

MICROBIOLOGICAL RESEARCH ON THE MUREȘ SEDIMENTS FROM OCNA MUREȘ AFFECTED BY POLLUTION

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Abstract: Five sediment samples from the Mureș river affected by pollution with sewage from the S.C. UPSOM S.A. Ocna Mureș was studied microbiologically. The following five ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, desulphofiers and iron-reducers. Some polluting, physico-chemical parameters, were also analysed: chlorides, sulphates, metals. The presence of all the five ecophysiological bacterial groups was registered in all the studied sediments. The descending ranking of their abundance was: aerobic mesophilic heterotrophs > ammonifiers > denitrifiers > desulfofiers ≡ iron-reducers. Based on the bacteria number of each ecophysiological groups, the bacterial indicators of sediment quality (BISQ) were calculated. The pollution strongly affected the bacterial potential of the studied sediments, as it was defined by the BISQ. The BISQ values were higher upstream (5.058), decreased at the polluting point, where three sewers carrying the sewage from the industrial enterprise empty into the river (4.258, 4.102 and 3.850, respectively) and came back close to the initial value (4.796) at 1 km downstream, certifying the regenerating capacity of the studied sediments. A positive correlation has been established between the enzymatic indicator, the bacterial indicator and the content of organic matter in the studied sediments. A strong negative correlation with a high statistical significance was established between the values of the two indicators (BISQ and EISQ), on one hand, and the content of the sediment in chlorides, sulphates, magnesium, sodium and iron, on the other hand.

Introduction

Decomposition and mineralization of organic matter are processes of great importance for the releasing of biogenic elements in the aquatic environments. A part of the organic matter which originate in phytoplankton and in zooplankton, enter into the dissolved organic phase phase of the water. The particulate phase is partly incorporated by the secondary consumers. The rest is converted in detritus and submitted subsequently to decomposition. Some of these compounds are incorporated by bacteria or microplankton, while others suffer further enzymatic decomposition. The compounds of little molecular weight resulted from the exoenzyme activities are rapidly metabolized by the heterotrophic bacteria. Thus, one can consider that the rate of the organic matter degradation is, probably, controlled by the first stage, that of the exoenzymatic hydrolyse [7].

In the aquatic ecosystems, the sediments are a clue link in the elements biocycle, the place where the mineralization processes of the organic substances undegraded in the water column is completed. The activity of the microorganisms on the substrates is carried out by means of enzymes catalyzing hydrolyses, oxido-reductions, as well as by means of some final products of the microbial metabolism. Malcolm and Stanley [5] consider the environment within a sediment is a complex function of many different factors, such as the major mineral matrix, the texture, the amount of organic carbon and the geographical location. According to the cited authors, the sediments have three major components: detrital material (derived from the erosion of the continents), biogenic material (formed by biological productivity) and authigenic material (formed *in situ*). The final character of a sediment is related to the relative proportions of these components.

The present paper completes the enzymological research carried out on the same sediments [8], for realize a better understanding of mechanisms by which the pollution of the Mureş river by the S.C. UPSOM S.A. from Ocna Mureş affects the microbial activity in the river sediments. The industrial enterprise S.C. UPSOM S.A. periodically spills into the river waste water containing chlorides, sulphates, natrium, manganese and other impurities resulted in the production processes. Thus, the enterprise is one of the most important source of the Mureş river pollution.

Materials and Methods

Microbiological and physico-chemical analyses were carried out on the 5 sediments, sampled from the river Mureş at 15.05.2001. The sample sites, located around the place where three sewer empty into the river the waste water from the enterprise S.C. UPSOM S.A., were appointed as follows: A – 100 m upstream from the mouths of the SC1 sewer; B – at the SC1 sewer mouth; C – at the SO2 sewer mouth; D – at the SHT3 sewer mouth; E – 1000 m downstream from the SHT3 sewer mouth. Between the three sewage mouths there are approximately 50 m. The sediments had a clayey consistence, without sand or gravel.

The following 5 ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs (agar plates), ammonifiers (peptone medium), denitrifiers (De Barjac culture medium) [13], desulphofiers (Van Delden medium) [2] and iron-reducers (Ottow modified medium) [11, 12]. Except the aerobic mesophilic heterotrophs (where we used the method of successive dilutions), the most probable number of bacteria was calculated according to the statistical table of Alexander [1].

