

Background Level of Pops in Ground Water Assessed on Chemical and Toxicity Analysis of Exposed Semipermeable Membrane Devices

Authors: Kočí, Vladimír, Ocelka, Tomáš, and Grabic, Roman

Source: Air, Soil and Water Research, 2(1)

Published By: SAGE Publishing

URL: <https://doi.org/10.1177/ASWR.S2128>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Background Level of Pops in Ground Water Assessed on Chemical and Toxicity Analysis of Exposed Semipermeable Membrane Devices

Vladimír Kočí¹, Tomáš Ocelka² and Roman Grabic²

¹Department of Environmental Chemistry, ICT Prague, Technická 5, 166 28 Prague 6, Czech Republic.

²Institute of Public Health Ostrava, National Reference laboratory for POPs, Partyzanske nam. 7, 702 00 Ostrava, Czech Republic.

Abstract: Persistent compounds are present around almost the entire world. The level of contamination in very old groundwater sources (Cenoman bedrock Mesozoic, approximately 100 millions year old) was assessed. This offers an information about realistic natural background. Together with chemical analysis a toxicity evaluation of sampled sites was performed. Semipermeable membrane devices were applied as a sampling system. Exposed SPMDs were analyzed both for chemical contain of POPs and toxicity properties. The chemical analyses of PAHs were made by HPLC-FLD, PCBs and OCPs were analysed by GC/MS/MS on GCQ or PolarisQ (Thermoquest). Toxicity bioassays on alga *Desmodesmus subspicatus*, bacteria *Vibrio fischeri* and crustacean *Daphnia magna* was performed. The results show very low contamination of groundwater with POPs with concentrations close to detection limits of applied analytical tools. Even this low contamination was possible to rank based on the obtained toxicity data. Toxicity proved to be a good parameter for determination of relative POPs contamination where concentration is near to detection limits and thus correct determination of all POPs cannot be undertaken. Although contamination levels were found to be very low, a secondary contamination of PCBs through the bedrock was observed. Organochlorine pesticides were found at a sampling site near a mouth of the ground watershed. Applied toxicity tests confirmed the presence of toxic substances and marked sites of higher contamination. Application of toxicological parameter V_{tox} allowed the ranking of assessed sites by their contamination level even in cases where concentrations of pollutants were near or under detection limits and it was not therefore possible to rank the sites on the basis of chemical parameters. Toxicity response of bioassays obtained on SPMDs exposed in clean groundwater can be used as a background toxicity values for further SPMD applications. Secondary contamination with PCBs and pesticides was detected in Cenoman groundwater. Toxicity evaluation of SPMD extract can be used as an effective tool for ranking of general level of water contamination.

Keywords: detection limits, natural background, POP, secondary groundwater contamination, SPMD, toxicity, V_{tox}

Introduction

The discharge of wastes containing persistent organic pollutants, POPs, is a growing environmental problem.¹ POPs cause serious health damage so their ambient transport is of current concern. POPs bioaccumulate easily, so even low concentrations of these substances can represent serious human health risks. POPs persist in environments and can be transported over long distances. Transport of POPs in ground water is of high concern, particularly in areas used as sources of drinking water.² It is preferable for underground water exploited commercially as mineral water not to undergo sophisticated water-treatment. Usually only the gas content is adjusted. A particular problem arises if POPs discharged into an environment make their way in to groundwaters which are then extracted for drinking purposes further downstream. If no specific treatments for their removal are applied, POPs can make their way in to drinking and/or mineral water.

Persistent compounds are present around almost the entire world. The level of contamination varies significantly between sites. With the development of modern analytical tools, POPs can be detected at very low concentrations, down to $\text{pg}\cdot\text{l}^{-1}$. Such low detection limits allow the detection of POPs in any chosen environmental matrix. This raises the question of what constitutes naturally occurring background POPs levels in, for example, drinking water sources. Having knowledge of this natural background level is highly important in order to determine whether a site or system has been secondarily

Correspondence: Vladimír Kočí, Department of Environmental Chemistry, ICT Prague, Technická 5, 166 28 Prague 6, Czech Republic. Email: vladimir.koci@vscht.cz



Copyright in this article, its metadata, and any supplementary data is held by its author or authors. It is published under the Creative Commons Attribution By licence. For further information go to: <http://creativecommons.org/licenses/by/3.0/>.

contaminated with POPs. In very old groundwater sources, such water from Cennoman bedrock (Mesozoic, approximately 100 millions year old), no artificial contamination of water is expected. Groundwater from such a ground watershed allows the possibility of determining expecting natural background POPs levels.

In recent years assessment of POPs has been linked with applications of an in-situ passive sampling approach.^{3,4} Passive dosimeters are usually applied to monitor water environments. Passive sampling offers many of advantages over standard sampling methods: the ability to record low levels of contamination (without the necessity for expensive pre-concentration of large volumes of water and analytical technique required for acceptable detection limits using standard sampling), accidental concentration variation of pollutants and limitations in determination of truly-dissolved (bio available) phase—all resulting in relatively low sampling and analytical costs.

One method of passive sampling is the use of semi-permeable membrane device, SPMD.⁵⁻⁹ An SPMD is a membrane filled with a triolein, a substance similar in its properties to fish fats. Various persistent organic pollutants are collected in the triolein.^{6,10,11} After exposition the SPMD is dialyzed and the final dialysate is then analyzed. Various organic solvents are used for preparation of dialysate. The choice of the solvent used for preparation of SPMD extract is very important for toxicity analysis and consequently for the choice of exposed organism. SPMD membranes have proved to be a highly effective dosimeter of hydrophobic, lipophilic organic contaminants of very low concentrations in water due to their bioaccumulation ability.¹²⁻¹⁴ Ambient toxicity of by SPMD sampled substances is of high concern as was reported for example by Turqut,¹⁵ Miglioranza et al.^{16,17} and Chaudhry et al.¹⁸ for organochlorine pesticides (OCP); by Tysklind et al.¹⁹ Harrad et al.²⁰ and Senthilkumar et al.²¹ for polychlorinated biphenyls; and Axelmann,²² Wittig²³ and Chaudhry²⁴ for polyaromates.

The main objective of this work was to determine a background level of various halogenated persistent organic compounds in a groundwater of Mesozoic bedrock which is a source of mineral water for an spa area in a Middle Bohemia. This Cennoman water is used for drinking purposes in large area of Czech Republic, especially in a form of packed table water. Since POPs are currently detected in

various environmental matrices the information of natural background concentrations of these substances is needed. Overall presence of POPs sampled using SPMDs can be evaluated based on toxicity testing of SPMD extracts. Because using of several solvents during preparation of SPMD extracts, express these extracts their own toxicity level. Determination of background toxicity level of SPMD extracts for further toxicity evaluation of in water present contaminants using SPMD sampling technique was specific objective of this work.

