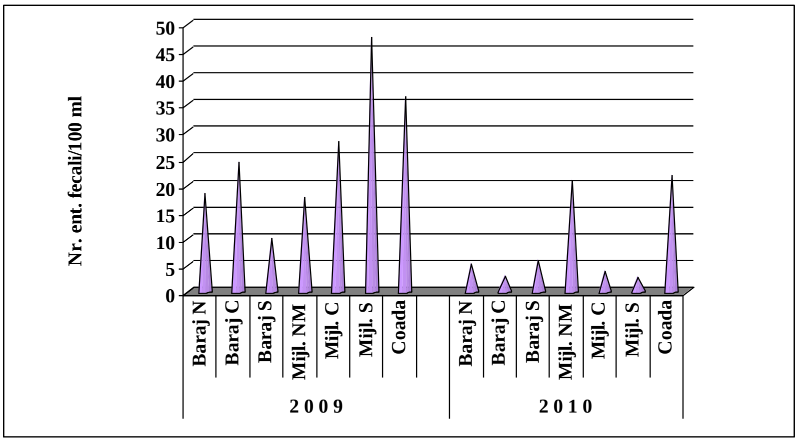


Fig. 36. The distribution of the mean annual values of fecal enterococci densities in Văliug lake throughout 2009 and 2010.

VI.3. The quantitative and qualitative variation of the sanitary bacteria

The sanitary bacteria in Secu lake presented higher numerical densities than those in Văliug lake. Fig. 37 illustrates the annual mean values and standard errors of total coliform bacteria, fecal coliforms and fecal enterococci in Secu lake water.

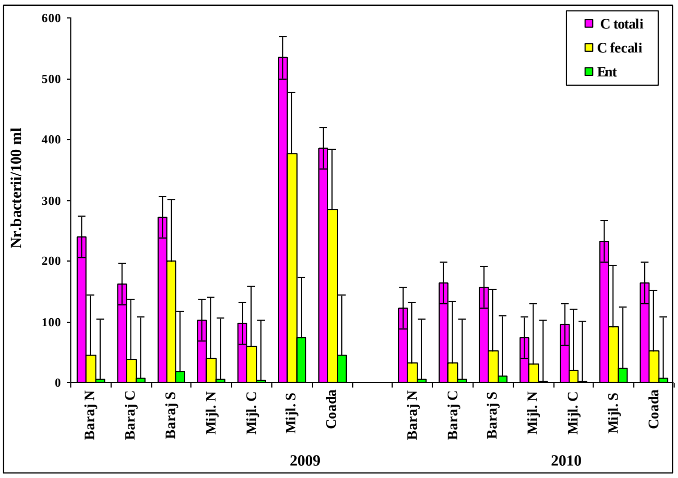


Fig. 37. The annual mean values and standard error values of the sanitary bacteria density in Secu lake.

Fig. 38 illustrates the annual mean values and standard errors of total coliform bacteria, fecal coliforms and fecal enterococci in Văliug lake water. Overall, the distribution of the sanitary bacteria throughout the two years was relatively homogeneous.

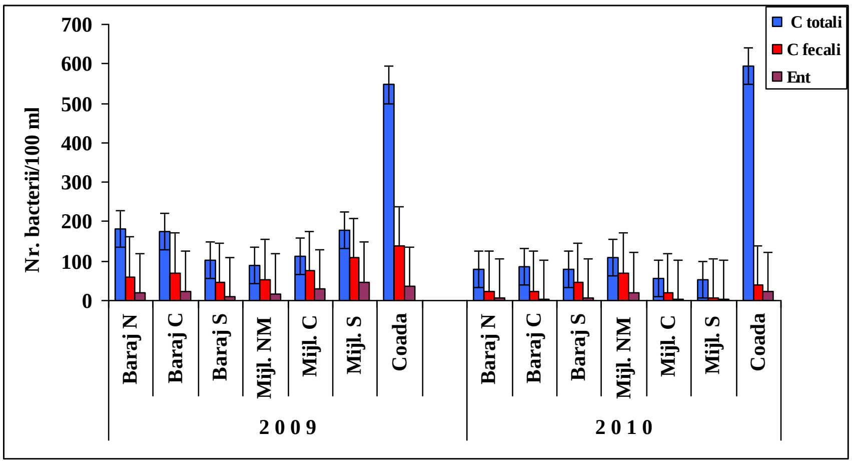


Fig. 38. The annual mean values and standard error values of the sanitary bacteria density in Văliug lake.

