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The Microtox® biological test: Application in toxicity evaluation of surface waters and sediments in Poland

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Abstract

Research into the suitability of Microtox® for the evaluation of toxicity of surface waters and bottom sediments in Poland was conducted. Water bodies of various pollution levels were tested, including the Odra River and its tributaries, the Lower Vistula River, the Kashubian Lake District and the Gulf of Gdańsk, using a Microtox® Model 500 analyser (Microbics Corporation, USA). The majority of tested surface water samples were found to be apparently non-toxic. However, 75% of the bottom sediment samples were found to be highly toxic (EC50<2%). These results indicate that the Microtox® test is suitable for evaluating the toxicity of bottom sediments, in which pollutants tend to accumulate. It seems, however, that Microtox® lacks the sensitivity to be of use in analyzing water quality. The relationship between the toxicity of analysed sediments and their organic content was examined.

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INTRODUCTION

Investigations of surface water and bottom sediment pollution levels are usually performed with chemical and physical methods, although these alone do not reflect water quality at the environmental level. Biological tests, which use organisms as indicators for the qualitative evaluation of the environment, are a valuable supplement to chemical and physical methods. In biological tests the organism acts as a ”reagent” where, as a result of their contact with the pollutant, various biochemical processes take place. In indicator organisms these processes result in noticeable symptoms such as morphologic body changes, illness or even death. Biological impacts can act as an initial screen of pollution indicating, whether more detailed chemical analyses are required (Cairns and Pratt 1991). Biological toxicity assessments of waterbodies have been performed for many years with such test organisms as algae (e.g. *Chlorella* sp**.**), daphnia (e.g. *Daphnia magna*), and fish (e.g. *Oncorhynchus mykiss*), as well as plants, earthworms, mussels and oysters. However these tests are both time and money consuming, and require ongoing culturing of test organisms (Nałęcz-Jawecki and Sawicki 1996).

One biological toxicity test is that of the EC50 value (Effective Concentration), which expresses the toxin concentration responsible for a 50% biological effect of a specific test, for example:, decrease in reproduction, various body deformations death.

Many modern biotests use selected bacterial strains in order to assess the toxicity of a sample rapidly, simply and reproducibly (Fernández et al. 1995). The biological toxicity test Microtox®, which uses luminescent bacteria, was applied for the first time in the USA in 1979. The luminescent bacteria *Vibrio fischeri* NRRL-B-11177 is used as the test organism because it is sensitive to a wide range of toxic organic and inorganic compounds. Distortion of bacterial cellular metabolism due to toxins causes a reduction in the intensity of emitted light, which can be measured using spectrophotometry (Płaza 2000). The toxicity is expressed as an EC50 value, which shows the toxin concentration that reduces the light intensity by 50% compared to the control sample.

The Microtox® method has been modified and is being distributed in many countries. This method is recommended in American, German and Polish Standards (Standard Methods (1995), ISO/DIS 11348 – Luminescent Bacteria Test, DIN 38412 – L 34 oraz PN-EN ISO 11348).

Environmental studies using the Microtox® system have been conducted in many scientific centres (Blaise and Ferard 2005, Krebs 2005). Fernández et al. (1995) evaluated surface water toxicity levels in the Tormes river in Spain, obtaining EC50 values of 4% to 81% (see toxicity criteria p. 8). Tung et al. (1990) and Greene et al. (1992) were the first to use the Microtox® test to assess sediments toxicity *in toto*. Tay et al. (1992) used Microtox® to study water and organic extracts as well as the solid phase (the whole sediment) to evaluate sediment toxicity. Their studies showed that the organic extracts and solid phase were much more toxic than water extracts of the tested sediments. Kwan and Dutka (1992) evaluated the toxicity of sediments from Lake Ontario with the Microtox® Solid-Phase Test (SPT), obtaining EC50 values in the range 0.03% to 7.56% (see toxicity criteria p. 8). The observed EC50 values for bottom sediments from the Po River in Italy were in the range 0.19% to 1.47%, and for organic extracts from those sediments 0.047% to 3.68% (Vigano et al. 2003).