Some physico-chemical characteristics of the sediment were also analyzed: pH, BCO5, CCOCr, organic matter, chlorides, sulphates [15]; magnesium, natrium, iron, copper, zinc, lead and manganese, by atomic absorbance spectroscopy.

Results and Discussion

The results of the physico-chemical and bacteriological analyses are presented in table 1 and fig. 1. We mention that from the enzymological results yet published [8] we have taken over only the enzymatic indicators of sediment quality, calculated according to the formula proposed by Muntean [10], in order to establish the correlation between the physico-chemical parameters and the microbial and enzymatic potential of sediments, defined by the values of bacterial (BISQ) and enzymatic (EISQ) indicators of sediment quality.

From the data in tab. 1 one can see a strong increase of the pH in the studied sediments. The high level of pH (8) is maintained even at 1 km downstream from the mouths of sewers where they empty into the river. The values of BCO5, CCOCr and the organic matter content don't vary significantly. On the other hand, at the mouths of the sewers strongly increase the concentration of sediments in chlorides sulphates and all the metals analyzed: magnesium, natrium, iron, copper, zinc, lead and manganese. It is normal, because the polluting enterprise produce NaOH, NaCl is another secondary product and the other metals are used as catalysts in the production process as well.

As we can see in fig. 1, in all the studied sediments was registered the presence of all the 5 ecophysiological bacterial groups. The mesophilic heterotrophic bacteria are the best represented. Their number is of an order of $10^7 \cdot g^{-1}$ sediment (dry matter) in the sample from the upstream site and in that situated 1 km downstream from the spill sewage mouths. The number decrease $\sim 10^3$ fold in the site C – the mouth of the SO2 sewer, which empties into the river the most polluted sewage. Otherwise, except the ammonifying bacteria, whose lowest number was registered in the site B, in the site C we registered the lowest number of all the other 4 bacterial groups.

The number of the other 4 ecophysiological groups (ammonifiers, denitrifiers, desulfofiers, iron-reducers) was much lower than that of the aerobic mesophilic heterotrophs.

Only upstream (site A), the ammonifiers reached values of $10^6 \cdot g^{-1}$ sediment (dry matter). In the other sites, the ammonifiers counted only 10^4 cells $\cdot g^{-1}$ sediment (dry matter). According to the criterion of cell abundance, followed: the denitrifiers – $10^4 \cdot g^{-1}$ sediment (dry matter) in the sites A, B and D, one order of magnitude higher in the site E and one order lower in the site C, the most polluted. Less represented were the desulfifiers and the iron-reducers. Both of the groups counted $10^2 \cdot g^{-1}$ sediment (dry matter), without significant differences between them, the lowest values registered also in the site C. The values registered are comparable with those reported by other researchers in different types of sediments [3, 4, 6, 14].

Based on the number of each ecophysiological groups, the bacterial indicators of sediment quality (BISQ) were calculated according to the formula proposed by Muntean [9]. The following values were obtained: site A – 5.058; site B – 4.258; site C – 3.850; site D – 4.102; site E – 4.796.

