Passive sampling and passive dosing: Novel approaches for the holistic assessment of marine sediment contamination by hydrophobic organic pollutants

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Anne Kreutzer, M. Sc.
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Dekan:

Prof. Dr. Sven Klimpel
Goethe-Universität Frankfurt am Main
Institut für Ökologie, Evolution und Diversität
Integrative Parasitologie und Tierphysiologie
Max-von-Laue-Str. 13, D-60438 Frankfurt am Main

Gutachter:

Prof. Dr. Henner Hollert
Goethe-Universität Frankfurt am Main
Institut für Ökologie, Evolution und Diversität
Evolutionsökologie und Umwelttoxikologie
Max-von-Laue-Str. 13, DE-60438 Frankfurt am Main

Prof. Dr. Jörg Oehlmann
Goethe-Universität Frankfurt am Main
Institut für Ökologie, Evolution und Diversität
Aquatische Ökotoxikologie
Max-von-Laue-Str. 13, DE-60438 Frankfurt am Main

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“What we know is a drop, what we don’t know is an ocean.”

- Isaac Newton

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List of Abbreviations

3,5-DCP	3,5-dichlorophenol
a	chemical activity
AC	activated carbon
Ace	acenaphthene
A. marina	*Arenicola marina*
Anth	anthracene
ASE	accelerated solvent extraction
BaA	benz[a]anthracene
BaP	benzo[a]pyrene
BCF	bioconcentration factor
BkF	benzo[k]fluoranthene
BPc	community bioturbation potential
BSH	Federal Maritime and Hydrographic Agency of Germany
C_{free}	freely dissolved concentration
Chr	chrysene
CIS	cooled injection system
C_{lipid}	concentration in the lipid
C_{total}	total concentration
CWA	Clean Water Act
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DGT	diffusive gradient in thin film
D. magna	*Daphnia magna*
DOM	dissolved organic matter
DW	dry weight
Ea_{50}	effective chemical activity
EC_{50}	effective chemical concentration
ECHA	European Chemicals Agency
EOM	extractable organic matter
EPA	Environmental Protection Agency
EPSM	equilibrium passive sampling methods

EqP	equilibrium partitioning
Fl	fluorene
Fluo	fluoranthene
GAC	granular activated carbon
GC-MS	gas chromatography–mass spectrometry
GES	Good Environmental Status
GF10	10 µm PDMS coated glass fiber
GF30	30 µm PDMS coated glass fiber
GM	growth medium
HCB	hexachlorobenzene
HF40	40 µm silicone tubing fiber
HOCs	hydrophobic organic compounds
HPLC	High Performance Liquid Chromatography
K_D	site-specific partitioning coefficient
K_{OW}	octanol-water partitioning coefficient
LOD	limit of detection
LOQ	limit of quantification
MDL	method detection limit
MeOH	methanol
MSFD	Marine Strategy Framework Directive
MQL	method quantification limit
Naph	naphthalene
NOEC	no-observed effect concentration
OCPs	organochlorine pesticides
OECD	Organisation for Economic Co-operation and Development
OSPAR	Oil Spill Prevention, Administration and Response
PAC	powdered activated carbon
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PDMS	polydimethylsiloxane
PE	polyethylene
Phen	phenanthrene
POM	polyoxymethylene
PSD	passive sampling device

Pyr	pyrene
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
R. subcapitata	*Raphidocelis subcapitata*
S_L	subcooled liquid solubility
SPMD	semipermeable membrane device
SPM	suspended particulate matter
SPME	Solid Phase Microextraction
SQG	sediment quality guidelines
TC	total carbon
TIC	total inorganic carbon
TOC	total organic carbon
WFD	Water Framework Directive

Abstract

The intensive use of the North Sea area through offshore activities, sand mining, and the spreading of dredged material is leading to increasing pollution of the ecosystem by chemicals such as hydrophobic organic contaminants (HOCs). Due to their toxicological properties and their ability to accumulate in the environment, HOCs are of particular concern. The contaminants partition between aqueous (pore water, overlying water) and solid phases (sediment, suspended particulate matter, and biota) within these systems. The accumulated contaminants in the sediment are of major concern for benthic organisms, who are in close contact with sediment and interstitial water. It is thus particularly important to better understand how contaminants interact with biota, as these animals may contribute to trophic transfer through the food web. Furthermore, sediments are a crucial factor for the water quality of aquatic systems. They not only represent a sink for contaminants but also determine environmental fate, bioavailability, and toxicity.

The Marine Strategy Framework Directive (MSFD) was introduced to protect our marine environment across Europe and includes the assessment of pollutant concentrations in the total sediment, which, however, rarely reflects the actual exposure situation. The consideration of the pollutant concentrations in the pore water is not implemented, although this is needed for the evaluation of bioavailability and risk assessment. For this reason, special attention is given to further development, implementation, and validation of pollutant monitoring methods that can determine the bioavailable fraction in sediment pore water. For risk assessment purposes, it is furthermore important to use biological indicators in addition to classical analytics to determine the effect of pollutants on organisms.

The main objective of this thesis was to gain insight into the pollution load and the potential risk of hydrophobic organic chemicals (HOCs) in the sediment of the North Sea and to evaluate these results with regard to possible risks for benthic organisms and the ecosystem. The following five aims are covered within these studies to gain a holistic assessment of sediment contamination:

1. Assessment of the pore water concentrations of PAHs and PCBs
2. Determination of the bioturbation potential by macrofauna analysis
3. Application of the SPME method on biological tissue
4. Assessment of recreated environmental mixtures in passive dosing bioassays
5. Development of SPME method for DDT in sediments

The thesis is comprised of three main studies supported by three additional studies. The first study aimed to assess the pollution state of sediments with HOCs in the North Sea and determined the bioturbation potential by macrofauna analysis. The bioavailability of a chemical is a key factor responsible for ecotoxicological effects and important for risk assessment approaches. The mobility of HOCs in sediments, and thus the bioavailability, depends on the pore water concentrations (C_{free}). Therefore, the pore water concentrations (C_{free}) in the North Sea sediment samples were determined using solid-phase microextraction (SPME). The chemical activities were calculated to assess the baseline toxic potential exhibited by the sediment. Moreover, a macrofauna analysis was aimed to identify whether there is a correlation between the bioturbation activities of the macrofauna and the C_{free} distribution in the sediment cores. The analysis of sediment cores showed that the contamination level of the North Sea is rather low and decreased towards the upper sediment layers, indicating that bans and regulations on these substances might have a positive impact. Nevertheless, "hotspots" of pollution could also be detected as two sampling stations situated near the coast of Helgoland are affected by the dumping of dredged material from the port of Hamburg. A direct correlation between bioturbation and sediment concentration could not be seen, but higher chemical loads might negatively affect the species abundance and bioturbation potential. Overall, our results contribute to an improved database for status description, assessment of the ecological status, and the detection and influence of possible stressors. This, in turn, will benefit the overall risk assessment and management of the MSFD.

In the second study, the passive sampling SPME approach was extended to the environmental compartment biota since the presence of biota plays an important role in the fate of contaminants in sediment. This study aimed to apply the SPME method to the environmental compartment biota as this approach opens new possibilities for studying the in-situ distribution and thermodynamics of HOCs in biota. The required equilibrium partitioning coefficients were determined for the lugworm *Arenicola marina*. In addition, the SPME approach was applied to assess the tissue concentrations of HOCs in lugworms from tidal flat sediments of the North Sea. The application of the method showed significantly high body residue levels of HOCs in the lugworms, which is probably site-specific as the sampling site was in close proximity to a refinery. Overall, the applied SPME method is considerably faster and less error-prone compared to conventional methods such as exhaustive extractions. Moreover, the HOC contamination in sediment and lugworms can be determined quickly, seasonally, and covering a wide area, making biological monitoring much more effective.

Studies three and four moved the focus on the risk that contaminated sediments may pose to organisms. Risk assessment of HOCs is difficult because maintaining a well-defined exposure concentration during aquatic toxicity testing is challenging due to limited water solubility and various loss processes such as volatilization, biodegradation, and sorption. The use of passive dosing techniques helps to overcome these challenges by delivering a well-controlled and solvent-free exposure. In these studies, the effects of polycyclic aromatic hydrocarbons (PAHs) on organisms of different trophic levels were evaluated. The growth inhibition test with the green algae *Raphidocelis subcapitata* as representative of the primary producers as well as the immobilization test with the water flea *Daphnia magna* as representative of the primary consumers were performed. Individual PAHs were investigated and their corresponding effect concentrations were determined. In addition, recreated PAH mixtures compositions of the North Sea sediments of study 1 were applied in the bioassays to obtain a more realistic test approach, as organisms are exposed to mixtures in the environment. The maximum exposure concentration of individual PAHs exhibited strong effects on both species. The comparison of the two trophic levels showed that the daphnids were less affected by the PAH mixture than the algae. Overall, this passive dosing approach can be applied for various substances and bioassays of different trophic levels, which is important as only a comprehensive battery of assays can provide a conclusive assessment of the impact on the ecosystem.

The fifth study focused on the development of a passive equilibrium sampling method for the detection of DDT in sediments, as it is important to further fill the gap in the analysis of priority pollutant classes in monitoring programs. This study aimed to implement the SPME approach for the organochlorine pesticide DDT and its metabolites, as no analytical methods are available for sediment pore water (C_{free}) contamination in particular. Therefore, the DDT partitioning coefficients were determined. The analysis showed that C_{free} was below the limit of quantification in the surface sediment of the North Sea. In addition to C_{free}, the total sediment concentrations (C_{total}) were determined by applying an exhaustive extraction method. This revealed that higher concentrations of DDT and its metabolites were found in deeper sediment layers, which could be attributed to the fact that the ban on DDT only came into force in 1977. Overall, the contamination with DDT of the surface sediment was low, but the higher concentrations of the deeper sediment might be remobilized when the sediment layers get disturbed by trawling or dredging.

Lastly, the efficiency of sediment remediation for organic contaminants using activated carbon (AC) was evaluated in the sixth study. Remediation of sediment through amendment with a strong sorbent such as AC has proven an effective in-situ method in reducing the concentrations of sediment-bound contaminants. The aim of this study was to evaluate the capping efficiency of powdered AC (PAC) against granular AC (GAC) using contaminated sediment from Oskarshamn harbor (Sweden). We observed that the application of GAC was less effective than PAC but reduced fluxes of high-molecular-weight PAHs. Altogether, PAC performed better than GAC, but adverse effects on the benthic community and transport of PAC to non-target areas are drawbacks that favor the use of GAC.

Overall, the thesis could clearly demonstrate the potential of novel techniques by assessing both the contamination level and the resulting risk executed by sediment-associated HOCs. We were able to show that the SPME technique is an efficient method for tracking the content of HOCs in sediment and biota. Moreover, the technique allows to evaluate the efficiency of sediment remediation with activated carbon. The SPME technique will become a green analytical technique for a variety of different applications in the future due to the flexibility of its design. The passive dosing method can then be used to introduce the determined C_{free} values of the SPME method into bioassays. In this way, natural mixture profiles can easily be reproduced in different toxicity bioassays, which in turn also represent different trophic levels and endpoints. Therefore, the combination of passive dosing and SPME is a promising approach to link the identified contamination with an understanding of biological effects and the resulting ecological impacts. The two approaches considerably improve and expand the monitoring framework for the MSFD and other programs and will lead to better-adapted measures to the prevailing situation, as regional conditions can be better addressed.

Zusammenfassung

Die Verschmutzung unserer Umwelt mit Schadstoffen ist ein immer größer werdendes, globales Problem, das ernsthafte Folgen nicht nur für Ökosysteme, sondern auch für die menschliche Gesundheit nach sich ziehen kann. Die Schadstoffe gelangen auf zahlreichen Eintrittspfaden in die Umwelt. Als Beispiele hierfür lassen sich unter anderem atmosphärische Ablagerungen, die Verschmutzung durch Schiffe bzw. industrielle Tätigkeiten wie Öl-, Gas- und Mineralexploration auf hoher See oder auch die (natürlichen) Einträge aus Flüssen nennen. Einmal in der Umwelt angekommen, werden sie über die Luft, das Wasser, den Boden oder das Sediment immer weiter transportiert. Schadstoffe kennen keine Grenzen, und unabhängig davon, wo sie in die Atmosphäre gelangen, haben sie Auswirkungen auf die globale Umwelt.

Sedimente sind von großer ökologischer Bedeutung, da sie sowohl Lebensraum als auch Nahrungsquelle für viele Tierarten darstellen. Die Schadstoffe verteilen sich im System zwischen wässrigen Phasen (Porenwasser und darüber liegendes Wasser) und festen Phasen (wie Sediment, Schwebstoffe und Biota). Die im Sediment akkumulierten Schadstoffe sind vor allem für benthische Organismen von großer Bedeutung, da diese in engem Kontakt mit dem Sediment und dem interstitiellen Wasser stehen. Vor diesem Hintergrund ist es umso wichtiger zu verstehen, wie Schadstoffe mit Organismen interagieren, da diese zum trophischen Transfer entlang der Nahrungskette beitragen. Darüber hinaus sind Sedimente ein entscheidender Faktor für die Wasserqualität von aquatischen Systemen. Sie stellen nicht nur eine Senke für Schadstoffe dar, sondern bestimmen auch über deren Verbleib in der Umwelt, die Bioverfügbarkeit sowie die Toxizität.

Die intensive Nutzung der Nordsee durch Offshore-Aktivitäten, Sandabbau und die Ausbringung von Baggergut führt zu einer zunehmenden Verschmutzung des Ökosystems durch Chemikalien wie hydrophobe organische Schadstoffe (HOCs). HOCs weisen zwar eine große strukturelle Vielfalt auf, jedoch können sie aufgrund ihrer hydrophoben, lipophilen und biologisch schwer abbaubaren Eigenschaften zu einer Gruppe zusammengefasst werden. Diese Eigenschaften sowie ihre Fähigkeit, sich in der Umwelt anzureichern, sind entscheidend für ihre weltweite Verbreitung und ihr Vorkommen in fast jeder Matrix (Böden, Sedimente, Wildtiere oder auch menschliches Gewebe).

Die Meeresstrategie-Rahmenrichtlinie (MSRL; 2008/56/EG) wurde im Jahr 2008 eingeführt, um unsere Meeresumwelt in ganz Europa zu schützen. Das Hauptziel der MSRL ist es, einen guten ökologischen und chemischen Zustand der EU-Meeresgewässer zu erreichen. Die

Umsetzung der Maßnahmen zum Schutz des benthischen Ökosystems vor der zunehmenden Belastung durch den Menschen erfordert dabei eine sorgfältige wissenschaftliche Bewertung des Zustands sowie der Funktionen des Meeresbodens. Die MSRL sieht bisher nur die Bewertung der totalen Schadstoffkonzentrationen (C_{total}) im Sediment vor, die jedoch nur selten die tatsächliche Situation widerspiegelt. Die Schadstoffkonzentrationen im Porenwasser (C_{free}) werden hingegen nicht berücksichtig, obwohl dies für die Bewertung der Bioverfügbarkeit und Risikobewertung notwendig wäre. Aus diesem Grund wird der Weiterentwicklung, Implementierung und Validierung von Analysemethoden, die den bioverfügbaren Anteil im Sedimentporenwasser bestimmen können, zunehmend besondere Aufmerksamkeit geschenkt. Für die Risikobewertung ist es darüber hinaus wichtig, neben der klassischen Analytik auch biologische Indikatoren einzusetzen, um die Effekte von Schadstoffen auf Organismen zu erfassen. Die Kombination von stoff- und wirkungsbezogenen Daten könnte wesentlich zur Etablierung von Instrumenten für eine ganzheitliche Bewertung und Überwachung der Umwelt beitragen.

Schwerpunkt dieser Arbeit war es, einen Einblick in die Schadstoffbelastung und das potenzielle Risiko hydrophober organischer Chemikalien (HOCs) im Sediment der Nordsee zu gewinnen und diese Ergebnisse im Hinblick auf mögliche Risiken für benthische Organismen sowie das Ökosystem zu bewerten. Um eine ganzheitliche Bewertung der Sedimentkontamination zu erhalten, erfolgte die Untersuchung auf Basis der folgenden fünf Ziele:

1. Bewertung der Porenwasserkonzentrationen von PAKs und PCBs
2. Bestimmung des Bioturbationspotenzials mittels Makrofauna-Analyse
3. Anpassung der SPME-Methode für biologisches Gewebe
4. Bewertung von künstlichen Umweltmischungen mittels ‚passive Dosing' Biotests
5. Adaptierung der SPME-Methode für das Pestizid DDT

Die vorliegende Dissertation setzt sich aus drei Hauptstudien zusammen, die durch drei weitere Studien ergänzt werden. Die erste Studie (Annex 1) hatte zum Ziel, den Belastungszustand der Sedimente mit HOCs in der Nordsee zu erfassen und das Bioturbationspotenzial mittels einer Makrofauna-Analyse zu bestimmen. Polyzyklische aromatische Kohlenwasserstoffe (PAKs) und polychlorierte Biphenyle (PCBs) gehören zu den häufigsten organischen Schadstoffen, die in der Nordsee nachweisbar sind. Ein erheblicher Teil dieser toxischen und persistenten Schadstoffe wird dabei im Sediment zurückgehalten. Die Bioverfügbarkeit einer Substanz ist ausschlaggebend für deren Auswirkungen auf die Umwelt, weshalb die Bioverfügbarkeit auch

als Schlüsselfaktor der Risikobewertung gesehen wird. Bei der Ermittlung der Gesamtkonzentration von Schadstoffen wird jedoch nicht berücksichtigt, dass Moleküle sorbiert, eingeschlossen oder sogar an Sedimentpartikel gebunden sein können und somit weniger mobil sind, als wenn sie im Porenwasser gelöst wären. Die Mobilität von HOCs in Sedimenten – und damit auch ihre Bioverfügbarkeit – hängt somit entscheidend von den Porenwasserkonzentrationen ab. Derzeit bietet die Messung der frei gelösten Porenwasserkonzentration (C_{free}) eine vielversprechende Alternative zur Messung der Gesamtkonzentration (C_{total}). Die Festphasenmikroextraktion (solid-phase microextraction; SPME) wurde als eine Methode der Probenahme zur Erfassung der bioverfügbaren Fraktion entwickelt. Die SPME-Methode ist eine nicht erschöpfende, passive Extraktionstechnik, die auf dem Prinzip der Gleichgewichtsverteilung beruht. Dazu wird ein Polymer in die zu untersuchenden Matrix (z. B. Sediment, Boden und Wasser) eingebracht, die organischen Schadstoffe in der Matrix verteilen sich dann durch Diffusion aus der Matrix in das Polymer bis sich ein chemisches Gleichgewicht einstellt. Anschließend kann die Konzentration im Polymer analytisch bestimmt werden und mit Hilfe von Verteilungskoeffizienten in C_{free} umgerechnet werden. Um die Belastung des Nordseesediments mit HOCs zu erfassen, wurden die Porenwasserkonzentrationen (C_{free}) mittels SPME bestimmt. Daraus konnten anschließend die chemischen Aktivitäten berechnet werden, um das toxische Potenzial (Basistoxizität) des jeweiligen Sediments zu bewerten. Darüber hinaus sollte eine Makrofauna-Analyse Aufschluss darüber geben, ob sich ein Zusammenhang zwischen den Bioturbationsaktivitäten der Makrofauna und der C_{free}-Verteilung in den Sedimentkernen feststellen lässt. Die Analyse der Sedimentkerne zeigte hierbei, dass die Kontamination der Nordsee an den untersuchten Stellen eher gering ist und die Konzentrationen in Richtung der oberen Sedimentschichten abnehmen. Dies könnte ein Hinweis darauf sein, dass sich existierende, gesetzliche Verbote und Vorschriften – wie etwa das weltweite Verbot von PCBs durch das Stockholmer Übereinkommen vom 22. Mai 2001 – bereits positiv auf die chemische Gesamtbelastung ausgewirkt haben. Dennoch konnten auch Verschmutzungs-"Hotspots" festgestellt werden, was darauf beruhen könnte, dass zwei der betreffenden Probenahmestellen in der Nähe von Helgoland unter dem Einfluss einer Verklappungsstelle für Baggergut aus dem Hamburger Hafen stehen könnte. Hinsichtlich der (möglichen) Korrelation von Bioturbation und Sedimentkonzentration konnte zwar kein direkter Zusammenhang festgestellt werden, jedoch kann sich eine höhere chemische Belastung generell negativ auf die Artenvielfalt und somit auch auf das Bioturbationspotenzial auswirken. Insgesamt tragen unsere Ergebnisse zu einer verbesserten Datenbasis für die Zustandsbeschreibung bei, die es ermöglicht potentielle

Stressoren frühzeitig zu erkennen. Dies wiederum könnte sich positiv auf die allgemeine Risikobewertung sowie die Umsetzung der MSRL auswirken.

In der zweiten Studie (Kreutzer et al. 2022; Annex 2) wurde die SPME-Methode auf das Umweltkompartiment Biota angewandt, da die Anwesenheit von Biota eine entscheidende Rolle für das Schicksal von Schadstoffen im Sediment spielt. Es ist bekannt, dass sedimentbewohnende Tiere die Freisetzung und Verteilung von HOCs durch ihre Bioturbationsaktivitäten, die Bewässerung des Sediments sowie durch Partikelvermischung beeinflussen. Zudem können sie HOCs entweder aus der wässrigen Phase oder über die Nahrung akkumulieren. Ziel dieser Studie war es, die SPME-Methode für das Umweltkompartiment Biota einzusetzen, da dieser Ansatz neue Möglichkeiten zur Untersuchung der in-situ-Verteilung von HOCs in Biota eröffnet. In einem ersten Schritt wurden erforderlichen Verteilungskoeffizienten wurden für den Wattwurm *Arenicola marina* bestimmt. Auf deren Basis konnten sodann in einem zweiten Schritt die exakten Gewebekonzentrationen in den bei Wilhelmshaven gesammelten Wattwürmern ermittelt werden. Dabei wurden deutlich höhere Konzentrationen gefunden als in der Literatur beschrieben. Dies könnte jedoch standortspezifisch sein und nur für die im Rahmen dieser Arbeit untersuchten Wattwürmer gelten, da die Probenahmestelle in unmittelbarer Nähe zu einer Raffinerie lag. Die hier angewandte SPME-Methode ist wesentlich schneller und weniger fehleranfällig als herkömmliche Methoden wie z. B. die erschöpfende Extraktion. Außerdem kann hiermit sowohl die HOC-Belastung in Sediment als auch in Wattwürmern schnell, saisonal und flächendeckend bestimmt werden, was die Analyse von Schadstoffen in der Umwelt wesentlich effektiver machen könnte.

In den Studien drei und vier (Annex 3 und Annex 4) wurde der Schwerpunkt der Untersuchung auf das – von Schadstoffen für Organismen ausgehende – Risiko gelegt. Die Risikobewertung von HOCs gestaltet sich schwierig, da die Aufrechterhaltung einer genau definierten Expositionskonzentration während aquatischer Toxizitätstests eine Herausforderung darstellen kann – insbesondere angesichts der begrenzten Wasserlöslichkeit von HOCs sowie verschiedener Verlustprozesse wie Verflüchtigung, biologischer Abbau und Sorption. Der Einsatz passiver Dosierungstechniken (passive dosing) kann dabei helfen, diese Herausforderungen zu überwinden, indem eine gut kontrollierte und lösungsmittelfreie Exposition erreicht wird. Passive Dosing basiert auf dem Prinzip einer konstanten Freisetzung der Prüfsubstanz in das wässrige Medium durch Gleichgewichtsverteilung, wobei ein Polymer (z. B. Silikon) mit der Prüfsubstanz beladen ist und als Reservoir dient. Diese Methode wurde

vorliegend genutzt, um die Auswirkungen von polyzyklischen aromatischen Kohlenwasserstoffen (PAKs) auf Organismen verschiedener trophischer Ebenen zu erfassen. Dementsprechend wurde zum einen ein Wachstumshemmungstest mit der Grünalge *Raphidocelis subcapitata* als Vertreter der Primärproduzenten sowie ein Immobilisierungstest mit dem Wasserfloh *Daphnia magna* als Vertreter der Primärkonsumenten durchgeführt. Das Testdesign der beiden Biotests wurde hierbei für ein miniaturisiertes Format in Mikrotiterplatten optimiert und Silikon O-Ringe wurden als passive Dosierungsphase verwendet. Nicht nur wurden einzelne PAKs untersucht und deren entsprechenden Wirkkonzentrationen bestimmt. Vielmehr wurden in den Biotests auch PAK-Gemische verwendet, um einen realistischen Testansatz zu erhalten – denn Organismen sind in der Umwelt hauptsächlich Gemischen ausgesetzt. Vor diesem Hintergrund wurden die PAK-Zusammensetzungen der einzelnen Nordseesedimente aus Studie 1 nachgebildet, um die Auswirkungen der kontaminierten Nordseesedimente auf Ebene der Organismen zu bewerten. Die Ergebnisse der Biotests mit den einzelnen PAKs zeigten, dass die maximalen Expositionskonzentrationen bei beiden Arten starke Effekte verursachten. Der Vergleich der beiden trophischen Ebenen nach Exposition mit den Mischungen deutete jedoch darauf hin, dass die Daphnien von dem PAK-Gemisch weniger beeinträchtig waren als die Algen. Da in diesen Studien nur eine kleine Anzahl an Substanzen in der Mischung vorhanden war, kann man davon ausgehen, dass die Gesamtheit eines Gemischs nachteilige Auswirkungen auf die Primärproduzenten hat und somit negative Folgen für das gesamte Ökosystem nach sich ziehen kann. Unsere Ergebnisse zeigen, dass der Passive Dosing-Ansatz grundsätzlich für verschiedene hydrophobe Substanzen und für Biotests verschiedener trophischer Ebenen angewendet werden kann. Dies ist vor allem in Hinblick auf eine umfassende Risikobewertung wichtig, da nur eine umfassende Testbatterie mit verschiedenen trophischen Ebenen und Endpunkten eine schlüssige Bewertung der Auswirkungen eines Schadstoffes auf das Ökosystem liefern kann.

Die fünfte Studie (Annex 5) befasste sich mit der Weiterentwicklung der SPME-Methode zum Nachweis von DDT in Sedimenten. Überwachungsprogramme wie die MSRL sind derzeit nicht in der Lage, die entscheidenden physikalisch-chemischen Parameter in hoher zeitlicher und räumlicher Auflösung zu bestimmen, um die Transport-, Austausch- und Verteilungsprozesse der verschiedensten chemischen Verbindungen umfassend zu beschreiben. Daher ist es von Bedeutung, die in Überwachungsprogrammen (noch) vorhandene Lücke an Methoden zur Analyse der bioverfügbaren Fraktion von prioritären Schadstoffklassen zu schließen. Die Schadstoffklassen der PAK und PCB wurden bereits im Rahmen von Studie 1 erfasst. Ziel der

fünften Studie war es, den SPME-Ansatz für das Pestizid DDT und seine Metaboliten zu implementieren, da insbesondere für die Kontamination des Sedimentporenwassers (C_{free}) keine Analysemethoden verfügbar sind. Die Umsetzung erforderte die Bestimmung der Polymer-Wasser-Verteilungskoeffizienten ($K_{PDMS:Wasser}$) von DDT und seinen Metaboliten, die dann zur Umrechnung der Polymerkonzentrationen in die entsprechenden Porenwasserkonzentrationen verwendet werden konnten. Die Bestimmung der Porenwasserkonzentrationen zeigte, dass C_{free} in den Oberflächensedimenten der Nordsee unterhalb der Nachweisgrenze lag. Zusätzlich zu C_{free} wurden auch die Gesamtsedimentkonzentrationen (C_{total}) durch Anwendung einer erschöpfenden Extraktionsmethode bestimmt. Dabei konnten wir zeigen, dass höhere Konzentrationen von DDT und seinen Metaboliten in tieferen Sedimentschichten vorlagen. Die höheren Gesamtkonzentrationen (C_{total}) von DDT und seinen Metaboliten in den tieferen Schichten könnten darauf zurückzuführen sein, dass das Verbot von DDT bereits im Jahr 1977 in Kraft getreten ist und die Oberflächensedimente daher nicht mehr so stark belastet sind. Ein eindeutiger Nachweis dahingehend, dass die Beschränkungen für das Pestizid DDT insgesamt erfolgreich gewesen wären, lässt sich durch unsere Ergebnisse jedoch nicht führen, da das betreffende Sediment nicht datiert worden ist. Insgesamt war die DDT-Belastung des Oberflächensediments gering, dennoch könnte DDT aus den tieferen Schichten wieder remobilisiert werden, falls die Sedimentschichten aufgewühlt werden, wie es etwa bei Schleppnetzfischerei oder Baggerarbeiten der Fall sein kann.

Schließlich wurde in der sechsten Studie (Annex 6) die Effizienz der Sedimentsanierung für organische Schadstoffe unter Verwendung von Aktivkohle (AC) bewertet. Die Sanierung kontaminierter Sedimente steht insbesondere in engem Zusammenhang mit der Notwendigkeit regelmäßiger Ausbaggerungen, bei denen große Mengen an kontaminierten Sedimenten anfallen können. Herkömmliche Bewirtschaftungs- und Sanierungsstrategien für kontaminierte Sedimente umfassen entweder Maßnahmen direkt vor Ort oder Verlagerungsmaßnahmen. Diese haben jedoch mehrere Nachteile, wie etwa begrenzte Raumkapazitäten, hohe Kosten sowie geringe bzw. fehlende Nachhaltigkeit und Umweltverträglichkeit. Die Sanierung von Sedimenten durch Zusatz eines starken Sorptionsmittels wie AC hat sich als wirksame in-situ-Methode zur Verringerung der Konzentrationen sedimentgebundener Schadstoffe erwiesen. Ziel dieser Studie war es, an kontaminiertem Sediment aus dem Hafen von Oskarshamn (Schweden) die Abdeckungseffizienz von pulverförmiger Aktivkohle (PAC) im Vergleich zu granulierter Aktivkohle (GAC) zu bewerten. Außerdem wurden die Auswirkungen der Resuspension auf die Schadstoffrückhaltung und die Integrität der AC-Abdeckung untersucht. Unsere Ergebnisse zeigen, dass die dünnschichtige Abdeckung mit PAC den Austausch

zwischen Sediment und Wasser unter Resuspension wirksamer reduzierte als unter statischen Bedingungen. Die Anwendung von GAC war insgesamt zwar weniger wirksam als die Anwendung von PAC, jedoch verringerte sie den Austausch hochmolekularer PAKs. Im Ergebnis schnitt PAC besser ab als GAC, allerdings ist diesbezüglich zu beachten, dass PAC nachteilige Effekte für die benthische Lebensgemeinschaft hat und auch in Nichtzielgebiete transportiert werden könnte. All diese Aspekte sprechen insgesamt eher für den Einsatz von GAC im Rahmen einer nachhaltigen Sedimentsanierung.

Mit unseren Ergebnissen konnten wir das Potenzial neuartiger Techniken deutlich aufzeigen, indem sowohl der Kontaminationsgrad als auch das daraus resultierende Risiko von sedimentassoziierte HOCs bestimmt wurden. Die SPME-Technik erwies sich als eine effiziente Methode zur Bestimmung der Belastung von Sedimenten und Biota mit HOCs. Des Weiteren konnte mit dieser Technik die Effizienz der Sedimentsanierung mit Aktivkohle erfasst und bewertet werden. In Zukunft könnte sich die SPME-Technik aufgrund ihrer Flexibilität zu einer umweltfreundlichen Analysetechnik für eine Vielzahl unterschiedlicher Anwendungen etablieren. Mit der passive Dosing Methode können die ermittelten C_{free}-Werte der SPME-Methode sodann in Biotests eingebracht werden. Auf diese Weise können natürliche Mischungsprofile leicht reproduziert werden und in diversen Biotests, die verschiedene trophische Ebenen und Endpunkte repräsentieren, getestet werden. Die Kombination von SPME und passive Dosing stellt somit einen vielversprechenden Ansatz dar, um mit Hilfe der Analyse von kontaminierten Sedimenten ein besseres Verständnis der biologischen Effekte von Schadstoffen zu erreichen. Beide Ansätze können den Überwachungsrahmen der MSRL (sowie anderer Programme) erheblich erweitern und verbessern. Im Ergebnis trägt die Verbindung beider Ansätze somit dazu bei, dass Maßnahmen ergriffen werden können, die nicht nur besser an die jeweilige Situation angepasst sind, sondern zugleich auch regionale Gegebenheiten berücksichtigen.

General Introduction

Anthropogenic pollution is not new – especially since the Industrial Revolution a vast majority of pollution on Earth has started. Man-made pollution is generally a byproduct of human activities such as consumption, industrial production, energy generation, waste disposal, and transportation. However, the nature and distribution of contaminants in the environment have changed in recent history as new compounds have emerged. The entry of contaminants into the environment can arise from numerous sources such as atmospheric deposition, pollution by ships, oil, gas and mineral exploration and exploitation, industrial activity as well as riverine inputs. When contaminants are released into the environment, they are transported by air, water, soil or sediment. Chemical pollution has no borders and no matter where the contaminants are released into the atmosphere they will have an impact on the global environment. In contact with biota uptake or association of contaminants may occur, allowing these compounds to be biologically transported and accumulated along trophic chains.

The implementation of measures to protect the benthic ecosystem from increasing human pressures requires a careful scientific assessment of the status and functions of the seabed. The Marine Strategy Framework Directive (MSFD) is the major European legal framework for implementing the ecosystem approach to manage human activities at sea. However, the MSFD merely includes the assessment of pollutant concentrations in the total sediment. The consideration of the pollutant concentrations in the pore water is not implemented, although this is needed for the evaluation of bioavailability and risk assessment. Therefore, special attention is given to further development, implementation, and validation of pollutant monitoring methods that determine the bioavailable fraction in sediment pore water. For risk assessment purposes it is furthermore important to use biological indicators in addition to classical analytics to determine the effect of pollutants on organisms. The integration of substance- and effect-related data will contribute significantly to the establishment of instruments for the assessment and monitoring of the ecological state of the environment.

The North Sea

The aquatic environment is composed of marine and freshwater ecosystems, with marine environments covering about 71 % of the Earth's surface and freshwater ecosystems accounting for less than 1 %. These regions produce about 50 % of the world's net primary production. (Häder et al. 2020). Marine ecosystems are exploited as a source of food, transport, and recreation. Anthropogenic activities have serious impacts on marine ecosystems, which are also increasingly affected by urban development and tourism, global change, and the unsustainable

use of aquatic resources (Beiras 2018). In addition, many marine pollutants enter the environment far upstream from the coasts.

The North Sea is located in the Atlantic Ocean in north-western Europe (Figure 1). It is surrounded by densely populated, highly industrialized countries (Belgium, Denmark, France, Germany, the Netherlands, Norway and the United Kingdom). Two large ports are situated on its coasts (Rotterdam and Hamburg). The North Sea has some of the busiest shipping lanes in the world, which, together with the impact of fisheries and hazardous substances, are highly important pressures for the North Sea region (OSPAR Commission 2010). The German Bight is the south-eastern bight of the North Sea, bounded by Denmark to the east and the Netherlands to the south, covering an area of around 40.459 km² of which 8.000 km² is the Wadden Sea. The Wadden Sea is one of the largest tidal flats in the world, divided into several tidal basins each connected to the North Sea by a tidal inlet. One of the largest basins is the Jade system near the city of Wilhelmshaven. The Wadden Sea is less saline than the North Sea because the seawater is diluted by rivers such as Elbe, Weser and Rhine. Suspended particulate matter (SPM) and associated contaminants transported by these rivers accumulate in the Wadden Sea. The adsorbed contaminants are then transported from east to west within the Wadden Sea (Laane et al. 2013). However, the Wadden Sea is not a permanent sink for contaminants, it rather functions as a source for contaminants to the sediment surface water above.

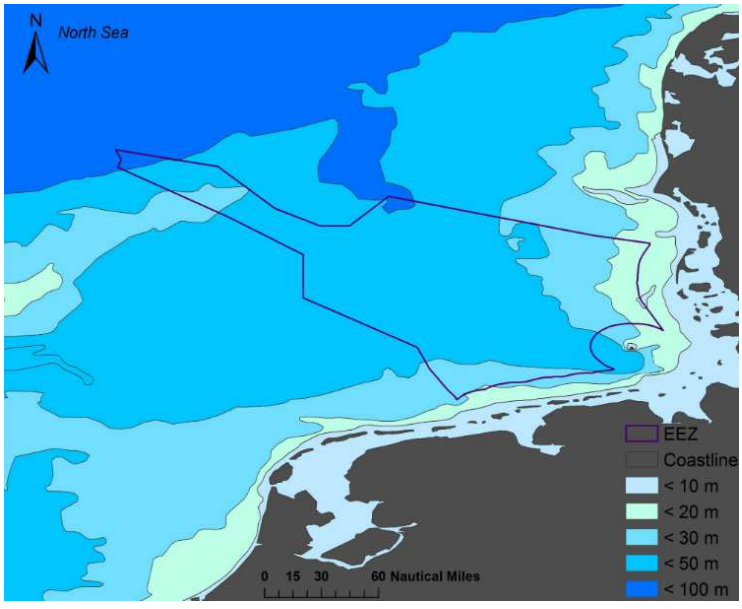

Figure 1: Region of the German North Sea with the German Exclusive Economic Zone (EEZ) and the corresponding water depths. Figure modified from Meyer and Kröncke (2019).

