



# Effects of sediment manipulation on freely dissolved concentrations of hydrophobic organic chemicals

Michiel T.O. Jonker

Institute for Risk Assessment Sciences, Utrecht University; P.O. Box 80177, 3508, TD Utrecht, the Netherlands



## HIGHLIGHTS

- Do freely dissolved concentrations ( $C_{free}$ ) of PAHs in sediments change *ex situ*?
- $C_{free}$  in field sediments increased up to a factor of 16 upon sample manipulation
- Simply freezing increased  $C_{free}$  up to 4 times and so did grinding
- Sample manipulations should be avoided in order not to overpredict risks

## ARTICLE INFO

### Article history:

Received 12 July 2020

Received in revised form

10 October 2020

Accepted 16 October 2020

Available online 22 October 2020

Handling Editor: Keith Maruya

### Keywords:

Freely dissolved concentration

Sediment

Freezing

Freeze-drying

Grinding

Storage

## ABSTRACT

The freely dissolved concentration ( $C_{free}$ ) of hydrophobic organic chemicals (HOCs) in pore water is an important parameter in the risk assessment and management of contaminated sediments and soils. It can be determined most conveniently through *ex situ* passive sampling, i.e., in the laboratory. For this purpose, samples are taken from the field and transported to the laboratory, where they are stored and possibly manipulated by sieving, freezing, drying and/or grinding. Although the objective of *ex situ* passive sampling often is to determine a  $C_{free}$  that reflects the metric under *in situ* conditions, hardly any information is available on possible effects of sample manipulation. Hence, the present study investigated the impact of freezing, freeze-drying, and grinding on  $C_{free}$  of HOCs in field sediments, as determined with solid phase microextraction (SPME). Freezing increased the  $C_{free}$  of 3- and 4-ring polycyclic aromatic hydrocarbons (PAHs) with up to a factor of 4, whereas for 5- and 6-ring PAHs hardly any effects were observed. Generally, additional freeze-drying did not further increase  $C_{free}$ , but subsequently grinding the samples (further) increased  $C_{free}$  of all PAHs with up to a factor of 4, leading to an overall maximum observed increase in  $C_{free}$  of a factor of 16. Probably, these effects are caused by a structural change in the sorption matrix, enhancing PAH availability. The results indicate that freezing, freeze-drying, and grinding prior to *ex situ*  $C_{free}$  determinations should be avoided, as these treatments may considerably increase the  $C_{free}$  of HOCs, leading to an overestimation of risks.

© 2020 The Author. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The freely dissolved concentration ( $C_{free}$ ) of hydrophobic organic chemicals (HOCs) in pore water of sediments and soils has been shown to better predict bioaccumulation and toxicity of HOCs in benthic organisms than total, solid phase-associated concentrations (Lydy et al., 2014). Hence,  $C_{free}$  is considered the most relevant exposure metric for benthic communities (Mayer et al., 2014) and an essential parameter in the risk assessment and management of contaminated sediments and soils (Greenberg

et al., 2014), e.g., when judging remediation necessity and effectiveness.

$C_{free}$  of HOCs can be determined through partitioning-based, non-depletive sampling with a specific polymer ('passive sampling'). The polymer is exposed to a sediment or soil slurry and HOCs present in the sample will partition from the pore water into the polymer, from which they can be extracted and analyzed. Passive sampling can be performed in the field (*in situ*) and in the laboratory (*ex situ*). The latter approach is often preferred, because it is faster, less laborious, and cheaper as compared to *in situ* sampling (Ghosh et al., 2014). *Ex situ* passive sampling requires that a sediment or soil sample be collected in the field and then transported to the laboratory for determination of  $C_{free}$ . In the laboratory,

E-mail address: [m.t.o.jonker@uu.nl](mailto:m.t.o.jonker@uu.nl).

the sample will be stored and possibly treated ('manipulated') before starting the passive sampling measurements. Storing can be done at room temperature, in a refrigerator (4–7 °C), or in a freezer (–20 °C), and samples may be manipulated by homogenization, sieving, drying and/or grinding.

