Accepted Manuscript

Comparing total and accessible concentrations of hydrophobic organic contaminants in sediments and suspended particulate matter in the Danube River

Michaela Belháčová-Minaříková[1], Ian Allan[2] and Branislav Vrana[1*]

[1]Masaryk University, Faculty of Science, RECETOX, Kamenice 753/5, 625 00 Brno, Czech Republic

[2]Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, NO-0349, Oslo, Norway

*Corresponding author:

Branislav Vrana
RECETOX
Faculty of Science
Masaryk University
Kamenice 753/5, pavilion D 29
625 00 Brno
Czech Republic
tel: +420 549 494 975
e-mail: vrana@recetox.muni.cz

Abstract

Contamination of aquatic ecosystems by hydrophobic organic contaminants (HOCs) is often assessed based on their concentrations in riverbed sediment and suspended particulate matter (SPM). However, total HOC concentration (C_{TOT}) in sediment or SPM is of limited value for evaluating exposure of benthic or pelagic organisms. The accessible HOC concentration (C_{AS}) presents a useful parameter quantifying the overall pool of HOC in sediment or SPM available for fast partitioning to the water phase or to biota. We applied ex situ sequential equilibrium partitioning with silicone elastomer sampler at a high sampler/SPM phase ratios to measure C_{AS} of HOC in SPM from the Danube River. We compared C_{TOT} and C_{AS} in SPM with those in surface layer sediment collected at the same sites in order to evaluate whether HOC monitoring in the two matrices provides equivalent information on environmental quality. At most sites, there was a good agreement and correlation of organic carbon (OC)-normalized C_{TOT} in SPM and sediment for polychlorinated biphenyls (PCBs) and a majority of organochlorine pesticided (OCPs). In contrast, C_{TOT} of polycyclic aromatic hydrocarbona (PAHs) in SPM were up to a factor 10 lower in SPM than in sediment. Site specific differences of OC -normalized C_{AS} concentrations in SPM and sediments were observed for PCBs and OCPs, with accessibility mostly lower in SPM than in sediment. SPM and riverbed sediment samples provide complementary, but not mutually interchangeable information on HOC contamination.

1   Introduction

Suspended particulate matter (SPM) is an important structural and functional element in aquatic ecosystems next to the water phase and the sediment. The chemical and geological composition of SPM depends on catchment area geology, land use, urbanization, state of wastewater treatment technology, season, the nutrient supply, the water discharge and the weather conditions (Schubert et al. 2015). SPM represents the most mobile fraction of sediments, but besides resuspended fine riverbed sediment particles, SPM may also contain a significant portion of phytoplancton and its degradation products (Sullivan et al. 2001). The quantity of SPM in the water phase plays an important role for the total load of organic and inorganic substances because a significant but varying portion of these substances are transported particle-bound (Cornelissen et al. 2005). Floods, dredging and ships may lead to a significant remobilization of sediments accompanied by increased contaminant levels in the water column (Eggleton and Thomas 2004).These considerations illustrate the complex function of SPM and sediment as sink, transport vehicle and source of particle-bound substances, including HOCs (Bartoszek and Gruca-rokosz 2019). The HOCs associate with SPM to the extent depending on their hydrophobicity (characterized by octanol-water partition coefficient K_{ow}) and the amount and type of SPM available. The transport of HOCs with a log K_{ow}>6 within the water column is mainly associated with the hydraulic remobilization of sediments and the subsequent transport and re-sedimentation of SPM (Liška et al. 2015).

Since SPM acts as a transportation vehicle for HOCs in the water column until it eventually settles at sites with a low water flow energy, it is assumed to match HOC contamination of freshly deposited riverbed sediment. Therefore, monitoring of HOCs for contaminant trend or compliance assessment in SPM has often been recommended as an alternative to bed sediments (Schubert et al. 2012).

Total concentrations (C_{TOT}) in SPM may be useful for the estimation of SPM-bound HOC loads or for monitoring spatial or temporal contamination trends. C_{TOT} are typically measured by applying conventional solvent extraction methods. However, from the risk assessment perspective, C_{TOT} in SPM or sediment has limited value for evaluating HOC exposure and related risk to benthic or pelagic organisms. This is because C_{TOT} does not distinguish between HOCs that are irreversibly adsorbed to particles and those which can easily partition to porewater or the water column, and thus C_{TOT} does not reflect contaminant bioavailability. On the other hand, soft extraction techniques, such as partitioning passive sampling, address HOC bioavailability in sediments (Greenberg et al. 2014; Jahnke, Mayer, and McLachlan 2012; Jonker et al. 2018; Lydy et al. 2015; Maruya et al. 2009; Witt et al. 2013). Smedes, Van Vliet, and Booij 2013 demonstrated that equilibrium passive sampling method based on equilibrating silicone passive sampler with sediment, enables estimation of HOC's freely dissolved porewater concentration (C_{free}), as well as their accessible concentration (C_{AS}) in sediment. The C_{free} represents compound's chemical activity, which plays a key role in environmental risk assessment (Reichenberg and Mayer 2006) while C_{AS} quantifies the pool of HOCs in sediment or SPM available for fast partitioning to the water phase or biota as a result of chemical activity gradient. While applying passive sampling at low (non-depletive) sampler-sediment ratio enables the estimation of C_{free} (Niehus et al. 2018; Allan et al. 2021), passive sampling at high (depletive) ratio provides an estimate of C_{AS} (Rusina et al. 2019; M. Belháčová-Mináříková et al. 2020).

