Advantages of algal toxicity bioassays for SPMD evaluation

Výhody řasových testů toxicity pro hodnocení SPMD

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Abstract

This paper focuses on monitoring of persistent organics pollutants (POPs) in the aquatic environment. POPs usually occur in trace concentrations, which are difficult to determine with conventional analytical methods.

A passive sampling method, using semipermeable membrane devices (SPMDs), in combination with algal toxicity bioassay and bacterial bioluminescence assay were used. *Scenedesmus subspicatus* is sensitive to variable concentrations of persistent organic pollutants contained in sample. An advantage of algal assay is low sensitivity to impurities originating from commercial triolen used for filling the SPMDs.

Introduction

Semipermeable membrane devices (SPMDs) represent a new integral, passive sampling methodology reflecting aquatic pollution. SPMDs are highly effective dosimeters for assessing the bioavailability of hydrophobic, lipophilic organic contaminants in water. These devises have several advantages over living organisms: they give reproducible results, they do not metabolise accumulated compounds, and they are durable in severely polluted environments. SPMDs can be used in various ecosystem compartments including sediment, water, soil and air.

Passive sampling offers a lot of advantages as compared to common sampling methods. The main disadvantages of point sampling methods are:

low sensitivity for trace levels of contamination (followed by expensive pre-concentration of large volumes of water and analytical technique needed for acceptable detection limits), accidental concentration variation of pollutants, limitations in determination truly-dissolved (bio available) phase – all resulting to high sampling and analytical costs. SPMDs are helpful in solution all the above-mentioned problems.

This work is related to a study already published in Czech Phycology. This study which was aimed at application of algal bioassays for toxicity evaluation of SPMD (Kočí, V., & al. 2002). This study tries to find limits for using of algal (*Scenedesmus subspicatus*) and bioluminescence bacterium (*Vibrio fischeri*) bioassays for SPMD samples toxicity evaluation. Therefore it is necessary to test samples from seemingly clean (nonpolluted with POPs) as well as from contaminated areas and to compare their toxicity.

SPMDs were exposed in four main rivers of the Czech Republic. They were selected to comprehend more and/or less polluted rivers flowing through differently industrialised areas with different level of contamination. Of course toxicity of those samples was compared to field blanks.

Materials and methods

Selected locations

Four locations in the Czech Republic were selected for the study (Fig. 1): Vltava River downstream near its embouchure, Labe River downstream on the border with Germany, Dyje and Morava Rivers downstream near the border with Slovakia. The Labe River is the biggest river in the Czech Republic with an average water flow rate of 293 m^3/s. It receives water including pollutants from the second biggest river Vltava (151 m^3/s). Both rives flowing through large cities receives treated wastewaters from numerous industrial and municipal wastewater treatment plants. The Dyje River (12.3 m^3/s) flows through the rural area and, therefore, it is not supposed to be polluted. Toxicity of sample obtained in such an area could serve approximately as natural background toxicity. The Morava River (59.6 m3/s) flows through the less industrial area as compared to Labe and/or Vltava Rivers. Therefore it is good to check whether the pollution is also lower.

Semi-permeable membrane device (SPMD) sampling

Semipermeable membrane device is designed for long-term monitoring of lipophilic, hydrophobic contaminants in aquatic environment. The membrane is filled with triolein, which is in properties similar to fish fat. Persistent organic pollutants such as: PAHs, PCBs, OCPs, PCDD/Fs are accumulated in the triolein. This bioconcentration of contaminants trough thin synthetic membrane is similar to that in biota tissue and it was mathematically described (HUCKINS & al. 1990, 1999).

SPMD sampler consists of flat thin walled tube of porous low-density polyethylene (LDPE). A porosity of the LDPE is 10^{-9} m and about the same all over the area. General dimension of the standard SPMD is: width 2.5 cm (lay-flat), overall length 91 cm, and thickness approximately 75 μm. The polyethylene membrane used in the SPMD methods mimics a biomembrane. The LDPE tube is filled inside with 1 ml of synthetic lipid triolein, 1,2,3-tri-[*cis*-9-octacenoyl]glycerol. This substance is found in most of organisms. The SPMD deployment is usually accomplished with metal, stainless steel protective container.

For convenient routine application it is necessary to provide appropriate Quality Control procedures. This is essential for both sampling and analysis, which generally involves: use of set of blanks for SPMD-contamination description during transport and handling at field, from used reagents, analytical process.

