This is the accepted manuscript version of the contribution published as:

Niu, L., Ahlheim, J., Glaser, C., Gunold, R., Henneberger, L., König, M., Krauss, M., Schwientek, M., Zarfl, C., Escher, B.I. (2021): Suspended particulate matter—A source or sink for chemical mixtures of organic micropollutants in a small river under baseflow conditions? *Environ. Sci. Technol.* **55** (8), 5106 – 5116

The publisher's version is available at:

http://dx.doi.org/10.1021/acs.est.0c07772

Suspended particulate matter – source or sink for chemical mixtures of organic micropollutants in a small river under baseflow conditions?

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TOC



Abstract

Suspended particulate matter (SPM) plays an important role on the fate of organic micropollutants in rivers during rain events, when sediments are remobilized and turbid runoff components enter the rivers. Under baseflow conditions, the SPM concentration is low and the contribution of SPMbound contaminants to the overall risk of organic contaminants in rivers is assumed to be negligible. To challenge this assumption, we explored if SPM may act as source or sink for all or specific groups of organic chemicals in a small river. The concentrations of over 600 contaminants and the mixture effects stemming from all chemicals in *in vitro* bioassays were measured in river water, SPM and surface sediment after solid-phase extraction or exhaustive solvent extraction. The bioavailable fractions of chemicals and mixture effects were estimated after passive equilibrium sampling of enriched SPM-slurries and sediments in the lab. Dissolved compounds dominated the total chemical burden in the water column (water plus SPM) of the river, whereas SPM-bound chemicals contributed up to 46% of the effect burden, even if the SPM concentration in rivers was merely 1 mg/L. The equilibrium between water and SPM was still not reached under low-flow conditions with SPM as a source of water contamination. The ratios of SPM-associated to sediment-associated neutral and hydrophobic chemicals as well as the ratios of the mixture effects expressed as bioanalytical equivalent concentrations were close to 1, suggesting that surface sediment can be used as a proxy for SPM under baseflow conditions when the sampling of a large amount of water to obtain sufficient SPM cannot be realized.

1. Introduction

Once introduced into a river, organic micropollutants are distributed among different river compartments, especially water, suspended particulate matter (SPM) and sediment. SPM provides a link between water and sediment and represents an effective vector for the transport and

accumulation of contaminants.^{1, 2} After being sorbed to SPM, the organic chemicals may undergo dynamic and advective processes like transport along the river, sedimentation and remobilization over time. A wide variety of factors, such as the physicochemical properties of chemicals, the characteristics of the hydrologic system and the external inputs, determine the distribution of contaminants between phases.³⁻⁵ This distribution also governs the exposure pathways of chemicals, including the bioavailability to the local organisms and their communities.^{1, 6} Therefore, considering the partitioning processes of pollutants, as well as their bioavailability would be beneficial for understanding the big picture of the risk caused by organic chemicals in aquatic systems.

The freely dissolved concentration represents the bioavailable fraction of chemicals associated with the uptake by and the adverse effects on aquatic organisms.⁷ In earlier studies, the measurement of dissolved chemicals in the water phase was mostly done after filtration with 0.45 or 0.7 μ m filters, followed by solid-phase extraction (SPE).^{5, 8} However, hydrophobic chemicals are likely to be sorbed to colloids (defined as particles between 1 nm and 1 μ m⁹) and dissolved organic carbon (DOC), which cannot be separated from the water phase by filtration. The conventional filtration method may thus overestimate the occurrence and effect of freely dissolved chemicals, especially for hydrophobic chemicals. Further studies have demonstrated that the freely dissolved concentration of chemicals could also be deduced with passive equilibrium sampling (PES) using polydimethylsiloxane (PDMS) as sampling phase.⁷ The comparison of dissolved concentrations obtained from different methods also allows the evaluation of the role of DOC and the identification of diffusive flux directions between SPM and water phase.¹

SPM represents the most mobile fraction of sediments. The contaminants sorbed to SPM can deposit and form bed sediment, which, in turn, can be resuspended as a source of SPM with the

change of hydrodynamic conditions.¹ Under dry weather conditions, the influence of sediment remobilization caused by agitation and the associated chemical inputs can be disregarded. Due to low SPM concentration, it is difficult to capture sufficiently large amounts of SPM in a river under low-flow conditions, and thus sediment is commonly sampled and resuspended in the lab to simulate the role of SPM in aquatic toxicity.¹⁰ Despite the physical connection of SPM and sediment, their chemical and toxicological profiles may differ as well as the bioavailability of particle-bound chemicals.¹¹

Previous studies addressing the distribution between aqueous and particulate phases were limited to small groups of chemicals, in particular hydrophobic chemicals.^{12, 13} In this study, a total of 642 chemicals and the mixture effects of all chemicals in water, SPM and sediment samples were characterized using an integrated target chemical screening and bioassay analysis. Our hypotheses were that (1) neutral and hydrophobic chemicals are at equilibrium between water and SPM in a river at baseflow, (2) surface sediment can serve as a proxy for SPM for these chemicals, and (3) charged or hydrophilic chemicals have distribution patterns between phases in a river that differ from those of neutral and hydrophobic chemicals.

2. Theory

At thermodynamic equilibrium, the distribution of chemicals between water, SPM and sediment (Fig. 1A) can be described by the partition constants (K_{oc}) between organic carbon (OC) in SPM or sediment and water (Eq. 1). The OC-bound fraction considers the readily desorbable chemicals in particles ($C_{i,OC}$). For simplicity we assumed that the DOC and the OC of particles have the same K_{oc} .

$$K_{\rm OC} = \frac{C_{\rm i,OC}}{C_{\rm i,w}} \tag{1}$$



Fig. 1. (A) Partitioning processes in a river system of water (w), suspended particulate matter (SPM) and sediment (sed) and (B) experimentally accessible measures in samples taken from a river. C: concentration; n: amount of chemicals; m: mass of samples; V: volume of sampled water; [DOC]: concentration of dissolved organic carbon in water phase; LVSPE: large volume solid phase extraction; SPE: solid phase extraction; i: any chemical i; *K*: partitioning constant; *D*: distribution ratio; PES: passive equilibrium sampling; PDMS: polydimethylsiloxane; OC: organic carbon; ASE: accelerated solvent extraction. All abbreviations are detailed in Table S1.

In a flowing river, even under baseflow conditions, we cannot assure that equilibrium is reached for chemicals between water, SPM and sediment. However, the distribution ratios between phases at the time of sampling can be measured by accessing the sample matrices with direct measurements or PES in the laboratory. The experimentally accessible measures determined in the laboratory are listed in Fig. 1B and summarized in Table S1.