Michniewicz and co-workers found luminescent bacteria to be sensitive in evaluation of the toxicity of pulp and paper effluents (Michniewicz et al. 2000). Gutierrez et al. (2002) applied the Microtox® system for screening wastewater discharges into wastewater treatment plants. The US Environmental Protection Agency (US-EPA) recommended *Vibrio fischeri* to study waters contaminated with toxic industrial chemicals (TICs) (Rogers et al. 2005). Boluda et al. (2002) tested the toxic effects of waters collected from irrigation channels in a Mediterranean wetland in Spain and found that Microtox® EC20 results were comparable with pollution indices gauged from analytical parameters.

The Microtox® SPT is much more sensitive than the basic test when analysing sediment extracts. In Waukegan Harbor (Illinois, USA) the Microtox® aquatic test was sensitive to only one sample, whereas the Microtox® SPT identified 50% of samples as toxic (Kemble et al. 2000).

Microtox® is the only bacterial test system that can be used for marine environment. Grant and Briggs (2002) used Microtox® to study sediments from around a North Sea oil platform, finding the bacteria to be very sensitive to samples polluted by hydrocarbons.

In Poland the Microtox® technique has not been commonly used. The first studies in this area were carried out by the Department of Environmental Studies of the Warsaw Medical Academy, in which well waters and wastewaters were examined (Nałęcz-Jawecki and Sawicki 1996). The Institute of Meteorology and Water Management analysed the adequacy of the Microtox® test for toxicity studies of wastewaters and surface waters from Warsaw and Gdańsk areas (Dmitruk and Wojciechowska 1996, Żelechowska et al. 1996). These studies showed that Microtox® can be used to assess the toxicity of wastewaters, particularly raw ones, and very polluted surface waters. To date there is no available literature on toxicity studies on bottom sediments in Poland conducted using the Microtox® test.

This paper reports on the adequacy of the Microtox® test in determining toxicity of waters and bottom sediments from rivers, lakes and marine sediments in Poland. Relationships between the toxicity levels of tested sediments and their organic content, expressed as loss on ignition, were investigated.

MATERIALS AND METHODS

These studies, testing toxicity of surface waters and bottom sediments using Microtox®, were initiated in the framework of a Polish-German project: International Odra Project (IOP) (Niemirycz et al. 1998, Niemirycz et al. 1999, Meyer 2002), and subsequently continued within grant KBN 6 P04G 027 19 (Niemirycz et al. 2002).

The study area covers the Odra River and its tributaries, the Lower Vistula, the Kashubian Lake District (Karlikowskie Lake, Karczemne Lake, Klasztorne Małe Lake, Goszyńskie Lake and Tuchomskie Lake) and the Gulf of Gdańsk (the geographical location of the control point being 19°06.8'E 54°30.3'N). Figure 1 shows the sample collection sites. Water and sediment samples were collected in 1998-2002, excluding winter months. Samples from the Odra River and its tributaries were collected in May and November 1998, June 1999, May and June 2000 and June 2002. Samples from the Vistula River were collected in May, August and September 2001 and March, May and July 2002. Samples from the Kashubian Lake District were collected in May and September 2001, and March, May and July 2002. Samples from The Gulf of Gdańsk were collected in June 2002. The total number of samples assessed for toxicity using the Microtox® system was 136 surface water samples and 98 sediment samples.

Water samples were collected from the subsurface, with no air bubbles, directly into dark glass vessels, which were stored in the dark at + 4°C. Tests on the water samples were conducted directly after they reached the laboratory.

Bottom sediment samples from the Odra River and its tributaries were collected with a steel scoop from the sediment surface. From the Vistula River, the Gulf of Gdańsk and lakes core sediments were collected with a Nurek Nemistö sampler (core diameter 5 cm and length up to 50 cm). Cores were divided into 5 cm layers (from 2 to 4 layers depending on the core length), with each layer tested individually. The samples were stored in tightly closed jars at –20°C Directly before testing them, the sediment samples were thawed (lyophilized) and homogenised.

The content of total organic substances in the sediments was determined in accordance with Polish Standard (PN-75 C-04616/01). The sediments were dried at 105°C and then ignited at about 600°C, and the organic content taken as the loss on ignition.