SPMD deployment

At all locations the protective shields with SPMDs were sunk in a stream without any touches to the bed or bank of the river. Field blanks (membranes that accompanied SPMDs exposed in water) consisted of one membrane for each location. The average water temperature at all four locations was 14.5-19.0°C. The exposure time lasted 29-30 days. In two cases the samplers were exposed longer than it is usual, that means 45 days. It caused a vandal, who destroyed the float, where the protective shroud with SPMDs was imbedded. A diver had to find these shields and therefore the exposure time was extended up to 45 days. All the SPMDs were successfully retrieved. Biofouling from the surface was removed and the membranes were transported at 10°C back to the laboratory.

In the laboratory, the SPMDs were rinsed with distilled water, quickly with acetone and air-dried. Each membrane was extracted with 250 ml of

hexane for 72 h, with solvent replacement after 24 h. The volume of the extract was reduced to 10 ml and 2.5 ml of this liquid was transferred into 2.5 ml of DMSO:aceton (1:1). Toxic effect of such prepared samples was determined.

BIOASSAY USED

Bioluminescence assay

A bioluminescent bacterium *Vibrio fischeri* is a well spread commercial toxicity test based on the inhibition bioluminescence activity of the marine bacteria as a result of adverse effect of a toxicant. The experiments were performed according to the standard procedure described by ISO11348 guideline. The samples toxicity was determined in a medium containing 2% of sodium chloride. As a test organisms reconstituted bacteria from a lyophilised reagent were used. Concentration of the carrier solvent (DMSO + acetone 1:1) was lower than 0.5%. The luminescence intensity was measured at 15°C at 5-, 15- and 30- min exposure times. Seven concentrations, excluding the control, were tested and each test was run in duplicate. The EC50 values were calculated using linear regression (log [concentration] vs. probit [perceptual inhibition of luminescence intensity]). Kalium dichromate was used as a standard toxicant, positive control, to check the sensitivity of the test system.

Algal growth inhibition test

This method is based on the biomass growth inhibition of freshwater algae *Scenedesmus subspicatus* as a result of toxic effect of a toxicant (ISO8692:1989). The experiments were carried out following the standard procedure. The macronutrient solution contains in one litter of deionised water: NaNO3 (467 mg), $Ca(NO_3)2 \cdot 4H_2O$ (59 mg), K_2HPO_4 (31 mg), $MgSO_4 \cdot 7H_2O$ (25 mg), $Na_2CO_3 \cdot 10H_2O$ (57 mg), $FeCl_3$ (1 mg) and 0.08 ml micronutrient solution (STAUB 1961). As a test organism algae pre-cultivated under the same conditions as the test was performed (27±2°C, continual light 6 000-10 000 lux) were used. Concentration of the carrier solvent (DMSO + acetone 1:1) was lower than 1.0%. The biomass growth was measured at 24-, 48-, 72- and 96-hours exposure times. Five concentrations, excluding the control, were tested. The EC50 values were calculated using

the non-linear regression. Kalium dichromate was used as a standard toxicant, positive control, to check the sensitivity of the test system.

Results

All the tests were provided under conditions described above. They were run in two duplicates using 5-8 sample concentrations and a control. Results of toxicity testing are summarized in following paragraphs.

Bioluminescence assay

All the samples inhibited the bioluminescence of bacteria *Vibrio fischeri* effectively. As was expected, the less toxic samples were from the Dyje River, both in the 1st and 2nd interval. It was closely followed by Morava 2nd SPMD sample. All the other samples (Labe1st,2nd, Vlatava1st,2nd and Morava1st) were a bit more toxic than Morava 2nd and by the same mail less toxic than Dyje samples. EC50 values of these five samples were almost identical.

Toxicity of field blanks was undesirably high. EC50 values were equal the most toxic samples from SPMDs exposed in rivers. Field blanks were even more toxic than some samples from exposed membranes, which should contain POPs (see Tab. 1). Otherwise field blanks are supposed to be less toxic than sample from exposed SPMD. Results from toxicity testing on *Vibrio ficheri* are summarised in Tab. 1 and Fig. 2.

Algal bioassay

In general, the sensitivity of *Scenedesmus subspicatus* to the toxicant mixtures in the SPMDs was lower or in the same range as that of bioluminescence assay. On the other hand, there were grater differences within individual samples than observed in bioluminescence assay. The shifting toxicity referees to the different industrial pollution in monitored areas and it accomplished our expectation.