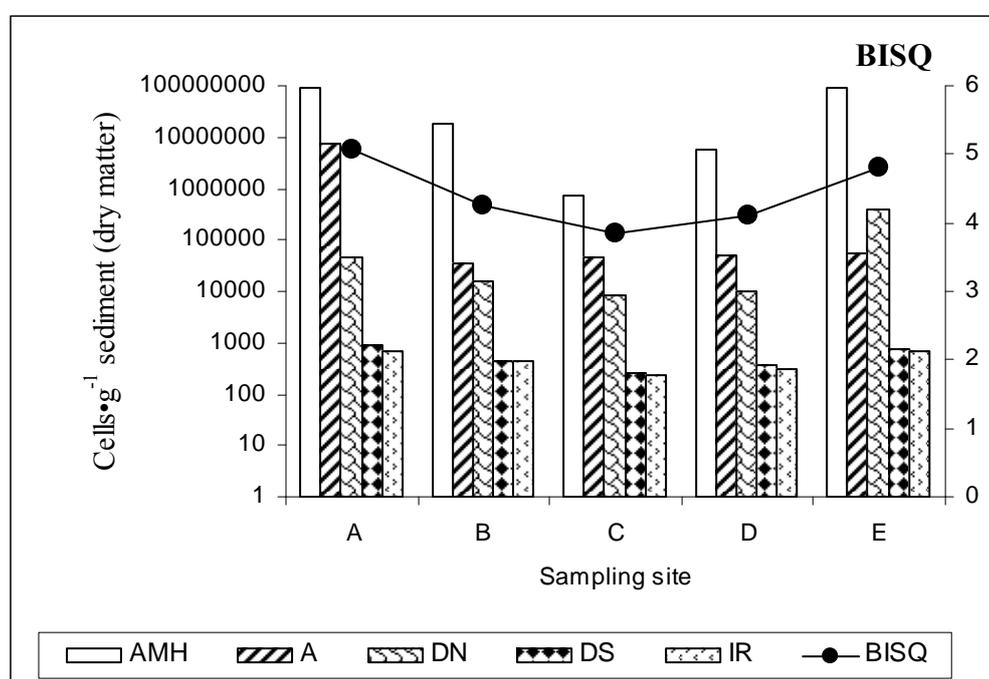


Fig. 1: Results of the microbiological analyses in sediments sampled from the 5 sites of the Mureș river affected by pollution. AMH = aerobic mesophilic heterotrophs; A = ammonifiers; DN = denitrifiers; DS = desulphifiers; IR = iron-reducers; BISQ = bacterial indicator of sediment quality.

The bacterial indicator of sediment quality offers a general overview on the microbial potential of sediments. As we can see, this potential is obviously lower in the sites where the sewage from the S.C. UPSOM S.A. enterprise in Ocna Mureș spills into the river. The lowest level of the microbial potential was registered in site C (BISQ = 3.850), where the most polluted sewer – SO₂ – empties into the river. At the mouths of the other two sewers (sites B and D), the BISQ also registered small values, only a few higher than 4. Only upstream (site A) the BISQ exceeded the value 5. A close value (4.796) was registered in the site E, 1 km downstream from the mouths of the three sewers. This indicates that, along a short section, the microbial potential (as well as the enzymatic one, as we can see in tab. 1) of the sediments from the Mureș river, very seriously affected by the pollution with sewage originated in the S.C. UPSOM S.A. enterprise Ocna Mureș, has been naturally recovered, at a level close to that registered upstream from the mouths of the sewers where they empty into the river. The fact is certifying the good autoregenerative capacity of the sedimentary microflora in the river.

The correlation between the analyzed parameters was also calculated. Because the correlation between the physico-chemical factors has not any relevance, only the correlation coefficients between every physico-chemical parameter, on one thing, and the BISQ and EISQ, on the other hand, are presented in tab. 1. As regard the positive correlation, as we can see, statistical significance ($p < 0.05$) has only the correlation between the two indicators (BISQ and EISQ), as well as between these ones and the organic matter content.

Table 1: Results of the physico-chemical, microbiological and enzymological analyses carried out on the sediments from the Mureş river. OM = organic matter ($\text{mg}\cdot\text{g}^{-1}$ sediment (dry matter)); EISQ = enzymatic indicator of sediment quality; BISQ = bacterial indicator of sediment quality; chemical parameters expressed in $\text{mg}\cdot\text{g}^{-1}$ sediment (dry matter).

Analysed parameter	Sampling site					Correlation coefficient (r) with EISQ	Correlation coefficient (r) with BISQ
	A	B	C	D	E		
pH	7.5	8.3	8.2	8.5	8	-0.673	-0.836
BCO5	5.42	4.33	4.04	5.5	5.62	+0.851	+0.675
OM	70	66	58	60	72	+0.887*	+0.922*
CCOCr	20.92	16.24	18.2	11.37	15.34	+0.168	+0.436
Chlorides	25.58	47.06	59.64	46.1	31.61	-0.990**	-0.982**
Sulphates	22.01	43.49	56.2	42.53	28.04	-0.990**	-0.982**
Magnesium	30.4	45.2	50.12	44.3	29.5	-0.978**	-0.964**
Natrium	12.8	27.3	36.4	31.4	20.5	-0.930*	-0.991**
Iron	0.038	0.075	0.094	0.071	0.052	-0.981**	-0.974**
Copper	0.016	0.041	0.056	0.049	0.037	-0.846	-0.941*
Zinc	0.058	0.089	0.124	0.088	0.074	-0.951*	-0.924*
Lead	0.006	0.009	0.014	0.012	0.008	-0.885*	-0.949*
Manganese	0.124	0.164	0.197	0.144	0.135	-0.949*	-0.857
EISQ	0.325	0.212	0.148	0.232	0.317	-	+0.958*
BISQ	5.058	4.258	3.850	4.102	4.796	+0.958*	-