Marine Strategy Framework Directive

Oceans can become sinks for many of river transported substances (Mustajärvi et al. 2017). Against this background, the EU has defined targets for nutrients and some chemicals in the Marine Strategy Framework Directive (MSFD; 2008/56/EC) to protect our marine environment. The MSFD has been enforced in the EU Member States since 2008 establishing a holistic and integrated approach for managing the marine environment across Europe. The main goal of the MSFD is to promote sustainable water use of rivers, lakes, estuaries, and coastal waters in order to achieve good ecological and chemical status of EU marine waters by 2020 (Van Hoey et al. 2010). The Good Environmental Status (GES) is defined as:

"The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions" - Article 3 MSFD

On the basis of eleven qualitative descriptors, GES is determined at the level of marine region or sub-region (Van Hoey et al. 2010). The descriptors relate to biodiversity, non-indigenous species, commercial fish species, food webs, eutrophication, sea floor integrity, hydrographical conditions, contaminants, contaminants seafood, marine litter, and energy inputs (including underwater noise). The Member States are obliged to introduce new ways to measure each descriptor as well as to establish baseline, targets, and indicators. The ecosystem approach is an essential foundation of the MSFD and is intended to ensure that the overall pressures arising from human activities are limited to a level compatible with achieving the GES. At the same time, the ability of marine ecosystems to respond to changes should not be affected. This approach should enable the sustainable use of marine resources today and for future generations. The Directive follows an adaptive management approach in which marine strategies must be kept up to date and reviewed every six years. The EU Commission updated the MSFD decision to determine GES in 2017. This update will be part of the European Green Deal and will directly contribute to its 2030 Biodiversity Strategy and the zero pollution ambition. Striving towards zero pollution is a priority for the EU (Montanarella & Panagos 2021), in particular by preventing the release of pollutants at the source. The implementation of measures under various EU directives to combat chemical pollution has already led to a reduction in the concentrations and impacts of some hazardous substances in the marine environment, such as polychlorinated biphenyls (PCBs), certain organochlorine pesticides, as well as a decrease of PAHs from oil spills (OSPAR Commission 2009). Nevertheless, these substances are very persistent and therefore still present in the marine environment.

Hydrophobic organic compounds (HOCs)

The growing demand and supply of new chemicals by the industrial society in recent decades is placing ever-increasing stress on the natural environment (Jaffé 1991). For instance, more than 22,000 unique chemicals are currently registered in Europe and from these, 2,343 are produced in large quantities above 1,000 tons per year (European Chemicals Agency 2021). Hydrophobic organic compounds (HOCs) are of particular concern due to their toxicological characteristics and their ability to accumulate in the environment. They enter the aquatic ecosystems via numerous pathways such as dumping, industrial discharge, eolian deposits, or bound to suspended particles in river runoff (Witt et al. 2013). Their extensive use in pesticides, petrochemicals, dioxins, flame retardants, preservatives and other products has resulted in ubiquitous environmental contamination (Nam & Kim 2002) and a substantial part is retained in the sediments (Witt et al. 2013). HOCs show a wide structural diversity, but can be grouped by their hydrophobic, lipophilic and poorly biodegradable properties. These properties determine the global distribution and their presence in almost every matrix (soils, sediments, wildlife, and human tissue). HOCs tend to accumulate in the fatty tissue of organisms as a result of continuous exposure to a contaminated environment (bioconcentration) and due to the ingestion of contaminated food or prey (biomagnification along aquatic food chains). The accumulation is of special importance for top predators (fish, seals and especially us humans), as they are end members of food chains originating from sediment-associated communities. Therefore, a reliable risk assessment is necessary not only for ecological reasons but above all for human health (Witt et al. 2013).

PAHs

The group of polycyclic aromatic hydrocarbons (PAHs) is a large class of organic compounds containing two or more fused aromatic rings consisting solely of carbon and hydrogen (Figure 2). Anthropogenic sources of PAHs include fossil fuel combustion, accidental oil spills and leakages, various forms of cooking, and during the production of petroleum-based products (Yuan et al. 2014). Whereas natural release pathways for PAHs into the environment are volcanoes and forest fires. Among the hundreds of known PAHs, the US Environmental Protection Agency (US EPA) has classified 16 PAHs as high-priority pollutants. These include: naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Anth), pyrene (Pyr), fluoranthene (Fluo), benzo[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BghiP), indeno[1,2,3-c,d]pyrene (Ind), and dibenz[a,h]anthracene

(DahA). These 16 PAHs are of environmental concern because of their mutagenic and carcinogenic properties to humans and other organisms (Benlahcen et al. 1997), as well as their widespread distribution and persistence in the environment (Witt et al. 2009). Because of their toxic potential, PAHs have been measured in a variety of environmental matrices, including water, sediment (soil), air and tissue samples. PAH contaminations rarely consist of single compounds but rather of a mixture of compounds. Traditional risk assessment and management, however, focus on the assessment of single compounds (Altenburger & Greco 2009), which can lead to an underestimation of the actual environmental risk, since non-toxic individual substances can become toxic in a mixture (Mayer & Reichenberg 2006, Smith et al. 2013b). It is therefore very important to understand the effects of mixtures on different organisms.

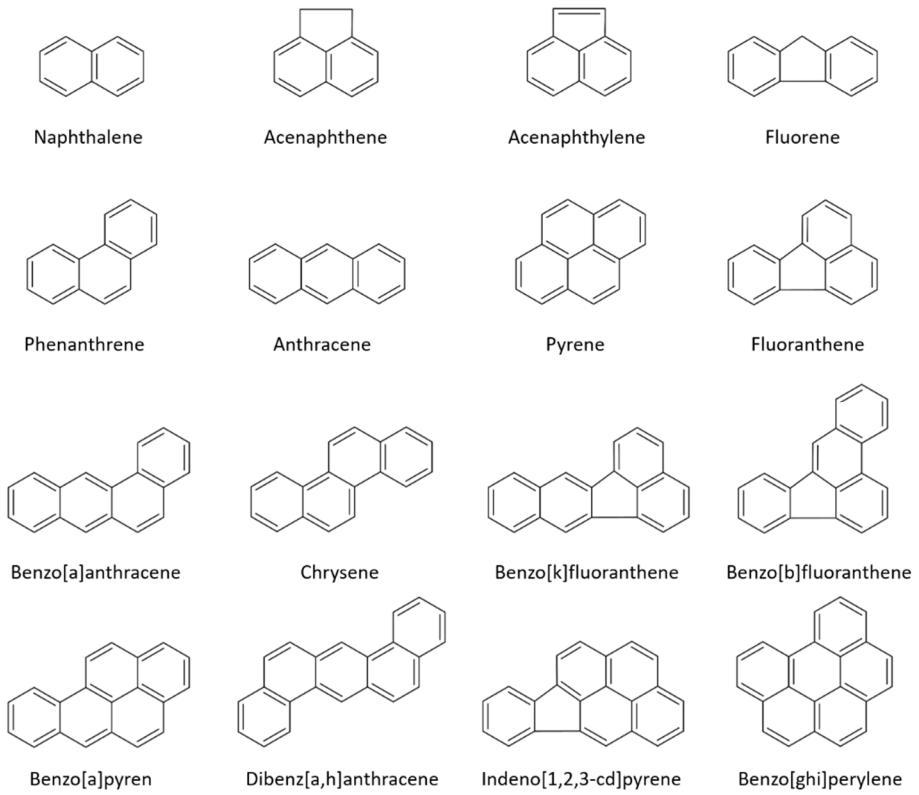

Figure 2: Names and structures of the 16 US EPA priority PAHs.

PCBs

Polychlorinated biphenyls (PCBs) are a group of man-made synthetic compounds consisting of two benzene rings, exhibiting ten possible chloral bonding sites (Figure 3). Depending on the position and degree of chlorination, 209 congeners are known, which are divided into dioxin-like (dl-PCB) and non-dioxin-like (ndl-PCB) PCBs. Twelve compounds (non-ortho PCBs and mono-ortho PCBs) that show molecular biological behavior similar to dioxins/furans are designated as dl-PCBs such as PCB 118. For the remaining PCBs, that do not resemble dioxins

in their toxicological behavior, a group of 6 indicator PCBs (PCB 28, 52, 101, 138, 153 and 180) was defined. PCBs have been used as coolant fluids, dielectrics and in open applications such as paint, buildings, installations and machinery until they were banned between 1970 and 1980 (Fent 2007, Weber et al. 2008). Despite being banned for several decades through the Stockholm Convention they remain a toxic legacy to the environment and our health (Needham & Ghosh 2019). They not only contribute to historical contamination but are still relevant to the current situation as they are still emitted into the environment from old condensers, elastic sealants and other building materials (Fent 2007).

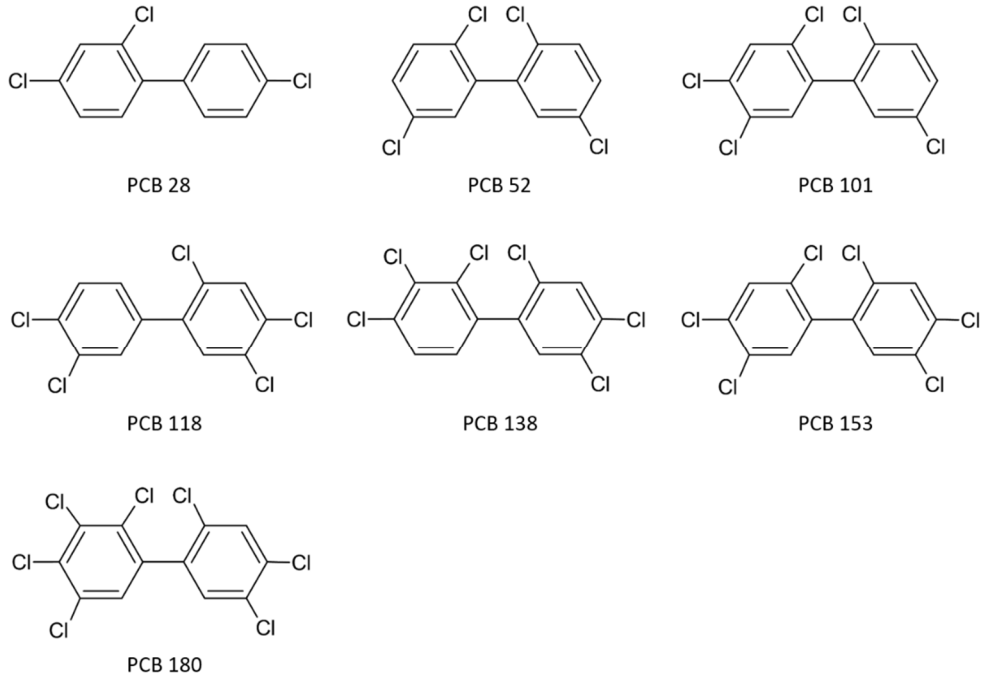

Figure 3: The 6 indicator ndl-PCBs (PCB 28, 52, 101, 138, 153 and 180) and the dl-PCB 118.

DDT

The organochloride dichlorodiphenyltrichloroethane (DDT) is probably the best known pesticide in the world, having been developed in the 1940s as the first modern synthetic insecticide (Jarman & Ballschmiter 2012). The commercially available DDT is a mixture of several closely related compounds since several combinations of ortho and para substituents are formed during the synthesis of DDT (Figure 4). The major component (77 %) is the desired p,p'-isomer, but impurities of the o,p'-isomeric impurity also occur in significant amounts (15 %) (World Health Organization 1979). Dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) account for the rest of the impurities in the commercial samples, which are also the major metabolites and environmental breakdown products. DDT was initially used to combat malaria, typhus, and other insect-borne human diseases among

both military and civilian populations (Van Den Berg et al. 2017). Furthermore, it was also effective for insect control in crop and livestock production, institutions, homes, and gardens (Jarman & Ballschmiter 2012). DDT is classified among the persistent organic pollutants (POPs) in the Stockholm Convention (2008). Although its use in agriculture has been banned worldwide since 2004 it can still be found in environmentally harmful concentrations in sediments due to its high persistence.

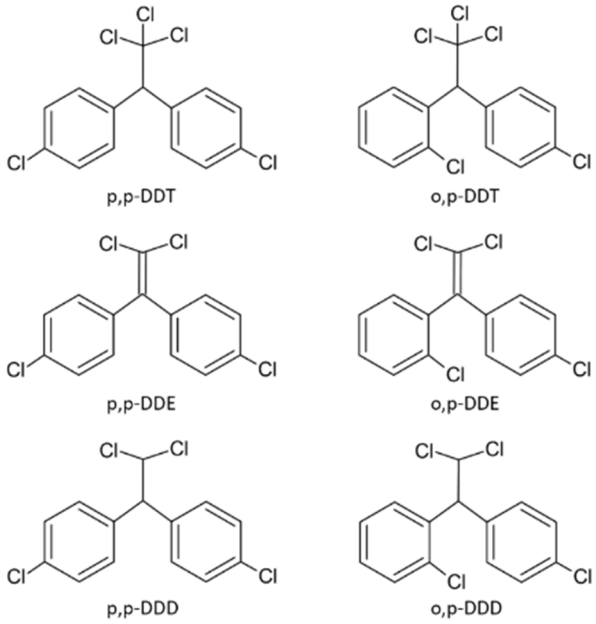

Figure 4: Structures of both DDT isomers and the metabolites DDD and DDE.

Sediments – an underestimated risk?

Aquatic sediments are complex dynamic matrices consisting of organic matter in various stages of decomposition, particulate mineral material of different sizes and chemical compositions, and inorganic material of biogenic origin (Chen & White 2004). They have great ecological relevance as they are both habitat and food sources for many species. Contaminants partition between aqueous (pore water and overlying water) and solid phases (sediment, suspended particulate matter and biota) within these systems (Figure 5) (Eggleton & Thomas 2004). The most problematic compounds for regulative issues are not primarily water-soluble but lipophilic, persistent and bioaccumulative substances. Dissolved compounds remain readily available to aquatic organisms in the free water column. At the same time, less soluble substances such as HOCs have a high affinity to adsorb to dissolved organic matter or particles and settle into the sediment, becoming less available for organisms (Nascimento et al. 2017). This sedimentation process of contaminants improves the habitat for pelagic organisms as their presence in the water column is decreased and thus, their toxicity potential (Eggleton & Thomas

2004). However, the accumulated contaminants in the sediment are of major concern for benthic organisms (Schuler & Lydy 2001). Therefore, a better understanding of how HOCs interact with sediment biota is crucial, as these organisms are in intimate contact with contaminated sediments and interstitial waters and these animals may contribute to trophic transfer through the food web (Christensen et al. 2002).

Legacy contamination can be remobilized due to natural events, e.g., floods, storms, currents and bioturbation (Hollert et al. 2014, Wölz et al. 2008), as well as human activities like trawling, dredging (Köthe 2003) and the deposition of dredged sediment can cause sediments to resuspend. Furthermore, resuspension can be caused by changes in environmental conditions such as pH, temperature, salinity and oxygen content, which can also lead to remobilization of contaminants (Eggleton & Thomas 2004). In these particular cases, sediment is transformed into a secondary source of pollution and poses a major problem for water quality in surface waters, especially in the context of the implementation of the EU Water Framework Directive (Kosmehl et al. 2007). Therefore, sediments are a very important factor for the water quality of aquatic systems as they not only represent a sink for contaminants but also by determining environmental fate, bioavailability and toxicity (Chen & White 2004, Mustajärvi et al. 2017).

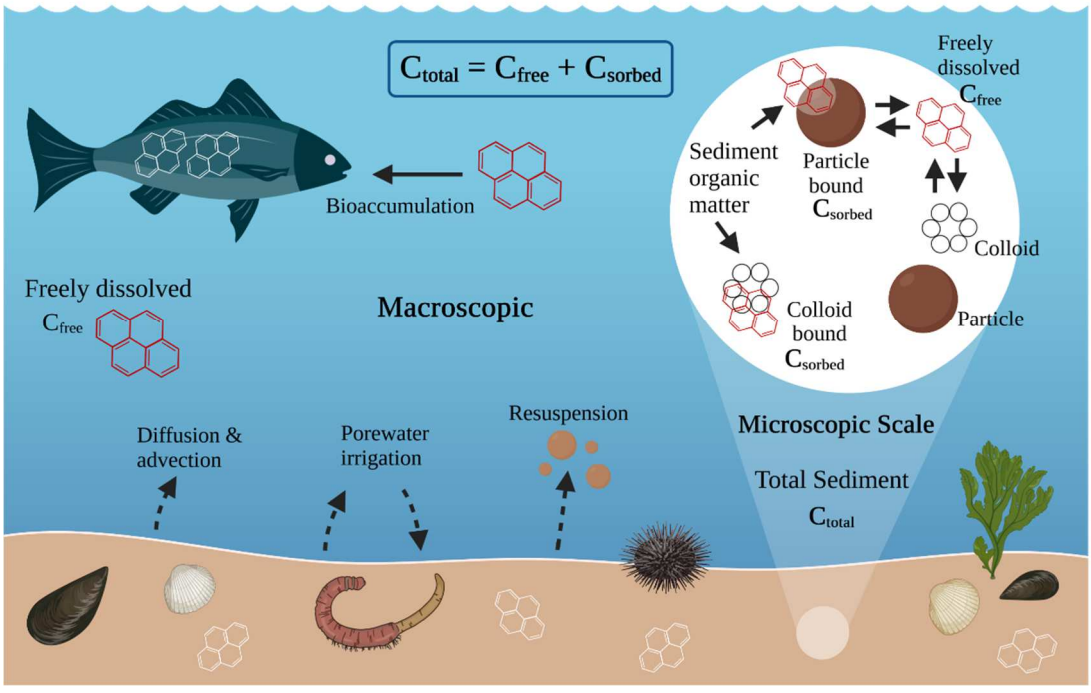

Figure 5: Conceptual view of contaminant cycling in the sediment-water system highlighting the central role of freely dissolved concentrations C_{free}. Scheme adapted from Mayer et al. (2014). Illustration was created with BioRender.

Distribution of contaminants within the sediment

Contaminants have different affinities for the various solid-phase fractions of the sediment. Most natural organic sorbents readily accept non-polar compounds such as organic pollutants, whereas charged particles (e.g., clays) form a tight association with neighboring water molecules excluding organic pollutants (Chen & White 2004). Organic contaminants preferentially partition to organic matter in sediment and dissolved organic matter (DOM) in pore water (Goossens & Zwolsman 1996). The contaminants composition of the pore water is determined by the redox potential, pH, and temperature and can differ significantly from that of the overlying water (Eggleton & Thomas 2004). Pore water concentrations (C_{free}) of the contaminants are more closely related to the toxicity of sediment than concentrations in bulk sediment, wherefore their assessment can help to determine the bioavailability and the toxic potential of the sediment (Ma et al. 2000). The magnitude of association between a compound and a particulate adsorbent is often expressed as the sorption partition coefficient (K_D) (Eggleton & Thomas 2004). Compounds with high octanol-water partitioning coefficients (K_{ow}) and low water solubility have a higher tendency to be associated with particulate matter (Chen & White 2004).

Remobilization of Contaminants

Historically contaminated sediments are now recognized as significant secondary sources of pollutants in many ecosystems, even after emission reductions of various volatile organic compounds (Gunnarsson et al. 1999, Josefsson et al. 2010, Mustajärvi et al. 2017). Contaminants are released from sediment to water by two major processes: (i) Diffusive flux of dissolved contaminants, and (ii) resuspension of contaminated particles (Latimer et al. 1999). Anthropogenic activities such as trawling and dredging, as well as the disposal of historically contaminated estuarine sediments, lead to significant disturbance of the sediments (Eggleton & Thomas 2004). Natural activities including currents, waves, wind energies, and storms can cause periodical remobilization of surface sediments (Calmano et al. 1993). In addition, the reworking and irrigation of sediments by benthic organisms (i.e., bioturbation) can significantly influence the physicochemical properties of the sediment and pore water and influence the fate of contaminants in the benthic environment (Kristensen et al. 2012).

Marine soft sediments are the most common habitats on Earth and represent some of the most functionally important ecosystems on Earth (Ellis et al. 2000). These habitats are characterized by a high diversity and biomass of invertebrate organisms exhibiting significant influence over the benthic sedimentary geochemical environments (Queirós et al. 2013). Macrofauna species

serve as a food source for larger benthic species, epifauna or demersal fish species, while feeding on smaller organisms such as meiofauna and bacteria, feces from all trophic levels, and on benthic and pelagic phytoplankton (Meyer & Kröncke 2019). These species are found in the upper layers of the sediment and at the water-sediment interface, and represent an important element of the benthic-pelagic coupling (Griffiths et al. 2017). The mixing of sediment and particulate material carried out during foraging, feeding, and burrow maintenance activities of benthic species is defined as bioturbation (Queirós et al. 2013, Reible et al. 1996). The reworking processes transport oxygen, nutrients and organic matter into deeper layers and increase the exchange processes between pore water and the water column (Meysman et al. 2006). A theoretical bioturbation measurement was first described by Solan et al. (2004), while Queirós et al. (2013) developed a classification for macrofaunal species depending on behavior, diet, and life stage and style. The community bioturbation potential (BPc) provides an estimate of the potential of a community to bioturbate rather than a direct measurement. The BPc, however, provides an opportunity to assess the extent to which benthic communities are likely to influence important ecosystem properties that underpin ecosystem functioning (Queirós et al. 2013).

The process of bioturbation can effectively incorporate contaminants into the sediment, leading to increased retention of pollutants in the sediment and reduced bioavailability. However, bioturbation can also induce increased mobilization of sediment-associated contaminants. The contaminant's fate in sediment has been attributed to the organism's mode of bioturbation, e.g., their feeding and burrowing strategies and intensity (Hedman et al. 2008, Josefsson et al. 2010). Depending on the organism's mode of bioturbation species that actively move particles within the sediment primarily relocate particle-associated contaminants by bringing them to the sediment-water interface, where they may desorb to the overlying water. Whereas other organisms create and continuously irrigate burrows with oxygenated water, which increases the exchange of solutes and dissolved contaminants between the pore water and the overlying water (Hedman et al. 2009). The bioturbation activity of macroinvertebrates can also change the partitioning of sediment-bound contaminants in different compartments such as sediment profile, pore water, and the water column (Hedman et al. 2008).

The most significant fraction of contaminants such as PAHs and PCBs are associated with sediments, benthic organisms, and pore waters, whereas concentrations in these compartments are several orders of magnitude greater than those in overlying water (Ciarelli et al. 1999). Josefsson et al. (2010) showed that the highest remobilization of HOCs occurred from the most

shallow sediment layers down to at least 10 cm due to bioturbation activity. Towards the end of the 20th century, the influence of bioturbation on the flux of contaminants from sediments became an increasingly important issue in the Baltic Sea due to the invasion of spionid polychaetes of the genus *Marenzelleria*. The burrowing depth of *Marenzelleria spp.* reaches up to 35-50 cm, which is much deeper than of other macrofauna species in the Baltic Sea. As a consequence, contaminants that were previously thought to be buried at safe depths can be remobilized (Josefsson et al. 2010).

In summary, numerous field and laboratory studies have shown a significant increase in the HOC concentrations in the overlying water after resuspension events providing evidence for the importance of sediment resuspension for HOC transport and internal recycling in marine ecosystems (Bogdan et al. 2002, Ko et al. 2003). Consequently, since aquatic sediments are not only an important sink for HOCs, a better understanding of contaminant transport processes from sediment to water is essential in making risk assessments and decisions about remedial actions (Hedman et al. 2009).

Remediation of Sediments

The management of contaminated sediments is closely related to the need for regular dredging activities to maintain navigational depth in ports and waterways, but also for remediation and flood protection (Förstner & Apitz 2007, Rulkens 2005). The problem with the dredging activities is the large quantities of contaminated sediment that are produced, e.g., around 100 to 200 million cubic meters per year in Europe (Bortone et al. 2004). Conventional management and remediation strategies for contaminated sediment include either on-site actions or relocation actions. However, they also have several disadvantages such as limited space capacity, high costs, low or lack of sustainability, and environmental compatibility (Akcil et al. 2015). Natural recovery and in-situ capping are remediation strategies that do not require dredging operations. However, dredging is followed by landfill disposal or dumping at sea, which are still the two most common management strategies worldwide.

Remediation of sediment through amendment with a strong sorbent has proven an effective in-situ method in reducing the concentrations of sediment-bound contaminants (Ghosh et al. 2011, Josefsson et al. 2012). Especially the use of activated carbon (AC) amendment is a promising approach for in-situ remediation as it represents a less intrusive and more cost-effective treatment (Ghosh et al. 2011). AC is a porous manufactured material with a high surface area and sorption affinity for HOCs that readily accumulate in sediments (Ghosh et al. 2011, Gustafsson et al. 2017). AC amendment increases sorption and sequestration of HOCs in the

sediment, thus reducing the aqueous concentrations, which in turn leads to lower bioavailability and bioaccumulation of HOCs in benthic organisms (Kupryianchyk et al. 2012). Several techniques have been used to place AC in the aquatic environment, e.g., the mixing of AC into sediments resulting in a homogenous distribution of AC in the sediment with high initial remediation effectiveness (Choi et al. 2014). However, this technique disturbs the contaminated sediment and its benthic community. No specialized equipment is needed to apply in-situ thin-layer capping, where AC is deposited on the sediment surface (Kupryianchyk et al. 2015). The AC thin layer will then be incorporated into the biologically active layer over time by bioturbation (Cornelissen et al. 2011). Ghosh et al. (2011) have proposed thin-layer capping with active sorbents such as AC as a promising alternative to dredging for sediment remediation.

Development of sediment monitoring and assessment tools

In aquatic systems, contaminated sediments are arguably among the most widespread and technically challenging ecological risks. The estimated cost of managing (i.e., remediating and monitoring) contaminated sediments is several billion US dollars worldwide (Burgess et al. 2013). The assessment of sediments becomes increasingly important in terms of (bio)remediation and decontamination of historically contaminated sites (Fent 2007). Several decision-making tools have therefore been developed in the last decades to assess the magnitude of the threat, as contaminated sediments have been recognized.

One of the first approaches was the sediment quality triad (SQT) to assess the risk associated with the sediments of concern (Long & Chapman 1985). However, the projected costs associated with the combined approach (chemical, bioassay, and infauna) have fueled the development of alternative approaches that use relatively simple and inexpensive measures to predict contaminated sediment risk (Burgess et al. 2013).

Biologically-based approaches, which assess contaminated sediments posing the greatest ecological risk, range from toxicity testing to benthic community analysis. Among the prevailing approaches was the development of the ‘sediment quality guidelines’ (SQGs), which are based on chemical-toxicity relationships for sediment (Mount et al. 2003). The sediment contamination and related parameters (e.g., organic carbon) are measured to predict adverse toxicological effects (Burgess et al. 2013). In addition, chemical-based SQGs provide a relatively inexpensive way to support contamination assessments. The SQGs can be categorized into two general forms: Empirical and mechanistic. The empirical form uses a database of matched sediment chemical and biological effects to derive sediment concentrations, whereas

the mechanistically-based form predicts the sediment toxicity based on the factors that influence the bioavailability of sediment-associated chemicals (Mount et al. 2003).

The physical-chemical concept of equilibrium partitioning (EqP) is thereby the main approach used and asserts that a contaminant's bioavailability is directly proportional to its chemical activity in sediment (Di Toro et al. 1991). The EqP method assumes that there is equilibrium between sediment and interstitial water and that the chemicals are distributed between interstitial water and sediment particles. The partitioning is determined primarily by the organic carbon content of the sediment (for non-ionic organics), the sulfide content (for metals), and the partitioning behavior of the individual chemicals. Therefore, EqP predicts bioavailability using partition coefficients between sediment particles and the interstitial water and allows an accurate estimation of the bioavailable concentration of a sediment pollutant and the potential resulting adverse effects (Burgess et al. 2013). The choice of a particular method influences the results of an investigation and thus the assessment of environmental risk. This can potentially lead to a measurement that may not be appropriate due to inaccurate data and wrong assumptions.

Risk Assessment of chemicals and contaminated sediments

One consequence of industrialization is vast quantities of toxic waste and effluents, resulting in a major source of water pollution when discharged in surface waters. Even though the overall water quality of major rivers might have improved since the 1960s due to stricter legislation in many countries – e.g., the Water Framework Directive (WFD) in EU countries, the European regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and the Clean Water Act (CWA) in the US – more than 60 % of the global aquatic ecosystems are degraded or unsustainably used (Mostert 2009). Sediment risk assessments have traditionally been based on sediment quality guidelines (SQGs). However, the issue of bioavailability is not well addressed by SQGs, since they are based on total sediment concentrations (Burton Jr 2002). Predicting a toxic effect based on total sediment concentrations is highly uncertain. Therefore, the equilibrium partition approach is a promising method to assess the bioavailable burden of contaminants in the sediment that reflect the field hazard potential and to identify chemicals of concern (Feiler et al. 2013, Hollert et al. 2003).

The chemical regulation is mainly built around the assessment of single compounds, as seen in REACH (EC 1907/2006) and the EU regulation on the use of Plant Protection Products (EC 1107/2009) (Mustajärvi et al. 2017). However, there is increasing evidence that mixtures cause toxic effects, even though the individual chemicals in the mixture are present below the

no-observed effect concentration (NOEC) (Kortenkamp et al. 2009). For instance, Smith et al. (2013b) could demonstrate that significant effects of an artificial PAH mixture were observed on the immobilization of *Daphnia magna*, even though the individual PAHs of the mixture had no or limited toxic effects.

Equilibrium passive sampling

There has long been a debate on how to define bioavailable concentrations. This debate mainly resulted from the effort to explain toxicity based on correlation with a concentration of a substance in the exposure medium. Exhaustive sediment extractions are proven to represent the total chemical concentrations (C_{total}) and have traditionally been used in risk assessment and risk management (Greenberg et al. 2014). However, the total concentration only rarely reflects the actual exposure situation (Mayer et al. 2003). This has led to an understanding of a bioavailable concentration as being a fraction of C_{total} that is (freely dissolved fraction) or can become (reversibly bound fraction) available for uptake. To assess this bioavailable fraction, Arthur and Pawliszyn (1990) introduced a solid-phase microextraction (SPME) technique applied as a passive sampling tool.

SPME is a non-depletive passive extraction technique using equilibrium partitioning to determine C_{free} in sediment pore water (Mayer et al. 2000). Equilibrium passive sampling (EPS) is performed by bringing a polymer in contact with the matrix of interest (e.g., sediment, soil, and water) (Lydy et al. 2014, Reichenberg et al. 2008, Vrana et al. 2005). The organic contaminants in the matrix then partition by diffusion from the matrix into the sampler until chemical equilibrium is reached between the polymer and the sampled matrix (Mayer et al. 2003). The concentration in the polymer ($C_{polymer}$) can be measured and linked to C_{free} by the respective partition coefficient ($K_{polymer}$) between the sampling phase and the aqueous phase of the compound. However, the precise determination of partitioning coefficients is important for passive sampling applications as it ensures an accurate determination of C_{free} and also comparability between studies (DiFilippo & Eganhouse 2010). Nevertheless, this passive sampling method offers a simple and less disruptive sampling approach than conventional pore water collection and measurement techniques, providing misleading information (Greenberg et al. 2014).

The application of passive sampling methods to investigate bioavailable contaminants in different environment compartments (sediment, water, air, biota, and soil) has become more important in the last decades. The physical size and shape and the type of polymeric sorbent

material used to construct the sampler are the two major characteristics defining a passive sampler (Lydy et al. 2014). There are two different configurations used: i) Thin films cut into sheets or strips or ii) coatings. Sheets and strips can vary in thickness and dimensions to accommodate the experimental design, while coatings, also of different thicknesses, can be applied to solid supports e.g., glass fibers and glass jars. The affinity (partition coefficient) is determined by the sorbent phase of the polymer, whereas the sorption capacity of the device is defined by the phase volume combined with the partition coefficient. Sampling phases such as polyethylene (PE) and polyoxymethylene (POM) are used in the form of films, and polydimethylsiloxane (PDMS) is typically used as coating (Lydy et al. 2014). Arthur and Pawliszyn (1990) firstly introduced SPME with coated glass fibers and quantification via thermal desorption. For sediments in this study, PDMS-coated glass fibers are applied as equilibrium samplers due to the buffering effect of the sediment (Figure 6). Passive sampling methods have a couple of advantages over traditional sampling methods: (i) They are time and cost-efficient while being less resource-intensive, (ii) the sample alterations during transport and storage are minimized, and (iii) no sample preparation is required for automated analysis. In addition, the detection limits are very low, especially for highly hydrophobic substances, which also allows the actual concentrations of contaminants in the water phase to be determined.

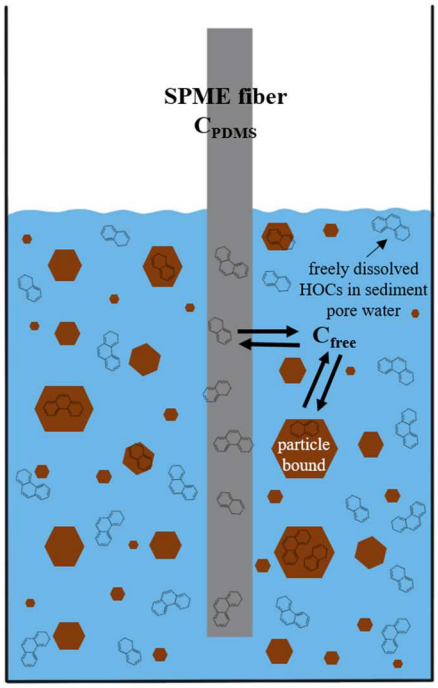

Figure 6: Principle of passive sampling in sediment with SPME fibers.

Chemical activity

Distribution, transport, and transformation processes, as well as the uptake of environmental contaminants, are determined by the direction and extent of chemical reactions, diffusion, and partitioning processes (Gilbert 2011). The concept of chemical activity is one thermodynamic concept that describes and predicts the fate and effect of a chemical in the environment (Reichenberg & Mayer 2006). The chemical activity (a), which is closely related to C_{free}, quantifies the energetic state of the chemical, determining the potential for ecologically relevant spontaneous processes such as diffusion and partitioning (Reichenberg & Mayer 2006, Schwarzenbach et al. 2016). It can be calculated by dividing C_{free} of a sparingly soluble compound in water by its respective subcooled liquid solubility (S_L) and the maximum chemical activity of a liquid compound is given to be one, where the minimum is zero (Gilbert 2011). Since bioaccumulation is proportional to chemical activity in the exposure environment, a better assessment of the bioaccumulation and toxicity of sediment-bound contaminants lies in the more accurate determination of the chemical activity of contaminants in this complex matrix (Lydy et al. 2014).

Baseline Toxicity

The partitioning of organic substances into the lipid membrane is a spontaneous process and is better described by the chemical activity than by the concentration in the exposure medium (Reichenberg & Mayer 2006). The sum of chemical activities is an indicator of the baseline toxic potential of a mixture since the baseline toxicity of mixtures is generally concentration additively (Di Toro et al. 2000). In particular, individual substances of a mixture are present below the threshold level of specific toxicity, but the underlying cumulative baseline toxicity of the mixture might determine the overall toxic effect (Escher et al. 2002). The concept of baseline toxicity was described by Ferguson (1939). Baseline toxicity, also called narcosis, is the minimal non-specific toxicity a hydrophobic compound can cause when crossing membranes (Escher & Schwarzenbach 2002). Reichenberg and Mayer (2006) compared effective concentration values (EC_{50}) of 29 different chemicals and three organisms (mouse, tadpole, algae). However, the EC_{50} values spanned several orders of magnitude, but after being normalized to the aqueous solubility of the corresponding chemical, the obtained effective chemical activity values (Ea_{50}) were relatively constant within the range of baseline toxicity (0.01 to 0.1). When looking at the PAHs and PCBs, their chemical activity increases with the number of fused benzene rings or with the level of chlorination. In a mixture, therefore, high molecular PAHs and PCBs can contribute most to the total chemical activity and thus also to

the baseline toxicity. In terms of risk assessment, the concept of baseline toxicity can better predict the risk to organisms based on C_{free}.

