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Bioanalytical Effect-Balance Model to Determine the Bioavailability of Organic Contaminants in Sediments Affected by Black and Natural Carbon

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Abstract

In sediments several binding phases dictate the fate and bioavailability of organic contaminants. Black carbon (BC) has a high sorptive capacity for organic contaminants and can limit their bioavailability, while the fraction bound to organic carbon (OC) is considered to be readily desorbable and bioavailable. We investigated the bioavailability and mixture toxicity of sediment-associated contaminants by combining different extraction techniques with *in vitro* bioanalytical tools. Sediments from a harbour with high fraction of BC, and sediments from remote, agricultural and urban areas with lower BC were treated with exhaustive solvent extraction, Tenax extraction and passive sampling to estimate total, bioaccessible and bioavailable fractions, respectively.. The extracts were characterized with cell-based bioassays that measure dioxin-like activity (AhR-CAFLUX) and the adaptive stress response to oxidative stress (AREc32). Resulting bioanalytical equivalents, which are effect-scaled concentrations, were applied in an effect-balance model, consistent with a mass balance-partitioning model for single chemicals. Sediments containing BC had most of the bioactivity associated to the BC fraction, while the OC fraction played a role for sediments with lower BC. As effect-based sediment-water distribution ratios demonstrated, most of the bioactivity in the AhR-CAFLUX was attributable to hydrophobic chemicals while more hydrophilic chemicals activated AREc32, even though bioanalytical equivalents in the aqueous phase remained negligible. This approach can be used to understand the fate and effects of mixtures of diverse organic contaminants in sediments that would not be possible if single chemicals were targeted by chemical analysis; and make informed risk-based decisions concerning the management of contaminated sediments.

Highlights

(1) First study of black carbon-bound sediment contaminants with cell-based bioassays.
(2) Bioanalytical equivalents as a measure of biological effects in different media.
(3) Highest bioactivity of contaminants bound to black carbon.
(4) Mixture effects of waterborne chemicals were negligible.
(5) Effect-balance model to differentiate effect contribution of HOCs bound to OC and BC.

Keywords (6): *In vitro* bioassays, equilibrium passive sampling, black carbon, bioavailability, mixture effect.

1. Introduction

1.1 Bioavailability of organic contaminants in sediment

Sediment contamination with hydrophobic organic contaminants (HOCs) is a worldwide problem and risk assessment of contaminated sediments remains a challenge. It is widely recognized that sorption of organic chemicals to different compartments within a sediment dictates their fate, availability and thus also their toxicological risks. The effects caused by sediment-associated contaminants are related to the bioavailable concentration rather than to the bulk chemical concentration (Hawthorne et al., 2007; Lu et al., 2011; Lydy et al., 2014) and it has been proposed that the bioavailable concentration of sediment-associated contaminants be considered in risk assessments of organic chemicals (Ghosh et al., 2014; Ortega-Calvo et al., 2015).

Polymer-based partitioning can be used to determine the freely dissolved, bioavailable fraction of sediment contaminants (Mayer et al., 2003; Cui et al., 2013; Mayer et al., 2014). Equilibrium-based passive sampling using polydimethylsiloxane (PDMS) has been used widely (Difilippo and Eganhouse, 2010; Mäenpää et al., 2010; Bielská et al., 2014) and will also be applied in this study. Furthermore, Tenax, a porous polymer resin, can be used as a sorptive phase for depletive extraction of sediment contaminants. While Tenax is an infinite sink for sediment contaminants, it is often used to sample the contaminant fraction that is easily and rapidly desorbed to the aqueous phase (Cornelissen et al., 2001; Landrum et al., 2007). Together with freely dissolved pore water concentrations of contaminants this makes up what is considered to be the bioavailable fraction of sediment contaminants (Ortega-Calvo et al., 2015). The rapidly desorbing fraction has been shown to be the main source of sediment pore water concentrations (Kraaij et al., 2002; You et al., 2007; Cui et al., 2010) and it has been correlated to chemical residues in organisms (Kraaij et al., 2002; You et al., 2007; Heijden and Jonker, 2009). A 24 h Tenax extraction has been used to accurately predict HOC bioavailability from sediments (Cornelissen et al., 2001; You et al., 2011; Lydy et al., 2015).

Hydrophobic organic contaminants have a high affinity to the total organic carbon (TOC) in sediments, which is made up of two major binding entities: amorphous organic carbon (OC) and black carbon (BC). The OC consists of biogenic origin carbon and the BC consists of highly condensed carbonaceous by-products and residues of incomplete combustion of fossil fuels and vegetation such as soot, coal and kerogen (Goldberg, 1986; Schmidt and Noack, 2000). Organic carbon makes up the bulk of TOC and often OC-normalized sediment contaminant concentrations have been reported according to the equilibrium partitioning theory (Di Toro et al., 1991). In contrast, BC has been shown to have much higher sorptive capacity than OC (Accardi-Dey and Gschwend, 2002; Jonker and Koelmans, 2002; Cornelissen et al., 2005) and to strongly decrease the bioavailability of sediment-associated contaminants (Knauer et al., 2007; Jia and Gan, 2014). Contaminants bound to OC are generally more bioavailable than contaminants bound to BC as the partition constant between OC and pore water (K_{OC}) can be several orders of magnitude smaller than the partition constant between BC and pore water (K_{BC}) (Di Toro et al., 1991; Jonker and Koelmans, 2002; Cornelissen et al., 2005). Therefore, OC is typically associated with the bioavailable fraction of sediment contaminants, while BC is associated with the inaccessible fraction.

Studies investigating the bioavailability of sediment contaminants in relation to OC and BC sorption have focussed on a few model compounds or chemical classes (Cornelissen and Gustafsson, 2003; Brändli et al., 2008; Jia and Gan, 2014). Whereas, in the environment, contaminants are present in

complex mixtures and the observed toxicity of a given sample most likely stems from the mixture effects. Bioanalytical tools allow the investigation of mixture toxicity of complex environmental samples (Jahnke et al., 2016) and have in the past been used to investigate the toxicity of whole sediment extracts. For example, mechanism-specific bioassays have been used to investigate the estrogenic and dioxin-like potential of sediments by Houtman et al. (2006) and Jung et al. (2012) while Kammann et al. (2004) used a combination of two toxicity assays to determine the genotoxic and teratogenic potential of sediments. Li et al. (2013) and Vethaak et al. (2016) used a battery of bioassays to investigate the toxicity and bioavailability of different chemical classes.

Here we apply bioanalytical tools, for the first time, to develop a comprehensive effect-balance of various sediment types. We used a set of extraction techniques to differentiate the biological effects of mixtures of chemicals associated with different compartments of the sediment (BC, OC and water). The goal was to develop an effect-balance model to assign the mixture effect to the slowly desorbing fraction associated with BC and to the readily desorbing fraction associated with OC. In addition, the role of the pore water in relation to the solid sediment phases was investigated. We compared a coal exporting harbour whose sediments contain relatively high fractions of BC with two sites that are dominated by OC.

1.2 Theoretical considerations

The combined effects of a chemical mixture in an environmental sample derived from bioassays have been expressed in bioanalytical equivalent concentrations (BEQ) (Escher and Leusch, 2012; Escher et al., 2012). The ratio of the effect concentration of a reference compound (EC_{ref}) in a certain bioassay to that of a sample is termed the BEQ_{bio} (Eq. 1). The EC_{ref} has units of mol/$L_{bioassay}$ while the sample ECs (EC_{sample}) are given in sample mass equivalent transferred to the bioassay ($kg_{sample}/L_{bioassay}$). The corresponding BEQs therefore have units of mol/kg_{sample}.

$$BEQ_{bio} = \frac{EC_{ref}}{EC_{sample}} \quad \textbf{(1)}$$

The BEQ concept allows a comparison between the measured effect and the effects that a mixture of known chemicals would elicit in the same bioassay. The BEQ_{chem}, derived from chemical analysis of a sample, is defined as the sum of the measured concentrations of known chemicals i, C_i, multiplied by their corresponding relative effect potencies (REP_i), which describe their effects in relation to a reference compound (Eq. 2).

$$BEQ_{chem} = \sum_{i=1}^{n} C_i \times \frac{EC_{ref}}{EC_i} = \sum_{i=1}^{n} C_i \times REP_i \quad \textbf{(2)}$$

If all bioactive chemicals are known and detected then $BEQ_{bio} = BEQ_{chem}$, but in reality there are many unknown bioactive chemicals and consequently typically $BEQ_{bio} > BEQ_{chem}$ and the difference is a measure of the effect caused by unknown chemicals. The advantage of using BEQ values as a measure of effect is that they are expressed in concentration units of the reference compound and thus they can be used in mass-balance models. However, the composition of the chemicals causing the BEQs might vary between the different compartments (sediment, water, OC, BC) due to differences in partition constants of different chemicals. Therefore partition constants related to BEQs are not thermodynamic constants but operationally defined distribution ratios.