The total coliform bacteria accounted for 61% (in 2009) and 72% (in 2010) of the sanitary indicators in the water of the Secu dam reservoir (Fig. 39).

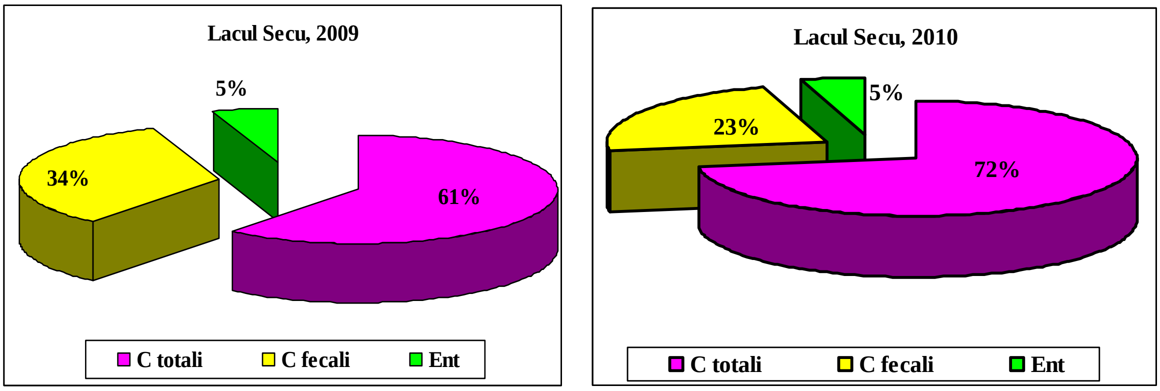


Fig. 39. The quantitative variation (%) of total coliform bacteria in the waters of Secu lake in 2009 and 2010.

The fecal enterococci, which indicate a recent fecal pollution, showed low values both in 2009 and in 2010 (5%).

The total coliform bacteria accounted for 65% (in 2009) and 78% (in 2010) in Văliug lake water, representing the largest group of sanitary bacteria. The fecal coliform bacteria, but also the fecal enterococci were more numerous in 2009 than in 2010 (Fig. 40).

The quantitative variation in percentage terms established between the three main types of bacteria indicates a large proportion of total coliform bacteria both for Secu lake and for Văliug lake.

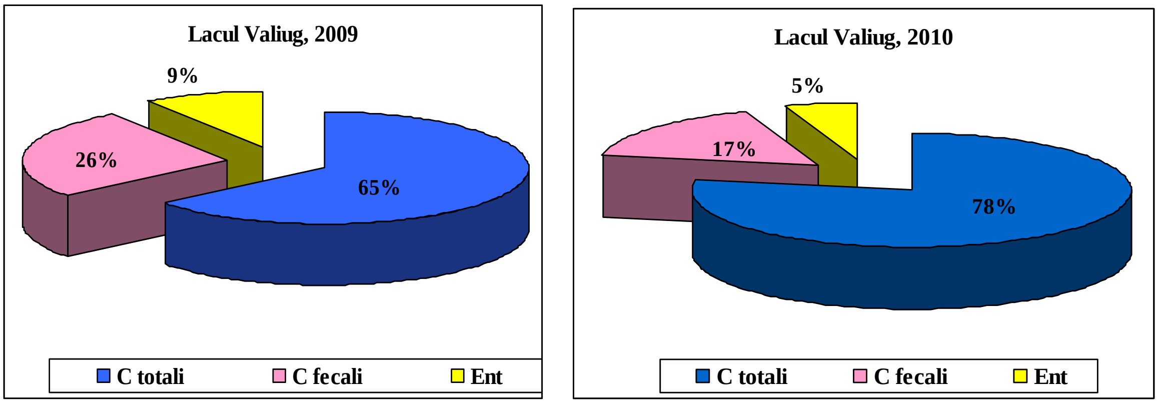


Fig. 40. The quantitative variation (%) of total coliform bacteria in the waters of Văliug lake in 2009 and 2010.

