ENZYMOLOGICAL STUDIES REGARDING THE PRESENT OF THE SEDIMENTS IN BEGA CHANNEL

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Summary: The sediment tests from the Bega canal were quantitatively and enzymologicaly studied. The following enzymological activities were determined: actual and potential dehydrogenase, catalase, urease and the reductive capacity of the FeIII. The sediment test was made in every season, during the year of 2006 in 4 drawing point levels Ghiroda, Uzina de apă downstream, Iosefin and Timişoara downstream. The studied enzymatic activities were taken under the form of complete values. Based on this the enzymatic indicator of the sediments` quality was determined (EISQ).

This indicator has seasonal variations and it depends on the drawing points of the tests. The recorded values for the studied enzymatic activities are very different, because of the existence in the drawing point of a certain stimulative or inhibitory element of the enzymatic activity. Waters containing a high level of urea, nitrate and nitrite have a stimulative effect on the urease activity.

The present and possible dehydrogenase activity has an important decrease in certain zones. This happens because the water from the Bega Canal has been cleaned from a toxic pollution found in the domestic and especially industrial waste waters (some heavy metals from specialized factories). Based on the EISQ values certain pollution sources were identified which have a destructive effect on the communities of the microorganism and organism from the water and the sediment in the Bega Canal.

Keywords: enzymatic activity, EISQ, sediments, Bega Canal

INTRODUCTION

The aquatic microorganism has an essential role in the conversion of vegetal and animal substances from the inorganic materia. The mineralization process returns the main nutrients to the photosynthesizing organism for the continuity of their activity. Overall, these products are part of the biochemical cycles, assuring the transformation and the recycling of the biogene elements in nature. Apart from this role, in the material tide and energy from the ecosystem, the role of the microorganism is also important regarding the transformation of the xenobiotic chemical substances, determined by their metabolic versatility (Colwell, 1980).

The sediments from the rivers always contain a larger number of microorganism than the water mass. The shallow layer of the silt is the most important. Huge quantities of organic substances are accumulated here through the sedimentation of the water mass. The sediment is a heterogenic system, in which different physical phases (solid, liquid, gaseous) and several biotic components (microorganism, small organism and enzymes) together with abiotic components (minerals, humic materials, organic and mineral units) are implied in the physical, chemical and biological process.

The enzymatic activity from the soil, respectively from the sediments, is controlled by four classes of enzymes: hidrolase, oxidoreductase, transferase and liase. Some of them (ureate) catalyse the reactions inside and outside the organism which synthesizes them. Others (proteinase) function only outside the cells, because of their big substrate. Enzymes like the dehydrogenasic ones are only functioning in the cells and are implied in important aspects of the metabolism (Gianfreda & Bollag, 1996).

The present rate, increased by industrialization, urbanization, modernization of the agriculture, the rapid growth of vehicles gives birth, in every country, to the intensification of the pollution process from the elements of the ecologic system. All this leads to physical, biological, chemical changes of the environment and of the water sources. It can damage the normal development of life on earth, especially the ability to work and health.

The aim of the present study is to emphasize, through enzymological studies, different characteristics of the sediments from the Bega Channel, during the year of 2006. Tests of the sediment from the Bega Channel have been made during the four seasons of 2006 (winter, spring, summer and autumn), at the level of the four drawing points, namely the Ghiroda Bridge, the water works, Iosefin Bridge, Timişoara downstream.

MATERIALS AND METHODS

The determination of the catalase activity from the sediments has been made by the following experiment: to the tests of active and inactive thermic sediments a buffer and watery solution of the substrate is added. After the incubation, the quantitative oxygenated water is determined the oxygenated water was not decomposed (Drăgan-Bularda, 2000). Catalase and non-enzymatic catalytic H_2O_2, which are thermostable (iron and manganese oxide, humical substances, clayey minerals) act according to the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2.$$

The determination of the dehydrogenase activity from the sediments is based on the following principle: $CaCO_3$ is added to the sediment (for the neutralization of the acid, formed during the incubation). TTC solution is also added (chloride of 2,3,5-trifeniltertrasol), serving as an acceptor of the hydrogene, transferred by dehydrogenase.

The H donors serve the preexisting organic substances in the soil or/and the added glucose. Toluene or other antiseptic is not added, because the dehydrogenase activity is due to the living microflora, capable of dissemination. The antiseptic substances inhibit the dehydrogenase activity; during the incubation, the TTC – which is a colourless compound – is reduced to a red formasan compound under the action of the transferred H by the dehydrogenase. The formasan can be extracted with the help of organic solvents (ethanol, methanol, acetone) and it can be photocolorimetrically determined (Casida et al., 1964). As long as the concentration of formasan is big, the dehydrogenase activity is high.

