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Toluene in Benthic Sediments in the Odra River after the 1997 Flood: an Attempt to Identify the Origin of Toluene

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Abstract

Transport and accumulation of large amounts of natural and man-made substances takes place in the Odra River. The river is a significant source of pollution for the Baltic Sea. The presence of toluene in the samples of benthic sediments collected after the flood in 1997 was an unexpected find made within the framework of the International Odra Project (IOP). At that time, the origin of toluene, which is a pollutant characteristic of benthic sediments, was not elucidated. Variation in toluene concentrations along the course of the river suggested that the compound might have been of microbial origin.

The aim of this study was to find the source of toluene identified in samples of benthic sediments in the Odra River basin after the 1997 flood.

Keywords: toluene, determination, benthic sediments, PT-TD-GC-MS, microbiological studies

Introduction

An aquatic environment is a complex ecosystem where many physico-chemical (e.g. mixing, sedimentation of suspended matter, adsorption, decomposition and mineralization of organic compounds) as well as microbial processes take place, particularly in the compartment of benthic sediments. A detailed knowledge of these processes is extremely important in reference to the development of general knowledge as well as environmental sciences.

The Odra River, one of the five longest rivers in Europe (854.3 km), is a transport and accumulation route of significant amounts of natural and man-made substances. The surface area of its river basin is 118.861 km^2, which is 89% in Polish territory, 6% in the Czech Republic and 5% in Germany. The Odra river mouth is a significant pollution source for the Szczecin Lagoon as well as for the Baltic Sea. The protection of both these bodies of water has been declared under the Helsinki Convention, therefore gaining knowledge about pollution levels in the Odra River and about processes undergoing in pollutants present in the riverine ecosystem is an extremely important task.

In the years 1997-2000, a research team from the Department of Analytical Chemistry, Chemical Faculty, Technical University of Gdańsk participated in the International Odra Project (IOP) [1]. A detailed description of the conducted research and the results obtained was presented in a number of publications [2-11].

The finding of toluene in benthic sediment samples collected immediately after the 1997 flood was an unexpected result of a study conducted by a research team from the Department of Analytical Chemistry. It was observed that toluene was present in almost all samples collected along the whole course of the river. Toluene

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concentrations in 69 analyzed sediment samples are shown in Figure 1 as histograms.

The concentrations measured in samples that had been collected during subsequent sample collections were definitely lower, and only samples of benthic sediments from the upper course of the Odra River contained toluene.

Neither a thorough analysis of anthropogenic emission sources nor literature search resulted in finding a plausible explanation for this unexpected phenomenon.

Therefore, it was concluded that the presence of toluene in sediment samples after the flood was connected to microbial activity. Two hypotheses can be considered here:

- hypothesis 1 - there are organisms that produce toluene during metabolic processes,
- hypothesis 2 – microorganisms decompose complex organic matter such as humic acids, which results in toluene release.

Experimental

Samples of Benthic Sediments from the Odra

This study used archival samples of sediments collected immediately after the flood. Benthic sediment samples were lyophilized and stored in glass containers closed with plastic screw caps. Samples had been stored in a refrigerator at 4 °C for five years prior to analysis. Based on previous research results, from among the available samples the ones with the highest toluene content were chosen. In order to reactivate the bacteria, water had been added to the samples that were left at room temperature for a week. Analysis took place afterwards.

A system for purging and concurrent retention of analytes on a solid sorbent (*Purge and Trap, PT*), constructed at the Department of Analytical Chemistry, Chemical Faculty, Technical University of Gdansk, was used [12,13].

The device was connected *on-line* with a gas chromatograph coupled with a mass spectrometer (Figure 2). Details of system's working conditions are specified in Table 1.

An Attempt to Verify the Microbial Origin of Toluene Present in the Investigated Samples of Benthic Sediments

The aim of this study was to investigate whether toluene content in samples increases after conditions stimulating microbial growth were created. In the experiment, seven sediment samples of highest toluene content were used. The samples were pooled and then split into four equal parts, labeled A, B, C and D. The types of sample pre-treatment are described in Table 2.

