Mixture risk drivers in freshwater sediments and their bioavailability determined with passive equilibrium sampling

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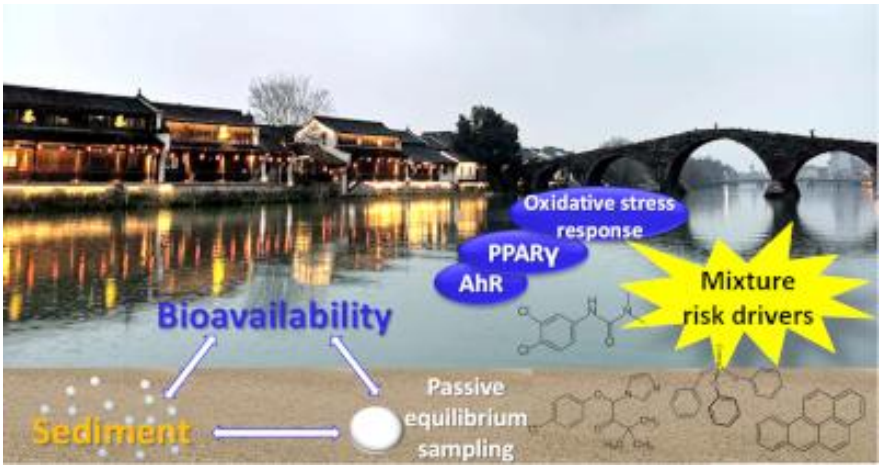
13 **TOC art**


Oxidative stress response
PPARγ
AhR
Mixture risk drivers
Bioavailability
Sediment
Passive equilibrium sampling

14

Abstract

The identification of mixture risk drivers is a great challenge for sediment assessment, especially when taking bioavailability into consideration. The bioavailable portion, which comprises the organic contaminants in pore water and the ones bound to organic carbon, was accessed by equilibrium partitioning to polydimethylsiloxane (PDMS). The exhaustive solvent and PDMS extracts were toxicologically characterized with a battery of *in vitro* reporter gene assays and chemically analyzed with liquid and gas chromatography coupled to high-resolution mass spectrometry. The bioavailable fractions of mixture effects and individual chemicals were mostly lower than 0.1, indicating that more than 90% of the substances are strongly bound and would not pose an immediate risk but could potentially be remobilized in the long term. Despite 655 organic chemicals analyzed, only 0.1%-28% of the observed biological effects was explained by the detected compounds in whole sediments, while 0.009%-3.3% was explained by bioavailable chemicals. The mixture effects were not only dominated by legacy pollutants (e.g., polycyclic aromatic hydrocarbon (PAHs) in the bioassay for activation of the aryl-hydrocarbon receptor (AhR) and oxidative stress response (AREc32)), but also by present-use chemicals (e.g., plastic additives for binding to the peroxisome proliferator-activated receptor γ (PPARγ)), with different fingerprints between whole sediments and bioavailable extracts. Our results highlight the necessity to involve different bioassays with diverse effect profiles and broader selection of contaminants along with bioavailability for the risk assessment of chemical mixtures in sediments.

Keywords: Sediment mixture; Risk driver; Bioavailability; *In vitro* bioassay; Chemical analysis

1. Introduction

Sediments are not only an important sink for hydrophobic contaminants introduced into the environment, but they are also a long-term source of pollution for the whole ecosystem. The contaminants distributing between sediments and water bodies may continuously pose a hazard on aquatic organisms and communities. Estimating sediment contamination is still challenging because the chemicals are present in complex mixtures. Risk assessment of sediment pollutants was traditionally based on routine instrumental analysis and the prior information on toxicity or predicted no effect concentrations of individual detected chemicals.[1, 2] However, there is the obvious limitation that chemical analysis could only shed light on a narrow portion of substances but not on all contaminants in the mixtures. As a consequence, the risk contaminated sediments poses may be underestimated. Effect-based methods using *in vitro* bioassays that are essential indicators of crucial steps of the cellular toxicity pathways have been recommended as a sensitive tool to introduce a mixture perspective in sediment quality assessment.[3, 4] They serve as a complement for the estimation of chemical mixtures addressing different toxicological endpoints, but are not directly linked to the ecological risks in sediments for organisms and communities. Application of batteries of *in vitro* assays allow one to identify which modes of action are affected and how large the mixture effects are, but it is not possible to identify which chemicals cause the specific effects. An integrated approach combing chemical and effect screening indicative of diverse compounds and toxicological endpoints has been shown powerful for monitoring sediment quality and may aid to identify mixture risk drivers.[5-7]

Bioavailability of sediment-associated chemicals determines the observed toxicity in sediment-dwelling organisms and has been proposed as a better indicator for the realistic environmental exposure in aquatic systems than the bulk concentration of sediment chemicals.[8, 9] Passive

equilibrium sampling (PES) using polydimethylsiloxane (PDMS) has become a promising alternative for extracting organic pollutants that are freely available for the uptake by aquatic biota or partition to other media.[8, 10] Many studies combined PES with chemical analysis to study the bioavailable concentrations[11-13] or with a battery of *in vitro* bioassays to test the related toxicity[3, 14, 15] of sediment extracts. Furthermore, Li et al.[6], Müller et al.[13] and Vethaak et al.[15] incorporated target chemical analysis and bioassays with PES technique to link the biological activities with total and bioavailable chemicals in sediments. Only a small set of target analytes were covered in these studies. The incorporation of bioavailability together with wide-scope chemical and biological screening of the complex sediment mixtures remains to be explored. We hypothesize that the risk profiles of bioavailable mixtures might be different from those of whole sediments and may even be specific in different bioassays, where different groups of chemicals act together.

Effectively interpreting the information from available analyses plays a vital role in the risk assessment of environmental pollution, and may support the identification of priority chemicals in chemical mixtures. Many previous studies discussed chemical burden and toxicological effects but did not link the two.[5, 16] In recent years, several mixture toxicity models, such as toxic unit (TU),[2] multi-substance Potentially Affected Fraction of species (msPAF)[17] and bioanalytical equivalent concentration (BEQ)[18] models, were established and successfully applied to bridge the gap between measured concentrations and adverse effects. Iceberg modelling is an extension of the BEQ approach, and it can not only identify the main drivers among thousands of pollutants that trigger the specific modes of action, but can also quantify how much of the experimental mixture effects can be explained by detected chemicals.[19] So far, iceberg modelling

has only been widely used in the prioritization of chemicals in water mixtures, but it has not yet been applied to investigate the sediment pollutants concerning total and bioavailable pollution.

