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CYTOTOXIC, MUTAGENIC AND GENOTOXIC EFFECTS OF SEDIMENTS FROM CZECH RIVER BASIN

Martin Beníšek[1], Klára Hilscherová[1], Henner Hollert[2], Luděk Bláha[1]

[1] Research centre for toxic compounds in the environment (RECETOX), Faculty of Science, Masaryk University, Kamenice 126/3, 625 00 Brno, Czech Republic, e-mail: benisek@recetox.muni.cz
[2] Department of Ecosystem Analyses, Institute for Environmental Research (Biology V), RWTH Aachen University, Worringerweg 1, D-52074, Aachen, Germany

Abstract: *This study summarizes effects of 14 sediments collected from rivers in Southern Bohemia (Czech Republic). After Soxhlet extraction, chemical analyzes of sediment extracts were performed and extracts were further tested for cytotoxicity and micronuclei induction with fish hepatoma cell line RTL-W1 and for mutagenicity by Ames fluctuation assay. Results from the toxicological experiments are compared with chemical analyses.*

Keywords: sediment, cytotoxicity, genotoxicity, mutagenicity

INTRODUCTION

Chemical contaminants present in the environment can negatively affect living organisms and cause either acute or chronic (sub-lethal) effects such as genotoxicity, carcinogenicity, immunotoxicity or endocrine disruption (Eisenbrand et al. 2002). Important group of chemicals with chronic effects are persistent organic pollutants like polychlorinated dioxins and furans (PCDDs/Fs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) or organochlorine pesticides (OCPs) (Jones, de Voogt 1999). These chemicals are often present in several environmental matrices.

In this study we studied effects of sediments extracts from Czech rivers using *in vitro* biotests and compared the results with chemical analyses of dominant contaminants.

METHODS

Chemical analysis. Sediments were collected from rivers in Southern Bohemia in 2008, freeze-dried and extracted by Soxhlet extraction. These organic extracts were used to determine residues of PCBs, PAHs and OCPs

using gas chromatography and were also used for *in vitro* experiments.

Cytotoxicity. Fish hepatoma cell line RTL-W1 was used to study cytotoxicity of sediment extracts by neutral red uptake assay in 96 well plates (48 h of incubation in 20 °C).

Genotoxicity (Micronucleus assay). RTL-W1 cells were further used to study genotoxicity by *in vitro* micronucleus assay. Cells were seeded on cover glasses plated on 6-well plates and exposed for 72 h. Then, cells were fixed and stained by acridine orange. Numbers of micronuclei in 2000 cells were evaluated in the fluorescence microscope, and induction factor (relative to the controls) was determined.

Mutagenicity (Ames fluctuation assay). The assay is similar to classical Ames test of the reverse mutations in histidin synthesis genes of *Salmonella typhimurium*. Two tester strains TA98 and TA100 were used to investigate both base pair substitution and frameshift mutation. After 10h of pre-incubation, bacteria were exposed to studied samples for 100 min in 24-well plates. Reversion indicator medium containing pH indicator (bromcresol purple) was added and equivalents were distributed into the 384-well plates and incubated for 48 h. Then, number of purple to yellow shifted wells (reverse mutations) was counted.

RESULTS

Tested sediments were from localities with variable pollution, from relatively clear localities to more contaminated localities (tab. 1).

Tab. 1: Contaminants concentrations in sediments extracts

Nr of sediment	1	2	3	4	5	6	7
Σ PCB (mg/filter)	0.612	1.832	0.904	0.578	0.312	2.314	0.526
Σ 16 PAH (ng/g)	25	505	402	1117	1951	1220	2730
Σ 28 PAH (ng/g)	34	661	538	1470	2542	1581	3631
Σ HCH (mg/filter)	1.256	0.602	0.744	1.01	1.19	0.888	1.26
Σ DDT (mg/filter)	2.046	2.164	4.142	3.134	7.544	4.244	1.54

Nr of sediment	8	9	10	11	12	13	14
Σ PCB (mg/filter)	0.182	0.584	1.802	1.762	0.656	0.836	0.67
Σ 16 PAH (ng/g)	60	1844	912	643	209	156	255
Σ 28 PAH (ng/g)	81	2385	1189	857	289	210	344
Σ HCH (mg/filter)	1.23	0.55	0.81	1.56	0.754	1.106	1.444
Σ DDT (mg/filter)	1.01	3.144	2.888	5.438	0.934	2.284	2.068

Legend: PCB – polychlorinated biphenyls, PAH – polycyclic aromatic hydrocarbons, HCH – hexachlorocyclohexane, DDT – dichlorodipheynltrichloroethane

Most of tested sediments were cytotoxic for fish RTL-W1 cells (number of viable cells was lower than 80 % of the control levels – IC 20). Lowest IC 20 was around 20 mg/ml (tab. 2). Only selected samples were studied for genotoxicity and one sediment from the contaminated locality (number 2) displayed significant induction of micronuclei (induction factor 1.5, details not shown).

Tab. 2: Cytotoxicity and mutagenicity of organic extracts of tested sediments

Nr of sed.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cytotoxicity IC20[1] (mg/ml)	100	30	25	20	37	36	50	50	45	45	32	100	95	25
Ames test (IF) TA98 + S9	1.9	1.7	2.5	2.6*	3.5*	2	2.2*	2	2.2	1.7	1.6	2.1	0.7	1
Ames test (IF) TA100+S9	1.4	2	2.7*	2	4.1*	3*	4.3*	2.7*	3*	2.5	2.4	1.9	2.1	2.2*

[1] IC 20 – concentration of sediment causing 20 % inhibition of cells viability compared to solvent control levels

* – sediments with statistically significant mutagenic potential according to Fisher test

All sediments were tested for mutagenicity in both tester strains and both with and without S9 fraction. No significant effects were seen when tested without S9 fraction (results not presented). Statistically significant effects were observed in samples with S9 fraction (induction factors are in table 2)

DISCUSSION

Freshwater sediments may serve as reservoirs of contaminants in the environment, which may later be released and affect biota. In our study, chemical analysis confirmed the presence of important POPs at various levels corresponding to low to moderate pollution when compared with other studies from the Czech republic (Hilscherova et al. 2001; Vondracek et al. 2001). *In vitro* biotests confirmed overall cytotoxicity of sediments from sites with higher chemical pollution. From the results of Ames fluctuation test, the highest induction factors (higher than 3) were observed in TA100 strain for sediments with highest PAH contamination. Similar correlation between the PAH contamination and mutations in TA100 strain (base pair mutation) was described also in a review of Chen (2004). Positive results for only few sediments tested by the strain TA98 then indicate possible high impact of PAHs or other compounds with base pare mutagenic activity for mutagenicity of tested sediments. Cytotoxic and mutagenic potential of sediments was described also in the study of Hollert et al. (2000). Only one of tested sediments was

found to be genotoxic in micronucleus assay (number 2) and only relatively weak induction factor compared to results from other study (Boettcher et al.) was seen for this sediment extract.

CONCLUSION

In conclusion, sediments extracts from moderately contaminated localities in Southern Bohemia had cytotoxic and mutagenic potential in *in vitro* assays and PAHs or similar compounds seem to be among those responsible for mutagenic potential of some tested sediments.

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