Degradation of chemicals during storage should be minimized, such that the concentrations occurring in the field are maintained. Therefore, storing sediment or soil samples at room temperature is discouraged (unless temperatures in the field are close to room temperature), as microbiological processes may intensify at higher temperatures. For this reason, storing samples in a refrigerator is common practice, but storing in a freezer will be even more effective in preventing any degradation of chemicals. For practical reasons, the practitioner may consider freeze-drying if the samples are frozen, because processing the resulting dry, powdered samples is more convenient than processing wet samples. After all, dry samples do not require dry weight determinations and are more simple to handle when preparing passive sampling equilibration systems (Jonker et al., 2020). To remove any lumps created during the freeze-drying process and to homogenize the sample, grinding in a mortar is a suitable and often applied final step in sample treatment. Alternatively, sieving may be performed with dried samples for homogenization purposes. This technique may however also be applied to wet sediment and soil samples, in order to remove e.g., stones, twigs, and leaves (Jonker et al., 2020).

Removing larger objects, particularly stones, is critical, because these may damage the passive sampler (in particular fragile Solid Phase Micro Extraction (SPME) fibers), which is applied for the  $C_{free}$  determination (Jonker et al., 2020). Sample manipulation by gentle sieving or manually picking out these objects ('size separation') thus is inevitable. Because  $C_{free}$  of HOCs is controlled by sorption of the chemicals to the organic carbon fraction, which is primarily associated with the fine fraction and not with stones, removing larger (no/low-carbon) objects probably will not substantially affect the metric. Whether the other sample storage and manipulation steps (i.e., freezing, freeze-drying, and grinding) can affect  $C_{free}$  of HOCs is largely unknown. Information on any effects is crucial though, since the objective of *ex situ* passive sampling often is to determine a  $C_{free}$ , which represents field conditions. In contrast to the 'size separation' step, freezing, freeze-drying, and grinding are not essential for a proper  $C_{free}$  determination and if the metric would be substantially affected by any of these treatments, they should not be applied.

The present study was performed to investigate if sample manipulation by freezing, freeze-drying, and grinding has an effect on  $C_{free}$  of HOCs in sediments. Five field-contaminated sediments were sampled and subsequently manipulated in the laboratory in different ways, after which  $C_{free}$  of native polycyclic aromatic hydrocarbons (PAHs) was determined using SPME fibers.

## 2. Materials and methods

### 2.1. Chemicals and passive samplers

Chemicals used were methanol (HPLC gradient grade; Biosolve, Valkenswaard, the Netherlands), acetonitrile (HPLC-S grade; Biosolve), sodium azide (extra pure; Merck, Darmstadt, Germany), and 2-methylchrysenes (99.2%; BCR, Geel, Belgium). Polydimethylsiloxane (PDMS)-coated, disposable SPME fiber (glass fiber core diameter 110  $\mu$ m, PDMS coating thickness 28.5  $\mu$ m) was obtained from Poly Micro Industries (Phoenix, AZ, USA). Prior to use, the fiber was cut into pieces of 5 cm length, which were washed three times for 30 min each with methanol and three times for 30 min each with Milli-Q water, respectively.

### 2.2. Sediments

Using a single manual grab sampler, the upper ~10 cm surface layer from five different sediments in the Netherlands was sampled. An overview of the characteristics of these sediments (sampling location, texture, likely PAH source, and organic carbon content) is presented in Table 1. Bioavailability in several of these sediments has been studied before (Muijs and Jonker, 2012).