Compartments in a sediment-water-system and relationships between them as well as sampling phases relevant for this study are shown in Fig. 1. Equation 3 describes the mass-balance of chemical i in the sediment system ($n_{i,sed}$).

$$n_{i,sed} = n_{i,OC} + n_{i,BC} + n_{i,w} + n_{i,residual} \quad \textbf{(3)}$$

$n_{i,OC}$ is the molar mass of i bound to OC and $n_{i,BC}$ is the molar mass of i bound to BC. The $n_{i,w}$ is the molar mass of i dissolved in the aqueous phase. The $n_{i,w}$ is the molar mass of i dissolved in the aqueous phase that is negligible in comparison to $n_{i,OC}$ and $n_{i,BC}$ for compounds with high K_{OC} and K_{BC} but it cannot be neglected for more hydrophilic chemicals. The $n_{i,residual}$ represents the amount of i bound to inorganic carbon and other 'non-binding' residuals. The amount of contaminants associated with this compartment is assumed to be negligible.

The total mass of the wet sediment ($m_{sed,ww}$) is the sum of the dry weight ($m_{sed,dw}$) and the pore water weight (m_w, or volume V_w, assuming a density of 1 kg L^{-1}), with the dry weight made up of OC, BC and residual solids (Eq. 4).

$$m_{sed,ww} = m_{sed,dw} + m_w = m_{OC} + m_{BC} + m_{residual} + m_w \quad \textbf{(4)}$$

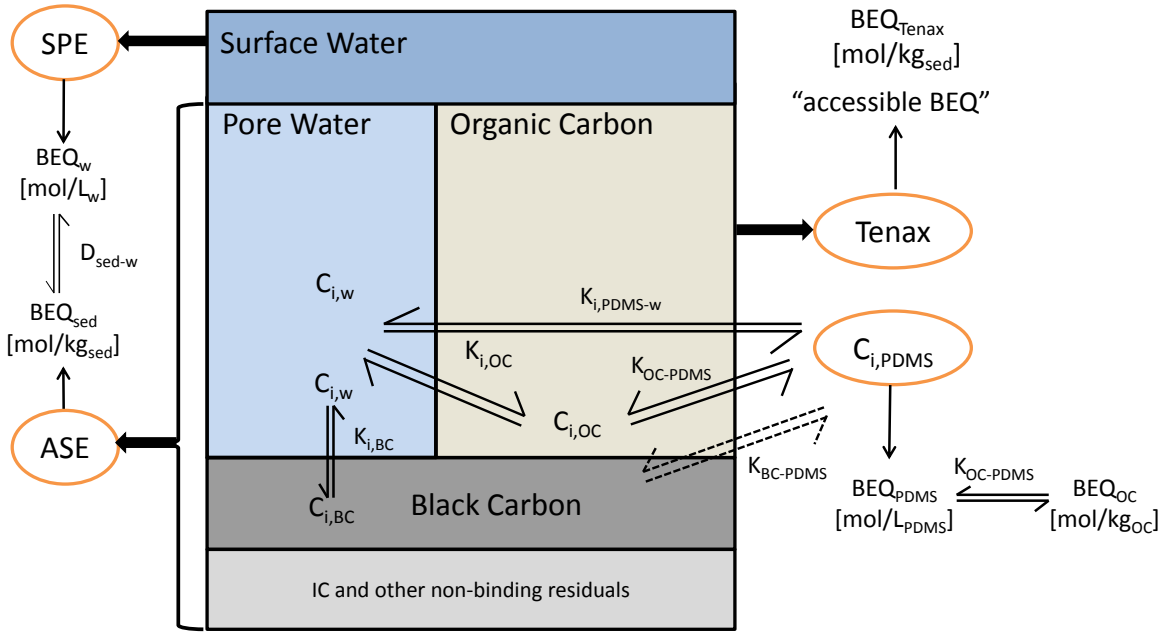


Figure 1: Conceptual model describing the relationships and equilibria in a sediment system. Orange circles represent extracts used in the study. Bold arrows ➡ refer to exhaustive extractions, two-way arrows ⇌ refer to equilibrium partitioning, while dashed arrows indicate limited exchange between phases. BEQ = bioanalytical equivalent concentration, SPE = solid phase extraction, ASE = accelerated solvent extraction, PDMS = polydimethylsiloxane extraction, BC = black carbon, OC = organic carbon, IC = inorganic carbon.

Equation 3 can be translated into total sediment contaminant concentrations (on a wet weight basis) by using the fraction of OC and BC (f_{OC} and f_{BC}). These are calculated as the mass of OC or BC

respectively, divided by the total sediment mass including pore water (Eq. 5). The resulting mass-balance describes sorption of organic compounds to the two major sorption sites in sediments: OC and BC and the remaining aqueous fraction.

$$C_{i,sed,ww} = \left(\frac{m_{OC}}{m_{sed,ww}}\right) \times \left(\frac{n_{i,OC}}{m_{OC}}\right) + \left(\frac{m_{BC}}{m_{sed,ww}}\right) \times \left(\frac{n_{i,BC}}{m_{BC}}\right) + \left(\frac{m_{w}}{m_{sed,ww}}\right) \times \left(\frac{n_{i,w}}{m_{w}}\right) = f_{OC,ww} \times C_{i,OC} + f_{BC,ww} \times C_{i,BC} + f_{w,ww} \times C_{i,w} \quad \textbf{(5)}$$

with the fractions f_y of compartment y related to the sediment wet weight (Eq. 6).

$$f_{y,ww} = \frac{m_y}{m_{sed,ww}} \quad \textbf{(6)}$$

For hydrophobic chemicals, the concentrations in the aqueous phase are negligible and the mass-balance reduces to Eq. 7 and fractions of wet sediment can be estimated as fractions of dry sediment (Eq. 8).

$$C_{i,sed,dw} = \left(\frac{m_{OC}}{m_{sed,dw}}\right) \times \left(\frac{n_{i,OC}}{m_{OC}}\right) + \left(\frac{m_{BC}}{m_{sed,dw}}\right) \times \left(\frac{n_{i,BC}}{m_{BC}}\right) = f_{OC,dw} \times C_{i,OC} + f_{BC,dw} \times C_{i,BC} \quad \textbf{(7)}$$

$$f_{y,dw} = \frac{m_y}{m_{sed,dw}} \quad \textbf{(8)}$$

and the $f_{y,dw}$ is related to $f_{y,ww}$ by Eq. 9.

$$f_{y,ww} = \frac{m_{sed,dw}}{m_{sed,ww}} \times f_{y,dw} \quad \textbf{(9)}$$

Li et al. (2013) showed that PDMS can be used to sample the bioavailable fraction of sediment contaminants and that bioassays can be applied to determine the effects of this fraction. Furthermore, a theoretical partition constant between OC and PDMS, $K_{OC\text{-}PDMS}$, was derived by Li et al. (2013) that is independent of the hydrophobicity of the chemicals (Eq. 10) and can be used to convert $C_{i,PDMS}$ to $C_{i,OC}$ (Eq. 11) and $BEQ_{i,PDMS}$ to BEQ_{OC} for compounds sorbed to the OC fraction of a sediment using (Eq. 12).