The concentration of a chemical i that is freely dissolved in an enriched and resuspended SPMwater PES system ($C_{i,free,SPM,PES}$) can be translated from the concentration in PDMS ($C_{i,PDMS,SPM}$) using the partition constant between PDMS and water ($K_{i,PDMS/w}$) (Fig. 1B, Eq. 2):

$$C_{i,free,SPM,PES} = \frac{C_{i,PDMS,SPM}}{K_{i,PDMS/w}}$$
(2)

For method comparison, we used two types of SPE for direct measurement of the aqueous concentration ($C_{i,w,(LV)SPE}$), i.e., the concentration of chemical i in water measured after conventional SPE ($C_{i,w,SPE}$ (ng/L)); or after large volume solid phase extraction (LVSPE) ($C_{i,w,LVSPE}$ (ng/L)). $C_{i,w,(LV)SPE}$ is actually the sum of the concentrations of freely dissolved ($C_{i,free,(LV)SPE}$) chemicals and those bound to DOC ($C_{i,DOC}$).¹⁴ $C_{i,free,(LV)SPE}$ can be derived from Eq. 3, where [DOC] is the DOC content in water (kg_{DOC}/L). A comparison between $C_{i,free,SPM,PES}$ and $C_{i,free,(LV)SPE}$ can tell us if the assumption that chemicals are at equilibrium between water and SPM under baseflow conditions is correct in practice.

$$C_{i,free,(LV)SPE} = \frac{C_{i,w,(LV)SPE}}{1 + [DOC] K_{oc}}$$
(3)

Since [DOC] was around 10⁻⁶ kg/L in the studied river water (Table 1), the DOC-associated fraction only contributes significantly (> 9%) to $C_{i,w,(LV)SPE}$ for chemicals with $\log K_{ow} > 5$ ($K_{ow} \approx K_{OC}^{11}$). The difference between $C_{i,free,(LV)SPE}$ and $C_{i,w,(LV)SPE}$ can be neglected for charged chemicals and chemicals with $\log K_{ow} \leq 5$. However, if chemicals with $\log K_{ow} \geq 7$ were detected in SPE extracts, they would be mainly bound to DOC, with chemicals of $\log K_{ow}$ between 5 and 7 in both phases.

The apparent distribution of chemicals between SPM and water ($D_{i,SPM,/w}$, L/kg) and SPM and sediment ($D_{i,SPM/sed}$, kg_{sed,dw}/kg_{SPM,dw}) at the time of sampling can be expressed with Eqs. 4 and 5.

$$D_{i,SPM/w} = \frac{C_{i,SPM}}{C_{i,w,SPE}}$$
(4)

$$D_{i,SPM/sed} = \frac{C_{i,SPM}}{C_{i,sed}}$$
(5)

 $C_{i,SPM}$ is the concentration of chemical i in SPM collected by metal filter (ng/g_{SPM,dw}), and $C_{i,sed}$ is the concentration of chemical i in sediment (ng/g_{sed,dw}).

The fraction of the SPM-bound chemical i in the total water column ($f_{i,SPM}$) can be estimated by Eq. 6, where m_{SPM} is the mass of SPM (g_{dw}) in a certain volume (V_w) of water (L).

$$f_{i,SPM} = \frac{C_{i,SPM} \times m_{SPM}}{C_{i,SPM} \times m_{SPM} + C_{i,w,SPE} \times V_w} = \frac{1}{1 + \frac{1}{D_{i,SPM/w}} \times \frac{V_w}{m_{SPM}}}$$
(6)

Bioanalytical equivalent concentrations (BEQs) are used to express the effect of a chemical mixture in concentration units by converting the effect concentrations of the mixture to the equieffective concentration of a reference compound.¹⁵ Accordingly, we can derive the effect-based distribution ratios D_{mixture}^{16} for chemical mixtures from the ratio of their BEQs, namely $D_{\text{mixture,SPM/w}}$ for distribution between SPM and water (Eq. 7) and $D_{\text{mixture,SPM/sed}}$ for distribution between SPM and sediment (Eq. 8). BEQ_{SPM} is the bioanalytical equivalent concentration of SPM mixtures collected by metal filters ($\mu g_{ref}/g_{SPM,dw}$), BEQ_{w,SPE} is the bioanalytical equivalent concentration of sediment mixtures ($\mu g_{ref}/g_{sed,dw}$).

$$D_{\text{mixture,SPM/w}} = \frac{\text{BEQ}_{\text{SPM}}}{\text{BEQ}_{\text{w,SPE}}}$$
(7)

$$D_{\text{mixture,SPM/sed}} = \frac{BEQ_{\text{SPM}}}{BEQ_{\text{sed}}}$$
(8)

The fraction of BEQ bound to SPM, f_{mixture,SPM}, can then be derived by Eq. 9.

$$f_{mixture,SPM} = \frac{1}{1 + \frac{1}{D_{mixture,SPM/w}} \times \frac{V_w}{m_{SPM}}}$$

3. Materials and methods

3.1. Sampling

The field sampling campaign was conducted on August 27, 2019 at two sampling sites (A and B) along the Ammer River, which is a tributary of the Neckar River, Germany. Site A (48°34′04.7″N, 8°53′30.7″E) is located upstream and Site B (48°31′34.7″N, 8°57′50.9″E) downstream of a wastewater treatment plant (WWTP). The average annual flow at the gauge of this river is 0.87 m³/s. Detailed information of the sampling sites and the WWTP were described in previous studies.^{2, 17}

A 293 mm Disc Filter Holder, Stainless Steel (Pall Corporation, Port Washington, NY, USA) with an effective filtration area of 587 cm³ was used for the separation of SPM in the field with metal filters of 6 μ m. To obtain enough SPM for analysis, approximately 2000 L of river water were filtered in total at each site. 2 L of filtered water were collected during filtration with amber glass bottles and enriched with SPE in the laboratory (Section 3.3). In parallel, a LVSPE device (Maxx Mess- und Probenahmetechnik, Rangendingen, Germany) equipped with a pre-filter and a preconditioned LVSPE cartridge was employed on site for an automatic sampling and extraction of the river water (40 L at Site A and 20 L at Site B).¹⁸ To trap the SPM applying the LVSPE, a steel cartridge containing a Soxhlet extraction thimble in a metal mesh was inserted before the prefilter and the cartridge. In addition, a composite sediment sample was generated from several sediment subsamples, which were collected with a clean stainless-steel shovel from the top 5 cm of the riverbed, avoiding big items and gravel, and transferred into a 500 mL amber glass jar. After collection, all the samples were kept cool in an icebox during transport to the laboratories at the University of Tübingen and the UFZ, Leipzig. Detailed sampling information is provided in Text S1.