### 2.3. Sediment treatments

Sediments were transported to the laboratory in 5 L buckets. Here, the PAH-containing sediments were gently sieved (2 mm), homogenized with a mechanical mixer, and stored in a refrigerator. From each bucket, four subsamples were taken and placed in 200 mL glass jars. One jar served as 'untreated, reference' sample and was used for PAH  $C_{free}$  determinations (see below) as such. The second jar was placed in the freezer (–20 °C) for two days, after which it was thawed and used for  $C_{free}$  determinations. The third jar was placed in the freezer for two days and then freeze-dried during 28 h. Due to the limited availability of sediment 5, this treatment was omitted for this sediment. The fourth jar was also frozen and freeze-dried, but the resulting dry material was additionally mortared manually for exactly 3 min, using a mortar and pestle. A schematic representation of these procedures is shown in Fig. 1.

### 2.4. $C_{free}$ determinations

$C_{free}$  in all samples was determined with SPME fibers in fourfold. The determinations were performed several years ago, when a standard protocol was not available yet. Therefore, the procedure slightly differed from the recently-published standard procedure (Jonker et al., 2020). Sediment samples representing 2 g of dry weight were brought into 7 mL amber glass vials and Milli-Q water containing 200 mg/L sodium azide was added to the level of 7 mL, leaving sufficient headspace for proper mixing. SPME fibers were

**Table 1**  
Characteristics of the Dutch field sediments investigated in the present study.

Sediment	Sampling location	Texture	Likely PAH source	Organic carbon % <sup>a</sup>
1	River Hollandsche IJssel near the city Gouda	Fine, clayey	Shipping and industrial activities	2.80 ( $\pm$ 0.15)
2	Canal in the vicinity of highway A4. Sampled from the side of the canal	Course, very sandy	Highway + unknown source (very high $C_{free}$ of PAHs)	0.30 ( $\pm$ 0.05)
3	Same as sediment 2, but sampled from the center of the canal	Fine, silty	Same as sediment 2	4.45 ( $\pm$ 0.38)
4	Ditch in a small village close to Utrecht	Clayey	Creosote-treated sheet piling	3.53 ( $\pm$ 0.10)
5	Ditch behind a gas station and carpool	Sandy, organic-rich	Exhaust gases, oil products, creosote-treated sheet piling	7.88 ( $\pm$ 1.10)

<sup>a</sup> Determined according to methods described in (Van der Heijden and Jonker, 2009).

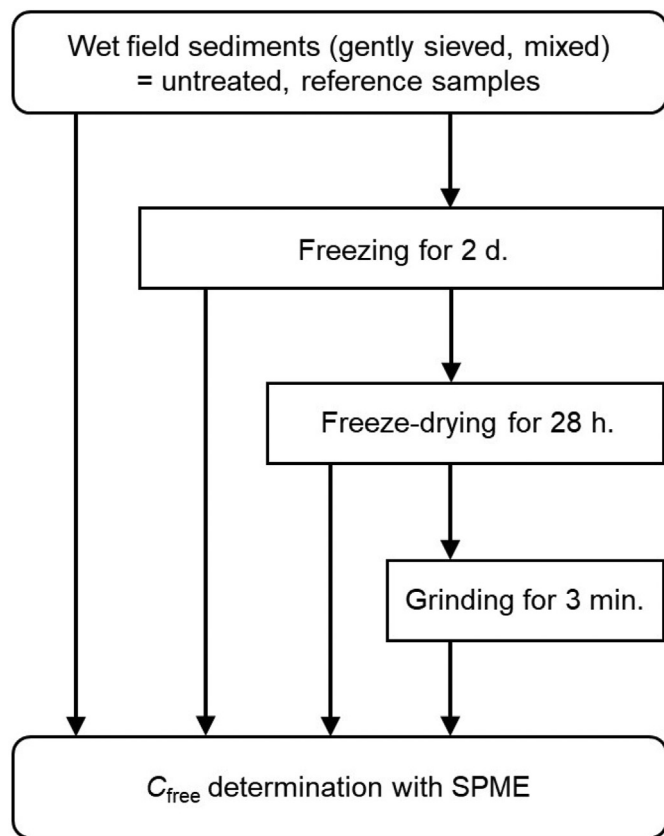


Fig. 1. Flow chart illustrating the sample processing sequence.

added and the vials were equilibrated on a rock and roller apparatus for 4 weeks at approximately 30 rpm. The fibers were then collected, cleaned with wet paper tissue, placed in autosampler vials containing acetonitrile and internal standard (2-methylchrysene), and extracted by vortexing for 1 min. PAH concentrations in all sampler extracts were analyzed by HPLC as described before (Jonker and Muijs, 2010).