Toxicity assessment of surface waters was conducted with the Microtox® test, to the recommended ISO Standard 11348:1998. This method utilizes the bioluminescent properties of *Vibrio fischeri*, in which a decrease in the

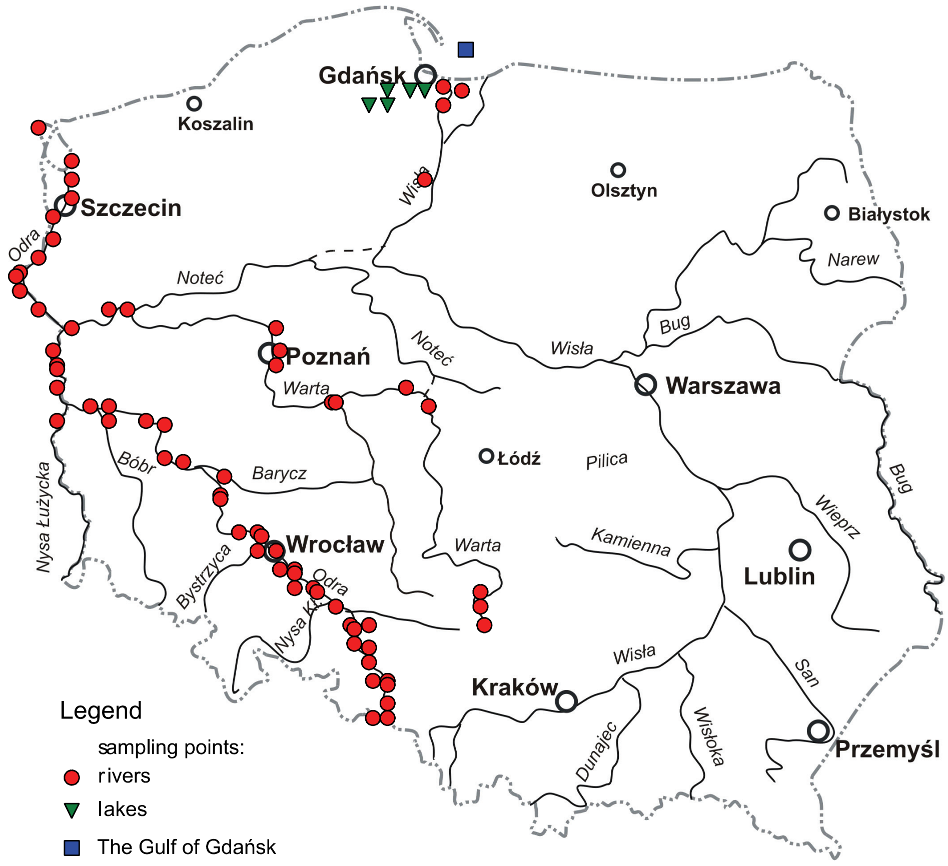

Fig. 1. Locations of surface water and bottom sediment sample collection points in Poland in 1998-2002 [research within IOP project and the individual grant KBN 6 P04G 027 19].

luminescence of the bacterium in an experimental sample (*Vibrio fischeri* in solution with the sample river water) is compared to that of a control (*Vibrio fischeri* in a 2% solution of NaCl). Toxic samples were defined as those in which the luminescence of the *Vibrio fischeri* decreased by 20% or more. If initial screening showed that samples were toxic, then a further test was performed to determine the EC50 value. The EC50 was defined as the sample concentration producing a 50% decrease in luminescence compared to the control sample. In some instances the initial screening result was not confirmed by the subsequent test; water samples in which a 20% decrease in luminescence was initially observed were classified as non toxic in the second test.

Evaluating the test results the following toxicity criteria, based on the EC50 value (15 minutes), were accepted (Vasseur et al. 1986):

- non-toxic samples EC50>100%,
- low-toxic samples 10%<EC50≤100%,
- toxic samples 1%≤EC50≤10%,
- highly-toxic samples EC50<1%.

EC50>100% is calculated by Microtox® software from the extrapolation of the test results.

The toxicity of the bottom sediments was evaluated with the Microtox® Solid Phase Test (SPT) according to the Microbics Corporation protocol (1995). The EC50 value, as a proportion of the sediment dry mass, was determined for each sample. The criteria of toxicity of the sediments were constructed based on the author's own data and published EC50 values (Tung et al. 1990, Kwan and Dutka 1992):

- non-toxic samples EC50≥2%,
- toxic samples 1%≤EC50<2%,
- highly-toxic samples EC50<1%.