Equilibrium passive dosing

Maintaining and confirming stable exposure concentrations is critical for assessing the toxicity of chemicals. Current guidelines for standard toxicity tests are generally not suitable for the large group of poorly water-soluble compounds, and often organic solvents are used to obtain a completely dissolved exposure concentration (Mayer et al. 1999, OECD 2002). Most of these standard tests are performed in static systems and the dissolved concentrations of these hydrophobic chemicals usually decrease during the experiment (Smith et al. 2010a). However, maintaining a well-defined exposure during aquatic toxicity testing is difficult due to the limited water solubility and various loss processes, including volatilization, biodegradation, and sorption (Mayer et al. 1999, Smith et al. 2010a, Smith et al. 2010b). In addition, the analysis of the freely dissolved concentrations (C_{free}) in the test medium is by no means straightforward and dynamic exposures can complicate the determination of robust toxicity test endpoints (Kiparissis et al. 2003, Stibany et al. 2017a).

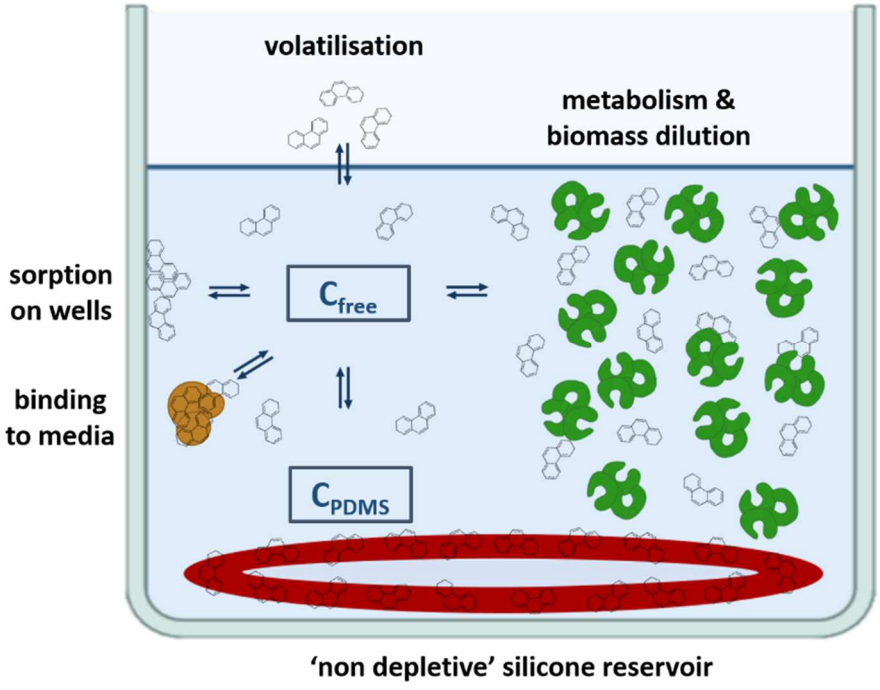

Figure 7: Principle of passive dosing with a non-depletive silicon reservoir (O-ring).

The use of passive dosing techniques helps to overcome these challenges by delivering a well-controlled and solvent-free exposure. Passive dosing is based on a constant release of the test substance into the aqueous medium by equilibrium partitioning, where a polymer (e.g., silicone) is loaded with the test substance and acts as a reservoir (Figure 7) (Mayer et al. 1999, Smith et al. 2010a, Smith et al. 2010b, Stibany et al. 2017a). Thus, a constant freely dissolved concentration can be maintained throughout the whole test period (Smith et al. 2010a). There is a whole range of suitable polymers used for passive dosing, but due to their chemically inert properties and bio-compatibility, mainly silicone and PDMS (e.g., in the form of silicone–cast vials, silicone rods, and sheets or O-rings) have been applied in the past (Gilbert et al. 2014, Mayer & Holmstrup 2008, Seiler et al. 2014, Smith et al. 2013a, Smith et al. 2010b, Smith et al. 2012, Vergauwen et al. 2015).

The test compound concentrations in the O-rings can be varied to apply a wide range of freely dissolved concentrations. Hence, passive dosing can be flexibly used for testing at HOC aqueous solubility, concentration-response testing, or chemical mixture toxicity (Smith et al. 2010a). Exposure to a mixture of chemicals allows for a more realistic testing approach as organisms in the environment are exposed to mixtures. In addition, the measured HOC composition of a specific location can be tested by recreating the mixture on the dosing phase and introducing it into the bioassay (Figure 8).

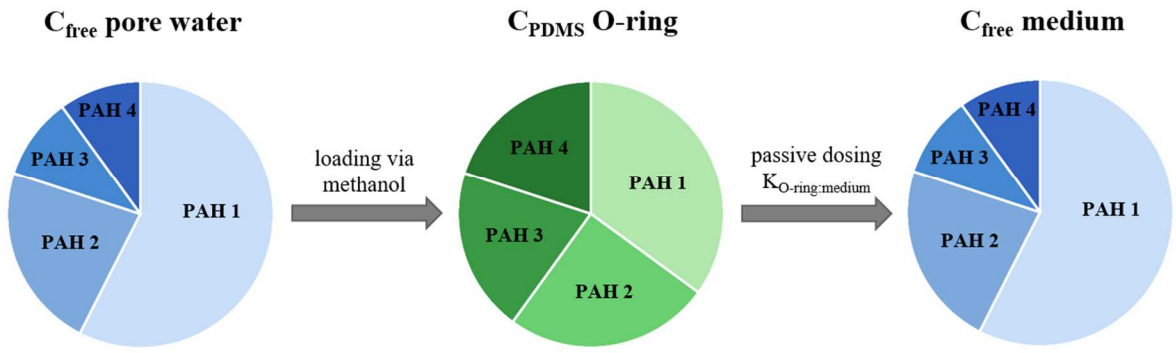

Figure 8: Principle of the recreation of a sediment-pore water concentration composition in bioassays using passive dosing. Figure adapted from Niehus et al. (2018).

Structure of this thesis & Aims

The overarching aim of this thesis was to gain insight into the pollution load and the potential risk of hydrophobic organic chemicals (HOCs) in the sediment of the North Sea and to evaluate these results with regard to possible risks for benthic organisms and the ecosystem (Figure 9). Contributing to an overall risk assessment and management of MSFD is difficult due to the intrinsic properties of HOCs. Wherefore, the assessment requires the application of novel methods. The steps towards a holistic assessment were taken through three studies (Annex 1-3). Three further studies are appended under "additional results" (Annex 4-6) and support the holistic assessment of sediment contamination. The following five aims are covered within these studies:

1. Assessment of the pore water concentrations of PAHs and PCBs
2. Determination of the bioturbation potential by macrofauna analysis
3. Application of the SPME method on biological tissue
4. Assessment of recreated environmental mixtures in passive dosing bioassays
5. Development of SPME method for DDT in sediments

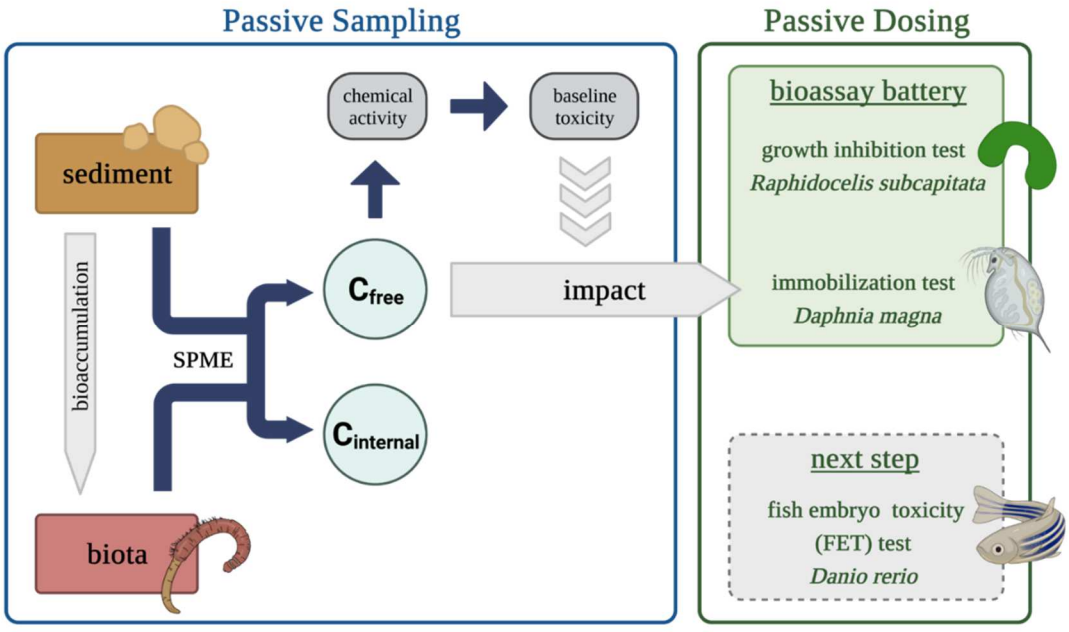

Figure 9: Overview of the main objectives of this thesis. The SPME method was applied to both sediments and biota to determine the pore water concentrations (C_{free}) and the internal tissue concentrations ($C_{internal}$), respectively. The obtained C_{free} mixture compositions were recreated and introduced into bioassays for two trophic levels using the passive dosing method. Illustration was created with BioRender.

The first study (Annex 1) aimed to investigate the pollution load of sediments with PAHs and PCBs in the North Sea. This was done by applying SPME to determine the freely dissolved pore water concentrations and chemical activities allowing to assess the baseline toxic potential

exhibited by the sediment. Additionally, a macrofauna analysis should identify whether there is a correlation between the bioturbation activities of the macrofauna and the C_{free} distribution in the sediment cores.

The aim of the second study (Annex 2) was to apply the SPME method to another environmental compartment in order to assess the pollution in a more comprehensive way. The foundation for the matrix-SPME method development was the determination of the specific partitioning coefficients of HOCs between the tissue of the lugworm *Arenicola marina* and the PDMS sampling phase. Afterwards, the SPME method could be applied to determine the internal tissue concentrations of the lugworm to assess the chemical distribution of HOCs in organisms.

In the third and fourth studies (Annex 3 and 4), the effects of PAHs on organisms of different trophic levels were investigated. The passive dosing method was applied to the growth inhibition test with the green algae *Raphidocelis subcapitata* and the immobilization test with the water flea *Daphnia magna*. For this purpose, the test design had to be optimized for application and practicability in a miniaturized format using well plates and PDMS O-rings were used as passive dosing phase. Individual PAHs were investigated and their corresponding effect concentrations were determined. In addition, the PAH mixture composition of the North Sea sediment was recreated and applied in the bioassays to assess the impacts of the contaminated North Sea sediments on the organism level.

The aim of the fifth study (Annex 5) was to implement the equilibrium passive sampling (EPSM) approach for the organochlorine pesticide DDT and its metabolites given the lack of analytical methods for sediment pore water (C_{free}) contamination in particular. To implement this approach, the polymer to water partition coefficients ($K_{PDMS:water}$) are required for translating polymer measurements into C_{free}. The determined $K_{PDMS:water}$ values were then applied to quantify equilibrium concentrations of DDT and its metabolites in pore water based on the analysis of fibers exposed ex-situ to field sediments.

Lastly, the sixth study (Annex 6) aimed to evaluate the efficiency of sediment remediation for organic contaminants using activated carbon (AC). This was done by applying thin-layer capping of powdered AC (PAC) and granular AC (GAC) on contaminated sediment cores. In addition, the effects of resuspension on contaminant retention and cap integrity were also studied.

The following manuscripts are part of this dissertation:

A1 Kreutzer A, Reininghaus M, Meyer J, Kröncke I, Seiler TB, Hollert H and Witt G: Application of equilibrium passive sampling to assess the influence of anthropogenic activities and bioturbation on the distribution of hydrophobic organic chemicals in North Sea sediment cores.

A2 Kreutzer A, Schacht SC and Witt G (2022):
Equilibrium passive sampling: A novel approach to determine internal tissue concentrations of hydrophobic organic compounds in biota.
Science of the Total Environment 824. 153764.
DOI 10.1016/j.scitotenv.2022.153764.

A3 Kreutzer A, Faetsch S, Heise S, Hollert H and Witt G:
Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to the microalgae *Raphidocelis subcapitata*.

A6 Rämö R, Bonaglia S, Nybom I, Kreutzer A, Witt G, Sobek A and Gunnarsson JS (2022):
Sediment Remediation Using Activated Carbon: Effects of Sorbent Particle Size and Resuspension on Sequestration of Metals and Organic Contaminants.
Environmental Toxicology and Chemistry.
DOI 10.1002/etc.5292.

General Discussion

Summary of the main findings

The aim of this thesis is to demonstrate the potential of new methodological approaches for assessing the pollutant load and the resulting risk in marine sediments. Furthermore, these methods will be critically reflected especially with regard to environmental risk assessment and management strategies. The first study of this thesis (Annex 1) assessed the pore water concentrations of PAHs and PCBs in North Sea Sediment and determined the bioturbation potential by macrofauna analysis. The applied equilibrium passive sampling approach with PDMS coated glass fibers enabled the detection of pore water concentrations down to extremely low concentrations (pg L^{-1}). The analysis of sediment cores showed predominantly decreasing pollutant concentrations in the upper sediment layers. This could indicate that bans and regulations on the investigated substances are having an effect on the total load. However, pollution "hotspots" were also discovered, as two sampling stations were affected by the dumping of dredged material from the port of Hamburg. The determination of site-specific distribution coefficients (K_D) indicated that the sorption strength of PAHs on sediment is stronger compared to PCBs. The macrofauna analysis revealed a decreasing trend in abundance, biomass, and taxa number, as well as bioturbation potential with sediment depth. A correlation between bioturbation activities and pore water concentrations in the sediment could not be established. However, a higher pollution load could have negative impacts on biodiversity and thus on the bioturbation potential.

In the second study (Appendix 2), the passive sampling SPME approach was extended to the environmental compartment biota. This SPME approach opens new possibilities for studying the in-situ distribution and thermodynamics of hydrophobic organic chemicals at trace levels in biota. The required equilibrium partitioning coefficients were determined for the lugworm *Arenicola marina*. Afterwards, the SPME approach was applied to assess the tissue concentrations of HOCs in lugworms from tidal flat sediments of the North Sea. The method application revealed considerably higher body residue values of HOCs in the analyzed lugworm compared to other data for the North Sea area. The high body concentrations are probably site-specific, as the sampling site was in direct proximity to a refinery that processes crude oil and refined fossil fuels. The new SPME method is considerably faster and less error-prone than conventional methods such as exhaustive extraction. In addition, the SPME method can be used to determine the HOC contamination in lugworms quickly, seasonally, and over a wide area. Hence, changes in the chemical load can be detected more quickly and better attributed to local events.

The third study (Annex 3) moved the focus on to the risk that contaminated sediments may pose to organisms. The applied passive dosing approach enabled toxicity testing of (highly) hydrophobic substances at aqueous solubility to assess toxicity at the maximum exposure concentration. The usage of PDMS O-rings allowed for a stable exposure concentration during the test duration. Furthermore, the application of the miniaturized format allowed a higher sample throughput and thus increases the statistical significance of the data. First single PAH substances were examined in the growth inhibition test with *Raphidocelis subcapitata* as representative of the primary producers. Thereafter, the PAH mixture compositions of the sampling stations of Annex 1 were recreated and applied in the bioassay. Some of the single PAHs (Naph, Ace, Phen, Fluo, BaP) exhibited the strongest effects with maximum growth inhibitions of > 97 % at saturation level. The recreated mixture showed strong growth-inhibiting effects even though only a small selection of the mixture was used for exposure. This indicates that the entirety of a native mixture would adversely affect primary producers and may thus have consequences for the entire ecosystem.

The additional results (Annex 4) expanded the investigations on possible risks to organisms caused by sediment-bound PAHs. The passive dosing approach was implemented in the immobilization bioassay with the water flea *Daphnia magna*, representing the trophic level of primary consumers. The miniaturized format in 6-well microtiter plates was easy to perform and resulted in reproducible data. The maximum exposure of individual PAHs exhibited strong effects on the immobilization rate of the daphnids, while the mixtures caused hardly any effects on the swimming behavior of the *Daphnia*. The comparison of the two trophic levels studied showed that the daphnids were less affected by the PAH mixture than the algae (Appendix 3). Nevertheless, it is important to include different trophic levels, as sensitivity to various substances can differ considerably and only a comprehensive battery of assays can provide a conclusive assessment of the impact on the ecosystem.

The additional results (Annex 5) focused on the development of an equilibrium passive sampling method to detect DDT in sediments. This required the polymer to water partition coefficients ($K_{PDMS:water}$) to translate polymer measurements into pore water concentrations. Therefore, the water and PDMS concentrations of DDT and its metabolites were determined using GC-MS. The determination of the DDT partition coefficients proved to be well feasible and the results were in the same order of magnitude as values already described in the literature for similar SPME methods. However, the degradation of DDT was not closely monitored, which could lead to uncertain partition coefficients. The distribution coefficients obtained were

then used to calculate DDT concentrations in North Sea sediments, but the surface sediments' bioavailable concentrations (C_{free}) were below the limit of quantification. In addition to C_{free}, the total sediment concentrations (C_{total}) were determined by applying an exhaustive extraction method. This revealed that higher concentrations of DDT and its metabolites were found in deeper sediment layers. Nevertheless, the success of the restrictions on the insecticide DDT cannot be clearly demonstrated in these sediment depth profiles, as the sediment has not been dated.

The additional results (Annex 6) evaluated the efficiency of sediment remediation for organic contaminants using activated carbon (AC). The capping efficiency of powdered AC (PAC) against granular AC (GAC) was compared using contaminated sediment from Oskarshamn harbor (Sweden). Furthermore, the effects of resuspension on contaminant retention and cap integrity were also examined. The thin-layer cap with PAC reduced sediment-water fluxes more effectively under resuspension than under static conditions. The application of GAC was less effective than PAC but reduced fluxes of high-molecular-weight PAHs. Overall, PAC performed better than GAC, but adverse effects on the benthic community and transport of PAC to non-target areas are drawbacks that favor the use of GAC.

Overall, the studies comprising this thesis can promote the improvement of a holistic assessment of pollution levels in marine ecosystems. The use of passive sampling and passive dosing methods will help to get a better understanding of contamination levels and the resulting risks. Against this background, the next chapter focuses on the relevance of the applied and newly developed methods from a holistic perspective in a critical evaluation and discussion.

SPME – a promising tool for monitoring and risk assessment?

EU-wide agreements and frameworks, such as MSFD, aim to improve and extend the monitoring framework and better link it with the understanding of biological effects and ecological impacts. An adequate exposure assessment can only be performed if the actual risk is known. The risk is posed mainly by the bioavailable fraction, which can have harmful effects on organisms. However, in order to be able to improve and expand the monitoring framework, it is also necessary to adapt the methods applied. In particular, the use of passive sampling methods based on the concept of equilibrium partitioning has gained importance in recent decades to determine bioavailable concentrations in different environmental compartments (sediment, water, air, biota, soil). Solid-phase microextraction (SPME) was developed by Arthur and Pawliszyn (1990) to overcome the limitations of the conventional extraction

methods. The SPME technique takes a leading position among microextraction methods (Merkle et al. 2015).

Detection of the bioavailable fraction of HOCs in sediment

The SPME approach is particularly suitable for hydrophobic organic pollutants, some of which are classified as priority substances by the Stockholm Convention (Stockholm Convention 2008) or the US EPA (US EPA 2014). The determination of PAHs and PCBs in sediments has already been carried out several times using SPME. Among others, Witt et al. (2013) and Niehus et al. (2019) were able to show that the method provides solid results both in situ and ex-situ. The ex-situ application can, for example, determine the vertical distribution of pollutants in different sediment layers. Hence, not only a spatial assessment of the pollutant contamination is obtained but also the historical input can be depicted. In study 1 (Annex 1), sediment cores from the North Sea were analyzed for PAH and PCB contamination using the SPME approach. We could demonstrate that the SPME method can detect freely dissolved pore water concentrations with sufficient accuracy down to extremely low concentrations (pg L^{-1}). The results presented in this thesis revealed that the contamination of the North Sea is relatively low. However, local hot spots in the area of a sediment dumping site with higher contamination levels were detected using this method. Compared to the North Sea surface sediment analysis of Niehus et al. (2019) the PCB concentrations are in the same order of magnitude for the North Sea sediments. In contrast, the sediment from the Wadden Sea area was less contaminated. However, the sum of PAHs was magnitudes higher compared to Niehus et al. (2019). This might indicate that the areas located on an axis towards the Elbe estuary are strongly influenced by suspended Elbe sediment due to the tidal sediment dynamics which is an important source of PAH input in this area. Another source could be the input of combustion-derived PAHs from central Europe and the transport of particle-bound PAHs over long distances. PCBs enter the North Sea through the Atlantic Ocean and the atmosphere, but also rivers, sewers, and sludge have a significant impact on the PCB input. Since the German Bight is an intensively used area in many aspects, the entries of HOCs are mostly diffuse and not attributable to single point sources.

Conventional sampling methods may omit pollutants of unknown toxicological relevance (Brack et al. 2009), but the SPME method can be designed to extract a wide range of contaminants from sediment (Greenberg et al. 2014). In study 5 (Annex 5), the SPME method was adapted for the detection of the priority pollutant class of organochlorine pesticides, in particular dichlorodiphenyltrichloroethane (DDT and its metabolites). Therefore, the

substance-specific partition coefficients between the PDMS of the fibers and the pore water were determined to assess the contamination load of North Sea sediments with DDT. However, in our case, the pore water concentrations of DDT and its metabolites were below the detection limits. The traditional exhaustive extraction method showed that DDT is still present in relatively high concentrations (ng g^{-1} DW) as a burden of the past. These results may lead to the assumption that DDT is bound so firmly to the sediment that it is no longer bioavailable for sediment organisms.

Altogether, the SPME method detects hazardous substances in very low concentrations with good precision. In addition, the pore water concentration (C_{free}) is an essential and universal parameter for the entire ecosystem as many other important parameters can be derived from it describing the diffusive exchange between the different environmental compartments (sediment-water-biota).

C_{free} – a key factor for other ecologically relevant parameters

The freely dissolved pore water concentration (C_{free}) can be used to calculate the chemical activity (a) of individual substances. The chemical activity was proposed as an exposure parameter for HOCs for two reasons: (i) It determines the partitioning into organisms and target membranes and (ii) baseline toxicity has been observed within a rather narrow chemical activity range of 0.01 to 0.1 (Mayer et al. 2014). The baseline toxicity is also of importance when assessing the risk of complex environmental mixtures. Even if the chemical activity of each compound in a mixture is below a toxicity threshold, the cumulative activities can cause an overall toxic effect (Escher et al. 2002). This, in turn, is important for the assessment of the hazard potential. In study 1 (Annex 1) the measured chemical activities of the North Sea stations were compared to the range corresponding to baseline toxicity. We concluded that, despite some elevated total PAH and PCB concentrations in these sediments, the bioavailability was limited because the estimated $\sum a$ was well below baseline toxicity levels. Furthermore, the higher molecular weight PAHs and PCBs were found to have a low contribution to C_{free} but contributed even more to the baseline toxicity. One reason for this may be that the baseline toxicity is not only driven by the concentration but also by the individual chemical properties of each analyte (e.g., water solubility, octanol-water distribution, vapor pressure, structure). However, only 16 PAHs and 7 PCBs were assessed in our study. Taking all PAHs, PCBs, and other chemical compounds together in a holistic screening, they might exhibit a potential 10 to 100 times higher than the well-known priority pollutants, which will lead to permanent harm to sediment organisms.

The site-specific distribution coefficient (K_D) of a substance between sediment and pore water can also be derived from C_{free}, indicating the sorption strength of the sediment. In general, the sorption of HOCs increases with increasing hydrophobicity of the analyte and the TOC content of the sediment (Chen & White 2004). The calculation of K_D of PAHs and PCBs in the North Sea sediments showed that, despite the higher log K_{OW} values of PCBs, they were more available than PAHs (Annex 1). This is in good agreement with the results of Bucheli and Gustafsson (2003) as well as Koelmans et al. (2006) who showed that the class of rigid planar PAH compounds has a higher affinity for soot than PCBs. These results do not only allow to draw conclusions on the sorption strength, and thus the availability of the sediment, but also on its content (e.g., soot, soot-like particles). Overall, this suggests that sediment composition is an essential parameter for the risk assessment of this compartment, as sediment with high soot content poses a lower risk than sediment with a low soot content. Consequently, a better understanding of contaminant transport processes from sediment to water is essential for risk assessment (Hedman et al. 2009). This knowledge will then help to assess and control whether sediment is a sink or a source of pollutants.

SPME application for biological samples

Sample preparation is one crucial step in the analysis of complex matrices such as biological samples. Tissue, plasma, as well as whole blood samples are complex mixtures and contain interfering components such as different ions, proteins, cells, and other compounds. The goal of sample preparation is to eliminate the interference of compounds with the sample matrices (Jalili et al. 2020). Conventional methods for preparing biological samples, such as liquid-liquid extraction (LLE), are lengthy, involve multiple steps, and require clean-up prior to analysis. Studies applying SPME in biological tissue and referred to as in-tissue passive sampling or lipid-rich matrix-SPME have been in the focus of research for the last few years but are still rare to date.

The SPME method can not only be quickly adapted to other substances but can also be applied to other matrices. The adaption is again based on the specific partition coefficients between the respective substance and the matrix to be determined. In study 2 (Annex 2) the partition coefficients for PAHs and PCBs were determined for the lugworm Arenicola marina to investigate the body burden of this sediment living species. The lugworm was chosen as a test organism because it is continuously exposed to contaminants in the sediment and is a quantitatively important deposit feeder at the base of the North Sea food web (Besseling et al. 2013). Furthermore, the lugworm has been recommended by Oslo-Paris Commission (1995) as

a monitoring organism. The worms are better suited as a bioindicator compared to mussels (Kaag et al. 1998) because they hardly absorb HOCs via gut fluid extraction due to the short digestive tract but mainly via the skin surface. This is why they are very similar to the silicone fibers used in this study. Kraaij et al. (2003) showed that the distribution between deposit-feeders and pore water is in agreement with equilibrium partitioning.

Our results have verified that SPME is a very sensitive tool that opens new possibilities for studying the in-situ distribution and thermodynamics of hydrophobic organic chemicals at trace levels in biota (Annex 2). Furthermore, the SPME fiber method is an adequate substitute for exhaustive extraction and one to prefer, since the SPME fibers can be injected directly into the tissue without the need for sample pre-treatment. Moreover, the HOC contamination in lugworms can be determined quickly, seasonally, and covering a wide area. This also allows sudden changes in chemical contamination to be detected more quickly and better attributed to local events.

The essential environmental compartments (sediment, water, and biota) can be covered by applying the SPME method which facilitates a direct comparison and provides a good overview of the fluxes within the system. In addition, future research should include assessments on different levels of biological organization in order to determine the risks organic contaminants may pose to an entire ecosystem. Thereby, biological monitoring will be more effective and precise and measures can be much better adapted to the prevailing situation.

Sediment remediation

Cleanup techniques for HOC contaminated sediments have been widely investigated in the last several decades (Maletić et al. 2019). In addition, the fate of the HOCs is determined by their physicochemical properties, which implies that they will be associated with fine-grained, organic-rich sedimentary material, making sediment an ultimate sink for these pollutants. However, contaminated sediments can also become secondary sources of pollution. Due to changed conditions in the aquatic system (flooding, acidification) sorbed pollutants are desorbed and return to the aqueous phase to pose a threat to aquatic organisms again (Eggleton & Thomas 2004). Hence, these sediments pose a challenge for remediation and management, as conventional dredging techniques are invasive, expensive, and sometimes ineffective or difficult to apply to large-scale sediment sites. Remediation of sediment through amendment with a strong sorbent such as activated carbon (AC) has proven an effective in-situ method in reducing the concentrations of sediment-bound contaminants (Ghosh et al. 2011, Josefsson et

al. 2012). In addition, the AC amendment represents a promising approach as it is less intrusive and a more cost-effective treatment (Ghosh et al. 2011).

The concentration of contaminants in pore water is one of the key factors known to influence the fate and movement of contaminants in or out of the sediment, as well as accumulation by organisms (Greenberg et al. 2014). Passive sampling devices are used to estimate freely dissolved contaminant concentrations (C_{free}) (Booij et al. 2016) or sediment-water fluxes often in a sediment remediation context (Allan et al. 2021). Therefore, the use of SPME techniques has great potential for monitoring the progress of remediation. The reduction of C_{free} assessed with passive samplers is already used as an acceptable endpoint for evaluating the efficiency of remediation strategies. Amendment of sediments with strongly sorbing materials, such as activated carbon (AC), has proven to be very efficient in reducing C_{free} of hydrophobic organic pollutants in sediments (Ghosh et al. 2011). In order to investigate possible differences between two AC types, we compared the capping efficiency of powdered AC (PAC) against granular AC (GAC) using contaminated sediment from Oskarshamn harbor (Sweden) (Annex 6). In addition, we investigated the effects of resuspension on AC thin-layer capping under controlled laboratory conditions. We could show that thin-layer capping with GAC was less efficient in reducing contaminant fluxes but offers an alternative to PAC in turbulent waters as it is less prone to resuspension. Furthermore, the benthic fauna was not affected by the application of GAC in contrast to the treatment with PAC, which reduced abundance. These promising results show that it is important to standardize and commercialize SPME methods for risk assessment and monitoring of contaminated sediment and incorporate these tests into legislation and laws (Maletić et al. 2019).

Implications for environmental management strategies

Until now, passive sampling methods have been used mainly to support research activities but have not found wide acceptance by the regulatory community for decision making (Mayer et al. 2014). However, the European Chemicals Agency (ECHA) published a report in which they recommended replacing direct sediment analysis with passive sampling devices (PSDs) (ECHA 2014). At the same time, the wide range of polymers available poses a major challenge for implementation in monitoring programs, as standardization of PSDs is being requested. Nevertheless, passive sampling methods allow the collection of many parameters that are essential for a comprehensive risk assessment.

The importance of sample preparation of environmental samples arises from the complexity and heterogeneity of the components. It is important to isolate and concentrate the analytes of

interest to achieve optimal sensitivity and selectivity, as opposed to interfering matrix components. Hence, the sampling and sample preparation is often the bottleneck of the analytical process and there is a need to minimize the number of steps to reduce both time and sources of error (Ridgway et al. 2007). Recent trends emphasize the move towards more environmentally friendly and green techniques which use smaller sample sizes and less or no solvents (Aulakh et al. 2005, Bojko et al. 2012).

The SPME technique is fast, sensitive, and cost-effective compared to traditional methods. Furthermore, it is an environmentally friendly method as the use of solvents is entirely unnecessary (Merkle et al. 2015). The samples do not have to be cleaned and only small sample quantities are needed (Hawthorne et al. 2009). The use of internal standards and reference substances can also be omitted. Coated fibers are ideally suited for on-site implementation because of their small size (Bojko et al. 2012). They are convenient to transport and deploy on-site. Low molecular weight or volatile compounds usually require a 100 µm PDMS coated fiber, whereas larger molecular weight or semi-volatile compounds can be extracted more effectively with 30 µm or 7 µm PDMS fibers (Aulakh et al. 2005). The technique with PDMS-coated fibers is a "soft" extraction technique in which only a small part of an analyte is removed from the investigated system, thus not disturbing the system and allowing repeatable extractions (Bojko et al. 2012).

These advantages offer the possibility to implement SPME for extensive sampling campaigns. Sampling can be done in larger numbers and on a smaller scale as large equipment for sampling is not required on-site. Moreover, the simplicity of the method enables sampling at remote places, such as Antarctica and other polar regions. The ex-situ performance of the SPME would already be possible on board the research vessel, as the fibers can not only be introduced directly into the matrix of interest for extraction on board but also be measured directly. Furthermore, the application of SPME is also suitable for developing countries where scientific progress is still difficult to accomplish. The method can be implemented locally with the simplest of resources, and the frozen fibers can be easily transported if no suitable analytical equipment is available locally. However, the final step in the implementation of SPME for field applications would be the full performance of the analysis process in the field using portable equipment (Bojko et al. 2012). By reducing errors associated with sample transport and possible changes during storage, a more accurate characterization of the system under investigation can be achieved (Ouyang & Pawliszyn 2006). The automation of the process would lead to further advantages such as lower time consumption, increased simplicity, lower probability of sample

contamination, and higher repeatability (Merkle et al. 2015). All these advantages would lead to better-adapted measures to the prevailing situation, as regional conditions can be better addressed. In addition, the more detailed data would also provide the opportunity to respond immediately to a source of contamination. For instance, timely counteractive measures could be initiated, developed, and evaluated.

Overall, the SPME method can considerably improve and expand the monitoring framework for the MSFD and other monitoring programs. It also offers the possibility to monitor all environmental compartments quickly and cost-effectively, generating a wide range of high-throughput data, which in turn would reduce uncertainties for modeling and extrapolation. In addition, the data can be used, with appropriate statistical analysis, to estimate the probability distribution of the chemical in space and time. These distributions can then be used to select suitable sites for subsequent sampling programs and risk assessment.

Moreover, the bioaccumulation of pollutants in fish tissue is of growing concern because of its potential impact on aquatic and human lives (Bojko et al. 2012). The present analytical methods, however, involve sacrificing the animal before the determination of the contaminants can be achieved. Consequently, a large number of fish was used to determine the concentration of the tissue leading to high variability between animals. The developed SPME methods coupled with LC-MS for in-vivo applications in living organisms can address these limitations, providing a non-lethal alternative to conventional methods. In addition, repeated sampling of the same animal is possible, which eliminates inter-animal variability. SPME also allows the detection of trace amounts of short-lived substances during biotransformation, thus, providing insight into degradation pathways (Merkle et al. 2015). Based on these findings, the identification and classification of PBTs (persistent, bioaccumulative, and toxic) could also be improved in regulatory settings, especially since the bioaccumulation study (OECD 305) is of increasing importance in regulatory chemical safety assessments.

Passive dosing – a promising tool for exposure assessment?

The exposure of organisms to HOCs in complex and heterogeneous media such as sediments is still difficult to characterize (Mayer & Holmstrup 2008). The best established and most promising approach is the equilibrium partitioning theory, where the partitioning is controlled by the chemical activity of the contaminant and can be described by distribution coefficients. The SPME method opens up the possibility of determining the concentration of freely dissolved contaminants (Birch et al. 2010), which represents the main risk of harmful effects on

organisms. However, the challenge of frameworks such as the MSFD is linking the identified contamination with an understanding of biological effects and ecological impacts.

Knowledge of the chemical concentration alone is not sufficient for predicting toxicity. Therefore, bioassays are performed with organisms from different tropical levels, as the use of a single species can be reductive for a correct evaluation of the toxicity level concerning the complexity of the ecosystem (Narracci et al. 2009). Aquatic testing of HOCs is still associated with scientific, conceptual, and technical challenges. If the concentration is set at the beginning of the assay, as is done by nominal dosing or the spiking with co-solvent, the results of the tests are often biased by poorly defined and declining dissolved exposure concentrations. This can be due to the limited water solubility and various loss processes, including volatilization, biodegradation, sorption to the plastic material of the microtiter plates, or uptake and metabolism by the test organisms (Mayer et al. 1999, Smith et al. 2010a, Smith et al. 2010b). Niehus et al. (2018) were able to show that spiking led to significantly lower or no effects compared to passive dosing, resulting in a striking underestimation of the risk of the tested chemical.

The exposure concentrations need to be continuously maintained throughout the test duration to overcome these challenges. This can be performed by regularly re-supplying the test substance through a renewal of the spiked medium (Seiler et al. 2014). Nevertheless, even with this approach, the uncertainties regarding the actual exposure concentration cannot be completely eliminated. The performance of a flow-through system is not available for all bioassays and is comparatively expensive in terms of equipment, work effort, and amounts of chemicals required. Most importantly, such systems are complicated to apply at a small scale, such as microtiter plates, which are essential for cell tests or assays with small organisms (Lammer et al. 2009). Hence, the passive dosing approach is promising for attaining stable and well-defined exposure concentrations during in-vitro bioassays (Smith et al. 2010b). For this purpose, the test chemical is loaded onto a polymer, which is then introduced into the bioassay (Smith & Jeong 2021). The polymer functions as a constant equilibrium partitioning source and test losses are compensated.

