

THE QUANTITATIVE VARIATION OF SOME ECOPHYSIOLOGICAL GROUP OF BACTERIA FROM ARIEȘ RIVER SEDIMENTS AFFECTED BY POLLUTION

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Abstract. Ten sediment samples from Arieș river sediments were studied microbiologically during the four seasons of the year 2008. The following four ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, nitrifiers and denitrifiers. The presence of all the four ecophysiological bacterial groups was registered in all the studied sediments. The studied bacterial groups present fluctuations according with the sampling season and the sampling points. The descending ranking of their abundance in the sampled sediments was: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, nitrifiers. Based on the bacteria number of the each ecophysiological group, the bacterial indicators of sediment quality (BIQS) were calculated and we observed its seasonal variations.

Keywords: ecophysiological groups, bacteria, sediments, Aries River, pollution

1. INTRODUCTION

In aquatic ecosystems and in sediments occurring processes of decomposition and mineralization of organic matter that are processes of great importance and in which are involved a variety of microbial population that are usually present in these habitats.

Nitrate it is essential for life in the biosphere because this element is included in the structure of nucleic acid and into the proteins. Although in nature, this element is present in very small quantities it could be find almost in invariable and inaccessible forms for animals and plants.

Animals are dependent by plants as a major source of nitrate. Vegetal, animal and microbial dead tissues are mineralized by proteolysis and amination, and transformed by nitrification in new accessible forms for animal and plants.

The aquatic microorganisms have an essential role in the conversion of organic matter produced by plants and animals into inorganic material. This is realized through complex processes that decompose the organic molecules into simple inorganic ones and so renew the essential nutrients like N and P, essential for the activity of photosynthesizing organisms.

The following four ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers and nitrifiers. Based on the bacteria number of each ecophysiological groups, the bacterial indicators of sediment quality (BIQS) were calculated. The pollution strongly affected the bacterial potential of the studied sediments, as it is defined by the BIQS.

The aim of our study is to realize a better understanding of mechanisms by which the pollution of the Arieș river affects the microbial population and activity in the river sediments. The hydrographic basin of Arieș River it is situated in the north-western part of the country, in the middle of the Apuseni Mountains. The Arieș River basin has an S form, a length of 131 Km and a medium width of 17, 5 Km. From the spring to the confluence with Mureș river, the Arieș covers almost 167 Km, with this length entering in the middle rivers category.

Arieș River water quality establishment is a major present interest, for human health, as well as for the flora and fauna. Pollution sources of the Arieș River are various, starting with mining activity, the atmosphere pollution, house holding activity, as well as the anthropic, uncontrolled factors etc.

The present paper completes the enzymological research carried out on the same sediments (Bodoczi, et al., 2008) for realize a better understanding of mechanisms by which the pollution of the Arieş river affects the microbial activity in the river sediments.

2. MATERIAL AND METHODS

The physico-chemical analysis consist in detection of some parameters: pH, nitrite (N-NO₂) and nitrate (N-NO₃), total ammonia (N-NH₄), sulphate (SO₄), and the presence of trace metals (copper, lead, zinc).

The mineralogical composition of the sediments has been established microscopically with chromatic objectives 4x (red), 10x (yellow) and 40x (blue) and a 10x ocular obtaining a 40, a 100, respectively, a 400 times magnification.

Microbiological analyses were carried out on sediments sampled from the Arieş river over 2008. Sediments were taken from the upstream and downstream of five major sampling sites in the following order: Abrud, Baia de Arieş, Sălcuia, Turda and Luncani.

The probable number of three groups of studied bacteria (amonifying, nitrifying and denitrifying) was performed through MTM (multiple tubes method) according to STAS 3001-91 (Cuşa, 1996; Dunca et al., 2004) in the case of ammonifiers was used a peptone medium, for nitrifiers bacteria in aerobe condition the Barjac culture media. For the determination of probable number of bacteria that produce the denitrification was used the Allen culture media with potassium azotate and glucosis in anaerob condition. The most probable number of the bacteria was calculated according to the statistical table of Alexander (1965).