In this study, mixtures of organic chemicals were exhaustively solvent-extracted from sediments and their bioavailable portions that could potentially be taken up by aquatic organisms were accessed through PES with PDMS.[3] We systematically used wide-scope target chemical analysis combined with a battery of *in vitro* bioassays indicative of different modes of toxic action (activation of the arylhydrocarbon receptor (AhR), binding to the peroxisome proliferator-activated receptor γ (PPARγ) and oxidative stress response (ARE)) as an integrated strategy to address the sediment-associated mixture toxicity. A total of 655 organic chemicals, covering 230 pharmaceutical and personal care products (PPCPs), 186 pesticides, 104 industrial chemicals, 16 plastic additives, 6 perfluorinated compounds, 17 food ingredients, 2 human metabolites, 7 natural compounds, 22 organochlorine pesticides (OCPs), 21 polycyclic aromatic hydrocarbons (PAHs), 4 polybrominated diphenyl ethers (PBDEs), 13 polychlorinated biphenyls (PCBs), 13 pyrethroids, 7 chlorobenzenes and 7 other halogenated compounds, were analyzed using liquid or gas chromatography coupled to high-resolution mass spectrometry (LC-HRMS and GC-HRMS). We aimed to (1) characterize the chemical and toxicological profiles of sediments, (2) elucidate the bioavailable fraction of chemicals and effects, (3) quantify cause-effect association between pollution load and toxicity, and (4) identify the risk drivers for the observed mixture effects of sediment-associated organic contaminants.

2. Materials and methods

2.1. Sampling

Surface sediments (0-20 cm) were collected at 5 sites in the Beijing-Hangzhou Grand Canal (BHGC, Hangzhou segment) and at 6 sites in the Qiantang River (QTR), China in January 2019 (Fig. S1). Starting from Beijing and ending in Hangzhou City with a total length of 1794 kilometers, the BHGC is the biggest canal in the world and has played a very important historical role as a traffic artery in China. The Hangzhou segment is the most southern section of BHGC and connects with the QTR, which is located in the Yangtze River Delta and serves as an indispensable drinking water source for local people. Detailed geographic information and the major anthropogenic pressures of each site can be found in Table S1. Three to 5 subsamples were collected with a stainless steel grab or shovel and combined ~~as~~ to one at each sampling site. The sediments were stored in aluminum foil bags and immediately transported to the laboratory in cooler bags. After manually removing stones and other big items, an aliquot of composite sediments was taken for physicochemical characterization. The fresh sediments were kept at 4 ºC up to 7 days before performing the PES experiments.

2.2. Physical chemical characterization of sediments

The water content (f_w, %) was measured by weighing an aliquot of sediments before and after freeze-drying (Table S1). The fraction of organic carbon ($f_{OC,dw}$, %) in sediments was determined with a modified Walkley-Black oxidation method (details are in Text S1) and an Elemental Analyzer (Vario EL cube) after acidification. The $f_{OC,dw}$ were expressed as the average value obtained from the two methods (Table S1).

2.3. Sample extraction

Accelerated solvent extraction (ASE) with Dionex ASE 350 (Thermo Fisher Scientific, CA, USA) was used to extract total chemicals in freeze-dried and sieved sediments according to a standard method with a few modifications[14, 20] (see Text S2).

A negligible-depletion PES with PDMS was applied to obtain the bioavailable fraction of contaminants in sediments based on the method established by Li et al.[3] The amounts of fresh sediments and PDMS and other details of PES can be found in Text S3 and Table S2.

All extracts were blown down to dryness, sealed and shipped from China to Germany. Full blow-down might have incurred partial loss of semi-volatile chemicals but was unavoidable due to transport regulations.

2.4. *In vitro* bioassays

In a previous study, Jahnke et al.[4] found that among the 7 bioassays they employed, the bioassays indicative of the activation of AhR (AhR CALUX), binding to PPARγ (PPARγ GeneBLAzer) and oxidative stress response (AREc32) were more sensitive, because no mixture effects were detected with the bioassays indicative of the effects on the estrogen, androgen, glucocorticoid and progesterone receptors. Therefore, in this study, a similar bioassay strategy, with AhR CALUX[21], PPARγ GeneBLAzer[22] and oxidative stress response (AREc32)[23] was selected accordingly for testing the total mixtures and PDMS extracts of sediment-associated pollutants. The routine cell culture and dosing procedures were conducted as those previously established[22, 24] and are detailed in Text S4.

2.5. Target chemical analysis

A total of 553 chemicals were quantified with LC-HRMS and 102 chemicals with GC-HRMS. Since the contaminants accumulated in sediments mainly originate from water bodies, the compounds typically targeted for water quality monitoring[19] were also included in this study apart from those previously detected in sediments. The analyzed chemicals covered 15 categories with a wide range of physicochemical properties and also included several transformation products (Table S3 and S4). The detailed conditions of the instrumental analysis are provided in Text S5. A 12-point calibration with standard mixtures, as well as solvent blanks, procedure blanks and quality control samples were run with every batch. The method detection limits (MDLs) were determined according to the guideline suggested by the U.S. EPA[25] (Table S5). The concentrations of target compounds in samples below the MDL were treated as zero in further statistical analysis.

2.6. Data evaluation for bioanalysis

The concentration unit of an extracted sample was expressed as relative enrichment factor (REF, $g_{sed,dw}/L_{bioassay}$ or $g_{PDMS}/L_{bioassay}$), which was calculated by multiplication of the enrichment factor (EF) and the dilution factor (DF) (Text S6). The concentrations causing 10% of the maximum effect (EC_{10}) or an induction ratio of 1.5 ($EC_{IR1.5}$) were further converted into BEQ_{bio} ($mol_{ref}/g_{sed,dw}$ or mol_{ref}/g_{PDMS}) (Text S6).[26] Benzo[a]pyrene served as positive reference compound for AhR (B[a]P-EQ), rosiglitazone for PPARγ (rosiglitazone-EQ) and dichlorvos for AREc32 (dichlorvos-EQ). The toxic unit for cytotoxicity (TU, $L_{bioassay}/g_{sed,dw}$ or $L_{bioassay}/g_{PDMS}$) was calculated as $1/IC_{10}$ (concentration causing 10% of inhibition of cell viability).

2.7. Iceberg modelling

The mixture effects of detected chemicals expressed as BEQ_{chem} ($mol_{ref}/g_{sed,dw}$ or mol_{ref}/g_{PDMS}) were calculated by summing up the product of the relative effect potency (REP_i) and the chemical concentration of single chemicals (C_i) (Text S7). The EC_{10}, $EC_{IR1.5}$ and IC_{10} values of the analyzed chemicals were obtained from the US EPA Tox21 database and other literature or were measured in house (Table S6). For cytotoxicity, the TU_{chem} ($L_{bioassay}/g_{sed,dw}$ or $L_{bioassay}/g_{PDMS}$) was calculated by summing up the product of the compound-specific TU_i and the chemical concentration C_i (Text S7). The contribution of known chemicals to the total biological effect and cytotoxicity was quantified by BEQ_{chem}/BEQ_{bio} and TU_{chem}/TU_{bio}. In addition, the compound-specific contribution of a detected chemical i to the known effect and cytotoxicity was further calculated by $BEQ_{chem,i}/BEQ_{chem}$ and $TU_{chem,i}/TU_{chem}$.