$$K_{i,OC-PDMS} = \frac{K_{i,OC}}{K_{i,PDMSw}} \approx K_{OC-PDMS} \quad \textbf{(10)}$$

$$C_{i,OC} = K_{OC-PDMS} \times C_{i,PDMS} \quad \textbf{(11)}$$

$$BEQ_{OC} = K_{OC-PDMS} \times BEQ_{PDMS} \quad \textbf{(12)}$$

The mixture effect of the total contaminants sorbed to a sediment ($BEQ_{sed,ww}$) as well as the fractions sorbed to OC and BC can be expressed by Eq. 13. The fractions of OC and BC can be measured directly and the OC sorbed contaminants can be sampled from the sediment using PDMS

partitioning. The last unknown variable, the bioanalytical equivalent concentration of contaminants sorbed to BC (BEQ_{BC}), can then be derived from the BEQ-balance given in Eq. 13.

$$\begin{aligned} BEQ_{sed,ww} &= f_{OC,ww} \times BEQ_{OC,ww} + f_{BC,ww} \times BEQ_{BC,ww} + f_w \times BEQ_w \\ &= f_{OC,ww} \times K_{OC-PDMS} \times BEQ_{PDMS} + f_{BC,ww} \times BEQ_{BC,ww} + f_w \times BEQ_w \\ &\left[\frac{kg_{OC}}{kg_{sed,ww}} \times \frac{L_{PDMS}}{kg_{OC}} \times \frac{mol}{L_{PDMS}} + \frac{kg_{BC}}{kg_{sed,ww}} \times \frac{mol}{kg_{BC}} + \frac{L_w}{kg_{sed,ww}} \times \frac{mol}{L_w} \right] \end{aligned} \quad \textbf{(13)}$$

For hydrophobic chemicals Eq. 13 can be simplified to Eq. 14, as the fraction of HOCs in the water phase is essentially negligible.

$$BEQ_{sed,dw} = f_{OC,dw} \times K_{OC-PDMS} \times BEQ_{PDMS} + f_{BC,dw} \times BEQ_{BC,dw} \left[\frac{kg_{OC}}{kg_{sed,dw}} \times \frac{L_{PDMS}}{kg_{OC}} \times \frac{mol}{L_{PDMS}} + \frac{kg_{BC}}{kg_{sed,dw}} \times \frac{L_w}{kg_{BC}} \times \frac{mol}{L_w} \right] \quad \textbf{(14)}$$

Assumptions made by this BEQ-balance (Eq. 14) are that: partitioning-based sampling with PDMS will sample contaminants that are mainly associated with the OC fraction of sediments and are in equilibrium with the pore water; and that the chemicals bound to BC do not partition to the PDMS. Most previous studies have implicitly made these assumptions, but since bioassays detect the mixtures of all chemicals the validity of this assumption can be checked.

1.3 Experimental approach

This work set out to experimentally test two hypotheses. The main assumption underlying the BEQ balances in Eq. 13 and 14 is that we can experimentally differentiate BEQ_{OC} and BEQ_{BC} by equilibrium partitioning to PDMS. To test this assumption we compare effects of PDMS and Tenax extracts. PDMS samples the equilibrium concentration in a non-depletive way while Tenax samples the water and OC bound chemicals in a depletive way. Depletive extraction could also be accomplished with larger volumes of PDMS (Smedes et al., 2013) but for a simpler experimental design we used Tenax according to Li et al. (2013). A 24 h Tenax extraction was applied to two sediments, one sediment containing next to no BC (<0.05%) and one containing an average fraction of BC (0.12%) for the sediments being studied. Our working hypothesis was that the bioavailable fractions extracted by the two methods would be the same for the sediment containing no BC, as all contaminants bound to OC are bioavailable, but would be different for the sediment containing appreciable amounts of BC.

We use two *in vitro* bioassays that target environmental pollutants at opposite ends of the hydrophobicity spectrum. Dioxin-like activity was quantified with the cell-based arylhydrocarbon receptor (AhR) chemically activated fluorescent expression (AhR-CAFLUX) assay (Nagy et al., 2002). Mainly hydrophobic chemicals activate the AhR. Oxidative stress, induced by more hydrophilic and reactive chemicals, was quantified by the activation of the adaptive stress response pathway. This pathway was probed with the AREc32 cell line (Wang et al., 2006) that measures the Nrf2-mediated activation of the antioxidant response element (ARE). Our hypothesis was that the full BEQ-balance (Eq. 13), including the water compartment, is required to describe the effects of mixtures of chemicals that cause oxidative stress. This is because many of the chemicals that induce oxidative stress are polar and thus typically have greater aqueous solubility. In contrast, the simplified dry

weight-based BEQ balance (Eq. 14) would be sufficient to describe the AhR assay because potent ligands of AhR are typically moderate to very hydrophobic.

2. Materials and Methods

2.1. Study area

Gladstone Harbour is located in the central coast of Queensland, Australia south of the Tropic of Capricorn (-23.829269, 151.250588). The harbour covers a total area of approximately 200 $km^2$and is sheltered by Curtis Island and Facing Island (Angel et al., 2010). Samples were taken from 14 sites in the greater Gladstone Harbour area in June 2013, covering the harbour area and all major estuaries, as well as four reference sites, chosen based on their distance from the main harbour area. A map with the sampling sites and more information can be found in the Supporting Information (Fig. SI 1). In order to compare the Gladstone Harbour sites (GH 1 to GH 14), classified as typical industrial sites, further samples representing different land-uses were taken. One site from the Brisbane River (BR) at the Oxley Creek outflow (-27.524, 152.994522) was sampled in October 2013, and two Fitzroy River samples (FR 1, -23.37512, 150.51404, and FR 2, -23.53534, 150.88101, sampled during the Gladstone sampling event) represents mainly urban areas. The Daintree River (DR, -16.251, 145.3067) was sampled in August 2013, representing a remote conservation area with minimal agriculture (i.e., 6% agriculture – ABS, 2010).

2.2 Sampling and sample preparation

Grab samples of surface water (2 – 3 L per site) were collected in solvent-washed amber glass bottles and were acidified to pH 3 with concentrated hydrochloric acid for sample preservation of samples and to maximise extraction efficiency (Escher et al., 2005). Surface water samples were filtered through a 1.6 µm glass fibre filter (GF/A, Whatman) to remove suspended solids and subsequently extracted by solid phase extraction (SPE). Surficial sediment samples were collected using a Van Veen grab sampler or with a stainless steel shovel if the site was accessible and stored in solvent-washed glass jars (700 mL, 4 jars per site). In the laboratory the sediment samples were homogenized in a stainless steel bowl. Approximately half of each sediment sample was preserved with 0.1% NaN_3 (dry weight basis) and stored at 4 °C for subsequent passive sampling experiments, while the other half was frozen at -20 °C until being freeze dried.

2.3 Solid Phase Extraction (SPE) of water samples

Water samples were extracted using SPE according to the methods described by Macova et al. (2011). The extracted chemicals include freely dissolved chemicals and chemicals bound to dissolved organic matter <1.6 µm (after filtration through a 1.6 µm glass fibre filter). Briefly, 2 – 3 L of surface water sample were extracted using 1 g OASIS® HLB solid phase cartridges (Waters, Australia). A 2 L aliquot of ultrapure water served as a process control and was treated in the same way as surface water samples. The cartridges were conditioned in a Visiprep manifold with 10 mL acetone/hexane (1/1), followed by 10 mL methanol and finally 10 mL of pH adjusted (pH 3) ultrapure water. The surface water sample was percolated under vacuum and the cartridges were dried for 2 – 3 h (or until dry). Dried cartridges were wrapped in aluminium foil and stored at -20 °C until elution. The polar fraction of the sample was eluted from the cartridge with 10 mL methanol and the non-polar fraction was eluted with 10 mL acetone/hexane (1/1). The solvents were percolated by gravity and vacuum was applied to draw any remaining solvent into the receiving test tubes. All eluates were

evaporated to dryness under a nitrogen stream and redissolved in 40 µL dimethyl sulfoxide (DMSO). Samples were stored at -20 °C in amber HPLC vials until further use.