VI. 4. Determination of the sanitary quality of waters in the two reservoirs (Secu and Văliug).

The most important condition of water potability is the absence of pathogenic enterobacteria. Generally, the laboratory tests investigate the microorganisms which occur naturally in humans and warm-blooded animals feces, which are indicators of fecal pollution of the water, their presence revealing the possible presence of pathogenic bacteria. From the point of view of numerical abundance of bacteriological sanitary indicators, the water quality classes can be established (table 2 and table 3).

Table 2. Determination of the sanitary water quality in Secu lake based on bacteriological indicators in 2009.

Sampling period	Bacteriological parameters (no./ml.)	Sampling points						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Spring	Total coliforms	240	164	272	102	100	550	390
	Fecal coliforms	48	39	200	48	76	42	285
	Fecal enterococci	6	8	18	6	3	7	40
Quality class		III	III	III	III	II	III	III
Summer	Total coliforms	270	182	242	98	99	575	357
	Fecal coliforms	52	39	211	51	78	49	290
	Fecal enterococci	2	7	12	9	4	6	15
Quality class		III	III	III	II	II	III	III
Autumn	Total coliforms	320	210	310	87	100	610	440
	Fecal coliforms	67	51	261	64	88	74	324
	Fecal enterococci	5	10	11	6	2	5	36
Quality class		III	III	III	II	II	III	III

1= North of the dam; 2= Center of the dam; 3= South of the dam; 4= Middle of the dam; 5= Middle-center of the dam; 6= Middle-South; 7= End (tail) of the lake

Table 3. Determination of the sanitary water quality in Văliug lake based on bacteriological indicators in 2009.

Sampling period	Bacteriological parameters (no./ml.)	Sampling points						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Spring	Total coliforms	110	142	98	85	91	118	389
	Fecal coliforms	54	64	28	38	67	96	112
	Fecal enterococci	15	19	8	12	20	37	21
Quality class		III	III	II	II	II	III	III
Summer	Total coliforms	156	165	103	90	102	120	420
	Fecal coliforms	65	67	37	46	70	100	128
	Fecal enterococci	12	20	10	18	21	45	26
Quality class		III	III	III	II	III	III	III
Autumn	Total coliforms	180	178	123	120	112	145	560
	Fecal coliforms	68	71	44	58	81	109	148
	Fecal enterococci	19	24	10	12	28	35	22
Quality class		III	III	III	III	III	III	III

1= North of the dam; 2= Center of the dam; 3= South of the dam; 4= Middle of the dam; 5= Middle-center of the dam; 6= Middle-South; 7= End (tail) of the lake

The water in Secu lake and also in Văliug lake in 2009 fell into the sanitary water quality class III (polluted) in most of the sampling points.

VII. WATER POLLUTION ASSESSMENT BY VARIOUS ECOTOXICOLOGICAL TESTS

VII. 1. The ecotoxicological test for pollution assessment - the *Vibrio fischeri* bioluminiscence testing.

In order to highlight the chemical pollutants load in the waters of the two lakes, Secu and Văliug, the *Vibrio fischeri* bacteria bioluminescence test was employed, according to SR ISO11348-1.

For this purpose, special liquid growth media were used to inoculate *Vibrio fischeri* bioluminescent species (Fig. 41). This was incubated, and then various concentrations of the tested waters were added, and luminescence was measured using the bioluminometer.

To test the luminescence, at least two chemical pollutants are needed to report the results. Therefore, two inhibition curves were carried out for two heavy metals (chromium and zinc). The first inhibition curve was performed for chromium using $K_2Cr_2O_7$. This chemical compound is frequently used in the protocol SR ISO11348-1 for the testing of waters loaded above the luminescence of *Vibrio fischeri.*