Bioassays are usually performed in well plates, but many passive dosing assays were performed in glass vials which indeed reduces the losses due to sorption compared to plastic. However, it is challenging to maintain a high throughput of tests when they are performed in glass vials, as incubation and measurement of individual glass vials require a great effort (Smith et al. 2010b, Stibany et al. 2017b). The most straightforward approach is to adapt the passive dosing device

to the test vessel and not vice versa (Niehus et al. 2018). For this reason, it is therefore very easy to adapt the passive dosing approach to all kinds of bioassays. The PMDS O-rings, which were used in this study (Annex 3 and Annex 4), can be applied for different bioassays carried out in well plates. They are made out of food-grade silicone available in various standardized sizes, and are easy to introduce and remove from the well (Smith et al. 2010b). Moreover, the O-rings enable a plate reader measurement during exposure. These O-rings can be loaded in large quantities and the storage in the freezer makes it possible to build up a stock for a flexible test start.

The PDMS O-rings can be used for testing single HOCs, but beyond that, passive dosing plays an important role in toxicity testing of HOC mixtures (Smith et al. 2010b). The exposure to a mixture of chemicals allows for a more realistic testing approach, as organisms in the environment are exposed to mixtures. Moreover, the measured HOC composition of a specific location can be tested by recreating the mixture on the dosing phase and introducing it into the bioassay. Another advantage of the passive dosing approach is that the test compound concentration in the O-rings can be varied to apply a wide range of freely dissolved concentrations. Hence, passive dosing can be flexibly used to test HOC aqueous solubility, concentration-response testing, or chemical mixture toxicity (Smith et al. 2010a).

Within the scope of this work, two bioassays were transferred into a miniaturized form applying passive dosing thus increasing the sample throughput of these tests (Annex 3 and Annex 4). Individual PAHs and realistic PAH mixtures were tested using the growth inhibition test with the green algae *R. subcapitata* and the immobilization test with the water flea *D. magna*. We could demonstrate that the toxicity of HOCs can be determined using the passive dosing approach. Performing the miniaturized format in microtiter well plates facilitated a higher sample throughput, which also increased the statistical power of the data obtained. Comparing the two trophic levels showed that the algae were more sensitive to the exposure of the recreated mixtures of the North Sea sediments than the daphnia. Therefore, it is even more important to consider several trophic levels and different endpoints (acute and chronic) for a holistic risk assessment.

Nevertheless, the O-rings used in this study have a spectrum of applications for non-polar hydrophobic compounds (Smith et al. 2010b). Hence, new polymers need to be developed that combine both the high affinity and diffusion properties for non-polar substances as well as a higher affinity for polar substances, which will extend the range that can be tested. Further optimization of the polymer architecture will also lead to faster release and allow testing of

volatile chemicals. Thus, the principle of passive dosing could be extended to other research areas, such as biodegradation studies. There are plenty of silicone devices in various shapes and sizes that would be suitable for most test designs.

However, the use of passive dosing in routine toxicity testing remains limited. The most likely reason for this is that it is a lengthy process to define standards and regulations for the regular application of passive dosing. Some scientists have already set an important basis for implementing passive dosing in bioassays by providing a comprehensive set of partition coefficients for different silicone types (Gilbert 2015, Smith et al. 2010a) and biota (Jahnke et al. 2014). Overall, the combination of passive dosing and SPME is a promising approach to link the identified contamination with an understanding of biological effects and the resulting ecological impacts.

Conclusion

Protecting benthic ecosystems from the consequences of increasing human impacts requires a detailed scientific assessment in order to apply appropriate measures. Given recent developments in the legislative framework for environmental monitoring risk assessment, there is an increasing demand for methods to determine the bioavailable fraction of pollutants, as this fraction is the main source of risk for organisms. The present thesis demonstrates the potential novel techniques by assessing both the contamination level and the resulting risk executed by sediment-associated HOCs.

In passive sampling, the complete bioavailable dissolved profile is captured in relation to the equilibrium partitioning ratios of the mixture constituents. On the other hand, passive dosing translates this mixture into the original dissolved levels via the same partitioning ratios and keeping them constant. These dissolved mixture profiles can then be reproduced in different toxicity bioassays that represent different trophic levels and endpoints.

The SPME technique has been shown to be a powerful method for tracking the content of HOCs in sediment and biota. It is expected that this technique will become a green analytical technique for a variety of different applications in the future due to the flexibility of its design. In order to evaluate good environmental status according to the MSFD, we recommended including sediment pore water concentrations as an indicator in MSFD monitoring programs. In addition, the adaptation of bioassays to passive dosing might offer the potential to optimize ecotoxicological testing of highly hydrophobic and poorly soluble substances towards a high level of reliability. The combination of SPME and passive dosing provides clear insights

compared to conventional methods for assessing pollutant exposure and should consequently be included in environmental risk assessment.

Future research will not only focus on validating and further improving the introduced methods for passive sampling and passive dosing but will also introduce novel sampling devices with a broader spectrum of applications. In order to be implemented in environmental monitoring and risk assessment, it will be crucial to seek consensus among science, management, and practitioners on the appropriate use of these techniques to support the management of contaminated sediments. Ultimately, this approach will foster consistent data sets, which could eventually be accepted as standard methods (e.g., by international organizations such as the OECD).

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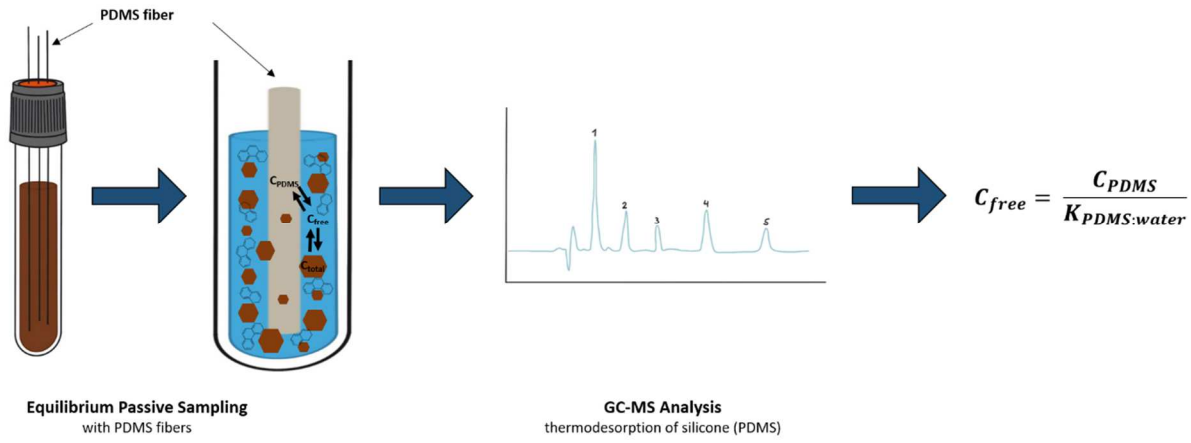
Appendix

Annex 1

Application of equilibrium passive sampling to assess the influence of anthropogenic activities and bioturbation on the distribution of hydrophobic organic chemicals in North Sea sediment cores



Application of equilibrium passive sampling to assess the influence of anthropogenic activities and bioturbation on the distribution of hydrophobic organic chemicals in North Sea sediment cores

Anne Kreutzer[a,b], Mathias Reininghaus[b], Julia Meyer[c], Ingrid Kröncke[c], Thomas-Benjamin Seiler[d], Henner Hollert[a] and Gesine Witt[b]

[a] Department Evolutionary Ecology and Environmental Toxicology, Institute of Ecology, Evolution and Diversity, Faculty Biological Sciences, Goethe University Frankfurt, Frankfurt 60438, Germany

[b] Department of Environmental Engineering, Faculty Life Sciences, Hamburg University of Applied Sciences, Hamburg 21033, Germany

[c] Senckenberg am Meer, Marine Research, Südstrand 40, 26382 Wilhelmshaven, Germany

[d] Ruhr District Institute of Hygiene, Rotthauser Straße 21, 45879, Gelsenkirchen, Germany

Abstract

The pollution state in the German Bight was investigated by determination of pollutant concentrations of sediment samples using equilibrium passive sampling. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were determined in the sediment pore water from four stations in the North Sea. The freely dissolved pore water concentration (C_{free}) was measured applying ex-situ Solid Phase Microextraction (SPME) by using PDMS-coated glass fibers followed by GC-MS analysis. The obtained results show that the contamination level of the North Sea is rather low. However, the stations close to the sediment-dumping site were higher contaminated. A macrofauna analysis showed that bioturbation activities were mostly present in the upper sediment layers, but a direct correlation between bioturbation and sediment concentration could not be shown. Overall, the contamination load was below baseline toxicity, but considering that several other priority pollutants will also contribute to the baseline toxicity, this can be considered as relatively high.

Introduction

The intensive use of the North Sea area through offshore activities, sand extraction and dredged material placement leads to increasing pollution of the ecosystem by chemicals such as hydrophobic organic contaminants (HOCs). Due to strong association of these HOCs to settling organic matter sediments act as sinks (Mustajärvi et al. 2017), which can be used to determine the current environmental status and for trend analysis. The rivers Rhine, Elbe and Weser are the main sources of contaminants in the Wadden Sea. Laane et al. (2013) reported that the input and concentration of most contaminants have significantly decreased in water, sediments, and organisms over the last three decades. However, sediment-borne HOCs remain a concern for ecosystems and human health due to their persistence, bioaccumulation potential and toxicity (Weber et al. 2008). In the North Sea, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are among the most common organic pollutants of concern, where a substantial part is retained in the sediments (Cui et al. 2013). These toxic and persistent pollutants are of special ecotoxicological concern as they are widely resistant to biodegradation and can accumulate in fatty tissues of organisms (OSPAR Commission 2009). Following the reduced emissions of several HOCs, e.g., polychlorinated biphenyls (PCBs), historically contaminated sediments are now recognized as a significant secondary contaminant source in many ecosystems (Gunnarsson et al. 1999, Josefsson et al. 2010, Mustajärvi et al. 2017).

The EU Marine Strategy Framework Directive (MSFD; 2008/56/EC) aims to protect the marine environment across Europe to achieve Good Environmental Status (GES) of the EU's marine waters by 2020. In May 2020, the strategy was adopted to the new EU Biodiversity Strategy for 2030 aiming to strengthen the protection of marine ecosystems and to restore them to achieve GES. It is therefore essential to assess the pollution load and investigate long-term changes in the environment so that pollutant concentrations are at a level that does not cause acute or chronic effects on the ecosystem. Monitoring programs determine residues in the abiotic environment as well as residues in selected organisms, which will provide an indication of the bioavailability of contaminants (Kaag et al. 1998, Wang et al. 2018). The bioavailability of a chemical is a key factor responsible for ecotoxicological effects of contaminants and important for risk assessment approaches since the total concentration of contaminants does not take into account that molecules might be sorbed, trapped or even bound to sediment particles and thus are less mobile than if dissolved in the pore water. The mobility of HOCs in sediments, and thus the bioavailability, depends on the pore water concentrations (Witt et al. 2010). Currently, the measurement of the freely dissolved pore water concentration (C_{free}) of moderately polar to nonpolar substances using equilibrium passive sampling methods (EPSMs) offers a promising alternative to the measurement of the total concentration (C_{total}) and is becoming established as an important endpoint for sediment quality and risk assessment (Mayer et al. 2014). Furthermore, the chemical activity (a), which is the contaminant's potential for spontaneous physicochemical processes, controls the bioaccumulation in benthic organisms (Kraaij et al. 2003) and the baseline toxicity (Reichenberg & Mayer 2006).

Macrofauna species are found in the sediment's upper layers and at the sediment-water interface, representing an essential element of the benthic-pelagic coupling (Griffiths et al. 2017). These species serve as a food source for larger benthic species, while they feed on smaller organisms, bacteria, phytoplankton and feces from all trophic levels (Meyer & Kröncke 2019). The mixing of sediment and particulate materials carried out during foraging, feeding and burrow maintenance activities of macrofauna species is defined as bioturbation (Queirós et al. 2013, Reible et al. 1996). This reworking and irrigation process of the sediment by benthic organisms can significantly influence the physicochemical properties of the sediment and pore water and influence the contaminants' fate in the benthic environment (Kristensen et al. 2012).

Bioturbation can effectively incorporate contaminants into the sediment, leading to increased retention of pollutants in the sediment and reduced bioavailability (Hedman et al. 2008). However, bioturbation can also lead to increased mobilization of sediment-associated

contaminants (Sun et al. 2018). The contaminants' fate in sediment has been attributed to the organisms' bioturbation mode, i.e., their feeding and burrowing strategies and intensity (Hedman et al. 2008, Josefsson et al. 2010). Depending on the organism's bioturbation mode, species that actively move particles within the sediment primarily relocate particle-associated contaminants by bringing them to the sediment-water interface, where they may desorb to the overlying water. Other organisms create and continuously irrigate burrows with oxygenated water, which increases the exchange of solutes and dissolved contaminants between the pore water and the overlying water (Hedman et al. 2009). Josefsson et al. (2010) showed that the highest remobilization of HOCs occurred from the most shallow sediment layers down to at least 10 cm due to bioturbation activity. One of the major factors limiting benthic communities and their bioturbation intensity is the food availability and the organic carbon (OC) intake (Hedman et al. 2008, Schückel et al. 2013). However, the highly productive shallow coastal shelf seas are affected by ongoing high levels of bottom trawl fishing, dredging and dumping, oil and gas extractions, as well as the discharge of the rivers (OSPAR Commission 2000). At the same time, anthropogenic pressure changes the hydroclimatic environment, which has affected the marine environment in return. Consequently, all considered pressures lead to seabed degradation, increasing water turbidity, nutrient enrichment, and decreasing riverine nutrient input, which is resulting in lower phytoplankton primary production leading to decreased food availability (Meyer et al. 2019).

In the present study, the freely dissolved pore water concentrations (C_{free}) for PAHs and PCBs were measured in North Sea sediment cores. The determination of C_{free} was performed using solid-phase microextraction (SPME) with PDMS coated glass fibers and analyzed via thermal desorption and GC-MS (Witt et al. 2013). Furthermore, the depth distribution of macrofauna was determined as well as their community bioturbation potential (BPc) within the sediment, and the species were categorized into functional groups following their biological traits. The objective was to determine (i) the C_{free} gradients of PAHs and PCBs in sediment cores by SPME and (ii) to calculate the chemical activities (iii) to assess the baseline toxic potential. The investigation of the (iv) site-specific distribution ratios (K_D) revealed the direction of the diffusive mass transfer between the sediment and the pore water. In addition, the (v) source of the PAHs in the North Sea sediment samples was identified. Furthermore, the macrofauna analysis should identify whether (vi) there is a correlation between the bioturbation activities of the macrofauna and the C_{free} distribution in the sediment cores.

Material and Methods

Study area

The North Sea has some of the busiest shipping lanes in the world, which, together with the impact of fisheries and hazardous substances (especially persistent organic pollutants), are highly important pressures for the North Sea region (OSPAR Commission 2010). The German Bight is the southeastern bight of the North Sea bounded by Denmark to the east and the Netherlands to the south and covers an area of around 40.459 km², of which 8.000 km² is the Wadden Sea. The Wadden Sea is one of the largest tidal flats in the world. It is divided into several tidal basins, each connected to the North Sea by a tidal inlet. One of the largest basins is the Jade system near the city of Wilhelmshaven.

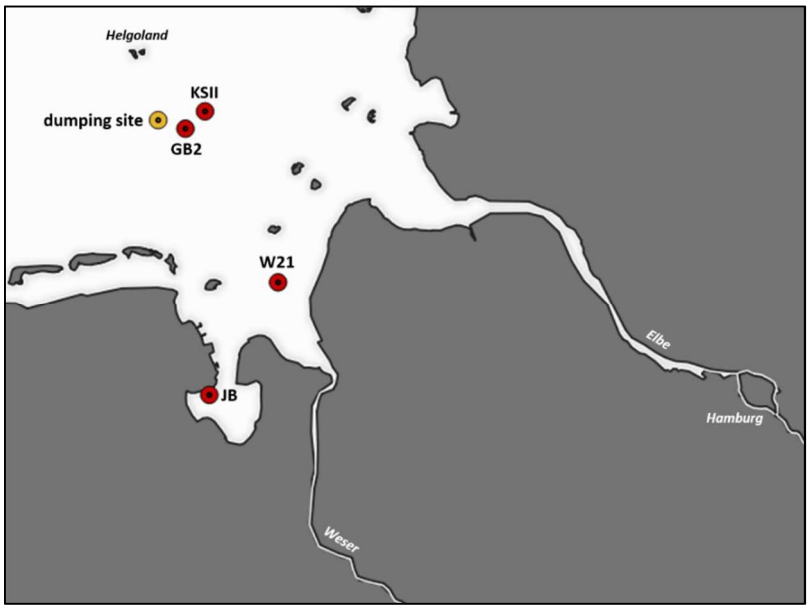


Figure 1: Sediment and macrofauna sampling stations in the North Sea (GB2 and KSII) and Wadden Sea (JB and W21) and the Elbe sediment-dumping site.

Sediment sampling

Sediment samples were collected at four locations across the German Bight in May 2018 on a cruise with the research vessel Senckenberg. Two sampling stations were located in the North Sea (GB2 and KSII), while the other two stations were located in the Wadden Sea (JB and W21) (see Table S1). Water depth ranged from 8 to 24 m and increased from south to north. The sediment cores were collected with a sediment corer (12.5 cm in diameter) from the area outlined by the sampling stations (Figure 1). There is an active sediment-dumping site near the two North Sea stations where sediment from the port of Hamburg is discharged. For the chemical analysis, sediment cores were cut into 2 cm thick slices and directly stored at -20 °C in pre-cleaned aluminum boxes until analysis. For the macrofauna analysis, sediment cores

were sliced into 5 cm layers. Afterwards, the sediment layers were washed in a sieve (1 mm mesh size) and the benthic organisms were fixed in 4 % buffered formaldehyde (buffer hexamethylenetetramine). Taxa were determined up to species level, counted, and weighed.

Total organic content and sediment analysis

The total organic content of the North Sea and Wadden Sea sediment was determined according to Leipe et al. (2017). Briefly, the total carbon (TC) was measured directly on dry samples using a Euro EA (HEKAtech GmbH, Germany) and the total inorganic carbon (TIC) was determined directly by H_3PO_4 removal of carbonates with a Multi EA 4000 (IR method; Analytic Jena). Then the total organic carbon (TOC) was calculated as TOC = TC – TIC.

The sediment analysis was performed at the institute Senckenberg am Meer (Wilhelmshaven). Briefly, the raw samples were desalted and the sand fraction was separated from the mud fraction using a sieve (0.063 µm). The dried sand was weighed and the gravel and mud content (> 2 mm) was determined by sieving. The SediGraph (SediGraph III 5120; Micromeritics Instrument Corporation, USA) was then used to determine the grain sizes < 63 µm. The analysis is performed by means of an X-ray beam and it detects the size of the particles according to their sedimentation rate (Webb 2004).

Equilibrium sampling with SPME

Freely dissolved pore water concentrations (C_{free}) of seven PCBs (PCB 28, 52, 101, 118, 138, 153, 180) and 16 PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[cd-1,2,3]pyrene, dibenzo[a]anthracene and benzo[ghi]perylene) were determined in sediment samples using a solid-phase microextraction (SPME) according to Witt et al. (2013). The experiments were conducted with commercially available glass fibers with a nominal PDMS coating of 10 µm (Fiberguide Industries, USA). The slightly deviating fiber properties identified by Witt et al. (2013) were used for calculations. The fibers were pre-cleaned by extracting three times with ethyl acetate using the ASE®350 (Thermo Scientific™ Dionex™, USA) at 50 bar and 130 °C. Solvent residues were removed by washing three times for 15 min with ultrapure water in an ultrasonic bath. The cleaned fibers were stored in ultrapure water at 4 °C until their application. For SPME, 7-10 g of the thawed and homogenized sediments were weighed into chromacol vials (Thermo Scientific, USA) and three fibers (10 cm in length) were placed in each vial through a cannula to pierce through the septum of the cap. The vials were shaken on an overhead shaker for 14 days at 20 ± 2 °C in darkness until equilibrium between sediment pore water and fibers was reached (Lang et al.

2015). After 14 days, the equilibrated fibers were removed, cleaned with ultrapure water and lint-free tissue, wrapped in pre-cleaned aluminum foil sheets (8 h at 300 °C) and stored at -20 °C until analysis.

Fiber analysis via thermal desorption in GC-MS

For the PAH and PCB measurements in the fibers, a GC-MS method from Lang et al. (2015) was used. Briefly, a gas chromatograph (GC 7890A) and a quadrupole mass spectrometer (MS 5975C; Agilent, USA) equipped with an automated liner exchange system (ALEX; Gerstel, Germany) was used. The empty liners were washed with hexane three times and cleaned at 250 °C for 19 minutes under helium flow in the GC injector to remove organic residues. Small glass beads (ø 0.75-1.0 mm; Carl Roth, Germany) were placed right above the notch in the glass liner (KAS4; Gerstel, Germany) to ensure the fiber stayed in position during thermal desorption. The fiber's exact length was measured for each equilibrated fiber and then placed in a cleaned glass liner. The loaded liner was transferred by MultiPurpose Sampler (MPS 2XL-Twister; Gerstel, Germany) into the cooled injection system (CIS) for thermal desorption (20 °C raised to 250 °C at 12 °C s^{-1}, then held for 15 minutes). The desorbed analytes were transferred in splitless mode onto the column (HP-5MS, 325 °C: 30 m x 250 µm x 0.25 µm; J&W Scientific, USA) and the GC program started (split vent set at 50 mL min^{-1} with MS transfer line temperature held at 310 °C). The native compounds were measured in selected ion mode (SIM). External standard calibrations of PAHs and PCBs (PAH Mix 9, PCB Mix 3; Dr. Ehrenstorfer, Germany) were used to quantify target analytes desorbed from the fibers. MassHunter software for quantitative analysis (version B.07.00; Agilent, USA) was used for peak evaluation.

Freely dissolved concentration (C_{free}) and chemical activity (a)

The concentration in the PDMS fiber coating (C_{PDMS}) was calculated as a ratio of the total mass (M_{PDMS}) of each analyte sorbed to the fiber and the coating volume (V_{PDMS}) of the fiber (Witt et al. 2013). Freely dissolved concentrations (C_{free}) of PAHs and PCBs were determined from the fiber coating concentrations (C_{PDMS}) and the compound-specific PDMS-to-water partitioning coefficients (K_{PDMS}), derived from Witt et al. (2009):

$$C_{free} = \frac{C_{PDMS}}{K_{PDMS:water}} \quad (1)$$

The chemical activities (a) are proportional to C_{free} and were calculated as follows, using the subcooled liquid solubility (S_L):

$$a = \frac{C_{free}}{S_L} \quad (2)$$

The used values are provided in the supporting information (Text S1, Table S2).

Total sediment concentration (C_{total})

The sediments were freeze-dried using a Christ freeze dryer (Alpha 1-4 LD plus). The total sediment extraction was performed according to Witt et al. (2009) with some modifications. Briefly, 2-4 g dried sediment was extracted using the ASE®350. The samples were introduced in the Dionex cell spiked with 100 ng of internal standards for all 16 EPA PAHs (PAH Mix 9 deuterated; Dr. Ehrenstorfer, Germany) and 7 PCBs (Mass-Labelled PCB Mixture MBP-D7; Wellington Laboratories, Canada) and extracted with 80 mL of acetone-hexane (v/v = 40/60) at 140 bar and 100 °C. Elemental sulfur was removed using activated copper, followed by solid-phase extraction with Al_2O_3 and silica in a two-stage Baker Bond system (J.T. Baker, USA) and a reduction of the extract volumes to 800 µL using a rotary evaporator (Heidolph, Laborata 4000 eff.).

For analysis, an aliquot sample of 1 µL was injected in a GC-MS (GC 7890A/MS 5975C; Agilent, USA) and the temperature was increased from 50 °C to 250 °C at 12 °C s^{-1}, then held for 15 minutes. The desorbed analytes were transferred splitless onto the column as the GC program started (split vent set at 50 mL min^{-1} with MS transfer line temperature held at 310 °C). Target analytes were the same as for SPME analysis. The recovery rates of internal standards ranged from 55 to 267 % for PAHs and from 28 to 162 % for PCBs in all extracts.

Site-specific partitioning coefficients (K_D)

The calculation of the site-specific partitioning coefficient (K_D) enables the assessment of the sorption strength between the sediment (C_{total}) and the pore water (C_{free}) of HOCs in the sediment-pore water system. K_D values are specific for each sediment and analyte, influencing the bioavailability of the substance in sediment. The K_D (L g^{-1} dw) values were determined as followed:

$$K_D = \frac{C_{total}}{C_{free}} \quad (3)$$

Community bioturbation potential (BPc) and trait groups

A theoretical bioturbation measurement was first described by Solan et al. (2004), while Queirós et al. (2013) developed a classification for macrofaunal species depending on behavior, diet, life stage and style. The community bioturbation potential (BPc) classification, according to Queirós et al. (2013), was used in this study. The BPc combines biomass and abundance data with information about the life traits of individual species or taxonomic groups, describing the modes of sediment mobility (Mi) and reworking (Ri) of each taxon (). These two traits regulate biological sediment mixing, a key component of bioturbation (Queirós et al. 2013, Solan et al. 2004). BPc provides an estimate of the potential of a community to bioturbate rather than a direct measurement. Hence, BPc offers an opportunity to estimate the extent to which benthic communities are likely to affect important ecosystem properties that underpin ecosystem functioning (Queirós et al. 2013). The BPc captures information about sediment particle reworking, wherefore pelagic species and those living on hard substrates were not included.

$$BPc = \sum_{i=1}^{n} \sqrt{\frac{Bi}{Ai}}\ x\ Ai\ x\ Mi\ x\ Ri \qquad (4)$$

Therefore, the macrofauna biomass (Bi) and abundance (Ai) of taxon *i* were used and each taxon *i* was classified into categorical scales of Mi (mobility) and Ri (sediment reworking) (Table S3). For each station, the mean abundance, mean biomass, mean BPc, are given per m^2, total taxa numbers are given per 0.0123 m^2. Trait groups were formed (e.g., B/SM biodiffusors with slow free movement through the sediment matrix) combining the mobility and the sediment reworking traits. This combination of Mi and Ri leads to 8 different functional groups. For each functional group, characteristic taxa with a percentage >1 % on the total BPc of each station were chosen.

Results and Discussion

TOC content and sediment analysis

The total organic content (TOC) of the surface sediment samples ranged from 0.20 % to 1.05 % (*w/w*, dry) (see Table S1). The sediment of the Wadden Sea stations gave TOC values below 0.6 %, whereas the North Sea sediment carried an organic content of around 1 %. The non-depletion criterion was calculated for all stations to ensure a sufficient buffering by the organic carbon during SPME. The safety factor to exclude possible depletion was met for all stations.

The sediment analysis showed that the sedimentary composition at stations GB2 and KSII were similar as mud and sand were clearly dominating (> 96 %; Figure S1). However, at the other

two stations (JB and W21) sand made up the largest proportion in the composition (92 % and 72 %, respectively). The particle-size distribution clearly showed that the mud fraction consisted mainly of very fine material (< 9.29 µm; see SI, Figure S2). The distribution of the respective particle sizes was very similar at stations KSII, GB2 and JB. At station W21, the distribution pattern deviated slightly, as this very fine fraction accounted for almost three quarters (70 %). However, the largest stones were also found at this station during sampling, but these were not taken into account in the sediment analysis.

Vertical distribution of C_{free} in North Sea sediment cores

The vertical distributions of PAHs and PCBs were estimated by analyzing sediment depth profiles at four North Sea stations. C_{free} ranged from 30 to 463 ng L^{-1} in North Sea sediments and from 15 to 205 ng L^{-1} in Wadden Sea sediments for the sum of 16 investigated PAHs (Figure 2). The sum of seven PCBs ranged from 0.01 to 0.09 ng L^{-1} in North Sea sediments and from 0.01 to 0.29 ngL^{-1} in Wadden Sea sediments, demonstrating a PAH predomination in all sediment samples.

In general, surface sediments represent the current status of sediment contamination. The surface sediment concentrations from the North Sea stations ranged from 33 to 232 ng L^{-1} for the sum of PAHs and PCBs. The station W21 exhibited the lowest TOC content (0.20 %) but showed the second-highest surface concentrations, indicating that the tidal sediment dynamics and the discharge of the Weser might strongly influence this area. The higher pollution loads observed at stations KSII and GB2 are in good agreement with the dredging and dumping activities taking place in this area, as Störmer et al. (2013) reported an active sediment dumping site 15 km south off the island of Helgoland, which is about 10 km distance to the North Sea sampling stations of this study (GB2 and KSII). In 2017, a dredged sediment volume of 1,460,000 t (dry matter) from the port of Hamburg was transported to the dumping site (HPA 2017). In this area, the current is influenced by east wind forcing (Staneva 2009), indicating that the dumped sediment may have drifted towards the North Sea sampling stations, as fine-grained sediment particles remain longer in the water phase compared to sandy particles. However, investigations by HPA (2017) showed that no dredged material-related increase of contaminant concentrations was detected in the surrounding stations within a field up to 12 km around the dumping site. In addition, some of the stations from the far field of the dumping site showed already higher sediment concentrations before the dumping activities started (HPA 2017). This might indicate that the areas located on an axis towards the Elbe estuary are strongly influenced by suspended Elbe sediment due to the tidal sediment dynamics, resulting in an

important source for PAH input in the area. The input of combustion-derived PAHs from central Europe and the transport of particle-bound PAHs over long distances might be another source. PCBs enter the North Sea through the Atlantic Ocean and the atmosphere, but also rivers, sewers and sludge have a great impact on the PCB input. As the German Bight is an intensively used area in many aspects, the entries of HOCs are mostly diffuse and not due to one point source.

The results also revealed that the 5-ring PAHs were present at lower concentrations in all samples than lower molecular weight PAHs. However, the 5-ring PAH concentrations increased with sediment depth at the North Sea stations, whereas at the Wadden Sea stations, all PAHs were decreasing with depth. The trend of increasing concentrations with increasing depth could reflect a higher anthropogenic influence during this time in the North Sea region, as there is a dumping site near these stations. The increased PAH concentrations could be the result of a few years ago when the dumping process was less controlled and monitored. The higher chlorinated PCBs were present at higher concentrations in the upper sediment layers and decreased at a sediment depth of about 8 cm at stations GB2, KSII and W21. In order to be able to assess the current load condition, the classification of the actual condition into the more recent pollution development is of interest. The sediment profiles showed different PCB distributions towards the sediment surface. The PCB concentration at station W21 was the highest of all stations, reached a peak between 2-4 cm, and then decreased with a reduction of layer depth. This high pollution may indicate a special burden of PCBs in the Weser estuary. At station KSII the sum PCBs was highest in the layer between 4-6 cm. According to Brockmeyer and Theobald (2016), the high HOC concentrations at station KSII are an indication of the high pollution of the former deposition of sewage sludge at this site. Furthermore, Brockmeyer and Theobald (2016) showed that the stations close to Helgoland Bight (KSII and GB2) are higher polluted compared to other sampling stations in the North Sea.

Compared to the North Sea surface sediment analysis of Niehus et al. (2019), the PCB concentrations are in the same order of magnitude for the North Sea sediments. In contrast, the sediment from the Wadden Sea was less contaminated. However, the sum of PAHs was magnitudes higher compared to Niehus et al. (2019). The North Sea's PAH surface sediment concentrations were up to 200 times higher, for the Wadden Sea up to 90 times higher. In addition, the determined C_{free} for PCBs were in the same order of magnitude for all sampling stations compared to a study of the Baltic Sea (Lang et al. 2015). However, C_{free} values of PAHs

were up to 27 times higher in the North Sea and up to ten times higher for the Wadden Sea (Lang et al. 2015). This again shows that the North Sea stations investigated in this study are probably influenced by a strong input of PAHs from the nearby dumping site.

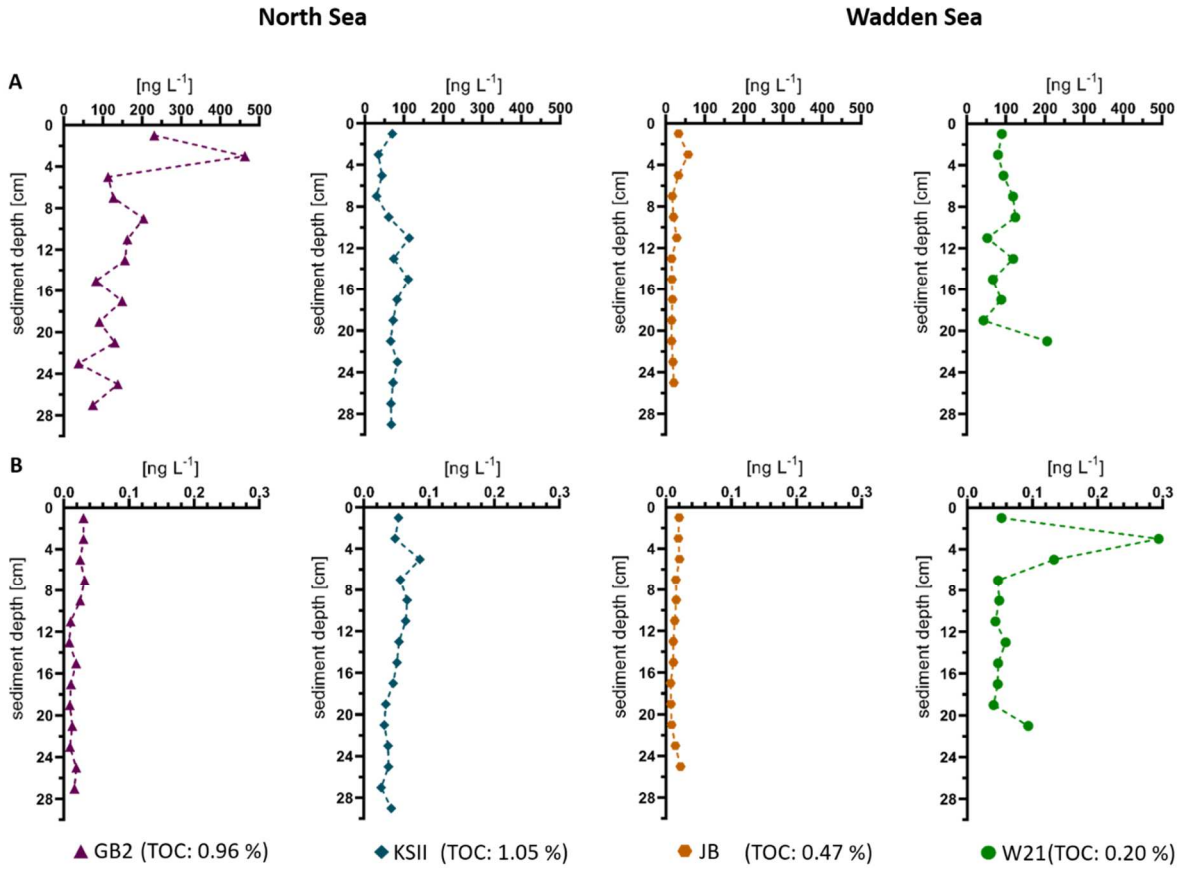


Figure 2: The sum of C_{free} of 16 PAHs (**A**) and seven PCBs (**B**) at the four sampling stations in the North Sea.

Chemical activities and baseline toxicity

The compositions of C_{free} of the surface sediments varied moderately between the North Sea and the Wadden Sea stations. However, the PAHs' general distribution was consistent as the lower molecular weight PAHs (phenanthrene, anthracene, fluoranthene and pyrene) prevailed. Even though the lower molecular weight PAHs dominated C_{free}, they only contributed a small portion to the chemical activities (Figure3). Higher molecular weight PAHs (benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene) accounted for less than 5 % to C_{free}, but contributed the most to the chemical activity (47 to 52 %) and thus to the baseline toxicity. In addition, PCBs contributed less than 1 % to C_{free}, with slightly higher concentrations for the lower molecular weight PCBs. However, the higher chlorinated PCBs in particular contributed up to 27 % to the chemical activity after C_{free} was being transformed. Hence, higher molecular PAHs and higher chlorinated PCBs contributed most to the baseline toxicity. This proves once again that the baseline toxicity is not only driven by the chemical concentration but also by the

individual properties of every single compound, which must be carefully taken into account for environmental risk assessment. Niehus et al. (2019) demonstrated that the Wadden Sea sediments accumulate especially PCBs and display a hotspot with high PCB concentrations. However, this difference between the North Sea and the Wadden Sea sediments could not be shown in this study, as PAHs were the dominating substances at all stations.