### 3. Results and discussion

#### 3.1. Effects of sediment manipulation on $C_{free}$

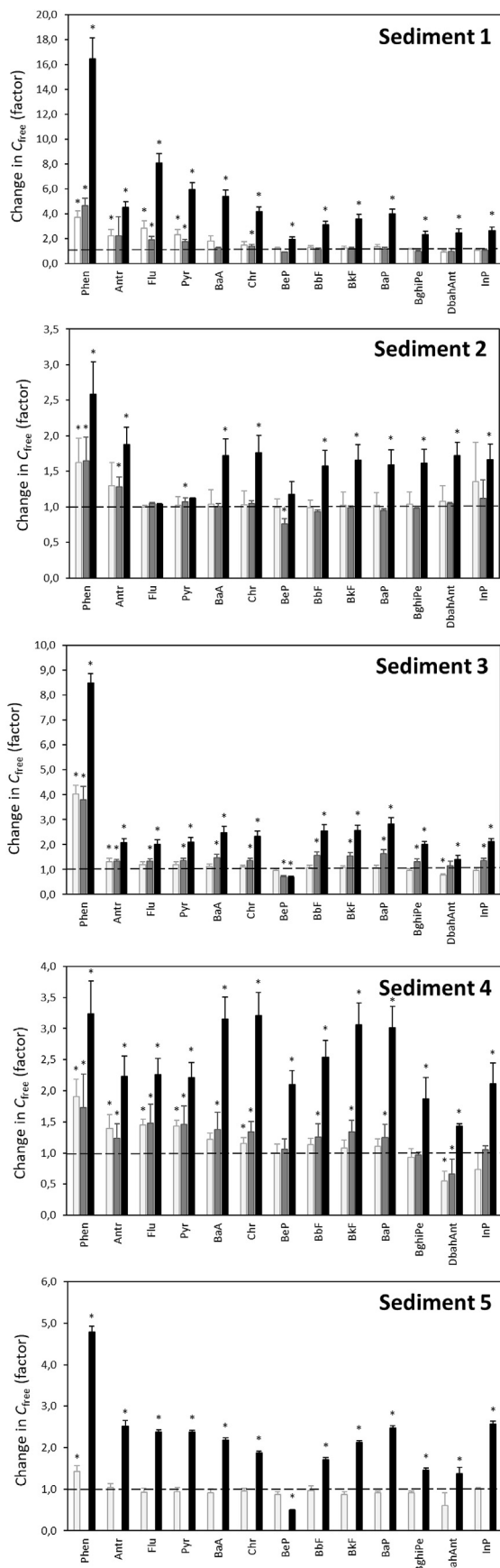
The results of the  $C_{free}$  determinations in the sediments are presented in Fig. 2. The Figure shows the effects of the different treatments (i.e., freezing, freezing/freezing-drying, and freezing/freezing-drying/grinding) on the  $C_{free}$  of the chemicals in a specific sediment, by normalizing the  $C_{free}$  values to those quantified in the untreated, reference sample. As such, the effect of a specific treatment is expressed as a factor, representing the change in  $C_{free}$  as a result of the treatment. In most of the cases, clear effects are observed, many of which are statistically significant (paired t-tests;  $P < 0.05$ ); however, the effects are different for the different sediments, chemicals, and treatments. The largest significant effects occurred for the 3-ring PAH phenanthrene as a result of the 'extensive treatment' (freezing/freezing-drying/grinding): the  $C_{free}$  of this chemical increased up to 16 times in sediment 1. For the 6-ring PAHs,  $C_{free}$  in all sediments significantly increased by a factor of approximately 1.5–3 as a result of this treatment.