Materials and Methods

Assessed groundwater area

Middle Bohemian Cennoman bedrock (Mesozoic, Cretaceous) is a source of high quality drinking water which is used at its southern extent, near Poděbrady town, Czech Republic, for spa purposes and for the treatment of commercially harnessed drinking water. The Cennoman bedrock soil structure is predominantly determined by sandstone layers. This Middle-European area is one of main sources of high quality mineral water. The direction of groundwater flow is from north to south and the direct length of the underground flow is 50 km. The Cennoman bedrock is approximately 100 millions years old; the age of the underground water is estimated (using the flow and geological conditions) at 1000 years. For assessment of groundwater contamination four underground boreholes of depth 350 meters were selected. The pressure of ground water in the selected boreholes was constant and sufficiently strong, ensuring a constant flow rate of sampled groundwater. Denomination of sampled boreholes in Czech geological system is (in north to south order): SK7C—Buda (most northern site); SK6C—Plazy; BPV1—Malý Vestec and BPV3—Poděbrady (most southern site) near the bank of Elbe River. Where Buda, Plazy etc are the names of municipalities where the boreholes are located. Exact coordinates of sampling sites are as follows: Buda (N 50°29'; E 14°59'), Plazy (N 50°24'; E 14°58'), Malý Vestec (N 50°13'; E 15°08'), Poděbrady (N 50°08'; E 15°07'). The north to south order of boreholes corresponds with the direction of groundwater flow. The sampling site Buda is approximately 10 km south of the northern extent of Cennoman bedrock. Allocation of sampling sites is shown in following Figure 1.

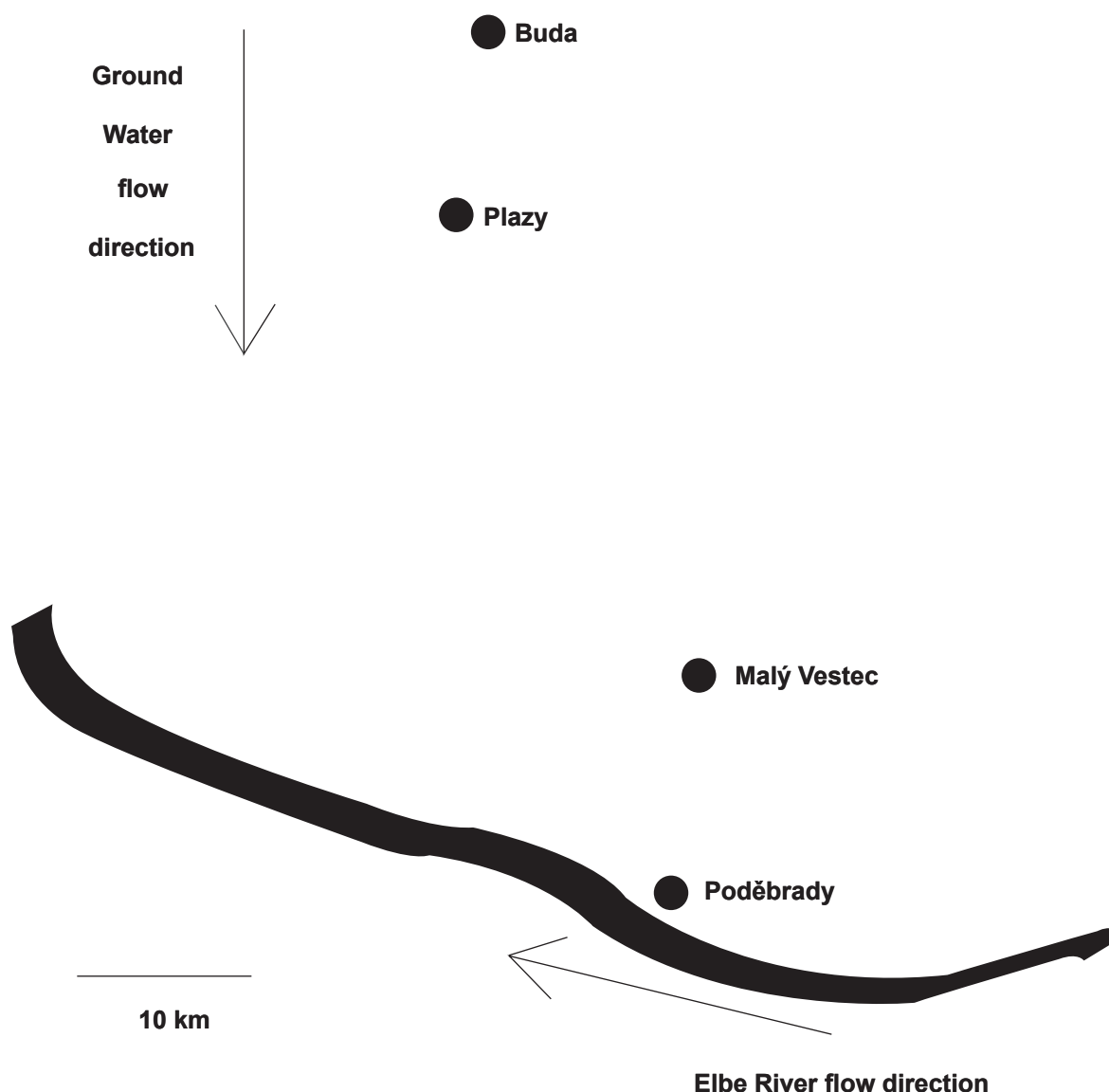


Figure 1. Scheme of the study area (Central Bohemia, Czech Republic). Map of Middle Bohemian Cennoman bedrock area. North-East from Prague. Direction of the ground water flow is from north to south. The approximate distance of the most northern site Buda from the end of Cennoman bedrock is 10 km, distance to next site Plazy is 8.8 km, following with interval 23.7 km to Malý Vestec site and finally interval to Poděbrady site is 9.3 km.

SPMD exposures and sampling

As the concentration of POPs in groundwater was expected to be low, passive sampling, namely a semipermeable membrane device system (SPMD), was chosen for contamination assessment. The passive sampling method represents the measurement of an analyte concentration as a weighted function of the sampling time (time averaged concentration). In this study (for given chemicals), the exposure is considered to be an integral contaminant response within a particular sampling period. The SPMD sampling tool is designed for long-term monitoring of lipophilic,

hydrophobic contaminants in aquatic and air environments. It can be viewed as a bridge between analytical chemistry and biomonitoring methods and is based on bioconcentration phenomenon.

A standard SPMD consists of a thin-walled nonporous tube with transient pores approximately 10^{-9} m in diameter, manufactured from low-density polyethylene (LDPE) filled inside by 1 ml of synthetic lipid—triolein (1,2,3-tri-[cis-9-octacenoil]glycerol) of high purity. General dimensions of the standard SPMD are: width 2.5 cm (lay-flat), overall length 91 cm, and thickness approx. 75 μ m.

SPMD sampling was performed as follows. Before sampling all membranes were immersed in hexane to remove monomers and others impurities for 24 hours, then placed in clean airtight steel cans and transported to sampling sites with transport-trip and field blanks. At the sampling point, SPMDs were placed in a perforated stainless steel container to protect the membranes against mechanical damage and to restrict water flow velocity at the membrane. Numbers of exposed SPMDs per site were decided using tested parameters and by the QA/QC aspect; 5 membranes per a site were used in this study. In addition, along with the deployed SPMD set, another set of SPMDs was exposed to ambient air during the deployment (trip/field blanks) at the sampling sites to monitor possible contamination from the air. Each container was equipped with a temperature logger (Tiny-Loggers, Intab, Stenkullen, Sweden) which registered water temperature every 15 minutes.

SPMD membranes were exposed in steel basins into which the ground water flowed at a constant rate throughout the exposure time. The intensity of flow was 0,001 L/s at Buda site; 0,011 L/s at Plazy site; 0,002 L/s at Malý Vestec site and 0,009 L/s at Poděbrady site. This flow of ground water at all sites sufficiently exchanged the water ensuring no changes of concentration of POPs due to the occurrence of sorption in SPMD. Duration of deployment was 33 days at the Buda, Malý Vestec and Plazy sites and 32 days at the Poděbrady site.