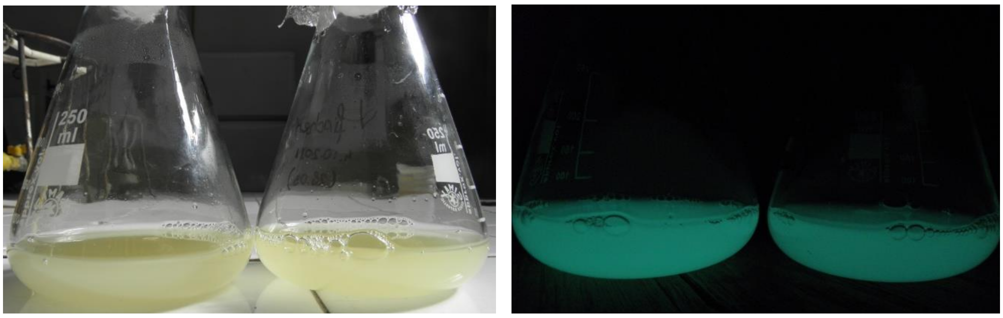


Fig. 41. *Vibrio fischeri* on a liquid growth medium in normal light and in the dark.

Based on the values measured by the bioluminometer, when the *Vibrio fischeri* bacteria were exposed for 15 minutes and 30 minutes respectively, with chromium (mg/l) ($K_2Cr_2O_7$), the inhibition curve was performed thus determining the percentage of the inhibitive effect (Fig. 42).

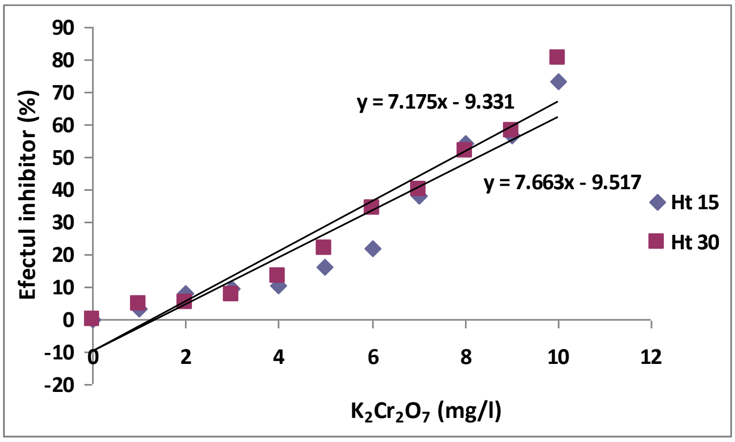


Fig. 42. The inhibition curve of $K_2Cr_2O_7$ used in the water testing protocol for the two reservoirs on the luminescence of *Vibrio fischeri*.

The second inhibition curve was carried out for zinc using $ZnSO_4$. This chemical compound is also frequently used in the protocol SR ISO11348-1 for the testing of waters loaded above the luminescence of *Vibrio fischeri.* Based on the values measured by the bioluminometer, when the *Vibrio fischeri* bacteria were exposed for 15 minutes and 30 minutes respectively, with zinc (mg/l) ($ZnSO_4$), the inhibition curve was performed thus determining the percentage of the inhibitive effect (Fig. 43).

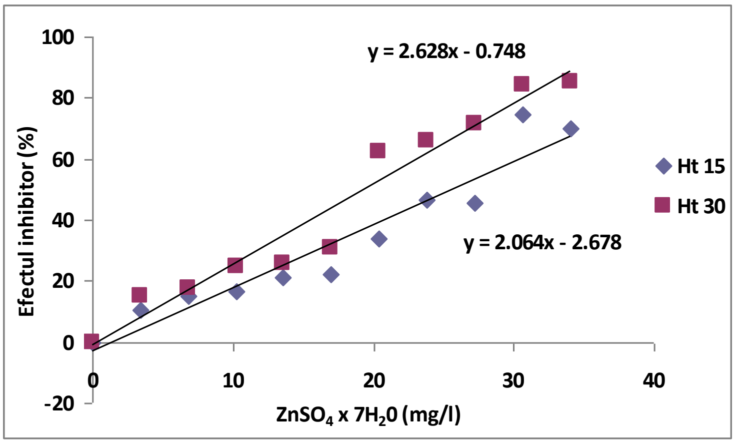


Fig. 43. The inhibition curve of $ZnSO_4$ used in the water testing protocol for the two reservoirs on the luminescence of *Vibrio fischeri*.

Based on the values and inhibition curves obtained, it can be stated that these reference substances ($K_2Cr_2O_7$ and $ZnSO_4$ x $7H_2O$) used in this test comply with and range within the admissible limits stipulated by standard SR ISO11348-1.