2.8. Mass and effect balance and bioavailable fraction

The molar amount ($n_{i,sed,ww}$) of contaminant i in sediment is the sum of the amount partitioned into OC, in the pore water and bound to other solids (residues). The concentration of contaminant i in whole sediment ($C_{i,sed,ww}$, $mol/g_{sed,ww}$) is defined by Eq. 1.

$$C_{i,sed,ww}=\frac{m_{OC}}{m_{sed,ww}}\times C_{i,OC}+\frac{m_{pw}}{m_{sed,ww}}\times C_{i,pw}+\frac{m_{residue}}{m_{sed,ww}}\times C_{i,residue,ww} \quad (1)$$

where $m_{sed,ww}$ is the wet mass of sediment (g_{ww}); m_{OC} is the mass of OC (g_{OC}); m_{pw} is the mass of pore water (g_w); $C_{i,OC}$ is the concentration of chemical i bound to OC (mol/g_{OC}); $C_{i,pw}$ is the concentration of chemical i dissolved in pore water (mol/g_w); $C_{i,residue,ww}$ is the concentration of chemical i bound to other solids ($mol/g_{residue,ww}$). More details are provided in Text S3.

The chemical concentrations in pore water and OC could be estimated by the measured C_{PDMS} and the partition coefficients ($K_{PDMS/w}$ and K_{OC}). It is not possible to derive BEQ_{pw} (BEQ in pore

185 water) from PDMS because we do not know the chemical composition of the samples and
186 $K_{PDMS/w}$ differs largely between chemicals, but it is possible to derive BEQ_{OC} because the
187 $K_{PDMS/OC}$ is very similar for all chemicals.[3]

188 $$BEQ_{OC} = \frac{BEQ_{PDMS}}{K_{PDMS/OC}} \quad (2)$$

189 Since the effects of hydrophobic compounds bound to OC were much larger than those in pore
190 water,[14] the BEQ_{pw} could be neglected in Eq. 3.

191 $$BEQ_{sed,ww} = \frac{m_{OC}}{m_{sed,ww}} \times BEQ_{oc} + \frac{m_{residue}}{m_{sed,ww}} \times BEQ_{residue,ww} \quad (3)$$

192 The chemical-specific $K_{PDMS/w}$ and K_{OC} for the chemicals analyzed in this study were
193 experimental data compiled from literature or predicted data from LSER and QSAR modelling.
194 The corresponding $K_{PDMS/OC}$ was calculated as the ratio of $K_{PDMS/w}$ and K_{OC}. The detailed
195 physicochemical properties and partition coefficients of individual chemicals, as well as the
196 criteria for the data selection are shown in Text S8, Table S4 and Fig. S2.

197 **2.9. Bioavailable fraction**

198 In this study, bioavailable chemicals were defined as chemicals that could readily desorb from
199 sediments (bound to OC and partitioned into pore water), which excludes those bound to residual
200 parts comprised of mineral particles and black carbon. The bioavailable fraction ($F_{bioavailable}$) of
201 individual chemicals was calculated with Eq. 4 and of effects expressed as BEQ with Eq. 5.

202 $$\text{Bioavailable fraction } F_{i,bioavailable,chem} = \frac{n_{i,OC} + n_{i,pw}}{n_{i,sed,ww}} = \frac{C_{i,OC} \times m_{OC} + C_{i,pw} \times m_{pw}}{C_{i,sed,ww} \times m_{sed,ww}} \quad (4)$$

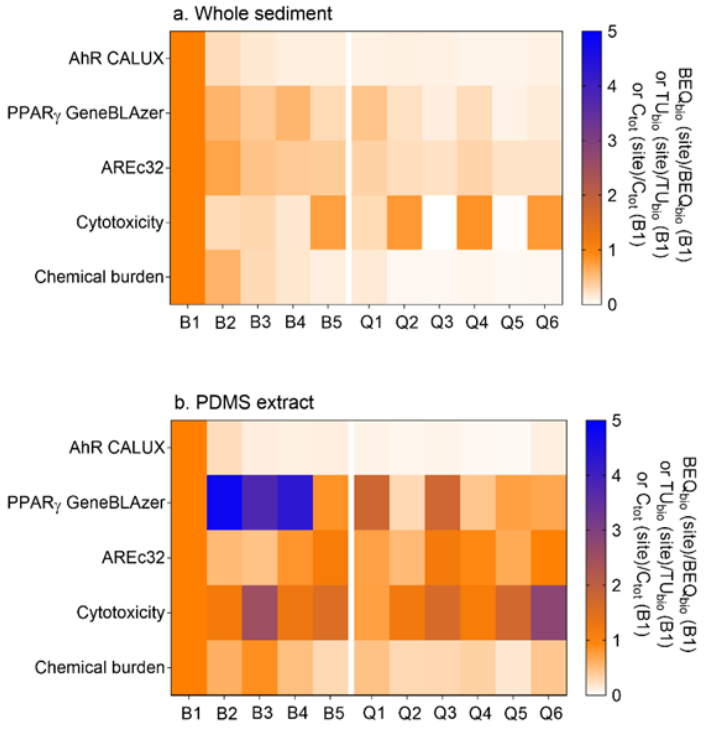
203 As for Eq. 3, $F_{bioavailable}$ can be simplified as Eq. 5.

Bioavailable fraction $F_{bioavailable,bio} = \frac{BEQ_{OC} \times m_{OC}}{BEQ_{bio,\ sed,ww} \times m_{sed,ww}}$ (5)

3. Results and discussion

3.1. Toxicological and chemical profiling of chemical mixtures in whole sediments

Representative concentration-response curves of sediment samples for the three *in vitro* bioassays are depicted in Fig. S3. The effect concentrations observed in exhaustive extracts varied between sites by a factor of up to 12, with the EC_{10} of 0.52-6.02 $g_{sed,dw}/L_{bioassay}$ in AhR CALUX, EC_{10} of 13.7-160 $g_{sed,dw}/L_{bioassay}$ in PPARγ GeneBLAzer and $EC_{IR1.5}$ of 24.7-108 $g_{sed,dw}/L_{bioassay}$ in AREc32 (Table S7). Among the three toxicological pathways, AhR-mediated activity was the most prominent due to the lowest EC_{10} quantified for most samples, which was consistent with that for sediments from other continents.[4] The compounds that can trigger the activation of AhR were reported to be dioxin-like chemicals and polycyclic aromatic hydrocarbons,[21] which are all very hydrophobic and therefore accumulate in sediments. The B[a]P-EQ_{bio} of whole sediment extracts ranged from 1.40×10^{-10} to 1.69×10^{-9} mol/$g_{sed,dw}$, the rosiglitazone-EQ_{bio} ranged from 4.14×10^{-12} to 3.70×10^{-11} mol/$g_{sed,dw}$ and the dichlorvos-EQ_{bio} ranged from 7.17×10^{-8} to 3.36×10^{-7} mol/$g_{sed,dw}$ (Fig. S4a). To compare the toxicological effects between different sampling sites, the BEQ values were further normalized to those at site B1, for which most of the bioassays showed the highest effect (Fig. 1a). The total sediment extracts showed similar spatial variation across all three bioassays, with observed effects higher at BHGC than at QTR and decreasing from up- to downstream. The ranges of BEQ of total sediment extracts in this study were in the middle or low levels when compared with other studies (Table S8). Results on cytotoxicity are discussed in Text S9 and the relative cytotoxicity depicted in Fig. 1 represents the data from AhR CALUX.