2.4 Accelerated Solvent Extraction (ASE) of sediments

Freeze-dried samples were extracted using accelerated solvent extraction (Thermo Scientific Dionex ASE 350), following the standard U.S. EPA method 3545 (1995). Six grams of freeze-dried sediment mixed with pre-cleaned diatomaceous earth were extracted in three static cycles, each lasting 5 min at 100 °C and 1500 psi using acetone/hexane (1/1) as the extraction solvent. Flush-volume and purge-time were 60% and 90 s, respectively. Process controls consisting of diatomaceous earth were treated in the same way as samples. The extracts were blown down to dryness under a nitrogen stream and redissolved in 60 µL DMSO. Samples were stored in amber HPLC vials at -20 °C.

2.5 Measurement of organic carbon and black carbon

For carbon analysis, the samples were ground to a fine powder in a ringmill. Approximately 0.5 g of the sample was weighed into a ceramic boat containing a nickel liner. Inorganic carbon (IC) was removed from the samples with 5-6% H_2SO_4 and the sediment OC content was subsequently measured by combustion on a LECO TruMac combustion analyser at 1300 °C. Black carbon was measured using a chemo-thermal oxidation method (Gustafsson et al., 1997) in which first OC was removed from the sample by oxidization at 375 °C in a muffle furnace for 18 h, followed by removal of IC through acid digestion as described above. Black carbon was then also determined in the sample by combustion on a LECO TruMAC combustion analyser at 1300 °C. Total carbon in the sample is defined as the dry weights of the total organic carbon (TOC) and total inorganic carbon (TIC) fractions (Eq. 15). Total organic carbon is defined as the sum of the amorphous organic carbon (OC) and black carbon (BC) fractions (Eq. 16).

$$f_{TC,dw} = f_{TOC,dw} + f_{TIC,dw}\left[\frac{g_{TC}}{g_{sed,dw}}\right] \quad \textbf{(15)}$$

$$f_{TOC,dw} = f_{OC,dw} + f_{BC,dw}\left[\frac{g_{TOC}}{g_{sed,dw}}\right] \quad \textbf{(16)}$$

2.6 Passive Sampling with PDMS

Polydimethylsiloxane (PDMS; Shielding Solutions Limited) discs (thickness of 0.0762 mm, diameter of 16 mm, and density of 1.17 kg/L) were used to extract the bioavailable fraction of sediment-associated contaminants. Only PDMS discs within the range of 21.71 mg ± 5% were used. Before use the PDMS discs were cleaned through sonication in methanol and acetone/hexane (1/1) for 30 min each, consecutively.

To sample the bioavailable fraction of sediment contaminants, wet sediment equivalent to 20 g of dry sediment was added to a 40 mL amber glass vial and brought up to a water content of approximately 70% using water from the same sampling site. One PDMS disc was added to each vial, keeping the sampler/sediment weight ratio at 0.001 for all sites. The amount of sediment used in the experiments was chosen to ensure negligible depletion (< 5%) (Mayer et al., 2003), detailed calculations can be found in the supplemental information (Section SI 2). Vials containing 20 mL

ultrapure water and a cleaned PDMS disc served as process controls. The vials were shaken at 150 rpm at room temperature. For each sediment sample, three replicate vials were prepared.

Equilibration times between the passive sampler and sediments with differing organic carbon contents ($f_{OC,dw}$ = 0.3 – 2.7%) were determined by measuring bioassay based uptake curves, using an established time-series method (Mayer et al., 2003; Smedes et al., 2013). Establishment of equilibrium was determined by measuring bioanalytical equivalent concentrations in the AhR-CAFLUX bioassay, which targets the effects of dioxin-like chemicals and is mainly triggered by very hydrophobic compounds that have slow uptake kinetics. Li et al. (2013) and Jin et al. (2013) have applied this method to establish time to equilibrium. The rationale behind applying BEQ is the following: if the mixture effect of all chemicals is constant, one can assume that all bioactive chemicals have reached equilibrium. Therefore the BEQ-based approach to assessing sampling kinetics is as legitimate as using the more traditional approach of a series of chemicals that might be present in some samples and not in others. Spiking a set of chemicals to samples is no alternative because the kinetics is likely to be different in native and spiked sediments, as Langdon et al. (2013) have shown in soils. An incubation time of 21 days was adequate, even for sediments with very low organic carbon content (Section SI 3). The samples were shaken for 21 days after which the PDMS discs were removed from the sediment, cleaned in ultrapure water, carefully wiped dry with lint-free tissue to remove any adhering sediment particles (Li et al., 2013) and transferred to a clean vial. PDMS discs were sonicated twice in 15 mL acetone/hexane (1/1), renewing and saving the solvent between each sonication. The two solvent extracts were combined and blown down under a nitrogen stream to dryness and redissolved in 40 µL DMSO for direct application in the bioassays. Excellent recovery of spiked PCDDs in lipid samples in previous work (Jin et al., 2013) confirmed the suitability of this passive sampler extraction method.

2.7 Tenax extraction

Solid phase extraction with Tenax beads was carried out according to You et al. (2007) and adapted by Li et al. (2013). Two sediments were chosen, sample DR with the lowest $f_{BC,TOC}$ (< 2%) and GH 10 with an average $f_{BC,TOC}$ (14%). Wet sediment equating 1 g of dry sediment was added to a 40 mL amber glass jar together with 0.25 g of Tenax resin (Sigma Aldrich; 11982). The jar was filled with 38 mL of ultrapure water and shaken at 150 rpm at room temperature for 24 h. Tenax was then collected from the surface of the water, washed with ultrapure water and extracted twice in 15 mL acetone/hexane (1/1). The extracts were combined, blown down to dry-ness and redissolved in 50 µL DMSO for direct application in the bioassays. Tenax resin shaken in ultrapure water for 24 h served as a process control.

2.8 *In vitro* bioassays

2.8.1 AhR-CAFLUX

Cells used for the assay are recombinant mouse hepatoma cells that were stably transfected with a dioxin-responsive enhanced green fluorescent protein (EGFP) reporter gene (Nagy et al., 2002). The cells respond to dioxins and dioxin-like chemicals that can bind to the AhR through the induction of EGFP in a time-, dose- and chemical-specific manner. The AhR-CAFLUX bioassay was performed according to Jin et al. (2013). Briefly, the cells were seeded in a black 96-well microtiter plate 24 h prior to exposure to the extracts. The sample extracts were then dissolved in assay medium and diluted serially (2-fold, 8 dilution steps) before adding to the assay plate. The samples were

incubated for 24 h at 33 °C, 5% CO_2 before relative fluorescent units (RFU) were measured by a FLUostar Omega plate reader (BMG Labtech). 2,3,7,8-TCDD served as a positive control and results were expressed as percentage of maximum 2,3,7,8-TCDD induction. The EC50 values were derived from log-logistic dose-response curves and the results were expressed as 2,3,7,8-TCDD equivalent concentrations (TCDD-EQ).

2.8.2 AREc32

The AREc32 stable cell line (Wang et al., 2006) is based on MCF-7 breast cancer cells and measures Nrf2 mediated activation of the Antioxidant Response Element (ARE) via a luciferase reporter transgene. The assay was performed following the protocol described in Escher et al. (2013). Briefly, the cells were seeded in a white 96-well microtiter plate 24 h prior to dosing them with sample extracts. The extracts were serially diluted (2-fold, 8 dilution steps) in assay medium and added to the cells. After 24 h of incubation at 37 °C and 5% CO_2 the luciferase response was quantified after lysing the cells and then adding the luciferase reagent (20 mM Tricine at pH 7.8, 2.67 mM $MgSO_4 \cdot 7H_20$, 0.1 mM EDTA, 33.3 mM DTT, 261 µM Coenzyme A, 530 µM ATP, 470 µM luciferin (Promega Cooperation,USA) and MilliQ water; the solution's pH was adjusted to 7.8 before use). The luminescence was quantified immediately after addition of the luciferase reagent using a FLUOstar Omega plate reader (BMG Labtech). t-Butylhydroquinone (tBHQ), a compound of moderate hydrophobicity (log K_{OW} = 2.57) which had been used before for water quality assessment (Escher et al., 2012), was chosen as a positive control and reference. The induction ratio (IR) of the luciferase is defined as the ratio of relative light units (RLU) of the sample divided by the average RLU of the controls (Escher et al., 2012). The assessment endpoint, $EC_{IR1.5}$, was derived from a linear concentration-IR regression through IR = 1. The resulting BEQs were related to tBHQ and expressed as tBHQ equivalent concentrations (tBHQ-EQ) (Escher et al., 2012).