RESULTS AND DISCUSSION

Toxicity assessment of the water environment

136 surface water samples were examined for total toxicity with the Microtox® test. 16 of them, originating from the Odra Basin, had toxic properties that were observed in an initial screening test (decrease in the luminescence of *Vibrio fischeri* >20%). Other tested river and lake water samples were deemed non toxic (the decrease in the luminescence of the *Vibrio fischeri* <20%). In cases where the screening test showed the water samples to be toxic, a further 'basic' test was performed to determine the EC50 values. The obtained EC50 values confirmed toxicity in only 4 samples, while the rest were non-toxic to the testing organism (EC50>100%). The toxic samples (1%≤EC50≤10%) were collected from surface waters of the Odra River middle course, close to the mouths of tributaries from the Bóbr and Nysa Łużycka (at 516.2 km and 542.4 km along the Odra River course respectively), and at a section of the Odra River in Krosno Odrzańskie (516 km) (where two pulp and paper mills are located on the left bank of the river). The observed EC50 values for these samples were in the range of 3.63% to 8.77%. Water samples close to the mouth of the Obrzyca River (at 469.4 along the Odra River course) were of low toxicity.

98 sediment samples from the bottoms of lakes, rivers and the sea were examined using the Microtox® SPT. These were observed to be much more

toxic than the overlying water samples (Table 1), with about 75% of the samples having toxic properties (EC50<2%).

Sediments from the Odra Basin had EC50 values ranging from 0.023% (close to the mouth section at Podjuchy, 730 km) to 30.37% (Kietz/Kostrzyn section, 617.6 km). Of the 55 sediment samples examined from this area, 73%

Table 1

The range of EC50 values for the tested sediments in the Microtox® SPT system

No	Research area	Number of study samples	Number of toxic samples (EC50<2%)	Range of EC50 values (%)
1	**Odra and its tributaries**	55	40	0.02-30.37
2	**Vistula River**	21	14	0.71-19.99
3	**Kashubian District Lakes**	18	15	0.11-16.25
4	**the Gulf of Gdańsk**	4	4	0.04-0.72

of them exhibited toxic features (EC50<2%) to *Vibrio fischeri*. Most of these were highly toxic, with EC50<1% (Fig. 2). Considering the mean EC50 values of sediments from the studied sections of the Odra Basin from 1998-2002, the most toxic sediments were found to originate from the upper Odra River tributaries at Kłodnica (94 km) and Kopanie (184 km) (Kłodnica and Kopanie both belong to the Upper Silesia industrial and urban area), from the central Odra River section at Frankfurt/Słubice (584 km) (where there are two pulp and

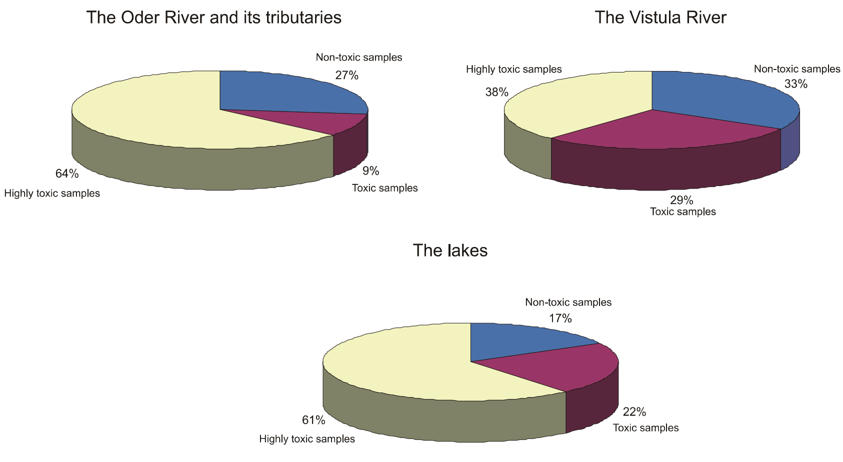

Fig. 2. Toxicity of river and lake sediments in Poland on the basis of Microtox® test results from 1998 to 2002.

paper mills, Eistenhűtten-Stad and Bad Muskau) and from regions close to mouth of the Odra River at Krajnik Dolny (690 km) and Szczecin (740 km) (as a result of high levels of pesticides in the river sediments (Niemirycz 2006)). High toxicity was also observed in sediment samples from the area around where the Warta River outlet empties into the Odra River and from the industrial area of Poznań (around 243.6 km of the Warta River course) (Fig. 3).

