BACTERIOLOGICAL STUDIES IN SEDIMENT FROM CERNA-BELARECA-DANUBE HYDROGRAPHIC SYSTEM (SW ROMANIA) WITH A ROLE IN ASSESSING POLLUTION

Marioara Nicoleta FILIMON[*], Aurica Breica BOROZAN[**], Vasile Daniel GHERMAN[***]

[*] *West University of Timişoara, Faculty of Chemistry-Biology-Geography, Department of Biology, Timişoara, Romania*
[**] *Banat University of Agricultural Sciences and Veterinary Medicine from Timisoara, Departament of Horticulture, Timişoara, Romania*
[***] *University of Polytehnic, Timisoara, Faculty of Hydrotechnic, Department of Microbiology, Timişoara, Romania*
Corresponding author: Marioara Nicoleta Filimon, Western University of Timişoara, Faculty of Chemistry-Biology-Geography, Department of Biology, 16 Pestalozzi, 300115 Timişoara, Romania, Tel.: 0040256592646, Fax:0040256592622, e-mail: nicoleta_filimon@yahoo.com

Abstract. This paper contains data about the microorganism population in sediment from Cerna-Belareca-Danube hydrographic system. Data allows us to identify possible pollution sources and determine their effect on certain eco physiological group of microorganisms. Bacteriological studies had in view to establish three eco physiological groups of microorganism: nitrifying bacteria, iron-reducing bacteria and anaerobic sulfate-reducing bacteria. Determination has been made using serial dilutions and specific culture environment for each eco physiological group. Based on absolute values of those 3 microorganism groups, the bacterial indicator of sediment quality has been established (BISQ). Sediment analyses have values between 1.274 (Orşova upstream) and 1.296 (Mehadia downstream) in spring. These are the values for winter: 1.286 (Topleţ downstream) and 1.310 (Orşova upstream). Additional contribution of organic sulfur compounds and microorganisms involved in the sulfur cycle have relatively close values for both seasons in Herculane sample point. Sampling points Mehadia downstream and Topleţ downstream represent the endpoint of BISQ. Lowest values had been recorded here in autumn and the highest values had been recorded in spring. BISQ lower values from Orşova harbor and Orşova shipyard is due to oil pollution, varnishes, paints, detergents and metals. BISQ has seasonal variations, depending on the sampling point of the studied aquatic ecosystem.

Keywords: bacterial indicator of sediment quality, sediment, pollution

INTRODUCTION

Water pollution decreases biomass production. Sometimes, certain microorganisms and organisms even disappear. If the phytoplankton has been strongly developed, it leads to ecosystem degradation due to lack of oxygen.

Decreased water quality is due to: industrial wastewater, sewage, various substances used in agriculture and organic substances which consist of rubbish, household waste and acquisition of fertilizers.

Rivers and streams provide a convenient route for liquid and solid industrial residues. Although waste disposal is strictly controlled in factories, a little accident is enough to destroy a river.

The composition of a microbial community in a given environment is strongly influenced by the environment's biogeographical and ecological properties, such as the dynamic food webs, the cycling of nutrients, and the presence of organic and inorganic matters [1, 2, 3]. Although many studies on environmental microbial compositions have been conducted, most of them focused on the microcosms found in solid substrates [16, 19], marine [10, 18], or freshwater [4, 9] ecosystems.

Decomposition and mineralization of organic matter are processes of great importance for the releasing of biogenic elements in the aquatic environments. The importance of microbial and enzymatic activity as an indicator of water and sediment pollution was frequently underlined [15, 14, 18].

Based on bacteriological determinations, this paper highlights pollution sources from the studied hydrographic system. The aim of this study is to assess pollution in the studied aquatic ecosystem, based on the bacterial indicator (established on 3 ecophysiological groups of bacteria), to identify possible sources of pollution and the effect they have on microorganism communities from sediment.

MATERIALS AND METHODS

Bacteriological analysis consisted in determining the number of nitrifying bacteria, iron-reducing bacteria and anaerobic sulfate-reducing bacteria.

For the 3 physiological groups undertaken into this study elective mediums were used. For the growth of nitrifying bacteria the Barjac culture medium. The nitrate freed following the nitrifying bacteria's activity can be evidenced through a blue color reaction with diphenylamine-sulfuric acid reactant [6].

Iron-reducing bacteria is cultivated on growth medium Ottow modified. The pink or red color indicates the presence of ferrous iron (II) in the presence of the color reactant αα-dipiridil [5].

Establishing the probable number of sulfate-reducing bacteria is achieved by multiple tube methods, using medium Starkey. Positive tubes have a black precipitate at the bottom (iron sulphide) [6].

Using Alexander's table, we established the most probable number of bacteria. These bacteria belong to three different eco physiological groups. Based on these values, the bacterial indicator of sediment quality has been established.