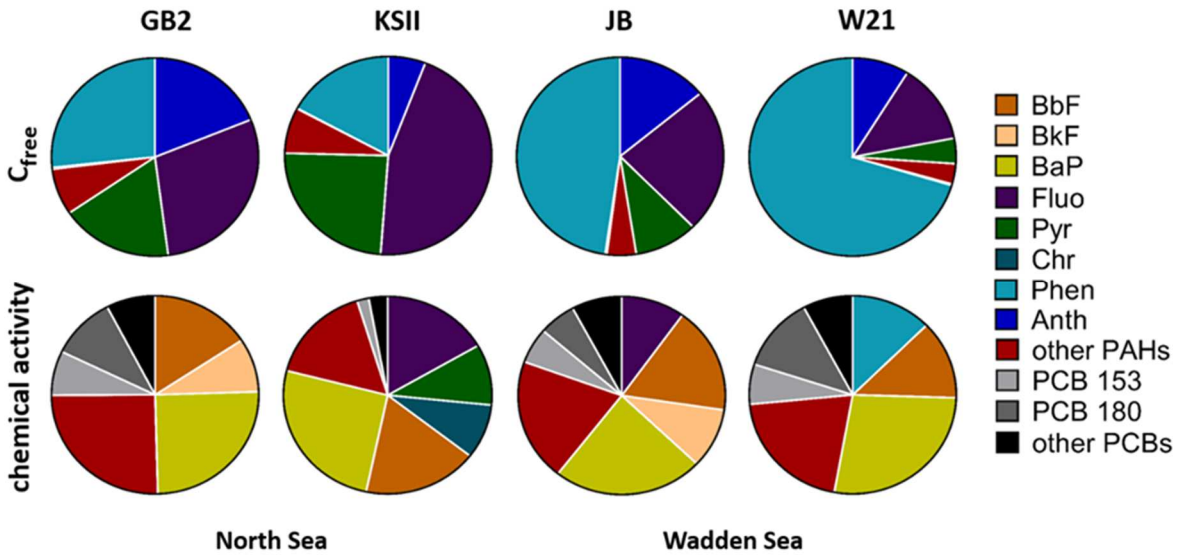


Figure 3: Composition comparison of Cfree and chemical activity for surface sediments (0-2 cm).

The baseline toxicity is the minimal toxicity a compound can cause when integrating into membranes, thus reducing their fluidity, and is of importance when assessing the risk of complex environmental mixtures. Even if the mixture's individual substances are below the threshold level of specific toxicity, the underlying cumulative baseline toxicity of the mixture might determine the overall toxic effect (Escher et al. 2002). The chemical activities of the sum of 16 PAHs and 7 PCBs are presented in Figure 4 as depth profiles. The contribution to the baseline toxicity varied considerably between the sampling stations but was below acute levels at all sites and sediment depths. The sum of chemical activities was higher in North Sea sediments compared to the Wadden Sea. However, the sum of chemical activities was ten to a hundred times below the acute baseline toxicity range. The chemical activity at the Wadden Sea stations decreased with depth indicating a rather recent input of PAHs and PCBs. In contrast, the chemical activity at the North Sea stations increased with depth, which would indicate major historical contamination. At stations GB2, KSII and JB, the chemical activities of the surface sediment were similar, while the chemical activity at station W21 was clearly higher. This could be due to the proximity to the River Weser and the harbor located at its mouth. Moreover, Niehus et al. (2019) determined chemical activities for North Sea surface

sediment at about the same range. Baseline toxicity at the four stations can be considered relatively high, although only a few priority compounds were analyzed in this study. Taking all PAHs, PCBs and other chemical compounds together, they might exhibit a potential 10 to 100 times higher than the well-known priority pollutants.

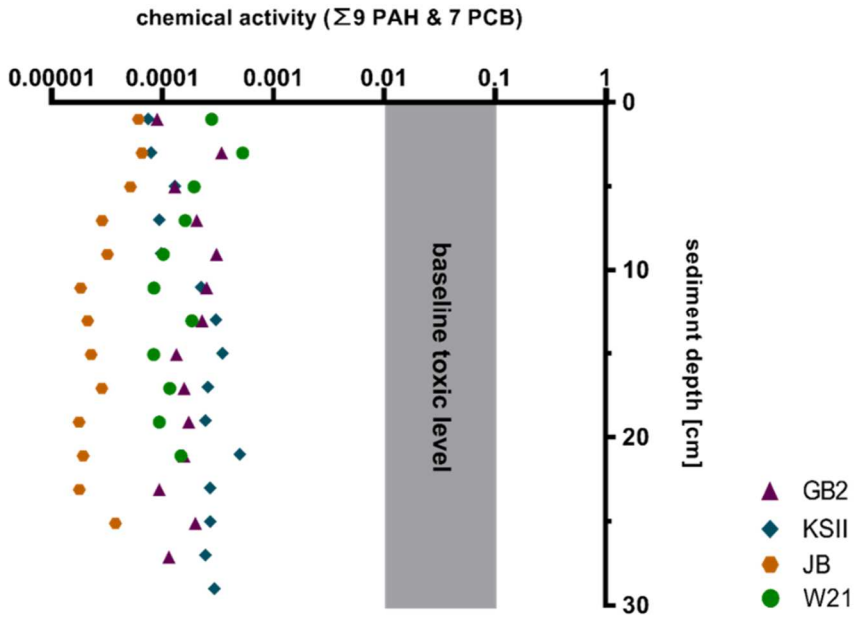


Figure 4: Baseline toxic level of sum chemical activities, calculated from 16 PAHs and 7 PCBs along a depth profile at North Sea sites.

Sorption to sediment

Site-specific distribution coefficients (K_D) were calculated to describe the equilibrium distribution of a chemical between sediment and pore water and to evaluate the sorption strength of the different sediment areas. In general, the sorption of HOCs increases with increasing hydrophobicity of the analyte and the TOC content of the sediment. The TOC content of the sediments was generally low between 0.96 % and 1.05 % in the North Sea and 0.20 - 0.47 % in the Wadden Sea. The correlation of log K_D for PAHs and PCBs against log K_{OW} allows an evaluation of regional differences in the sorptive strength for HOCs in the North Sea sediments (Figure 5).

The K_D values of each PAH from all stations followed a linear regression near the 1:1 ratio (r-values between 0.79 and 0.93), indicating that the distribution of PAHs between sediment and pore water was similar to that of octanol to water (log K_{OW}). K_D values at the Wadden Sea stations were lower, showing weaker sorption and hence indicating that the PAHs are better available. For the analyte benzo[ghi]perylene, the K_D values were higher compared to the 1:1 ratio, which indicates that the sorption to the sediment was stronger (station KSII and GB2).

The same correlation was also performed for PCBs in the sediments. All correlations between K_D and K_{OW} were below the 1:1 ratio, showing weaker sorption to the sediment and better availability of the PCBs. However, the correlation for Wadden Sea sediments was better compared to the North Sea as both correlation coefficients for Wadden Sea sites were 0.99, whereas the r-values for the North Sea stations were 0.71 (GB2) and 0.97 (KSII). The better correlation and stronger sorption at the North Sea stations GB2 and KSII could be caused by the dumping material. At these stations, the original pattern of the sediment might be disturbed by a stronger input of PAHs bound to soot particles from the port area of the Elbe.

In general, the sorption strength of PAHs to sediment is stronger compared to PCBs, which is in good agreement with Bucheli and Gustafsson (2003). They showed that the rigidly planar compound class of PAHs has higher affinities to soot sorbent than PCBs. Jonker and Koelmans (2002) could also show that planar contaminants have very high affinities to soot and soot-like materials due to enhanced sorption in the narrow pores. The strong sorption can result in heavily reduced aqueous concentrations for both types of chemicals and will lead to a significantly lower uptake by aquatic organisms. Consequently, there will be a reduced potential of ecotoxicological effects (Jonker & Koelmans 2002).

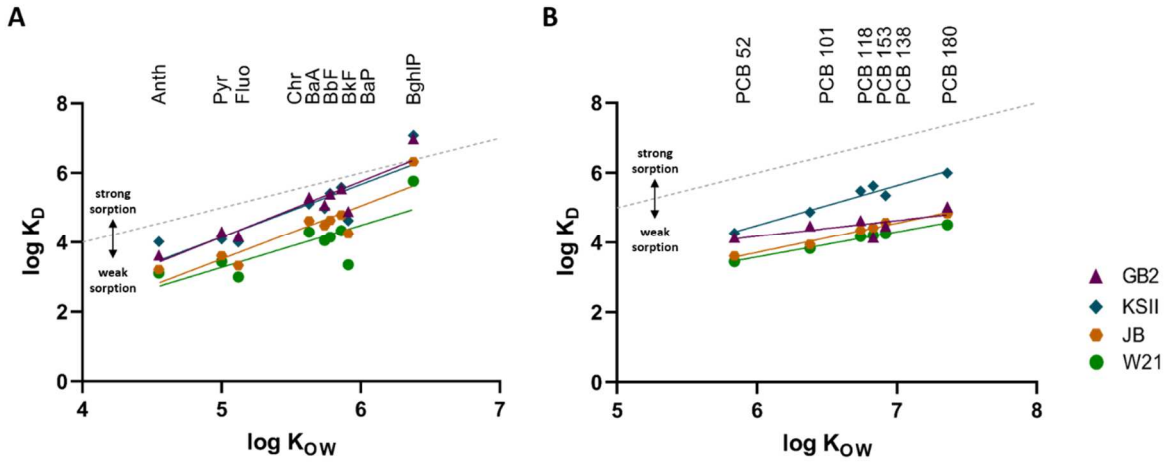


Figure 5: Correlation of site-specific distribution ratios (K_D) (surface sediment layer, 0-2 cm) of the North Sea and log K_{OW} of PAHs (A) and PCBs (B).

Source identification based on the composition of PAHs

The source of PAHs can be identified by individual PAH ratios, and whether they are from fuel-combustion (pyrolytic) or crude oil (petrogenic) contamination (Biselli et al. 2005, Tam et al. 2001). The determination of the phenanthrene/anthracene and fluoranthene/pyrene ratio allows the estimation of the source of contamination. A ratio of phenanthrene/anthracene < 10 and fluoranthene/pyrene > 1 indicates that the contamination was caused by combustion processes

(pyrolytic origin) (Benlahcen et al. 1997), whereas petrogenic pollution is characterized by a higher phenanthrene/anthracene ratio as petroleum often contains more phenanthrene relative to anthracene (Tam et al. 2001). The ratios from the present study are summarized in Table S4. Pyrolytic or petrogenic zones can be identified by plotting the fluoranthene/pyrene (fluo/pyr) ratio against the phenanthrene/anthracene (phen/anth) ratio. The zones defined by high Fluo/Pyr ratios and low Phen/Anth are characteristic of pyrolytic PAHs (top left quadrant), and low Fluo/Pyr ratios and high Phen/Anth are characteristic of petrogenic PAH (bottom right quadrant). The other two quadrants may be indicative of a mixed source of PAHs (Figure S3).

The PAH distribution and concentration ratios indicated a predominantly pyrolytic input in the North Sea sediments. The source distribution at station KSII was the most scattered of all stations. One sample was in the top mixed zone due to the phen/anth ratio being greater than 10 (station KSII 6 - 8 cm) and four samples exhibited fluo/pyr ratios smaller than 1 relating them to the bottom mixed zone (station JB 10 - 12 cm, 12 - 14 cm, 22 - 24 cm; station KSII 4 - 6 cm). This pattern could also be seen in sediments from the Fladen Ground oilfield in the northern North Sea, where most samples also belong to the pyrolytic zone and are dominated by the heavier, more persistent PAHs (Ahmed et al. 2006). In addition, Biselli et al. (2005) also found a pyrolytic origin of the PAH contamination for the sediment of the North Sea. Although PAHs can undergo degradation by photooxidation in surface waters, dissolution, evaporation and through microbial activity in sediment, the ubiquitous distribution of PAHs indicates that accumulation phenomena dominate degradation processes in sedimentary matrices (Ahmed et al. 2006). The OSPAR Commission (2009) reported that the levels of metals, PCBs and PAHs in the environment are generally decreasing but still give rise to pollution effects at coastal locations.

Bioturbation potential analysis

The macrofauna analysis of all four North Sea stations identified a total of 28 species from six different phyla. The class of Polychaeta (Annelida) was the dominant class with 17 identified species followed by four different bivalves (Mollusca). Two species each were found in the sediment for the classes Malacostraca (Arthropoda) and Ophiuroidea (Echinodermata). The phyla Phoronida and Nemertea were represented with one species each. The razor clam *Ensis directus*, which was only found at station KSII, was excluded in all bioturbation analyses as it could not be assigned to a specific sediment layer due to its body length. Furthermore, the razor clams buried themselves deeper into the sediment while the sediment core was divided into different layers.

The species abundance in the different sediment layers differed clearly between the sampling stations (Figure S4). The macrofauna analysis showed that only at station KSII species were found in sediment depths up to 20 cm, while species lived only in the upper 10 cm at station GB2. In the first sediment layer, the number of species and abundances were highest at station KSII (14 species, 130 individuals per 0.025 m^2), where the polychaete *Scalibregma inflatum* and the bivalve *Nucula nitidosa* exhibited the highest number of individuals (57 and 37 individuals, respectively). Station GB2 showed a high species richness with 10 different species, but in total only 28 individuals were found in the first 5 cm of the sediment, where a third of the total abundance was accounted for the bivalve *Nucula nitidosa* (9 individuals). At station JB, the abundance amounted to 102 individuals from 8 species and the polychaetes *Aphelochaeta marioni* and *Pygospio elegans* (38 and 34 individuals, respectively) dominated the abundance in the upper sediment layer. In contrast, the sampling station W21 exhibited a low species richness (3 species) with 26 individuals in total. The first sediment layer was dominated by the polychaete *Pygospio elegans* (21 individuals).

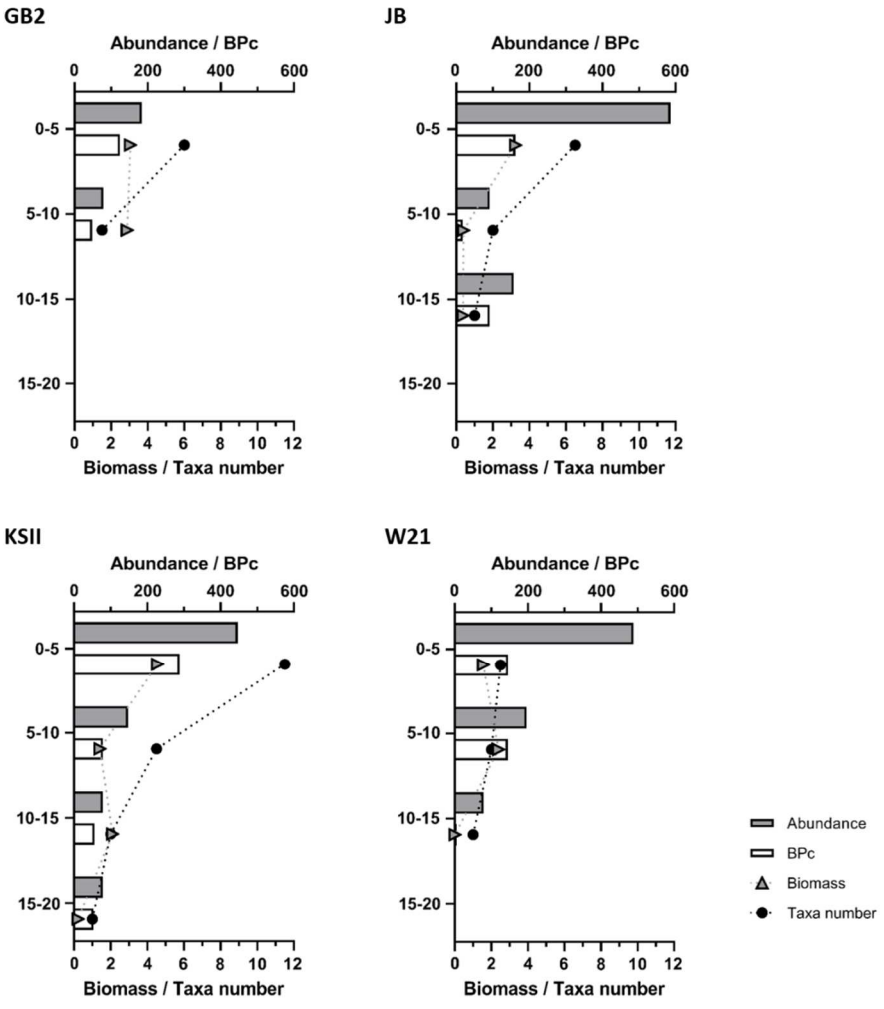


Figure 6: Mean abundance, biomass, and BPc per 1 m^2 and total taxa number per 0.0123 m^2 per sampling station in the North Sea.

At all stations, a decreasing trend of the mean BPc, abundance, biomass and taxa number was detected with sediment depth (Figure 6). Dauwe et al. (1998) already mentioned this declining trend as they found a highly functional macrofaunal diversity in the North Sea sediment due to the higher quality and quantity of TOC, which led to the highest abundances and biomass in the upper sediment layer. However, a steeply decreasing gradient of the macrofaunal abundance and biomass was reported, showing that TOC also influences the vertical distribution and bioturbation activity and intensity (Dauwe et al. 1998). In addition, the abundance and species composition are strongly depending on the food availability, which in turn is determined by the current and sedimentation of fine-particulate organic material. Food availability is one of the main limitings in all benthic ecosystems (Rosenberg 1995, Schückel et al. 2013). Meyer et al. (2018) reported a decreasing trend in the North Sea for phytoplankton primary production and biomass.

Table 1: Percentage of functional groups and their characteristic taxa of the total bioturbation potential of each station; asterisks (*) indicate <1 %. The main bioturbators are shown in **bold**.

Functional group	**Characteristic taxa**	**%**			
		GB2	**KSII**	**JB**	**W21**
S/FT	*Phoronis spp.*	1.4	*	2.0	
S/LM	***Aphelochaeta marioni***			**20.1**	
	Ophiura albida	12.3	*		
	Abra alba	9.2	9.8		
	Kurtiella bidentata	8.3	*	1.7	
S/SM	***Nucula nitidosa***	**23.2**	**26.4**		
S/FM	*Corophium arenarium*			*	
U/FT	*Pygospio elegans*			9.3	10.0
U/LM	*Ampharete spp.*		1.9	7.9	*
	Streblospio benedicti			5.7	
	Notomastus latericeus		1.8		
B/SM	***Scoloplos armiger***		*		**69.3**
	Nephtys hombergii			**41.5**	**19.9**
	Oligochaeta			10.1	
	Phyllodoce rosea	2.4	*		
	Nemertea	2.0	*	1.1	
B/FM	***Scalibregma inflatum***	**21.2**	**52.6**		
	Hediste diversicolor	**19.7**			
	Total	99.5	96.3	100.0	99.6

The functional group of biodiffusors (B/SM; see Table S3 for trait definitions) contributed the most of the total BPc for the Wadden Sea stations JB and W21, represented by the polychaetes *Scoloplos armiger* and *Nephtys hombergii* (Table 1). The North Sea stations were also dominated by a biodiffusor functional group (B/FM) represented by the polychaetes *Scalibregma inflatum* and *Hediste diversicolor*. However, at the North Sea stations, the functional group of surficial modifiers was also stronger represented by the bivalve *Nucula nitidosa*. This is in good agreement with Kröncke et al. (2004), who reported that the macrofauna community of the inner North Sea stations is dominated by the subsurface deposit-feeding polychaete *Scalibregma inflatum* as well as *Nucula nitidosa*.

Establishing a link between the macrofauna analysis and the chemical analysis, the bioturbation potential (BPc) together with the total sediment concentration (C_{total}) and the freely dissolved pore water concentration (C_{free}) along the sediment depth profiles of all sampling stations are displayed in Figure 7. The sum of the BPc is decreasing with depth at all sampling sites. Morys et al. (2017) showed that most organisms were found in horizons of the highest chlorophyll concentrations. The main food for benthic macrofauna is deposited phytoplankton, which can be measured by using chlorophyll as a tracer. The distribution of chlorophyll within the sediment is again dependent on its sedimentation, degradation and sediment mixing (Morys et al. 2017). The bioturbation potential at the sediment surface at station KSII is about three times higher compared to the other sites. All sediment cores, except for stations GB2 and W21, showed homogeneous PAH and PCB concentration distributions in the upper sediment layers, indicating that sediment transformation processes such as bioturbation may be responsible. Undisturbed sediments serve as a sink for HOCs and exhibit a normally distributed chemical profile according to the historical input of HOCs in the environment. For example, the PCB concentration first increases with depths until a maximum is reached and then decreases again with depths according to the temporal industrial use. However, no increase in PAH and PCB pore water concentrations (C_{free}) was observed at depths, where no bioturbation occurred due to anoxic conditions. This might indicate that the sediment at all stations was disturbed by anthropogenic activities such as sediment dumping or trawling. In contrast, the total sediment concentration of the stations GB2 and KSII increased clearly with depth. The contamination level remained stable in JB and W21, which could be due to the low TOC content, as this reduces the binding of organic pollutants to the sediment particles.

A direct comparison of GB2 and KSII showed that station KSII was the one with the lower C_{free} contamination and higher bioturbation potential. In contrast, the chemical load at station GB2 was significantly higher and the bioturbation potential in the surface sediment layer was clearly lower. The sediment analysis showed that the sediment composition of both stations is very similar and consisted mainly of sand and mud (Figures S1 and S2). The particle size distribution was also very similar, from which it can be concluded that the sediment of both stations is the same and is thus also preferred by a similar community of organisms. However, this was not reflected in the abundance and especially in the bioturbation potential. Therefore, it can be assumed that this difference in the macrofauna is not influenced by the sediment composition, but rather by the higher chemical load at station GB2. Moreover, station GB2 is closer to the dumping site and might be also more affected by anthropogenic influences such as bottom trawl fishing. In addition, the sediment at station GB2 is extremely anoxic and hydrogen sulfide (H_2S) is clearly present. However, small-scale flow conditions can cause lower H_2S concentrations at station KSII, which in turn can also influence the macrofaunal composition. In addition, Oehler et al. (2015 a, b) showed similar sediment profile trends for surface sediments of the Helgoland Mud Area of the benthic oxygen and nitrogen fluxes as well as the biogenic silica cycle.

In general, the benthic biomass decreases with increasing distance from the coast in the southern North Sea (Rehm & Rachor 2007). However, increased biomass in the area of the island of Helgoland was described by Duineveld et al. (1991) and Rehm and Rachor (2007). A mass occurrence of e.g. small tube-living species might cause significant changes in the abundance-based community structure but may affect BPc less than the biomass of large individual species, as bioturbation is always related to the biomass of organisms (Meyer et al. 2019). In this study, mainly small species, e.g., polychaetes, were found in the sediment of the investigated stations. However, Meyer et al. (2019) proposed that the calculation of the BPc includes the most important functional traits that are important for biogenic mixing and the influence of macrofauna on sediment structure. Moreover, while the BPc successfully predicts particle distance transport, the prediction of bioturbation depth, activity, and the biodiffusive transport is limited (Queirós et al. 2015). Mustajärvi et al. (2019) demonstrated that the bioturbation activities of organisms might increase the sediment-to-water flux by up to one order of magnitude. Furthermore, bioturbation enhances the loss of hydrophobic sediment-associated contaminants due to infaunal activity caused by different processes such as the release to overlying water, microbial degradation as well as bioaccumulation and metabolism by the macrofauna itself (Timmermann et al. 2011).

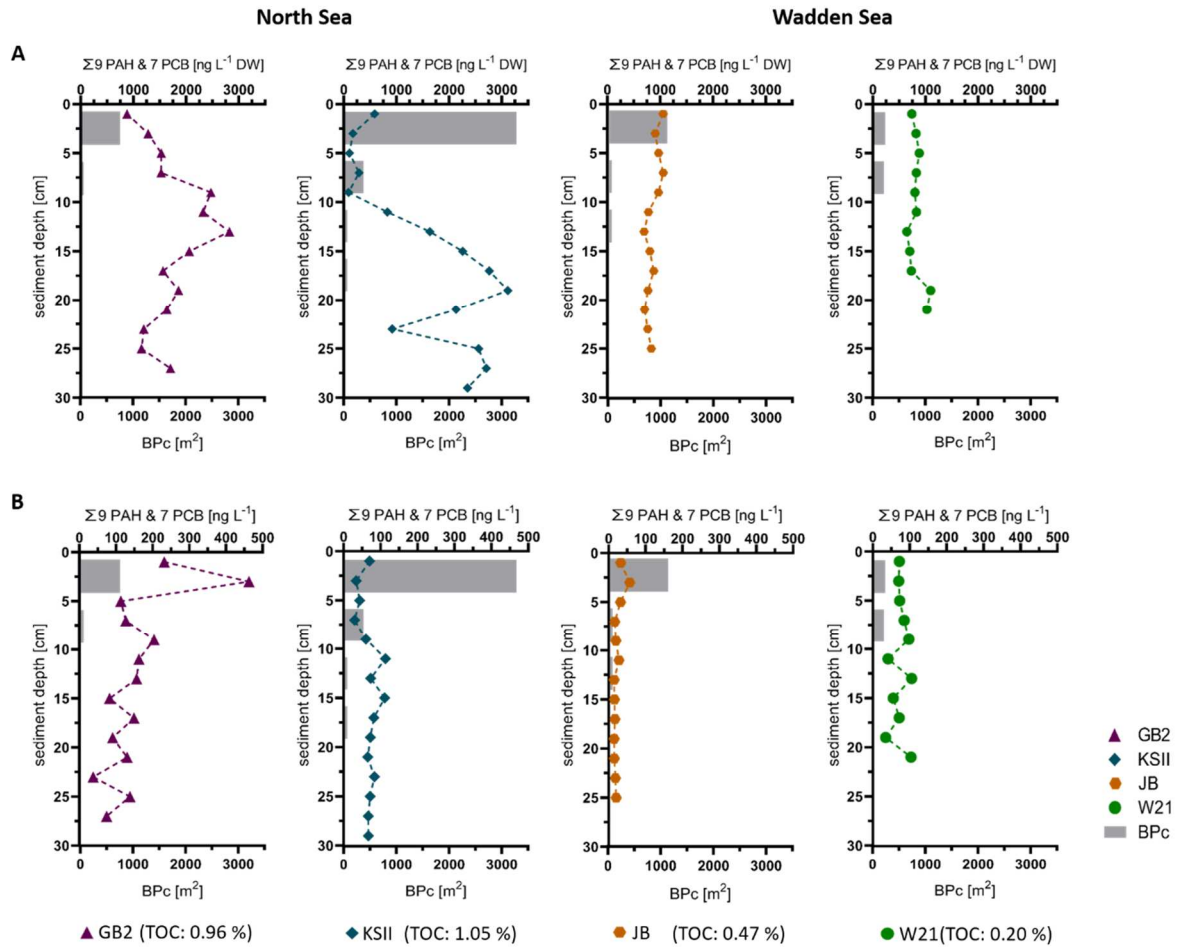


Figure 7: Sum of C_{total} (**A**) and sum of C_{free} (**B**) of PAHs and PCBs as well as the sum of the bioturbation potential (BPc) along the sediment depth profile.

Conclusion

The study demonstrated that the equilibrium passive sampling approach for measuring the freely dissolved pore water concentrations can be performed with sufficient accuracy down to extremely low concentrations (pg L^{-1}) and is applicable for a wide range of sediment-bound HOCs in marine waters such as the North Sea. The pollutant analyses in the sediment cores predominantly revealed decreasing pollutant concentrations in the upper sediment layers, which indicates that bans and regulations of the investigated substances are making an impact. However, the dumping site might have a negative impact as higher sediment concentrations have been found at nearby stations (GB2 and KSII). A direct correlation between bioturbation and sediment concentration could not be seen, but higher chemical loads might negatively affect the species abundance and bioturbation potential. The results of the present study contribute to an improved database for status description, assessment of the ecological status and the detection and influence of possible stressors. This will benefit the overall risk assessment and management of the MSFD.

Acknowledgements

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Supporting Information

Supplementary Table 1

Table S1: Sampling stations, coordinates, TOC content (%) and water depth [m].

	Station	Coordinates		TOC [%]	Water depth [m]
		North	East		
North Sea	GB2	54°01'58.7"N	8°03'32.7"E	0.96	24
	KSII	54°04'03.4"N	8°07'32.2"E	1.05	19
Wadden Sea	JB	53°29'53.0"N	8°08'25.1"E	0.47	8
	W21	53°43'33.1"N	8°22'24.1"E	0.20	11

Supplementary Figure 1

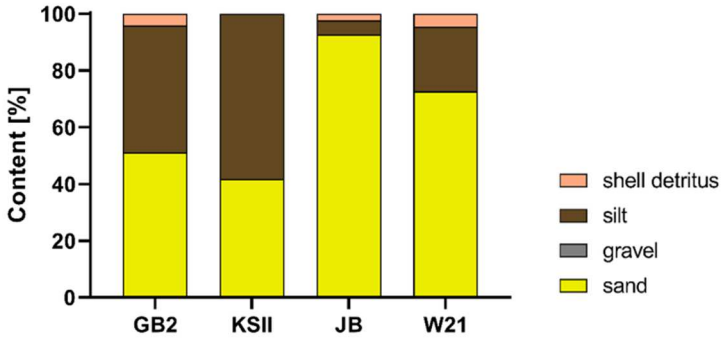


Figure S1: Sedimentary composition at the four sampling stations separated in shell detritus (> 2 mm), gravel (> 2 mm), sand (< 2 mm) and mud.

Supplementary Figure 2

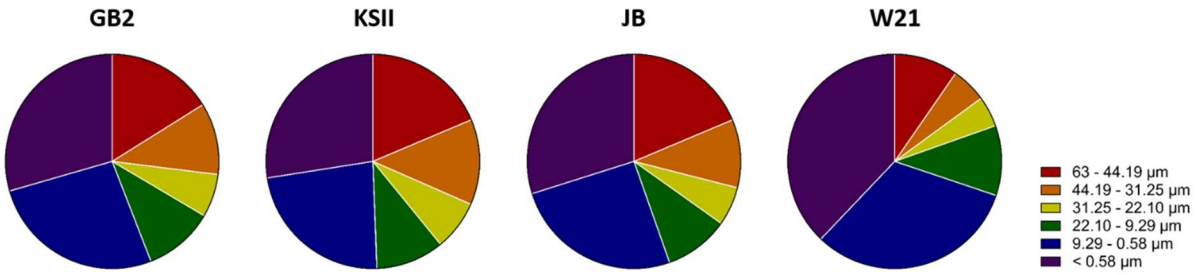


Figure S2: Particle-size distribution [%] of the mud fraction for the four sediment samples using the SediGraph analysis.

Supplementary Text 1 and Table 2

Text S1: Calculation of the chemical activity

The chemical activity was calculated using the following equation:

$$a = \frac{10^{\gamma} * 0.01807 * C_{free}}{M} \cong \frac{C_{free}}{S_L} \qquad \textbf{(S1)}$$

Where γ is the log aqueous activity coefficient, C_{free} in pg L^{-1}, M the molar mass (Mackay et al. 2006) and 0.01807 L the molar volume of water at 20 °C.

Table S2: Activity coefficients γ and molar masses (M) for the calculation of the chemical activity using equation S1.

Substance	γ	M (pg mol^{-1})
Phen	6.78	1.782E+14
Ant	6.75	1.782E+14
Fluo	7.27	2.023E+14
Pyr	7.32	2.023E+14
BaA	8.31	2.283E+14
Chr	8.34	2.283E+14
BbF	8.92	2.523E+14
BkF	8.86	2.523E+14
BaP	8.99	2.523E+14
PCB 28	8.00	2.5754E+14
PCB 52	8.53	2.9199E+14
PCB 101	9.36	3.2643E+14
PCB 118	9.53	3.2643E+14
PCB 153	10.32	3.6088E+14
PCB 138	10.21	3.6088E+14
PCB 180	11.19	3.9532E+14

Supplementary Table 3

Table S3: Abbreviations (A) and Scores for mobility (Mi) and sediment reworking (Ri) traits for benthic taxa and in the southern North Sea according to Queirós et al. (2013).

score	Mi	A	Ri	A
1	living in a fixed tube	*FT*	-	
2	limited movement	*LM*	surficial modifiers	*S*
3	slow free movement through sediment	*SM*	upward/downward conveyors	*U*
4	free, three-dimensional movement	*FM*	biodiffusors	*B*
5	-		regenerators	*R*

Supplementary Table 4

Table S4: Ratios for phenanthrene/anthracene and fluoranthene/pyrene for source analysis.

Station	Ratio	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24	24-26	26-28	28-30
GB2	**Phen/Anth**	3.49	4.00	4.09	4.52	4.08	4.89	3.41	3.84	3.09	4.44	2.79	4.46	3.71	3.22	
	Fluo/Pyr	1.31	1.32	1.36	1.28	1.28	1.23	1.24	1.22	1.16	1.22	1.19	1.23	1.17	1.17	
KSII	**Phen/Anth**	6.86	5.07	2.07	11.25	2.98	7.76	4.02	4.80	4.27	3.36	4.80	1.69	4.27	5.18	5.09
	Fluo/Pyr	1.50	1.58	0.54	1.18	1.21	1.35	1.69	1.44	1.64	1.65	1.85	1.37	1.55	1.47	1.64
JB	**Phen/Anth**	4.21	2.79	3.92	4.44	3.52	1.99	1.83	3.75	2.12	3.07	2.31	2.44	2.46		
	Fluo/Pyr	1.42	1.35	1.41	1.42	1.43	0.76	0.95	1.33	1.20	1.12	1.19	0.78	1.25		
W21	**Phen/Anth**	2.47	4.02	3.12	2.86	2.84	2.85	3.25	3.82	3.01	3.02	2.13				
	Fluo/Pyr	4.56	1.16	1.37	1.17	1.30	1.33	1.28	1.19	1.21	1.02	1.33				

Supplementary Figure 3

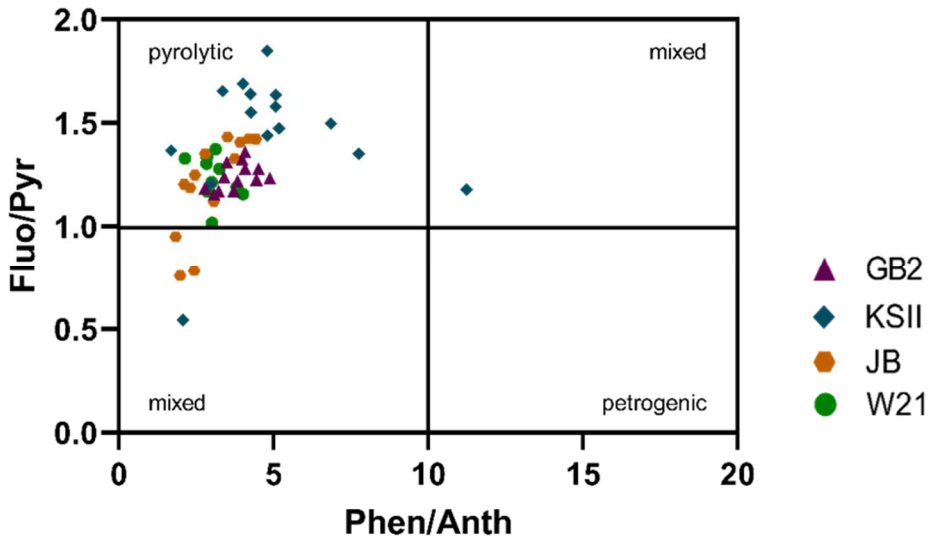


Figure S3: PAH concentration ratios to assess the sources of PAHs in the North Sea. Plot of Fluo/Pyr ratios against Phen/Anth ratios for each station and sediment layer.

