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## INTEGRATED APPROACH - THE EFFECTIVE TOOL FOR POLLUTION LEVEL CONTROL OF SEDIMENTS FROM LAKE TURAWSKIE

### KOMPLEKSOWA OCENA STOPNIA ZANIECZYSZCZENIA OSADÓW DENNYCH JEZIORA TURAWSKIEGO

**Abstract:** Lake Turawskie, an artificial reservoir on the Mała Panew River, was selected for a preliminary project financed by the Province Environment Protection Fund in Opole (Poland). The aim of this project was to assess the ecological state of this lake, and testing aqueous extracts from bottom sediments for toxic effects was one of the approaches. The toxicity of aqueous extracts of sediments was assessed applying the measurements of bioluminescence inhibition of *Vibrio fischeri* bacteria. In addition, analyses of organic compounds in sediment extracts obtained by aqueous and subsequent dichloromethane extraction were performed. The chromatograms from coupled gas chromatography - mass spectrometry (GC/MS) indicated a very complex composition of the examined dichloromethane extracts. The GC/MS non target screening analyses were conducted on a set of selected samples as an attempt to identify chemical substances responsible for the observed toxicity effects. However, the differences in sediment toxicity were not reflected in the results of the GC/MS analyses and it was not possible to correlate sediment toxicity with specific organic compounds.

**Keywords:** gas chromatography-mass spectrometry, organic compounds, lake sediments, Microtox test, *Vibrio fischeri*

Lake sediments contain thousands of substances of natural and anthropogenic origin. Taking into account that an unknown number of them are toxic, the presence of some compounds may have a negative influence on an aquatic ecosystem. Therefore, it is very important to obtain reliable information about the toxicity of the lake's sediments.

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Chemical analysis provides only a part of the knowledge necessary to evaluate and assess the toxic potential of compounds for wildlife and humans. This is due to the different bioavailability of forms in which pollutants exist in the environment and their different biological activities. Furthermore, complex interactions between different environmental chemicals are not completely understood and considered [1]. A complementary approach, taking into account the above-mentioned facts, can be the application of biotests. Bioassays provide data about the effect, without pinpointing the substances and the potential source. Therefore, a procedure is necessary for providing toxicity data, as well as identification of the compounds causing the effects [2]. An integrated approach based on a parallel application of bioassays and chemical analysis is the most promising tool for the assessment of environmental pollution [2-5].

Lake Turawskie is an artificial reservoir on the Mała Panew River. Lake Turawskie was selected for the preliminary project from among 14 objects of the Odra basin in terms of the large-scale program called "The ecological state of barrier lakes in the Odra river basin and works conducted towards its improvement". The aim of the project, financed by the Provincial Environment Protection Fund in Opole, is the evaluation of the ecological state of Lake Turawskie, to obtain valuable information to serve in the planning and selection of a method for its remediation. The necessity of conducting a full inquisitorial campaign of Lake Turawskie results from the lack of authoritative information on the subject of its pollution, participation and influence on specific types of pollutants during the process of eutrophication.

The sources of pollution for Mała Panew River waters are supposed to be mainly agricultural activity, municipal waste waters and industrial wastes. Industry, concentrated in the upper and central part of the Mała Panew River basin, includes mining and metallurgy of silver, zinc and lead, manufacture of cellulose, chemical production (dyestuff for the textile industry, explosives), ferrous metallurgy and glass-works. Due to this fact, the Mała Panew River supplies Lake Turawskie with polluted water and a large quantity of sediments contaminated with heavy metals [6, 7].

In a previous study on 154 Lake Turawskie samples surface water, underground water and bottom sediments were analysed for in total 33 chemical and physicochemical parameters (pH, conductivity, dissolved oxygen, biological oxygen demand (BOD<sub>5</sub>), chemical oxygen demand (COD), chloride, sulfate, dissolved silica, ammonia nitrogen, nitrate(V) nitrogen, nitrite(III) nitrogen, Kjeldahl nitrogen, phenols, anionic detergents, total iron, mercury, lead, copper, nickel, zinc, cadmium, manganese, total chromium, chromium(VI), magnesium, sodium, potassium, calcium, alkalinity, total hardness, turbidity, total content of solutes, and suspended matter). As seen, the chemical analysis includes the standard water quality parameters, heavy metals, and some organic components [8]. In addition, GC/MS target analysis of PCB congeners, organochlorine pesticides, and PAH was performed on this large set of samples [9].