The determination of the urease activity from the sediments consists of the following: toluene is added to the soil (in order to prevent the proliferation of the microorganism) and the watery substance is added to the enzymatic substrate (urea). During the incubation, the urea hydrolytically divides the urease action. The formed ammonium is extracted with a KCI solution and it quantitatively determines itself through colorimetry and nesslerization (Drăgan-Bularda, 2000).

As long as the concentration of starch and mercury iodide is big, the urease activity is high. The microbial reduction of the trivalent iron can be used as an ecotoxicological test. This is how the effects of polluted substances (organic solvents, hydrocarbons, pesticides, heavy metals) can be evidenced on microbial population from the soil or from the aquatic sediments. The iron reductive microorganism is heterotrophic and anaerobic or optional anaerobic. Like this the Fe III can be reduced to Fe II. This process can be quantitatively estimated by the determination of the photocolorimetric or spectrophotometric intensity (Drăgan-Bularda, 2000).

Based on the complete value of the enzymatic activity, from every studied sample, the enzymatic indicator of the sediments` quality, according to the reckonings of Muntean (1995 - 1996) can be made as follows:

$$EISQ = 1/n \sum V_r (i) / V_{max} (i)$$

where: EISQ = the enzymatic indicator of the sediments` quality,
n = the number of activities,
V_r (i) = the real individual value,
V_{max} (i) = the theoretical maximum individual value.

RESULTS AND DISCUSSIONS

The bacteriological and enzymological research made on the sediment tests, taken from the Bega Channel, on the section of Ghiroda Bridge – Timişoara downstream 2 km, had in view the establishment of possible pollution source points, which can appear and also the influences on the vegetal and animal organism from this aquatic ecosystem.

The enzymological studies gathered the determination of the following enzymatic activities : the present and possible dehydrogenase, urease activity and the reductive capacity of the Fe III. The decomposed organic materia, at the level of the sediments, is mostly made by microorganism, namely the bacteria. These are the last links from the trophic chains at the level of the aquatic ecosystem. Without this, the level of the aquatic ecosystem would remain organic and inorganic materia, not decomposed and impossible to use by other partners of the trophical chain (vegetal and animal organism).

The dehydrogenase activity of the sediment is considered as a global indicator of the microorganisms` biological activity. It has been already used as an ecotoxicologic test for the evaluation of the pollution effects on the microbiote, but not from the soil (Popa, 1999).

The present and possible dehydrogenase activity as well as the reductive capacity of Fe III, can be used at the level of the aquatic sediments as ecotoxicologic tests (organic and hydrocarbon pollution, detergents, mineral pollution – metals Cu, Pb, Zn, Mo, Cr).

These activities are determined by proliferate living microorganism, capable of growth and multiplication. Therefore as long as the two enzymatic activities are reduced to the level of the aquatic sediment, the number of the living microorganism is reduced. A disturbing factor got into the ecosystem: an organic or inorganic pollution source, which led to the reduction of the number of microorganism and finally at the reduction of the enzymatic activity.

The catalase is an enzyme gathered in the sediment. It keeps its activity for a long time, especially in the form of different mineral and organic substrates. Its activity depends on the quantity of the humus, the environments` pH and on the number of microorganism. The capacity to participate at the decomposer of certain xenobiotic compounds from the industry and agriculture is remarkable. This is the reason why in the case of pollution with several substances, the immediate effects on the living microorganism are mitigated in a way, and late in the case of activities with gathered enzymes.

The phosphotase, just like the catalase, is gathered at the level of the sediments and keeps its activity a long time. These activities are less influenced by the immediate variation of external factors (temperature, pollution with several substances), which can be immediately seen on the living microorganism and is successfully reflected on the dehydrogenase activity.

The determination of the urease activity has been effected in order to form an image about the quantity of urea from the studied ecosystem. The decomposal of the urea by the urease is more intense, because the quantity from the environment is bigger. The urease activity can also give information about possible faecal gene pollution. This enzymatic activity together with hygienic and sanitary indicators allow us an appreciation of the faecal gene pollution in the studied aquatic ecosystem.

The pollution of the aquatic environment is a topical interest. Among the other classic methods of determining the pollution, there are enzymological methods like: the present and possible dehydrogenase activity and the reductive capacity of Fe III, which can be successfully used. Enzymatic activities offer us information about the present microbial activity from the sediments. The dehydrogenase activity is the result of the action from the living microorganism and the capacity of proliferation. There is a strong relationship between the number of microorganism from the sediments and the dehydrogenase activity.