The number of aerobic mesophilic heterotrophs was determined on plates bullion agarized medium (Atlas, 2004). After incubation the number of colonies in each Petri dish was read, the average of the parallel samples values was calculated from the most significant dilution and it was multiplied with the reverse value of the respective dilution.

3. RESULTS AND DISSCUSION

The evaluation of the sediment quality of the Arieş river at the sampling sites were made on the base of physico-chemical, mineralogical and microbiological analysis. The presence or the absence of an organism in an aquatic media is in close relationship with the biotic and abiotic factors

of that environment. On the other hand, the quality of freshwaters from source to watering is increasingly being affected by human activities, thus the conditions of existence for most of the aquatic organisms being changed. Under these new conditions, an alteration of the initially established ecological relations between the community members can be observed, as compared to the situation encountered in other, similar, less affected ecosystems which we used as reference.

In this work samples were taken from 5 sampling sites located in upstream and downstream of the main cities that the river passes through. The physico-chemical and biological characteristics of the sediments were determined, with the goal of elaborating an original method for monitoring their quality. The 5 sampling points were established taking into consideration the river's characteristics, the influence exerted by its tributaries and the pollution sources. The polluting chemical elements discovered in the water of Arieş river are given in table 1.

Heavy metal salts found in the water and in the sediments, represent a very serious form of pollution for surface waters due to their toxicity and stability. They can induce disorders of the biological balance, with negative consequences over the various uses of water.

The environmental changes have a strong influence upon the river dynamics, leading to river instability, and thus to changes in the erosion, transport and accumulation processes, in the sediments dynamic, heavy metal transport and geographical distribution throughout the river basin (Macklin, 1996).

Heavy metals accumulate in the sediments through complex physical and chemical adsorption mechanisms depending on the nature of the sediment matrix and the properties of the adsorbed compounds (Maher and Aislabie, 1992; Ankley et al.; 1992 Leivouri, 1998; Marin et al., 2010).

Arieş catchment has the highest metal potential from the Apuseni Mountain. There are about 20 ore deposits, the most important ones being in exploitation at Roşia Montana and Roşia Poieni. Sediments are relatively more contaminated than surface waters in the Arieş river, particularly for Cu and Zn, whose concentrations increase downstream of mining-affected tributaries, suggesting their importance as a source of metals to channel sediments in the Arieş river. Data collected from the Arieş River has indicated that the degree of pollution is dependent upon the nature of mine waste, the hydrological link between mines and local rivers, and the local physico-chemical environment.

The main source of Cu in the water of Arieş River is the Roşia Poieni quarry, located at 12 km upstream from Baia de Arieş, and, to a lesser degree, the mining works from this area.

The increase of Zn quantity in Abrud and Baia de Arieş sampling area (table 1) is caused by the input of effluents that originate in the settling ponds of Sartasului Valley.

The sedimentary formation from the Arieş River are varied. In Abrud upstream they are represented by sandstones, laminated conglomerates and shales. Lithologically one can notice a predominance of conglomerates,

macroconglomerates, sandstones, clays and limestones in flysch facies (Iancovici et al., 1976; Chira, 2000).

Baia de Arieş is represented by the igneous bodies consisting of, andesites, and the predominance of quartz, kaoline, micas, feldspar, gypsum and alunite. The magmatic products are accompanied by polymetallic, Au, Ag and Cu mineralized which contribute significantly to the increase of the local and regional geochemical background, due to the large area of distribution and the alteration phenomena accompanying them (Ghergari et al., 1991).

Table 1. The values of some physico-chemical parameters registered in the Arieş river water over the year 2008