Sediment samples that were apparently non-toxic (EC50≥2%) were collected from the upper Odra River, close to the mouths of tributaries at Nysa Kłodzka, Oława and Olza, the central part of the Odra River in Krosno Odrzańskie (516 km) and Bytom Odrzański (416 km), from tributary mouths in Kaczawa, and from the lower Odra River at Kostrzyń (617.6 km). Non-toxic samples were also observed from the upper part of the Warta River at Zawiercie (801 km) (Fig. 3).

Toxicity values of bottom sediments collected from the main course of the Odra River between 1998 and 2000, as determined by the Microtox® SPT, do not demonstrate any clear trends (Fig. 4). Lowest sample toxicities were observed in samples collected in November 1998, and highest toxicity was recorded from sediments collected in May/June 2000, probably due to secondary pollution from the mud after the flood of 1997.

The EC50 values for sediment samples from the section of the Lower Vistula River close to its mouth ranged from 0.082% (from the left bank at Kiezmark) to 22.761% (from the right bank at Kiezmark). 21 sediment samples were examined, and 67% of them had toxic properties (EC50<2%), of which 38% were highly toxic (EC50<1%) (Fig. 2). The mean toxic values for the sediment samples from the Lower Vistula River (Kiezmark, 926 km and Kwidzyn, 867.6 km) collected in 2001 and 2002 indicate that these sediments were non-toxic (EC50≥2%) (Fig. 3).

The EC50 values reported here for bottom sediments in Poland indicate much higher toxicity than in those of the Po River in Italy (Virgano et al. 2003).

18 sediment samples from lakes were tested, with EC50 values ranging from 0.025% (Karczemne Lake) to 16.25% (Klasztorne Małe Lake). These values are comparable to those observed for Lake Ontario sediments tested by Kwan and Dutka, 1992. In comparison to the river sediments, the majority of lake samples were seen to be toxic (83%), with EC50 values<2%. Among the toxic samples more than 50% were very toxic, with EC50 values <1% (Fig. 2).

The mean EC50 values for lake sediments collected in 2001 and 2002 showed the most toxic sediments to *Vibrio fischeri* to be those collected from Tuchomskie Lake, having water quality outside any defined class (WIOŚ 2000). Non-toxic sediments (EC50≥2%) were observed in Klasztorne Małe Lake (Fig. 3).

4 sediment samples from the Gulf of Gdańsk were also analysed, with EC50

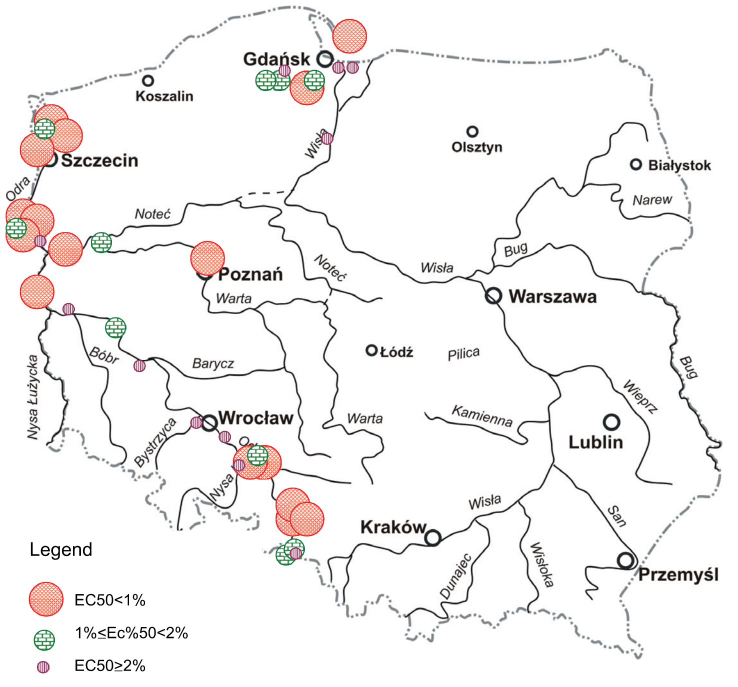

Fig. 3. Mean toxicity values for Polish bottom sediments tested using the Microtox® system from 1998 to 2002.