After sampling, each sampler was rinsed with drinking water; the SPMDs were placed in a clean airtight steel can. Periphyton, minerals and rough particulates were then removed from membrane surfaces with a clean cloth and the membranes rinsed with clean water. Exposed membranes were preserved frozen at $-18\text{ }^{\circ}\text{C}$ until analyzed.

Chemical analysis

The following chemical parameters were monitored: 1) all detectable tri-deca polychlorinated biphenyls (PCBs), 2) 12 of 16 U.S. EPA monitored polyaromatic hydrocarbons (PAHs): phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluorantene, benzo[k]fluorantene, benzo[a]pyrene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene, indeno[1,2,3,-c,d]pyrene, 3) a group of organochlorine pesticides (OCP): hexachlorobenzene (HCB) and isomers of hexachlorocyclohexane (HCH), DDE, DDD and DDT.

Exposed SPMDs were dialyzed with hexane (suprapure quality, MERCK) for 3 days using 2 solvents, resulting in a 200 ml fraction. After dialysis the ^{13}C -labelled isotopic internal standards ($^{13}\text{C}_6$ gama HCH and $^{13}\text{C}_{12}$ ppDDE for OCPs and mono to deca $^{13}\text{C}_{12}$ labeled PCBs (3, 15, 31, 52, 118, 153, 180, 194, 206, 209)—Wellington laboratories, CIL) were added to the extract and analyzed in accordance with available (accredited) laboratory methods. The solvent of the aliquot for determination of PAHs was changed to methanol and analyzed by HPLC-FLD.

Aliquot for PCBs and OCP analysis was cleaned on a column filled with silica gel deactivated by H_2SO_4 . Recovery standard ($^{13}\text{C}_{12}$ labeled PCB 80) was added and sample volume was adjusted to 100 μl of n-heptane. PCBs and OCPs were analyzed by GC/MS/MS on GCQ and PolarisQ, respectively. The MS/MS parameters were optimized in similar way to that described elsewhere.²⁵ The DB5 ms (30 m \times 0,25 mm \times 0,25 μm) column was used for chromatographic separation. All results of analysis were evaluated as the concentration per SPMD. Evaluation of ambient concentration was then performed using known uptake rates for the particular conditions (temperature) and compound.

This calculation was performed using equation (1) which is derived from a complex equation describing uptake kinetics.^{5,6}

$$C_w = \frac{C_{SPMD} * V_{SPMD}}{R_s * t} \quad (1)$$

where C_w is ambient truly dissolved contaminant concentration in water, C_{SPMD} is concentration in SPMD, V_{SPMD} is overall volume of the SPMD, R_s effective sampling rate, and t is the time of exposure (sampling time). The effective sampling rates (R_s) were derived in accordance with Kathleen and Gale.²⁶ For bioassays testing aliquots were transferred into acetone-DMSO (1:1) mixture.²⁷ A good solubility and low background toxicity was thus ensured. These aliquots were used to construct dilution series for bioassays.

Toxicity testing

For predicting the impact of contamination on the ecology of the receiving surface water body, it is necessary to determine the toxicity of the contaminated water. The toxicity of a POPs contaminated effluent depends on the amounts and

types of the individual compounds present; however, the concentration-toxicity relationships may be nonlinear even for pure compounds. Mixtures of compounds pose greater problems because the toxicity of a mixture is not simply linked to individual toxicities of components of the mixture.

The SPMD extracts were tested on chlorococcal alga *Desmodesmus subspicatus* (earlier *Scenedesmus subspicatus*) and luminescent bacteria *Vibrio fischeri* for toxicity evaluation and crustacean *Daphnia magna*. These organisms were chosen due to their frequent application in environmental monitoring projects.

Algal bioassays were provided with *Desmodesmus subspicatus*, strain BRINKMANN 1953/SAG 86.81 (obtained from Culture Collection of Autotrophic Organisms, The Institute of Botany, Czech Acad. Sci., Trebon) based on ISO 8692²⁸ with following alterations. Due to the small amount of SPMD dialysate obtained, 96 wells microplates with 0.3 ml of suspension medium in 6 replicates were used. Monospecific algal cells were cultured for several generations in a defined medium containing a range of concentrations of the tested SPMD aliquot, prepared by mixing appropriate quantities of nutrient concentrate, demineralized water and an inoculum of exponentially growing algal cells. An initial cell density of 10^4 cells per millilitre was used. The test solutions were incubated for a period of at least 96 hours, at a light intensity of $60 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and temperature 27°C . Cell density in each suspension was measured every 24 hours, microplate reader BIOTEC was used to measure the optical density at 750 nm. The cell density determination was based on the optical density (cell density calibration curve). Inhibition was measured as a reduction in growth and growth rate, relative to control cultures grown under identical conditions.²⁹

Tests with bioluminescent bacterium were carried out following the standard procedures (ISO 11348).³⁰ The samples were tested in a medium containing 2% NaCl and about 10^7 cells of bacteria were reconstituted from the lyophilised reagent (Bruno Lange, *Vibrio fischeri* NRLL-B-11177). Each test was performed in 6 to 10 duplicates and with a negative control. The luminescence was measured using the LMZ II tube luminometer (Immunotech, Beckman Coulter Company) at 5-, 15- and 30-min exposure times. The concentration of the original SPMD triolein (mg/L/day), which caused a 50% reduction in light

production after exposure for 5(or 15) minutes, was designated as the 5(15)-min EC50.

The crustacean bioassay is based on the immobilization of *Daphnia magna* STRAUS. Procedures recommended by ISO 6341³¹ compliant with national and international standard methods (e.g. OECD, EEC, U.S. EPA, ASTM guidelines) were followed. Due to the small number of tested SPMD membranes, a miniaturized test design was applied. The volumes of tested solutions were 20 ml, where the tests in 50 ml beakers were realized. A single daphnia requires at least 5 ml of tested media to allow for free movement. As such, the number of treated animals per replicate was reduced accordingly.

Toxicity evaluation

The basic aim of SPMD sampling and of further analysis and interpretation is to provide suitable data, which can then be used for the evaluation of ambient conditions. For this reason, the final data from SPMD monitoring has to be comparable not only to similar data sets but even with real quality of the assessed environment. Extracts or lipid rinsed from exposed SPMDs contain chemicals present across the whole or a fractional volume of the sampler. After transferring the enriched SPMD extract to an appropriate carrier solvent, aliquots are used for assays. Endpoint EC50 represents a specific mass of SPMD lipid per unit volume of carrier solvent that elicits a toxic response. Thus the presentation of merely EC50 of exposed triolein is insufficient for a final conclusion of how “toxic” the ambient area is. Determined EC50s of SPMD extracts were used for determination of V_{tox} values.³² The parameter V_{tox} allows the comparison of the toxicity of samples obtained from SPMDs with different durations of exposition, different sites, projects and laboratories. The value of V_{tox} represents a volume of media which is theoretically needed for dilution of all toxicants absorbed in one membrane during one average day of deployment to obtain solution with chosen effective concentration, for example EC50. The higher the value of V_{tox} , the bigger the volume of toxicants absorbed and thus the higher the contamination of the sampled site. The following formula (2) defines V_{tox} , where (m) is the concentration of extracted membranes in solvent mixture expressed as number of membranes in ml of solvent mixture ($\text{pcs}\cdot\text{ml}^{-1}$), (d) is duration of deployment of the membrane during sampling (days) and EC50 ($\text{ml}\cdot\text{L}^{-1}$) is

effective concentration of extract on a chosen organism.³²

$$V_{tox}(50) = \frac{1}{m \cdot EC50 \cdot d} (L \cdot d^{-1}) \quad (2)$$

It is principally impossible to correlate toxicity with chemical composition of a mixed sample, as in the case of aliquots from SPMDs containing POPs. The response of an organism to a complex sample is somewhat more complicated than the addition and/or elimination of toxic influence of individual compounds. It is necessary to determine with varying synergism and antagonism, changes in the chemical and physical condition of the environment along with changes in the state of every organism. Keeping this in mind, we compare the course of the main contaminant groups with the course of toxicity expressed as V_{tox} . V_{tox} has an opposite use in expressing toxicity in comparison to the EC50. The value of V_{tox} and the toxicity of evaluated sample are in direct proportion. This means that a higher V_{tox} indicates a higher toxicity.