<i>Sampling points</i>	<i>Physico-chemical parameters</i>	<i>Annual media in upstream</i>	<i>Annual media in downstream</i>
Abrud upstream and downstream	pH	7.317	7.237
	Ammonia (N-NH ₄)mgN/l	2.292	3.565
	Nitrites (N-NO ₂) mg N/l	0.029	0.031
	Nitrates (N-NO ₃) mg N/l	2.8	4.175
	Sulphates (SO ₄) mg/l	163.125	314.7
	Copper mg/l	0.237	0.084
	Lead mg/l	0.001	0.001
	Zinc mg/l	0.643	0.835
Baia de Arieş upstream and downstream	pH	6,8	6,625
	Ammonia (N-NH ₄)mgN/l	0,238	0,425
	Nitrites (N-NO ₂) mg N/l	0,017	0,017
	Nitrates (N-NO ₃) mg N/l	2,237	3,05
	Sulphates (SO ₄) mg/l	73,45	90,3
	Copper mg/l	0,008	0,018
	Lead mg/l	-	0,001
	Zinc mg/l	0,111	0,197
Sălcuia upstream and downstream	pH	8,2	7,8
	Ammonia (N-NH ₄)mgN/l	0,197	0,302
	Nitrites (N-NO ₂) mg N/l	0,082	0,06
	Nitrates (N-NO ₃) mg N/l	2,8	3,75
	Sulphates (SO ₄) mg/l	8,6	67,46
	Copper mg/l	0,006	0,003
	Lead mg/l	-	0,001
	Zinc mg/l	-	0,043
Turda upstream and downstream	pH	8	7,65
	Ammonia (N-NH ₄)mgN/l	0,352	0,597
	Nitrites (N-NO ₂) mg N/l	0,03	0,032
	Nitrates (N-NO ₃) mg N/l	2,625	3,075
	Sulphates (SO ₄) mg/l	97,4	103,5
	Copper mg/l	0,010	0,019
	Lead mg/l	0,003	0,005
	Zinc mg/l	0,042	0,055
Luncani upstream and downstream	pH	8,06	7,375
	Ammonia (N-NH ₄)mgN/l	0,457	0,865
	Nitrites (N-NO ₂) mg N/l	0,127	0,422
	Nitrates (N-NO ₃) mg N/l	4,275	5,85
	Sulphates (SO ₄) mg/l	103,05	132,45
	Copper mg/l	0,006	0,069
	Lead mg/l	0,001	0,002
	Zinc mg/l	0,043	0,086

In Salciua sampling sites from lithological point of view, the low terrace deposits are constituted of from clayey sands, sands with gravels, sands with gravels and boulders and totally subordinated silty sands. At the superior part, is developing, with lenticular aspect and having a small thickness, sandy clays and clays with gravels elements (Iancovici et al., 1976; Chira, 2000).

The inferior basin of the Aries river (Turda and Luncani sections), as result of the selective erosion of the contact between the resistant formations of Mesozoic limestones and ophiolites and the Neogene sedimentary formations, was carved by the Arieş, whose valley progressively enlarging. They consist of sarmatian and quaternary sediments (sands, clays, gravels, volcanic tuff) represented as terraces and meadows (Diaconu, 1971; Popescu, 1977, Ghergari et. al., 1991; Chira, 2000). Due to their high content of highly soluble salts, they exert a strong influence on the chemistry of Aries River in these sections.

Tabel 1 shows that the values of heavy metal salts' concentration are higher in the upstream sampling points as compared with those registered in the downstream of the river. It can be affirmed that these elevated values are a consequence of the pollutants' accumulation as a result minig activities.

The mineralogical composition of the studied sediment samples it is represented in table 2, where can be observed that the diameters of the mineral particles are between 2 µm and 0.4 mm. Also, it has been observed that in all sediment samples the quartz is predominantly and it was detected in high percentage. The highest values were registered in Turda sampling point 87 %. Hornblende and iron oxide and hydroxide are also presents in each analyzed samples. In Turda and Luncani sampling sites there has been detected trace of opaque minerals.

The amonification it is a process that takes place in the superficial layers of the aquatic sediments, where the organic matter is submitted and forms the detrital materia. In Arieş river sediments the ammonifiers bacteria (AM) are find in a big number wich demonstrate the pressence at the level of these sediments of increased quantities of nitrate. The numerical distribution of these bacteria in Aries river sediments is represented in figure 1.

The ammonifiers bacteria are present in great number in warm seasons of summer and autumn. The maximum values are reached in autumn. This high values in autumn can be explained by the accumulation of vegetal or animal organic matter at the end of the vegetation period, and more, there are due by the high temperature of the water which stimulate the microorganisms development and activities in these habitats.