2.8.3 Calculation of Relative Enrichment Factors

EC_{50} or $EC_{IR1.5}$ values, expressed in terms of the relative enrichment factor (REF) of the sample were derived from dose-response curves from both bioassays. The REF of the sample (Eq. 17) is the product of the dilution factor of the bioassay (Eq. 18) and the enrichment factor of the sample (Eq. 19). The enrichment factor for the water ($L_{water}/L_{extract}$) as well as sediment ($kg_{sed,ww}/L_{extract}$) extracts are defined as the amount of water/sediment extracted by the final volume of the extract and were calculated according to Eq. 19. The dilution factor of each bioassay was calculated using Eq. 18.

$$\mathrm{REF} = \mathrm{dilution\,factor}_{\mathrm{bioassay}} \times \mathrm{enrichment\,factor}_{\mathrm{sample}} \quad \textbf{(17)}$$

$$\mathrm{dilution\,factor}_{\mathrm{bioassay}} = \frac{\mathrm{volume\,of\,extract\,added\,to\,bioassay}}{\mathrm{total\,volume\,of\,bioassay}} \quad \textbf{(18)}$$

$$\mathrm{enrichment\,factor}_{\mathrm{sample}} = \frac{V_w \,\mathrm{or}\, m_{sed,ww}}{V_{extract}} \quad \textbf{(19)}$$

Thus, BEQs are expressed as the amount of reference compound per litre of water for SPE extracts, per kilogram of wet weight sediment for sediment and Tenax extracts and per litre of PDMS for PDMS extracts.

3. Results and Discussion

3.1 TOC and BC concentrations

The fractions of TOC per dry-weight sediment, $f_{TOC,dw}$, ranged from 0.4 to 2.7%. The corresponding fraction of BC, $f_{BC,dw}$, was < 0.05 to 0.3% (SI, Table SI 2). The BC constituted between < 2 to 24% of the TOC of the samples, with the median being 9% (Fig. 2 and SI Table 2). Nine % BC content was reported by Cornelissen et al. (2005) as a median of 300 literature values of sediments from around the world. So, despite the fairly small sample size (n = 18) the investigated sites represent a wide and representative variation of $f_{BC,TOC}$ (Fig. 2 and SI, Table 2). Although the 14 Gladstone Harbour (GH) sites are geographically close to each other they exhibit highly variable BC/TOC ratios. This is likely to be due to the natural heterogeneity of sediments as well as the diverse uses of adjacent land including uses as a coal loading bay, shipping, recreation etc.

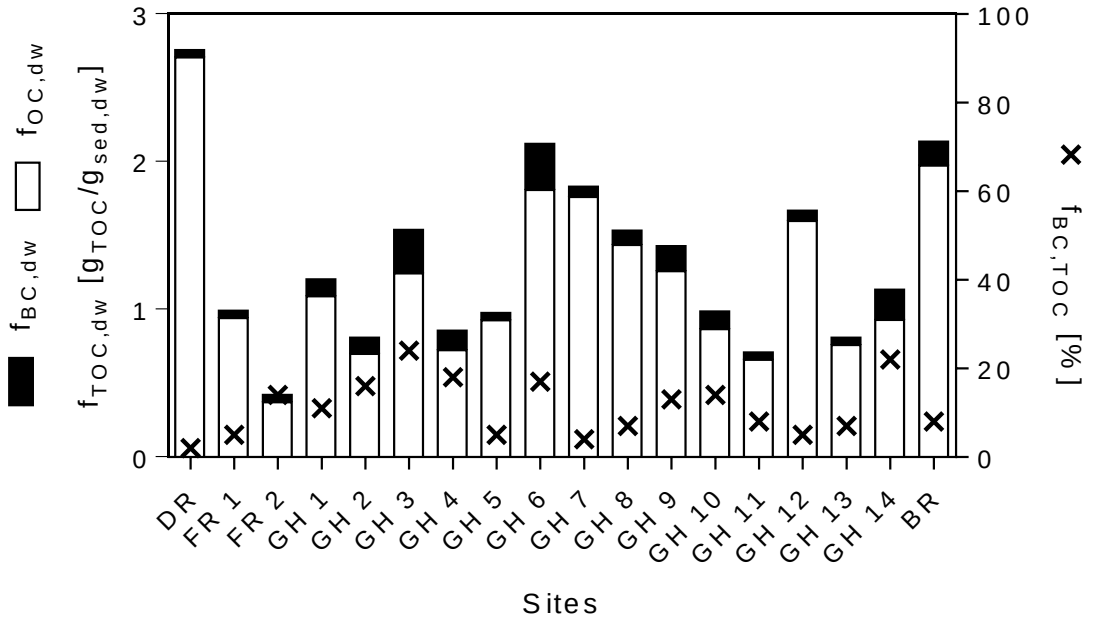


Figure 2: Total organic carbon content ($f_{TOC,dw}$) of sediment samples (bars, left y-axis) divided into fractions of black carbon ($f_{BC,dw,}$ solid shading) and organic carbon ($f_{OC,dw}$, no shading) as well as the fraction of BC in TOC ($f_{BC,TOC}$) ('x', right y-axis).

3.2 Equilibrium passive sampling of HOCs from sediment pore water

The time to attain steady state for the passive sampler with the sediment slurry was determined for five sediments with differing organic carbon contents ($f_{OC,dw}$ = 0.3 – 2.7%). Time to reach the steady state ($t_{95\%}$) was 8 – 19 days, based on the AhR-CAFLUX bioassay. Details on passive sampling kinetics can be found in the supplemental information (SI 3). Depletion of all sediments was below 5% for contaminants active in the AhR-CAFLUX and AREc32 bioassays (SI, Table S3). With attainment of steady state and non-depletion two important criteria of equilibrium passive sampling were fulfilled.

3.3 Validation of BEQ-balance model assumptions

The main assumption for the simplified BEQ-balance model in Equation 14 is that the fraction of HOCs in the aqueous phase is low to negligible. This assumption can be tested by deriving an apparent BEQ-based distribution constant between HOCs in sediment (including pore water) and in the overlying water.

From the BEQ of the total sediment extracts (BEQ_{sed}) and the bioanalytical equivalents of the surface water samples (BEQ_w) a bioassay-based distribution constant ($D_{sed\text{-}w}$) was calculated (Eq. 20).

$$D_{sed-w}\left[\frac{L_w}{kg_{sed,ww}}\right] = \frac{BEQ_{sed}}{BEQ_w}\left[\frac{\frac{ng}{kg_{sed,ww}}}{\frac{ng}{L_w}}\right] \quad \textbf{(20)}$$

Surface water samples are not an ideal proxy of pore water, as they contain dissolved OC bound HOC fractions. In addition, organic contaminants in the water column and pore waters are very unlikely to be in chemical equilibrium with one another. Passive sampler-derived pore water concentrations would be a better proxy of sediment pore water concentrations, but due to the remoteness of the sampling locations such passive sampling was not possible.

