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Bacterial Luminescence Test Screening of Highly Polluted Areas in the Odra River

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Water samples collected from the Odra River in May 1998 were tested for toxicity using ToxAlert 10 system based on luminescence inhibition of freeze-dried bacteria (*Vibrio fischeri*). Base on this toxicity assessment, the three highly polluted area in the Odra River were identified (Brzeg Dolny, Nysa Kłodzka, Mała Panew). Toxicity at Brzeg Dolny was linked to volatile chloroorganic compounds, at Nysa Kłodzka to nutrients and at Mała Panew to metals most probably zinc and lead.

Surface water contains thousands of substances of natural and anthropogenic origin. Many of them are toxic, and their presence may have a negative effect on aquatic ecosystems. In the traditional approach parameters such as chemical and biochemical oxygen demand (COD and BOD), total organic carbons (TOC), or absorbable organohalogens (AOX), are used to assess the potential impact of contaminated water on the aquatic environment. The ecological risk of polluted waters is not described well by these parameters. While chemical analysis enables identification and quantitation of organic pollutants, it does not provide sufficient information to assess the ecological hazard, since it is not possible to investigate all the possible substances and their interactions in bodies of water. Consequently, chemical analysis results should be supported by toxicity determination.

Most of the aquatic assays like *Chlorella, Daphnia and Fish* are time consuming, expensive and require large sample volumes. During the last decade, rapid microassay methods to quantify contaminants in ecosystems has been developed.

Microassays using luminescent bacteria as a biosensor has been shown to be effective and of practical use (Qureshi et al. 1998). This

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test is based on inhibition of natural luminescence of bacteria *Vibrio fischeri* (Bulich 1986). Sample toxicants (organic and/or inorganic) interfering with the organisms' metabolism can reduce light output. The results obtained with this method strongly correlated with data of other aquatic test methods (Schultz et al. 1998).

This paper describes use of luminescence freeze-dried bacteria (*Vibrio fischeri*) ToxAlert 10 system for screening of the Odra River to identify highly contaminated regions. Attempts to relate toxic effects to the toxic agents responsible are also presented.

MATERIALS AND METHODS

Water samples were collected from the Odra River in May 1998. Locations of sampling sites are presented in Figure 1. Samples were collected in glass containers, stored in a cool and dark place, and analysed for toxicity immediately after delivery to the laboratory (not longer than fifth days).
Toxicity bioassays were performed with ToxAlert 10 system (Merck), which includes freeze-dried *Vibrio fischeri* bioluminescent bacteria. The osmolality of all samples was adjusted to 2 % NaCl for optimal reagent performance (ToxAlert 10 ...). The change in bioluminescence was measured after 15 minutes and expressed as percentage of inhibition. Sample inhibition over 20% was regarded as a acute toxicity indicator.

Purge-and-trap technique (PT) was used to isolate and preconcentrate volatile organic compounds (organosulphur compounds, hydrocarbons and organohalogen compounds) from water samples (10 mL) (Wardencki et al. 2000). A home-made PT unit was connected to a gas chromatograph (8000 series, Fisons Instruments) and a mass spectrometric detector (MS) working in full scan mode (10-450 amu).

RESULTS AND DISCUSSION

Water samples collected from the Odra River in May 1998 were analysed for toxicity using the ToxAlert 10 system. During the sampling period, water level in the Odra River was high (the level which can cause flooding). It follows from the graph presented in