Subsequently, the testing of water samples from the two lakes, Secu and Văliug, was conducted. Similar to the case of the reference substances, for these samples too the exposure was carried out at 15 minutes and 30 minutes respectively.

After the 15 minutes and 30 minutes exposure, respectively, the relative luminescence units (RLU) were read by means of the bioluminometer.

Based on the relative luminescence units (RLU) recorded by the luminometer, graphics were generated where these values were clearly lower in Secu lake (Fig. 44) compared to the Văliug reservoir (Fig. 45).

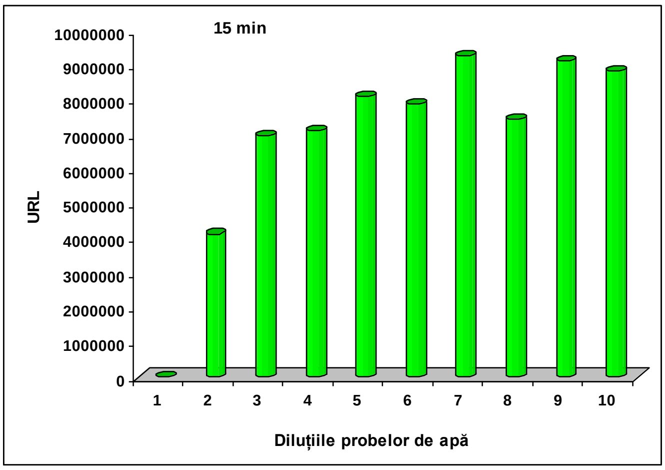


Fig. 44. The relative luminescence units (RLU) of the *Vibrio fischeri* strain at 15 minutes exposure in the water samples collected from Secu lake.

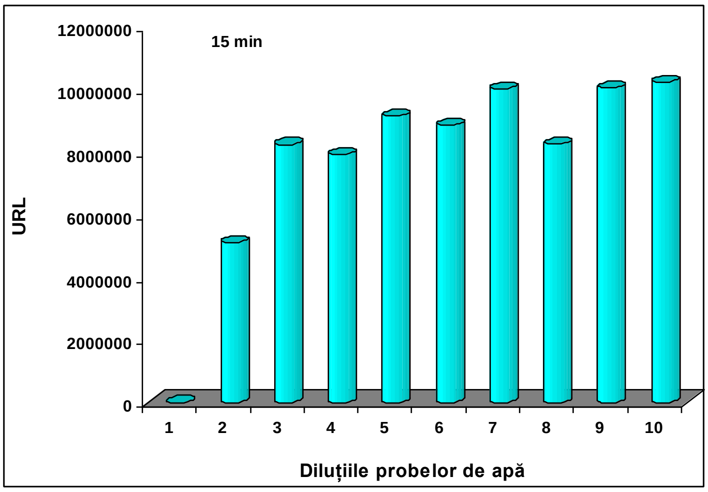


Fig. 45. The relative luminescence units (RLU) of the *Vibrio fischeri* strain at 15 minutes exposure in the water samples collected from Văliug lake.

For the correct interpretation of the luminescence test, the exposure was extended to 30 minutes and then the relative luminescence units (RLU) were read by means of the bioluminometer.

Based on the relative luminescence units (RLU) recorded by the luminometer, after a 30 minutes exposure, graphics were generated where these values showed a slight decrease both in Secu lake (Fig. 46) and in the Văliug reservoir (Fig. 47).

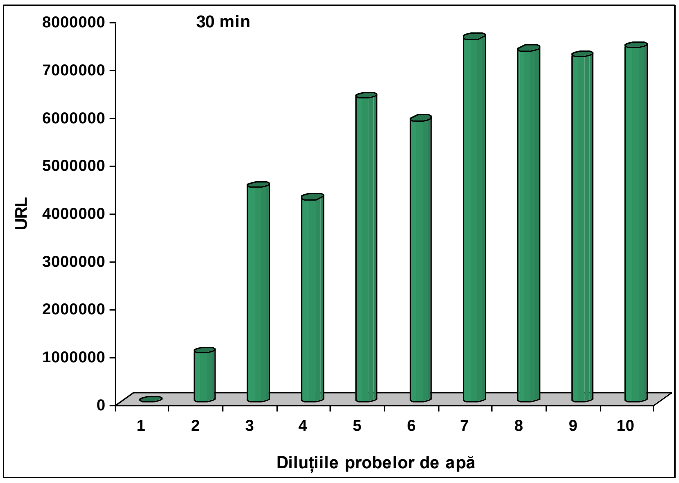


Fig. 46. The relative luminescence units (RLU) of the *Vibrio fischeri* strain at 30 minutes exposure in the water samples collected from Secu lake.