3.2. Physicochemical characterization of samples

The physical parameters of water, including temperature, pH, electrical conductivity and turbidity were measured on site during sampling or in the lab according to the method reported in the studies of Guillet et al.¹⁹ and Glaser et al.¹⁷ (Table S2). The mass concentration of SPM in water [SPM], the [DOC], the OC content of SPM [OC, SPM] and sediment [OC, sediment] and the water content of sediment were also determined (details are given in Text S1) and the results are tabulated in Table 1. It is noteworthy that the [DOC] was twenty times higher than the [OC, SPM]. Hence, [SPM] was highly enriched prior to the PES experiments to make sure that the chemicals bound to SPM could be characterized.

Sample	Parameter	Site A	Site B
Filtered river water (< 0.45 µm)	[DOC] ^a (mg _{OC} /L)	1.63±0.18	2.33±0.03
Suspended particulate matter (> 6 µm)	$[SPM]^b (mg_{dw}/L)$	1.05	0.95
	$[OC, SPM]^{c} (g_{oc}/g_{SPM,dw})$	0.105 ± 0.001	0.112±0.002
	[OC, SPM] (mg _{oc} /L)	0.110	0.106
Sediment (< 630 µm)	$[OC, sed]^d (g_{OC}/g_{sed,dw})$	0.029 ± 0.001	0.041 ± 0.0004
	Water content m_w/m_{sed}^e (%)	46.4%	55.3%

Table 1. Selected physicochemical characteristics of water and particle samples.

^a[DOC]: dissolved organic carbon content; ^b[SPM]: mass concentration of suspended particulate matter in water; ^c[OC, SPM]: organic carbon content of SPM; ^d[OC, sed]: organic carbon content of sediment; ^em_w/m_{sed}: mass weight of water/mass weight of dry sediment.

3.3. Extraction of water and particle samples

The SPE was used for extracting and concentrating the chemicals from 2 L of filtered river water with 500 mg HR-X cartridges (Chromabond, Macherey-Nagel, Düren, Germany). The elution of freeze-dried cartridges from LVSPE was conducted according to Schulze et al.¹⁸ The dried SPE extracts were all dissolved in methanol. The accelerated solvent extraction (ASE) method coupled with clean-up procedures established previously²⁰ was modified and used for the exhaustive extraction of SPM, LVSPE filter and sediment. Triplicate experiments were conducted for sediment and SPM samples, while all water was extracted with one SPE column because our previous work demonstrated the high repeatability of the SPE extraction.^{21, 22} The detailed pretreatment of water and particle samples, as well as the clean-up procedures are described in Text S2.

3.4. Passive equilibrium sampling of SPM and sediment

Depletive PES was used to extract the bioavailable fractions of pollutants in homogenized SPM and sediment. A preliminary uptake kinetics experiment of PDMS with sediment was performed to identify the time for attaining equilibrium. After wiping dry with tissue, the PDMS sheets were extracted twice with ethyl acetate. The combined extracts were evaporated and reconstituted in $500 \,\mu\text{L}$ of ethyl acetate. The sum of freely dissolved and OC-bound chemicals was used to indicate the bioavailable fractions of chemicals in particles. All PES experiments were performed in triplicate. More information on PES is given in Text S3 and Table S3, and the uptake kinetics experiments are described in Text S4, Fig. S1–S2 and Table S4.

3.5. Target chemical analysis

The target chemical analysis included 642 chemicals of 15 categories with diverse physicochemical properties and was performed with LC-HRMS and GC-HRMS. The names, classification and physicochemical properties of the analytes and the internal standards are listed in Table S5 and S6. The detailed information on instrumental conditions, as well as the method detection limits (MDLs) can be found in our previous study.¹¹ The concentrations of chemicals in samples were blank-corrected for further analysis.

PDMS was used to estimate the bioavailability of neutral chemicals with $\log K_{ow} \ge 3$.²³ Therefore, the analyzed contaminants were grouped into two categories: (1) neutral and hydrophobic chemicals, which comprised neutral chemicals with $\log K_{ow} \ge 3$ and (2) charged or hydrophilic chemicals, which comprised charged chemicals or those with $\log K_{ow} < 3$.

3.6. Bioanalysis and data evaluation

The panel of *in vitro* bioassays used in this study had already been used in previous studies on sediments¹¹ and river water.¹⁵ The targeted modes of action included the activation of the aryl hydrocarbon receptor (AhR) with AhR CALUX, the binding to the peroxisome proliferator–activated receptor gamma (PPARγ) with PPARγ GeneBLAzer and the oxidative stress response

with AREc32 assay. Detailed methods were described by Neale et al.²⁴ and König et al.²⁵ In addition, the photosynthesis inhibition was measured with the combined algae test.²⁶ The algae assay was only dosed with water samples because particle samples did not trigger the photosynthesis inhibition in our preliminary experiments because herbicides were not present in particles at sufficiently high levels in a previous study.²⁷ The viability of cell lines and the growth inhibition of algae were concurrently recorded.

To identify which chemicals are the drivers for the observed effects and cytotoxicity of chemical mixtures, mixture effects predicted for the identified and quantified chemicals (BEQ_{chem}) or the predicted toxic unit (TU_{chem}) for cytotoxicity were compared to the measured biological effects (BEQ_{bio}) or toxic units (TU_{bio}) for cytotoxicity of the corresponding samples. The effect concentrations and compound-specific relative effect potencies (REP) regarding these four biological endpoints were compiled previously^{11, 26} and are reprinted with permission in Table S7 from Niu, L. L.; Carmona, E.; Konig, M.; Krauss, M.; Muz, M.; Xu, C.; Zou, D. L.; Escher, B. I., Mixture Risk Drivers in Freshwater Sediments and Their Bioavailability Determined Using Passive Equilibrium Sampling. Environ. Sci. Technol. 2020, 54, (20), 13197-13206. Copyright (2020) American Chemical Society and reprinted with permission in Table S8 from Glauch, L.; Escher, B. I., The Combined Algae Test for the Evaluation of Mixture Toxicity in Environmental Samples. Environ. Toxicol. Chem. 2020, 39, (12), 2496-2508. Copyright (2020) The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals LLC on behalf of SETAC.