Results and Discussion

Chemical analysis

The level of groundwater contamination is very low. The presented tables summarize obtained data for polyaromatic hydrocarbons, polychlorinated biphenyls and selected organochlorine pesticides. Table 1 shows approximate sums of main contaminant groups. Parameters found below the quantification limit were taken into account as values of LOQ. This approach was chosen to obtain the maximum value that can be found (due to toxicological relevance). These values are only approximate as the concentrations of many assessed compounds were below the detection

limit. In such cases the detection limit value was used for counting the sum of a contaminant group. A more detailed overview on present concentrations of all analyzed substances is summarized in Tables 2, 3, 4 where a concentration below detection limit is marked with the symbol “<”. Differences in LOQ are caused by different sampling times and temperatures. Extracted aliquots were the same.

In Table 2 where concentrations of PAHs are presented, the most frequently occurring contaminant of all those analyzed was phenanthrene with the highest concentration being 13.9 ng.l⁻¹ at the Buda borehole. The concentration of phenanthrene was highest at the Buda sampling site, close to the point where the flow enters the underground Cennoman bedrock. The concentration of phenanthrene declined over subsequent sampling sites to a minimum value of 0.094 ng.l⁻¹ at Poděbrady. Similar overall trends of decreasing concentration were observed in all other evaluated PAHs. This decrease in concentration is most likely caused by sorption to solid surfaces along with a partial degradation. As a source of PAHs we expect atmospheric deposition and slow migration with solid particles on which these hydrophobic substances are readily binded.

No such decrease in concentration as that observed with PAHs was evident in the case of values of PCBs summarized in Table 3. The highest value for the sum of all congeners was just 168 pg.l⁻¹, and the lowest observed value was that at the Poděbrady sampling site at only 22 pg.l⁻¹. As such, is it necessary to consider why the total PCB content does not follow a similar decline to that of PAHs. At the Plazy sampling site the value is higher than that at Buda—the initial source of flow. The explanation for this can be found in congener profiles of PCBs Figure. 2 demonstrates that a similar PCB composition is present at sites Buda,

Table 1. Measured Overview of contaminant concentrations in evaluated sites. Sum of PAHs, PCBs, HCH, HCB and DDT concentrations for every site. All values are expressed in pg.l⁻¹.

Compound	Buda	Plazy	Malý vestec	Poděbrady
Sum of PAHs	16159	4380	2807	179
Sum of PCBs	95	168	72	22
Sum of HCH	220	140	220	470
HCB	7.9	8.3	8.3	2.2
Sum of DDT	4.2	4.1	3.9	26.7

Table 2. PAH concentrations detected in assessed profiles. All concentrations are expressed in ng.l^{-1} . HPLC-FLD detection system was used.

Compound	Buda	Plazy	Malý vestec	Poděbrady
Phenanthrene	13.9	1.3	1.6	0.094
Anthracene	0.91	0.17	0.26	<0.010
Fluoranthene	0.92	0.99	0.42	0.052
Pyrene	0.37	0.33	0.43	0.033
Benzo[a]anthracene	0.059	0.45	0.067	<0.008
Chrysene	<0.030	0.34	0.030	<0.008
Benzo[b]fluoranthene	<0.038	0.34	<0.038	<0.009
Benzo[k]fluoranthene	<0.036	0.18	<0.036	<0.009
Benzo[a]pyrene	<0.035	0.15	<0.035	<0.009
Benzo[g,h,i]perylene	<0.064	0.13	<0.064	<0.016
Dibenzo[a,h]anthracene	<0.079	<0.079	<0.079	<0.026
Indeno[1.2.3.-c.d]pyrene	<0.055	<0.055	<0.055	<0.018

Malý Vestec and Poděbrady. A different congener profile was observed at the Plazy sampling site. It is evident, therefore, that there is a source of groundwater contamination at this location. Thus demonstrating that PCB surface contamination can enter a groundwater watershed even in deep Cenomanian bedrock. The anthropogenic contamination of this area is realistic, because the PCBs were commercially produced up to year 1984 in the Spolana Neratovice company located several tens of km from on the sampling site.

In contrast to that seen in both previously discussed groups of POPs, a different pattern of contamination was observed for organochlorine pesticides (see Table 4). Concentrations of pesticides were almost constant (with exception of gamma HCH at Plazy) at Buda, Plazy and Malý Vestec but significantly different at the Poděbrady site. The most abundant compound was gamma HCH with a starting value of 220 pg.l^{-1} at Buda site, dropping down to 140 pg.l^{-1} at Plazy, increasing downstream at Malý Vestec back to 220 pg.l^{-1} and reaching a final concentration of 470 pg.l^{-1} at the end of groundwater flow at Poděbrady. A significant increase in total DDT concentration was observed at Poděbrady. This concentration reached a final value of 26.7 pg.l^{-1} at this location, having remained almost constant previously, at a value of 4 pg.l^{-1} .

An opposite situation was observed with HCB. This contaminant diminished in its concentration at the Poděbrady site to a value of 2.2 pg.l^{-1} after

maintaining an almost constant value of 8 pg.l^{-1} previously. The phenomenon whereby, after maintaining an almost constant value, the pesticide concentration rapidly changes at Poděbrady can be explained by presence of Elbe River near the sampling borehole. The Elbe River basin comprises the largest agriculture areas in Czech Republic, where the use of pesticides is very common. As such, the observed pesticide pattern is not surprising, with gamma HCH dominating and HCB levels being around one order of magnitude lower, together with DDT and their metabolites. It is noteworthy that, from the level of contamination, the found concentrations are negligible for toxicological evaluation. On the other hand, this data can be used for future reference regarding background POPs levels.

Results of toxicity testing

In this study the results of toxicity tests are presented by EC50 of aliquot from one whole membrane, its hillslope and 95% confidence intervals. These intervals show how well experimental data fit the sigmoid curve chosen as a standard course of toxicity in a relationship dose (concentration)—response. Final interpretation of toxicity in assessed groundwater is presented with the use of the previously outlined V_{tox} concept.

The pattern of toxicity along groundwater flow is apparently similar in bioassays with algae and crustaceans, as shown in Table 5. From the

Table 3. Concentrations of PCB congeners detected in assessed profiles. All concentrations are expressed in $\text{pg}\cdot\text{L}^{-1}$. Analysis was made by GC/MS/MS on GCQ and PolarisQ, respectively.