As a supplement to the previously obtained large set of analytical target parameter data, the present study was designed to search by GC/MS non target screening analysis for previously unrecognized toxic organic compounds using a comparatively small number of sediments preselected by toxicological testing.



## Materials and methods

### Lake Turawskie sediment samples

The drilling campaign in the bottom of the Lake Turawskie was carried out in the period between June and September 2004. The sediment samples were collected from 34 sediment cores (from 0.07 to 8.00 m in length). In total 154 samples were tested using: gas chromatography coupled to mass spectrometry (PCB congeners, organochlorine pesticides, PAH), inductively coupled plasma - atomic emission spectrometry (Cr, Zn, Cu, Ni, V, Fe, Mn, Al, Li), electrothermal atomic absorption spectrometry (Cd, Pb), hydride generation atomic absorption spectrometry (As), cold vapour atomic absorption spectrometry (Hg), trueness of which was examined by appropriate reference materials analyses, ie SRM 1941a (Organics in Marine Sediment, NIST) in the case of PCBs and PAHs, and MESS-2 (Marine Sediment, NRCC) in the case of heavy metals [9].

The next step of the sample testing was the performance of a series of measurements allowing for an assessment of acute toxicity of all samples taken [9].

This report presents results of the GC/MS non target screening analysis and toxicological testing of 11 selected sediments sampled at 6 points and 1 to 3 different depths [m]: TZB 6 (0.00÷0.60), TZB 8 (0.00÷0.13), TZB 25 (0.00÷0.35), TZB 25 (0.35÷0.70), TZB 53 (1.00÷2.00), TZB 53 (2.00÷3.00), TZB 76 (0.00÷1.00), TZB 76 (2.00÷3.00), TZB 81 (0.00÷0.35), TZB 81 (0.35÷2.00) and TZB 81 (2.00÷3.00).

### Acute toxicity tests of aqueous extracts with bioluminescent bacteria

Freeze-dried sediments were mixed with a four-times greater volume of water and shaken (24 h). After centrifugation (10 min/3000 rpm) and filtration (0.45 µm pore diameter fiberglass filters, Millipore), pH and specific conductivity were measured using a pH-metric electrode EPP-3 (Elmetron) and a waterproof multipurpose instrument, CX - 401 (Elmetron, and conductometric sensor Type CD-2, No. 1530), respectively. As a result of this process, clear and colourless aqueous extracts of sediments were obtained. The extracts were subjected to toxicological studies against selected indicating organisms.

Acute toxicity was determined using the Microtox® Model 500 (Microtox®, Strategic Diagnostics Inc., USA). As bioindicator organisms, the bioluminescent bacteria from the comma bacillus group (*Vibrio fischeri* class) were applied. Toxicity measurements of aqueous eluates obtained from sediments were conducted in accordance with the requirements of the International Standard Organization (ISO) (PN-EN ISO, 2002). The pH of the samples was measured and, when necessary, adjusted to pH 6.0÷8.0 using NaOH or HCl. Tests were carried out according to the Basic Test Protocol of Microtox with four concentrations and one control in each test and a measurement of the inhibition of bioluminescence of freeze-dried *Vibrio fischeri* bacteria after 30 min. The obtained data was used to calculate the  $EC_{20}$  and  $EC_{50}$ , which are the median sample concentrations that cause, respectively, a 20% and 50% reduction in bacteria bioluminescence. Internal quality control tests using zinc sulphate ( $ZnSO_4 \cdot 7H_2O$ ) were run periodically during the study [10, 11].  $THI_{50}$  values were evaluated on the basis of the formula [12]:  $THI_{50} = 100/EC_{50}$



