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Original Research

Preliminary Study on Toxicity of Aquatic Ecosystems in Bug River Basin

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Abstract

Toxicity of water, sediment and sewage in the Bug River Basin was assessed in 2001 (two campaigns) as part of an international monitoring program originating at the ECE/UN (Economic Commission for Europe/United Nations) Convention on the protection and use of trans-boundary waters. Using standardized testing procedures, a battery of tests including plants, bacteria, rotifers and crustaceans were exposed to water, and sediment and sewage samples were collected from monitoring points within the basin. Surface waters within the basin were classified as non-toxic, with the possible exception of samples collected from tributary rivers in the upper Bug River basin and in Terespol. Many of the sewage samples appeared toxic for indicator organisms. The reaction of indicator organisms (toxicity effect) to exposure in a determined sample differed greatly. Comparing the results of the ecotoxicological studies of surface waters and the sewage discharged into these waters, one can observe a distinct influence of the poor ecotoxicological quality of the sewage on the deterioration of the recipient body, i.e. the surface waters. Sediment samples indicated a rather high toxicity for a significant portion of samples, yet these effects were very close in the presence of indicator organisms. Ecotoxicological monitoring perfectly complements chemical monitoring and gives us the possibility to evaluate the toxic impact of pollution on the environment.

Keywords: sediments and river water quality, toxicity, microbiotests, Bug River

Introduction

In the past, water and sediment pollution monitoring in Poland, as well as in developed countries, was mainly based on chemical analysis (chemical monitoring). The chemical diversity of pollutants (organic, organic and organometallic compounds) as well as their quantities (it is estimated that the environment is a sink for hundreds of thousands of substances of anthropological origin) makes such chemical monitoring very expensive [1]. It requires a monitoring network of highly-specialized laboratories endowed with the appropriate equipment operated by highly qualified specialists.

Considering the financial aspects that limit the number of possible designations of identified (known) environmental pollutants (the range of the examined compounds, the frequency of the analyses, and the number of monitoring points) as well as the analytical aspects (impossibility or difficulties of identifying all the pollutants present in the environment, and of the development of appropriate procedures necessary to designate those pollutants) it should be

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stated that complete knowledge about the level of chemical contamination in the environment is difficult to realize. Unfortunately, even a complete monitoring database does not allow one to directly determine all of the biological effects that take place in an examined ecosystem resulting from a particular composition of pollutants.

The traditional chemical approach to the evaluation of environmental pollution has recently been complemented by biological methods (biomonitoring, biological monitoring) for the last couple of years and, quite recently, by ecotoxicological methods as well [2-3].

Biomonitoring (monitoring of biological quality, according to the Water Framework Directive) consists of defining the presence of species, resources and biodiversity, i.e. it is a *de facto* description of the state of biota in the examined ecosystem. Changes within the composition of species present, the resources or the biodiversity caused by environmental pollution may be observed over a prolonged period of time. From the perspective of monitoring, this does not enable early prevention of negative effects of pollution.

In the 1960s, scientists began to use living organisms (fish, crustaceans, algae) to assess pollution levels mainly in water environments. The tests were, however, time-consuming; they required high capacity samples, while their reproducibility was low.

At the end of the 1970s in the United States of America and later in Western Europe, standard rapid ecotoxicological tests underwent significant development [4-8]. Using these methods, pollution levels were assessed based on the specific, exhaustive reaction of standard indicating organisms (plants, bacteria, crustaceans, etc.) to the complex mixture of toxic substances present in the studied environmental compartment [9-12]. Such an approach enables the acquisition of complex information about the potential influence of pollutants (considering their mutual interactions) on the living part of an ecosystem. The answer, depending on the applied indicating organism and the character of the test (acute or chronic toxicity, mutagenicity, etc.) can be obtained in a few minutes (e.g. a test using *Vibrio fischeri* bacteria), or after several days (with other organisms, e.g.: *Daphnia magna Thamnocephalus platyurus, Heterocypris incongruens*). These tests do not require high volume samples and are developed according to ISO, OECD standards or a procedure developed by the producer of tests. Microbiotests are also characterized by high reproducibility and repeatability.