The effects of freezing and freezing/freezing-drying on  $C_{free}$  are much smaller as compared to the effects of the extensive treatment, and are primarily observed for the 3- and 4-ring PAHs

(phenanthrene – chrysene), for which increases in  $C_{free}$  with up to a factor of 4 are observed. For the 5- and 6-ring PAHs (benzo[e]pyrene – indeno[123-cd]pyrene), hardly any effects of these treatments are visible (barring several exceptions, in particular in sediment 3, where significant increases up to a factor of 1.6 are seen). However, although small, it is important to stress that effects are actually observed for these treatments. After all, this indicates that simply freezing (and freezing/freezing-drying) can cause an increase in  $C_{free}$ . In the presently investigated sediments, the maximum increase is a factor of four, but larger increases in other sediments or soils cannot be excluded. Interestingly, in most cases, the impact of both treatments was similar. This suggests that freezing, and not the freeze-drying process, is responsible for causing the increase in  $C_{free}$ , as freeze-drying in addition to freezing generally did not further increase  $C_{free}$ .

Considering the differences in effects observed for different sediments, both in intensity and in terms of 'PAH fingerprint' (i.e., relative differences between the different PAHs), the effects of the treatments will most probably be related to the OC content or type (i.e., PAH source – soot, diesel, creosote, coal, or oil) of the sediment. In other words, the observed differences most likely will be caused by differences in the way and the extent of binding of the chemicals to the matrix. This implies that by simply freezing (and thawing) a sediment, the binding strength of (3 and 4-ring) PAHs can be decreased. The change appears irreversible, because after freeze-drying and resuspension in water, similar  $C_{free}$  values, thus sorption strengths, were determined. Generally, differences in (OC-normalized) HOC sorption affinity for soils and sediments are explained by differences in the chemical nature of the matrix, such as the degree of aromaticity or the content of black carbon or oil (Cornelissen et al., 2005). However, a change in the chemical composition as a result of freezing is impossible. Therefore, the sorption matrix most likely will be physically changed, e.g., due to structural 'damage' caused by ice crystals, which may break open the matrix and expose inner sorption sites, enhancing the potential for PAH desorption and consequently increasing  $C_{free}$ . Apparently, the influence of this hypothesized structural change is the largest for the relatively less hydrophobic (3- and 4-ring) PAHs. The exact cause of this chemical size/hydrophobicity-dependent behavior is unclear. Presumably, grinding has a similar (physical) effect, leading to an increased desorption surface or reduced diffusion path lengths for the sediment-bound PAHs that are sampled by the polymer. Previously, Ter Laak et al. (Ter Laak et al., 2007) also observed a reduction in the sorption of 3- and 4-ring, but not 5- and 6-ring PAHs to a specific soil (containing weathered tar particles as the PAH source), after grinding the sample. Because the uptake kinetics of all PAHs in SPME fibers were higher in the ground sample, the authors explained this phenomenon by differences in redistribution kinetics and hypothesized that for the less hydrophobic PAHs equilibration processes might still be ongoing.

In the present study, grinding, even though performed for only 3 min, generally had a larger impact than freezing (up to a factor of 4). Assuming a physical effect, this observation could be explained by a higher level of energy introduced. Therefore, here a relation with the PAH source would be expected even more, as crushing e.g., coal particles would be expected to enhance PAH availability more than grinding (liquid) PAH-containing creosote or diesel. However, although the exact PAH source was not determined for the test sediments, this characteristic is unlikely to explain the trends in the observed manipulation effects, as sediments 2 and 3 most probably have the same PAH source (they were sampled from the same canal, only 2–3 m apart), but display different 'PAH fingerprints' (see Fig. 2), thus responded to the treatments differently. It should be mentioned though, that sediment 2 has a 10 times lower OC content than sediment 3, and much higher (20–90 times)  $C_{free}$  values