The Dyje samples exhibited the lowest toxicity in the test, followed by the Labe, Morava and Vltava SPMD samples. In the Fig. , there is shown considerable difference of EC50 within individual samples. It accentuates the sensitivity of *Scenedesmus subspicatus*.

However, due to technical reason (limited amount of the sample) the values of EC50 of field blanks were not determined. The highest tested concentrations didn't reach significant inhibition of the algal growth (see Fig.).

Discussion

Commercially available triolein, which is the SPMD filled with, contains 5% of impurities. Methyl-oleate probably represents the major part of its 5% contaminant. During the SPMD deployment in a river, the methyl-oleate diffuses into the exterior of the membrane and here it can be rinsed away with river flow. When the SPMD membrane is not exposed in water body (field blank), the methyl-oleate cannot rinse away from the surface and thus finally it occurs in the tested sample.

High toxicity of field blanks to *Vibrio fischeri* was probably caused by mentioned impurities in triolein. Therefore it is not advisable to use bioluminescence assay for field blanks evaluation as a quality control of SPMD sampling. On the other hand, there was observed no toxic effect of those same field blanks to *Scenedesmus subspicatus*. Even if there were tested higher concentrations tested in comparison with to the bioluminescence bacterium assay. It means that, in contract to *Vibrio fischeri*, *Scenedesmus subspicatus* is not sensitive to impurities contained in commercial triolein and to contaminants, which got into the sample during its preparation or SPMD installation (Tab. 2 and Fig. 3). It is a great advantage of toxicity test on *Scenedesmus subspicatus*.

Bioluminescence assay presents one advantage over the algal test. It required smaller amount of the sample. However, it cannot markedly distinguish different toxicity of different samples. Simply, bioluminescence bacterium *Vibrio fischeri* is not sensitive to variable concentration of POPs in tested solution (see Fig. 2) whereas green algae *Scenedesmus subspicatus* is able to distinguish individual samples markedly.

Conclusion

The application of algal bioassay with SPMD passive sampling method should be recommended as sensitive tool for monitoring of persistent organic pollutants in the aquatic environment. Green algae *Scenedesmus subspicatus* is, in contrast to *Vibrio fischeri*, able to distinguish low differences of samples toxicity. Also, impurities contained in triolein or impurities which contaminate sample during test preparation are not too toxic for this alga.

References

HUCKINS, J.,N., TUBERGEN, M.,W., & MANUWEERA, G.,K., (1990) Semipermeable membrane devices containing model lipid: a new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential. Chemosphere 20, pp. 533-552.

HUCKINS, J.N., PETTY, J.D., ORAZIO, C.E., LEBO, J.A., CLARK, R.C., GIBSON, V.L., GALA, W.R., & ECHOLS, K.R.: (1999) Determination of Uptake Kinetics (Sampling Rates) by Lipid-Containing Semipermeable Membrane Devices (SPMDs) for Polycyclic Aromatic Hydrocarbons (PAHs) in Water. ENVIRON.SCI.TECHNOL. 33:, pp.3918-3923.

ISO 11348 (1998) Water quality- Determination of the inhibitory effect of water samples on the ligh emission of *Vibrio fischeri* (Luminiscent bacteria test).

ISO 8692:1989 Water quality. Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*.

KOČÍ, V., MLEJNEK, M., OCELKA, T., KOCHÁNKOVÁ, L. (2002): Application of algal bioassay for toxicity evaluation of SPMD. Czech Phycology 2, pp.129-137

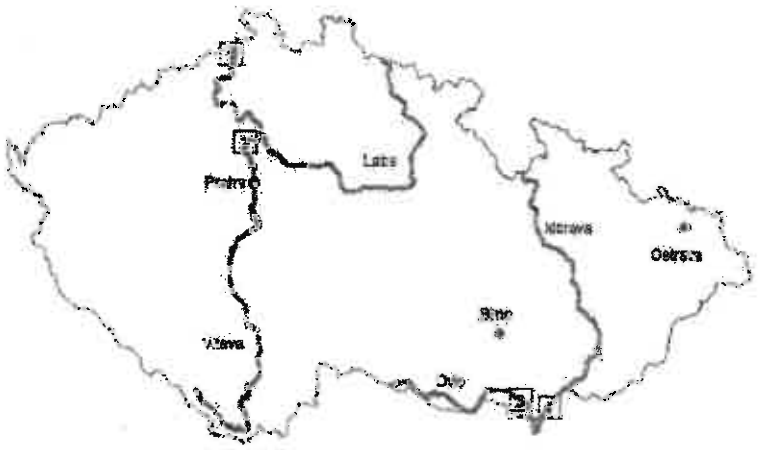

Fig. 1 - Semipermeable membrane device (SPMD) deployment locations: 1-Labe River downstream in Hřensko, 2-Vltava River downstream in Zelčín, 3-Dyje River downstream, 4-Morava River downstream.