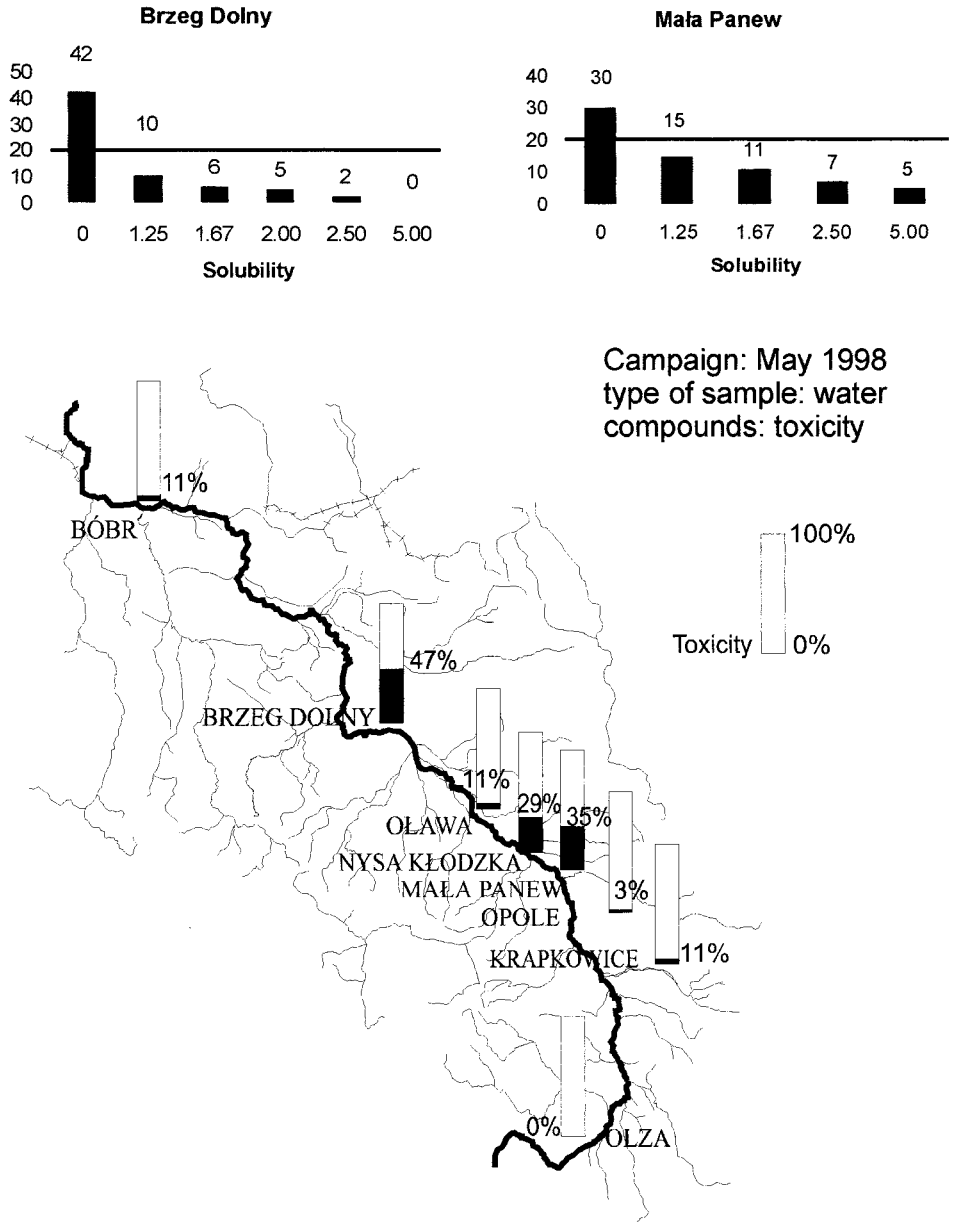

Figure 1. Water sample toxicity in the Odra River in May 1998.

Figure 1 that inhibition of three water samples exceed 20% (Brzeg Dolny, Nysa Kłodzka, Mała Panew).

The most toxic sample was collected near Brzeg Dolny (47% inhibition). The Rokita Chemical Plant, included on the list of 80