The reference compound in the AhR CALUX was benzo[a]pyrene, rosiglitazone in the PPAR γ GeneBLAzer, dichlorvos in the AREc32 and diuron in the algae assay. The ratios BEQ_{chem}/BEQ_{bio} and TU_{chem}/TU_{bio} are a measure of the fraction of effects explained by the detected chemicals. The

effect drivers can be prioritized according to the effect contribution of a certain chemical i $(BEQ_{chem,i}/BEQ_{chem})$ or the cytotoxicity contribution $(TU_{chem,i}/TU_{chem})$. Detailed data evaluation processes are described in Text S5.

4. Results and discussion

4.1. Dissolved and particle-bound chemicals

The pollutant concentrations in water agreed very well between SPE after filtration ($C_{i,w,SPE}$) and LVSPE ($C_{i,w,LVSPE}$) (Text S6, Fig. S3A and B). However, the concentrations in SPM were consistently higher when determined with LVSPE than after filtration with a metal filter (Fig. S3C and D, Fig. S4-S5). The particle cut-off in LVSPE filters was not well defined and the uncertainty of SPM mass in LVSPE filters was high due to the smaller volume of filtered water (40/20 L) associated with a very low SPM mass (19.1 to 42.1 mg) collected with the LVSPE instrument. Therefore, we interpret that the systematic deviation is likely due to the underestimation of the SPM mass in the LVSPE experiment. Further evaluation will only focus on the samples filtered with the metal mesh.

A total of 41% of analyzed chemicals (n=266) were detected in SPE extracts, with the concentrations ranging from 10^{-4} to 10^4 ng/L (Table S6, Fig. 2A and Figs. 3A and 3B).

The mass concentrations of individual chemicals were converted into molar concentrations to be summed up for further comparison. The sum of dissolved concentrations of all detected chemicals was 12 times higher at Site B (32.1 nmol/L), i.e. downstream of the WWTP, than at Site A (2.59 nmol/L), upstream of the WWTP (Fig. S6A). The industrial chemicals and the pharmaceuticals and personal care products (PPCPs) accounted for over 50% of the sum of chemical concentrations in the water phase (Fig. S6B). Similar high concentrations of PPCPs were also reported in a

previous study in the same river section.²¹ Specifically, sucralose (a sweetener) was the most abundant chemical at Site A and 1H-benzotriazole (a corrosion inhibitor) at Site B (Table S10). In general, the detected numbers and the concentrations of charged or hydrophilic chemicals in the water phase were larger than those of neutral and hydrophobic chemicals. The sum of dissolved concentrations of neutral and hydrophobic chemicals were 0.081 nmol/L (Site A) and 0.577 nmol/L (Site B) (Fig. S6C), and those of charged or hydrophilic chemicals were 2.93 nmol/L (Site A) and 34.0 nmol/L (Site B) (Fig. S6E). They neutral and hydrophobic chemicals only contributed to 2–3% of the total detected chemicals in the water phase and were mainly composed of PPCPs and pesticides (Fig. S6D), while charged or hydrophilic chemicals were mainly comprised of PPCPs and industrial chemicals (Fig. S6F). This is in line with the cognition that hydrophilic compounds prefer to remain in the water phase.²⁸

The freely dissolved concentrations of neutral and hydrophobic chemicals derived from SPE and PES methods are compared in Figs. 3A–C. Only half of the chemicals with $\log K_{ow}$ of 3–6 that were detected by both methods fell between 10:1 and 1:10 lines (Fig. 3A). 76% of the number of detected chemicals had freely dissolved concentrations calculated from PES that were higher than those from SPE, with the median ratio $C_{free,SPE}/C_{free,SPM,PES}$ of 0.32 ($\log(C_{free,SPE}/C_{free,SPM,PES}) = -$ 0.5). This indicates that most of these chemicals were introduced into the river with particles, resulting in disequilibrium with an ongoing flux from SPM to water. When plotting the log ratio of $C_{free,SPE}$ to $C_{free,SPM,PES}$ against the $\log K_{ow}$ of individual chemicals (Fig. 3C), there appeared to be an upward trend with increasing hydrophobicity. All the chemicals with 3 < $\log K_{ow}$ < 5 had the ratio of $C_{free,SPE}/C_{free,SPM,PES} \leq 1$, indicating a flux from SPM to water. In contrast, many chemicals with $\log K_{ow} > 5$ had a ratio > 1, which could mean that there is a flux from the dissolved phase to

SPM for the more hydrophobic chemicals. It is also clear that the concentrations bound to DOC cannot be neglected any more for $C_{w,SPE}$ of chemicals with $\log K_{ow} > 5$.



Fig. 2. (A) The concentrations of freely dissolved, (B) particle-bound and organic carbon (OC)-bound chemicals in the river at the two sampling sites A and B. SPE: solid phase extraction; SPM: suspended

particulate matter; PES: passive equilibrium sampling; n_{neutral} refers to the number of neutral and hydrophobic chemicals and n_{other} refers to the number of charged or hydrophilic chemicals. In Fig. A, the triangles facing up in dark blue refer to neutral and hydrophobic chemicals and the triangles facing down in light blue refer to charged or hydrophilic chemicals. In Fig. B, the triangles facing up in olive green refer to neutral and hydrophobic chemicals in bulk SPM or sediment, the triangles facing down in light green refer to charged or hydrophilic chemicals in bulk SPM or sediment and the triangles facing up in orange refer to neutral and hydrophobic chemicals bound to OC.



Fig. 3. (A) Comparison of the concentrations of neutral chemicals with $\log K_{ow} \ge 3$ in surface water estimated by solid phase extraction (C_{SPE}) and passive equilibrium sampling with suspended particulate matter (C_{free,SPM,PES}); (B) the relative frequency distribution of C_{SPE}/C_{free,SPM,PES}; (C) the relationship between the ratio of C_{SPE} (or C_{free,SPE}) to C_{free,SPM,PES} and the K_{ow} of chemicals. The red dashed line in (B) and (C) refers to a ratio of 1 and the blue dashed line to the median of the logarithms of the ratios, which corresponds to a ratio of 0.32.

