

Effect-directed analysis of mutagenic polyaromatic compounds in sediment extracts



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Introduction

Environmental matrices like sediment extracts often contain a broad range of toxicants that can exhibit different hazardous effects like mutagenic effects. Effect-directed analysis is a valuable approach to identify these compounds in complex mixtures using different kinds of fractionation methods and bioassays. Mutagenic effects in environmental samples were not only observed for the well known Polycyclic Aromatic Hydrocarbons (PAHs) like benzo(a)pyrene but also for their alkylated derivatives and the medium polar to polar Polycyclic Aromatic Compounds (PACs), e.g. Nitro-PAHs. To determine mutagenic effects in sediment samples influenced by different

industrial and communal effluents samples were collected 2006 at three different "hot spots" in the river Elbe downstream of the industrial region of Pardubice (Czech Republic) and in its tributaries Biliina downstream of Most (Czech Republic) and in the creek Spittelwasser (Germany) in the heavily polluted industrial area of Bitterfeld. Upstream the sampling site near Pardubice (P) chemical industry and a refinery, and near Most (M) textile, chemical and petrochemical industry are present whereas the Bitterfeld (S) area is known to be heavily contaminated mainly by the former production of a broad range of chemical products.

fraction no.	eluting compounds
1	alkanes
2	alkanes, sulphur, PCBs with 2 or 4 chlorines in <i>ortho</i> -position, PCNs with 3 chlorine atoms
3	naphthalene, biphenyl, PCBs with 1 or 2 chlorines in <i>ortho</i> -position, PCNs with 3 to 5 chlorine atoms
4	non- <i>ortho</i> -chlorinated PCBs, PCDDs/Fs
5	Small-sized PAHs like acenaphthylene with more than two aromatic rings
6	PAHs with three aromatic rings (anthracene)
7	PAHs with four aromatic rings (pyrene)
8	PAHs with four aromatic rings (chrysene)
9	PAHs with five aromatic rings (benzo[a]pyrene)
10	PAHs with six aromatic rings (benzo[ghi]perylene)
11	PAHs with seven aromatic rings (coronene)
12	mainly mononitro-PAHs
13	(hydroxy-)quinones, keto-, dinitro-, hydroxyl-PAHs, N-heterocycles with rising polarity
14-18	

Table 1: Fractions and characterising compound groups.

Sample preparation

Freeze-dried and sieved (< 63 µm) sediment samples were extracted using pressurised liquid extraction (PLE) and cleaned using a PLE-assisted dialysis procedure with a semipermeable membrane [1]. Fractionation was performed on coupled and automatically switched normal-phase HPLC-columns separating compounds according to their polarity, planarity and size of the aromatic system in one run [2]. 18 fractions were gained characterised by a range of model compounds as described in table 1. Fraction Most 10 characterised by benzo[a]pyrene was further fractionated using reversed-phase HPLC yielding 11 subfractions separated according to their lipophilicity.

All fractions, their parent extracts/fraction and reconstituted samples prepared using equal amounts of each fraction of the respective sample were tested for mutagenic effects in the Ames fluctuation assay according to Reifferscheid (TA98) [3] and Xenometrix (TA 100) [4].

Mutagenic effects subfractions of Most fraction 10

Mutagenic effects of Most fraction 10 were mainly distributed between subfractions M10-5 to 8 containing benzofluoranthenes, benzo(e)pyrene and perylene (M10-5) and benzo(a)pyrene (M10-6). Additionally, compounds with mass weight 266 and 278 were detected in these fractions. Interestingly, not only indirect mutagenic effects were observed: M10-7 was much more mutagenic with than without metabolic activation as well as mutagenic effects of M10-5 were nearly equal with and without activation. Effects of the reconstituted samples were in the same range like those determined for the parent fraction indicating a good compound recovery and the absence of suppressing or toxic effects.

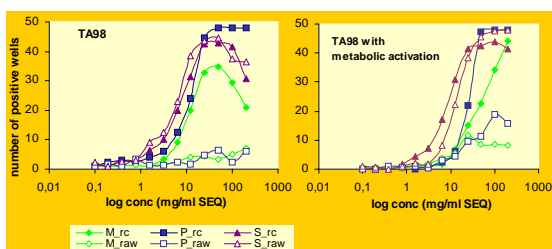


Fig. 1: Dose-response curves of the Pardubice (P), Most (M) and Bitterfeld (S) cleaned raw extracts (raw) and reconstituted samples (rc).