226

227 **Fig. 1.** Biological equivalent concentrations (BEQ_{bio}), cytotoxicity unit (TU_{bio}) and chemical burden (C_{tot})
228 normalized to Site B1 for (a) whole sediments and (b) PDMS extracts from Beijing-Hangzhou Grand
229 Canal (BHGC, B1-5) and Qiantang River (QTR, Q1-6).

230

231 64% (420) of the measured chemicals were detected at least once in whole sediments, with 157
232 chemicals detected at all sampling sites (Table S5). The concentrations of semi-volatile
233 chemicals might have been underestimated because no extraction recovery standards could be
234 added prior to extraction in order to avoid false positive responses in the bioassays. The
235 concentrations of the semi-volatile chemicals were still reported because the same extracts
236 underwent chemical analysis and bioassays, hence any detected chemicals should contribute to
237 the mixture toxicity. The mass concentrations of 420 detected chemicals were converted to molar

concentrations and summed up in compound classes. The cumulative molar concentrations of the 15 classes of chemicals in exhaustive extracts ranged from 0.93 to 21.2 nmol/$g_{sed,dw}$ (Fig. S5a). In the light of chemical composition, PAHs were the most abundant group (up to 12.9 nmol/$g_{sed,dw}$ at site B1), followed by PPCPs (up to 2.05 nmol/$g_{sed,dw}$ at site B1) and industrial chemicals (up to 1.05 nmol/$g_{sed,dw}$ at site B1) (Fig. 2a). The top three chemical groups accounted for 74%-89% of the total chemical burden in whole sediments. In terms of spatial variation, the total extracted samples from BHGC showed higher cumulative chemical burden than those from QTR, which was consistent with that found with bioassays. QTR is a broader and deeper river than BHGC and the sampling sites were mostly further away from urban areas. The water quality of this river might be only slightly influenced by agriculture, nearby constructions and small industrial plants located in suburban areas.

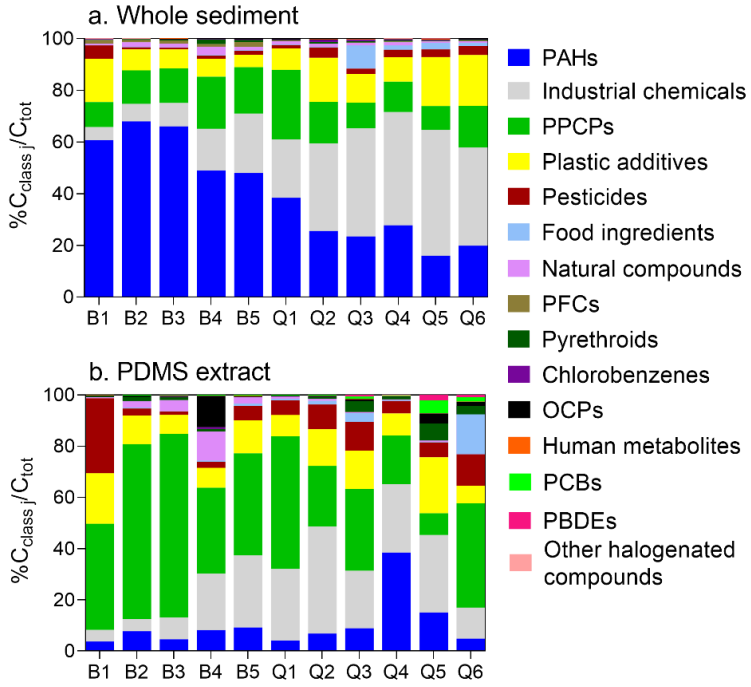


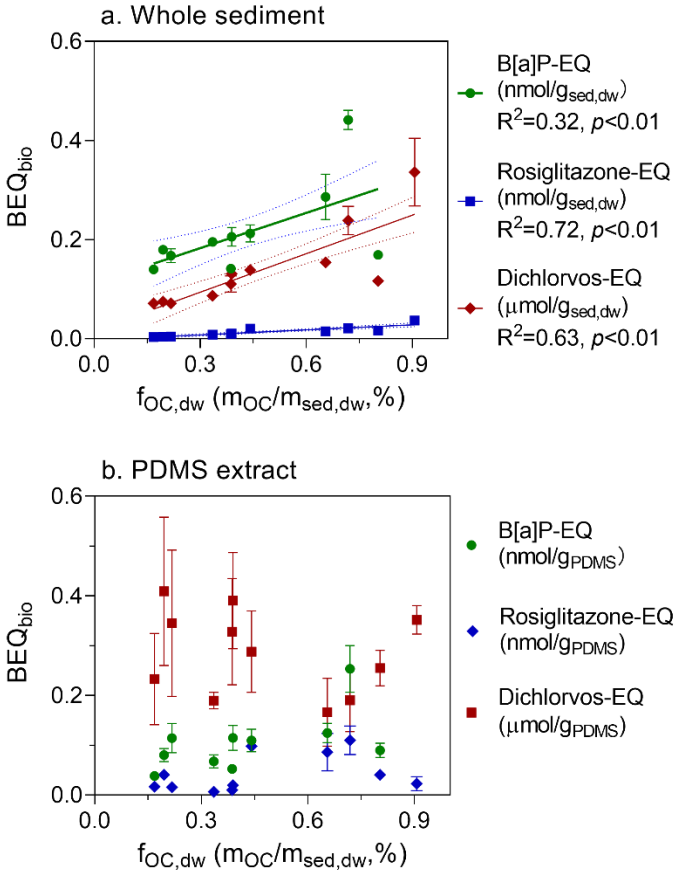
Fig. 2. Classes of chemicals detected in (a) whole sediments and (b) PDMS extracts from Beijing-Hangzhou Grand Canal (BHGC, B1-5) and Qiantang River (QTR, Q1-5). PAHs: polycyclic aromatic

hydrocarbons; PPCPs: pharmaceuticals and personal care products; PFCs: perfluorinated compounds; OCPs: organochlorine pesticides; PCBs: polychlorinated biphenyls; PBDE: polybrominated diphenyl ethers.