Table 2. The mineralogical composition of the Arieş river sediments

<i>Sampling point</i>	<i>Principal mineral elements</i>	<i>Percentage (%)</i>	<i>Particles diameter</i>	<i>Predominant particle dimensions</i>
Abrud	Quartz	70%	2-80 µm	2-10 µm
	Orto-pyroxenes	8%		
	Iron oxides and hydroxides	20%		
	Feldspar	2%		
Baia de Arieş	Quartz	58%	0.01-0.4 mm	0.05-0.1 mm
	Hornblende, peroxides	30%		
	Iron oxides and hydroxides	2%		
	Biotite	10%		
Luncani	Quartz	72%	0.01-0.3 mm	0.01-0.1 mm
	Hornblende, peroxides	23%		
	Iron oxides and hydroxides	3%		
	Biotite	2%		
	Feldspar and opaque mineral trace			
Salciua	Quartz	65%	0.01-0.3 mm	0.05-0.1 mm
	Hornblende	30%		
	Biotite	2%		
	Opaque minerals	1%		
	Trace of iron oxides and hydroxides and chlorite	2%		
Turda	Quartz	87%	0.01-0.27 mm	0.08-0.2 mm
	Hornblende, peroxides	9%		
	Iron oxides and hydroxides	3%		
	Biotite and opaque minerals trace	1%		

The decomposition of organic matter takes place partially in the water column, but most mineralization processes occurring in sediments. By these reason sediments are considered important to the average of mineralization processes.

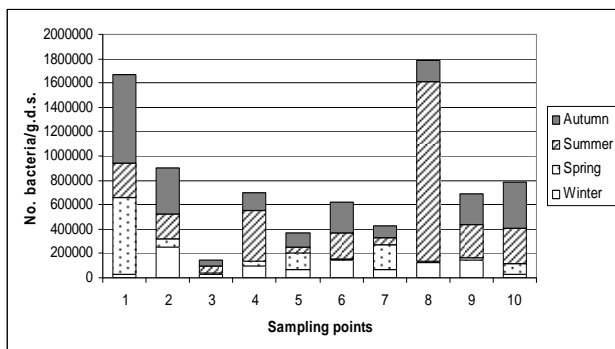


Figure 1. The numerical distribution of ammonifiers (AM) in Arieș river sediments over 2008 (1 – Abrud upstream; 2 – Abrud downstream; 3 – Baia de Arieș upstream; 4 – Baia de Arieș downstream; 5 – Sălciua upstream; 6 – Sălciua downstream; 7 – Turda upstream; 8 – Turda downstream; 9 – Luncani upstream; 10 – Luncani downstream)

According with our results the ammonifiers bacteria in Arieș river sediments are numerical well represented (10^5 - 10^6 /g.d.s.). The maximum number was registered in autumn in Abrud upstream and Turda downstream sampling points. The minimum values was about 10^4 and was achieved in spring in Baia de Arieș upstream sampling point (where was 11.111 bacteria/g.d.s.), Luncani upstream (13.553 bacteria/g.d.s.), Turda downstream (15.248 bacteria/g.d.s.) also in the winter in Luncani upstream (26.970 bacteria/g.d.s.). The reduced number of the ammonifiers bacteria in Abrud downstream and Baia de Arieș upstream comparing with those registered in Abrud upstream may be due to the presence in a high content of heavy metal salts and the chemical composition modification of the river water as a result of the confluence with the tributaries Abrud and Valea Șesei, both, containing mining wastewaters.

Considering that the mine-waste slurry deposited in tailings dam represent important sources of sediments in Arieș riverbed, a higher contamination of sediments was registered in downstream of Abrud and Baia de Arieș sampling points due by the significant enrichment of these sediments with pollutant trace metals resulted from this contaminants. Sediments can action as significant deposits of pollutant metals and also it is possible that the erosion of banks in this section of Arieș valley to remobilize this materials offering in this way the appreciation of the potential threatening

represented by the erosion on the quality of water and sediment.

At a seasonal analysis it can be observed variation in large limits of ammonifiers bacteria which realize the reduction of organic compounds with nitrate at NH_3 , nitrate being nitrified at least or stabilize different reaction with nitrate soil compounds.