Comparing the results achieved from the two lakes, following a 30 minutes exposure, it is also noted that these values were relatively low in Secu lake compared to Văliug lake.

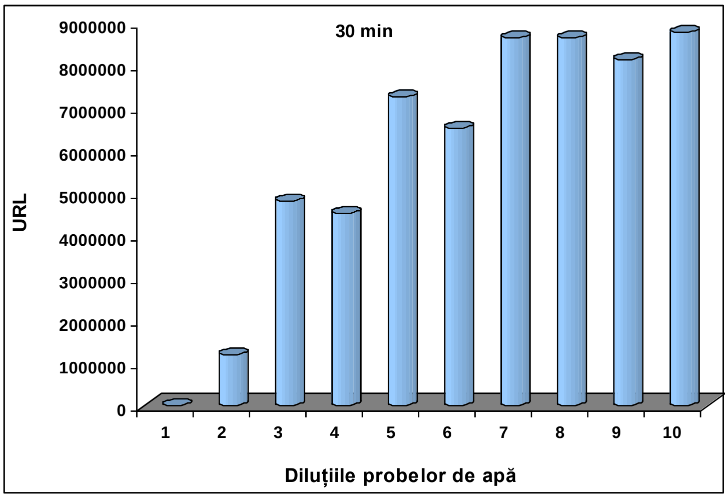


Fig. 47. The relative luminescence units (RLU) of the *Vibrio fischeri* strain at 30 minutes exposure in the water samples collected from Văliug lake.

However, these findings indicate an absence of pollution with hazardous chemical substances both in Secu lake and in the Văliug lake. Due to its properties, *Vibrio fischeri* came to be used in the ecotoxicity tests just like the luminiscent bacterium *Photobacterium phosforeum* which is used for the assessment of water quality for household waters and household wastewaters in the German standard of 1992 DIN 38412 Part 341 (Hoffmann et al., 2003).

The values of the two reference substances used ($K_2Cr_2O_7$ and $ZnSO_4$ x $7H_2O$) ranged within the admissible limits set up by the standard SR ISO11348-1.

The findings indicate the absence of pollution with hazardous chemical substances both in Secu lake and in Văliug lake.

VIII. MONITORING AND ASSESSMENT OF WATER QUALITY IN THE TWO DAM RESERVOIRS OF SECU AND VĂLIUG

In order to monitor and assess the water quality of the two reservoirs, Secu and Văliug, which supply drinking water to the town of Reșița, the following aspects need to be taken into consideration:

* Periodic dredging of the lakes is an efficient solution to delay lake succession, time evolution of the aquatic ecosystem, and maintenance of the climax. The succession trend of the biocenoses depends on the ratio between the accumulation and distribution of energy in the external environment.

* Stopping deforestation in these lakes area and outside the perimeter of the lake. Cutting the forest on the slopes of the lakes generates sawdust, among other known ecological damages. Sawdust, together with garbage from anthropogenic sources (chalets, guest houses, restaurants, sewage drainage, uncivilized tourists) and other organic substances from the vegetation layer infiltrating the aquatic basins determine, as overall effects, the decreased dissolved oxygen concentration, proliferation of pathogenic and saprophytic microorganisms, increased water turbidity, and affects the filtrating aquatic organisms, prevents fish breathing by clogging of the gills, covers some benthonic species on the substrate and kills them.

* Raising the awareness of local authorities for the monitoring and conservation of these valuable ecosystems both for the environment, and for public health.

* Compliance with the lakes protection perimeters and a more careful study on the release of building permits for projects that do not fall within the effective environmental legislation.