To provide further insight into the site-specific pollution patterns, the top 20 specific chemicals with high contribution to the total chemical burden of exhaustive extracts from each sampling site are tabulated in Table S9. PAHs and industrial chemicals, as well as some plastic additives like triphenyl phosphate, tris(1-chloro-2-propyl)phosphate and bis(2-ethylhexyl) phosphate prevailed chemical contamination in whole sediments at most sites. BHGC is still used as a transport channel nowadays. Therefore, it is expected that the major pollutants here are PAHs and related compounds, which are emitted from fuel combustion in ships' engines. Due to the phasing out of some brominated flame retardants, organophosphate flame retardants and plasticizers were extensively produced and applied worldwide. This could explain the high levels of tris(1-chloro-2-propyl)phosphate and triphenyl phosphate, which were also found in similar concentration ranges in sediments from other sites.[27, 28] Diphenyl sulfone, which is used as dye, intermediate for plastic products and thermal paper coating, was predominant in exhaustive samples at most sites from QTR, with a contribution of up to 18% at site Q5. The paper mill close to sites Q4 and Q5 might be the potential source. No other studies reported the dominance of diphenyl sulfone in aquatic systems, indicating a site-specific occurrence here. Many other industrial chemicals used as rubber additives, such as 2-(methylthio) benzothiazole and the transformation product 2-benzothiazolesulfonic acid were also found frequently and in high concentration in analyzed sediments. This might be related to the materials from tires attached to ship bodies and road run-off during rain events. Similar to the high detection of pyrethroid insecticides in global sediments,[2, 29] permethrin and bifenthrin were also found in more than 90% of the total extracted sediment samples at BGHC and QTR. This is in line with the fact that

permethrin and bifenthrin are among the most used pyrethroid insecticides worldwide.[30] In addition, the concentrations of permethrin and bifenthrin were found to be higher in urban than in agricultural areas on a global scale,[29] which was in agreement with our finding that their concentrations in whole sediments were higher at BHGC than at QTR. It is noteworthy that some chemicals that are now restricted or prohibited in China, like persistent organic pollutants (POPs) and pesticides, could still be detected in sediments with high frequency. This indicates the essential role of sediments as a long-term reservoir of various pollutants. With the economic development and increasing urbanization, the pollutants including PAHs, OCPs, phthalate esters and PBDEs were also detected in sediments from the same areas during previous studies. A detailed comparison is shown in Table S10.

Organic carbon plays a vital role in the environmental fate and toxicological risk of contaminants.[14] In this study, the influence on the variance of pollutant occurrence caused by different sources should not be obvious as BHGC and QTR are two rivers connected to each other. Therefore, it was expected that the biological responses and chemical concentrations of the exhaustive sediment extracts would depend on OC content, as shown in Fig. 3a and S6a. For example, the activity of binding to PPARγ elicited the strongest correlation between $BEQ_{bio,sed,dw}$ and $f_{OC,dw}$, in which 72% of the variance was explained by OC (Fig. 3a).



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294 **Fig. 3.** The biological equivalent concentrations (BEQ_{bio}) of (a) whole sediments and (b) bioavailable
295 sediment mixtures from PDMS extracts for AhR CALUX, PPARγ GeneBLAzer and AREc32 plotted
296 against the fraction of organic carbon ($f_{OC,dw}$). The BEQ_{bio} of B1 were excluded in (a) and (b) because
297 they were so high (Fig. S4a) that would drive the regression.

298

299 **3.2. Toxicological and chemical profiling of bioavailable contaminants in sediments**

300 The *in vitro* activity profiles of PDMS-associated contaminants are shown in Table S11 and Fig.
301 S4b. The EC_{10} ranged from 1.02 to 38.6 $g_{PDMS}/L_{bioassay}$ in AhR CALUX and 4.72 to 109
302 $g_{PDMS}/L_{bioassay}$ in PPARγ GeneBLAzer and the $EC_{IR1.5}$ ranged from 22.2 to 61.0 $g_{PDMS}/L_{bioassay}$ in
303 AREc32. The B[a]P-EQ_{bio} of PDMS extracts ranged from 3.82×10^{-11} to 9.66×10^{-10} mol/g_{PDMS},
304 the rosiglitazone-EQ_{bio} ranged from 6.58×10^{-12} to 1.10×10^{-10} mol/g_{PDMS} and the dichlorvos-EQ_{bio}

ranged from 1.66×10^{-7} to 4.09×10^{-7} mol/g_{PDMS}, which were generally lower than those from other studies (Table S8). A nearly 1:1 relationship between $BEQ_{bio,sed,dw}$ and $BEQ_{bio,PDMS}$ was found here and in previous work of Jahnke et al.[4] (Fig. 4). This suggests that PDMS may have-a similar binding capacity as the sediment particles, with the more contaminated sediments, the higher bioavailable concentrations. Similar to the exhaustive sediment extracts, the bioavailable effects observed in different bioassays varied among sites (Fig. 1b). The activation of AhR caused by PDMS compounds was also found to be higher upstream than downstream, whereas the effects of binding to PPARγ and oxidative stress response showed no spatial trend.

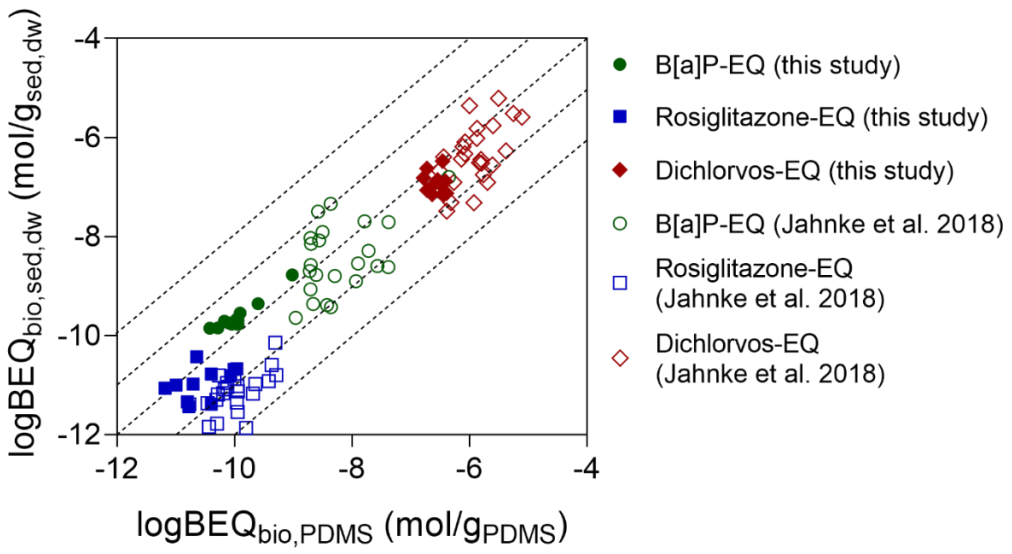

Fig. 4. Relationship between $BEQ_{sed,dw}$ (mol/$g_{sed,dw}$) and BEQ_{PDMS} (mol/g_{PDMS}). The BEQs (bioanalytical equivalent concentration) were recalculated with the EC_{10} and $EC_{IR1.5}$ in Jahnke et al.[4] and this study.