### Preparation of sediment extracts for organic analysis

Freeze-dried sediment was mixed with a four-times greater volume of water and shaken (24 h). After centrifugation (10 min/3000 rpm) and filtration (0.45  $\mu\text{m}$ , fiberglass) the aqueous sediment-extract was shaken (10 min) with 1  $\text{cm}^3$   $\text{CH}_2\text{Cl}_2$ . The dichloromethane-extract was reduced to 300  $\text{mm}^3$  and used for GC/MS-analysis directly, as well as after separation into fractions of increasing polarity by silica gel chromatography (after [13], modified). Borosilicate glass columns (12 mm i.d., 79 mm height, Baker) were dry packed with 2 g silica gel (Baker, type 70245) held between two PTFE frits. The silica was activated for 15 h at 180°C before use. The sample solutions were adjusted to 600  $\text{mm}^3$  with *n*-pentane, and elemental sulfur was removed by addition of activated copper powder. 500  $\text{mm}^3$  of the samples were taken from the supernatant and separated by liquid-solid chromatography over silica columns into fractions:

1. fraction: 5  $\text{cm}^3$  *n*-pentane;
2. fraction: 8.5  $\text{cm}^3$  *n*-pentane/  $\text{CH}_2\text{Cl}_2$  (95/5 v/v);
3. fraction: 5  $\text{cm}^3$  *n*-pentane/  $\text{CH}_2\text{Cl}_2$  (90/10 v/v), then 5  $\text{cm}^3$  *n*-pentane/ $\text{CH}_2\text{Cl}_2$  (40/60 v/v);
4. fraction: 20  $\text{cm}^3$   $\text{CH}_2\text{Cl}_2$ .

The fractions were concentrated to 50  $\text{mm}^3$ . Final assays were performed by GC/MS-analysis on an HP 5890 gas chromatograph (280°C interface temperature), equipped with on-column injector, retention gap (2.5 m x 0.53 mm), and a BPX-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$  film) coupled to a VG 70SE mass spectrometer (EI<sup>+</sup>, 70 eV, 200°C source temperature), scanning from m/z 500 to m/z 35 at 0.9 s cycle time with 0.2 s interscan delay. Temperature programmed analyses (60°C, 3 min hold, 5°/min heating rate to 280°C, 10 min hold) were run by injection of 1  $\text{mm}^3$  sample with helium as the carrier gas at ~35 cm/s linear velocity.

### Results and discussion

This paper reports on the toxicity assessment and the analysis of organic compounds exemplified on 11 sediment samples collected at different depths from bores made at the bottom of Lake Turawskie. The ecotoxicological data set was compiled using sediment extracts, and GC/MS analysis was performed on dichloromethane extracts prepared from the tested aqueous solutions.

In Table 1 measurement results are presented of acute toxicity (using the *Vibrio fischeri* bacteria) determined for sediment samples collected from analytical bores made in the Turawski basin. In this table there were additionally placed Toxicity Impact Index ( $THI_{50}$ ) values.

An evaluation of the ecotoxicological quality of analysed sediment samples was conducted on the basis of a classification system, developed within the scope of the ARGE-Elbe project [14]. This system classifies sediment samples from a I-V ecotoxicological quality classification on the basis of a percentage value (PE) of the observed toxic effect (Table 2). In this case the percentage effect is luminescence inhibition.

Table 1

Acute toxicity measurement results with *Vibrio fischeri* bacteria conducted for aqueous extracts of sediment samples collected from analytical bores made in the Turawski basin ( $EC_{20(50)}$  - the concentration of a sample that causes 20 or 50% of the maximal inhibition of bioluminescence; low values indicate high toxicity and high values lack of toxicity,  $THI_{50}$  - Toxicity Impact Index)

No. sample	Bore-hole depth [m]	Luminescence inhibition [%]	$EC_{20}$ (0.5 h) [%]	$EC_{50}$ (0.5 h) [%]	Class of toxicity	$THI_{50}$
TZB 6	0.00÷0.60	99	3	5	V	20.00
TZB 8	0.00÷0.13	52	24	76	IV	0.13
TZB 25	0.00÷0.35	59	20	60	IV	1.67
TZB 25	0.35÷0.70	89	5	16	V	6.25
TZB 53	1.00÷2.00	43	15	100	III	1.00
TZB 53	2.00÷3.00	72	23	49	V	2.04
TZB 76	0.00÷1.00	99	3	8	V	12.50
TZB 76	2.00÷3.00	100	0	0	V	-
TZB 81	0.00÷0.35	100	0	0	V	-
TZB 81	0.35÷2.00	100	11	18	V	5.56
TZB 81	2.00÷3.00	46	28	95	III	1.05