Bacterian indicator of the sediment quality (BISQ) was calculated using the calculation formula proposed by Muntean [11]: $BISQ = 1/n \times \Sigma \log_{10} N$, where: BISQ - bacterian indicator of the sediment quality; n - number of ecophysiological groups; N - number of bacteria appertaining to each ecophysiological group.

There were 8 sediment samples. The sampling has been performed in 2008, spring and autumn.

RESULTS

Using serial dilutions, specific culture environments and Alexander's table, we established the most probable number of bacteria, which belong to three different eco physiological groups: nitrifying bacteria, iron-reducing bacteria and anaerobe sulfate-reducing bacteria.

Although these bacteria are involved in the biogeochemical cycle of three different elements N, Fe and S, the latter two groups are considered water pollution indicators. Based on absolute values determined for each eco physiological group, the bacterial indicator of sediment quality has been established (BISQ).

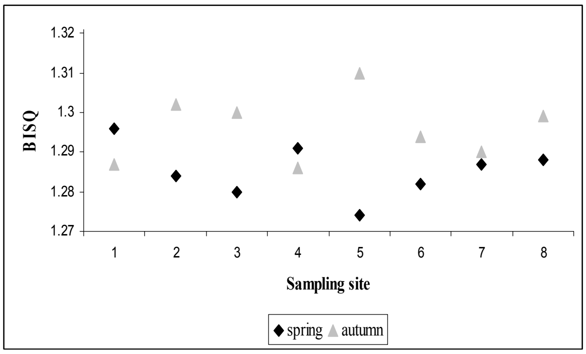

Figure 1. Captures aspects of BISQ seasonal variations in sediment samples taken from Cerna-Belareca-Danube hydrographic system.

Analyzing the bacterial indicator of sediment quality in Belareca-Cerna-Danube hydrographic system, we found values between 1.274 (Orşova upstream) and 1.296 (Mehadia downstream) in spring. Winter values are 1.286 (Topleţ downstream) and 1.310 (Orşova upstream) in winter. We should note that in spring there are relatively close values. Lower values are recorded in autumn.

Higher values in autumn may be attributed to additional contribution of organic substances at the end of the vegetation season. A strange situation has been recorded in Mehadia downstream. This is due to the fact that there are higher BISQ values in spring than in autumn. Here maximum value is recorded in spring.

Causes may be: rain, which brings an additional contribution of organic substances, especially iron compounds, causing an increase of iron-reducing bacteria. Therefore, it is an increased BISQ value. Relatively close values of the bacterial indicator at Herculane sampling point, may have the following causes: water discharge in Cerna riverbed from specific mineral, thermal springs in the area (particularly sulfur springs), provide an additional contribution of organic sulfur compounds, but also the microorganisms involved in biogeochemical cycles of sulfur.

Sulfate reduction bacteria are mentioned here. The contribution of domestic wastewater from many tourist complexes in the area is an important factor of biological pollution. On a seasonal study, we found higher values in autumn at the sampling point Cerna-Belareca downstream. This is due to the contribution of organic substances from the end of vegetation period.

At the sample point Topleţ downstream an unusual situation has been discovered. There are higher values in spring than in winter. The only plausible explanation is the frequent flood in spring around this area. Household waste water may be a pollution source, since the city has no cleaning station.

Flow phenomenon is also common and provides an additional contribution of household wastes. With a flood, all domestic and industrial wastes come from upstream into the riverbed. At the sampling point Orşova upstream, we found higher values in spring. Riverbanks have large quantities of iron compounds from rain and flow phenomena, which overflow into the riverbed. The sample point located in Orşova downtown, recorded close values in the two seasons. It shows the existence of pollution sources operating continuously and steadily, providing a surplus of wastewater and industrial waste. The presence of recreational craft in the area (ship, boat) provides accidental or maybe not accidental oil pollution, affecting the number of microorganisms and their activity in water and sediment.

At Orşova sample point we found similar values of bacterial indicator in the two seasons. Pollution in this area is present through chlorides, fats, detergents. Ship or boat replenishment with oil, may accidentally get into the river bed, which is another possible source of pollution. It has a constant intensity throughout the year, reducing the number of microorganisms.

This is translated by BISQ close values in the 2 seasons of 2008. There are average values. Sampling point located near Orşova Shipyard is a significant source of pollution for the aquatic environment. Ship crafting has a specific role in supplying pollution by: varnish, paint, metals, electrodes, grit (quartz, metal residues) or washing the ship before launching it to the water. These pollution factors act permanently and consistently throughout the year, reducing the number of microorganisms.

DISCUSSION

A comparative analysis of bacterial indicator of sediment quality in two seasons, namely spring and autumn of 2008, at the Cerna-Belareca-Danube hydrographic system we established that at almost every sampling point values are higher in autumn with two exceptions: Topleţ downstream and Mehadia downstream.