Ecotoxicological monitoring perfectly complements chemical monitoring and gives early warning in cases of danger, which, as a result, limits or eliminates negative influences on the biota [8, 13] and is used in the USA, Canada and a number of European countries. The European Commission – through the Water Framework Directive – demands that EU member states reach satisfactory ecological conditions for surface and ground waters by 2015, but does not directly stress the necessity of biotests application. In subordinate acts, such as Guidelines on the Monitoring and Assessment of Transboundary Rivers [14], the European Union declares that the assessment of water quality in river basins and the ecological functioning of water ecosystems requires integration of the following elements:

- physico-chemical analysis of water, suspension, sediments, and living organisms,
- ecotoxicological assessment,
- biological review.

In 2001, within the framework of the project "Pilot project for the application of monitoring directions and assessing the quality of transboundary watercourses in the Bug River basin," as part of an international monitoring program originating at the ECE/UN (Economic Commission for Europe/United Nations) Convention on the protection and use of trans-boundary waters, ecotoxicity of the water, sediment and sewage in the Bug River Basin were assessed.

This paper presents the results of the ecotoxicity study in the Bug River basin and gives some remarks about the possible benefits of applying microbiotests in management systems of river basins.

Materials and Methods

Bug River Basin Characterization

The Bug River basin is situated on the territory of northwestern Ukraine, southwestern Belarus and middleeastern Poland. In its course, the Bug collects waters from a few physiographic regions (Fig. 1). Presently, it flows into the Narew, a tributary of the Vistula, in the area of Zegrzyńskie Lake. The total length of the Bug (from its sources in Ukraine up to the inflow to Zegrzyńskie Lake) is 772 km. The Bug river basin is a region poorly urbanized and industrialized with a small anthropogenic transformation. The Bug basin rivers have a draining character.

The main problems identified in the Bug River basin were:

- high concentration of biogenic pollution and eutrophication processes;
- high concentrations of organic pollution;
- bad sanitary state of the waters;
- high variability of flow, at low levels causing deterioration of water quality and at high levels increasing erosive processes and presenting a flood hazard;
- lack of identification of the presence of toxic pollution, especially pesticides.

Sample Collection

Sixteen surface water samples and 11 sediment samples from the Bug River basin were collected on 17-20 April, 2001, and 22 surface water samples, 6 sediment samples and 11 samples of wastewater on 2-22 October, 2001. Samples were stored in glass containers and transported to the laboratory in portable coolers, at a low temperature. Toxicity tests were conducted no later than within 24 hours from the time of sample collection.

Sampling sites during the ecotoxicological survey are given in Fig. 1.

Ecotoxicological Studies

Toxicity of the surface water and sewage were analyzed using the following tests:

- Rotoxkit F – (*Brachionus calyciflorus*) end point – the percentage of mortality; implementation was in accordance with recommendations from the producer of the Standard Operational Procedure (MicroBioTests Inc., Nazareth, Belgium).
- Thamnotoxkit F (*Thamnocephalus platyurus*) end point – the percentage of mortality; implementation was in accordance with recommendations from the producer (MicroBioTests Inc., Nazareth, Belgium).
- Daphtoxkit F magna™ (*Daphnia magna*) end point – the percentage of mortality; implementation was in accordance with recommendations from the producer (MicroBioTests Inc., Nazareth, Belgium).
- Test on duckweed (*Lemna minor*) end point: the percentage of mortality of the plants (ISO/CD 20079 : 2001), for the analysis *Lemna minor* was collected from a collector characterized by clean water and exemplary health [15].