Supplementary Figure 4

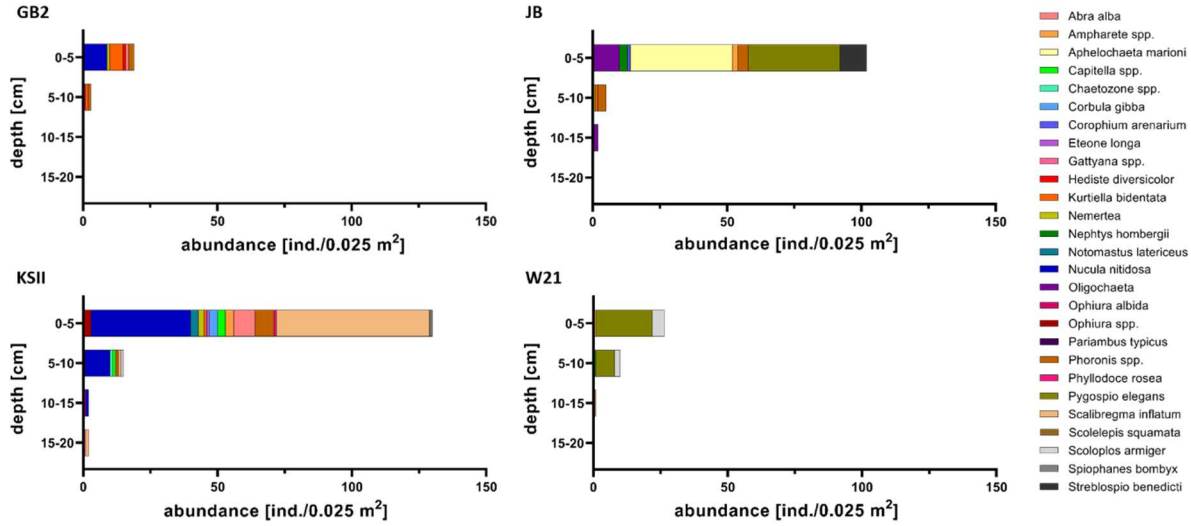
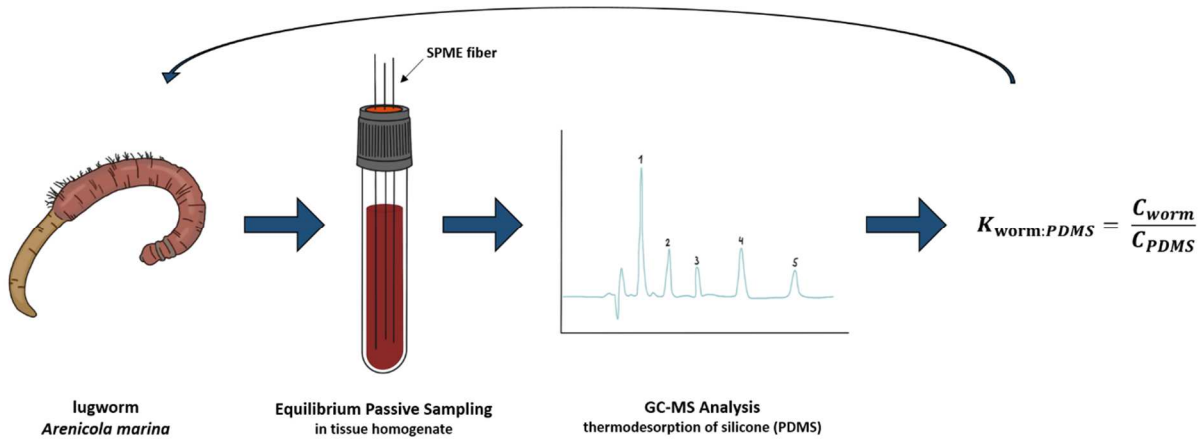


Figure S4: Species abundance per 0.025 m^2 in the different sediment layers of the North Sea sampling stations.

Equilibrium passive sampling: A novel approach to determine internal tissue concentrations of hydrophobic organic compounds in biota



Equilibrium passive sampling: A novel approach to determine internal tissue concentrations of hydrophobic organic compounds in biota

Anne Kreutzer[a,b], Susann-Cathrin Schacht[c] and Gesine Witt[b]

[a] Department Evolutionary Ecology and Environmental Toxicology, Institute of Ecology, Evolution and Diversity, Faculty Biological Sciences, Goethe University Frankfurt, Frankfurt 60438, Germany

[b] Department of Environmental Engineering, Faculty Life Sciences, Hamburg University of Applied Sciences, Hamburg 21033, Germany

[c] Agilent Technologies Deutschland GmbH, Hewlett-Packard-Str. 8, Waldbronn 76337, Germany

Abstract

Equilibrium passive sampling has been applied in numerous abiotic environmental matrices. This approach was extended to biological material. In this work, a passive equilibrium sampling method for the measurement of HOCs in biota was developed as an innovative alternative because classical exhaustive extraction techniques are time-consuming and error-prone. The newly developed method is based on the well-proven SPME fiber method for sediment. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were used as model lipophilic organic pollutants. Partition coefficients of PAHs and PCBs between the lugworm tissue and the PDMS sampling phase were determined. Polydimethylsiloxane (PDMS) coated glass fibers were directly inserted in homogenized lugworm tissue and glass fibers were analyzed using gas chromatography coupled to mass spectrometry. The method application on lugworms from tidal sand flats near Wilhelmshaven showed that the mean body residue values of PCBs (4 µg g^{-1}) and PAHs (256 µg g^{-1}) were about five times higher for PCBs and more than 22 times higher for PAHs compared to literature data for the North Sea area. This high level of contamination might be a consequence of the oil processing refinery located in direct proximity to the sampling site. This novel approach of applying the SPME method to biota will make biological monitoring more effective and holistic, because seasonally and area-wide changes in all environmental compartments can be recorded quickly.

Introduction

Sediments have great ecological relevance as they are both habitat and food source for a great number of species. The analysis of sediments has been proven to be an efficient tool to identify environmental impacts as sediments tend to retain contaminants coming from land and water columns. In addition, the environmental risk-analysis approach uses studies on sediment quality to contribute to a proper characterization in decision-making procedures for its management (Ramos-Gómez et al. 2011). When entering the aquatic environment, hydrophobic organic contaminants (HOCs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) can sorb to suspended material in the water column and then be deposited on the sediment surface. Consequently, the availability of these contaminants to benthic invertebrates is a major environmental concern (Schuler & Lydy 2001). Therefore, a better understanding of how HOCs interact with sediment biota is particularly important, as these

organisms are in intimate contact with contaminated sediments and interstitial waters, and these animals may contribute to trophic transfer through the food web (Christensen et al. 2002).

The presence of biota plays an important role in the fate of contaminants in sediment, especially sediment-dwelling animals are known to affect the release and distribution of HOCs because of their bioturbation activities, irrigation and particle mixing (Gordon et al. 1978, Schaffner et al. 1997). The accumulation of HOCs by benthic organisms can occur either from the aqueous phase or dietary exposure (Lamoureux & Brownawell 1999). However, the importance of water exposure pathways decreases relative to sediment ingestion. Sediment ingestion has been reported to be a significant or primary route of uptake of particle-associated contaminants (Morales-Caselles et al. 2008). The exposure to contaminants is increased by particle selectivity in food selection, and bioavailability is enhanced by the animals' attempt to solubilize food from ingested particles (Jørgensen et al. 2008). Kaag et al. (1997) showed that the feeding mode of benthic invertebrates clearly influences the accumulation of sediment-bound lipophilic contaminants as sediment-feeding species accumulate organic contaminants to higher concentrations than filter-feeders. Also, the ability of a hydrophobic contaminant to cross a biological membrane depends to a large extent on the type of organic material with which the contaminant is associated. Freely dissolved contaminants are fully bioavailable, whereas those associated with dissolved organic material (DOM) have a reduced bioavailability (Voparil & Mayer 2000).

Arthur and Pawliszyn (1990) introduced a solid-phase microextraction (SPME) technique applied as a passive sampling tool to assess the bioavailable fraction of contaminants. The use of polydimethylsiloxane (PDMS) as sampling phase has been applied in numerous sampling devices of different formats, for example, PDMS sheetings (thin-film microextraction) and PDMS-coated glass fibers (solid-phase microextraction, SPME). Furthermore, these sampling techniques have been used for human biological fluids as well as diverse environmental matrices such as water and soil/sediment compartments (Jahnke et al. 2009). Studies applying SPME in biological tissue and referred to as in-tissue passive sampling or lipid-rich matrix-SPME are in the focus of research in the last few years but still rare to date. According to Ossiander et al. (2008), they performed the first study applying SPME by immersing the sampling phase (PDMS coated fibers) directly in biological tissue with high-fat content to measure fluoranthene, PCBs and HCB (hexachlorobenzene). They demonstrated that the sample was not depleted and that the equilibrium was reached within a very short time. The PDMS partitioning properties were not modified, which was also confirmed by a

comprehensive study by Jahnke and Mayer (2010). The determination of partition coefficients of PCBs and OCPs (organochlorine pesticides) between lipids of different trophic levels (model lipids) and PDMS was the primary focus of Jahnke et al. (2008), suggesting the use of generic corrected and uncorrected partition coefficients, generated from different pure oils when measuring HOCs in C_{PDMS} from biota for the conversion into C_{lipid} (concentration in the lipid). One classical approach of Kraaij et al. (2003) to assess the body burden of HOCs in benthic organisms is to multiply the freely dissolved concentration (C_{free}) in the water by the appropriate bioconcentration factor (BCF).

This study focuses on the improvement and replacement of classical exhaustive extraction techniques. For this purpose, a passive equilibrium sampling method (matrix SPME) for the measurement of the HOC fraction in biota was developed and tested. The newly developed method is based on a well-proven SPME fiber method and allows direct measurement, which is fast, robust and easy to perform. Further advantages of this method are that no clean-up steps of the sample are needed, no error-prone modeling or BCFs are required, as only the previously determined partition coefficients are sufficient.

Many organisms have been used for biomonitoring in the marine environment. Various studies have shown a close relationship between sediment contamination and bioaccumulation of HOCs in different benthic organisms (Jahnke et al. 2014a, Jahnke et al. 2012, Jahnke et al. 2014b, Schäfer et al. 2015). Suspension feeding bivalves, such as the mussel *Mytilus edulis,* are often used to estimate the water quality as they primarily filter the water. Sediment-feeding organisms such as the lugworm *Arenicola marina* are much better suited to capture the impact of HOCs in sediment (Kaag et al. 1998). Particularly, Kaag et al. (1997) have shown that the lugworm *Arenicola marina* accumulates organic contaminants to higher concentrations than filter feeder animals. Therefore, the lugworm *A. marina* was selected for this study, as it is the largest and quantitatively most important deposit-feeding member of the infauna of tidal flats in the Wadden Sea and it prefers sandy sediment with low organic content (Timmermann & Andersen 2003).

In the present study, the determination of the partition coefficients of HOCs between the lugworm *A. marina* and the PDMS sampling phase was the foundation for the matrix-SPME method development. Therefore, (i) the total tissue concentration of HOCs was measured, (ii) the lipid content of the tissue was determined and (iii) equilibrium passive sampling experiments with the biological material were performed. This novel approach to determine

internal tissue concentrations of hydrophobic organic compounds in biota shows promising results for assessing the chemical distribution of HOCs in organisms.

Material and Methods

Test organism *A. marina*

The lugworm *Arenicola marina* was chosen as a test organism because it is continuously exposed to contaminants in the sediment and is a quantitatively important deposit feeder at the base of the North Sea food web (Besseling et al. 2013). The large polychaete worm is commonly found on intertidal areas on both sides of the North Atlantic and tolerates a wide range of particle sizes and salinities (Gordon et al. 1978, Morales-Caselles et al. 2008). It forms 20 to 30 % of the benthic biomass in the western part of the Dutch Wadden Sea and it can be locally present in densities up to 100 individuals per square meter. Adults are approximately 20 - 25 cm long and 1 cm in diameter, whereby the front end is thicker and the rear end, containing the gut, is approximately half in diameter. The body is ringed or segmented. The head end is blackish-red, bears no tentacles or bristles and merges into the thicker red middle part. The worm lives in a U-shaped burrow and ingests large amounts of sediment in the subsurface and pumps extensive amounts of water through its feeding tunnel resulting in extensive bioturbation processes to depths of 15 cm or more (Christensen et al. 2002). *A. marina* ingests up to 20 times its body weight of wet sediment per day (Cadée 1976, Weston & Mayer 1998). The feeding rate is influenced by seasonal cycles as in summer the reworking process is one order of magnitude higher than in winter. This cannot exclusively be explained by temperature differences, because food availability and quality also seems to be an important factor (Cadée 1976, Timmermann & Andersen 2003). Furthermore, the lugworm has been recommended by Oslo-Paris Commission (1995) as a monitoring organism. The lugworm is better suited as a bioindicator compared to mussels, as it hardly absorbs HOCs via gut fluid extraction due to its short digestive tract, but mainly via the skin surface, which makes it very similar to the silicone fibers used in this study. In addition, the lugworm is not able to metabolize PAHs. Kraaij et al. (2003) showed that the distribution between deposit-feeders and pore water is in agreement with equilibrium partitioning.

Sampling of *A. marina*

For SPME method development, samples of *A. marina* were collected at three stations from tidal sand flats near Cuxhaven (Germany) in August 2009 (Figure 1) (Table S1). The lugworms were excavated with a shovel and placed in a bucket with seawater for 30 h to allow the worms

to empty their gut. Afterwards, the worms were dabbed dry using lint-free tissue and frozen in pre-cleaned aluminum boxes.

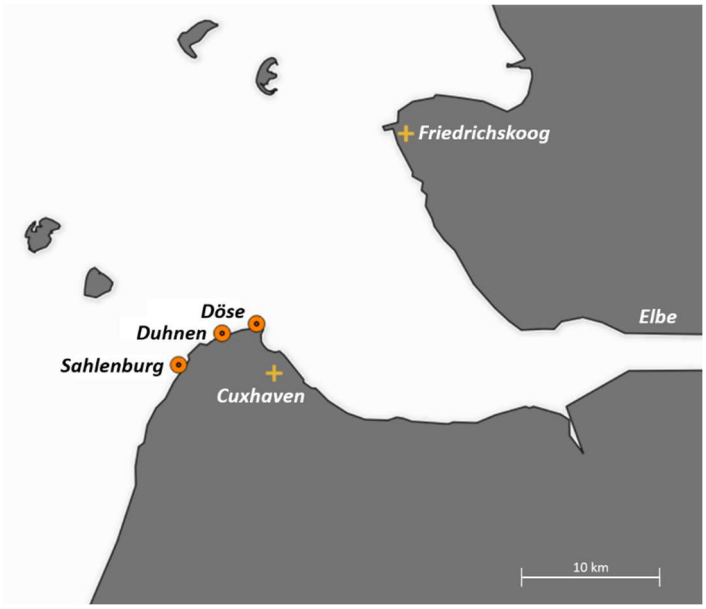


Figure 1: Lugworm sampling stations at Cuxhaven, Germany (North Sea).

Sample preparation

The frozen lugworms were thawed, combined to one bulk sample and intensively homogenized with pestle and mortar. The tissue sample was divided into three aliquots (30 g each) and spiked with a mix standard consisting of 16 EPA PAHs (PAH Mix 9 deuterated; Dr. Ehrenstorfer, Germany; Table S2) in three concentrations (sample 1, 2 and 3), which resulted in final extract concentrations of 100, 500 and 1000 pg μL^{-1} PAHs. The spiked tissue was shaken on a roller-shaker to allow the worm material to equilibrate with the standard PAH solution for 24 h. Each sample was then divided into two halves, whereas one was used for the SPME fiber experiments and the other one for extraction experiments.

Classical approach: Exhaustive worm extraction (C_{total})

An exhaustive extraction was performed to determine the total (bulk) concentration in the worm tissue. This step is necessary for the calculation of the distribution coefficients (K), which in turn are needed for the application of the SPME fiber method. For the exhaustive extraction, the spiked worm material (sample 1, 2 and 3) was divided into triplicates (10 g each) to allow for replicate evaluation at a later stage and then freeze-dried at -35 °C and 0.023 mbar using a Christ freeze dryer (Alpha 1-4 LD plus, Martin Christ GmbH). The water content of the worm material was determined to be approximately 80 %, resulting in 1 g dry weight of spiked worm material per concentration sample.

The total extraction was performed according to Witt et al. (2009) with some modifications. Briefly, 1 g dried worm material was extracted using the ASE 2000 system (Dionex™, USA). The samples were introduced in the Dionex cell and extracted with 80 mL of acetone-hexane (v/v = 40/60) at 140 bar and 100 °C. The lipid content of the worm material was determined as an intermediate step while processing the worm samples. Therefore, the obtained extracts were reduced nearly to dryness using a rotary evaporator (Laborota 4000 efficient; Heidolph Instruments, Germany) and then nitrogen evaporation until no change in weight was detectable. This weighed amount is called extractable organic matter (EOM) and is almost equal to the lipid content. Afterwards, the EOM was re-dissolved in an acetone-hexane mixture using an ultrasonic bath treatment. Elemental sulfur was removed using copper, followed by filtration using a Baker Bond system (J.T. Baker, USA) with Teflon frits and reduced to a maximum of 250 µL. The clean-up step was performed using a Hitachi LaChrom Elite HPLC system (pump L-2130; VWR Hitachi, Germany) using a silica gel column (LiChrospher Si 60-5; Merck, Germany). The extracts were again reduced to a maximum of 500 µL and the exact volume was determined using a microliter syringe (Hamilton, USA).

For the PAH and PCB concentration analysis in the extracts ($C_{total(AM)}$), a sample volume of 1 µL was injected into the GC-MS (GC 7890A/MS 5975C; Agilent, USA) and the inlet PTV temperature was raised from 20 °C to 250 °C with 12 °C s^{-1} (hold for 15 min). The desorbed analytes were transferred splitless onto the column and the GC oven temperature was programmed as follows: ramped from 50 °C to 190 °C with 1 °C min^{-1} (hold for 3 min) and ramped to 300°C ramped with 5 °C min^{-1} (hold for 10 min). Target analytes were the same as in SPME analysis. For quantification purposes, a ten-point internal/external standard calibration curve of PAHs (PAH Mix 9, PAH Mix 9 deuterated, Dr. Ehrenstorfer; Table S2) and PCBs (PCB Mix 3, Dr. Ehrenstorfer; Mass-Labelled PCB Mixture MBP-D7, Wellington Laboratories; Table S3) was generated by using the same analytical conditions as for the samples. Validity of the ten-point calibration curve was checked on a regular basis by re-running three levels (low-, mid-, high-calibration level) in line with the sample batches as QA/QC (see Table S4).

The target compounds of this study are the seven indicator PCBs (PCB 28, 52, 101, 118, 138, 153, 180) and the 16 US EPA PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[cd-1,2,3]pyrene, dibenzo[a]anthracene and benzo[ghi]perylene). The total worm concentrations ($C_{total(AM)}$) of

PAHs and PCBs were determined using the internal standard calibration method. Therefore, the PAH and PCB contents on dry weight basis were calculated by dividing the analyte's mass (M_A) in the sample extract by the dry weight mass of the worm material (M_{AM}) (Eq. 1). Afterwards, $C_{total(AM)}$ was normalized to the lipid content (EOM) of the worms.

$$C_{total(AM)} = \frac{M_A}{M_{AM}} \quad (1)$$

New approach: Development of the SPME fiber method

For the SPME fiber experiment, commercially available glass fibers with a nominal PDMS coating of 10 µm (Fiberguide Industries, USA) were used. The slightly deviating fiber properties identified by Witt et al. (2013) were used for PDMS concentration calculations. Fiber properties are described in Supplemental Information, Table S5. The fibers were pre-cleaned by extracting twice with methanol and ultrapure water in an ultrasonic bath. For SPME, 5 g of each spiked lugworm tissue material was weighed into chromacol vials (Thermo Scientific, USA) and the vials were closed with a septum containing cap (chromacol rubber/PTFE seal, 12 mm, Thermo Scientific). Three fibers (10 cm in length) were placed in each vial through a cannula to pierce through the septum of the cap. The vials were shaken on an overhead shaker for 2 days at 20 ± 2 °C in darkness until equilibrium between worm material and fibers was reached.

One reason for this choice of time period was the assumption that lipid-rich material equilibrates with the PDMS material in a very short time. Ossiander et al. (2008) investigated the uptake kinetics for two classes of chemicals (Fluoranthene and PCBs) in harbor porpoise blubber and the earthworm. Depending on the lipid content of the matrices, equilibrium times between 10 s and 60 min were achieved in PDMS fibers of 10 – 50 µm coating thicknesses. In addition, Jahnke et al. (2009) confirmed a rapid equilibration within the range of hours in lipid-rich fish (i.e., eel) by using PDMS thin-films of multiple thicknesses (140 – 620 µm). Both studies conducted a static uptake experiment, which leads to the assumption that a dynamic shaking of the *Arenicola marina* tissue might accelerate the uptake into the PDMS. Furthermore, the use of a significantly lower PDMS thickness (10 µm), which exhibits a higher area-to-volume ratio of the sampling phase, also implies faster uptake kinetics compared to Jahnke et al. (2009). A second reason was to prevent the fibers from biofouling, which may easily occur at room temperature without disinfection. During the experiment, the vials were regularly checked visibly and nasally for biofouling and odor. Finally, the fibers were removed from the vial,

cleaned with ultrapure water and lint-free tissue, wrapped in pre-cleaned aluminum foil sheets (8 h baking out at 300 °C) and stored at -20 °C until analysis.

PDMS fiber coating concentrations ($C_{PDMS(AM)}$)

The PAH and PCB concentrations in the PDMS fiber coatings ($C_{PDMS(AM)}$) were determined using a GC-MS method from Lang et al. (2015). Briefly, a gas chromatograph (GC 7890A; Agilent) and a quadrupole mass spectrometer (MS 5975C; Agilent, USA) equipped with an automated liner exchange system (ALEX; Gerstel, Germany) was used. Prior to loading, the glass liners (KAS4; Gerstel, Germany) were plugged with deactivated glass wool, rinsed three times with hexane and pre-heated at 250 °C for 19 minutes under helium flow in the GC injector in order to remove organic residues. The exact fiber length was measured for each equilibrated fiber and then placed in a cleaned glass liner. The loaded liner was transferred by MultiPurpose Sampler (MPS; Gerstel, Germany) into the cooled injection system (CIS) for thermal desorption (50 °C raised to 250 °C at 12 °C s^{-1}, then held for 15 minutes). The desorbed analytes were transferred in splitless mode onto the column (HP-5MS, 30 m x 250 µm x 0.25 µm; J&W Scientific, USA) and the GC oven temperature was programmed as follows: (1) 60 °C (hold for 15 min), (2) ramped to 195 °C with 15 °C min^{-1} (hold for 2 min), (3) ramped to 225 °C with 15 °C min^{-1} (hold for 0 min), (4) ramped to 260 °C with 5 °C min^{-1} (hold for 0 min) and (5) ramped to 300 C with 20 °C min^{-1} (hold for 10 min), whereby the transfer line temperature was kept constantly at 310 °C. The native compounds were measured in selected ion mode (SIM). External standard calibrations of PAHs and PCBs (PAH Mix 9, PCB Mix 3; Dr. Ehrenstorfer, Germany) were used to quantify target analytes desorbed from the fibers.

The PDMS concentrations ($C_{PDMS(AM)}$) were calculated by dividing the total mass (M_{PDMS}) of each analyte sorbed to the fiber, which was detected with mass spectrometry, by the coating volume (V_{PDMS}) of the fiber:

$$C_{PDMS} = \frac{M_{PDMS}}{V_{PDMS}} \quad (2)$$

Calculation of partition coefficients of HOCs ($K_{total/lipid:PDMS(AM)}$)

The compound-specific partition coefficients ($K_{total/lipid:PDMS(AM)}$) between the tissue of *Arenicola marina* (AM) and the PDMS material were calculated as the ratio of the total HOC concentrations in the worm tissue, derived from extract measurements and normalized to the

lipid content ($C_{total/lipid(AM)}$) and the concentration in the PDMS coating ($C_{PDMS(AM)}$), derived from the SPME fiber experiments:

$$K_{total/lipid:PDMS(AM)} = \frac{C_{total/lipid(AM)}}{C_{PDMS(AM)}} \quad (3)$$

SPME method application

Individuals of the lugworm *A. marina* were collected from tidal sand flats near Wilhelmshaven (53°37'51.8"N 8°05'51.7"E, Germany; air-line distance to first sampling station: 50 km) in July 2018 (Figure 2). The lugworm preparation for freezing was performed as described before. The lipid content of the lugworms was determined as EOM by extracting the freeze-dried lugworm homogenates three times with 10 mL n-hexane each by placing it in an ultrasonic bath for 15 minutes. The extracts were filtered through glass fiber filters using a Baker Bond system (J.T. Baker, USA). Extract volumes were evaporated under a nitrogen stream nearly to dryness until no change in weight was detectable.

Figure 2: Lugworm sampling stations at Wilhelmshaven, Germany (North Sea).

Four lugworms were examined for their HOC load via SPME. Since lugworms are characterized by their bipartite physique, also samples of their abdomen and their tail were analyzed separately using SPME. For sample preparation, the frozen lugworms were blended, transferred into chromacol vials (Thermo Scientific, USA) and three cleaned fibers were placed in each vial. In contrast to the described fiber experiments for method development, the worm material was given only 16 hours for equilibration as the equilibrium state was already reached.

The total PAH concentrations in the worms ($C_{total(AM)}$) were calculated using the compound-specific partition coefficients ($K_{total/lipid:PDMS(AM)}$) between the tissue of *A. marina* (AM) and the PDMS material. Afterwards, $C_{total(AM)}$ was normalized to the lipid content (EOM) of the worms (see equation 3).

Results and Discussion

SPME fiber method development: Outcome and observations

Good sample preparation is essential for chemical analysis as the detection accuracy is dependent on the sample preparation. In this study, the homogenizing step of the sample with pestle and mortar proved to be slightly difficult. Although the sample was treated intensively, the homogeneous pulpy mass still contained some larger worm pieces that could hardly be crushed. This could have been prevented by adding quartz glass to allow effortless homogenization. Using a blender was impractical because the amount of worm material was too small. The SPME experiments using 10 µm PDMS fibers proved to be practicable without any complications. Considerations beforehand indicating that biofouling would affect the SPME fiber properties throughout the sample preparation and experiment were not of any concern during the entire processing time. Adhering tissue did not alter the fiber surface in terms of adsorbed unremovable particles resulting in disturbance of chromatographic analyses or overestimation of the HOC concentrations. The duration of sample processing and SPME equilibration appeared to be sufficient and did not deteriorate the sample treatment quality and analytical process. Biofouling was not notable in differences in odor or visual appearance of the worm homogenate and no alterations on the fiber surfaces were observed. Ossiander et al. (2008) also demonstrated that the procedure of immersing the fiber directly in tissue neither depleted the sample of analytes nor modified the partitioning properties of the PDMS. The extraction method using an ASE followed by sample clean-up via the Baker Bond system and HPLC proofed suitable for the analysis of the total HOC concentrations in the worm material.

Quality control and assurance

The relative standard deviations (RSD) of the fiber analyses ranged between 3.0 - 12.5 % for PAHs (n = 3) and 7.2 and 12.5 % for PCBs (n = 9), whereas the RSD of the extract analyses were between 0.43 - 21.5 % for PAHs (n = 3) and 11.1 and 23.3 % for PCBs (n = 9). The results of the SPME analyses showed approximately 10 % lower RSDs compared to the conventional extraction technique. The insufficient homogenization of the worm material might account for the higher RSDs in the extract analyses but plays a minor role in the fiber experiments.

For quality assurance of the SPME fiber experiment, at least three fibers were tested and the GC liners were checked for chemical purity. Cleaned and unexposed fibers served as the analytical blanks and target compounds were not detected in any procedural blank. External standard solutions (PAH-Mix 9 and PCB-Mix 3, Dr. Ehrenstorfer) were used for quantification. Mean standard recoveries of the target compounds were found to be 92 ± 12 %. Method detection and quantification limits (MDLs and MQLs) were calculated by adding three times (MDL) or ten times (MQL) of the standard deviation to the average PAH and PCB mass in the blank fibers. Conversion of the masses into fiber coating concentrations (C_{PDMS}) (pg μL^{-1}) resulted in MQLs between 3.6 to 8.9 pg μL^{-1} for PAHs and 3.2 to 7.2 pg μL^{-1} for PCBs. The MDLs were lower than 2.0 pg μL^{-1} for all target compounds. MQL based on freely dissolved concentrations (MQL_{free}) was lower and decreased with increasing PDMS-to-water partition coefficient (K_{PDMS}).

The quality assurance of the extracts was performed using an internal and external standard solution (PAH Mix 9, PAH Mix 9 deuterated, Dr. Ehrenstorfer) and PCBs (PCB Mix 3, Dr. Ehrenstorfer; Mass-Labelled PCB Mixture MBP-D7, Wellington Laboratories). Mean standard recoveries from internal and external PAH and PCB target compounds were 90 % ± 15 %. MQLs were between 11 and 24 pg μL^{-1} and 5 and 18 pg μL^{-1} for PAHs and PCBs, respectively. MDLs were lower than 10 pg μL^{-1} for all target compounds. Target PAHs and PCBs were not detected in any procedural blank.

Partition coefficients of PAHs and PCBs

The partition coefficients (log $K_{total/lipid:PDMS(AM)}$) between the total HOC concentrations in the worm material normalized to lipid and PDMS material for spiked PAHs and native PCBs as mean values (n = 9) were calculated (Table 1). Correlation between the measured concentration in the PDMS material ($C_{PDMS(AM)}$) and the total measured concentrations in the tissue normalized to the lipid content ($C_{total/lipid:PDMS(AM)}$) for all 7 native PCBs yielded a correlation coefficient of $R^2 = 0.815$. For the individual PAHs correlation coefficient values of $R^2 > 0.9$, except for acenaphthylene ($R^2 = 0.71$) as well as benzo[b]fluoranthene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene ($R^2 > 0.82$).

The equilibrium partitioning coefficients present the possibility for converting equilibrium sampling data from many environmental matrices into a more informative form (Jahnke et al. 2008). Jahnke et al. (2008) also showed that the equilibrium partitioning of neutral lipophilic environmental contaminants into the lipids of three investigated species, representing three trophic levels, would be very similar. Higher concentrations in the organisms would indicate

biomagnification, similar levels would indicate equilibrium partitioning whereas lower concentrations would hint at a kinetic uptake situation or metabolism (Jahnke et al. 2008).

Table 1: Distribution coefficients between the total concentrations in the lugworm material normalized to lipid and PDMS material for spiked PAHs and natural PCBs (n = 9) and the relative standard deviations (RSD).

compound	log $K_{total/lipid:PDMS(AM)}$ [L kg^{-1}]	± RSD [%]
Acy	0.66	16.5
Ace	0.64	13.2
Fl	0.65	15.7
Phen	0.80	10.9
Anth	0.75	12.1
Fluo	1.00	8.1
Pyr	1.09	8.6
BaA	1.17	11.5
Chr	1.40	13.4
BbF	1.56	12.5
BkF	1.50	5.9
BaP	1.75	8.0
InP	1.80	7.6
BghiP	1.91	4.4
PCB 28	0.75	13.1
PCB 52	0.70	12.8
PCB 101	0.74	13.5
PCB 118	0.84	8.2
PCB 138	0.80	7.4
PCB 153	0.85	6.6
PCB 180	0.82	11.0

The established lipid-rich matrix-SPME method using PDMS-coated glass fibers and lugworm tissue enables a faster and low-budget approach to determine the concentrations of complex matrices. In addition, this ´green´, solvent-free and automated method could replace traditional solvent techniques for the measurement of HOCs in biological material (see Table S6 for a detailed comparison of the two techniques).

Method application

The average lipid content (EOM) of three lugworms (W1-W3) was determined to be 3.16 ± 0.18 % (Table S7). The determination of the HOC concentrations were conducted for four lugworm samples (L1-L4), where two worms were divided into the abdomen and the tail part (L3 and L4). Stollberg et al. (2021) showed that the Biota-SPME method is suitable for lipid contents greater than one percent. The analysis in the lugworm tissue revealed a nearly equal load of the individual pollutants (256 ± 55 µg g^{-1} for PAHs and 4 ± 0.9 µg g^{-1} for PCBs) (Figure 3; Table S8). The concentrations of the individual substances also showed a similar

distribution pattern. Benzo[a]pyrene dominated the chemical load for all PAHs, while PCB 180 showed the highest concentrations of all PCBs in all samples. In addition, it can be seen that the higher molecular weight PAHs and the higher chlorinated PCBs, with exception of PCB 28, accounted for the largest proportion of chemical load in the lugworm tissue. Since many HOCs are known to be carcinogenic to humans and other mammals, it is important to investigate the fate of HOCs in the environment and identify sites where they can accumulate in significant concentrations. In particular, higher molecular weight compounds have a greater tendency to bioaccumulate.

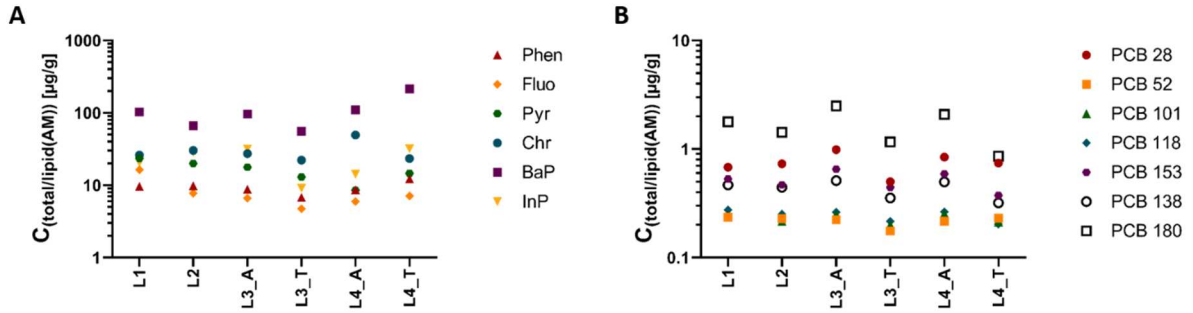


Figure 3: Lugworm tissue concentrations of PAHs (A) and PCBs (B) in the different lugworm samples. The whole lugworm was sampled for samples L1 and L2, whereas for samples L3 and L4, the worms were divided into abdomen (A) and tail (T) parts. Only the six most abundant PAHs were included for this analysis (A).

In general, the chemical load of PAHs and PCBs differed in their composition marginally (Table S8). All samples were dominated by the higher molecular weight PAHs, whereas BaP was followed by chrysene and InP (Figure 4). The contamination with BaP in the lugworms was between 24 and 63 % of the total HOC concentration (L2 and L4_T, respectively) indicating a high bioaccumulation level. The ratio of Chr was between 22 and 49 % in the lugworms L1, L2, L3_T and L4_A, while InP was the second-highest proportion in L3_A and L4_T (both 32 %). The percentage of PCBs was below 2.2 % in all lugworm samples. However, the PCB concentrations of the abdomen part were marginal higher compared to the tail part. The subdivision of the worm into abdomen and tail for a differentiated examination of the total HOC concentrations showed that no differences could be detected. Therefore, the whole worm itself can be examined in the future.

The mean PAH body residue values of this study (mean value: 256 µg g^{-1}) were considerably higher compared to literature data. Kaag et al. (1998) reported PAH levels below 12 µg g^{-1} (North Sea, Netherlands) and Ramos-Gómez et al. (2011) described PAH concentrations of up to 3.5 µg g^{-1} (Spain and Canary Islands). The PCB concentrations assessed by Mattig et al. (1996) of approximately 0.8 µg g^{-1} for the sum of 8 PCB congeners in *A. marina* tissue from

the Wadden Sea (North Sea, Germany), which is about 5 times lower than in the body burden level of this study. The lugworm *A. marina* is less sensitive compared to other species and can tolerate PAH-contaminated environments and accumulate PAHs (Morales-Caselles et al. 2008). This is clearly shown by the significantly higher body residues of PCBs and especially PAHs in the lugworms in this study. The high body concentrations are probably site-specific and might indicate a high level of sediment contamination. But the sediment concentrations could not be measured as sediment samples of this site were not available. However, the sampling site was located in the area of the Wilhelmshaven harbor and in direct proximity to a refinery which processes crude oil and refined fossil fuels. Since PAHs represent typically up to 60 % of oil constituents, the high body residues of the lugworm might be a consequence of the oil processing at this location (Dupuis & Ucan-Marin 2015).

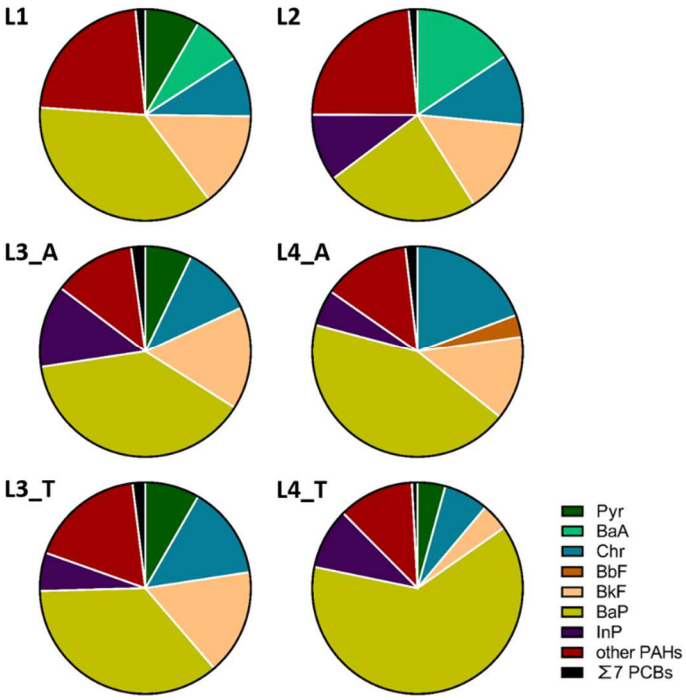


Figure 4: Comparison of the composition of $C_{total/lipid(AM)}$ between the lugworm samples. The whole lugworm (L1 and L2) and abdomen (A) and tail (T) parts were sampled of lugworm L3 and L4.