Compound	Buda	Plazy	Malý Vestec	Poděbrady
PCB19	<0.34	<0.34	0.34	<0.29
PCB18	0.98	0.72	0.91	0.30
PCB17	0.46	0.33	0.33	0.16
PCB27 + 24	<0.20	<0.20	<0.20	<0.16
PCB16 + 32	0.46	0.46	0.39	0.16
PCB26 + 25	0.63	0.42	<0.21	<0.27
PCB28 + 31	2.2	1.7	1.9	1.1
PCB33	0.79	1.0	0.65	0.47
PCB22	0.63	0.73	0.42	<0.32
PCB37	<0.20	<0.20	<0.20	<0.44
PCB54	<0.74	<0.74	<0.87	<0.49
PCB53	0.37	<0.37	<0.49	<0.38
PCB51	<0.37	0.74	<0.49	<0.38
PCB45	<0.23	<0.23	<0.3	<0.23
PCB46	<0.41	<0.41	<0.55	<0.41
PCB52	1.6	1.0	1.2	0.64
PCB49	0.57	0.57	<0.46	<0.51
PCB48 + 47	1.2	2.9	0.44	<0.49
PCB44	0.65	0.32	<0.32	<0.36
PCB42	<0.29	0.29	<0.39	<0.44
PCB41 + 64 + 71 + 72	0.89	0.98	0.53	0.85
PCB74	0.49	0.59	0.39	0.49
PCB70	0.78	0.78	0.78	0.61
PCB80 + 66	0.91	0.69	0.80	0.74
PCB60 + 56	0.80	0.69	0.57	<0.51
PCB81	<0.12	<0.12	<0.25	<0.57
PCB77	<0.25	<0.25	<0.25	<0.93
PCB104	<0.49	<0.49	<0.59	<0.24
PCB95	2.0	2.2	1.6	0.88
PCB84 + 89 + 92	1.4	0.96	0.96	0.31
PCB101	3.0	3.1	1.7	0.93
PCB99 + 113	0.83	<0.41	<0.55	<0.41
PCB119	<0.41	<0.28	<0.41	<0.76
PCB97	0.41	<0.41	<0.41	<0.41
PCB87	0.46	0.34	<0.34	1.1
PCB110	2.1	1.6	1.4	0.69
PCB123	<0.37	<0.37	<0.37	<0.87
PCB118	0.99	1.4	0.74	<1.1
PCB114	<0.41	<0.41	<0.41	<0.96

(Continued)

Table 3. (Continued)

Compound	Buda	Plazy	Malý vestec	Poděbrady
PCB105	0.45	<0.45	<0.45	<1.4
PCB126	<0.34	<0.34	<0.51	<1.9
PCB155	<0.57	<0.57	<0.69	<0.51
PCB148	0.91	1.4	1.3	<0.51
PCB151	2.2	2.4	1.8	0.51
PCB135 + 144	2.0	1.8	1.7	0.61
PCB149	7.3	8.3	4.7	1.3
PCB153 + 168	10.9	16.8	10.1	1.9
PCB130	3.0	3.2	1.8	0.83
PCB163 + 164	2.2	5.7	3.7	<1.6
PCB138	3.8	8.0	3.1	1.5
PCB158	<0.51	1.7	<0.067	<1.6
PCB128	1.2	1.4	0.69	<0.55
PCB167	<0.67	<0.67	<0.90	<2.9
PCB156	0.90	2.2	<0.90	<2.9
PCB157	<0.67	<0.67	<0.90	<2.3
PCB169	<0.79	<0.79	<1.1	<4.7
PCB188	<1.1	<1.4	<1.7	<1.2
PCB179	1.7	1.7	1.7	<0.83
PCB176	<0.55	<0.55	<0.83	<0.83
PCB178	0.39	1.2	<0.59	<0.59
PCB187	3.7	8.8	3.5	0.95
PCB183	2.0	3.7	1.6	0.78
PCB174	3.7	8.8	1.8	0.88
PCB177	1.4	4.7	1.0	<0.59
PCB171	0.78	2.2	0.78	<0.59
PCB180	7.6	24.7	7.2	1.9
PCB191	<0.67	<0.67	<0.90	<2.1
PCB170	7.2	18.0	5.4	<0.90
PCB189	<0.67	<0.9	<1.1	<3.5
PCB202	0.34	1.0	<0.67	0.67
PCB201	<0.67	0.67	<0.67	0.84
PCB199	2.0	7.1	<1	<0.841
PCB203 + 196	1.3	4.4	1.3	<0.84
PCB194	2.3	3.7	2.3	<1.2
PCB205	<1.4	<1.9	<2.3	<2.3
PCB208	<6.1	<6.1	<8.1	<6.1
PCB206	<14	<14	<16	<5.1
PCB207	<8.1	<8	<10	<6.1
PCB209	<14	<18	<18	<8.1

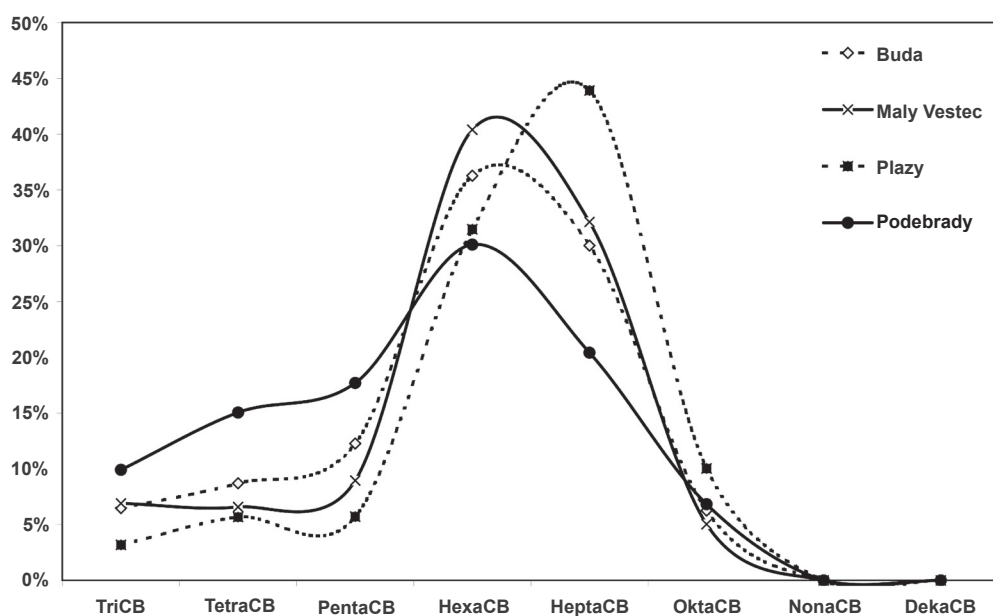


Figure 2. Relative distribution of the sum of PCBs at different sampling profiles. The different relative distribution, called “fingerprint”, refers to different source of contamination. This is evident especially for Plazy bore hole, where new anthropogenic contamination of PCBs was detected. The percent value is proportion in sum of PCBs which are 94.6 pg.l^{-1} at Buda; 168.2 pg.l^{-1} at Plazy; 71.5 pg.l^{-1} at Malý Vestec and 22.1 pg.l^{-1} at Poděbrady site.

starting V_{tox} value at the Buda site, it increases at Plazy, drops down at Malý Vestec to a value similar to that at Buda and further decrease at Poděbrady. The course of sampling site toxicity to bacterium is similar across the first two sites to the results of tests with the alga and daphnia. Compared to the Buda site, toxicity is higher at Plazy and later drops down again (in relation to

algae and daphnids more significantly) at Malý Vestec. Contrary to other bioassays, the investigation of bacterium response demonstrates a significant increase of toxicity at the Poděbrady site. This is related to the proximity of this site to the Elbe River, whose river shores are utilized intensively for agriculture, with the attendant frequent application of various pesticides.