* - $p > 0,01$

** - $p > 0,001$

On the other hand, a strong negative correlation has been established between the two indicators and the content of sediments in chlorides, sulphates (in both the cases, $r = 0.990$ with EISQ, the highest value, and $r = 0.982$ with BISQ, respectively). A high statistical significance has also the negative correlation between the BISQ, on the one hand, and the content of sediment in magnesium, natrium and iron, on the other hand, as well as between the EISQ and the content of sediments in magnesium and iron, respectively. In all these cases, $p < 0.01$. A negative and also statistical significant ($p < 0.05$) correlation has been established between the two indicators

All the results presented show the strong inhibitory effect of the high concentrations of the chemical parameters analyzed (chlorides, sulphates, metals) on the microbial and enzymatic activities in the sediments affected by pollution. The high natural autoregenerative capacity of these sediments is also underlined. These capacity is illustrated by the high values of the enzymatic and bacterial indicators at 1 km downstream from the mouths of the sewers where they empty into the river, values very close to those registered upstream from the polluting site.

Conclusions

The chemical analyses showed a high concentration of the sediments in chlorides, sulphates, magnesium, sodium, copper, zinc, lead and manganese, at the mouths of the sewers where they spill into the river the sewage originated in S.C. UPSOM S.A. Ocna Mureş.

The presence of all the five bacterial ecophysiological groups was detected in all the five sediments analysed. The number of the bacteria differed, the descending ranking of their abundance being: aerobic mesophilic heterotrophs > ammonifiers > denitrifiers > desulfifiers \equiv iron-reducers.

The values of the bacterial indicators of sediment quality indicate a strong decrease of the microbial potential of the sediments at the sites where the sewage from the S.C. UPSOM S.A. Ocna Mureș spills into the river. The lowest level of the microbial potential is registered in the site C, where the sewer SO₂ spills into the Mureș river: BISQ = 3.850, as compared to the site A situated upstream (BISQ = 5.058), and to the site E situated 1 km downstream (BISQ = 4.796). This last value, close to that registered upstream, exhibits a good autoregenerative capacity of the sedimentary microflora.

A positive statistically significant correlation has been established between the bacterial and enzymatic indicators of sediment quality, as well as between the two indicators and the organic matter content in sediments. A strong negative correlation with a high statistical significance was established between the values of the two indicators (BISQ and EISQ), on the one hand, and the content of the sediment in chlorides, sulphates, magnesium, sodium and iron, on the other hand.

The results obtained exhibited the strong inhibitory effect of the high concentrations of the chemical parameters analysed on the microbial activities in sediments. The observations certified the validity of the enzymatic and bacterial indicators as efficient tools for estimating the intensity of the microbial activities in sediments, for monitoring the effect of the polluting factors on these natural habitats.