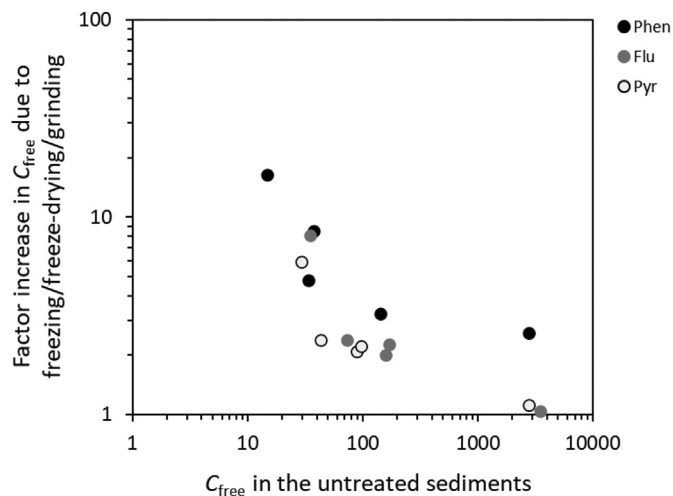
(data not shown). This sediment therefore has an inherent high PAH availability and the treatments may have been less effective in increasing it further. Actually, this hypothesis may play a role in explaining the effect of the extensive treatment observed for several 3- and 4-ring PAHs, as the magnitude of the effect is inversely related to  $C_{free}$  of these PAHs in the untreated sediments (see Fig. 3). For the other PAHs and treatments; however, clear relationships as those shown in Fig. 3 are not observed. Likewise, a relationship between the magnitude of the observed effects and OC content is also absent: the largest effects are found for sediment 1, having the second lowest OC content, whereas generally the lowest effects are observed for sediment 2, which has the lowest OC content. Unraveling the exact mechanisms underlying the increased  $C_{free}$  values would therefore require additional sediment characterization analyses, such as quantifying the black carbon and NAPL content, (change in) particle size distribution, total solid phase-associated PAH concentrations (enabling the calculation of sorption coefficients), and/or PAH desorption kinetics. However, such analyses were beyond the scope of the present study, which merely aimed to identify possible effects of manipulations on  $C_{free}$  and to provide practical advice on whether or not to apply these manipulations.

### 3.2. Implications

The present study demonstrates that certain storage conditions and sediment manipulation steps can significantly increase  $C_{free}$  of HOCs. Although observed in the laboratory, the effects may also be environmentally relevant. After all, certain soils and sediments may get frozen in the winter. Admittedly, in the field the matrices generally are not suddenly exposed to  $-20\text{ }^{\circ}\text{C}$  and may freeze more ‘gradually’, but the formation of ice crystals, which hypothetically may damage the OC matrix, will occur anyway. In this respect, freezing in the field does not differ from freezing in a freezer and upon defrosting in spring, the availability of native contaminants may be enhanced. Whether or not repetitive (yearly) freeze-thaw cycles are able to progressively increase  $C_{free}$  of HOCs remains to be investigated. Likewise, the present laboratory results may be extrapolated to environmental manipulations or geo-engineering activities, such as rolling, heavy truck use or perhaps even dredging, which all induce physical stress or crush the matrix. Possibly, these activities are therefore also able to affect the availability of HOCs in the respective soil or sediment systems.

Regarding *ex situ*  $C_{free}$  determinations in the laboratory, the implications of the present results are straightforward. Soil or sediment samples taken from the field should not be stored in the freezer, but kept in a refrigerator. Further, they should not be freeze-dried or ground. All these sample treatments may potentially increase the availability of target chemicals and the final results may thus not reflect actual conditions in the field. This way, risks of the contamination may be overestimated, (partly) nullifying the merits of determining  $C_{free}$  in the risk assessment procedure (Greenberg et al., 2014).