Monitoring programs should therefore also consider the sediment-water-biota fluxes in their entirety, in order to gain a better understanding of the chemical load of all compartments. Especially since Kaag et al. (1998) showed that the concentrations in the lugworms were not directly proportional to the concentrations in the sediments. This clearly shows that sediment-feeding benthic animals should be included in monitoring programs, as they can provide additional values compared to the sediment analysis alone. However, the sampling of lugworms for environmental monitoring should be performed in a restricted period relative to the

reproduction cycle as lugworms show seasonal fluctuation in the body residue levels of contaminants (Kaag et al. 1998).

The BCF is an important concept in environmental risk assessment as it provides quantitative information on the ability of a pollutant to be taken up by organisms from water. However, it is a variable that depends on different ecological and biological conditions. In this study, bioaccumulation factors could not be determined as sediment samples from the lugworm sampling site were not available. However, the same SPME method can be also applied for assessing the chemical load of sediment pore water. Kaag et al. (1997) showed that the accumulation factors of PAHs and PCBs appeared to be related to the log K_{ow} and that the factors increased with increasing log K_{ow}, until a maximum was reached. At higher log K_{ow}'s (> 5 – 6) the accumulation potential decreased again. However, the log Kow is not the only factor determining the accumulation factors, as molecular size and structure may result in different rates of accumulation and elimination but the manner in which different compounds are bound to the sediment matrix may also be influenced (Kaag et al. 1997). In addition, Oliver (1987) demonstrated that sediments with a high organic content have the lowest concentration factors, indicating a lower bioavailability of contaminants. In conclusion, the risk for benthic invertebrates cannot be assessed by using chemical equilibrium partitioning alone. Food sources and feeding habits have also been taken into account, especially when contaminants with a log K_{ow} value of 5-6 are considered (Kaag et al. 1997).

Conclusion

This study has verified that the SPME method is a very sensitive tool that opens new possibilities for studying the in-situ distribution and thermodynamics of hydrophobic organic chemicals at trace levels in biota. Using the SPME approach, a holistic investigation of the environmental compartments water, sediment and biota of different environments can be conducted. The applied SPME method is considerably faster and less error-prone compared to conventional methods. Moreover, the HOC contamination in sediment and lugworms can be determined quickly, seasonally and covering a wide area, making biological monitoring much more effective. Changes in the chemical contamination can thus be recorded more quickly and better attributed to local events. Furthermore, future research should also include assessments on different levels of biological organization in order to determine the risks these organic contaminants may pose to an entire ecosystem.

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Supporting Information

Supplementary Table 1

Table S1: Sampling coordinates at Cuxhaven and Wilhelmshaven, Germany (North Sea).

Station	Coordinates	
	North	East
Sahlenburg	N53° 52.008'	E8° 35.065'
Duhnen	N53° 53.400'	E8° 38.551'
Döse	N53° 53.630'	E8° 40.521'
Wilhelmshaven	N53° 37.863'	E8° 05.730'

Supplementary Table 2

Table S2: Analyte composition of the PAH standards

PAH 9 Mix	PAH 9 Mix deuterated
Acenaphthene	Acenaphthene D10
Acenaphthylene	Acenaphthylene D8
Anthracene	Anthracene D10
Benz[a]anthracene	Benzo(a)pyrene D12
Benzo(k)fluoranthene	Benzo(b)fluoranthene D12
Benzo[a]pyrene	Benzo(g,h,i)perylene D12
Benzo[b]fluoranthene	Benzo(k)fluoranthene D12
Benzo[ghi]perylene	Benzo[a]anthracene D12
Chrysene	Chrysene D12
Dibenzo(a,h)anthracene	Dibenzo(a,h)anthracene D14
Fluoranthene	Fluoranthene D10
Fluorene	Fluorene D10
Indeno[1,2,3-cd]pyrene	Indeno(1,2,3-c,d)pyrene D12
Naphthalene	Naphthalene D8
Phenanthrene	Phenanthrene D10
Pyrene	Pyrene-d10

Supplementary Table 3

Table S3: Analyte composition of the PCB standards

PCB Mix 3	Mass-Labelled PCB Congener Mix
2,2',3,4,4',5'-Hexachlorobiphenyl	2,4,4'-Trichloro($^{13}C_{12}$)biphenyl
PCB No. 101	2,2',5,5'-Tetrachloro($^{13}C_{12}$)biphenyl
PCB No. 118	2,2',4,5,S'-Pentachloro(13C12)biphenyl
PCB No. 153	2,3',4,4', 5-Pentachloro($^{13}C_{12}$)biphenyl
PCB No. 180	2,2',3,4,4',S'-Hexachloro($^{13}C_{12}$)biphenyl
PCB No. 28	2,2',4,4',S,5'-Hexachloro($^{13}C_{12}$)biphenyl
PCB No. 52	2,2',3,4,4',S,5'-Heptachloro($^{13}C_{12}$)biphenyl

Supplementary Table 4

Table S4: Concentration levels of the ten-point calibration curve. Three calibration levels (low-, mid-, high) were used for validation of the ten-point calibration curve and are asterisked (*).

concentration [pg μL^{-1}]
5
10*
20
40
60
80*
120
160
180*
200

Supplementary Table 5

Table S5: Important fiber parameters and fiber geometry calculated for 1 cm fiber length; GF: fiber with glass core and PDMS coating; HF: PDMS tube/hollow fiber; R=r+s (* mean values for 12 replicates, from Witt et al., 2013).

name	coating thickness* s [µm]	inner radius r [µm]	outer radius R [µm]	fiber length L [cm]	PDMS-volume V [µL cm^{-1}]	surface area A [cm^2]	A/V ratio [cm^2 cm^{-3}]
GF10	12.7	103.6	116.3	1	0.0877	730.7	8329
GF30	26.7	53.6	80.3	1	0.1123	504.5	4492
HF40	48.1	85.3	133.4	1	0.3305	838.2	2536

Supplementary Table 6

Table S6: Comparison of the extraction method with the newly developed SPME fiber method.

	Exhaustive extraction method	**SPME fiber method**
time	time and cost consuming (more process steps and more chemicals necessary)	faster (less preparation and process steps)
expandable materials / equipment	freeze-drying system, ASE (Accelerated Solvent Extraction system) or soxhlet, solvents	SPME fibers, immersion blender/pestle and mortar, overhead shaker
sample preparation	sample clean-up using HPLC system or SPE, solvents, rotary evaporator	no sample clean-up necessary
analytical measurement	direct GC-MS measurement from evaporated extracts	indirect GC-MS measurement from fibers using partitioning coefficients (K) for
intended result	total concentration in the worm material	total concentration in the worm material

Supplementary Table 7

Table S7: Mean lipid contents of three lugworm samples each measured six times and the standard deviations (SD).

sample	**mean lipid content [%]**	**SD**
W1	3.35	0.08
W2	2.96	0.08
W3	3.17	0.10
total	3.16	0.18

Supplementary Table 8

Table S8: Lugworm tissue concentrations of PAHs and PCBs in the different lugworm samples. The whole lugworm was sampled for samples L1 and L2, whereas for samples L3 and L4, the worms were divided into abdomen (A) and tail (T) parts. Only the six most abundant PAHs were included for this analysis.

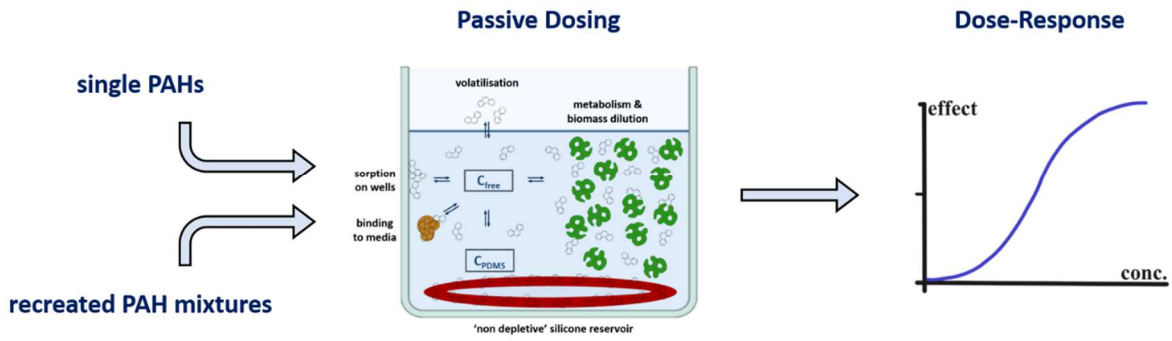
	L1 [µg g^{-1}]	**L2** [µg g^{-1}]	**L3_A** [µg g^{-1}]	**L3_T** [µg g^{-1}]	**L4_A** [µg g^{-1}]	**L4_T** [µg g^{-1}]
Phen	9.62	9.80	8.78	6.76	8.64	12.23
Fluo	16.35	7.76	6.62	4.71	5.94	7.09
Pyr	23.51	19.99	17.77	12.98	8.52	14.57
Chr	26.10	30.25	27.31	22.17	49.31	23.44
BaP	102.46	66.19	96.21	55.88	110.91	214.67
InP	20.56	28.66	31.56	9.09	14.15	31.81
PCB 28	0.68	0.73	0.98	0.50	0.84	0.74
PCB 52	0.24	0.23	0.22	0.18	0.22	0.23
PCB 101	0.25	0.22	0.25	0.20	0.25	0.21
PCB 118	0.27	0.25	0.26	0.22	0.26	0.20
PCB 153	0.53	0.47	0.65	0.44	0.59	0.37
PCB 138	0.47	0.44	0.51	0.35	0.50	0.32
PCB 180	1.78	1.42	2.49	1.16	2.08	0.86

Supplementary Table 9

Table S9: Proportions of the individual HOCs in the total concentration of the respective worm samples.

	L1 [%]	L2 [%]	L3_A [%]	L3_T [%]	L4_A [%]	L4_T [%]
Acy	0.48	1.44	0.17	2.11	0.33	1.64
Ace	0.61	3.61	0.60	0.59	0.70	0.75
Fl	0.49	0.66	0.49	0.60	0.48	0.69
Phen	3.42	3.52	3.53	4.34	3.38	3.58
Anth	0.24	0.16	0.28	0.39	0.24	0.31
Fluo	5.81	2.78	2.66	3.02	2.33	2.08
Pyr	8.36	7.18	7.14	8.34	3.34	4.27
BaA	7.56	15.64	0.99	1.39	1.23	0.65
Chr	9.28	10.86	10.97	14.24	19.31	6.87
BbF	3.82	3.79	3.69	4.85	3.41	1.84
BkF	14.51	14.48	15.86	16.11	12.95	4.18
BaP	36.42	23.76	38.64	35.89	43.44	62.90
InP	7.31	10.29	12.67	5.84	5.54	9.32
BghIP	0.20	0.47	0.16	0.34	1.46	0.06
PCB 28	0.24	0.26	0.40	0.32	0.33	0.22
PCB 52	0.08	0.08	0.09	0.11	0.08	0.07
PCB 101	0.09	0.08	0.10	0.13	0.10	0.06
PCB 118	0.10	0.09	0.11	0.14	0.10	0.06
PCB 153	0.19	0.17	0.26	0.29	0.23	0.11
PCB 138	0.17	0.16	0.21	0.23	0.20	0.09
PCB 180	0.63	0.51	1.00	0.75	0.82	0.25
Sum	100	100	100	100	100	100

Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to the microalgae *Raphidocelis subcapitata*



Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to the microalgae *Raphidocelis subcapitata*

Anne Kreutzer[a,b], Sonja Faetsch[c], Susanne Heise[c], Henner Hollert[a], and Gesine Witt[b]

[a] Department Evolutionary Ecology and Environmental Toxicology, Institute of Ecology, Evolution and Diversity, Faculty Biological Sciences, Goethe University Frankfurt, Frankfurt 60438, Germany

[b] Department of Environmental Engineering, Faculty Life Sciences, Hamburg University of Applied Sciences, Hamburg 21033, Germany

[c] Department of Biomedical Engineering, Faculty Life Sciences, Hamburg University of Applied Sciences, Hamburg 21033, Germany

Abstract

Risk assessment of hydrophobic organic compounds (HOCs) is difficult because maintaining a well-defined exposure during aquatic toxicity testing is challenging due to the limited water solubility and various loss processes such as volatilization, biodegradation and sorption. Passive dosing techniques help to overcome these challenges by providing a well-controlled and solvent-free exposure. In this study, the algal growth inhibition test (DIN EN ISO 8692) was converted into a miniaturized passive dosing setting. For this purpose, biocompatible O-rings were used as substance reservoirs and loaded with polycyclic aromatic hydrocarbons (PAHs). The growth inhibition of the microalgae *Raphidocelis subcapitata* induced by single PAHs (log K_{OW} 3.24–5.91) was investigated. In addition, recreated PAH mixtures were tested representing field compositions of the pore water North Sea sediments. Some of the single PAHs revealed strong growth inhibiting effects on the algal growth, while the recreated mixture compositions had slightly lower effect on the growth inhibition in the highest concentrations. Overall, the toxicity of the PAHs generally increased with the maximum chemical activities (a_{max}) of the PAHs and the inhibition data could be fitted with one maximum chemical activity response curve. Therefore, the miniaturized passive dosing approach appears as a promising practical and economical method that can be used for toxicity testing of the different trophic levels to improve comprehensive risk assessment.

Introduction

Substance-specific information on aquatic toxicity is essential for comprehensive risk assessment and management of contaminated water resources. The partitioning passive sampling approach is already well established as it can quantify hydrophobic organic chemicals (HOCs), i.e. polycyclic aromatic hydrocarbons (PAHs), from water samples at trace concentrations (Brack et al. 2017). PAHs are entering the aquatic environment from both natural and anthropogenic sources and due to their widespread distribution, there is a need to assess, and mitigate where necessary, the risks these substances pose to aquatic life (Bragin et al. 2016). The assessments of the bioavailable fraction in terms of environmental fate, exposure, and effects of these compounds are urgently needed. However, maintaining a well-defined exposure during aquatic toxicity testing is difficult due to the limited water solubility and various loss processes including volatilization, biodegradation and sorption (Mayer et al. 1999, Smith et al. 2010a, Smith et al. 2010b). In addition, the analysis of the freely dissolved concentrations (C_{free}) in the test medium is by no means straightforward and dynamic exposures

can complicate the determination of robust toxicity test endpoints (Kiparissis et al. 2003, Stibany et al. 2017a). The use of passive dosing techniques helps to overcome these challenges by delivering a well-controlled and solvent-free exposure. Passive dosing is based on a constant test substance release into the aqueous medium by equilibrium partitioning, where a polymer (e.g. silicone) is loaded with the test substance and acts as a reservoir (Mayer et al. 1999, Smith et al. 2010a, Smith et al. 2010b, Stibany et al. 2017a).

The challenge for comprehensive risk assessment is to use a high-throughput, easy handling and cost-efficient method for bioassays in order to be able to conduct an area-wide inventory. The application of the passive dosing bioassay was therefore performed in polystyrene well plates, despite the sorption losses. Polydimethylsiloxane (PDMS) O-rings are ideal as dosing phase for assays in multi-well plates as they are made of food-grade silicone and are commercially available in multiple standardized sizes (Stibany et al. 2017a). Therefore, O-rings are compatible with many different aquatic toxicity test systems as they also allow a plate reader measurement during exposure. PDMS O-rings have proven versatile for the passive dosing approach of HOCs in environmental and toxicological testing (Bragin et al. 2016, Gilbert et al. 2014, Mayer & Holmstrup 2008, Mayer et al. 1999, Niehus et al. 2018, Seiler et al. 2014, Smith et al. 2010b, Smith et al. 2012).

The test compound concentration in the O-rings can be varied to apply a wide range of freely dissolved concentrations. Hence, passive dosing can be flexibly used for testing at HOC aqueous solubility, concentration-response testing or chemical mixture toxicity (Smith et al. 2010a). Exposure to a mixture of chemicals allows for a more realistic testing approach, as organisms in the environment are exposed to mixtures. In addition, the measured HOC composition of a specific location can be tested by recreating the mixture on the dosing phase and introducing it into the bioassay.

For this purpose, the free dissolved concentrations (C_{free}) measured in the surface sediment pore water of four sites in the German Bight (North Sea) were reconstructed in their respective composition. Two stations were located close to the island Helgoland and are influenced by a dredged material dumping site from the port of Hamburg. The contamination level was higher compared to the other two sites located in the Wadden Sea area (Kreutzer et al. in prep.). The recreated PAH mixture from all four stations was applied to silicone O-rings and due to the equilibrium partitioning between the O-rings and the test medium, the same composition as in the sediment pore water was obtained. Niehus et al. (2018) and Rojo-Nieto et al. (2012) have already demonstrated a successfully reproduced PAH mixture in bioassays using passive

dosing, wherefore this approach is a promising tool for introducing defined mixtures into toxicity tests.

In the current study, the algal growth inhibition test with the microalgae *Raphidocelis subcapitata* was adapted for the first time to a passive dosing format in multi-well plates. Individual PAHs as well as realistic PAH mixtures were tested. The algal growth inhibition test was selected for the present study as it is required for environmental risk assessment of chemicals (European Union 2006). Furthermore, the unicellular microalgae have a much larger surface area to volume ratio compared to daphnia and fish resulting in much faster bioconcentration kinetics and allowing equilibrium concentrations to be reached in the test organism within a shorter period of time (Stibany et al. 2017b).

The main objectives of this study were: (1) to optimize the application and practicability of passive dosing using PDMS O-rings in a miniaturized format using well plates; determination of toxicities of (2) single PAHs and (3) recreated PAH mixtures and, (4) the comparison of the study data with earlier literature toxicity data for PAHs using passive dosing or conventional dosing methods and other organisms.

Material and Methods

Chemicals and materials

Food-grade silicone O-rings with an outer diameter of 14.4 mm, inner diameter of 9.6 mm, mass of 223 ± 1.98 mg (n = 20) and a density of 1.2 g cm^{-3} (ORS-0096-24, Altec, Cornwall, United Kingdom) made of polydimethylsiloxane (PDMS) were used as passive dosing device. As test vessels, 24-well flat-bottom polystyrene plates (Costar® Flat Bottom Cell Culture Plates, Corning®) were used. The following 11 PAHs were selected based on the sixteen High Priority Pollutants by the Environmental Protection Agency (EPA): naphthalene (Naph; ≥ 98.5 %), acenaphthene (Ace; ≥ 98.5 %), fluorene (Fl; ≥ 99 %), phenanthrene (Phen; ≥ 99.5 %), anthracene (Anth; ≥ 99 %), pyrene (Pyr; ≥ 97.5 %), fluoranthene (Fluo; ≥ 98 %), chrysene (Chr; > 98 %), benz[a]anthracene (BaA; ≥ 98.5 %), benzo[k]fluoranthene (BkF; ≥ 99 %) and benzo[a]pyrene (BaP; ≥ 96 %; all substances were obtained from Sigma). 3,5-Dichlorophenol (97 %; Sigmal Aldrich) was used as positiv control.

Preparation of O-rings

The cleaning and loading process of the silicon O-rings was performed according to Smith et al. (2010b). Briefly, O-rings were cleaned overnight in ethyl acetate, followed by three times overnight in methanol. Finally, O-rings were rinsed three times overnight in ultrapure water. Cleaned O-rings were then stored in ultrapure water.

A loading approach was applied based on partitioning from a methanol standard solution where individual PAHs were dissolved to saturation level (Booij et al. 2002). Methanol (MeOH) was used as loading solvent since PAHs are sufficiently soluble. The minimum volume of PAH loading solution for one O-ring was calculated to prevent the loading solution from becoming depleted during the loading step (Smith et al. 2010a). The loading of one O-ring required at least 2 mL of loading solution, for acenaphthene at least 2.1 mL.

$$V_{MeOH} = \frac{K_{silicone:MeOH} * V_{silicone}}{\frac{1}{fraction_{MeOH} - 1}} \quad (1)$$

The saturated solution was diluted in steps of two (1:1 (V1), 1:2 (V2), 1:4 (V3), 1:8 (V4) and 1:16 (V5) to obtain a dose-response curve. O-rings were loaded in these solutions at 21 °C and allowed to equilibrate for 72 h on an orbital shaker (150 rpm). The O-rings were removed, wiped dry and rinsed three times with a small volume of ultrapure water for 1 h to remove any residual methanol. The dried O-rings were wrapped in pre-cleaned aluminum foil and stored at -20 °C.

Calculation of exposure concentration in test medium

The release of the PAHs from the methanol solutions to PDMS O-rings and then to the aqueous phase can be calculated by applying Equations (2) to (4):

$$C_{PDMS} = \frac{C_{MeOH}}{K_{MeOH:PDMS}} \quad (2)$$

$$C_{water} = \frac{C_{PDMS}}{K_{PDMS:water}} \quad (3)$$

where C_{MeOH}, C_{PDMS} and C_{water} is the PAH concentration in the methanol stock solution, PDMS and water, respectively and $K_{MeOH:PDMS}$ and $K_{PDMS:water}$ are the corresponding partition coefficients between these phases (Table S1).

Since the solubility of PAHs in salty medium is decreased due to the salting-out effect, the salt concentration of the algae medium has to be considered. The Setschenow (1889) equation was used to calculate the predicted concentration in algae medium.

$$C_{iw,salt}^{sat} = C_{iw}^{sat} * 10^{-K_i^S * [salt]_{total}} \quad (4)$$

Where $C_{iw,salt}^{sat}$ (mg L^{-1}) is the concentration in the algae medium at saturation, C_{iw}^{sat} (mg L^{-1}) the concentration in H_2O, K_i^S (L mol^{-1}) the Setschenow constant and $[salt]_{total}$ (mol L^{-1}) the salt concentration in the algae medium (0.00114 mol L^{-1}). Setschenow constants were taken from Xie et al. (1997), Jonker and Muijs (2010) and Rojo-Nieto et al. (2012).

Recreation of mixtures

The recreation of PAH mixtures was performed according to Niehus et al. (2018). The concentration in methanol (C_{MeOH}) was calculated using the compound-specific partitioning coefficients from Smith et al. (2013a) and Gilbert (2011). The PAH composition was taken from a passive sampling ex-situ experiment with sediment from four North Sea stations (GB2, KSII, W21, JB; see Figure S1) (Kreutzer et al. in prep.). In total, 10 PAHs were solved in methanol at the respective concentrations and the derived solution was diluted in steps of two (1:1 (V1), 1:2 (V2), 1:4 (V3), 1:8 (V4) and 1:16 (V5). O-rings were loaded in these solutions for 72 h and treated liked those for the single PAH O-rings. Chrysene was omitted in the mixture due to crystal formation on the O-rings in the single substance bioassays.

Culture of *Raphidocelis subcapitata*

The mircoalgae *Raphidocelis subcapitata* was obtained from the University of Göttingen (Culture Collection of Algae, SAG; strain number 61.81). Algae cultures are maintained in Kuhl-medium (Kuhl & Lorenzen 1964) at 20 ± 1 °C with at a dark:light regime of 8:16 hours. Light intensity is set to 68 - 95 µE m^{-2} s^{-1} (6000 – 9000 lux), following the ISO norm.

Preculture and calibration

The freshwater growth inhibition assay was performed according to DIN EN ISO 8692 (2012), with the deviation that it was carried out in 24-well plates. 4 days prior to each test, a pre-culture was started by transferring 2 mL of a 3 months old maintenance culture into 100 mL growth medium (GM) (ISO 8692:2012) (Table S2). The pre-culture was kept under constant light exposure on a magnetic stirrer, set to 200 rpm. For the bioassay, the pre-culture was diluted with GM to a concentration which would result in a final cell density of 10^4 cells mL^{-1} in the test wells to allow exponential growth during the test phase. Cell concentrations were

determined by fluorescence measurement, applying a previously determined relationship between fluorescence and cell density.

Performance of the algal growth inhibition test

The passive dosing well plates were prepared for pre-equilibration the day before the test. For this, four O-rings of each concentration (V1-V5) and four cleaned O-rings (control) were added to the 24-well plate (one ring per well). The O-rings which had been loaded with different concentrations were arranged in alternating order on the well plate. The design of the negative control plate was different, as blank O-rings were added only to half of the wells. 1600 µL of ultrapure water and 200 µL of 10 times concentrated growth medium were added to all wells. 3,5-dichlorophenol (3,5-DCP) was used as positive control. Based on a 3,5-DCP stock solution (100 mg L^{-1}), concentrations of 6 (D1), 4.6 (D2), 3.6 (D3), 2.7 (D4) and 2.1 (D5) mg L^{-1} were prepared. The positive control plate was arranged as follows: Column 1 served as control, column 2-6 were filled with 3,5-DCP concentrations D1 – D5. The test was initiated by adding 200 µL of algae test-culture to the upper three rows, resulting in 3 replicates (row A – C) (Table S3). The last row (row D) served as blanks for exposure confirmation. 200 µL of the growth medium was added instead of algae. The prepared well plates were then placed on a horizontal shaker (MTS 2/4, IKA®-Werke GmbH & CO. KG) at 20 °C with 250 rpm under continuous light for 72 hours. The well plate design and the needed volumes are presented in the supporting information (Figure S2).

The fluorescence of the controls and the test vessels was measured at 465 nm excitation, 680 nm emission. All well plates were covered with non-sterile polyester films (Adhesive Film for Microplates, VWR) to reduce volatilization of the PAHs. Algal growth was determined by fluorescence measurements of all plates at test start (t_0) and after 24 (t_1), 48 (t_2) and 72 h (t_3). All data were corrected for background fluorescence measured in pure algal medium. The following performance criteria should be met for the algae in the control wells for the test to be considered valid: The average growth rate must be at least 1.4 per day; the mean coefficient of variation must not exceed 5%; the pH value over the duration of the test must not increase by more than 1.5.

Data treatment

The endpoint in this assay was the growth rate inhibition and was calculated according to DIN EN ISO 8692 (2012). Therefore, the specific growth rate (μ, d^{-1}) was calculated from the algal biomass measured as fluorescence (N, unitless) by the following equation:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t} \quad (5)$$

where N_t and N_0 are the algal biomass at sampling time (t, d) and the start of the experiment, respectively. Then the growth inhibition (I_μ), i.e., the relative reduction of the treatment growth rate compared to the control growth rates was calculated as follows:

$$I_\mu = 1 - \frac{\mu_{treatment}}{\mu_{control}} \quad (6)$$

The inhibition growth rates were then plotted as a function of the measured freely dissolved concentrations for the passive dosing assays. The effect-response relationship was fitted to a dose-response-inhibition model with variable slope (four-parameter logistic curve) using GraphPad Prism (version 8.4.0, San Diego, USA) and the EC_{50} values were calculated using this model.

Results and Discussion

Controls

The freshwater growth inhibition test with *R. subcapitata* met all validity criteria as specified in the guidelines. An acceptable increase of the final pH was observed (range, 8.66 – 9.14), which had no discernible effect on the algal growth over the test. The control with unloaded O-rings had no growth-inhibiting effect, but slight growth-promoting effects were observed in comparison to the control group without O-rings (Figure 1). To test whether the difference between the two control groups was statistically significant, a Shapiro-Wilk normality test was first performed on the growth rates. The data for the control group without O-ring showed a normal distribution whereas the data for the control group with O-ring was not normally distributed. Therefore, a non-parametric significance test had to be performed on the data. Since the distribution of the growth rate data with and without O-ring was different, one requirement of the Mann-Whitney U test was not fulfilled. Therefore, a Median test was performed that is not dependent on the shape of the distributions. The median test performed resulted in a p-value of 0.1797. With regard to this value, no significant difference between the two groups can be assumed. The growth constant k of 0.047 h^{-1} with O-rings was only marginally higher than the

growth constant without O-rings (0.045 h^{-1}). This slight growth-promoting effect was also observed by Stibany et al. (2017b) who worked with the same algae and O-rings and by Niehus et al. (2018) who work with the same O-rings but with different algae. The similar growth constants of the two control groups also showed that the promoting effect was negligible in the passive dosing tests with PAHs as it was not statistically significant.

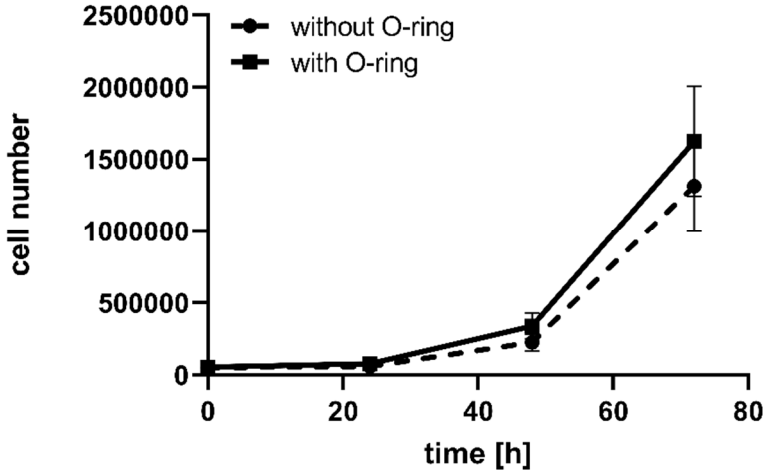


Figure 1: Exponential growth of *R. subcapitata* in the control groups with and without cleaned silicone O-rings.

Exposure confirmation

The confirmation of exposure in aquatic tests is crucial, especially when tested in plastic vessels. The analytical determination of the PAHs concentration in the medium proved to be difficult, because despite immediate fixation with methanol, the PAHs were so volatile that they were below the detection limit of the HPLC. Therefore, the saturated concentration in the test medium was not directly measured in the test medium, but calculated using the Setschenow equation (eq. 4) (Table 1). The dilution concentrations (i.e., dilutions 1:2, 1:4, 1:8, 1:16 and 1:32) were subsequently calculated using these calculated values. This approach was selected as several studies already demonstrated that the passive dosing method allows exposure at saturation level (Butler et al. 2013, Niehus et al. 2018, Stibany et al. 2017b).

Table 1: Octanol-water coefficients (log K_{OW}) and solubilities for PAHs in fresh water (S_{water}) and algae medium (S_{medium}) calculated with the Setschenow equation (eq. 4). The calculated solubility (S_{medium}) corresponds to the maximum freely dissolved concentration (C_{sat}) in algae medium.

	Log K_{ow}[a]	S_{water} [mg L^{-1}]	ks [L/mol]	$S_{medium} \triangleq C_{sat}$ [mg L^{-1}]
Naph	3.24	31.5	0.23	31.4811
Ace	4.07	3.8	0.26	3.7974
Fl	4.18	1.9	0.28	1.8986
Phen	4.46	1.1	0.272	1.0992
Anth	4.55	0.045	0.326	0.0450
Pyr	5.00	0.132	0.32	0.1319
Fluo	5.12	0.265	0.339	0.2648
Chr	5.63	0.006	0.336	0.0060
BaA	5.74	0.011	0.354	0.0110
BkF	5.86	0.0008	0.32	0.0008
BaP	5.91	0.0038	0.32	0.0038

[a] Mackay et al. (2006)

Passive dosing with single PAHs

The algal growth inhibition tests based on passive dosing with single PAHs showed that five PAHs (Naph, Ace, Phen, Fluo, BaP) had the strongest effects, with maximum growth inhibitions of > 97 % at saturation level (Figure 2). For fluorene and pyrene, a 95 % growth inhibition was observed at the highest exposure concentration. Moderate effects were observed for anthracene and benz[a]anthracene with 72 % and 63 %, respectively. Chrysene and benzo[k]fluoranthene showed maximum effects below 30 % inhibition at saturation level. However, the exposure data showed a complete concentration-response curve only for fluorene, phenanthrene, pyrene and benzo[a]pyrene, thus allowing a reliable calculation of EC_{50} values only for these PAHs (Table 2). The determination of the EC_{50} of chrysene was not possible since the maximum growth inhibition at saturation level did not exceed 8 %. All EC_{50} were below the exposure concentrations corresponding to the maximum attainable solubility in the test medium, except for the EC_{50} value of BkF. The structure of the PAHs appears to play an important role regarding the acute toxicity to *R. subcapitata*, as the toxicity increases with increasing molecular size. Standard deviations were low where good dose-response relationships were observed, indicating good reproducibility of the passive dosing tests.

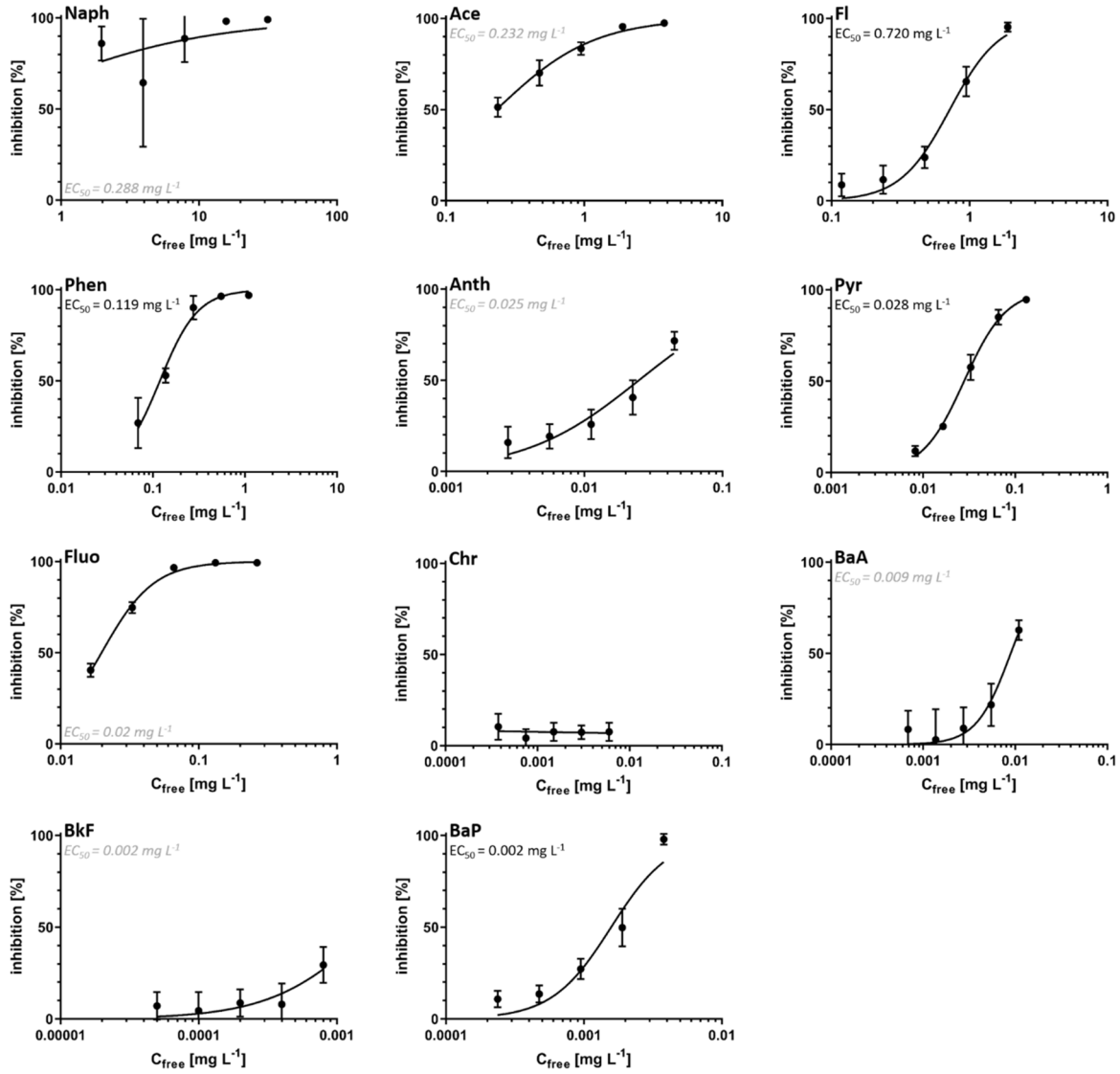


Figure 2: Growth inhibition test with *R. subcapitata* for naphthalene (Naph), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Anth), pyrene (Pyr), fluoranthene (Fluo), chrysene (Chr), benz[a]anthracene (BaA), benzo[k]fluoranthene (BkF) and benzo[a]pyrene (BaP) using passive dosing. Plotted symbols are mean values (well replicates, n = 3) and their standard deviations. The EC_{50} values marked in black represent the reliable calculated values, whereas the *grey italic* values are considered not reliable.