The nitrifiers bacteria (NB) assure the final process of organic nitrate mineralization from the substrate level. Nitrification is the biological process of amoniac oxidation formed as result of ammonification. Exposed to the nitrification processes in which in a first case in the presence of nitritbacteria the amoniac is oxidated resulting nitrit. In a next stage, in the presence of the nitrate bacteria, nitrit is oxidated resulting nitrate.

The nitrifiers bacteria are present in also water and sediments, their maximum density being achieved in the upper level of sediments, at the contact between water and sediments. Being sensible at the level of water oxygenation, the number of these bacteria in sediments offers important data about the water circulation in aquatic basins.

The lowest values in case of nitrifiers was achieved in winter in Luncani upstream and downstream were this group of bacteria reach to 10^3 . The maximum values were reached in Abrud downstream (13.207 bacteria/g.d.s.) in summer can be corelated with the high contents of the nitrates and ammonia in the water at the level of this sampling point. The numeric variation limits for NB has large variation from 10^3 in the winter to 10^5 in the summer. It can see that the number of this bacteria in summer is grater but still apropiate by those from autumn.

The number of nitrifiers bacteria in the analysed sediment samples is about 10^4 . The numeric distribution of NB in Arieș river sediments are represented in figure 2.

Denitrification is an important way in which the atmospheric nitrate forms, being the last process in the biogeochemical cycle of the elements. Denitrification is one of the most efficient way of nitrate reduction in residual waters with a high content in nitrates, blocking in this way the pollution and the eutrophication of water courses.

Denitrification is a process that takes place in water and sediments with low aeration and where increased quantities of organic matter accumulate, especially nitrates (Strong and Fillery, 2002). The denitrification process may be stimulated in the case when in water are overflowed fertilizers especially those that grow the concentration of the azotats (Zarnea, 1984).

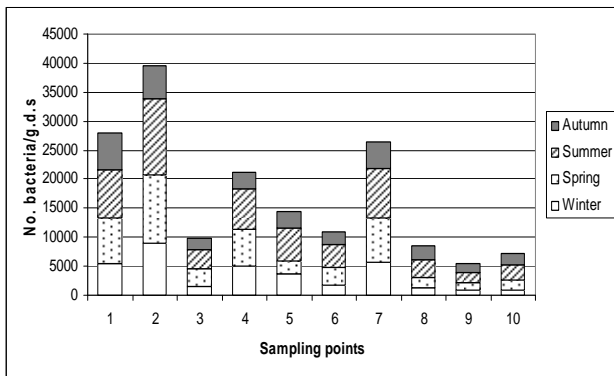


Figure 2. The numerical distribution of nitrifiers bacteria (NB) in Arieș river sediments over 2008 (1 – Abrud upstream; 2 – Abrud downstream; 3 – Baia de Arieș upstream; 4 – Baia de Arieș downstream; 5 – Sălciua upstream; 6 – Sălciua downstream; 7 – Turda upstream; 8 – Turda downstream; 9 – Luncani upstream; 10 – Luncani downstream)

The denitrifiers bacteria (DN) are present at the level of the studied sediments in each season (Fig. 3) in $10^4 - 10^5$ bacteria/g.d.s. The lowest values was registered in Baia de Arieș downstream sampling point in winter (3.454 bacteria/g.d.s.) with can be explained as a consequence of the precence of the pollutant substances in the river and which has a negative influence over the bacterial growth and development. The highest values was registered in Sălciua upstream sampling point in autumn (167.053 bacteria/g.d.s.).