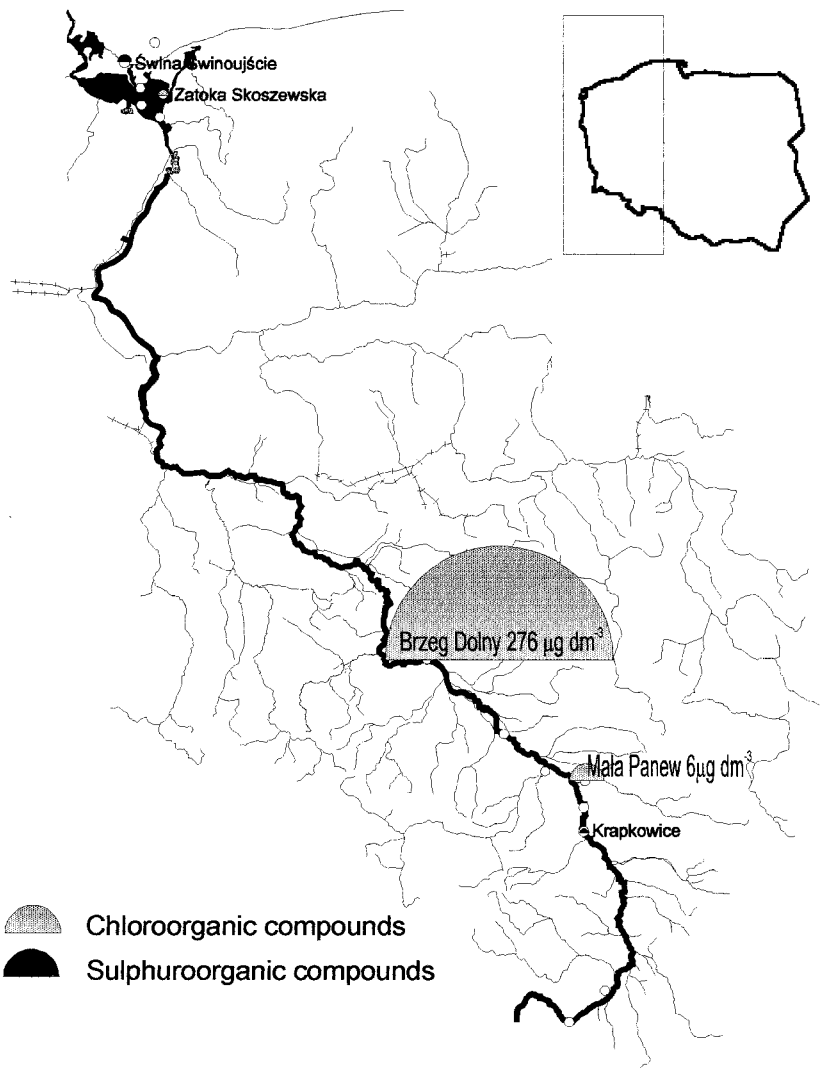

Figure 2. Concentration of volatile organochlorine compounds (VOCl) in the Odra River in May 1998.

plants of the highest environmental concern in Poland, is located in this city. According to the Provincial Inspectorate for Environmental Protection (Wojewódzki Inspektorat Ochrony Środowiska - WIOŚ) in

Wrocław, the "Rokita" plant emits free Cl_2, as well as aliphatic and aromatic hydrocarbons (Reports ..., 1997). Phenol and chlorophenols were found in environmental samples from the vicinity of this plant. Of all the plants situated in the former voivodship of Wrocław, the Rokita chemical plant released the largest amounts of industrial waste (ca. 27% of these wastes belonging to the very harmful class).

The toxicity data were in good agreement with concentrations of volatile organochlorine compounds (VOCl) in water samples from Brzeg Dolny (unpublished data). The VOCl studies were carried out using the same water samples as for the toxicity studies.

Figure 2 shows changes in VOCl concentrations in water samples along the Odra River. VOCl concentration in Brzeg Dolny was very high (total VOCl : 276 μg dm^{-3}). Trichloroethene (274.2 μg dm^{-3}) and tetrachloroethene (1.6 μg dm^{-3}) were identified in this water sample.

Taking into account that EC_{50} for trichloroethene in the Microtox test (comparable to ToxAlert in the USA) (Richardson and Gangolli, 1994) is 176 ppm, it can be assumed that VOCl is primarily responsible for the toxicity of this sample.

The next sample with increased toxicity (35%) was collected from Mała Panew River. In the VOCl group, only low concentrations of chlorobenzene (6 μg dm^{-3}) were found. According to the Provincial Inspectorate for Environmental Protection in Opole (Reports ..., 1997), concentrations of zinc (Zn) and lead (Pb) exceed many times the respective maximum allowable concentrations (MAC) in the Mała Panew River (Reports ..., 1997). Zinc and lead processing plants are located in the area, and most probably these two metals are primarily responsible for the toxic effects observed.

The third sample in which increased toxicity was observed came from the Nysa Kłodzka area (29% inhibition). Nysa Kłodzka River is strongly polluted with nitrites and phosphates (Reports ..., 1997).

The toxicity data were compared with concentration of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and Cl-, N-and P-pesticides determined in the same water sample (unpublished data). No correlation with any of these classes of compounds could be found. It should be emphasized, however, that the PAH, PCB and pesticide levels found in the samples were low.

In summary, ToxAlert 10 is a fast and sufficiently sensitive tool to screen the toxicity of surface waters. Highly polluted areas in the Odra River were found based on the toxicity assessment. Increased toxicity

generally correlated well with data on the Odra River pollution, coming from other sources.

Evaluation of the degree of environmental pollution is typically based on chemical studies. They can be carried out in several different ways, including determination of total parameters, speciation analysis based on instrumental methods, biomonitoring and bioanalytics. None of these individual tools yield complete information, which can only be obtained by using an optimal combination of the methods. Rapid biological tests can effectively reduce the costs of monitoring by limiting the number of samples that require detailed analysis only to those which are shown to be toxic (inhibition over 20%). Preliminary environmental screening studies can also benefit from the use of biological tests.

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