High $D_{sed\text{-}w}$ values, as observed for the AhR-CAFLUX bioassay (that ranged from 3300 (GH 14) – 144,700 $kg_{sed,ww}/L_w$ (GH 8); Fig. 3), indicate that the chemicals responsible for the measured effects were mostly very hydrophobic chemicals which partition into the sediment, leaving the BEQ in the water phase small to negligible. As expected, the $D_{sed\text{-}w}$ values for the AREc32 bioassay were lower, at all sites, than those of the AhR-CAFLUX bioassay, ranging between 500 (FR 1) and 6800 $kg_{sed,ww}/L_w$ (GH 13) (Fig 3). For the AREc32 bioassay it is thus possible that the measured effects were caused by both polar compounds and HOCs. Therefore, in the BEQ-balance model for the AREc32 bioassay, the water phase taken was into account (Eq. 13).

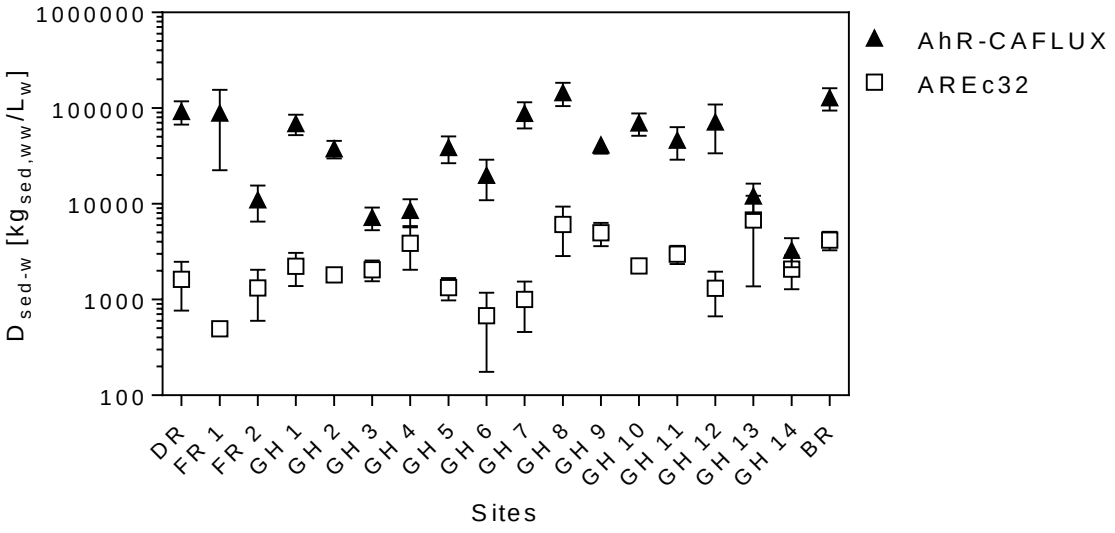


Figure 3: Bioassay-based distribution constant between sediment and surface water ($D_{sed\text{-}w}$) values for the AhR-CAFLUX and AREc32 bioassays.

3.4 Total bioaccessible vs. bioavailable fraction

To investigate the differences between the bioavailable and the bioaccessible fractions samples DR and GH 10 were chosen, as they represent two differing OC and BC contents. For the AhR-CAFLUX bioassay, the rapidly desorbing fraction sampled from DR and normalized to OC was in the same order of magnitude as the bioavailable fraction normalized to OC (Table 1, Tenax/PDMS ratio of 1.39). This reflects the very low level of BC (< 2%) and sorption of HOCs mainly to OC in this sediment. For sample GH 10, with a BC content of 14%, the rapidly desorbed fraction was approximately 13-fold higher than the bioavailable fraction (Table 1, Tenax/PDMS ratio of 12.6). This shows that BC, in sample GH 10, is a relevant sorption phase for HOCs, making them less available for equilibrium partitioning.

A comparison of BEQs of sample DR of bioaccessible sediment contaminants to total extractable sediment contaminants showed that the total sediment contamination was bioaccessible (Table 1, Tenax/ASE ratio of 0.99). In contrast, the bioaccessible fraction of sample GH 10 was smaller than the total sediment BEQs (Tenax/ASE ratio of 0.11), indicating a high percentage of HOCs bound tighly to BC, making them unaccessible in the short term.

The same extracts were also tested in the AREc32 bioassay, which monitors the adaptive stress response pathway and is activated by chemicals that are mainly chemically reactive and more hydrophilic than the chemicals that activate the AhR-CAFLUX bioassay (Martin et al., 2010; Escher et al., 2012). No clear indication of different binding fractions could be found for the two investigated sediments based on the AREc32 results. For both DR and GH 10 sediments the Tenax extractable fraction was substantially greater than the PDMS extractable fraction, whereas the Tenax extractable and the total extractable fractions were in the same order of magnitude. This indicates that most of the chemicals extracted from these sediments were associated with the rapidly desorbing fraction of the sediment and therefore accessible in a 24 h Tenax extraction. However, interferences of the Tenax resin itself were detected in the process control and could not be resolved through multiple cleaning steps of the resin before use, indicating that compounds in the resin itself induce oxidative stress in the AREc32 bioassay. Therefore results for this bioassay should be regarded with caution. Using the AREc32 bioassy on Tenax extracts is still useful as it highlights that the AhR-CAFLUX bioassay is a better choice to investigate the binding of chemicals to BC and that chemicals present in the water phase may play a more important role in the AREc32 bioassay.

Table 1: Comparison of PDMS, Tenax and ASE extractable AhR-CAFLUX and AREc32 BEQs for samples DR and GH 10. Mean values ±standard deviation.

AhR-CAFLUX TCDD-EQ		DR	GH10	Process Control PDMS [µg/L_{PDMS}] Tenax [µg/L_w] ASE [µg/$kg_{sed,dw}$]
PDMS	[µg/kg_{OC}]	294 ± 142	16 ± 7	0.6 ± 0.4
Tenax	[µg/kg_{OC}]	408 ± 99	205 ± 22	
Tenax	[µg/$kg_{sed,ww}$]	5.6 ± 1.3	0.7 ± 0.1	0.6 ± 0.1
ASE	[µg/$kg_{sed,ww}$]	5.6 ± 0.1	6.2 ± 1.6	0.008 ± 0.003
Ratio	Tenax/PDMS	1.39 ± 0.75	12.6 ± 5.5	
Ratio	Tenax/ASE	0.99 ± 0.24	0.11 ± 0.03	
AREc32 tBHQ-EQ		**DR**	**GH10**	**Process Control** PDMS [mg/L_{PDMS}] Tenax [mg/L_w] ASE [mg/$kg_{sed,dw}$]
PDMS	[mg/kg_{OC}]	266 ± 47	340 ± 68	92 ± 12
Tenax	[mg/kg_{OC}]	703 ± 33	4249 ± 262	
Tenax	[mg/$kg_{sed,ww}$]	9.6 ± 0.4	15.2 ± 0.9	12 ± 1
ASE	[mg/$kg_{sed,ww}$]	13 ± 3.9	12.8 ± 1.6	1 ± 0.2
Ratio	Tenax/PDMS	2.6 ± 0.5	13 ± 2.6	
Ratio	Tenax/ASE	0.7 ± 0.2	1.2 ± 0.2	

3.5 BEQ-balance for hydrophobic contaminants (AhR-CAFLUX)

The BEQ_{sed} values of the exhaustive sediment extract in the AhR-CAFLUX bioassay were calculated for all 18 samples from the EC_{50} values (SI, Table SI 5) using Equation 1. The ww-normalised BEQ_{sed} values are listed in SI, Table SI 6. As compounds that induce the AhR are typically hydrophobic, the simplified mass-balance excluding the aqueous phase was applied and the $BEQ_{sed,ww}$ were converted to $BEQ_{sed,dw}$ (Fig. 4) using $f_{sed,dw}$ (SI, Table SI 2).