Lai et al.¹ used a similar PES method with filtered water but not resuspended SPM to find that the freely dissolved PAHs accounted for 6.5–38% of the SPE extracts in the Yangtze River, China, which is a much larger river with high flow rate. This fraction was lower than that obtained in this study (28–63%). This is reasonable because the impact of continuous resuspension and disturbance on chemical equilibrium was much less in a river at lower flow velocities, leading a shorter time to reach equilibrium. In addition, they observed a similar increase of the ratio of $C_{w,SPE}$ to $C_{free,SPM,PES}$ with increasing K_{ow} for the case of PAHs. Qin et al.²⁹ also found a pronounced disequilibrium of hydrophobic PAHs between SPM and water in the Lake Chaohu, China. The disequilibrium pattern was also found for PFCs in Tokyo Bay, Japan, where the influx of fresh river water or the surface runoff might be possible reasons.¹²

Müller et al.² applied the partitioning theory to compare the measured and the predicted distribution ratios of neutral chemicals in the same river section during storm events. Similar to their findings, we also observed that equilibrium had been attained for triphenylphosphate (Cfree.SPE/Cfree.SPM.PES=1.4 at Site A and 0.9 at Site B) and tri(butoxyethyl) phosphate (Cfree.SPE/Cfree.SPM.PES=0.7 at Site B) between SPM and water during dry weather periods. In the case of isoproturon, the ratios $C_{\text{free,SPE}}/C_{\text{free,SPM,PES}}$ were 4×10^{-4} at Site A and 4×10^{-3} at Site B, which indicates that isoproturon was introduced by SPM and continued to partition into water during dry weather. This is in contrast to the findings during storm events, where isoproturon was close to equilibrium between water and SPM.² Isoproturon is an herbicide and also used as biocide in urban applications and hence may have multiple inputs into rivers and may also enter in its dissolved form during a storm event. The partitioning of chemicals between SPM and water is controlled by various factors, such as river discharge and the characteristics of SPM.²⁸ It varies between chemicals depending on their sources and physicochemical properties. To avoid filtration artifacts, previous studies also proposed to estimate the truly dissolved concentration of chemicals based on the concentrations in the solid or OC phase with partition constants.³⁰ Our findings imply that this partitioning method should be scrutinized since the equilibrium of some chemicals in rivers may not be reached even at low flow velocities.

Fewer chemicals were detected in SPM (n=120, 19% of the total analyzed chemicals) and sediment (n=156, 24% of the total analyzed chemicals) samples (Table S9), with the concentrations ranging from 10^{-3} to 10^5 ng/g_{dw} (Table S6 and Fig. 2B). The total chemical concentrations in SPM were 59.8 nmol/g_{dw} (Site A) and 58.8 nmol/g_{dw} (Site B) and were 50.1 nmol/g_{dw} (Site A) and 90.6 nmol/g_{dw} (Site B) (Fig. S7A) in sediment. The concentrations of neutral and hydrophobic chemicals detected in SPM and in sediments were generally within a factor of 10, while the SPM-

bound charged or hydrophilic chemicals had lower concentrations than in sediment (Fig. S8). The similarity of neutral and hydrophobic chemicals (p = 0.07) and the difference of charged or hydrophilic chemicals (p < 0.0001) in SPM and sediment were further evidenced by a ratio paired t-test. It is noteworthy that although the total chemical concentrations in SPM and sediment were similar, their compositions were quite different, with industrial chemicals prevailing in SPM but PAHs in sediment (Fig. S7B). Due to their high hydrophobicity, PAHs were the major group among neutral and hydrophobic chemicals in both SPM and sediment (Fig. S7D). Industrial chemicals were major components of the group of charged or hydrophilic chemicals in particles (Fig. S7F). Sediments could reflect a long-term pollution source of several years, whereas SPMcontaminants have different sources and are in exchange with the water phase. SPM eventually settles and turns into bed sediment. In contrast, a similar composition but higher concentration in SPM than in sediment was observed for perfluorinated compounds (PFCs) in Tokyo Bay, Japan¹² and Pearl River, China.³¹ The preferential sorption on SPM was also reported for estrogens in Yangtze Estuary³², for PAHs (polycyclic aromatic hydrocarbons) in Yangtze River¹ and for PAHs, organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Chaohu, China.³³ Even though the number of detected neutral and hydrophobic chemicals and charged or hydrophilic chemicals were similar in both bulk SPM and sediment extracts (Fig. 2B), neutral and hydrophobic chemicals contributed more to sediment- (> 96%) than SPM-associated pollution (< 40%) (Fig. S7C and S7E). Despite the smaller particle size and larger specific surface area of SPM, the lower abundance of neutral and hydrophobic chemicals indicates that the chemicals on SPM are more labile and exchangeable and are mainly controlled by dissolved compounds given the similar chemical compositions between SPM and

water phase. In addition, other contributions of less polluted particles, e.g., calcite precipitates, to SPM other than to sediment is likely another reason for this observation.

The $\log D_{SPM/w}$ and $\log D_{SPM/sed}$ were calculated by Eqs. 4 and 5. Neutral and hydrophobic chemicals showed significantly different distribution patterns between SPM and sediment and between SPM and water when compared to charged or hydrophilic chemicals. The compound-specific logD_{SPM/sed} values ranged over five log-units (Fig. 4A), with the median logD_{SPM/sed} of -0.2 (5th and 95th percentile of -1.4 and 1.8 at Site A) and -0.4 (5th and 95th percentile of -1.0 and 1.3 at Site B) for neutral and hydrophobic chemicals and -0.05 (5th and 95th percentile of -0.2 and 1.1 at Site A) and 1.1 (5th and 95th percentile of -0.3 and 2.9 at Site B) for charged or hydrophilic chemicals. This indicates that hydrophobic chemicals have a similar affinity to particles of SPM and sediment and/or are closer to equilibrium, whereas hydrophilic chemicals exchange more readily between SPM and water and do not accumulate in sediments. Some of them might also not be very persistent. The distribution ratios of chemicals between SPM and water were also evaluated, with the logD_{SPM/w} ranging from 0 to 8 (Fig. 4B). The sorption of neutral and hydrophobic chemicals (5th percentile of 3.1, 50th of 5.3 and 95th of 6.3 at Site A, and 5th percentile of 2.7, 50th of 5.6 and 95th of 6.2 at Site B) on SPM was much stronger than charged or hydrophilic chemicals (5th percentile of 2.3, 50th of 3.9 and 95th of 5.0 at Site A, and 5th percentile of 1.4, 50th of 3.2 and 95th of 4.7 at Site B).