In this study, we applied ex situ sequential equilibrium partitioning with silicone passive sampler at a high sampler/SPM phase ratios with the objectives to: 1) estimate C_{AS} of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in SPM sampled at 6 sites along the Danube River during the Joint Danube Survey 3 in 2013; 2) compare C_{TOT} and bioavailability (C_{AS}) in SPM with the corresponding C_{AS} in riverbed sediments collected at the same sites and previously characterized by multi-ratio equilibrium passive sampling (M. Belháčová-Mináříková et al. 2020).

2 Materials and methods

2.1 The sampling campaign/site characterization

The Joint Danube Survey 3 (JDS 3) took place between August and September 2013 by the expedition ship Argus (Liška et al. 2015). Sampling of SPM investigated in this study was performed at 6 sampling locations along the Danube River from the border

between Austria and Germany in the upstream river stretch down to the river delta in Romania (Fig. 1, Table 1).

2.2 **Depletive equilibrium passive sampling in SPM**

In depletive extraction mode, SPM and sampler are equilibrated at a sampler/SPM phase ratio m_P/m_{SPM} that is sufficiently high to result nearly complete depletion (D) of the accessible fraction of HOC from the SPM sample by partitioning to silicone. D is the ratio of a HOC in the sampler and its total amount in the system (N_{SPM} is amount in SPM) following the capacity ratio:

$$D = \frac{N_P}{N_P + N_{SPM}} = \frac{m_P K_{PW}}{m_P K_{PW} + m_{SPM} f_{OC} K_{OC}}$$ (1)

where K_{PW} is the polymer/water partition coefficient, f_{OC} is the organic carbon content in SPM and K_{OC} is the organic carbon/water partition coefficient.

To design the SPM extraction experiment, a priori estimates of m_P/m_{SPM} were calculated according to (Smedes, Van Vliet, and Booij 2013) to result in a D value close to 1 (depletive extraction), assuming that for the accessible HOC fraction K_{OC} value is of the same order of magnitude as K_{PW}.

The accessible concentration of HOC in SPM ($C_{AS:SPM}$) is calculated as:

$$C_{AS:SPM} = \frac{N_{P:1} + N_{P:2}}{m_{SPM}}$$ (2)

where $N_{P:1}$ and $N_{P:2}$ are HOC amounts extracted by the first and the second silicone piece used for sequential SPM extraction.

The accessibility (F_{AS}) of HOCs is expressed as a fraction of the accessible/releasable concentration from the total concentration in SPM.

$$F_{AS} = \frac{C_{AS}}{C_{TOT}}$$ (3)

2.3 SPM sampling and ex situ handling

SPM samples were collected using a continuous centrifuge approach at the sampling sites in the middle of the stream on board of the ship Argus, except for the two most upstream sampling sites were collected by a centrifuge installed in a car. The centrifuge was a Z61H from Carl Padberg Zentrifugenbau GmbH,(Germany) operating at a cylinder speed of 17000 rpm. Sampling typically took from 30 minutes to several hours, depending on the concentration of SPM in water. Preservation was attained through keeping the samples in the dark and refrigerated or on ice during transportation at 4° C (ISO 5667-15 2009).

Water content of each SPM sample was determined from the mass loss of homogenized sample after drying at 105° C to constant weight. Freeze-dried sediment subsamples were homogenized in a ball mill and after removal of carbonates with hydrochloric acid, the organic carbon content (f_{OC}) was measured using an elemental analyzer (Vario TOC Cube, Elementar, Germany) as CO_2 after combustion at 1000° C.

In a Rockeval 6 instrument for pyrolysis analysis, about 80 mg of freeze-dried and homogenized SPM was heated up to 650 °C in anoxic atmosphere and by monitoring released carbon, the amorphous, more labile organic carbon content (f_{AOC}) was determined (Poot et al. 2009). In the following oxidation stage, heating up to 850°C, the residual carbon fraction was measured (f_{BC}), containing the more inert carbon types, like soot and black carbon. These results are listed in Supplementary Information 1, SI 1, including the total organic carbon content (f_{OC}), calculated as the sum of f_{AOC} and f_{BC}.