Table 2

Ecotoxicity classification (PE - percentage effect, WFD - EU Water Framework Directive) of sediments formulated within the *ARGE-Elbe* project [14]

Class of toxicity	Values of PE	Environmental state (in respect of WFD)
I	≤ 15%	very good
II	> 15% PE ≤ 30%	good
III	> 30% PE ≤ 50%	moderate
IV	> 50% PE ≤ 70%	weak
V	> 70%	bad

Lake sediments contain thousands of substances of natural and anthropogenic origin. A certain number of them are toxic and may constitute a risk for an aquatic ecosystem. Compounds were identified by comparing their mass spectra with those of own, library, and literature data [15], and by taking into account gas chromatographic retention behaviour.

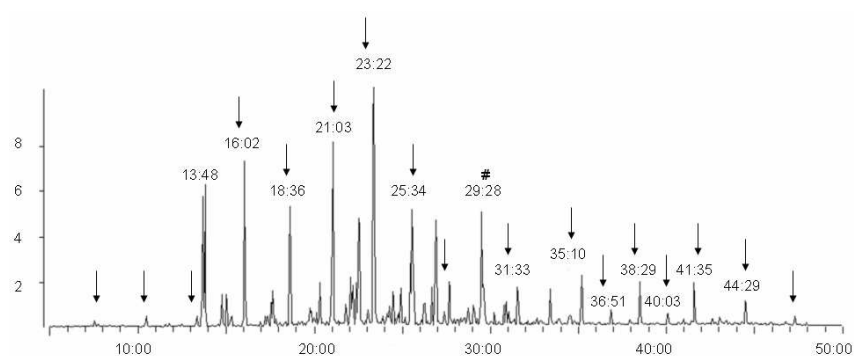


Fig. 1. GC/MS total ion chromatogram (background corrected, retention time - min) of TZB 6, fraction 1,  $n-C_{11}$ -  $n-C_{31}$  (arrows), 18-norabietan (#), other peaks mainly branched alkanes, alkylcyclopentanes, alkylcyclohexanes

Aqueous extraction of the sediment samples was applied to preferentially enrich a polar bioavailable organic fraction, assumed to cause the toxic effects observed. The dichloromethane extracts of these aqueous phases, however, contained a majority of less-polar organic compounds commonly found in moderately polluted limnic sediments. The total ion chromatograms (denoted as RI33 in the corresponding figures) as well as reconstructed traces of characteristic ion mass to charge ratios ( $m/z$ ) of a given sediment extract fraction of different sediments were very similar, so large differences between samples of different toxicity classes were not apparent. An exemplary overview of the organic compounds the fractions of the sediment extracts are typically composed of is given in Figures 1-3a using the arbitrarily chosen sediment TZB 6. In addition to bio- and geochemical markers an anthropogenic contribution was visible in *n*-alkane patterns (TZB 6 fraction 1, Fig. 1), phenylalkanes and di-*iso*-propylnaphthalenes [16] (TZB 6 fraction 2). Pollution with chlorinated pesticide residues and PCB was low, while PAH-contents were high (TZB 6 fraction 3). Diarylhydrocarbons, in part known from styrene/ $\alpha$ -methylstyrene- and xylene-chemistry, were characteristic components of these sediments, and therefore, may form specific anthropogenic marker compounds.