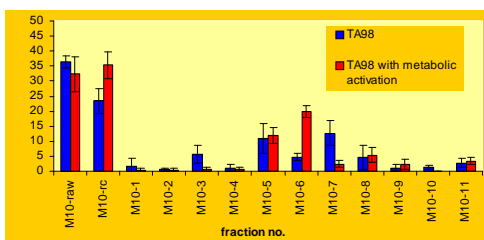


Fig. 3: Mutagenicity patterns of fractions obtained from the subfractions of Most (M) fraction 10 that is characterised by PAHs with mass weight 252 like benzo[a]pyrene. Concentrations in the assay were equal to 0.4 g/ml sediment. Maximum number of positive wells is 48. raw: cleaned raw extract, rc: reconstituted sample.

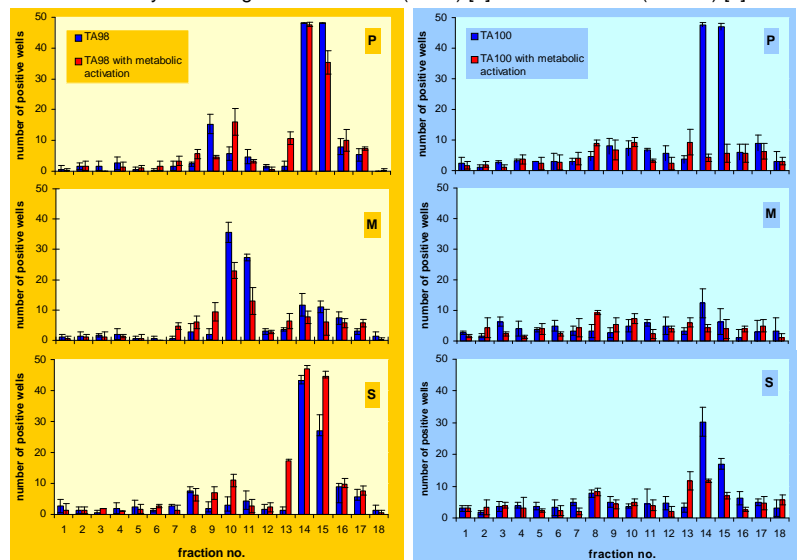


Fig. 2: Mutagenicity patterns of fractions obtained from Pardubice (P), Most (M) and Bitterfeld (S) extracts after fractionation using tester strains TA98 (left) and TA100 (right) with and without metabolic activation. Concentrations in the assay were equal to 0.2 g/ml sediment. Maximum number of positive wells is 48.

Mutagenic effects of cleaned extracts and reconstituted samples

Mutagenic effects observed for the Most and Pardubice cleaned raw extracts with and without metabolic activation were significantly lower than for the reconstituted samples (fig. 1) indicating a suppression by sample compounds that may be removed during fractionation. In contrast, both dose-response curves observed for the Bitterfeld cleaned raw extract and reconstituted sample were nearly equal. The Bitterfeld cleaned raw extract as well as the Bitterfeld and Most reconstituted samples exhibited cellular toxicity at the highest concentrations.

Mutagenic effects of fractions

Whereas fractions 1-9 were non or only slightly mutagenic esp. Most fractions 10 and 11 characterised by PAHs with five and six fused rings (tab. 1) and coeluting compounds (e.g. alkylated PAHs, S- and O-heterocyclic PACs) showed high mutagenic effects using TA98 with but also without metabolic activation (fig. 2). Because PAHs need to be metabolised to be mutagenic also non identified direct acting compounds may be present in these fractions. The highest mutagenic effects were observed for the Bitterfeld and Pardubice fractions 14 and 15 with and without metabolic activation and for both tester strains. These fractions are characterised by medium polar compounds like dinitropyrenes that are known to be potent mutagenic substances, keto- and hydroxy-PAHs, quinones and N-heterocyclic PACs.

Outlook

To identify single mutagenic compounds fractions will be further fractionated, tested for mutagenic effects and chemically analysed. Synthetic solutions of identified single compounds will be tested in the Ames fluctuation assay to confirm their mutagenic potential.

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- [4] Flückiger-Iser S et al. 2004, Mut. Res. 558, 181-197