2.4 Passive sampling of SPM

AlteSil™ translucent silicone rubber (SR) sheets 0.5 mm thick (Altec, UK) were used as passive samplers of HOCs in wet SPM. The SR samplers were made by cutting strips of about 1.5-1.8 g weight with a corresponding surface area about 50-59 cm^2. Before use, the samplers were extracted with ethylacetate for 24 h to remove possible additives and low molecular weight polymers and subsequently stored in a clean glass jar (with aluminium foil in the lid) until use.

SR strips were brought into contact with 1-3 g wet SPM samples from the Danube (Table 1). The phase ratio was calculated using Eq. 1. For passive sampling, SPM was shaken with SR in 50 mL glass centrifuge tubes (cleaned in the muffle furnace at 550 °C) for 4 weeks, shaken on an orbital shaker at 150 rpm. Moreover, 30 mL of MiliQ water and sodium azide (1 g L^{-1}) to prevent biodegradation, were added. Two-step sequential extraction was applied to assure complete depletion of accessible HOCs from SPM samples (SI 2). Following extraction, SR strips were removed and rinsed with MilliQ water, dried and were ready for HOC extraction.

2.5 Determination of HOC in passive samplers

Recovery internal standards (RIS: PCB 4, 29, 185 and D_8-naphthalene, D_{10}-phenanthrene, D_{12}- perylene) were dosed on the surface of SR sampler sheets prior to extraction (SI 3). SR samplers of all sizes were 8 h Soxhlet extracted in 100 mL of

methanol. Extracts were reduced by evaporation on water bath using a Kuderna-Danish apparatus to less than 2 mL. After addition of 20 mL hexane the extract was azeotropically transferred to hexane during evaporation to ~1 mL in a Kuderna-Danish apparatus. The extracts were quantitatively split in two aliquots. The 20% extract aliquots for analysis of PAHs were further purified on a activated silica gel (dried a 160 °C, 12 h) over a glass column using 40 mL diethyl ether and 10 mL acetone elution. After addition of p-terphenyl as syringe internal standards (SIS), the volume of extracts was further reduced to approximately 0.7 mL under a gentle flow of nitrogen and quantitatively transferred to cone-shaped mini vials. Final volume was adjusted to 0.5 mL. Thereafter, samples were analyzed using a GC-MS/MS method for PAHs. The 80% extract aliquots for analysis of hexachlorobenzene (HCB), OCPs, such as dichlorodiphenyltrichloroethane (DDT) and analogues and PCBs were purified by using glass column containing 8 g silica gel modified with concentrated sulphuric acid (44%, w:w), eluted with 30 mL of hexane-dichloromethane, 1:1 (v:v) mixture. After addition of SIS (PCB 121), the eluates were Kuderna-Danish concentrated down to ~1 mL, quantitatively transferred to cone-shaped mini vials. Final volume was adjusted to 0.1 mL under a gentle nitrogen flow. Afterwards, instrumental analysis for indicator PCBs and OCPs were performed as described in SI 4. Mass of individual SR sheets was recorded after extraction and evaporation of solvents in a fume cupboard (12 h).

2.6 Determination of total HOC concentration in SPM

Total HOC concentrations in each SPM was determined in subsample by 8 h Soxhlet extraction of freeze-dried SPM (from 1.2 to 5 g dry weight according to available sample mass) in 100 mL of dichloromethane (Smedes and De Boer 1997); after addition of RIS - the same as for SR samplers. After Kuderna-Danish solvent reduction to ~2 mL and addition of ~100 mg activated copper powder, the extracts were placed in an ultrasonic bath for 30 min to remove sulphur. Subsequently, the extracts were treated and analyzed in a similar way as for SR samplers.

2.7 Instrumental analysis

All analyzed target compounds together with their physical-chemical properties are collected in SI 5.

PCBs, OCPs, PeCB and HCB were analyzed using a 7890B gas chromatograph (Agilent, USA) equipped with a 60 m x 0.25 mm x 0.25 µm HT-8 capillary column (SGE Analytical, UK) coupled to Agilent 7000B QQQ MS-MS operated in EI+ mode. At least 2 MRM transitions were recorded for each compound analyzed. Three microlitre of extract were injected in pulsed splitless mode at 280 °C. Helium was used as carrier gas at the flow of 1.5 ml min^{-1}. The GC temperature program started at 80 °C (1.5 min hold), ramped 40 °C min^{-1} to 200 °C (18 min hold), and finally ramped 5 °C min^{-1} to 305 °C.