Therefore the reductive capacity of Fe III and the dehydrogenase activity are considered possible parameters of showing the level of the pollution. In the tables from below the values of the enzymatic activities are restored from the level of the sediment tests taken in winter, spring, summer, autumn in 2006.

The present dehydrogenase activity has little variations from one test to the other, from upstream to downstream. The report between the high and low value is of 4, 69. Therefore in this season there were no factors with a huge impact in a certain drawing point.

The reductive capacity of Fe III has relatively low values at the level of the four drawing points, meaning a greater sensibility of the iron reductive bacteria towards the possible pollution sources present along the river.

The highest urease value for the drawing point Timişoara downstream emphasizes the presence in the area of some important pollution sources with organic origins, containing lots of urea probably from human and animal dejection.

Table 1. The enzymological studies of the sediments from the Bega Channel in the winter season (ADA – the actual dehydrogenase activity (mg formazan/3 gs.u), PDA – the potential dehydrogenase activity (mg formazan/3 gs.u), R Fe III – the reduction of Fe III (mg Fe II/ 3 g.s.u.), CA – the catalase activity (mg H_2O_2/ 3 g.s.u.), UA – the urease activity (mg NH_4/3 g.s.u.)).

Sampling sites	Enzymatic activity				
	ADA	PDA	R Fe III	CA	UA
Ghiroda Pod	1,375	1,512	0,2292	0,425	0,375
Uzina de apă downstream	0,525	0,950	0,1276	0,170	1,125
Iosefin Pod	0,475	0,875	0,1327	0,252	0,987
Timişoara downstream	2,150	2,450	0,1128	0,110	1,467

Table 2. The enzymological studies of the sediments from the Bega Channel in the winter season (ADA – the actual dehydrogenase activity (mg formazan/3 gs.u), PDA – the potential dehydrogenase activity (mg formazan/3 gs.u), R Fe III – the reduction of Fe III (mg Fe II/ 3 g.s.u.), CA – the catalase activity (mg H_2O_2/ 3 g.s.u.), UA – the urease activity (mg NH_4/3 g.s.u.)).

Sampling sites	Enzymatic activity				
	ADA	PDA	R Fe III	CA	UA
Ghiroda Pod	1,275	1,787	0,0408	0,425	1,474
Uzina de apă downstream	1,762	1,775	0,0402	0,467	1,196
Iosefin Pod	0,450	1,062	0,0307	0,510	1,454
Timişoara downstream	0,375	0,825	0,0388	0,340	1,525

Table 3. The enzymological studies of the sediments from the Bega Channel in the winter season (ADA – the actual dehydrogenase activity (mg formazan/3 gs.u), PDA – the potential dehydrogenase activity (mg formazan/3 gs.u), R Fe III – the reduction of Fe III (mg Fe II/ 3 g.s.u.), CA – the catalase activity (mg H_2O_2/ 3 g.s.u.), UA – the urease activity (mg NH_4/3 g.s.u.)).

Sampling sites	Enzymatic activity				
	ADA	PDA	R Fe III	CA	UA
Ghiroda Pod	1,737	1,137	0,0419	0,297	1,082
Uzina de apă downstream	1,700	1,125	0,0448	0,170	1,719
Iosefin Pod	0,150	0,187	0,0301	0,310	2,357
Timişoara downstream	0,125	0,485	0,0509	0,935	1,936

Table 4. The enzymological studies of the sediments from the Bega Channel in the winter season (ADA – the actual dehydrogenase activity (mg formazan/3 gs.u), PDA – the potential dehydrogenase activity (mg formazan/3 gs.u), R Fe III – the reduction of Fe III (mg Fe II/ 3 g.s.u.), CA – the catalase activity (mg H_2O_2/ 3 g.s.u.), UA – the urease activity (mg NH_4/3 g.s.u.)).

Sampling sites	Enzymatic activity				
	ADA	PDA	R Fe III	CA	UA
Ghiroda Pod	1,900	2,487	0,1384	0,425	3,627
Uzina de apă downstream	2,050	1,787	0,1306	0,212	3,169
Iosefin Pod	0,250	0,975	0,1469	0,340	0,660
Timişoara downstream	2,187	3,575	0,3258	0,350	1,175

The actual and potential dehydrogenase activity has huge variations in summer. For instance the report between the higher and lower value is 13,89 for the present dehydrogenase.