REFERENCES

1. Alexander, M., 1965, Most probable-number method for microbial populations, in Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E., Clark, F.E. (eds.), "Methods of Soil Analysis", Ed. Am. Soc. Agron., Madison: 1467-1472.
2. Allen, O.N., 1957, *Experiments in Soil Bacteriology*, Third Ed., Ed. Burgess, Minneapolis: 31.
3. Crișan, R., Muntean, V., Pașca, D., Moldovan, O., Rajka, G., 2001, Studiul microflorei bacteriene de pe paramentul barajului Drăgan (jud. Cluj), *An. Univ. Oradea, Biol.*, **8**: 307-314.
4. Kulikov, A.S., Sadchikov, A.P., Maksimov, V.N., 1989, Obshchciaya aktivnost' bakterii sedimentatsionnogo detrita, izmerennaya s pomoshchiu fluorestseindiatsetata, *Mikrobiol. J.*, **5**: 7-11.
5. Malcolm, S.J., Stanley, S.D., 1982, The sediment environment, in Nedwell, D.B., Brown, C.M. (eds.), *Sediment Microbiology*, Ed. Acad. Press, London: 1-14.
6. Meyer-Reil, L.-A., 1983, Benthic response to sedimentation events during autumn to spring at a shallow water station in the western Kiel Bight. 2. Analysis of benthic bacterial populations, *Mar. Biol.*, **77**: 247-256.
7. Meyer-Reil, L.-A., 1987, Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments, *Appl. Environ. Microbiol.*, **53**: 1748-1755.
8. Millea, C.L., 2002, Cercetări enzimologice asupra unor sedimente din râul Mureș afectate de poluare, *Stud. Cercet., Biol.*, **7**: 7-12.
9. Muntean, V., 1995-1996, Bacterial indicator of mud quality, *Contrib. Bot.*: 73-76.
10. Muntean, V., Crișan, R., Pașca, D., Kiss, S., Drăgan-Bularda, M., 1996, Enzymological classification of salt lakes in Romania, *Int. J. Salt Lake Res.*, **5**, (1): 35-44.
11. Ottow, J.C.G., 1968, Evolution of iron-reducing bacteria in soil and the physiological mechanism of iron reduction in *Aerobacter aerogenes*, *Z. Allg. Mikrobiol.*, **8**: 441-443.
12. Pârvu, R., Stanciu, E., Lorinczi, F., Kiss, S., Drăgan-Bularda, M., Rădulescu, D., 1977, Iron-reducing capacity of soil micromycetes, in *Fourth Symp. Soil Biol.* (Cluj-Napoca, 1977), Ed. Ceres, București: 149-154.
13. Pochon, J., 1954, *Manuel technique d'analyse microbiologique du sol*, Ed. Masson, Paris: 59-60.
14. Poremba, K., Lochte, K., Rheinheimer, G., 1993, Mikrobiologische Untersuchungen im norwegischen Sognefjord, *Ber. Inst. Meereskd. Univ. Kiel*, **242**: 90-99.
15. *** STAS 4706/1988, Ape de suprafață - Categori și condiții tehnice de calitate.

CERCETĂRI MICROBIOLOGICE ASUPRA UNOR SEDIMENTE DIN RÂUL MUREȘ (ZONA OCNA MUREȘ), AFECTATE DE POLUARE**(Rezumat)**

Au fost efectuate analize microbiologice și fizico-chimice asupra a 5 eșantioane de sedimente prelevate din râul Mureș în zona de deversare a apelor reziduale depozitate în batalul S.C. UPSOM S.A. din Ocna Mureș. S-au determinat următoarele grupe fiziologice de bacterii: heterotrofe aerobe, amonificatoare, denitrificatoare, desulfocatoare și fier-reducătoare. Pe baza numărului de bacterii care aparțin celor 5 grupe fiziologice determinate în sedimente s-a calculat indicatorul bacterian al calității sedimentelor (BISQ). Potențialul microbial al sedimentelor analizate, așa cum este el definit de valorile BISQ este sensibil mai scăzut în punctele în care se deversează sewagele cu ape reziduale. Cercetările microbiologice confirmă rezultatele analizelor enzimologice asupra aceluiași sedimente, date publicate. Între indicatorii enzimatici și cei bacterieni ai calității sedimentelor s-a stabilit existența unei corelații pozitive statistic semnificative. O corelație negativă puternică, statistic foarte semnificativă s-a înregistrat între valorile celor doi indicatori, pe de o parte și conținutul sedimentelor în cloruri, sulfați, magneziu, sodiu și fier, pe de altă parte. Se atestă astfel puternicul efect inhibitor pe care concentrațiile crescute ale parametrilor chimici analizați le au asupra activității microbiene în sedimente. Valorile relativ ridicate ale indicatorilor bacterieni și enzimatici în sedimentul prelevat la 1 km aval de zona de deversare, apropiate de cele înregistrate în sedimentul prelevat în amonte de această zonă, reflectă o bună capacitatea autoregenerativă a sedimentelor râului.