41.5% of the targeted chemicals showed concentrations above MDLs in PDMS extracts at more than one sampling site, with 62 chemicals found at all sites (Table S5). The sum molar concentrations of all chemicals in PDMS extracts were in the range of 5.91 to 33.3 nmol/g_{PDMS}. The number of detected substances and their cumulative concentrations in PDMS at BHGC was higher than that at QTR (Fig. S5b). The spatial variation observed on chemical burden of PDMS

samples agreed generally well with that of total sediment extracts (Fig. 1b). In contrast, the contribution of PAHs was lower in PDMS extracts than in whole sediment extracts. PPCPs (up to 21.0 nmol/g_{PDMS} at site B3), industrial chemicals (up to 4.42 nmol/g_{PDMS} at site Q1) and plastic additives (up to 6.58 nmol/g_{PDMS} at site B1) dominated the bioavailable sediment contaminants (Fig. 2b). These three compound groups represented 54%-88% of the sum chemical concentrations in the bioavailable portion of sediments. 6-Acetyl-1,1,2,4,4,7-hexamethyltetralin (tonalide), a fragrance compound, was found to be the most abundant chemical at BHGC, with the contribution of up to 52% at site B3, which is a park surrounded by residential areas. There were several chemicals that were detected in PDMS extracts but not in bulk sediments. This might be attributed to the lower mass of sediment samples used for ASE than for PES and the different enrichment factors during analysis. A correlation was also found between bioavailable concentration and $f_{OC,dw}$ (Fig. S6b). This is could be explained by more chemicals falling below the MDL at low contamination levels. As expected, no relationship between $f_{OC,dw}$ and biological effect induced by PDMS extracts was observed (Fig. 3b).

Chemical concentrations in PDMS extracts can be well linked to those bound to OC and freely dissolved concentrations via PDMS-OC and PDMS-water partition ratios at equilibrium.[8] To obtain the $K_{PDMS/OC}$ values for the calculation of BEQ_{OC}, the correlations between experimental log$K_{PDMS/w}$ with logK_{ow} and logK_{OC} with logK_{ow} based on neutral chemicals with log$K_{ow} \geq 3$ previously established were further refined (Fig. S7a). The slopes of the linear regressions of log$K_{PDMS/w}$ to logK_{ow} and logK_{OC} to logK_{ow} were close to 1; therefore, the slopes were fixed to 1 and the derived $K_{PDMS/OC}$ was 0.82 (Fig. S7b). Given the variations of OC and chemicals, we eventually used an equal $K_{PDMS/OC}$ and $K_{OC/PDMS}$ of 1 for the estimation of $F_{bioavailable}$, which was of the same order of magnitude as that used in previous studies ($K_{OC/PDMS}$ = 2).[3, 14] Considering

the practical application of PDMS for sediment analysis,[31] PDMS may not be applicable for ionized chemicals or chemicals with low K_{ow}. Therefore, only the $F_{bioavailable,chem}$ of non-ionized chemicals with log$K_{ow} \geq 3$ (n=211) were evaluated and discussed in this study. However, it is interesting to note that charged and hydrophilic chemicals were also detected in PDMS extracts (detailed discussion is in Text S10).

As shown in Fig. 5 and Table S12, the $F_{bioavailable,bio}$ were 0-0.006 in AhR CALUX, of 0.003-0.043 in PPARγ GeneBLAzer and of 0.005-0.018 in AREc32. The $F_{bioavailable,chem}$ varied greatly between different chemicals. The range of $F_{bioavailable,chem}$ calculated with chemical-specific $K_{PDMS/OC}$ was similar to that with the consensus value of 1 (median of 0.020-0.221 *vs.* 0.030-0.200 between sites) (Table S13). To keep consistency and reduce the bias caused by the uncertainty of $K_{PDMS/OC}$, we focused on the data calculated from the consensus $K_{PDMS/OC}$ of 1 in the following discussion. Due to the large variation of $K_{PDMS/w}$ between different chemicals (Fig. S2), the chemical-specific $K_{PDMS/w}$ were used for the estimation of C_{pw}.

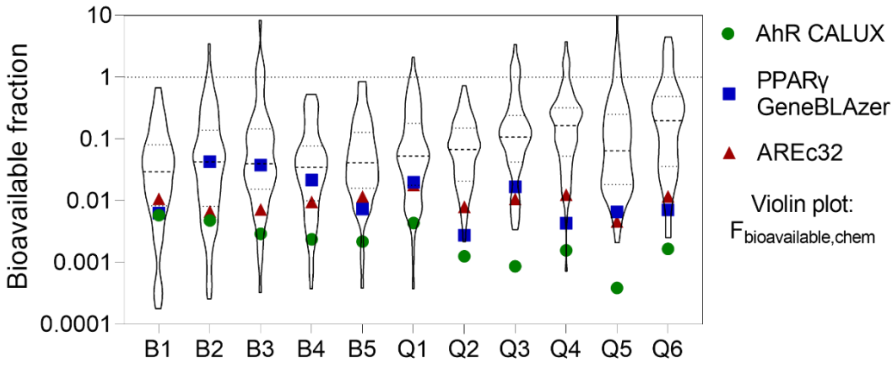

Fig. 5. Effect- ($F_{bioavailable,bio}$) and concentration-based ($F_{bioavailable,chem}$) bioavailable fractions (Eq. 3 and 4) of sediment-associated neutral chemicals with log$K_{ow} \geq 3$.

The $F_{bioavailable,chem}$ of most chemicals were higher than $F_{bioavailable,bio}$ (Fig. 5), with the $F_{bioavailable,chem}/F_{bioavailable,bio}$ ratio of 4-125 (median of 17) in AhR CALUX, 1-38 (5) in PPARγ GeneBLAzer and 2-17 (6) in AREc32. This is counterintuitive because the bioassays captured the entire pollutant mixtures including those present below MDLs in instrumental analysis and unknown chemicals. However, strongly bound chemicals that are not bioavailable are often very hydrophobic and could therefore be highly bioactive. The variance of bioavailability between bioanalysis and chemical analysis was observed to be the largest regarding AhR activity, especially at QTR. It might be due to the very hydrophobic compounds that activate AhR, such as PAHs, were strongly sorbed to black carbon (BC) or other non-OC sites in sediments.[14] The $F_{bioavailable,bio}$ at BHGC were much closer to $F_{bioavailable,chem}$ than those at QTR. The ranking of the toxicity exerted by the exhaustive-extracted mixture and the bioavailable portion was not always consistent. For example, the total sediment extract from site B2 posed the second highest oxidative stress response, whereas the PDMS extract showed the second lowest response, resulting in a much lower $F_{bioavailable,bio}$ than at other sites.

The concentrations of neutral chemicals with $\log K_{ow} \geq 3$ that were bound to BC and mineral surfaces were further calculated with the mass balance model (Eq. 1). The $C_{residue}$ were smaller for lower C_{sed} than for higher C_{sed} (Fig. S8) and median residual fractions ranged from 0.86 to 0.97 between sites (Table S14). This indicates that only a small portion of active compounds is readily available for partitioning or uptake, while the majority of mixture toxicity is not bioavailable and relatively safe for benthic organisms and human health in the short term. This is consistent with the observation made by Bräunig et al.,[14] in which the effect levels of extracts from sediment, water and PDMS were simultaneously determined. They found that the

bioavailable fraction of mixtures in sediments could be significantly decreased by a higher BC content.