Next, samples were treated as follows:

- each of four samples (A, B, C and D) was split into12 equal parts, each weighing ca. 1 g, and placed in sterile glass vials,
- water was added to six vials from each 12-vial series A, B, C and D,

Table 1. Working conditions of a PT-TD-GC-MS system.

System elements	Working conditions
Gas chromatograph	TRACE GC, *Thermo Quest.*
Column	RTX – 624 Restek Corporation, 60m x 0,32mm ID fused silica: D_f – 1,8 µm: 6% cyanopropylphenyl, 94% dimethylpolyoxosilane
Detector	Mass spectorometer (SCAN: 10 – 450).
Injector	Purging vessel connected to sorption microtrap; Purging gas: argon at 20 m^3min^{-1}; Purge time: 5 min.
Microtrap	Sorbent: 80 mg Tenax TA/30 mg Carbosieve III; Desorption temperature: 250°C for 60 sec.
Carrier gas	Helium: 100 kPa, ~2 cm^3min^{-1}.
Temperature settings	35°C for 2min, 5°C min^{-1} to 100°C, 10°C min^{-1} to 240°C, 240°C for 10 min.

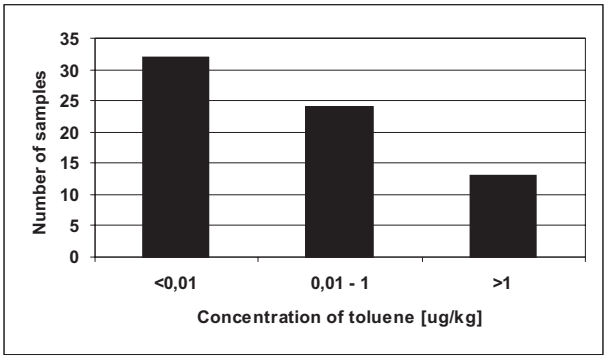

Fig. 1. Toluene concentrations determined in 69 samples of benthic sediments.

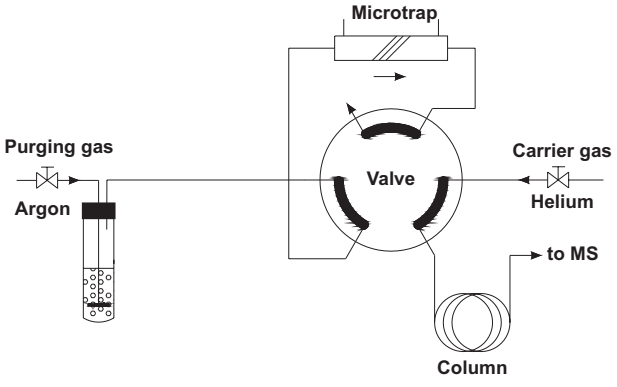

Fig. 2. A schematic diagram of a PT-TD-GC-MS system [12,13].

Table 2. Sample pre-treatments applied to the investigated benthic sediments.

Sample labeling for microbiological analysis	Pre-treatment
A	Sterilization
B	Purging of toluene by extraction (washing out) with a stream of inert gas (48 hrs)
C	Purging of toluene by extraction (washing out) with a stream of inert gas (48 hrs) and a 15-min extraction in a ultrasonic bath
D	Blank (no pre-treatment)

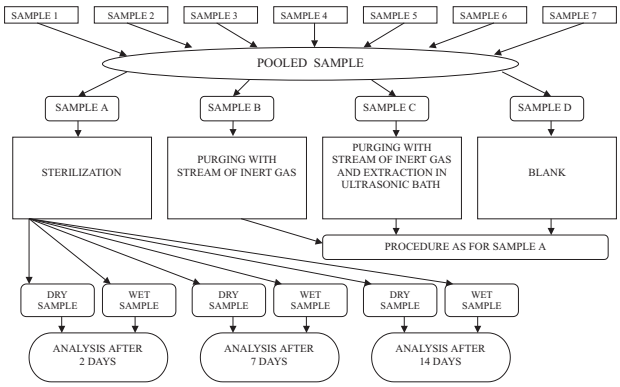

Fig. 3. Procedure flowchart of archival sample handling during toluene content determinations in benthic sediments.