The OC-bound concentrations of neutral and hydrophobic chemicals in SPM and sediment were calculated from the concentrations in PDMS extracts and the $K_{OC/PDMS}$ (Table S6). The sum of OC-bound chemical concentration was 10.5 mmol/goc (Site A) and 5.7 mmol/goc (Site B) in SPM and 65.1 (Site A) and 87.2 mmol/goc (Site B) in sediment (Fig. S9A). A higher level of OC-bound contamination in SPM than in sediment was found by Gong et al.³⁴ for endocrine disrupting

chemicals (EDCs) in Pearl River, China, which used the OC content in particles to normalize the detected concentrations to OC basis. PAHs contributed the most to the bioavailable parts of SPM and sediment (Fig. S9B). The median bioavailable fractions (F_{bioavailable,chem}, Eq. S6) of detected chemicals calculated based on their chemical concentrations were 0.061 (Site A) and 0.066 (Site B) in SPM, and 0.048 (Site A) and 0.104 (Site B) in sediment (Fig. S10 and Table S11). This is in accordance to our previous observation¹¹ that only a small fraction of chemicals was readily desorbed from sediment. Overall, considering the similarity in chemical concentrations and dominant groups, surface sediment may act as a proxy for river SPM for neutral and hydrophobic chemicals under baseflow conditions.



Fig. 4. Distribution ratios (*D*) of individual contaminants (Eq. 4 and 5) and chemical mixtures (Eq. 7 and 8) (A) between suspended particulate matter (SPM) and sediment ($D_{SPM/sed}$) and (B) between SPM and water phase ($D_{SPM/w}$). The dashed lines in the violin plots represent the 50th percentiles and dotted lines the 25th and 75th percentiles of the data. The dashed line in A marks $D_{SPM/sed}$ of 1.

4.2. Toxicity and risk drivers of chemical mixtures

Compared to the revised effect-based trigger (EBT) values for mixtures of organic micropollutants in surface water proposed by Escher and Neale³⁵, the BEQs of water extracts were all far below the trigger values for the three cell-based in vitro bioassays, with B[a]P-EQ_{bio} of 19.2-29.8 ng_{B[a]P}/L against the EBT-B[a]P-EQ of 250 ng_{B[a]P}/L, Rosiglitazone-EQ_{bio} of 4.2-10.3 ng_{Rosiglitazone}/L against the EBT-Rosiglitazone-EQ of 1.2 µg_{Rosiglitazone}/L, and Dichlorvos-EQ_{bio} of 1245–2207 ng_{Dichlorvos}/L against the EBT-Dichlorvos-EQ of 1.4 mg_{Dichlorvos}/L (Table S13). The 2h Diuron-EQ_{bio} at both sites and the 24h Diuron-EQ_{bio} at Site A derived from the combined algae test were below the EBT (EBT-DEQ_{2h IPAM} = $0.08 \ \mu g_{Diuron}/L^{26}$), whereas the 24h diuron-EQ_{bio} at Site B (251 ng_{Diuron}/L) was 3.6 times higher than the proposed EBT (EBT-DEQ_{24h IPAM} = 0.07 $\mu g_{\text{Diuron}}/L^{26}$). An overview of the concentration-responses curves of the extracted chemical mixtures in water, SPM, sediment and PDMS are given in Fig. S11–S14. The effect concentration that caused 10% of the maximum effect (EC₁₀) or the induction ratio of 1.5 (EC_{IR1.5}) and 10% of the maximum inhibitory concentration, as well as the calculated BEQ_{bio} and TU_{bio} are tabulated in Table S12-S13. A related discussion on the cytotoxicity in the three *in vitro* bioassays and the growth inhibition of algae are given in Text S7 and Fig. S15.

Iceberg modeling can help to quantify the contribution of detected chemicals to the observed effects of an extract and thus identify chemicals driving the biological effects. Among the 266 detected chemicals in the dissolved phase, less than 15% (n=19–38) activated the specific effects in *in vitro* bioassays used in this study (Table S7-S9). In total, these bioactive chemicals explained up to 0.1% of the observed AhR effect, 4% of the PPAR γ effect and 5% of the oxidative stress response (Fig. S16). The extrapolated total number of chemicals in water would be expected to range from 460–67,000 for these three assays (Table S16), which was estimated based on the known number of bioactive chemicals and observed effects (Text S8). This extrapolation assumes

that the distribution of the effect potencies is similar in the groups of detected and non-detected chemicals, but it could also be that there are fewer undetected chemicals with higher relative effect potency that were either not captured by the chemical analysis or were not active in the bioassays as single compounds. The highest extrapolated number of bioactive chemicals was found for the AhR assay, which is triggered for example by polycyclic aromatic hydrocarbons (PAH). Only 21 PAH were included in the chemical analysis, but more than 100 PAHs exist in the environment and many have not been identified nor tested as individual chemicals in the bioassays. Solubility is often an issue with toxicity testing of very hydrophobic compounds, but they can still contribute to mixture effects.³⁶

An earlier study on small streams in Germany incorporating nearly 400 chemicals also found similar low fractions of the three effects explained by the quantified chemicals.¹⁵ The 28 detected and active chemicals explained 29–68% of the photosynthesis inhibition on algae, evidencing a more specific effect than the three other *in vitro* bioassays, which is in agreement with previous findings in the same river.³⁷ Consistently, the extrapolated number of chemicals inhibiting photosynthesis was only 40–130 chemicals.

The BEQ_{chem} of individual chemicals analyzed in water are listed in Table S14 and S15. The types of chemicals contributing to the total BEQ_{chem} were quite diverse for the water samples (Fig. S17). The groups of PPCPs (such as clobetasol, climbazole and telmisartan) were identified as the key toxicants for the AhR activation at Site B, while benzo[b]fluoranthene alone explained 38% of the AhR activation of water extract from Site A (Table S17). These chemicals did not rank highly according to their dissolved concentrations (Table S10), but they were identified as effect drivers by iceberg modeling due to their high effect potencies. Neale et al.¹⁵ identified diclofenac as the risk driver during rain events in small streams. However, telmisartan contributed the most (over

60%) to the observed PPAR γ effect in this study. In the case of oxidative stress response, pesticides, PAHs and industrial chemicals were identified as key toxicants in water samples. Similar to the finding on the same river in a study conducted in 2017,²⁶ terbutryn was the dominant mixture effect driver for photosynthesis inhibition, followed by atrazine at Site A and isoproturon at Site B.