**Fig. 2.** Changes in the freely dissolved concentrations ( $C_{free}$ ) of PAHs, as a result of different sediment manipulations: freezing (white bars), freezing/freeze-drying (gray bars), and freezing/freeze-drying/grinding (black bars). All effects are expressed as a factor, relative to the  $C_{free}$  values in the untreated, reference sediments, as indicated by the horizontal dashed lines. Gray bars are missing for sediment 5, due to the limited availability of this sediment. Error bars represent standard deviations of replicated measurements. Asterisks indicate statistically significant differences as compared to the untreated, reference sediments.



**Fig. 3.** Relationship between the increase in the freely dissolved concentrations ( $C_{free}$ ) of three PAHs in the five test sediments as a result of the extensive treatment (freezing/freezing-drying/grinding) and the chemicals'  $C_{free}$  in the untreated, reference sediments.

### CRediT authorship contribution statement

**Michiel T.O. Jonker:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization.

### Declaration of competing interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

Stephan van der Heijden is kindly acknowledged for his practical

assistance.

### References

- Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* 39 (18), 6881–6895.
- Ghosh, U., Kane Driscoll, S., Burgess, R.M., Jonker, M.T.O., Reible, D., Gobas, F., Choi, Y., Apitz, S.E., Maruya, K.A., Gala, W.R., Mortimer, M., Beegan, C., 2014. Passive sampling methods for contaminated sediments: practical guidance for selection, calibration, and implementation. *Integrated Environ. Assess. Manag.* 10 (2), 210–223.
- Greenberg, M.S., Chapman, P.M., Allan, I., Anderson, K.A., Apitz, S.E., Beegan, C., Bridges, T.S., Brown, S.S., Cargill, J.G., McCulloch, M.C., Menzie, C.A., Shine, J.P., Parkerton, T.F., 2014. Passive sampling methods for contaminated sediments: risk assessment and management. *Integrated Environ. Assess. Manag.* 10 (2), 224–236.
- Jonker, M.T.O., Muijs, B., 2010. Using solid phase micro extraction to determine salting-out (Setschenow) constants for hydrophobic organic chemicals. *Chemosphere* 80 (3), 223–227.
- Jonker, M.T.O., Burgess, R.M., Ghosh, U., Gschwend, P.M., Hale, S.E., Lohmann, R., Lydy, M.J., Maruya, K.A., Reible, D., Smedes, F., 2020. Ex situ determination of freely dissolved concentrations of hydrophobic organic chemicals in sediments and soils: basis for interpreting toxicity and assessing bioavailability, risks, and remediation necessity. *Nat. Protoc.* 15, 1800–1828.
- Lydy, M.J., Landrum, P.F., Oen, A.M., Allinson, M., Smedes, F., Harwood, A.D., Li, H., Maruya, K.A., Liu, J., 2014. Passive sampling methods for contaminated sediments: state of the science for organic contaminants. *Integrated Environ. Assess. Manag.* 10 (2), 167–178.
- Mayer, P., Parkerton, T.F., Adams, R.G., Cargill, J.G., Gan, J., Gouin, T., Gschwend, P.M., Hawthorne, S.B., Helm, P., Witt, G., You, J., Escher, B.I., 2014. Passive sampling methods for contaminated sediments: scientific rationale supporting use of freely dissolved concentrations. *Integrated Environ. Assess. Manag.* 10 (2), 197–209.
- Muijs, B., Jonker, M.T.O., 2012. Does equilibrium passive sampling reflect actual in situ bioaccumulation of PAHs and petroleum hydrocarbon mixtures in aquatic worms? *Environ. Sci. Technol.* 46 (2), 937–944.
- Ter Laak, T.L., Barendregt, A., Hermens, J.L.M., 2007. Grinding and sieving soil affects the availability of organic contaminants: a kinetic analysis. *Chemosphere* 69 (4), 613–620.
- Van der Heijden, S.A., Jonker, M.T.O., 2009. PAH bioavailability in field sediments: comparing different methods for predicting in situ bioaccumulation. *Environ. Sci. Technol.* 43 (10), 3757–3763.