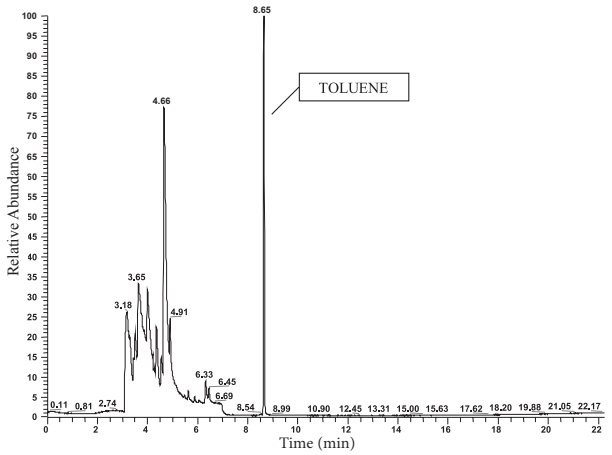

Fig. 4. A chromatogram obtained from the archival sample of benthic sediments using the PT-TD-GC-MS technique.

- vials were tightly closed and left at room temperature, not protected from natural light, until analyzed,
- analyses were conducted sequentially on days 2, 7, and 14; from each series A, B, C and D, 2 dry and 2 wet samples were analyzed by using a PT-TD-GC-MS *(Purge and Trap – Termal Desorption – Gas Chromatography – Mass Spectrometry)* technique.

A detailed flowchart of handling procedure for benthic samples during toluene determinations with the use of a PT-TD-GC-MS system is shown in Figure 3.

Toluene Determination in Biological Samples

Microbiological analysis consisted of culturing sediment samples on different solid media such as agar and *Sabourouda* medium. The aim of these tests was to identify the microorganisms present in benthic sediment samples.

Next, it was tested whether the microorganisms cultured in media are capable of producing toluene. The bacteria and fungi that had grown in media were transferred separately into sterile vials containing appropriate medium, and left undisturbed for 3 days to be later analyzed.

Results

Benthic Sediment Samples from the Odra

The presence of toluene was observed in the archival samples of benthic sediments collected after the 1997 flood. Except for toluene, practically no other substances were identified in these samples. A typical chromatogram is shown in Figure 4.

An Attempt to Confirm the Microbial Origin of Toluene

Toluene content values determined in samples prepared according to the flowchart in Figure 3 are shown in Figure 5. The amount of toluene was estimated from the appropriate peak area on the chromatogram.

The obtained results may give basis to the following conclusions:

- in samples from series B and C, in which toluene was purged with a stream of inert gas prior to incubation, the presence of toluene had been detected again at a level comparable to a blank from series D,
- as incubation time increased, the toluene content in samples decreased down to the level approaching a detection limit of analytical procedure,
- the kinetics of toluene decrease was different for dry and wet samples.

Toluene Determination in Biological Samples

The following microorganisms were cultured and identified in the investigated samples of benthic sediments:

- fungus from genus *Aspergillus*,

- fungus from genus *Microsporum*,
- fungus from genus *Mucor*,
- fungus from genus *Penicillium*,
- fungus from genus *Trichophyton*,
- fecal streptococci (*Enterococci*),
- bacteria from species *Pseudomonas aeruginosa*,
- sulfite-reducing bacteria from genus *Clostridia*.

During the analysis of specific microorganisms a number of volatile organic compounds was detected (Table 3). These compounds were identified by comparing their spectra with those of reference materials contained in the NIST library.

The presence of toluene was observed in only one sample containing a fungus from genus *Trichophyton.* In the same sample, a large amount of different organic compounds such as, aliphatic and aromatic hydrocarbons, ketones, aldehydes and alcohols, was detected. The chromatogram obtained from the sample is presented in Figure 6.

Conclusions

Characterization of Chosen Sediments

- Toluene detection in the archival samples of benthic sediments that had been stored for 5 years at ca. 4°C after lyophilization, may be an indirect proof of its microbial origin.
- The hypothesis of microbial origin may also be supported by the fact that no other compounds were identified in the investigated sediment samples.
- High numbers of both fecal *E. coli* and fecal bacteria from genus *Streptococcus* indicate the high pollution level of sediments with municipal sewage.