Table 4. Concentration of pesticides detected in assessed profiles. All concentrations are expressed in pg.l^{-1} . Analysis was made by GC/MS/MS on GCQ and PolarisQ, respectively.

Compound	Buda	Plazy	Malý vestec	Poděbrady
alfaHCH	<5.7	<6.8	<7.1	<6.8
betaHCH	<7.6	<8.7	<8	<8.4
gamaHCH	220	140	220	470
deltaHCH	<7.2	<8.3	<8	<13
HCB	7.9	8.3	8.3	2.2
opDDE	<1.8	<1.8	<1.8	3.7
ppDDE	4.2	4.1	3.9	11.0
opDDD	<2.3	<2.3	<2.3	<1.3
ppDDD	<2.4	<2.4	<2.4	4.4
opDDT	<3.4	<3.4	<3.4	<3.3
ppDDT	<3.8	<3.8	<3.8	7.6

Increased pesticide concentration as found at this site is presented in Table 5.

The course of toxicity to algae and crustaceans from the Buda site through Plazy and Malý Vestec to the Poděbrady site is similar to that of the total PCB concentration at the scale of pg.l^{-1} . This similarity is evident across the course of toxicity to bacterium except at the Poděbrady site. Bacteria were apparently more sensitive to the sample from Poděbrady site, where pesticides were more abundant. No similarity between patterns of toxicity of all three testing organisms and contamination by PAHs is found. It is presumed, therefore, that contamination of water with PAHs concentrations below 16 ng.l^{-1} is of no toxic significance.

Although the toxicity at all sampling sites was determined, it was not possible to conclude that the ground water was toxic. The concept of V_{tox} is able to express a value for toxicity even if the SPMD membrane was exposed at a natural, clean and apparently non-toxic site. If the value of V_{tox} increases above an empirically determined critical level, then the site can be considered toxic even for organisms living on site (Kočí et al. 2004). All V_{tox} values presented in this study are under this empirical critical level and, thus, no toxic

conditions occur at any of these sites. The empirical critical level of V_{tox} is a task for future research. At present few sets of comparable data are available, so work such as this can help to set a base for its overall determination. One benefit of V_{tox} is in its ability to rank sites based on bioassays even in cases where the sites are basically non toxic. Conversely, V_{tox} based on SPMD sampling can be used for ranking sites of extremely high toxic properties, to which conditions no living organism can be exposed.

The practical potential of V_{tox} was appreciated in comparing the contamination of sites with low contamination, where, based on chemical analysis, it was not possible fully rank different sampling sites (as too many chemical parameters were near or under detection limits). Bioassays marked effectively those sampling sites where significant contamination occurs: increased toxicity to all organisms of aliquot from SPMD exposed at the Plazy site correlate with higher contamination of PCBs found there; sensitivity of the bacterium bioassay to aliquot from SPMD deployed at the Poděbrady borehole point to increased contamination by pesticide-like compounds. In addition, it was evident from the bioassay that although toxic contaminants were present and their toxic level

Table 5. Results of toxicity evaluation of exposed SPMDs performed on alga *Desmodesmus subspicatus*, crustaceans *Daphnia magna* and bacterium *Vibrio fischeri*. Expositions of organisms were 7day in algal *D. subspicatus* bioassay, 48 hours in test with crustacean *D. magna* and 30 minutes with bacterium *V. fischeri*.

Parameter	Buda	Plazy	Malý vestec	Poděbrady
<i>Desmodesmus subspicatus</i>				
EC50, ml.l^{-1}	10.0	5.0	9.6	24.2
95% CI EC50	3.3 to 30.5	4.4 to 5.7	3.6 to 25.4	6.7 to 87.3
Hillslope	2.722	1.136	1.13	0.581
V_{tox50} , l.d^{-1}	0.003	0.006	0.003	0.001
<i>Daphnia magna</i>				
EC50, ml.l^{-1}	9.7	1.5	10.8	11.7
95% CI EC50	7.7 to 12.3	1.0 to 2.2	10.7 to 10.9	8.2 to 16.9
Hillslope	1.83	1.73	4.63	1.61
V_{tox50} , l.d^{-1}	0.003	0.020	0.003	0.003
<i>Vibrio fischeri</i>				
EC50, ml.l^{-1}	0.94	0.54	1.8	0.07
95% CI EC50	0.67 to 1.32	0.38 to 0.78	1.4 to 2.3	0.06 to 0.08
Hillslope	1.38	0.950	1.46	1.39
V_{tox50} , l.d^{-1}	0.032	0.056	0.017	0.443

was determined, no acute toxicity can be expected at the sampling sites as the values of V_{tox} did not exceed the critical level.

Two parallel SPMDs were deployed for toxicity testing at the Poděbrady sampling site. These membranes were used for determination of the repeatability of chosen bioassays for this study. Summarized data of these parallel bioassays are presented in Table 6. The dose-response curve of algal bioassays was not a typical sigmoid curve, and thus its 95% confidence intervals of EC50 are wide. Such an event is not unusual in toxicity testing. It should not be taken to mean that the experiment should be repeated. This is seen in the similar EC50 values of both parallel membranes. Values of V_{tox} of parallel membranes fit well. Improvements are necessary in the crustacean *Daphnia magna* investigation. This organism is relatively large and so requires a greater volume of tested media together with a higher consumption of SPMD aliquot. The volume of an SPMD sample is always limited (especially in a case of samples with low toxicity, where it can not be extensively diluted), so development of miniaturized bioassay would be beneficial.

Table 6. Comparison of toxicity response in parallel testing performed on parallel SPMDs exposed in Poděbrady sampling site bore hole. Expositions of organisms were 7day in algal *D. subspicatus* bioassay, 48 hours in test with crustacean *D. magna* and 30 minutes with bacterium *V. fischeri*.

Parameter	SPMD A	SPMD B
<i>Desmodesmus subspicatus</i>		
EC50, ml.l ⁻¹	25.8	22.6
95% CI of EC50	7.3 to 90.6	6.1 to 84.1
Hillslope	0.52	0.65
V_{tox50} , l.d ⁻¹	0.00121	0.00138
<i>Daphnia magna</i>		
EC50, ml.l ⁻¹	9.8	13.6
95% CI of EC50	7.9 to 12.1	8.5 to 21.8
Hillslope	1.86	1.36
V_{tox50} , l.d ⁻¹	0.00319	0.00230
<i>Vibrio fischeri</i>		
EC50, ml.l ⁻¹	0.068	0.073
95% CI of EC50	0.058 to 0.081	0.063 to 0.084
Hillslope	1.39	1.39
V_{tox50} , l.d ⁻¹	0.457	0.430

However, for a rigid explanation, in terms of general application of chemical parameters to *toxicity tests*, the limitations of used models must be considered. The model of prediction for given chemical parameters and toxicity is valid only for the observed system (the underground water of this study). Limitations are mainly due to the complex behavior and relationships of chemical parameters to toxicity response at various concentrations. Thus, the model can be used at the concentration level as well, related to the observed system.