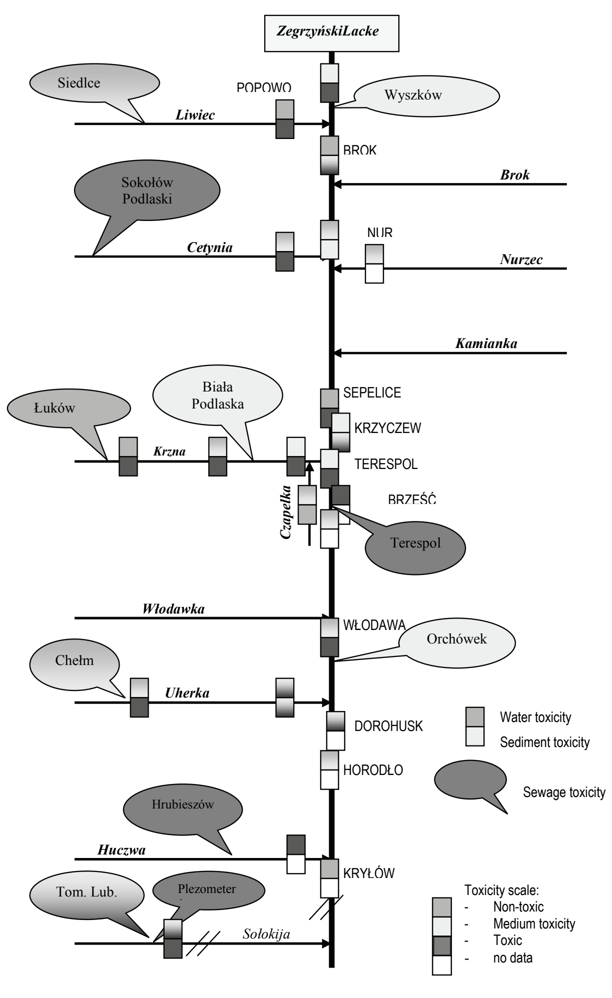

Fig. 1. Comparison of the results of ecotoxicicological studies of surface waters, sewage and sediment.

- ToxAlert 100® (*Vibrio fischeri*) end point: the decline of luminescence after 30 minutes of the bacteria's incubation in an examined sample; implementation was in accordance with the standard procedure PN-EN ISO 11348-2:1998 (Merck, Germany).

The toxicity of sediments with the application of the following tests:

- Ostracodtoxkit F *Heterocypris incongruens*, end point – the percentage of mortality, implementation was in accordance with recommendations from the producer (MicroBioTests Inc., Nazareth, Belgium).
- Test on white mustard (*Sinapis alba*), end point: the percentage of growth inhibition (ISO 11269-1:1993) [16], the white mustard originated from a collection of the Herbapol company; the quality if supervised and controlled by their laboratories.
- ToxAlert 100®, (*Vibrio fischeri*), end point: the decline of luminescence after 30 minutes of the bacteria's incubation in an examined sample; implementation was in accordance with PN-EN ISO 11348-2:1998 standards (Merck, Germany).

Chemical Studies

The sediment samples were studied with relation to the presence of heavy metals (Pb, Zn, Cr, Cu, Cg, Hg, Ni, Co), macroelements (Fe, Mg, Al, Mn) and ions (NH_4^+, Cl^-, SO_4^{2-}, NO_3^-, NO_2^-). Determination was conducted within the regular framework of regular monitoring by laboratories from the Voivodeship Inspectorates of Environmental Protection with the application of validation methods and quality control systems, which were required by these laboratories.

Results and Discussion

Ecotoxicity of Surface Waters

Ecotoxicological studies of surface waters in the Bug River basin were performed using the battery of tests with five organisms (rotifers *Brachionus calyciflorus,* crustaceans *Thamnocephalus platyurus* and *Daphnia magna,* pop. common duckweed *Lemna minor,* bacteria *Vibrio fischeri*).

In the spring of 2001, Terespol and Krzyczew were the sampling points in the Bug River with a toxicity effect towards *Vibrio fischerii, Thamnocephalus platyurus* and *Brachionus calyciflorus* higher than 20% (see location on the Fig. 1).

For all of the water samples, collected from the tributaries of the Bug River, toxicity effects below 20% were observed toward the examined organisms (except Krzna river).

In the autumn of 2001, nearly all of the samples from the Bug River show toxicity effects between 20-50% for bacteria *Vibrio fischerii*. The highest toxicity effects were observed in two points (Dorohusk and Włodawa) for *Brachionus calyciflorus* and for all indicator organisms in Terespol (below the sewage discharge point from the treatment plant in Terespol).

Table 1. Ecotoxicological classification system used to evaluate the quality of water, sediment and sewage in the Bug River basin.

Toxicity class	Numerical Value of the PE Indicator			Colour code
	water	sediment	sewage	
I	PE < 20% for all organisms	PE ≤ 40% for all analyzed organisms	PE ≤ 30% for all analyzed organisms	green
II	PE < 30% for every fourth organism	40% <PE < 50% for one organizm	PE ≤ 50% for all analyzed organisms	green-yellow
III	PE > 30% for at least two organisms	40% <PE < 50% for two organisms	50% < PE < 100% for one of the analyzed organisms	yellow
IV	PE > 40% for at least two organisms	PE > 50% for one organism	PE > 100% for one of the analyzed samples and PE > 50% for at least one of the analyzed organisms	yellow-red
V	PE > 50% for at least one of the analysed organisms and PE close to 50% for at least one of the remaining organisms	PE > 50% for two organisms	PE > 100% for two organisms	red

PE – the analyzed effect for a determined organizm [%].