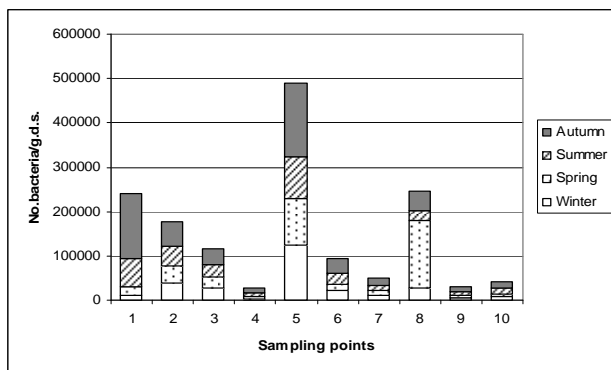


Figure 3. The numerical distribution of denitrifiers bacteria (DN) in Arieș river sediments over 2008 (1 – Abrud upstream; 2 – Abrud downstream; 3 – Baia de Arieș upstream; 4 – Baia de Arieș downstream; 5 – Sălciua upstream; 6 – Sălciua downstream; 7 – Turda upstream; 8 – Turda downstream; 9 – Luncani upstream; 10 – Luncani downstream)

Results were compared with those from literature. According to Jones (1971), due by the high content of the organic matter and of the anaerob condition this sediments has a contents of $10^5 - 10^{10}$ DN/g.d.s. which indicate a high potential of denitrification. Denitrification and nitrification are two processes that can be used to treat the water

resulted by house-holding activities which a rich content of organic substances, also is a process which favorize the reduction of N content of the effluents resulted by the epuration sations of rezidual waters, respectivley, reduce the eutrophication risk of the receptor basins.

It can be consenned (Fig. 4) that the nitrificators bacteria are present in increased numbers in the sampled sediments, their number is higher in Abrud downstream and Turda upstream probably due by the high content of pollutant substances resulted as a consequence of the anthropic influence of urbanization and industrial development, and also by the presence at the level of water and sediments of a high contents of organic matter and residues which represent the supplier of high amounts of particularly nitrate substrates.

Between the three studied bacteria from the nitrogen cycle at the level of Arieș river sediments over 2008, we may confirm that well represented by numerical point of view are the amonificators bacteria which indicate a high mineralization of organic substances with nitrate, followed by the denitrifactors and by the nitrificators bacteria.

For the mezophilic aerob heterotroph bacteria (HA) determination was used an agarized meat beef extract culture media.

In Arieș river sediment samples the HA (Fig. 4) represent the most numerous group from the ecophysiological studied bacteria.

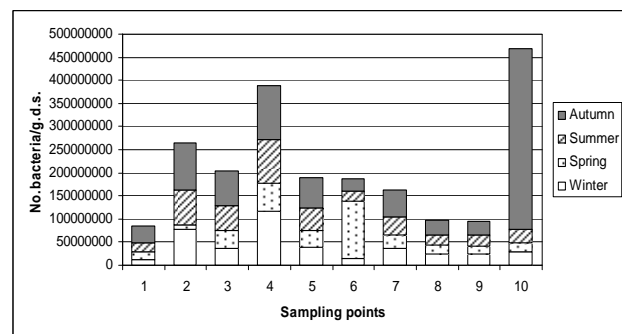


Figure 4. The numerical distribution of mesophilic aerob heterotroph bacteria (HA) in Arieș river sediments over 2008 (1 – Abrud upstream; 2 – Abrud downstream; 3 – Baia de Arieș upstream; 4 – Baia de Arieș downstream; 5 – Sălciua upstream; 6 – Sălciua downstream; 7 – Turda upstream; 8 – Turda downstream; 9 – Luncani upstream; 10 – Luncani downstream)

The maximum values were registered in Luncani, Baia de Arieș and Abrud downstream sampling points. The highest values were registered in autumn in the majority of the sampling points. This may be due by increased air and water temperature in summer time which has as consequence an enhancing bacterial activity in

sediments. The lowest values registered in Abrud upstream sampling point for every sampling season can be explained as a consequence of the river pollution with wastewaters resulted from mining activity and these bacteria sensitivity to the existent substances in the water composition as it can be observed in the case of the analyzed physico-chemical parameters.

The seasonal fluctuation can be observed in each sampling point. The minimum values were registered in Baia de Arieş upstream sampling point for each sampling seasons, and the maximum values in Turda downstream in autumn. It can be observed an intensification of HA in case of sampling points situated in downstream of the river course this could be explained as a result of a reduced water flow, a wide river band which favours the processes of sedimentation thus influencing the growth and density of microorganisms.