Tab. 1 - Toxicity of samples from exposed SPMDs and from Field blanks in bioluminiscence assay. Exposure time: 15 min.

Profile	Period 1	Period 2	Field blank
	EC 50 (15 min.), [$V_{L[ml]}/L$][a]		
Labe (95% CI)[b]	0.33 (0.24 - 0.45)	0.72 (0.63 - 0.90)	0.33 (0.28 - 0.34)
Vltava (95% CI)	0.38 (0.32 - 0.44)	0.50 (0.42 - 0.63)	0.28 (0.22 - 0.37)
Morava (95% CI)	0.35 (0.23 - 0.55)	0.63 (0.54 - 0.72)	0.31 (0.26 - 0.35)
Dyje (95% CI)	0.70 (0.55 - 0.93)	0.75 (0.54 – 1.1)	0.29 (0.25 - 0.33)

[a] Concentration are based on the amount of SPMD triolein.
[b] CI, confidence interval.

Tab. 2 - Toxicity of the SPMDs samples in algal assay. Exposure time: 96 h.

Profile	Period 1	Period 2
	EC 50 (15 min.), [$V_{L[ml]}/L$][a]	
Labe (95% CI)[b]	1.1 (0.72 – 1.6)	1.3 (0.44 – 3.78)
Vltava (95% CI)	0.26 (0.067 - 0.99)	0.81 (0.59 – 1.1)
Morava (95% CI)	0.35 (0.064 – 1.8)	0.99 (0.03 – 31)
Dyje (95% CI)	1.7 (1.6 – 1.9)	3.9 (1.53 – 9.9)

[a] Concentration are based on the amount of SPMD triolein.
[b] CI, confidence interval.

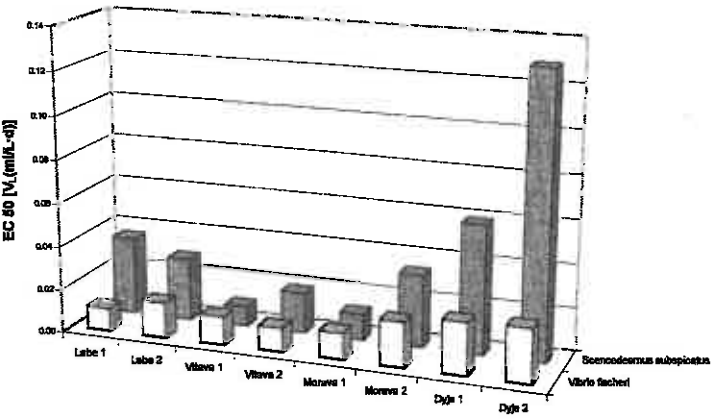

Fig. 2 - A comparison of monitored profiles toxicity and sensitivity of used bioassays.

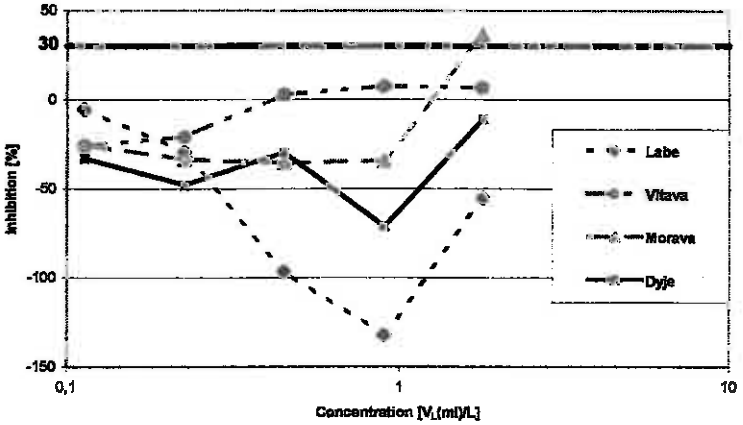

Fig. 3 -Dose-response curves of field blank samples. Test organism *Scenedesmus subspicatus*. Exposure time: 96 h.