Two SPMD membranes exposed in parallel were evaluated with toxicity assays to check comparability. The values of EC50 together with values of V_{tox} obtained on these parallel membranes were well comparable.

PCA Analysis

The PCA analysis was additionally performed on all uncensored data. We understand uncensored data to be data below the lower sensitivity limit of measurements, which can be called “low-censored” or simply “censored” data. The score plot explains 92.9% variance of analysed samples; the first component explains 74.6% variation, the second one 18.3% (see Figure 3). Loading vectors form three clusters of variables: one for PAHs, one for HCHs and DDT together with *Vibrio fischeri*, and the final of PCB, with HCB. Dominating sum HCHs (sum of alfa to gama compounds) and DDT (DDT and their metabolites) correlate with *Vibrio fischeri* while the PCBs groups (mainly tetra-octaCB) exhibit correlation with *Daphnia magna* and *Desmodesmus subspicatus*. PAHs exhibit no correlation, even if they are in importantly higher concentration in compare to other contaminants, what shows on lack of metabolic activation during bioassays. Clusters can be explained by various sensitivity of those organisms to those compounds which is probably given by chemical structure (molecule sizes), as well as log Kow.

Conclusions

An SPMD sampling system was used for monitoring of primary and secondary groundwater contamination of Cennoman bedrock. Chemical parameters such as PAHs, PCBs and OCPs concentrations together with the toxicities of samples to *Desmodesmus subspicatus*, *Daphnia magna* and *Vibrio fischeri* were determined. Although contamination levels were found to be very low, a secondary contamination of PCBs

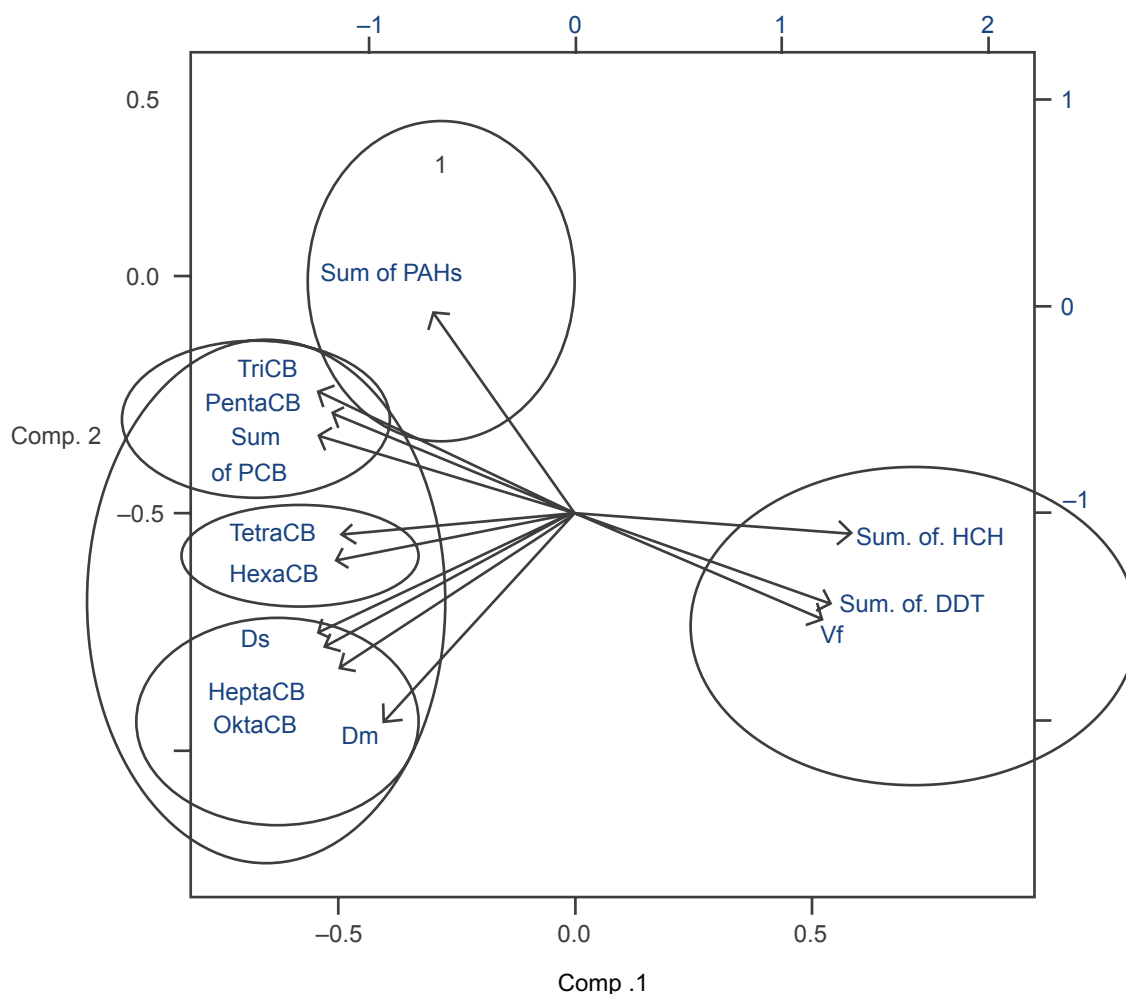


Figure 3. PCA of analytical results and bioassays responses from SPMD extracts obtained from Cennoman groundwater from Buda, Plazy, Malý Vestec and Poděbrady sites in Czech Republic. Grouping of toxicity responses expressed as *Vtox50* values for bacterium *Vibrio fischeri* (*VibFisch... Vtox50*), crustacean *Daphnia magna* (*DaphMag... Vtox50*) and alga *Desmodesmus subspicatus* (*DesSub... Vtox50*) was done with polyaromates (Sum of PAHs), pesticides (Sum of HCH, Sum of HCB, Sum of DDT) and sums of biphenyls groups (TriCB, TetraCB, PentaCB, HexaCB, HeptaCB and OktaCB).

through the bedrock was observed. The PCBs sink down through bedrock and secondarily contaminate sources of thousand years old water in the aquifer.

Organochlorine pesticides were found at a sampling site near a mouth of the ground watershed. Applied toxicity tests confirmed the presence of toxic substances and marked sites of higher contamination. Application of toxicological parameter *Vtox* allowed the ranking of assessed sites by their contamination level even in cases where concentrations of pollutants were near or under detection limits and it was not therefore possible to rank the sites on the basis of chemical parameters. Pesticides, namely the sum of HCHs and DDTs (DDTs and their metabolites) exhibited correlation with *Vibrio fischeri*.

The applied bioassays showed no correlation with concentrations of PAHs under a value of 16 ng.l^{-1} . Changes in PCB concentration from 22 to 168 pg.l^{-1} induced proportional changes in toxicity response of all test organisms. The measured HCH concentration of 470 pg.l^{-1} together with DDT concentration 26.7 pg.l^{-1} caused significant response in bacterium, although crustaceans and algae show no sensitivity to this level of pesticides.

Acknowledgements

We kindly acknowledge Eva Žáčková and Pavel Sysel for their field work support and for making sampling feasible under difficult conditions. Colleagues from National reference laboratory for POPs (IPH Ostrava) are kindly acknowledged for

their excellent analytical work. Madeleine Hunter is acknowledged for her language support. This work was supported by the Research Plan grant MSM 6046137308 from the Ministry of Education, Youth and Sports of the Czech Republic.