Chromatographic analysis for PAHs was performed using a high performance Agilent 7890 GC system (Agilent, Germany). Analytes were separated on a 60 m DB-5MS column (0.25 mm I.D., film thickness: 0.25 µm; Agilent J&W, USA) and interfaced with

MS/MS Triple Quadrupole 7000B MS (Agilent, Germany). Detection was performed in selected ion monitoring mode (SIM), temperature of ion source was 320 °C and quadrupole temperature was 150 °C. A 1 µL-sample was injected in splitless mode at 280 °C. Helium was used as carrier gas at a constant flow of 1.5 mL min^{-1}. The GC oven temperature program was as follows: initial oven temperature of 80°C (1 min hold), then ramped at 15 °C min^{-1} to 180 °C and then at 5 °C min^{-1} to 310 °C (20 min hold).

2.8 Quality assurance and control (QA/QC)

The recovery of target compounds in the procedures described above was assessed by addition of RIS to all analyzed samples (SI 3). A solvent blank, i.e. pure solvent spiked with RIS, was included in every batch consisting of nine samples to check for any interferences or contamination from solvents and glassware. Non-exposed SR samplers were analyzed to observe any contamination coming from the applied SR material. Procedural recovery samples, i.e. non-exposed SR samplers spiked with a mixture of investigated compounds were also included to assess analytes recoveries. The recovery results showed ranging between 70% and 106%, however lower recoveries were observed for more volatile procedural standards (D_8-naphthalene and to a lesser extent D_{10}-phenanthrene and PCB 4) subjected to evaporation in some cases.

3   Results and Discussion

3.1 Comparing total concentrations of HOCs in SPM and sediment

For a better comparison of HOC concentrations in SPM samples with those measured in corresponding upper layer riverbed sediment samples, we normalized the data to total organic carbon content (OC). Since SPM, representing the actual contamination status of upstream river, after deposition create river bed sediment, it´s logical to compare total contaminant levels in SPM ($C_{TOT:SPM,OC}$) with those in sediment ($C_{TOT:SED,OC}$) collected at the same site. $C_{TOT:SPM,OC}$ of PCBs, PeCB, HCB, DDT and its metabolites (DDX) and PAHs were compared with HOC $C_{TOT:SED,OC}$ measurements in sediments collected during JDS 3 campaign (M. Belháčová-Minaříková et al. 2020).

The sum of total OC normalized concentrations ($C_{TOT:SPM,OC}$) of the seven analyzed PCB congeners ranged between 90 and 953 µg kg^{-1}. The highest PCB concentration was observed in the central Danube at site JDS 38 (likely related to the PCB input from the Tisza river (Kočan et al. 2001).This is in a good agreement with elevated PCB concentrations found in sediment sample collected at the same site (M. Belháčová-Minaříková et al. 2020). The $C_{TOT:SPM,OC}$ pattern of individual PCB congeners contains higher proportion of heavier PCB congeners (PCB 138, PCB 153 and PCB 180), especially a di-ortho substituted non-coplanar congener, PCB 153.

$C_{TOT:SPM,OC}$ of pentachlorobenzene (PeCB) ranged from 7 to 40 µg kg^{-1} with its maximum value at JDS 6, while hexachlorobenzene (HCB) ranged from 16 to 190 µg kg^{-1} and the highest concentration was observed at JDS 14, downstream

Bratislava, which corresponds well with observations in sediment (M. Belháčová-Minaříková et al. 2020).

The summed $C_{TOT:SPM,OC}$ of DDT and its metabolites (DDx, 6 compounds) ranged from 1 to 840 µg kg^{-1}at sites JDS 6 and JDS 68, respectively. The highest observed concentrations were for 4,4'-DDD and 4,4'-DDE isomers towards the river delta at JDS 68. This is in full agreement with our measurements in sediments (M. Belháčová-Minaříková et al. 2020) as well as with the findings during previous Danube surveys (JDS 1 and JDS 2). According to Umlauf (2014) the longitudinal concentration profiles in sediment suggest DDx releases into the lower Danube originating from left bank sources and tributaries, especially Arges, Siret andPrut.

For most PAHs, the highest $C_{TOT:SPM,OC}$ concentrations were observed at JDS 38, close to the urban agglomeration around Belgrade. At JDS 38, the maximum $C_{TOT:SPM,OC}$ of PAHs were mostly observed with 4 and more condensed aromatic rings (fluoranthene, pyrene, chrysene, benzo[*b*]fluoranthene and benz[*a*]anthracene). A specific spatial $C_{TOT:SPM,OC}$ trend was observed for almost all PAHs, with elevated concentrations in the middle Danube and lower concentrations further downstream. Overall, $C_{TOT:SPM,OC}$ of PAHs varied between 61 and 2832 µg kg^{-1} for dibenz[*a,h*]anthracene (JDS 22) and fluoranthene (JDS 38), respectively.