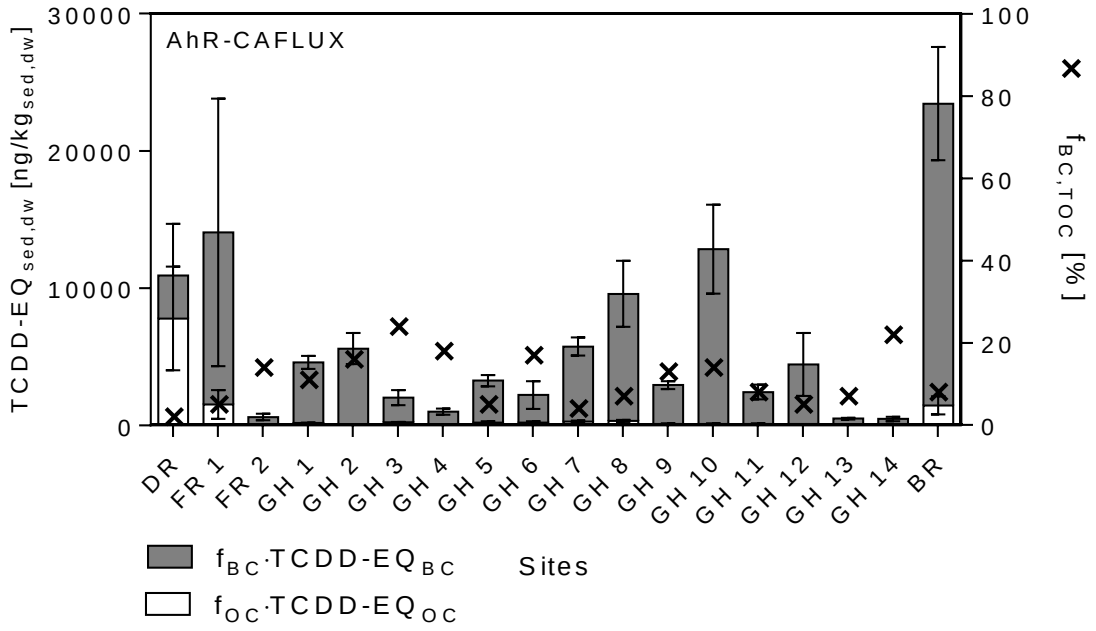
The contribution of organic carbon bound $f_{OC} \cdot BEQ_{OC}$ to total $BEQ_{sed,dw}$ was calculated with the simplified mass-balance (Eq. 14) using the fraction of OC, the BEQs derived from the PDMS extract and the $K_{OC\text{-}PDMS}$ derived by Li et al (2013). This is depicted in Figure 4 as the white portion of the bar representing the TCDD-EQ_{sed} in the AhR-CALFUX bioassay. The contribution of the BC bound fraction $f_{BC} \cdot BEQ_{BC}$ was calculated by subtracting BEQs contributing to the OC fraction from the total BEQs (black portion of the bars in Fig. 4).

There were clear differences between sites, with site BR (an estuary in vicinity to urban dwellings) showing the highest activity and the reference sites GH 13 and 14 showing the lowest overall activity. Overall GH sites showed lower AhR-CAFLUX activity compared to DR, FR 1 and BR. Sites GH 10, in the Calliope River, and GH 8, at the entrance to the marina, showed similar overall activity as site DR. The contribution of readily available, OC-bound, BEQs to the activity of the total sediment extract in the AhR-CAFLUX bioassay was markedly higher for site DR (71% of total BEQ, Fig. 4) than

all other sites investigated (1 – 12% of total BEQ). Consequently, f_{BC}·BEQ_{BC}, the fraction of BEQs bound to BC and unavailable for partitioning, was lowest for site DR (29%) and markedly higher (over 88%) for all other sites. Site DR contained the smallest amount of BC ($f_{BC,TOC}$ < 2%), thus leaving a large portion of activating HOCs bound to OC and thus available for partitioning (Fig. 4). Site DR is a remote site where the land-use is dominated by conservation with minimal agricultural activity, far from industrial input, therefore having a low amount of BC. However, herbicides (Kroon et al., 2012), pesticides (Magnusson et al., 2013) and unwanted pesticide by-products (Holt et al., 2008) may contribute to the large AhR-CAFLUX effects found at this site. Furthermore, due to low BC content a high amount of these activating compounds are available for partitioning and may thus cause adverse effects on organisms in this catchment.

Site FR 1 showed higher activation of AhR compared to the river mouth of the same river, FR 2 (57.3 km downstream; Fig. 4), while the contribution of f_{BC}·$TCDD\text{-}EQ_{BC}$ to total effect was comparable for both sites (FR 1, 89%, and FR 2, 91%, Fig. 4). For most Gladstone Harbour sites more than 90% of activating compounds appeared to be bound to BC and were thus unavailable for partitioning using PDMS based equilibrium passive sampling. This was also the case for the reference sites GH 13 and 14, however, they showed overall lower total AhR-CAFLUX activity compared to the other GH sites. Site BR, situated in a large urban estuary, showed the highest overall activity of the samples investigated, but due to 0.2% BC at this site 94% of the total BEQs were not available for equilibrium partitioning.

Braendli et al. (2008) have shown that small amounts of activated carbon can decrease the available concentration of PAHs in soil/water suspensions by 99% and Lohmann et al. (2005) showed that BC adsorption in harbour sediments was responsible for over 80 – 90% of the total PAH sorption and more than 90% of the sorption of certain PCDDs in two different sediments. These results are comparable to the harbour and estuary sediments investigated in this study (Figure 4), all of which had detectable BC contents.



498
499 **Figure 4:** Bioanalytical equivalents of the exhaustive (ASE) sediment extract (TCDD-$EQ_{sed,dw}$) and
500 contributions of organic carbon (OC) bound f_{OC} TCDD-EQ_{OC} (calculated from PDMS equilibrium
501 sampling) and black carbon (BC) bound f_{BC} TCDD-EQ_{BC} (calculated with Equation 14) contaminants to
502 total BEQs in the AhR-CAFLUX bioassay. The 'x' and right y-axis show the ratio of BC to OC, $f_{BC,TOC}$.

503 ## 3.6 BEQ-balance for more hydrophilic contaminants (AREc32)

504 Concentrations of HOCs have been shown to be close to equilibrium between sediment pore water
505 and surface water in many instances, especially in well-mixed environments (van Noort and
506 Koelmans, 2012), making surface water a good proxy for sediment pore water. The sites investigated
507 in this study can be considered well-mixed and therefore BEQ_w was used as a proxy for the pore
508 water BEQ in Eq. 13.

509 Our results showed that the contribution of the BEQ_w remained negligible in the overall mass-
510 balance with contributions of BEQ_w being well below 1% for all sites (Fig. 5). Site FR 2 showed the
511 highest contribution of BEQ_w (0.25%), despite showing one of the lowest toxicities. `This could
512 indicate fairly recent contamination or perhaps local discharge of activating compounds into the
513 water phase at this site. The majority of chemicals that were bioactive in the AREc32 bioassay were
514 associated with BC (64 – 93%) while 7 – 36% were associated with OC. The lowest contribution of BC
515 associated BEQs (f_{BC}·BEQ_{BC}) was for samples GH 6 and 7 (both 64%) while highest contributions were
516 found for samples GH 3 and 4 (93%). Generally, the relative proportions of bioactive chemicals
517 associated with BC and OC were similar for the AREc32 and the AhR-CAFLUX bioassays with most
518 bioactivity associated with the BC of the sediment. However, for samples GH 6 and 7 around 64% of
519 bioactive chemicals were associated with the BC, perhaps reflecting a higher percentage of polar
520 compounds at these sites, which would be more readily available and associated with the OC. For
521 sample DR the trend was reversed compared to the AhR-CAFLUX bioassay, with a higher percentage
522 (72%) of bioactive compounds associated with the BC. Overall, it can be seen that the GH sites
523 showed similar bioactivity in the AREc32 bioassay compared to the DT, FR and BR sites. This

difference between the GH sites was more pronounced for the more hydrophobic chemicals bioactive in the AhR-CALFUX bioassay.