Therefore the minimal value from Timişoara downstream emphasizes the presence of a pollution source, reducing the number of microorganism. The higher value of the urease activity at the level of the Iosefin Bridge drawing point, very close to the last point, confirms the presence in the area of a pollution source with compounds containing lots of nitrogen (urea, nitrate, nitrite).

In autumn, at every drawing point level we have relatively high values for those five enzymatic activities. The causes may be: the water temperature has been growing with a stimulating effect on the growing number of microorganism and their enzymatic activities; the contribution of vegetal and animal organic materia from the end of the vegetation period.

Values are growing from upstream to downstream along the river. In most cases the higher value can be reached at the drawing point level from Timişoara downstream. The higher values are probably determined by the contribution of organic or inorganic materia from the water cleaning station in the country. Based on the complete value of the enzymatic activities from every studied sample, enzymatic indicators are taken into account from the sediments` quality, according to the reckonings of Muntean (1995 - 1996): $EISQ = 1/n \sum V_r(i) / V_{max}(i)$

Where EISQ = enzymatic indicator of the sediments` quality; n = number of the activities; Vr (i) = the real individual value; Vmax (i) = the theoretical maximum individual value.

Based on the EISQ values we can have a complete image concerning the activity of the microorganism at the level of the sediments. The enzymatic indicator of the sediments` quality (EISQ) has variations according to the drawing point of the tests and the season in which the tests have been done. The higher values of the enzymatic activities at the level of the drawing tests in spring, is worth to be noticed.

This can be seen in the limits of the disturbing factors, making the pollution possible at the level of the aquatic ecosystem and their bigger influence in summer. Also, the high values from spring can be the cause of the rain, which determined a higher contribution of organic and inorganic compounds in the water mass, followed by the streaming process.

The drawing tests came from an area where the shores are not dyked. The enzymatic activity from the level of the sediments, evidenced by the EISQ values (the enzymatic indicator of the sediments quality) had values between 0,179 and 0,435. Therefore we can say that the enzymatic activity of the sediments from the Bega Channel is relatively moderate. At the level of almost every drawing point we can say that the low values appear in winter or spring and the high values in summer or autumn.

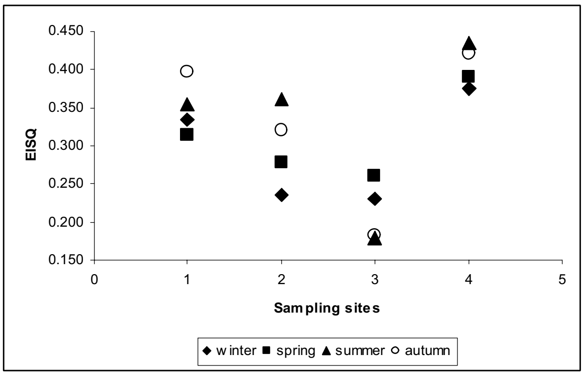

Figure 1. The seasonal variations of the EISQ from the sediment at the drawing tests in the Bega Channel during 2006.

The highest values are recorded at the drawing point level from Timişoara downstream. These high values recorded downstream can be determined by the lower speed of flow, which favors the sediment process, stimulating the growing number of bacteria and the enzymatic activity. The lowest values of the EISQ at the level of the tests from the sediment in the Bega Channel were recorded at the drawing point level Iosefin Bridge.

These lower values are seemingly determined by the existence in the area of a powerful pollution source which stops the growing number of bacteria, the reduction and inhibit of the enzymatic activity. The enzymatic indicator of the sediments quality is growing from upstream to downstream, with the exception of the drawing point Iosefin where the pollution source reduced the enzymatic activity.

A straight reduction in every season from the first drawing point Ghiroda Bridge to Iosefin Bridge is exceptional. At the drawing level Timişoara downstream the highest value is recorded for EISQ in every season. The enzymologic information obtained from the tests in the sediment taken from the Bega Channel is under the form of EISQ. Compared with the imformation from a specific literature, the values are lower than the values obtained at the Crişul Alb River (Filimon & Drăgan-Bularda, 2005)and are similar, as value, with the Mureş River (Muntean et al., 2004) on certain sections.

CONCLUSIONS

The proposed enzymatic activities represented in every studied sediment test, emphasized that there was no point where the pollution might be major and make the microorganism totally disappear.

The reduction of the dehydrogenase activity along the river, from upstream to downstream and the pollution sources are correctly amplified.

The relatively high values at the level of the 2 last drawing points for the urease activity in both seasons, show the presence of a pollution source with organic polluting containing lots of urea.

The enzymatic indicator of the sediments` quality has variations according to the drawing point of the tests. The highest values are recorded at the level of the 2 last drawing points.

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