During this time period (autumn), more than a 20% effect (mainly for *Vibrio fischerii* and *Brachionus calyciflorus*) was observed with relation to two of the five examined organisms in the upper tributary rivers (Sołokija, Huczwa and Uherka).

The sensitivity of indicator organisms according to the composition of pollutants contained in the analyzed surface water samples is different. However, these organisms can be organized in the following manner (beginning with high sensitivity organisms): rotifers *Brachionus calyciflorus* = bacteria *Vibrio Fischer* > crustacean *Thamnocephalus platyurus* = crustacean *Daphnia magna* > duckweed *Lemna minor*.

In this study, it was observed that Lemna minor had low sensitivity to the composition of the chemical compounds in the determined water samples.

Ecotoxicity of the Sediments

Ecotoxicological studies of sediments in the Bug River basin were performed using a battery of tests with the three organisms in question (crustacean *Heterocypris incongruens*, pop. white mustard *Sinapis alba*, bacteria *Vibrio fischeri*). Sediment samples examined in the spring of 2001 caused toxicity in the examined indicating organisms within a range of 50-100%, whereas in the autumn of 2001 these values were slightly lower (30-90 %).

It is interesting that in almost all of the samples, the observed effect for organisms used to determine sample toxicity were similar. This indicates that the application of bioindicators designates a similar sensitivity towards the specific composition of pollutants appearing in sediments within the Bug River basin.

The sediments are built from materials with a differing structure and nature (mineral and organic matter). As such, they pose as a good sorption material for contaminants introduced to ecosystems from anthropogenic origin. Bounded pollutants, cumulated in sediments are a source of information regarding events which occurred in the past in the basin.

Assessment of Sewage Toxicity

Ecotoxicological studies of sewage dumped into the surface waters of the Bug River basin from treatment plants have been performed using a battery of tests with five organisms in question (rotifers *Brachionus calyciflorus*, crustaceans *Thamnocephalus platyurus* and *Daphnia magna, Lemna minor* pop. common duckweed, bacteria *Vibrio fischeri*).

Different from the sediments, it can be observed that for nearly all of the sewage samples, the toxicity effect, observed towards the tested organisms, differed greatly. For example, the toxicity effect for sewage from the treatment plant in Biała Podlaska achieved 100%, 50%, 40%, 0% and 21% of effects determined towards *Brachionus calyciflorus, Thamnocephalus platyurus* and *Daphnia magna, Lemna minor* and *Vibrio fischeri* appropriately.

The least sensitive test found in the sewage samples originating from the treatment plants located in the Bug River basin was the *Lemna minor* test.

The sensitivity of indicator organisms in the case of pollutant composition fund in sewage samples changes in the following manner: *Brachionus calyciflorus* rotifers= *Thamnocephalus platyurus* crustacean> *Vibrio fischeri* bacteria = *Daphnia magna* crustacean > *Lemna minor* duckweed and this alignment is similar to the order presented for surface waters. In terms of the sewage samples, *Thamnocephalus platyurus* indicates a high sensitivity. Similar conclusions regarding the sensitivity of indicator organisms can be found in works from other authors [17].

Using a very simplified classification system, similar to the system used in the ARGE–Elbe project (Table 1), the quality of water, sediment and sewage was evaluated in the Bug River basin [18], collected in autumn.

Results of the conducted evaluation of water, sediment and sewage samples is compared and presented in Fig. 1.

Comparing the results of ecotoxicity studies on surface waters and sewage spilled into these waters, one can discern a distinct influence of sewage quality on the state of surface waters. The obtained results indicate that the application of microbiotests in monitoring most of all sewage but also surface waters, can allow us to better evaluate the threat to aquatic ecosystems coming from the quality of sewage directed into it, and to better manage waters in the basin by the responsible institutions (administrations).