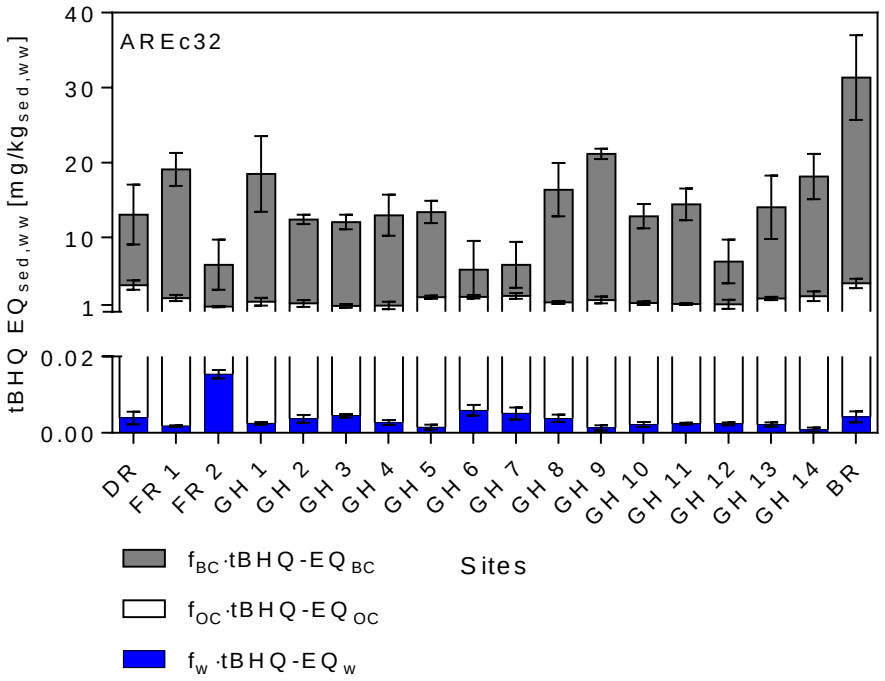


Figure 5: Bioanalytical equivalents of the exhaustive sediment extract (tBHQ-$EQ_{sed,ww}$) and contributions of organic carbon bound (f_{OC}·tBHQ-EQ_{OC}, calculated) and black carbon bound (f_{BC}·tBHQ-EQ_{BC}, Equation 13) contaminants as well as water phase BEQs (f_w·tBHQ-EQ_w, calculated) to total BEQs in the AREc32 bioassay.

3.7 Implications for sediment risk assessment

Results from this study showed that the overall toxicity of the whole sediment often poorly reflects the actual, bioavailable toxicity. The most contaminated site in this study, site BR that was influenced mainly by urban and industrial runoff, showed lower effects of available HOCs compared to the less overall polluted site DR, situated in a remote conservation area. However, lacking the influence of BC at the DR site, a higher proportion of the contaminants may be bioavailable, potentially posing a threat to the aquatic environment. In this context BC present in sediments, even at very low levels, can have a positive effect in reducing the bioavailability of HOCs to sediment-dwelling organisms. Previous studies have shown that reduction of the bioavailability of sediment contaminants through the presence of BC also decreased the risk of exposure to sediment dwelling-organisms, because it ultimately decreases the sediment pore water concentration of HOCs and uptake of these is believed to be mainly via the water phase (Cornelissen et al., 2005; Koelmans et al., 2006). This concept has also been used to artificially decrease bioavailability of HOCs by introducing manufactured carbonaceous materials to contaminated sediments (Zimmerman et al., 2004; Ghosh et al., 2011; Janssen and Beckingham, 2013), which in addition to natural carbonaceous materials further reduce the bioavailability of HOCs and contaminant flux into the water-column. Ortega-Calvo et al. (2015) proposed that when assessing the risks associated with contaminated sites the fraction of a chemical available for uptake into an organism within a given time span should

549 be considered. Using the methods described in this paper can give a good overview of bioavailability
550 of HOCs in the presence of differing carbon contents at contaminated sites and could help to make
551 informed management decisions.

552 ## 3.8 Conclusions

553 Many soil and sediment guidelines still evaluate toxicity based on total contaminant concentration,
554 although it is widely accepted that total concentrations often show little or no relation to actual
555 toxicity to benthic organisms. This study showed that the bioavailability of bioactive sediment
556 contaminants was greatly decreased in the presence of BC. While this has been shown previously in
557 several other studies with selected individual compounds or compound classes, the novelty of this
558 study was to demonstrate this concept for a complex environmental sample, containing not only one
559 class of contaminants but a multitude of contaminants in mixtures and investigating the effects using
560 cell-based bioassays. A modelling approach was used to include BC-bound HOCs in combination with
561 *in vitro* bioassays to be able to compare the induction potential of bioavailable fractions as well as
562 slowly desorbing fractions. This method allows the differentiation of total contaminants and black
563 carbon-/organic carbon-bound contaminants and could potentially be applied when investigating
564 the hazard potential of sediments at industrially influenced sites with higher BC.

565 The benefit of using bioassays over chemical analysis for this evaluation is that the activity of an
566 unknown mixture of contaminants can be determined, while chemical analysis gives exact
567 concentrations but may miss contaminants contributing to the mixture effect. Especially when
568 dealing with diverse sources of contamination from agriculture, industry and mining as in this study,
569 the non-targeted bioanalytical tools can give a comprehensive picture of the contaminant burden
570 and will allow a prioritization of sites for further investigation and possibly identification of bioactive
571 pollutants. Mechanism-specific bioassays can furthermore give information about the chemical
572 classes present in a complex matrix such as sediments. A specific value of this work is the linkage to
573 the bioavailability of hydrophobic contaminants.

574 A range of factors including the type of BC and contaminants present, the presence of dissolved
575 organic carbon, temperature and pH may change the sorption to BC. Therefore, this framework can
576 only provide an initial estimate of the partitioning of contaminants in sediment. No concentrations
577 of contaminants were derived but effect-scaled concentrations (BEQs) and these provide valuable
578 insights on mixture toxicity of natural samples.

579

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Figure Captions

Figure 1: Conceptual model describing the relationships and equilibria in a sediment system. Orange circles represent extracts used in the study. Bold arrows ➡ refer to exhaustive extractions, two-way arrows ⇌ refer to equilibrium partitioning, while dashed arrows indicate limited exchange between phases. BEQ = bioanalytical equivalent concentration, SPE = solid phase extraction, ASE = accelerated solvent extraction, PDMS = polydimethylsiloxane extraction, BC = black carbon, OC = organic carbon, IC = inorganic carbon.

Figure 2: Total organic carbon content ($f_{TOC,dw}$) of sediment samples (bars, left y-axis) divided into fractions of black carbon ($f_{BC,dw,}$ solid shading) and organic carbon ($f_{OC,dw}$, no shading) as well as the fraction of BC in TOC ($f_{BC,TOC}$) ('x', right y-axis).

Figure 3: Bioassay-based distribution constant between sediment and surface water ($D_{sed\text{-}w}$) values for the AhR-CAFLUX and AREc32 bioassays.

Figure 4: Bioanalytical equivalents of the exhaustive (ASE) sediment extract (TCDD-$EQ_{sed,dw}$) and contributions of organic carbon (OC) bound f_{OC} TCDD-EQ_{OC} (calculated from PDMS equilibrium sampling) and black carbon (BC) bound f_{BC} TCDD-EQ_{BC} (calculated with Equation 14) contaminants to total BEQs in the AhR-CAFLUX bioassay. The 'x' and right y-axis show the ratio of BC to OC, $f_{BC,TOC}$.

Figure 5: Bioanalytical equivalents of the exhaustive sediment extract (tBHQ-$EQ_{sed,ww}$) and contributions of organic carbon bound (f_{OC}·tBHQ-EQ_{OC}, calculated) and black carbon bound (f_{BC}·tBHQ-EQ_{BC}, Equation 13) contaminants as well as water phase BEQs (f_w·tBHQ-EQ_w, calculated) to total BEQs in the AREc32 bioassay.

Table Captions

Table 1: Comparison of PDMS, Tenax and ASE extractable AhR-CAFLUX and AREc32 BEQs for samples DR and GH 10. Mean values ±standard deviation.