Disclosure

The authors report no conflicts of interest.

References

- Vallack HW, Bakker DJ, Brandt I, et al. Controlling persistent organic pollutants—what next? *Environ. Toxicol Pharmacol.* 1998;6:143–75.
- Gerth J, Förstner U. Part IV, Fitness for Aquatic Systems: Long-term Forecast—Key to Groundwater Protection. *ESPR.* 2004;11:49–56.
- Kot A, Zabiegala B, Namiesnik J. Passive sampling for long-term monitoring of organic pollutants in water. *Trends Anal Chem.* 2000;19:446–59.
- Gorecki T, Namiesnik J. Passive sampling. *Trends Anal Chem.* 2002;21:276–91.
- Huckins JN, Manuweera GK, Petty JD, Mackay D, Lebo JA. Lipid-Containing Semipermeable Membrane Devices for Monitoring Organic Contaminants in Water. *Environ Sci Technol.* 1993;27:2489–96.
- Huckins JN, Petty JD, Orazio CE, Lebo JA, Clark RC, Gibson VL, Gala WR, Echols KR. Determination of Uptake Kinetics (Sampling Rates) by Lipid-Containing Semipermeable Membrane Devices (SPMDs) for Polycyclic Aromatic Hydrocarbons (PAHs) in Water. *Environ Sci Technol.* 1999;33:3918–23.
- Herve S, Prest HF, Heinonen P, Hyötyläinen T, Koistinen J, Paasivirta J. Lipid-Filled Semipermeable Membrane Devices and Mussels as Samples of Organochlorine Compounds in Lake Water. *ESPR.* 1995;2:24–30.
- Petty JD, Jones SB, Huckins JN. An approach for assessment of water quality using semipermeable membrane devices (SPMDs) and bioindicator tests. *Chemosphere.* 2000;41:311–21.
- Bridges Ch, Little E, Gardiner D, Petty J, Huckins J. Assessing the Toxicity and Teratogenicity of Pond Water in North-Central Minnesota to Amphibians. *ESPR.* 2004;11:233–9.
- Prest HF, Jacobson LA, Wilson M. Passive water sampling for polynuclear aromatic hydrocarbons using lipid-containing semipermeable membrane devices (SPMDs): Application to contaminant residence times. *Chemosphere.* 1997;35:3047–63.
- Vrana B, Paschke A, Popp P, Schüürmann G. Use of Semipermeable Membrane Devices (SPMDs). Determination of Bioavailable, Organic, Waterborne Contaminants in the Industrial Region of Bitterfeld, Saxony-Anhalt, Germany. *ESPR.* 2001;8:27–34.
- Rantalainen AL, Cretney WJ, Ikonomou MG. Uptake rates of semipermeable membrane devices (SPMDs) for PCDDs, PCDFs and PCBs in water and sediment. *Chemosphere.* 1999;40:147–58.
- Rastall A, Neziri A, Vukovic Z, Jung Ch, Mijovic S, Hollert H, Nikcevic S, Erdinger L. The Identification of Readily Bioavailable Pollutants in Lake Shkodra/Skadar Using Semipermeable Membrane Devices (SPMDs), Bioassays and Chemical Analysis. *ESPR.* 2004;11:240–53.
- Kočí V, Ocelka T, Mlejnek M, Grabic R. Efficiency Assessment of Wastewater Treatment Plant Based on SPMD Sampling. *Centr Eur J. Chem.* 2004;2:91–112.
- Turqu C. Uptake and Modeling of Pesticides by Roots and Shoots of Parrotfeather (*Myriophyllum aquaticum*). *ESPR.* 2005;12:342–6.
- Miglioranza KSB, González Sagrario MA, Aizpún de Moreno JE, Moreno VJ, Escalante A, Osterrieth M. Agricultural Soil as a Potential Source of Input of Organochlorine Pesticides into a Nearby Pond. *ESPR.* 2002;9:250–6.
- Miglioranza KSB, Aizpún de Moreno JE, Moreno VJ. Land-based Sources of Marine Pollution: Organochlorine Pesticides in Stream Systems. *ESPR.* 2004;11:227–32.
- Chaudhry Q, Schröder P, Werck-Reichhart D, Grajek W, Marecik R. Prospects and Limitations of Phytoremediation for the Removal of Persistent Pesticides in the Environment. *ESPR.* 2002;9:4–17.
- Tysklind M, Bosveld ATC, Andersson P, Verhallen E, Sinnige TL, Rappe Ch, Berg M. Inhibition of Ethoxyresorufin-O-deethylase (EROD) Activity in Mixtures of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Polychlorinated Biphenyls EROD Activity as Biomarker in TCDD and PCB. Risk Assessment. *ESPR.* 1995;2:211–6.
- Harrad SJ, Smith DJ. Bioaccumulation Factors (BAFs) and Biota to Sediment Accumulation Factors (BSAFs) for PCBs in Pike and Eels. *ESPR.* 1997;4:189–93.
- Senthilkumar K, Kannan K, Subramanian A, Tanabe S. Accumulation of Organochlorine Pesticides and Polychlorinated Biphenyls in Sediments, Aquatic Organisms, Birds, Bird Eggs and Bat Collected from South India. *ESPR.* 2001;8:35–47.
- Axelmann J, Broman D, Näf C, Pettersen H. Compound Dependence of the Relationship log KOW and log BCFL—A Comparison Between Chlorobenzenes (CBs) for Rainbow Trout and Polycyclic Aromatic Hydrocarbons (PAHs) for Daphnia. *ESPR.* 1995;2:33–6.
- Wittig R, Ballach HJ, Kuhn A. Exposure of the Roots of *Populus nigra* L. cv. Loenen to PAHs and its Effect on Growth and Water Balance. *ESPR.* 2003;10:235–44.
- Chaudhry Q, Blom-Zandstra M, Gupta SK, Joner E. Utilising the Synergy between Plants and Rhizosphere Microorganisms to Enhance Breakdown of Organic Pollutants in the Environment. *ESPR.* 2005;12:34–48.
- Grabic R, Novák J, Pacáková V. Optimization of a GC-MS/MS Method for the Analysis of PCDDs and PCDFs in Human and Fish Tissue. *J High Resol Chromatogr.* 2000;23:595–9.
- Kathleen AM, Gale RW. Investigation of Distribution of Organochlorine and Polycyclic Aromatic Hydrocarbon Compounds in the Lower Columbia River Using Semipermeable Membrane Devices. Portland, Oregon: U.S. Geological Survey. Water-Resource Investigation Report. 1999;99–4051.
- Johnson BT, Huckins JN, Petty JD, Clark RC. Collection and detection of lipophilic chemical contaminants in water, sediment, soil, and air—SPMD-TOX. *Environ Toxicol.* 2000;15:248–52.
- ISO 8692. Water quality. Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*. 1989. p. 11.
- Lukavský J. Microprocedure for standard marine algal bioassay (ISO 10253). *Algol Stud.* 2001;101:137–47.
- ISO 11348. Water quality. Determination of the inhibitory effect of water samples on the ligh emission of *Vibrio fischeri* (*Luminiscent bacteria* test); 1998. p 20.
- ISO 6341. Water Quality. Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea). International Organisation for Standardisation; 1982. p. 9.
- Kočí V, Mlejnek M, Kochánková L. Toxicological evaluation of exposed SPMD membranes. *Centr Eur J Chem.* 2003;1:28–34.