The ratio of $C_{TOT:SPM,OC}$ and $C_{TOT:SED,OC}$ shows the differences in HOC content in SPM and sediment (Fig. 2). The log($C_{TOT:SPM,OC}$/$C_{TOT:SED,OC}$) of a compound close to zero indicates the same HOC concentration both compartments, whereas the log($C_{TOT:SPM,OC}$/$C_{TOT:SED,OC}$) higher or lower than zero means a deviation in HOC composition in SPM and sediments. At most sites, there was a good agreement of OC-normalized C_{TOT} in SPM and sediment for PCBs and a majority of OCPs (Fig. 2, SI 7). In contrast, C_{TOT} of most PAHs at all sites except JDS 68 in SPM were up to a factor 10 lower in SPM than in sediment. Moreover, a good correlation between $C_{TOT:SED,OC}$ and $C_{TOT:SPM,OC}$ was found for PCBs, HCB and most DDx (except 2,4'-DDT and 4,4'-DDT), confirming the same spatial profile of pollution in SPM and the riverbed sediment (SI 7). In contrast, such correlation was absent for PAHs (SI 7). For all investigated HOCs there was no trend of the log($C_{TOT:SED,OC}$/$C_{TOT:SPM,OC}$) ratio with compound hydrophobicity (Fig. 3), confirming similar partitioning properties of both sampled matrices.

3.2 Comparing accessibility of HOCs in SPM and sediment

Further investigation using data from partitioning passive sampling was done to show whether the observed differences in HOC concentrations in SPM and sediment are related to differences in HOCs accessibility. The m_P/m_{SPM} phase ratio applied as described in section Depletive equilibrium passive sampling in SPM, was designed sufficiently high to result nearly complete depletion of accessible HOCs from SPM. To assure that the passive sampling was fully depletive, a two-step extraction with SR at the set m_P/m_{SPM} was applied (SI 2). Extracts from the two subsequent sampling steps were analyzed separately. In most samples and for the majority of HOCs, the yield of the second extraction step was much smaller than in the first step, which indicates that a two-step extraction was sufficient to extract the easily accessible pool of HOCs (SI 2). The HOC accessibility was then expressed as fraction F_{AS} of the accessible/releasable concentration from the total concentration in sediment (Eq. 3).

Accessibility of the HOCs under investigation varied between compound classes and sites without a clear pattern (SI 11).The highest accessibility was observed for PCB congeners with four or more chlorine atoms per molecule, with F_{AS} values ranging between 20 and 30%. The median F_{AS} of less chlorinated PCB congeners ranged from 15 to 21%. The accessibility of PeCB was very low with median F_{AS} of 6%. On the other hand, median F_{AS} of HCB was 18%, with elevated values at sites JDS 6 and JDS 22. The median F_{AS} of DDx varied from 0 to 23%.

In our previous study accessibility of HOCs has been characterized using multi-ratio equilibrium passive sampling (M. Belháčová-Minaříková et al. 2020), with $C_{AS:SED}$ as one of the testing endpoints. Since the same type of silicone was applied in both studies (i.e. Altesil™) for passive sampling of HOC for sediment and SPM from the same sampling sites, and the sample processing was performed in the same laboratory, mutual comparison of HOC accessibility in both sampled matrices is possible.

Analogically to total concentrations (discussed in section Comparing total concentrations of HOCs in SPM and sediment) the ratio of $C_{AS:SPM,OC}$ and $C_{AS:SED,OC}$ shows the differences in accessible HOC content in SPM and sediment (Fig. 4). At most sites, OC-normalized C_{AS} in SPM were equal or lower than in riverbed sediment from the corresponding site (Fig. 4 and SI 12). A good correlation between $C_{AS:SED,OC}$ and $C_{AS:SPM,OC}$ was found only for PCB 28, and DDx except 2,4'-DDT and 4,4'-DDT, (SI 9). For all investigated HOCs there was no trend of the log($C_{AS:SED,OC}/C_{AS:SPM,OC}$) ratio with compound hydrophobicity (SI 10), confirming similar partitioning properties of both sampled matrices. With a few exceptions (HCB and 4,4'-DDE at JDS 22) the accessible fraction F_{AS} of investigated HOCs (PCBs, PeCB, HCB and DDx) in SPM was lower than in sediment (SI 12).

3.3 What causes the different apparent accessibility of HOCs in SPM and sediment?

In our previous study (M. Belháčová-Minaříková et al. 2020) contaminant levels in sediment porewater ($C_{W:0}$) in sediments collected at the sites investigated in this study were compared with those in the water column ($C_{W:W}$) in the upstream Danube stretch. For PCBs and DDx, in most cases log($C_{W:W}/C_{W:0}$) values were positive, indicating compound fugacity in the water column equal or higher than fugacity in sediment. Thus, the data indicated either equilibrium between sediment and water, or potential for compound deposition from water to sediment. This is in apparent contrast with the above observations of OC-normalized in SPM ($C_{AS:SPM,OC}$) equal or lower than in riverbed sediment ($C_{AS:SED,OC}$) from the corresponding site, indicating a HOC fugacity in water column lower than in sediment. However, one has to consider that HOC concentrations in SPM and sediment were compared after data normalization to a common organic carbon composition. Although that was necessary to avoid incorrect conclusions due to natural or technically-based differences in sample composition (Schubert et al. 2012), a potential bias in such comparison may be related to different qualitative composition of organic carbon in SPM and sediment. In other words, one cannot assume a priori the same composition and sorptionproperties of bed sediments and SPM.