Before comparing the biological effects between SPM and sediment, we compared the effects in the three bioassays used for particle samples before and after clean-up. As shown in Fig. S18, the clean-up procedure did not substantially alter the biological effects triggered by samples. To stay consistent with and make it comparable to chemical analysis, we only refer to the extracts with clean-up in the following.

The BEQ_{bio} of specific effects (Table S13) agreed within a factor of 10 between sediment and SPM (Fig. 5) and were very similar between sites for a given bioassay. For bulk extracts, the B[a]P-EQ_{bio} were very similar for sediment and SPM with 27.5–27.8 μ g_{B[a]P}/g_{sed,dw} and 11.7–15.8 μ g_{B[a]P}/g_{sPM,dw}. In contrast, the Rosiglitazone-EQ_{bio} were ten times higher in SPM (0.10–0.13 μ g_{Rosiglitazone}/g_{SPM,dw}) than in sediment (0.01 μ g_{Rosiglitazone}/g_{sed,dw}), which is consistent with mainly wastewater-derived chemicals activating the PPAR γ receptor. Oxidative stress response did not yield a clear picture with the Dichlorvos-EQ_{bio} being very similar at site A between SPM (38.3 μ g_{Dichlorvos}/g_{SPM,dw}) and sediment (43.3 μ g_{Dichlorvos}/g_{sed,dw}), but differing largely at site B between SPM (68.6 μ g_{Dichlorvos}/g_{SPM,dw}) and sediment (945 μ g_{Dichlorvos}/g_{sed,dw}).



Fig. 5. Comparison of bioanalytical equivalent concentrations (BEQ_{bio}) of chemical mixtures in suspended particulate matter (SPM) and sediment and in their organic carbon (OC) fractions. Data are from Table S13. The error bars are standard errors (standard deviation/number of replicates) calculated from triplicate experiments.

The OC-bound chemicals in SPM showed ten times lower potency than those in sediment regarding the activation of AhR, whereas ten times higher in terms of the binding to PPAR γ (Fig. 5). The BEQ values of OC-bound chemicals in SPM and sediment were much closer to each other for the oxidative stress response (Dichlorvos-EQ_{bio}) than the other two biological endpoints. The bioavailable fractions (F_{bioavailable,bio}, Eq. S8) calculated based on the BEQ_{bio} values of chemical mixtures in SPM and sediment were within the same range as the F_{bioavailable,chem} (Fig. S10 and Table S11).

Among the chemicals detected in bulk SPM and sediment and their bioavailable fractions, 19-24% (n=23-35) of them activated AhR, 2-6% (n=3-7) activated PPAR γ and 19-26% (n=23-36)

triggered the oxidative stress response (Table S9). Compared to the water phase, more of the observed oxidative stress response was explained by chemicals detected in bulk particles and the bioavailable parts (8–19% in bulk SPM, 3–85% in bulk sediment and 2–18% in PDMS of particle slurries), whereas less effect was explained in the case of binding to PPAR γ (0.01–0.02% for SPM, 0.02–0.06% for sediment and 0.1–0.6% in PDMS of particle slurries) (Fig. S16). The observed effects in particles and their PDMS extracts were attributable to fewer chemicals than those in water (Fig. S19). However, the extrapolated number of total chemicals in particles showed no significant difference from that in water (34–75,007 *vs.* 460–67,000). PAHs were expected to be the driving group for the AhR effect and oxidative stress response in both SPM and sediment samples due to their high concentrations and high REPs (Table S17). Similar to our previous study on Chinese sediments,¹¹ triphenylphosphate was also identified as a priority contaminant in SPM and sediment regarding the binding to PPAR γ .

The distribution patterns of chemical mixtures between SPM and sediment and between SPM and water were further explored and compared with those of individual chemicals (Fig. 4). The $D_{\text{mixture,SPM/sed}}$ of AhR CALUX (0.57–0.42) were closer to 1 than those of the other two assays (10.4–11.6 for PPAR γ GeneBLAzer and 0.07–0.9 for AREc32). This is in a good agreement with the chemical analysis, which indicated that the distribution of neutral and hydrophobic chemicals between SPM and sediment was much closer to equilibrium than that of charged or hydrophilic chemicals. The distribution of chemical mixtures between SPM and water was quite comparable among bioassays, with $\log D_{\text{mixture,SPM/w}}$ of 5.6–5.9 for AhR CALUX, of 4.0–4.5 for PPAR γ GeneBLAzer and 4.5 for AREc32. The chemical mixtures showed similar $D_{\text{SPM/w}}$ (Eq. 7) to the median values of individual neutral chemicals (Fig. 4). A range of 3–5 (AhR) and 3–4 (ARE) for effect-based $\log D_{\text{mixture,SPM/w}}$ were reported previously at the same sampling sites during a storm

event.² The $D_{\text{mixture,SPM/w}}$ estimated from the AhR effect in that study was lower than in the present study, which might result from the lower BaP-EQ_{bio} of SPM during a storm event.² This is counterintuitive because the SPM in a rain event is expected to be considerably impacted by the mobilization of surface sediment from the riverbed,¹⁷ which would be expected to affect hydrophobic chemicals that are the major activators of AhR. Potentially, other sources of SPM and other chemicals might become relevant under different hydrological conditions. Another possible reason is that other chemicals that are not predominant in sediment might be the mixture effect drivers in SPM for the activation of AhR during a storm event.

4.3. Fate of chemicals in the whole river system

Due to the mobility and transferability of SPM between water column and sediment, its existence and amount may influence the bioavailability and biological impacts of pollutants to aquatic organisms.³⁸ In recent years, the importance of SPM for the behavior and fate of waterborne contaminants has been recognized with studies that have exclusively analyzed chemicals but not effects.^{39, 40}