To enable the comparison of bioavailability with other studies, the $K_{PDMS/OC}$ value of 1 was also employed to recalculate the $F_{bioavailable,bio}$ in other studies with the available EC or BEQ data (Table S15). Similarly, a small $F_{bioavailable,bio}$ was also found in marine and river sediments with various AhR assays (0.001-4.20) and AREc32 (0.009-0.332) assays.[3, 4, 6, 14, 15] However, higher $F_{bioavailable,bio}$ was found in sediments from Brisbane, Australia regarding oxidative stress response (0.33-1.72). It should be pointed out that the OC contents in Brisbane sediments were 1.6%-12.9%, which were much higher than those reported in other ~~and this~~ studies as well as in the present case. Given the 1:1 ratio of $BEQ_{bio,sed,dw}$ and $BEQ_{bio,PDMS}$, we deduced that $F_{bioavailable,bio}$ is highly controlled by $f_{OC,dw}$. In addition, it has been documented that the variability of the sampling site, chemical physicochemical property, sediment type, OC characteristics and ageing time could all result in different proportionality between sediment particles and bioavailable portion.[9, 32] In addition, the low contaminant concentrations in sediments measured in the present study might also be responsible for the low bioavailability since the sorption of BC rather than OC is more relevant in this case.[33] The bioavailability of individual chemicals was generally similar to those reported in literature for permethrin[11], PBDEs[12] and PAHs[15], around or more than 90% of which were not readily bioavailable. This is also in line with the finding by Lohmann et al.[34] that the hydrophobic chemicals bound to BC could contributed between 80%-90% or even more than 90% to the total concentrations detected in Boston and New York Harbor sediments even though the BC was 10 times lower than the OC content.

3.3. Linkage of biological and chemical analysis

Iceberg modelling is effective for linking biological effects to target compounds and identifying the risk drivers in complex mixture.[19] The BEQ approach for iceberg modelling applies to low effect levels of chemicals in mixtures with the same and different modes of action.[35] Among the detected chemicals in whole sediments, 74 substances can activate AhR, 19 can activate PPARγ and 84 can activate oxidative stress response, while in PDMS extracts, 43 can trigger AhR, 7 can trigger PPARγ and 56 can trigger oxidative stress response (Table S16).

The BEQ and TU of total sediment extracts derived from biological and chemical analysis are compared in Fig. 6a and 6b. Specifically, the contributions of known chemicals to the observed effects were 0.1%-9.3% in AhR CALUX, 0.1%-0.3% in PPARγ GeneBLAzer and 0.8%-28.4% in AREc32 (Fig. S9). Oxidative stress response is an indicator downstream of the molecular initiating event.[36] A higher contribution of quantified chemicals to the observed oxidative stress response (up to 12%) than to the other toxic endpoints was also found in untreated wastewater.[24] The small contribution of identified chemicals to the observed mixture effects indicates that there is still a large number of unidentified chemicals responsible for the mixture biological effects. A similar large portion of unknown adverse effects was also identified in sediments from European river basin based on TU and multi-substance Potentially Affected Fraction of species (msPAF) models, which used a battery of 6 sediment contact tests for toxicity assessment.[17] Rocha et al.[37] also found that less than 5% of the induction in AhR assay could be explained by measured PAHs in sediments from reservoirs along the Tietê River and the Pinheiros River, Brazil using a similar BEQ concept. In contrast, PAHs alone made up 41% of the observed AhR-mediated potencies in sediments from Lake Tai Basin, China (with additional clean-up procedure for total extracts),[6] 84% of the effects in sediments from the west coast of South Korea[18] and even 118%

of the effects in sediments from River Elbe Estuary, Germany (with additional clean-up procedure for total extracts).[38]

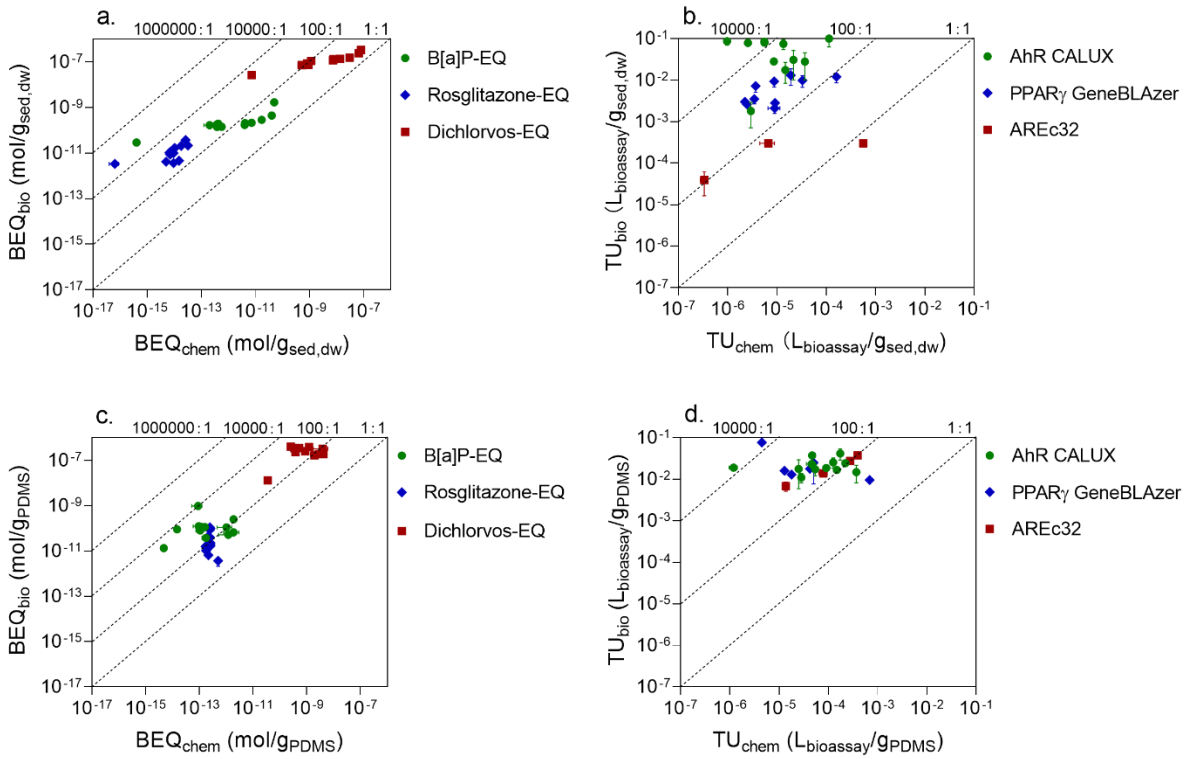

Fig. 6. Comparison of biological equivalent concentration (BEQ) and cytotoxicity unit (TU) from bioanalysis and chemical analysis in (a and b) exhaustive and (c and d) PDMS extracts of sediments.