It is generally accepted that the amorphous organic carbon is dominantly responsible for reversible partitioning sorption (Allen-King, Grathwohl, and Ball 2002). In the work of Rusina et al. (2019) the fraction of amorphous organic carbon (AOC) in the middle Danube stretch represented on average 20% of OC content. Besides SPM contained higher OC than riverbed sediments, the Rockeval analysis showed also somewhat higher average percentage of 45% AOC in SPM. Unfortunately, Rockeval analysis was not available specifically for sediments from JDS sites where SPM was collected. However, based on the above, we hypothese that accessible concentrations expressed on AOC basis in SPM ($C_{AS:SPM,AOC}$) would also likely be lower than the accessible concentrationsin sediment ($C_{AS:SED,AOC}$).

The apparent difference in OC-based accessible concentrations in SPM and sediment can be explained by another SPM parameter that renders its properties different from riverbed sediments. The Rockeval pyrolysis revealed that all SPM samples contained elevated labile organic carbon fraction (pyrolysing with a maximum signal at 340 °C), which was not present in riverbed sediment samples analysed by Rusina et al. (2019). Most likely, SPM contains lipid and other organic matter from the suspended plancton present in the water column. Smedes et al. (2020) have recently shown that phytoplancton mostly contains lower thermodynamic level of HOCs than the surrounding aqueous phase because of acombination of slow diffusive HOC uptake to algal cells and their growth dillution. Presence of an amorphous OC fraction with lower chemical activity of HOC than the surrounding water column or the „aged" riverbed sediment seems to be aplausible explanation for the lower HOC levels in SPM.

4 Conclusions

The study demonstrated the utility of partitioning passive sampling approach for measuring accessibility for a range of HOCs in SPM along the Danube River. Ex-situ extraction of SPM with silicone can be executed within a reasonable time frame of several weeks for HOCs. Both riverbed sediment and SPM provided comparable information on total and accessible concentrations of micropollutant HOC in the Danube River with an acceptable difference of less than an order of magnitude. The study shows that the exposure of Danube organisms to HOCs in water column and the upper sediment layer is well comparable. The apparent difference in organic-carbon based accessible concentrations is most likely caused by a different quality of the organic matter contributing to sorption of HOCs in the compared matrices. Future investigation of HOC sorption to suspended freshwater phytoplankton may provide an experimental evidence explaining lower organic-carbon based HOC concentrations in SPM than in riverbed sediment.

5 Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

CZ.02.1.01/0.0/0.0/17_043/0009632) provided infrastructure for sample processing, instrumental analysis and data analysis.

Authors' contributions

Ian Allan designed the passive sampling-SPM equilibration experiment and performed the SPM passive sampling and reviewed the manuscript. Michaela Belháčová-Minaříková performed sample processing, data analysis and was a major contributor in writing the manuscript. Branisalv Vrana interpreted the data and was involved in writing the manuscript. All authors read and approved the final manuscript.

References

Allan, Ian J., Violette Raffard, Alfhild Kringstad, and Kristoffer Næs. 2021. "Assessment of Marine Sediment Remediation Efficiency with SPME-Based Passive Sampling Measurement." *Science of the Total Environment* 756 (February): 143854. https://doi.org/10.1016/j.scitotenv.2020.143854.

Allen-King, Richelle M., Peter Grathwohl, and William P. Ball. 2002. "New Modeling Paradigms for the Sorption of Hydrophobic Organic Chemicals to Heterogeneous Carbonaceous Matter in Soils, Sediments, and Rocks." *Advances in Water Resources* 25 (8–12): 985–1016. https://doi.org/10.1016/S0309-1708(02)00045-3.

Bartoszek, Lilianna, and Renata Gruca-rokosz. 2019. "Ecohydrology & Hydrobiology Characteristics and Origin of Suspended Matter in a Small Reservoir in Poland," no. 2018. https://doi.org/10.1016/j.ecohyd.2019.05.003.

Belháčová-Minaříková, M., F. Smedes, T.P. Rusina, and B. Vrana. 2020. "Application of Equilibrium Passive Sampling to Profile Pore Water and Accessible Concentrations of Hydrophobic Organic Contaminants in Danube Sediments." *Environmental Pollution* 267. https://doi.org/10.1016/j.envpol.2020.115470.