In this study, the contribution of SPM-associated organic contaminants to the total water column in a river ($f_{i,SPM}$; Eq. 6 and $f_{mixture,SPM}$ in Eq. 9) was estimated based on both the chemical concentration and the biological effect. As shown in Fig. 6, the $f_{i,SPM}$ of neutral and hydrophobic chemicals ranged from 10^{-3} to 1 and those of charged or hydrophilic chemicals were more variable ranging from 10^{-5} to 1. Because of the low mass concentration, the role of SPM has been ignored for a long time in water quality assessment, especially under dry weather conditions. However, in this study, we found that even though the SPM mass contributed only around a fraction of 10^{-6} to the total water column of the river (1.05 and 0.95 mg/L of SPM, assuming a density of ~1 kg/L in

water), the contribution of individual SPM-associated contaminants was always by several orders of magnitude higher than 10^{-6} (Fig. 6). The median $f_{i,SPM}$ were 0.18 (Site A) and 0.26 (Site B) for neutral and hydrophobic chemicals and 0.008 (Site A) and 0.002 (Site B) for charged or hydrophilic chemicals. This is in accordance with earlier studies that dissolved forms of contaminants dominated the chemical burden in water column, with the contribution of 97-98% for PFCs in Tokyo Bay, Japan¹², Liao River, China and Taihu Lake, China⁴¹ and of 12–98% for EDCs in the Northern Aegean Sea of Greece¹³, the Yangtze Estuary of China³² and the Pearl River Delta of China⁴². However, it is noteworthy that the $f_{i,SPM}$ of some neutral chemicals detected in this study were over 50%, especially for PAHs. Previous studies also found that the long chain PFCs bound to SPM could contribute to 100% of their total concentrations detected in the water column.³⁹ The contribution from SPM-loading was also up to 72% for PAHs in the urban stretch of the River Tiber $(81.4 \text{ m}^3/\text{s})^3$, up to 88% for estrogens in the Yangtze Estuary³², up to 96% for PBDEs in the Yellow River, China⁴³ and 81% for tetrabromobisphenol A in Taihu Lake and its tributaries, China.⁴⁴ This might be related to the high sorption capacity of SPM for organic chemicals, but we cannot exclude that the high contribution of SPM-bound chemicals to the total pollution load might also be caused by the high SPM mass concentration due to the unknown flow rate of these rivers.

In the case of the effect-based $f_{mixture,SPM}$ (calculated from BEQ with Eq. 9 that is analogous to Eq. 6), the SPM-bound chemical mixtures accounted for 0.46 (Site A) and 0.27 (Site B) of the AhR activation, 0.03 (Site A) and 0.009 (Site B) of the binding to PPAR γ and 0.03 (Site A and B) of the oxidative stress response observed in total water column. The higher contribution of SPM to the AhR effect is reasonable due to the high affinity of hydrophobic chemicals to particles.

Our results indicate that even under dry weather conditions SPM is a crucial vector from a toxicological perspective. The contribution of SPM should be taken into account for the water quality assessment to provide a better and accurate understanding of the fate and risk of chemicals in aquatic systems.



Fig. 6. Fractions of individual contaminants (f_{i.SPM}, Eq. 6) and chemical mixture effects (f_{mixture,SPM}, Eq. 9) in suspended particulate matter (SPM) to the total river water column. The dashed lines in the violin plots represent the 50th percentiles and dotted lines the 25th and 75th percentiles of the data.

The fate of contaminants in aquatic systems is not only governed by the distribution between phases, but also depends on the input sources and the degradability of chemicals. During dry weather periods, the discharge of WWTP might have a greater impact on the chemical and effect burden of the river than other disturbing factors.²¹ Site B was located downstream of a WWTP and accordingly had a higher pollution burden with higher detection frequency of chemicals, especially in terms of charged or hydrophilic chemicals, which was also indicated by the dominance of

wastewater markers¹⁵ like 1H-benzotriazole, sucralose, 5-methyl-1H-benzotriazole, diclofenac, carbamazepine, caffeine and sulfamethoxazole at Site B (Table S10). Consistently, the sum of chemical concentrations in water at Site B were 12 times higher (Fig. S20) and the effects were1.6–15 times higher than those from Site A (Fig. S21). The impact of the WWTP effluent on the water quality was also found in the same river section in previous studies.^{8, 21}

There was a smaller difference of the particle-bound chemical levels and effects (Fig. S20 and S21) between Site A and Site B. SPM-bound contaminants were apparently less affected by the WWTP, because WWTPs can effectively eliminate hydrophobic contaminants through sorption and sedimentation of solids. Similar levels at both sites were also found for PAHs during a rain event.¹⁷ Consistently, there was no significant difference on the mixture effect drivers identified in SPM and sediment mixtures between the two sites (Table S17).

The experimental results led us to reject our initial hypothesis (1) that neutral and hydrophobic chemicals are at equilibrium between water and SPM in a river at baseflow. However, we corroborated the two other hypotheses that (2) the sediment can serve as proxy for SPM at baseflow condition, but more work is required on hydrophilic and charged chemicals because (3) they showed differences in SPM-water and sediment-water distributions from neutral and hydrophobic chemicals and their association with condensed phases is not negligible. The risk posed by the various organic contaminants in aquatic systems may be underestimated or misunderstood without taking the role of SPM into consideration. Chemicals bound to SPM may significantly impact the fate and risks of pollutant mixtures in river. Especially when it comes to hydrophobic chemicals and mixture effects, a comprehensive assessment of water quality should include SPM, which would increase the accuracy of risk estimation for aquatic environments.

ASSOCIATED CONTENT

Supporting information

The supporting information is available free of charge at https://pubs.acs.org/doi....

Additional information on sampling sites, experimental methods, data evaluation method, physicochemical properties of samples, uptake kinetics experiments, comparison of LVSPE and filtration methods on water monitoring, chemical and toxicological profiles of water, SPM and sediment samples and cytotoxicity related results (pdf). Excel file with all experimental data.

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Author contributions

Beate I. Escher and Lili Niu designed the study; Clarissa Glaser, Beate I. Escher and Lili Niu performed the sampling campaign; Marc Schwientek gave advice for the sampling campaign; Lili Niu performed the ASE and PES experiments; Maria König and Lili Niu performed SPE and the

bioassay experiments, Clarissa Glaser measured the physicochemical properties of samples; Jörg Ahlheim, Roman Gunold, Luise Henneberger, Christiane Zarfl helped with the organization of sampling and experiments; Lili Niu, Roman Gunold and Martin Krauss conducted the chemical analysis with LC and GC instruments; Beate I. Escher developed the data evaluation and the iceberg modeling; Lili Niu evaluated all the chemical and bioassay data, performed the iceberg modelling; Lili Niu and Beate I. Escher wrote the manuscript; all authors reviewed the manuscript.

All authors have given approval to the final version of the article.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The present study was partially funded by the German Research Foundation DGF (Collaborative Research Centre 1253 CAMPOS (Project P1: Rivers) DFG grant SFB 1253/1 2017). Lili Niu is supported by a Humboldt postdoctoral fellowship from the Alexander von Humboldt Foundation. We gratefully acknowledge access to the platform CITEPro (Chemicals in the Environment Profiler) funded by the Helmholtz Association. The authors thank Amelia Lewis, Aleksandra Piotrowska and Rita Schlichting.

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