The IBQS values represented in figure 5 change depending on sampling season. Usually, the lowest values were reached in winter and spring. The explanation is that the low temperatures in winter reduce the microbial activity in sediments. A parallel evolution but higher, was registered in summer and in autumn.

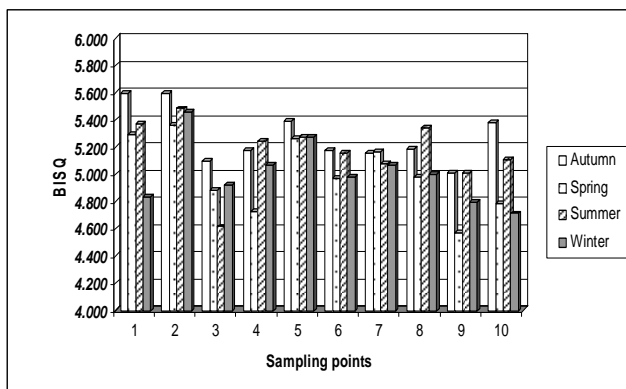


Figure 5. Seasonal variation of the bacterial indicator of sediment quality from Arieş river sediments over 2008 (1 – Abrud upstream; 2 – Abrud downstream; 3 – Baia de Arieş upstream; 4 – Baia de Arieş downstream; 5 – Sălciua upstream; 6 – Sălciua downstream; 7 – Turda upstream; 8 – Turda downstream; 9 – Luncani upstream; 10 – Luncani downstream)

At a seasonal analysis the lowest value was registered in spring Baia de Arieş downstream, and the maximum in Baia de Arieş upstream. In autumn, the minimum value was registered in Turda upstream sampling point, and the maximum for this sampling season in Abrud upstream. In winter the minimum value was registered in Sălciua downstream and the maximum in Baia de Arieş upstream.

The high values of the bacteriological indicator of the sediments in Arud, Baia de Arieş and Luncani sampling points may be due by the presence in the water of the clorure, carbonates and different salts wastes, respectivley, due by the organic matter charges that determine a growth bacterial indicator as a result of an intense microbial activity.

The seasonal distribution of bacteria implied in the bacterial cycle of nitrogen in Arieş river sediments differ a lot from one group of bacteria to another. In this way we may observe the development in a large number on ammonifier bacteria comparing with the rest of studied bacterial groups for each sampling season. Meanwhile, it can be observed the increased values of AM in summer followed by those from autumn.

The numerical growing of these bacteria in hot seasons especially in autumn, are due by the decomposition and mineralization of organic wastes by these bacteria (Ailiesei and Jâpa, 1995). Another explanation may be the high temperature which favours the microorganisms development and in the same time enhance their activity, respectively, the accumulation of vegetal or animal origin detritic material, or the organic pollutants resulted by overflows which demonstrate the existence of high quantities of particularly nitrate substrate (Herlea et al., 1995).

The nitrifiers bacteria are represented numerically by reduced values comparing with the second other studied bacteria. This group has a maximum activity in the autumn, meanwhile the minimum was registered in the winter when temperature are low, decreases the water temperature and they all have a negative impact on the microorganisms activity and development in water and sediments as well. The denitryfiers bacteria present visible seasonal oscillation. At the sediments level the maximal values was registered in spring and autumn.

4. CONCLUSIONS

The ammonifying, nitrifying, denitrifying and mesophilic aerobe heterotrophic bacteria were determined numerically in all the sediment samples.

A direct correlation between the microbial development inhibition and the pollution degree of the sediment specific to each sampling point was observed (in the mountainous zone due to mining industry and on the inferior course due to urban wastes).

The bacterial indicator of sediment quality (BIQS) presents seasonal variations and also

variations according with the sampling points.

The temperature and organic substances dissolved in the water had a major role in the development of the microorganisms population, influencing the numerical values registered during the four seasons. The polluting substances that enter in the river have a negative impact on some bacterial populations.

From biological and microbiological point of view, the study of this water is essential in order to find the impact ways and to apply the suitable control methods for rehabilitation and maintaining of Arieş river water quality at a good and very good potential.

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