Belháčová-Minaříková, Michaela, Foppe Smedes, Tatsiana P. Rusina, and Branislav Vrana. 2020. "Application of Equilibrium Passive Sampling to Profile Pore Water and Accessible Concentrations of Hydrophobic Organic Contaminants in Danube

Sediments." *Environmental Pollution* 267: 115470. https://doi.org/10.1016/j.envpol.2020.115470.

Cornelissen, Gerard, Örjan Gustafsson, Thomas D. Bucheli, Michiel T O Jonker, Albert A. Koelmans, and Paul C M Van Noort. 2005. "Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen in Sediments and Soils: Mechanisms and Consequences for Distribution, Bioaccumulation, and Biodegradation." *Environmental Science and Technology* 39 (18): 6881–95. https://doi.org/10.1021/es050191b.

Eggleton, Jacqueline, and Kevin V. Thomas. 2004. "A Review of Factors Affecting the Release and Bioavailability of Contaminants during Sediment Disturbance Events." *Environment International* 30 (7): 973–80. https://doi.org/10.1016/j.envint.2004.03.001.

Greenberg, Marc S., Peter M. Chapman, Ian J. Allan, Kim A. Anderson, Sabine E. Apitz, Chris Beegan, Todd S. Bridges, et al. 2014. "Passive Sampling Methods for Contaminated Sediments: Risk Assessment and Management." *Integrated Environmental Assessment and Management* 10 (2): 224–36. https://doi.org/10.1002/ieam.1511.

ISO 5667. 2009. Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples. *International Standard Organisation*, issued 2009.

Jahnke, Annika, Philipp Mayer, and Michael S. McLachlan. 2012. "Sensitive Equilibrium Sampling to Study Polychlorinated Biphenyl Disposition in Baltic Sea Sediment." *Environmental Science and Technology* 46 (18): 10114–22. https://doi.org/10.1021/es302330v.

Jonker, Michiel T.O., Stephan A. Van Der Heijden, Dave Adelman, Jennifer N. Apell, Robert M. Burgess, Yongju Choi, Loretta A. Fernandez, et al. 2018. "Advancing the Use of Passive Sampling in Risk Assessment and Management of Sediments Contaminated with Hydrophobic Organic Chemicals: Results of an International Ex Situ Passive Sampling Interlaboratory Comparison." *Environmental Science and Technology* 52 (6): 3574–82. https://doi.org/10.1021/acs.est.7b05752.

Kočan, Anton, Jan Petrík, Stanislav Jursa, Jana Chovancová, and Beata Drobná. 2001. "Environmental Contamination with Polychlorinated Biphenyls in the Area of Their Former Manufacture in Slovakia." *Chemosphere* 43 (4–7): 595–600. https://doi.org/10.1016/S0045-6535(00)00411-2.

Liška, Igor, Franz Wagner, Manfred Sengl, Karin Deutsch, and Jaroslav Slobodník. 2015. *Joint Danube Survey 3: A Comprehensive Analysis of Danube Water Quality*. http://www.danubesurvey.org/results.

Lydy, Michael J., Amanda D. Harwood, Samuel A. Nutile, and Peter F. Landrum. 2015. "Tenax Extraction of Sediments to Estimate Desorption and Bioavailability of Hydrophobic Contaminants: A Literature Review." *Integrated Environmental Assessment and Management*. https://doi.org/10.1002/ieam.1603.

Maruya, K.A., E.Y. Zeng, D. Tsukada, and S.M. Bay. 2009. "A Passive Sampler Based on Solid-Phase Microextraction for Quantifying Hydrophobic Organic Contaminants in Sediment Pore Water." *Environmental Toxicology and Chemistry* 28 (4): 733–40. https://doi.org/10.1897/08-322R.1.

Niehus, Nora Claire, Sabine Schäfer, Christel Möhlenkamp, and Gesine Witt. 2018. "Equilibrium Sampling of HOCs in Sediments and Suspended Particulate Matter of the Elbe River." *Environmental Sciences Europe* 30 (1): 12302–18. https://doi.org/10.1186/s12302-018-0159-8.

Poot, Anton, Joris T.K. Quik, Harry Veld, and Albert A. Koelmans. 2009. "Quantification

Methods of Black Carbon: Comparison of Rock-Eval Analysis with Traditional Methods." *Journal of Chromatography A* 1216 (3): 613–22. https://doi.org/10.1016/j.chroma.2008.08.011.

Reichenberg, F., and P. Mayer. 2006. "Two Complementary Sides of Bioavailability: Accessibility and Chemical Activity of Organic Contaminants in Sediments and Soils." *Environmental Toxicology and Chemistry* 25 (5): 1239–45. https://doi.org/10.1897/05-458R.1.