* The most important, yet the most vulnerable aspect is the enforcement of environmental laws. European and country-tailored legislation exists and it is reliable, but, as I pointed out in the previous chapters, the interest groups similar to mafia organizations with international political branches enforce the law in a discretionary manner, for their own interests, with disastrous consequences. If the laws were observed "ad literam", the pollution problems with the entire avalanche of negative effects would be considerably reduced to the limits of self-regulation and natural self-remediation of these valuable ecosystems.

* To ensure the drinking water quality for the town of Reșița and the infrastructure protection, it is first necessary to ensure the protection of water sources (Secu and Văliug reservoirs), observing the stages of water cleaning and sanitation, maintaining the functionality of the public drinking water supply system.

* According to the water safety plan and recommendations of the World Health Organization, the ongoing monitoring of physico-chemical and microbiological parameters required by the law is necessary to prevent any risk related to the presence of pathogens.

GENERAL CONCLUSIONS

This study conducts for the first time a comprehensive research of microbial ecology of the Secu and Văliug reservoirs. The main research goals were to establish the ecological status of water and sediment (by determining the ecological groups of bacteria), and the water sanitary status (by determining the bacteriological indicators), to assess the enzymatic activity in the sediment of the two reservoirs (by quantitatively analyzing four enzymatic activities and by qualitatively analyzing nine enzymatic activities), to study the effect of pollution on the growth and viability of the *Vibrio fischeri* strain, to assess the impact of mixed pollution on the bacterial populations and the potential associated risks to human health due to the improper use of water.

The ecophysiological groups of bacteria. The microorganisms in the bacterial cycle of nitrogen and iron examined in this thesis were quantitatively assessed in the samples collected from both reservoirs, Secu and Văliug. The organic substances dissolved in water and the temperature play an important role in the development of microbial populations influencing the numerical values recorded throughout the two years of sampling.

The ecophysiological groups of bacteria examined in this thesis (aerobic heterotrophic bacteria - AHB, ammonifying bacteria - AMB, nitrite- NiB and nitrate-bacteria - NaB, denitrifying bacteria DNB and iron-reducing bacteria FeR) revealed quantitative variations depending on the sampling year and on the sampling points. The high values are specific to the warm summer seasons when samples were collected, due to causes related to the temperatures in these seasons and to the increased organic matter intake of plant and animal origin.

The bacteria in the 6 ecophysiological groups examined showed a different numerical abundance for each group, as follows: AHB 10^7–10^8 cells, AMB were on an average about 10^5–10^6 cells, NiB were about 10^2 cells, NaB were 10^3, DNB were 10^4–10^5 cells, and the FeR bacteria were of the order of 10^2–10^3 cells. Of the bacteria involved in the biological cycle of nitrogen, the best represented in numerical terms were AMB, and the smallest number belonged to NiB.

The BIWQ values showed fluctuations depending on the sampling points. The highest BIWQ values were recorded in the sampling points located in the middle-south part (BIWQ = 4.251), and the end (tail) of the lake respectively, in case of the Secu reservoir (BIWQ=4.213). In case of the Văliug reservoir, the maximum BIWQ values were recorded in the sampling points of the central part of the dam (BIWQ=4.952). The BIWQ values decrease in the sampling points located towards the end (tail) of the Văliug lake.

The enzymatic activities - determined in the sediments of the two reservoirs - Secu and Văliug - represent an effective assessment tool for the diversity of microbiota involved in the biogeochemical cycles. The knowledge of enzymatic activity intensity represents an important and new research method in water protection, enabling the characterization of the contamination degree of aquatic ecosystems, i.e., it provides the ability to maintain water quality within good or even very good limits.

The quantitative enzymatic activities: the actual dehydrogenase activity (ADA), the potential dehydrogenase activity (PDA), the phosphatase activity (PA) and the catalase activity (CA) presented seasonal and sampling points-related variations. Depending on the sampling points, the intensity of enzymatic activities varies according to the examined type of enzyme.

The EISQ values showed fluctuations depending on the sampling points. The highest EISQ values (0.246) were recorded in the sampling points located in the middle-south part of the Secu reservoir. The EISQ values measured in Văliug lake were much higher than those recorded in Secu lake.

The qualitative enzymatic activities of the examined enzymes show intensity variations depending on the examined enzyme and on the sampling points.