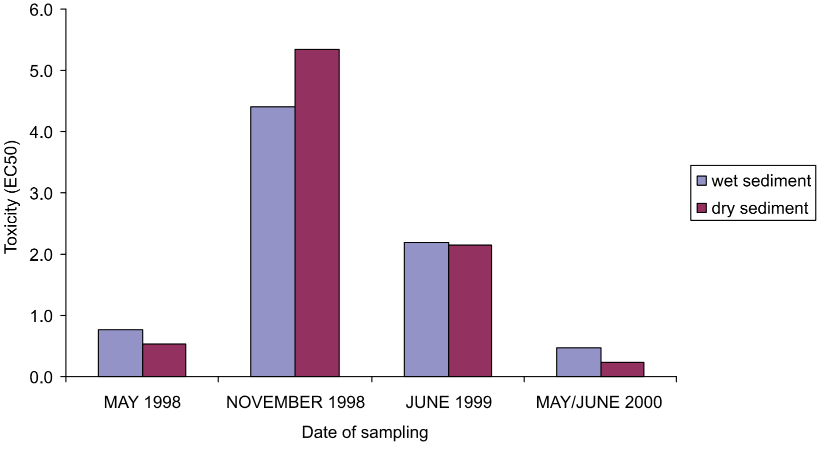

Fig. 4. Toxicity values for bottom sediments (wet and dry) from the main course of the Odra River evaluated using the Microtox® system from 1998 to 2002.

values ranging from 0.041% (at a depth of 0-10 cm) to 0.725% (at a depth of 30-40 cm) (Fig. 3). As a result of these high toxicity levels it would seem appropriate to conduct further sediment studies in the Gulf of Gdańsk.

Influence of organic content on bottom sediment toxicity

The relationship between the toxicity (1/EC50) of river and lake sediments and their organic content were analysed. An increase in sediment toxicity with increasing organic content was observed for sediments collected in May/June 2002 from Odra River tributaries, sections close to the mouth of the Vistula River in Kiezmark (its right bank), and from Karczemne Lake (R=0.55-0.93) (Fig. 5). No other samples showed a significant correlation between the two.

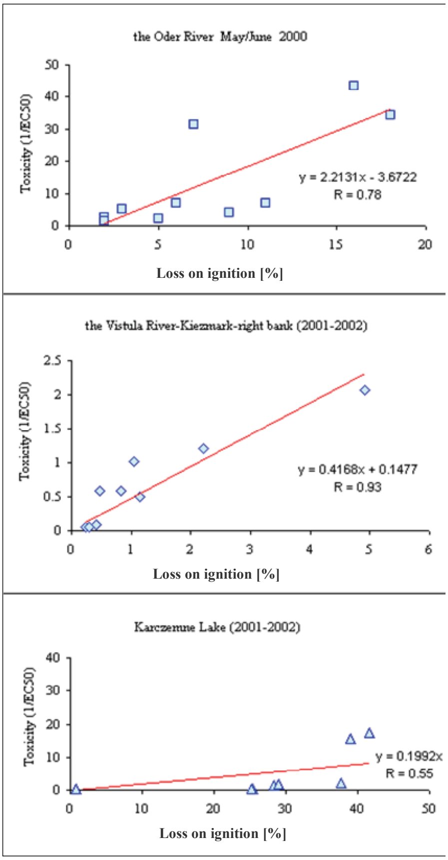

Fig. 5. Toxicity levels of river and lake sediments in relation to their organic content.

CONCLUSION

Toxicity tests conducted on surface fresh and marine waters in Poland between 1998 and 2002 using the Microtox® system resulted in no evidence of toxicity to the test organism, *Vibrio fischeri*. However, the majority of sediment samples from the same locations were observed to be toxic (EC50<2%), and even highly toxic (EC50<1%), using the Microtox® Solid Phase Test. In some cases the toxicity of the sediment was significantly correlated to the organic content.

The results illuminated the presence of sediments toxic to the bacterium *Vibrio fischeri* in a number of regions in Poland. These areas are:

- close to the mouth of the Odra River at Krajnik Dolny and Szczecin,

- the central part of the Odra River, in the Frankfurt/Słubice region, and the upper part of the Odra River, at Kłodnica and Kopanie,
- the Warta River, in the region of Poznań, and at its outlet to the Odra River,
- Tuchomskie Lake,
- the control point in the Gulf of Gdańsk.

Further tests on sediments are recommended at the above study areas, in order to consider their toxicity towards organisms higher up the trophic chain.

The Microtox® test is recognized as a cheap and fast bioindicator method for evaluating toxicity in the natural environment. The results presented here indicate that this test is insufficient for evaluating surface water quality, whereas it can be used for bottom sediment tests.

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