Rusina, Tatsiana P., F. Smedes, Maja Brborić, and Branislav Vrana. 2019. "Investigating Levels of Organic Contaminants in Danube River Sediments in Serbia by Multi–Ratio Equilibrium Passive Sampling." *Science of the Total Environment* 696: 1–10. https://doi.org/10.1016/j.scitotenv.2019.133935.

Schubert, Birgit, Peter Heininger, Martin Keller, Evelyn Claus, and Mathias Ricking. 2012. "Monitoring of Contaminants in Suspended Particulate Matter as an Alternative to Sediments." *TrAC - Trends in Analytical Chemistry*. Elsevier. https://doi.org/10.1016/j.trac.2012.04.003.

Schubert, Birgit, Peter Heininger, Martin Keller, Mathias Ricking, Evelyn Claus, Peter Heininger, Martin Keller, and Evelyn Claus. 2015. "Monitoring of Contaminants in Suspended Particulate Matter as an Alternative to Sediments." *Trends in Analytical Chemistry* 36: 58–70. https://doi.org/10.1016/j.trac.2012.04.003.

Smedes, Foppe, and Jacob De Boer. 1997. "Determination of Chlorobiphenyls in Sediments - Analytical Methods." *TrAC - Trends in Analytical Chemistry*. https://doi.org/10.1103/PhysRevD.64.127501.

Smedes, Foppe, Jaromír Sobotka, Tatsiana P. Rusina, Pavla Fialová, Pernilla Carlsson, Radovan Kopp, and Branislav Vrana. 2020. "Unraveling the Relationship between the Concentrations of Hydrophobic Organic Contaminants in Freshwater Fish of Different Trophic Levels and Water Using Passive Sampling." *Environmental Science and Technology* 54 (13): 7942–51. https://doi.org/10.1021/acs.est.9b07821.

Smedes, Foppe, L. Alexander Van Vliet, and Kees Booij. 2013. "Multi-Ratio Equilibrium Passive Sampling Method to Estimate Accessible and Pore Water Concentrations of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls in Sediment." *Environmental Science and Technology* 47 (1): 510–17. https://doi.org/10.1021/es3040945.

Sullivan, B. E., F. G. Prahl, L. F. Small, and P. A. Covert. 2001. "Seasonality of Phytoplankton Production in the Columbia River: A Natural or Anthropogenic Pattern?" *Geochimica et Cosmochimica Acta* 65 (7): 1125–39. https://doi.org/10.1016/S0016-7037(00)00565-2.

Umlauf, Gunther. 2014. "Semivolatile Organic Compounds in Water, Suspended Particulate Matter, Sediments and Biota in the Danube." In *The Danube River Basin. The Handbook of Environmental Chemistry, Vol. 39*, edited by Igor Liska. Springer Berlin Heidelberg. https://doi.org/https://doi.org/10.1007/698_2014_3.

Witt, Gesine, Susann-Cathrin Lang, Dagny Ullmann, Gotja Schaffrath, Detlef Schulz-Bull, and Philipp Mayer. 2013. "Passive Equilibrium Sampler for in Situ Measurements of Freely Dissolved Concentrations of Hydrophobic Organic Chemicals in Sediments." *Environmental Science & Technology* 47 (14): 7830–39. https://doi.org/10.1021/es400395t.

Figure captions

Fig. 1: Map of the Danube showing the sites at which SPM and riverbed sediment were sampled during JDS 3 in 2013 and further investigated by ex-situ passive sampling. Details of sampling at each site are shown in Table 1

Fig. 2. Comparison of the ratio (log units) of the total organic carbon normalized concentration in sediment ($C_{TOT:SED,OC}$) (Michaela Belháčová-Minaříková et al. 2020) versus total organic carbon normalized concentration in SPM ($C_{TOT:SPM,OC}$) collected at sampling sites (JDS 6, 14, 22, 38, 62 and 68; Table 1). Meaning of compound name abbreviations is explained in SI 5. Sampling sites correspond to the sampling stations with sediment monitoring activities during JDS 3 survey

Fig. 3. Ratio (log units) of organic carbon normalized total PCBs, OCPs, DDX and PAHs concentrations in sediment and SPM versus log K_{OW} at site JDS 62 in the Danube River

Fig. 4. Comparison of the ratio (log units) of the accessible organic carbon normalized concentration of PCBs, OCPs and DDx in sediment ($C_{AS:SED,OC}$) (Michaela Belháčová-Minaříková et al. 2020) versus their total organic carbon normalized concentration in SPM ($C_{AS:SPM,OC}$) collected at JDS 3 survey

Table captions

Table 1: SPM sampling sites in the Danube River. Sampling of SPM was carried out by a flow-through centrifuge from the Argus ship cruising downstream the river during August and September 2013. Sampling sites correspond to the sampling stations with parallel riverbed sediment sampling during JDS 3 survey (M. Belháčová-Minaříková et al. 2020)