An Attempt to Confirm the Microbial Origin of Toluene in the Investigated Samples of Benthic Sediments

- A repeated appearance of toluene in samples from series B and C may prove its microbial origin.
- In the investigated samples a decrease of toluene content was observed in dry and wet sediments. This might have resulted from:

- toluene production only by the microorganisms that were at a specific growth phase,
- the presence of microorganisms decomposing toluene (biodegradation) besides toluene-producing organisms,
- the occurrence of conditions inhibiting toluene--producing microorganisms, e.g. lack of oxygen.

- A much faster decrease in toluene content was observed in wet sediments as compared to dry sediments. This phenomenon might have been caused by better growth conditions for toluene-decomposing microorganisms due to humidity.

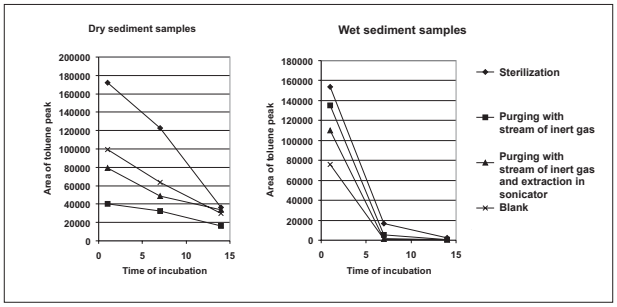

Fig. 5. Plots of toluene concentrations in the investigated benthic sediments.

Table 3. Volatile organic compounds identified in microbiological samples.

Type of organism	Compound
Bacteria	Dimethyl-disulfide
	Trimethylsulfide
	2,2-Dimethylhexane
	2,2,3,3-Tetramethylbutane
	2,4-Dimethyl-1-heptene
Fungi	Ethyl acetate
	2-Methyl-1-propanol
	Acetic acid
	3-Methyl-1-butanol
	2-Methyl-1-butanol
	1,1-Diethoxyethane
	7-Octene-4-ol
	2,4-Dimethyl-1-heptene

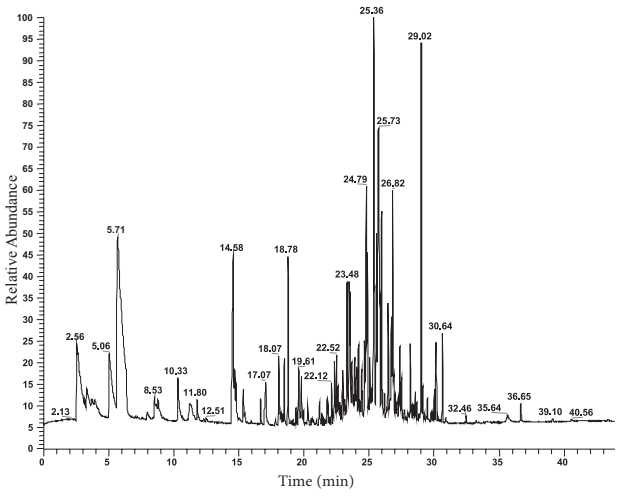

Fig. 6. A chromatogram obtained from the sample containing a fungus from genus *Trichophyton* using the PT-TD-GC-MS technique.

Determination of Toluene Content in Biological Samples

- The obtained results allow us to exclude bacteria as a potential toluene source. Based on the analysis of microbiological samples, the presence of toluene at higher levels than background concentration was detected in one sample only - this sample contained a fungus from genus *Trichophyton*. The analytical results suggest that toluene might have been a product of fungal metabolism.
- High content of various alcohols in the analyzed microbiological samples proved that fermentation took place because the decomposition of organic compounds present in culturing medium results in the production of alcohols.

General Conclusions

- This study proved the usefulness of the PT-TD-GC-MS technique for the analysis of benthic sediments as well as microbial samples.
- It is highly likely that the investigated benthic sediments from the Odra are of microbial origin; however, more research is necessary in order to confirm the above hypothesis because the obtained results are not explicitly conclusive.

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