The sediment samples were also analyzed with relation to the presence of heavy metals (Pb, Zn, Cr, Cu, Cg, Hg, Ni, Co), macroelemnts (Fe, Mg, Al, Mn) and ions (NH_4^+, Cl^-, SO_4^{2-}, NO_3^-, NO_2^-). An illustration of the chemical tests shows that the most polluted sediments are those from the Cetynia River in Białobrzegi and from the Liwiec River in Gwizdały. Sediments from the Krzna River in Strzyżew and Porosiuki and those from the Bug River in Nur, Brok and Popowo are slightly less polluted. Sediments from the Czapelka River in Starzynka seem to be chemically clean. Unfortunately, the analysis of the sediments did not include indication of organic compounds. Sediments collected in the Bug River basin indicated, for a significant portion of the samples, a rather high toxicity. In between toxicity (an average value from determined toxicity parameters for analyzed organisms) and reflective parameters of the chemical load of the sediment samples (the sum of the content of relative concentration level of an indicated parameter in relation to the average value of this parameter indicated in all of the analyzed samples) there should appear a strict relationship. A condition for the appearance of such a relationship is that the spectrum for the indicated physiochemical parameters should reflect elements which in reality pollute the analyzed element of the environment and indicates toxicity for the test organisms.

Analyzing the relationship (Fig. 2) between toxicity parameters and the chemical load of the analyzed sediment samples can be differentiated using three groups:

- three points (Bug-Włodawa, Bug-Terespol, Krzna-Neple) indicating a higher ecotoxicity from the expected pollutant level in the compound resulting from chemical parameters;

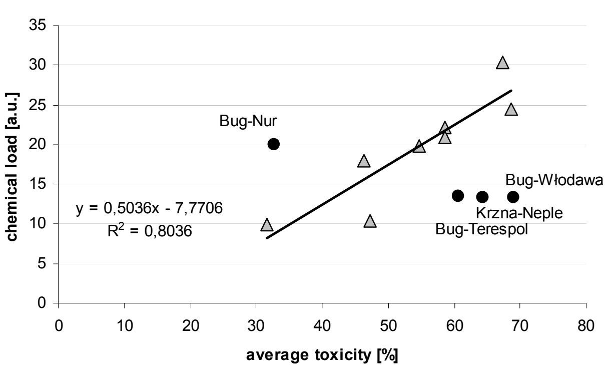

Fig. 2. The relationship between sediment toxicity from the Bug River basin and their level of chemical pollution.

- one point (Bug-Nur) indicating a high chemical load with a simultaneous low toxicity;
- a collection of points (grey triangles), which indicate a relationship between analyzed chemical parameters and sample toxicity (determination coefficient $R^2 = 0.8036$).

The high level of chemical pollution in sediments at the Bug-Nur point is caused by a somewhat higher (two-fold) content of Mg and Zn from the average in the analyzed samples. Such a pollution composition should not influence the toxicity of a sample.

A rather high toxicity in samples collected at Bug-Włodawa, Bug-Terespol and Krzna-Neple suggests that substances other than those monitored have an influence on the level of pollution of the analyzed samples. A monitoring parameter verification process should be conducted at these sites, including:

- an inventory of pollutants disposed in the water near these sites;
- an independent attempt at identifying substances responsible for such a high toxicity of the analyzed sediment samples.

The obtained results indicate that the use of biotests for evaluating the quality of sediments allows for the classification of these sediments from the perspective of realistically appearing threats within for the aquatic environment, because the response of the test organism on the folded composition of pollutants is a complex response including the bioavailability of the substance and the interaction between the components of their pollutant composition.

Conclusions

The results of the pilot studies on the ecotoxicity of the Bug River ecosystem show that:

- Generally, the surface water samples did not pose any toxicity threat toward the examined organisms, which means that water in the Bug River basin can be deemed non-threatening.
- The quality of sewage discharged in surface water differed greatly. Comparing the results of the ecotoxicological studies of surface waters and the sewage discharged into these waters, one can observe a distinct influence of the poor ecotoxicological quality of the sewage on the deterioration of the recipient body, i.e. the surface waters.
- The majority of the analyzed sediment samples indicated a toxicity effect towards test organizms. Pollutant deposition appears in sediments introduced over many years into aquatic ecosystems in the Bug River basin. These pollutants can be a potential source of secondary pollution in the aquatic ecosystems.
- Taking into account the sensitivity of biotests, *Brachionus calyciflorus, Thamnocephalus platyurus* and *Vibrio fischerii* would be preferable for monitoring water and sewage in the Bug River basin.

A study of the correlation between toxicity and chemical pollution of sediments allows us to obtain additional information regarding the environment. This information can be important for the process of basin management and show that chemical analysis should be supported by biological methods (ecotoxicological study). The studies performed confirm the usefulness of microbiotests with regard to ecotoxicological control and the monitoring of sewage disposition into surface waters and can be an appropriate tool for evaluating their potential impact on surface waters.

Acknowledgements

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