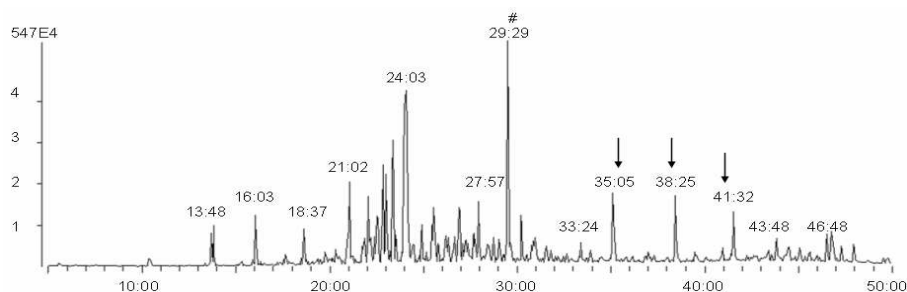


Fig. 2. GC/MS total ion chromatogram (background corrected, retention time - min) of TZB 6, fraction 2, *n*-alkenes  $C_{23}$ ,  $C_{25}$ ,  $C_{27}$  (arrows), 18-norabietan (#) and related diterpenoid geochemical marker compounds, mono- and diaromatic hydrocarbons

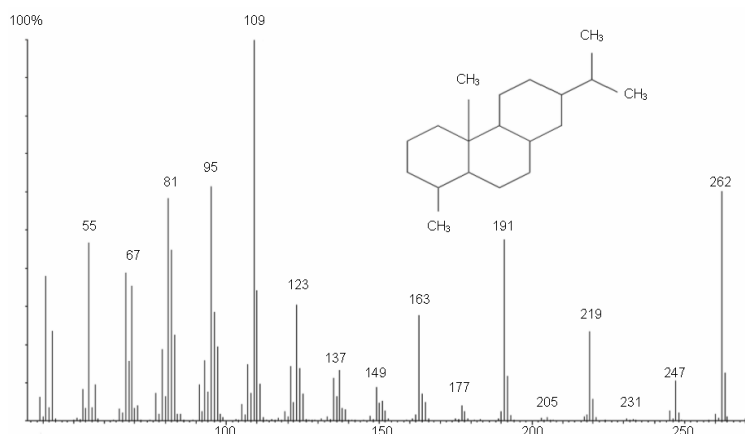


Fig. 2a. Mass spectrum of 18-norabietan (from peak at retention time 29:29 min)

A more polar fraction with sedimentary aldehydes, methylketones, esters, PAH-ketones and quinones was usually contaminated with omnipresent compounds, like phthalates, and with 2-ethylhexyl-3,5,5-trimethylhexanoate of obscure origin.

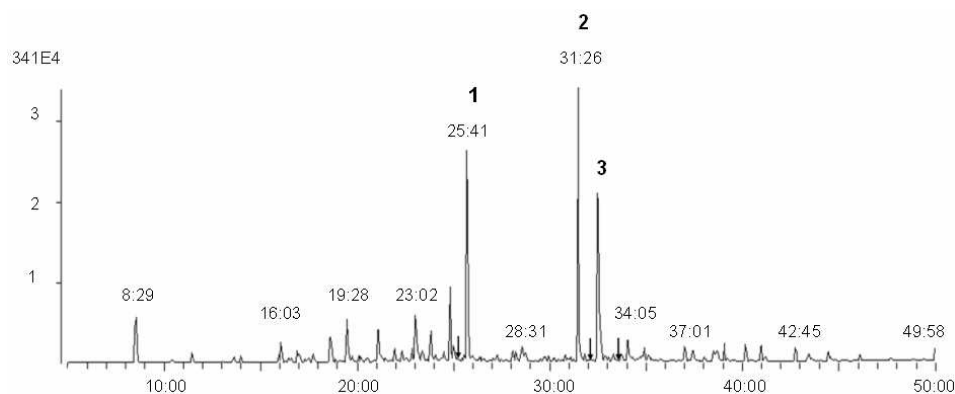


Fig. 3. GC/MS total ion chromatogram (background corrected, retention time - min) of TZB 6, fraction 3, polycyclic aromatic compounds (1 - phenanthrene, 2 - fluoranthene, 3 - pyrene), traces of chlorinated hydrocarbons (HCH and *o,p*- and *p,p*-DDE, arrows), geochemical marker compounds