Comparing obtained values for BISQ in sediment from Cerna-Belareca-Danube hydrographic system with values recorded in other river sediments from Romania, we established the following: compared to sediments taken from Crişul Alb, maximum values of BISQ are higher [7]; as compared to sediments taken from Mureş River, values are lower [13] values from River Jiu are lower [8] and sediments from River Aries have smaller values [12]. In conclusion we can say that pollution in the studied hydrographic system is lower than pollution from Crişul Alb and is similar to pollution from Mureş, Arieş and Jiu rivers.

Bacterial indicator of sediment quality varies seasonally, depending on the sampling point from Belareca- Cerna- Danube hydrographic system. Organic iron compounds in Belareca riverbed stimulates growth of microorganisms in spring, recorded by an increased BISQ value.

Additional organic sulfur compounds and microorganisms involved in the sulfur cycle have relatively close results in the two seasons for Herculane sample point. Lower values of BISQ at the sample points Orşova harbor and Orşova Shipyard is due to oil pollution, varnishes, paints, detergents and metals.

REFERENCES

[1] Becks, L., Hilker, F.M., Malchow H., Jergens, K., Arndt, H., (2005): Experimental demonstration of chaos in a microbial food web, Nature, 435: 1226–1229.
[2] Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L., Lilley, A.K., (2005): The contribution of species richness and composition to bacterial services, Nature, 436: 1157–1160.
[3] Bowman, J.P., McCammon S.A., Dann, A.L., (2005): Biogeographic and quantitative analyses of abundant uncultivated g-proteobacteria clades from marine sediment, Microbial Ecology, 49: 451–460.
[4] Casamayor, E.O., Schafer, H., Baneras, L., Pedro´s-Alio, C., Muyzer, G., (2000): Identification of and spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis, Applied and Environmental Microbiology, 66: 499–508.
[5] Drăgan-Bularda, M., (2000): Microbiologie generală-Lucrări practice, Cluj-Napoca, pp. 197.
[6] Dunca, S., Ailiesei, O., Nimitan, E., Stefan, M., (2007): Microbiologie aplicată, Demiurg Publishing House, Iaşi, pp 270-271; pp. 283-284.
[7] Filimon, M.N., Drăgan-Bularda, M., (2007): Seasonale study regarding the faecal pollution of the water from the Crişul Alb river, Studia Universitatis Babeş-Bolyai, Biologia, LII(1): 93-101.
[8] Filimon, M.N., Borozan, A.B., Radu, F., (2008): Bacteriological and enzymological study of the sediments from the middle course of the Jiu riverl, Annales of the University of Craiova, Universitaria Craiova, XXXVIII/B: 164-171.
[9] Humayoun, S., Bano, N., Hollibaugh, J.T., (2003): Phylogenetic composition of the bacterioplankton from an alkaline, hypersaline lake, Mono Lake, California, Applied and Environmental Microbiology, 69: 1030–1042.
[10] Irigoien, X., Huisman, J., Harris, R.P., (2004): Global biodiversity patterns of marine phytoplankton and zooplankton, Nature, 429: 863–867.
[11] Muntean, V., (1995-1996): Bacterial indicator of mud quality, Contribuții Botanice, 32: 73-76.
[12] Muntean, V., (2007): Bacterial and enzymatic indicators of water and sediment pollution in the Arieş River, Studia Universitatis Babeş-Bolyai, Biologia, LII(1): 87-92.
[13] Muntean, V., Ştef, L.C., Brăgan-Bularda, M., (2005) Cercetări microbiologice asupra unor sedimente de pe cursul mijlociu al râului Mureş, Studia Universitatis Babeş-Bolyai, Biologia, L(2): 175-181.
[14] Noble, R.T., Weisberg, S.B., Leecaster, M.K., McGee, C.D., Ritter, K., Walker, K.O., Vainik, P., (2003): Comparison of beach bacterial water quality indicator measurement methods, Environmental Monitoring and Assessment, 81: 301-312.
[15] Papp, J., Fodorpataki, L., (2002): Evaluation of the organic pollution of the Mureş River on the study of the indicator microflora, Contribuții Botanice, 37: 231-237.
[16] Stach, J.E.M., Maldonado, L.A., Masson, D.G., Ward, A.C., Goodfellow, M., Bull, A.T., (2003): Statistical approaches for estimating Actinobacterial diversity in Marine Sediments, Applied and Environmental Microbiology, 69: 6189–6200.
[17] Stef, L.C., Muntean, V., Dragan-Bularda, M., (2004): Microbiological research on the Mureş sediments from Ocna Mureş affected by pollution, Contribuții Botanice, 39: 227-232.
[18] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., (1997): The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Research, 25: 4876–4882.
[19] Walker, J.J., Spear, J.R., Pace, N.R., (2005): Geobiology of microbial endolithic community in the Yellowstone geothermal environment, Nature, 434: 1011–1014.

Submitted: 18 April 2010
Accepted: 27 April 2010

Analele Universității din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433