The compound-specific contribution of individual chemicals in whole sediments to the total BEQ_{chem} and TU_{chem} was further evaluated (Fig. S10a-c and Fig. S11a-c), showing considerable variability between different bioassays and sampling sites. The group of PAHs was recognized as the mixture effect drivers in total sediment mixtures for the activation of AhR (66%-100% of B[a]P-EQ_{chem}) and oxidative stress response (66%-99% of dichlorvos-EQ_{chem}), while plastic additives (58%-98% of rosiglitazone-EQ_{chem}) for the binding to PPARγ. It was expected that PAHs were the key toxicants in AhR CALUX and AREc32 assays because of their higher REPs and elevated concentrations. Polychlorinated dibenzofurans (PCDFs) were identified as the

major contaminants in sediments from the Pohang Area, Korea.[39] PCDFs were not included in the present study, but they would have been captured in the measured mixture effects. Even though the biological effects and chemical burden of total sediments extracts were found to be higher at BHGC than at QTR, more diverse chemicals, including industrial chemicals and plastic additives, responsible for the mixture effects were found at QTR than at BHGC.

The detailed site-specific top 20 driving chemicals for the observed biological responses induced by total sediment extracts are tabulated in Table S17. Basically, the mixture risk drivers in exhaustive sediment extracts were in line with those we found according to chemical screening, with the chemicals belonging to PAHs, PPCPs, industrial chemicals and plastic additives contributing more to the total BEQ_{chem}. However, there are some compounds, such as diphenyl sulfone and 6-acetyl-1,1,2,4,4,7-hexamethyltetralin, that were detected with high frequencies and concentrations, but contributed only little to BEQ_{chem} due to their lower biological activities or being inactive in the bioassays applied in this study. In addition, it is also worth to pay attention to the substances detected at low concentrations. For example, the concentrations of the herbicide diuron were lower in sediments from BHGC and QTR than in those from European river mouths.[2] However, the contribution of diuron to the B[a]P-EQ_{chem} of exhaustive sediment extracts ranked highly in the risk list (up to 11%) because of its high REP in AhR CALUX. Similar cases were the pesticide 2,4-dichlorophenoxyacetic acid and the food ingredient 2-Amino-3-methyl-3H-imidazo[4,5-f]quinolone. This indicates that not only chemicals with high concentration, but also those with high REP should be of great concern in the risk assessment of sediments.

For bioavailability-associated estimation, the EC and IC data of chemical mixtures and the concentrations of chemicals detected in PDMS were directly used in iceberg modelling to avoid

463 the uncertainty caused by the partition coefficients of mixtures and single chemical. The BEQ_{chem}
464 were around 1-4 orders of magnitude lower than the BEQ_{bio} for all three bioassays (Fig. 6c and
465 6d). In comparison with exhaustive sediment extracts, the identified chemicals explained less
466 effect of PDMS extracts in AhR CALUX (0.009%-2.8%) and AREc32 (0.06-2.2%), but more in
467 PPARγ GeneBLAzer (0.2%-3.3%) (Fig. S9). The fewer bioactive chemicals detected in PDMS
468 extracts and the low detected concentrations may explain the smaller fractions of explainable
469 effects when compared to bulk sediments. Thousands or even more of both detected and
470 bioactive chemicals would be needed to explain 100% of the observed effects in PDMS samples
471 (Fig. S10). The fractions of the explained effects for bioavailable chemical mixtures at QTR
472 were higher than those from BHGC with respect to AhR and PPARγ activities. In biological
473 analysis, we found that the PDMS extracts from site B1 showed higher B[a]P-EQ_{bio} than those
474 from other sampling sites. However, the contribution of identified chemicals to the observed
475 AhR-mediated response was the lowest at site B1, indicating more unquantified bioactive
476 chemicals at site B1 than at other sites.

477 Despite fewer numbers of detected chemicals activating the three endpoints, the distribution of
478 chemicals in PDMS extracts responsible for the effects and cytotoxicity was more variable than
479 that in exhaustive samples (Fig. S11d-f, S11d-f and Table S18), except for the effect of binding
480 to PPARγ. Fewer chemicals in PDMS samples than in total extracts could explain more effects in
481 PPARγ GeneBLAzer. That suggests the bioavailable chemicals have a higher potency in
482 triggering the effect responsible for binding to PPARγ. The majority of chemicals with high
483 contribution to the activation of AhR fell into the group of PAHs (9.4%-95%), which resembled
484 that in total extracts. This is in agreement with the predominance of PAHs to AhR-mediated
485 potency found in bioavailable extracts of sediments from the Lake Tai Basin, China[6] and the

North Sea, the South-western Baltic Sea and the Western Mediterranean.[15] It is interesting to note that although the sum of bioavailable PAHs concentrations was the highest at Q4, the contribution to BEQ_{chem} (40%) was at an intermediate level among all samples. It highlights the importance of considering the REP of single chemicals for a realistic risk assessment. In addition to plastic additives, PPCP and pesticide groups were also identified with PPARγ GeneBLAzer assay as effect drivers in the bioavailable portion of sediments. In the case of oxidative stress response, PPCPs were also the key toxicants besides PAHs. It is noteworthy that pesticides were not considered as priority pollutants in exhaustive sediment extracts; however, they contributed considerably to the toxicological effects activated by bioavailable mixtures.

3.4. Implications for sediment risk assessment

In this study, we gave a comprehensive overview on the chemical and toxicological profiles of sediment mixtures including a large range of contaminants. Chemical occurrence alone is not sufficient, but the potency of individual components needs to be considered, too, to estimate their contribution to the mixture risk. Iceberg modelling showed the limitation of the commonly applied toxic unit concept, where only detected chemicals with available toxicity data could be included the in the mixture risk prediction. If we could define effect-based trigger values for sediments in a similar way as has been proposed for surface water,[40] bioassays could contribute to sediment risk assessment.

Our results highlight the necessity to involve different bioassays with diverse profiles of effects and a large number of contaminants as different lines of evidence for in the risk assessment of chemical mixtures in sediments. Non-target analysis has a great potential to identify new chemicals for the expansion of the chemical list. With the expansion of chemical and bioassay

screening for samples from diverse sites and scenarios, a priority list of key toxicants and related bioassays is warranted for future routine sediment monitoring. It is also worth to conduct chemical screening along with bioassays, whose information would be bridged together into an integrated picture by mixture models and thus help to identify the priority contaminants that are urgently needed for remediation.

Given the inconsistent profiles of concentration and risk between the whole sediments and the bioavailable fractions, we also clearly demonstrated that it is imperative to incorporate bioavailability in effect- and chemical-based diagnosis of sediments. Future studies are needed to take bioavailability into consideration for setting up trigger values and sediment quality guidelines.

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, physicochemical properties and partition coefficients of analyzed chemicals, effect- and chemical-related results, comparison of BEQs and chemical concentrations with other studies, discussion on cytotoxicity, bioavailable fractions derived based on biological and chemical analysis toxicity data and results for iceberg modelling.

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Author contributions

Lili Niu and Beate I. Escher designed the study; Lili Niu, Chao Xu and Deliang Zou lead the sampling campaign and performed the passive sampling experiments; Deliang Zou performed the ASE experiments; Lili Niu performed the PDMS extraction; Lili Niu and Maria König performed the bioassay experiments; Lili Niu, Martin Krauss and Melis Muz conducted the chemical analysis with LC and GC instruments; Eric Carmona helped with the use of target screening software; Beate I. Escher conceived the data evaluation and developed the iceberg modelling; Lili Niu evaluated all the chemical and bioassay data and 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.

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