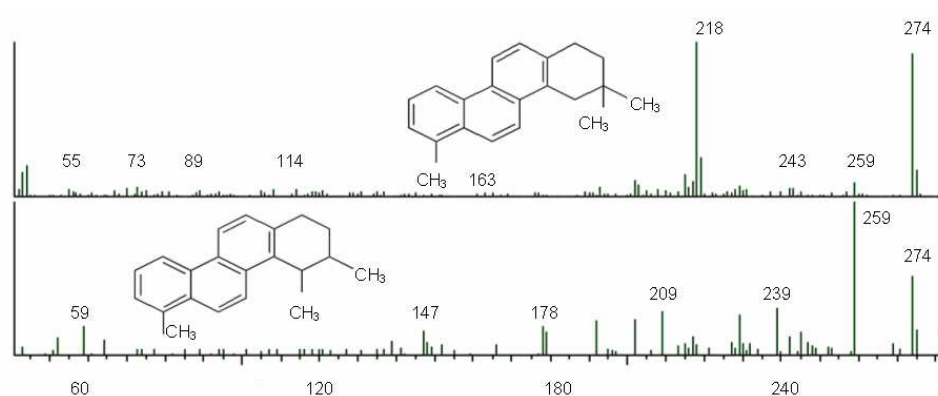


Fig. 3a. Mass spectra of the triterpenoid geochemical marker compounds 3,3,7-trimethyl-tetrahydrochrysene (upper) and 3,4,7-trimethyl-tetrahydrochrysene (lower)

## Conclusions

As a consequence of the very complex composition of the sediment extracts produced by water treatment and subsequent dichloromethane extraction, it was not possible to correlate the observed toxicity effects with specific organic pollutants in the sediments. A more selective sediment work up accompanied by toxicity testing in each step may help to produce more complete information about the chemical causes of toxicological effects. However, non target screening analysis of complex sediment

extracts provides characteristic patterns of marker compounds [17] reflecting a man-made and natural influence.

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## KOMPLEKSOWA OCENA STOPNIA ZANIECZYSZCZENIA OSADÓW DENNYCH JEZIORA TURAWSKIEGO

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**Abstrakt:** Turawski zbiornik retencyjny został wytypowany do projektu pilotażowego spośród 14 obiektów zlewni Odry w ramach programu: „Stan ekologiczny jezior zaporowych w dorzeczu Odry i działania na rzecz jego poprawy”. Celem projektu jest ocena stanu ekologicznego Jeziora Turawskiego dla uzyskania niezbędnych informacji mających służyć do zaprojektowania i wyboru metody jego remediacji. Jezioro Turawskie jest nizinnym zbiornikiem retencyjnym na rzece Mała Panew. Potencjalnymi źródłami zanieczyszczeń wód Małej Panwi jest działalność rolnicza, ścieki komunalne i odpady przemysłowe. Działalność przemysłowa (również w przeszłości) w zlewni rzeki obejmuje między innymi: eksploatację i hutnictwo srebra, cynku i ołowiu, produkcję celulozy, produkcję chemiczną w (tym barwników dla przemysłu włókienniczego i materiałów wybuchowych), hutnictwo żelaza oraz hutnictwo szkła. Celem przedstawionych badań była ocena poziomu zanieczyszczenia osadów jeziora oraz poszukiwanie korelacji pomiędzy oszacowaną toksycznością a wynikami analiz chemicznych (na przykładzie wybranych próbek). W ekstraktach z próbek osadów oznaczano między innymi związki organiczne. Do izolacji związków o charakterze polarnym zastosowano ekstrakcję rozpuszczalnikiem (dichlorometan). Do rozdzielania związków wykorzystano chromatografię gazową, a następnie analizowano je w detektorze spektrometrii mas (GC-MS; tryb pracy: SCAN). Badania ekotoksykologiczne przeprowadzono, korzystając ze standardowego testu bakteryjnego *Microtox*® wykorzystującego zjawisko bioluminescencji bakterii z rodzaju przecinkowców (gatunek *Vibrio fischeri*).

**Słowa kluczowe:** chromatografia gazowa, związki organiczne, osady dennie, *Microtox*®, *Vibrio fischeri*