The oligase activity (maltase, sucrose, lactase and cellobiase) was significant in all sediment samples examined from both reservoirs, Secu and Văliug, in the Caraș -Severin county.

The polyase activity (amylase, dextranase, glicogenase, cellulase and inulinase) indicates their presence in almost all sampling points, except the glicogenase activity.

The bacteriological sanitary indicators examined were represented by the total coliform germs, by fecal coliforms, and by the fecal enterococci group. Each of the examined groups showed seasonal fluctuations and numerical oscillations according to the sampling points. The high levels of these indicators are specific to the summer season, when high water temperatures favor the growth and activity of microorganisms. The values of the sanitary indicators are higher in the case of sampling points located in the northern and southern part, but also at the end of the lakes, due to anthropogenic influences and to urbanization. The nature of fecal pollution in the waters of the two reservoirs was determined based on the FC/FE ratio.

In terms of sanitary water quality assessment, Secu lake water falls within class III of sanitary quality (polluted) in most of the sections. Only in points 4= middle-north and 5= middle-center, the sanitary quality of the water was classified as class II - slightly polluted.

The sanitary quality of Văliug lake water falls within Class III of sanitary quality (polluted) in all examined sections. This lake was increasingly affected by anthropization, which is reflected also in the fecal pollution recorded in all examined sections.

The statistical processing proves the existence of some functional connections between the examined sanitary indicators in the two reservoirs. Significant correlations have been established between the three groups of sanitary bacteria, while the most powerful correlations between them and the physico-chemical indicators were related to the amount of organic matter, temperature and dissolved oxygen (but in this case the correlation was negative).

The pollution of the waters in the two lakes, Secu and Văliug, with domestic waste waters adversely affect the water quality, having an inhibitive effect on the microbial growth and activity.

The ecotoxicological testing with Vibrio fischeri. The bacterial bioluminescence of the *Vibrio* sp. and *Photobacterium* sp. is the expression of cellular respiration. Therefore, the physico-chemical and biological factors affecting the cellular integrity also affect the luminescence emitted by them. The measurement of the bacterial luminescence is thus an important, sensitive and rapid water toxicity assessment technique.

The values of the two reference substances used ($K_2Cr_2O_7$ and $ZnSO_4 \times 7H_2O$) ranged within the admissible limits set up by the standard SR ISO11348-1.

Following the analysis of the water samples collected from the Secu and Văliug lakes, regarding the luminescence of the *Vibrio fischeri* strain recorded after a 15 minutes exposure, the maximum number of relative luminescence units was recorded, of 12000000 RLU for the Văliug and 10000000 RLU for the Secu reservoir.

After a 30 minutes exposure of the *Vibrio fischeri* strain to the water samples in the Secu and Văliug reservoirs, a decrease of the relative luminescence units to 8000000 RLU was noticed for the water samples in Secu lake and to 9000000 RLU for the water samples collected from Văliug lake.

The findings indicate the absence of pollution with hazardous chemical substances both in Secu lake and in Văliug lake.

The bioluminescence testing is very sensitive to low concentrations of toxic compounds present in the environment. Consequently, this test cannot be employed for anthropogenic pollution.

Final conclusion. The results of this research highlight the current water quality in the two lakes (Secu and Văliug) under the influence of anthropogenic factors acting cumulatively and changing the quality of the hydrographic basin, especially in sectors exposed to anthropogenic pollution.

The anthropogenic activities represented mainly by tourism and logging negatively and evidently affect the ecosystems, while significantly higher values of sanitary bacteria densities occur in populated areas (southern shore of Secu lake and eastern shore of Văliug lake).

Comparing the two lakes based on sanitary and physico-chemical data, it can be noticed that Secu lake has a bacterial load higher for all groups of bacteria, and, as a general note, it can be said that the upstream Văliug lake is "cleaner" than the downstream Secu lake.

In conclusion, the two reservoirs - Secu and Văliug - should be preserved and maintained very strictly, and, also, they should be constantly controlled and monitored as their water is intended for drinking purposes. The reduction of anthropogenic pollution around and within the monitored aquatic ecosystem of the two reservoirs Secu and Văliug depends on their environmental potential.

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