Since this is the first time that passive dosing O-rings were used to introduce a large set of PAHs in a multi-well fresh-water algae test, the comparison with results from literature is limited. In a study by Bragin et al. (2016), EC_{50} values were determined for *R. subcapitata* for at least four PAHs relevant in this study, but three of the tests were performed only as passive dosing limit tests targeting the aqueous solubility of these substances, as the solubility of these substances was very low. For fluorene the EC_{50} value of 1.05 mg L^{-1} was reported, which is about one third higher compared to this study (0.72 mg L^{-1}). Niehus et al. (2018) published data with the marine algae *Phaeodactylum tricornutum* applying the same test design for five different PAHs (Naph, Ace, Fl, Phen, Fluo). Comparing the EC_{50} values of the two algae

species, *R. subcapitata* appears to be more sensitive to exposure to most PAHs than *P. tricornutum*, as the obtained EC_{50} values in that study are up to six times higher. However, in the case of fluorene, the EC_{50} value of *R. subcapitata* is about twice as high as that of *P. tricornutum*. Other aquatic toxicity data found on PAHs found in literature were based on tests in which the test medium was spiked with carrier solvents and thus no continuous maintenance of test substance concentrations were applied. In this study, the use of the passive dosing approach allowed for a constant controlled exposure of higher molecular weight PAHs such as BaP, which are normally more difficult to test in aquatic toxicity bioassays. Especially as Niehus et al. (2018) showed that lower EC_{50} values are obtained using the passive dosing approach since the dose-response curve shifted to the right due to losses using the spiking approach.

Table 2: Effect concentration at 50 % growth inhibition for PAHs using passive dosing. EC_{50} values were calculated with a dose-response-inhibition model using GraphPad Prism (version 8.4.0, San Diego, USA). The EC_{50} values marked in black represent the reliable calculated values, whereas the *grey italic* values are considered not reliable.

PAH	**EC_{50} (mg L^{-1})**	**95 % confidence interval (mg L^{-1})**
Naph	*0.29*	*0.0021 to 40.49*
Ace	*0.23*	*0.20 to 0.27*
Fl	**0.72**	**0.64 to 0.81**
Phen	**0.12**	**0.10 to 0.14**
Anth	*0.025*	*0.019 to 0.033*
Pyr	**0.028**	**0.026 to 0.030**
Fluo	*0.020*	*0.019 to 0.021*
Chr	---	---
BaA	*0.0090*	*0.0073 to 0.011*
BkF	*0.0018*	*0.00067 to 0.0048*
BaP	**0.0016**	**0.0013 to 0.0019**

Chemical activity

The chemical activity (a) is a parameter that not only quantifies the concentration of a compound, but also the energetic state which determines the potential for spontaneous physicochemical processes, such as partitioning into organisms. The sum of chemical activities is an indicator of the baseline toxic potential of mixtures (Reichenberg & Mayer 2006). Even though the chemical activity of each individual compound in a mixture may be below a toxicity threshold, the cumulative activities may cause an overall toxic effect (Escher et al. 2002).

The chemical activity (a) of PAHs in saturated water corresponds to the activity of PAHs in their pure, solid crystal state (a_{max}) (Engraff et al. 2011). The a_{max} for each PAH was estimated

at 22 °C (T = 295 °K) from its reported melting point (T_m, °K) using Walden's rule (Mayer & Holmstrup 2008, Smith et al. 2010a).

$$a_{max} = \exp(6.8 \left[1 - \frac{T_m}{T}\right]) \quad (7)$$

The sensitivity of an organism to the substances can be described by the effective chemical activity (Ea_{50}), which can be calculated by plotting the inhibition rate against the maximum chemical activity (Figure 3). The correlation was fitted with a sigmoidal concentration–response curve with variable slope. In this study, the effective chemical activity causing 50% lethality (Ea_{50}) was determined to be 0.009 with a 95 % confidential interval of 0.007 to 0.01 and R^2 of 0.81. Bragin et al. (2016) determined an Ea_{50} value of 0.08 for the substance fluorene for *R. subcapitata*. The Ea_{50} value for the substance BaP was greater than 0.018. These values are in the same range as the Ea_{50} value of this study. For the marine algae *Phaeodactylum tricornutum* a Ea_{50} of 0.14 was determined by Niehus et al. (2018). Ea_{50} values have been identified for species from other trophic levels: For example *Daphnia magna*, an value of 0.038 was identified (Smith et al. 2010a). Mayer and Holmstrup (2008) reported an Ea_{50} of 0.058 for the springtail *Folsomia candida*. For *Danio rerio*, an Ea_{50} value of 0.089 was indicated by Seiler et al. (2014). The comparison of the different values indicates that the microalgae *R. subcapitata* is more sensitive to PAHs than the other species. The Ea_{50} value of this study is nearly in the range of effective chemical activities for the onset of baseline toxicity between 0.01 and 0.1 (grey area in Figure 3), which were calculated from various published effective concentration values (EC_{50}) for algae, fish, tadpoles and mice (Calamari et al. 1983, Ferguson 1939, Könemann 1981, Mayer & Reichenberg 2006, Reichenberg & Mayer 2006).

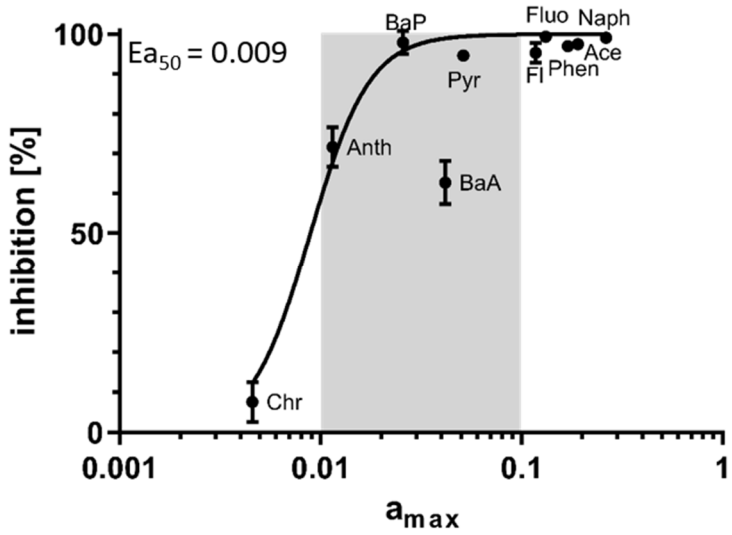


Figure 3: Growth rate inhibition of *R. subcapitata* after exposure to single PAHs at saturation level plotted against the chemical activity (R^2 = 0.81). Error bars represent the standard deviation of the inhibition between replicates and the grey area represents the baseline toxic potential (between 0.01 and 0.1). Fitted with a log (inhibitor) vs. response variable slope (four parameters) model with Graph Pad Prism 8.4.0.

Passive dosing with PAH mixtures

The third aim of this study was using passive dosing for the application of realistic mixtures of ten PAHs in the miniaturized algal growth inhibition test. Therefore, the actual proportions of each compound found in the real sediment samples were reproduced. Rojo-Nieto et al. (2012) and Niehus et al. (2018) have already shown that the replication of a realistic mixture is possible using the passive dosing approach. The maximum differences between target and measured proportion were 3 % for chemical activities of phenanthrene (Rojo-Nieto et al. 2012) and up to 13 % (Niehus et al. 2018). These results show a good reproducibility opening up the possibility of using passive dosing for toxicity tests with complex mixtures of HOCs. In this study, the O-ring loading approach was used according to Niehus et al. (2018).

The recreated mixtures were investigated to show whether the analytical determined C_{free} composition of the four North Sea stations (GB2, KSII, JB, W21) had an effect on the algal growth (Figure 4). The field concentration at station KSII inhibited the growth of *R. subcapitata* up to 95 %, whereas stations GB2, JB and W21 showed growth inhibition effects up to 56 %, 49 % and 33 %, respectively. Stations GB2 and KSII exhibited the lowest EC_{50} values (34.02 ng L^{-1} and 31.35 ng L^{-1}, respectively; see Table S4), whereof KSII had the higher growth-inhibiting effect at the highest mixture concentration (95 %). The EC_{50} value of station JB (78.55 ng L^{-1}) was highest indicating that the growth-inhibiting effect of the mixture was lowest. However, the dose-response curves of stations JB and W21 (EC_{50}: 48.21 ng L^{-1}) were not fully developed. In this case, the calculations of the mean effective concentrations (EC_{50})

depend on estimating maximum values for the increase. As a result, relatively small EC_{50} values were determined.

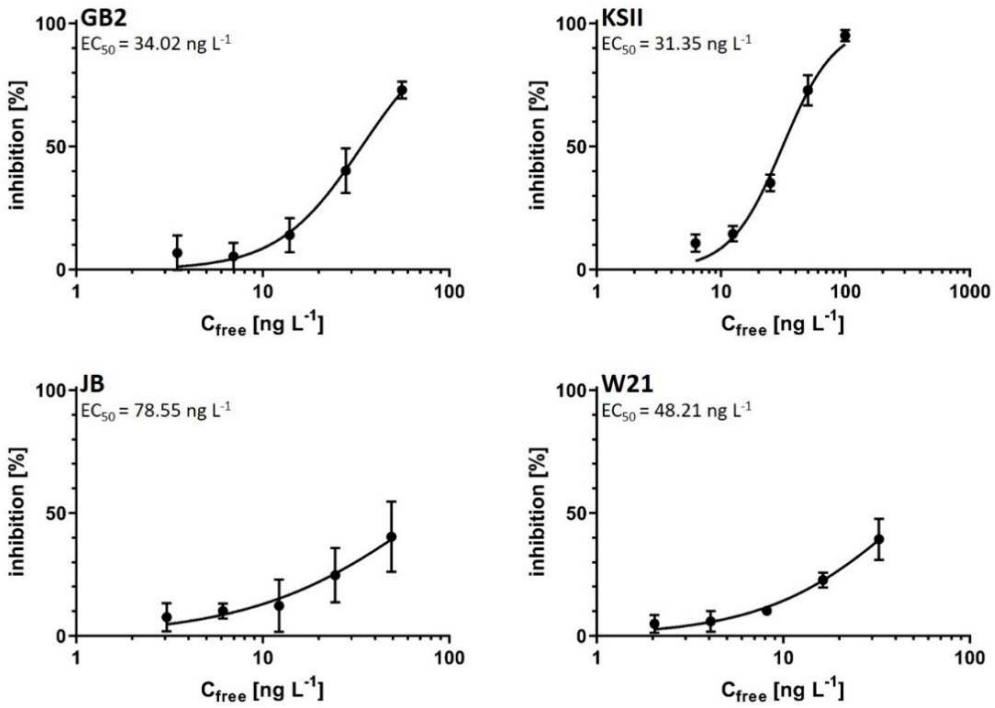


Figure 4: Growth inhibition test with *R. subcapitata* with recreated PAH mixtures (∑ 10 PAHs) of North Sea sediment samples using passive dosing. Plotted symbols are mean values (well replicates, n = 3) and their standard deviations.

The analytical analysis of the C_{free} of these four North Sea stations carried out by Kreutzer et al. (in prep.) showed that stations GB2 and KSII are higher contaminated because they are close to a sediment-dumping site of Hamburg harbor. When comparing the obtained EC_{50} values with the measured C_{free} values it can be clearly seen that station KSII with the highest PAH concentration (99.91 ng L^{-1}) also had the smallest EC_{50} value. Followed by station GB2 with the second highest C_{free} value of 55.84 ng L^{-1} and second smallest EC_{50} value. This correlation was not transferable for stations JB and W21, because although the PAH concentration at station JB (49.03 ng L^{-1}) was higher compared to W21 (32.69 ng L^{-1}), the EC_{50} value of JB indicated a lower sensitivity of *R. subcapitata* to the mixture. Smith et al. (2013b) showed that the solubility of the PAHs in the mixture did not influence each other and that the solubility was thus additive which is in line with the well-established concept of concentration addition for mixture toxicity of chemicals with a similar mode of action. They were also able to demonstrate that non-toxic individual substances can become toxic in a mixture. However, PAHs with a high melting point, which are not toxic individually, can develop acute toxicity in a mixture if together a sum of chemical activities of 0.01-0.1 is reached (Smith et al. 2013b). In this study, the results show that the realistic mixtures of the North Sea stations strongly

influenced the algal growth of *R. subcapitata*. However, in the real world, other compounds may also contribute to the toxicity of these sites. Mixtures that cover the entire spectrum could thus represent the real toxicity on the basis of passive dosing.

Conclusion

This study demonstrated that the application of the passive dosing approach had some important benefits for the testing of HOCs in aquatic media. This approach enables toxicity testing of (highly) hydrophobic substances at aqueous solubility to assess toxicity at the maximum exposure concentration. The use of PAH loaded silicone O-rings facilitated well defined toxicity testing with good reproducibility in the growth inhibition test over 72 h. Passive dosing is able to compensate for most losses and is the better alternative for aquatic toxicity testing of hydrophobic organic compounds in terms of risk assessment. The recreated mixture showed already strong effects with the small selection of substances indicating that the entirety of a mixture would have adverse effects on primary producers and may thus have consequences for the entire ecosystem. Toxicity showed a close relationship between the toxicity of hydrophobic organic compounds and their chemical activity. The use of the miniaturized format in 24-well microtiter plates facilitate a higher sample throughput and thus increase the statistical power of the data obtained for the toxicity assessment as an increased number of test repetitions can be performed in a short time. This passive dosing approach can be applied in bioassays of different trophic levels to identify the risks that these organic pollutants may pose to an entire ecosystem or could be used to attribute measured toxicities to effective chemicals.

Acknowledgements

This work was part of the NOAH-project funded by the German Federal Ministry of Education and Research (Contract No. 03F0742D). We thank Anne Below for the algae maintenance and practical support during the algal bioassay. Further we thank the Senckenberg am Meer team, especially Dr. Ingrid Kröncke and Dr. Julia Meyer, for the support during the sediment sampling campaign.

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Supporting Information

Supplementary Table 1

Table S1: Equilibrium partitioning ratios utilized to prepare the passive dosing O-rings. Given are the equilibrium partitioning ratios for PDMS:water (Smith et al. 2010b), PDMS:O-ring (Gilbert 2011) and MeOH:PDMS (Smith et al. 2010a) in L L^{-1}.

substance	$K_{PDMS:water}$ [L L^{-1}]	$K_{PDMS:O\text{-}ring}$ [L L^{-1}]	$K_{MeOH:PDMS}$ [L L^{-1}]
Naph	1074	1.489	2.410
Ace	4222	1.404	1.730
Fl	5436	1.477	2.330
Phen	9338	1.437	3.160
Anth	12195	1.475	3.080
Fluo	19627	1.436	3.370
Pyr	23720	1.366	3.050
BaA	62627	1.488	3.920
Chr	54357*	1.492	4.070
BkF	67920*	1.490	3.847
BaP	185549	1.494	3.920

*partition coefficients were calculated using the following equation: $\log K_{PDMS:water} = 0.691 \log K_{OW} + 0.686$ ($r^2 = 0.985$) with a log K_{OW} of 5.86 (Chr) and 6.00 (BkF).

Supplementary Figure 1

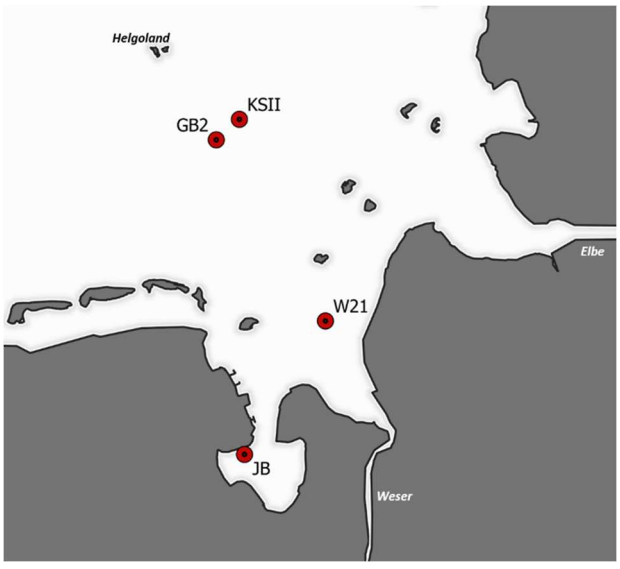


Figure S1: Sediment sampling stations in the North Sea (GB2, KSII, JB and W21).

Supplementary Table 2

Table S2: Mass concentrations of nutrients in the growth medium

stock solution	nutrients	mass concentration stock solution	mass concentration growth medium
1: macronutrients	NH_4Cl	1.5 g L^{-1}	15 mg L^{-1} (N: 3.9 mg L^{-1})
	$MgCl_2 * 6 H_20$	1.2 g L^{-1}	12 mg L^{-1} (Mg: 2.9 mg L^{-1})
	$CaCl_2 * 2 H_20$	1.8 g L^{-1}	18 mg L^{-1} (Ca: 4.9 mg L^{-1})
	$MgSO_4 * 7 H_2O$	1.5 g L^{-1}	15 mg L^{-1} (S: 1.95 mg L^{-1})
	KH_2PO_4	0.16 g L^{-1}	1.6 mg L^{-1} (P: 0.36 mg L^{-1})
2: Fe-EDTA	$FeCl_3 * 6 H_2O$	64 mg L^{-1}	64 µg L^{-1} (Fe: 32 µg L^{-1})
	$Na_2EDTA * H_2O$	100 mg L^{-1}	100 µg L^{-1}
3: micronutrients	H_3BO_3	185 mg L^{-1}	185 µg L^{-1} (B: 32 µg L^{-1})
	$MnCl_2 * 4 H_2O$	415 mg L^{-1}	415 µg L^{-1} (Mn: 115 µg L^{-1})
	$ZnCl_2$	3 mg L^{-1}	3 µg L^{-1} (Zn: 1.4 µg L^{-1})
	$CoCl_2 * 6 H_2O$	1.5 mg L^{-1}	1.5 µg L^{-1} (Co: 0.37 µg L^{-1})
	$CuCl_2 * 2 H_2O$	0.01 mg L^{-1}	0.01 µg L^{-1} (Cu: 3.7 ng L^{-1})
	$Na_2MoO_4 * 2 H_2O$	7 mg L^{-1}	7 µg L^{-1} (Mo: 2.8 µg L^{-1})
4: $NaHCO_3$	$NaHCO_3$	50 g L^{-1}	50 mg L^{-1} (C: 7.14 mg L^{-1})

Supplementary Figure 2

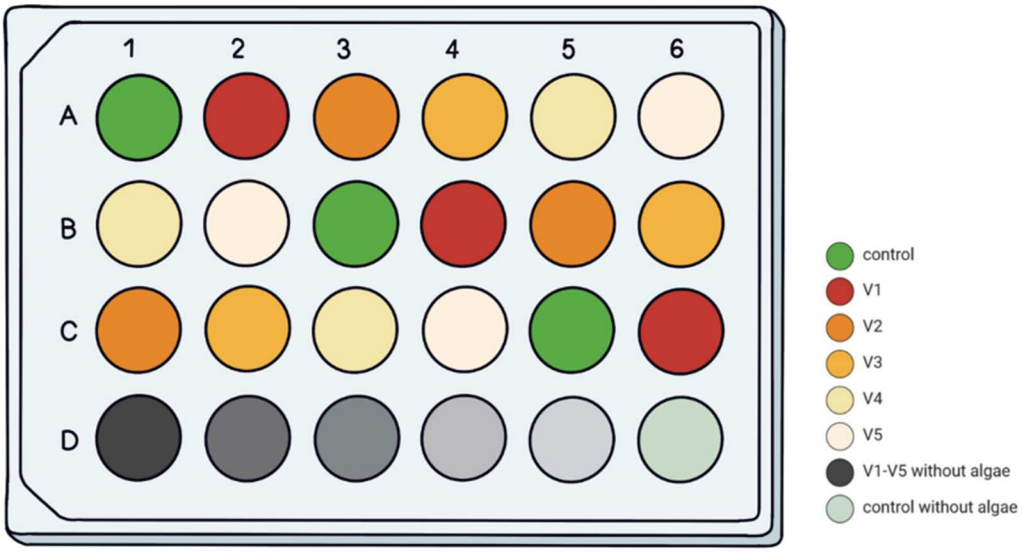


Figure S2: Layout for the algae growth inhibition test in 24-well plates. Five concentrations (V1-V5) and a control (cleaned O-ring) are presented. In addition, the five concentrations as well as a control with a cleaned O-ring were tested without algae for analytical purpose.

Supplementary Table 3

Table S3: Scheme of the volumes needed for each well in the algae growth inhibition test.

	ultrapure water [µL]	10-fold medium [µL]	1-fold medium [µL]	algae 10^5 [µL]	suspension algae mL^{-1}
V1 – V5	1600	200	---	200	
Control	1600	200	---	200	
V1 – V5 (without algae)	1600	200	200	---	
Control (without algae)	1600	200	200	---	

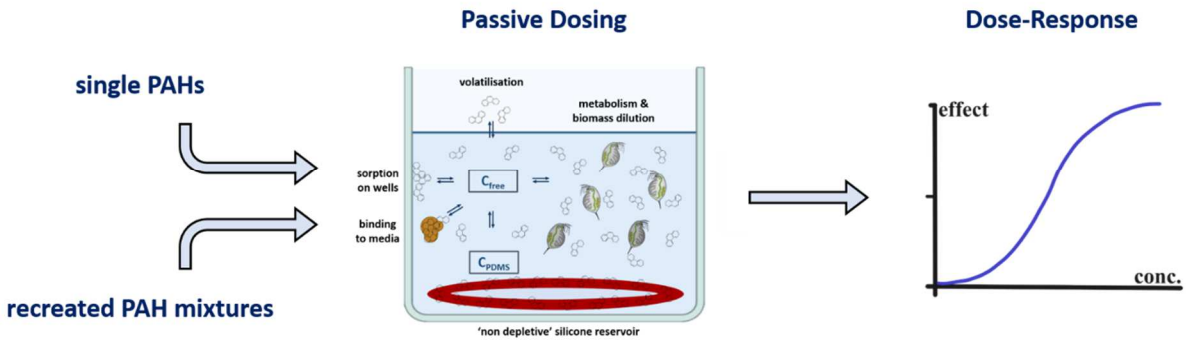
Supplementary Table 4

Table S4: Effect concentration at 50 % growth inhibition for recreated PAH mixtures of the North Sea using passive dosing. EC_{50} values were calculated with a dose-response-inhibition model using GraphPad Prism (version 8.4.0, San Diego, USA).

station	EC_{50} ($ng\ L^{-1}$)	95 % Confidence interval ($ng\ L^{-1}$)
GB2	34.02	30.04 to 38.53
KSII	31.35	28.45 to 34.55
JB	78.55	41.48 to 148.7
W21	48.21	37.26 to 62.38

Additional Results

Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to water flea *Daphnia magna*



Passive dosing: Assessing the toxicity of individual PAHs and recreated mixtures to water flea *Daphnia magna*

Anne Kreutzer[a,b], Silja Denise Kröger[b], Jessica Sachse[b] and Gesine Witt[b]

[a] Department Evolutionary Ecology and Environmental Toxicology, Institute of Ecology, Evolution and Diversity, Faculty Biological Sciences, Goethe University Frankfurt, Frankfurt 60438, Germany

[b] Department of Environmental Engineering, Faculty Life Sciences, Hamburg University of Applied Sciences, Hamburg 21033, Germany

Aims

The North Sea is one of the busiest maritime areas and the intensive use of this area through offshore activities, dredged material placement and maritime traffic leads to increasing pollution of the ecosystem by chemicals (OSPAR Commission 2010). Hydrophobic organic contaminants (HOCs), including polycyclic aromatic hydrocarbons (PAHs), are among the most common organic contaminants of concern. Due to their strong hydrophobicity, HOCs sorb to organic material and have their final destination in sediment (Cui et al. 2013). As particle-bound pollutants, they can bioaccumulate in aquatic and sediment-dwelling organisms through ingestion, respiration and direct contact.

Bioavailability is a key factor in ecotoxicological effects of contaminants and important for risk assessment approaches (Reichenberg & Mayer 2006). However, total exhaustive extractions of contaminants do not consider the importance of the bioavailable fraction. The mobility of HOCs, and consequently bioavailability, depends on their pore water concentrations (Witt et al. 2010). Currently, the freely dissolved concentration (C_{free}) is established as an important endpoint for sediment quality and risk assessment. Solid-phase microextraction (SPME) was applied according to Witt et al. (2013) to examine the spatial and temporal distribution of C_{free} of PAHs in sediment cores sampled in the North Sea (see Annex 1). The determined C_{free} values are representative of the actual pollution situation on-site and can be used to recreate realistic PAH mixtures found in North Sea sediments. These mixtures can then be applied in bioassays to study their ecotoxicological effects. Since nominal dosing often exhibits significant losses (e.g. due to sorption or volatilization) (Smith et al. 2010a, Smith et al. 2010b), the passive dosing method was used to overcome these difficulties and to obtain stable exposure concentrations. The PAHs are released into the aqueous medium via passive diffusion from biocompatible polydimethylsiloxane (PDMS) O-rings, which serve as reservoirs for PAHs. The chemicals distribute between the silicone (C_{PDMS}) and medium (C_{free}) in the test vessels until thermodynamic equilibrium is reached.

This study aims to develop a miniaturized passive dosing immobilization test with *Daphnia magna* using well plates. The toxicity of selected single PAHs on *D. magna* was assessed in a modified test design according to OECD Guideline 202 (2004). Furthermore, the recreated mixtures of the sediment samples from the North Sea were tested to estimate the risk posed by the actual pollution situation on site. The toxicity data from this study were then compared with the results for the green algae *R. subcapitata* (see Annex 3) and with further literature data.

Material and Methods

Chemicals and materials

Food-grade silicone O-rings with an outer diameter of 14.4 mm, inner diameter of 9.6 mm, mass of 223 ± 1.98 mg (n = 20) and a density of 1.2 g cm^{-3} (ORS-0096-24., Altec, Cornwall, United Kingdom) made of polydimethylsiloxane (PDMS) were used as passive dosing device. As test vessels, 24-well flat-bottom polystyrene plates (Costar® Flat Bottom Cell Culture Plates, Corning®) were used. Methanol (hypergrade for LC-MS, VWR) and ethyl acetate (ROTISOLV® ≥ 99.8 %, Pestilyse®, Carl Roth) were used as solvents. The following ten PAHs were selected: naphthalene (Naph; ≥ 98.5 %), acenaphthene (Ace; ≥ 98.5 %), fluorene (Fl; ≥ 99 %), phenanthrene (Phen; ≥ 99.5 %), anthracene (Anth; ≥ 99 %), pyrene (Pyr; ≥ 97.5 %), fluoranthene (Fluo; ≥ 98 %), benz[a]anthracene (BaA; ≥ 98.5 %), benzo[k]fluoranthene (BkF; ≥ 99 %) and benzo[a]pyrene (BaP; ≥ 96 %; all substances were obtained from Sigma).

Preparation of O-rings

The preparation process of the O-rings was performed according to Smith et al. (2010b) and (Booij et al. 2002) as described in Annex 3. The saturated solutions of the single PAHs (Ace, Fl, Phen and Fluo) were diluted in steps of two (1:1, 1:2, 1:4, 1:84, 1:16 and 1:32) to obtain a dose-response curve. The recreation of PAH mixtures was performed according to Niehus et al. (2018) as described in Annex 3. Therefore, the freely dissolved concentrations (C_{free}) of the surface sediment pore water of four North Sea sites (GB2, KSII, W21, JB) were reconstructed in their respective compositions (see Annex 1 for analytical sediment analysis). In total, ten PAHs were solved in methanol at the respective concentrations and the derived solutions were diluted in steps of two (1:1, 1:2, 1:4, 1:84, 1:16 and 1:32). O-rings were loaded in these solutions for 72 h and treated as the single PAH O-rings.

Immobilization assay with *Daphnia magna* using passive dosing

The immobilization assay was performed with *Daphnia magna* (clone 5) according to OECD Guideline 202 (2004) „*Daphnia sp.*, Acute Immobilization Test“. The test design was slightly modified to miniaturize the test. Therefore, 6-well plates were used to perform the passive dosing method with O-rings. In addition, the test duration was extended from 48 hours to seven days, since the daphnids responded merely to the exposure of single PAHs after 48 hours.

Daphnia culture

Daphnia magna was maintained in Elendt-Medium (M4) at 20 ± 1 °C with a light-dark rhythm of 16:8 h, 10 individuals per 800 mL glass vessel. Daphnids were fed every day with 1 ml of the algae *Chlorella vulgaris* and additionally one day per week with 1.5 mL of yeast (0.1 g / 10 L M4 medium). Once a week, the Elendt-Medium was replaced, while the neonates were separated from the adult daphnids.

Controls and Test Solutions

Potassium dichromate ($K_2Cr_2O_7$) is recommended as reference substance according to OECD Guideline 202 (2004). The substance copper sulfate ($CuSO_4$; 3.1 mgL^{-1}) was applied as a possible substitute for potassium dichromate, as this is classified as very toxic and carcinogenic. To determine whether copper sulfate is a suitable alternative to potassium dichromate, the EC_{50} values of the two substances were determined employing three replicates using six concentrations. The comparison revealed that copper sulfate is a suitable alternative to potassium dichromate and therefore, the following daphnid tests were performed with copper sulfate.

Performance

The day before test initiation, the passive dosing well plates were prepared for pre-equilibration. Therefore, two O-rings of each concentration were placed in one well, in total four wells per dilution concentration were prepared (Figure 1). Afterwards, 5 mL of dilution medium according to OECD Guideline 202 (2004) was added (Table S1) and the well plates were shaken overnight for ensuring equilibrium state. In total, six different concentrations of single PAH substances and PAH mixtures were tested, as well as a positive control (PC) containing $CuSO_4$ (3.1 mg L^{-1}) and a negative control (NC) with cleaned O-rings. Five neonate daphnids per replicate (max. 24 h after hatching) were transferred into each testing well and the well plates were covered with a non-sterile polyester film (Adhesive Film for Microplates, VWR) to prevent evaporation of the test medium. The neonates were incubated at 20.0 ± 1.0 °C and after seven days the immobile daphnids were counted. The immobilization was determined a non-ability to swim within 15 seconds. Daphnids were considered to be immobilized even if they could still move their antennae. The O_2 level (MultiLine®, Multi 3630 IDS, WTW) was measured after seven days. According to OECD Guideline 202 (2004), a test was considered valid if the mortality of the negative control was ≤ 10 % until 48 h post-exposure and the concentration of dissolved oxygen was more than 3 mg L^{-1} at the end of the test. In the final

step, a mixed sample of all test wells from each dilution factor (100 µL per well) was transferred into a 1.5 mL GC vial and 500 µL of methanol was added to stabilize the sample.

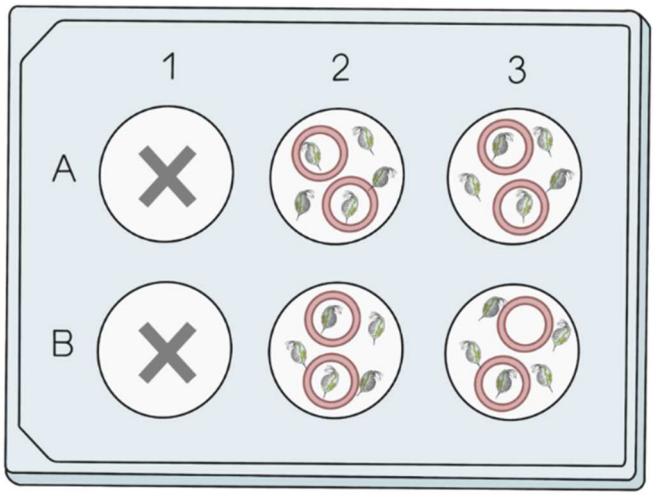


Figure 1: Exemplary experimental plate setup with loaded O-rings. Each well contained two loaded O-rings and five daphnids.

Exposure confirmation

For exposure confirmation, all dilution samples were measured using a HPLC VWR HITACHI Chromaster system (5310 Column oven; 5160 Pump, 5260 Autosampler) equipped with a fluorescence detector (FD; 5440 FL Detector) and a reverse phase 18 column (HI-5PAH2-100A, Hichrom with Guard Catridge Holder, Hichrom). A volume of 10 µL of each sample was injected into the column with a flow rate between 2 – 3 mL min^{-1}. Acetonitrile (ROTISOLV® HPLC, Carl Roth) and ultrapure water were used as the mobile phase: 100 % ultrapure water between t = 0 and 1 min, a gradient from 60 to 10 % ultrapure water between t = 2 and 19 min and 100 % ultrapure water from 20 to 22 min (Table S2). PAH concentrations were quantified using a six-point external standard calibration curve (PAH-Mix 9; 10 mg L^{-1}; Dr. Ehrenstorfer) and the signal integration was performed using the Chromaster System Manager (Version 1.2; VWR HITACHI).

Data treatment

The endpoint in this essay was the determination of the immobilization rate according to OECD Guideline 202 (2004). Therefore, the percentages of the immobilization of *D. magna* were calculated and plotted against the freely dissolved concentrations (C_{free}). The concentration and immobility were fitted with a sigmoidal concentration-response curve model with variable slope (GrapPad Prism, version 8.4.0, San Diego, USA).

Results and Discussion

Passive dosing with single PAHs

The immobilization assay with *D. magna* fulfilled all validity criteria, as the dissolved oxygen concentration was constantly above 3 mgL^{-1} and no more than 10 % of the daphnids in the control groups were immobilized, confirming the biocompatibility of the test system with O-rings. The daphnids exposed to the positive control substance $CuSO_4$ exhibited an immobilization rate of 50 % during all tests. The passive dosing experiment with the four selected PAHs showed that a complete dose-response curve was obtained (Figure 2). All four PAHs induced immobilization rates of > 96 % at the highest exposure concentration. EC_{50} values were calculated (Table 1) using a dose-response-inhibition model with variable slope using (GraphPad Prism). All EC_{50} were below the exposure concentrations corresponding to the maximum attainable solubility in the test medium. Standard deviations are low where good dose-response relationships were observed, indicating good reproducibility of the passive dosing tests. The EC_{50} values of fluorene and phenanthrene were in the same range (0.182 $mgL^{-1}$and 0.186 mgL^{-1}, respectively), while the effective concentration of acenaphthene (0.382 mgL^{-1}) was about twice as high (Table 1). The highest effect on the immobilization of the daphnids was exhibited by fluoranthene (0.115 mgL^{-1}). The structure of the PAHs appears to play an important role regarding the acute toxicity to *D. magna*, as the toxicity increases with increasing molecular size.

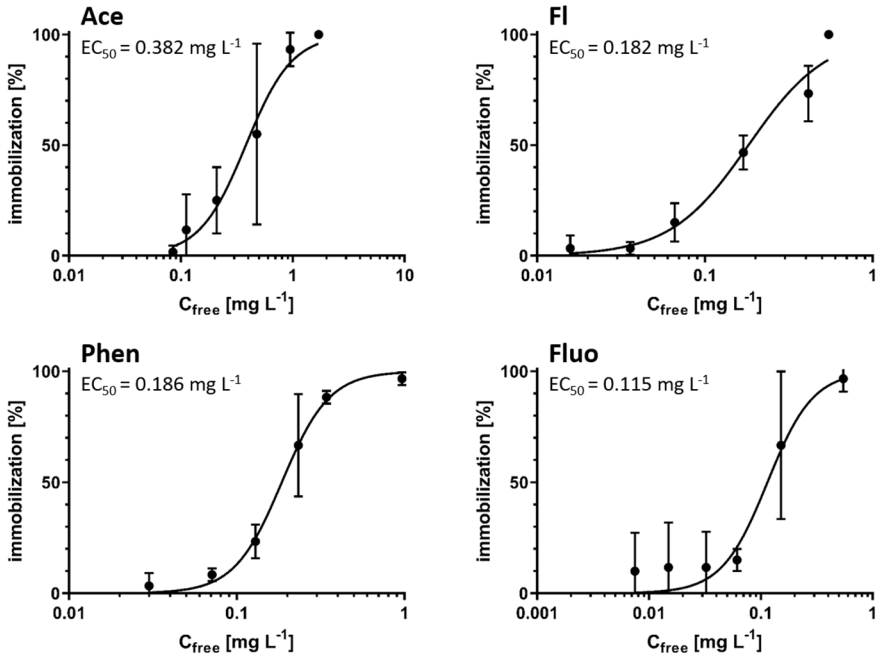


Figure 2: Immobilization assay with *D. magna* for acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen) and fluoranthene (Fluo) using passive dosing. Plotted symbols are mean values (well replicates, n = 3) and their standard deviations.

In this study, it is the first time that passive dosing O-rings were used to introduce a set of PAHs in a multi-well daphnid immobilization test, wherefore the comparison with literature data is limited. Munoz and Tarazona (1993) determined a 48 h EC_{50} for Acenaphthene of 1.275 mgL^{-1} in a nominal dosing bioassay, which is about 4 times higher compared to the EC_{50} of this study. Ha et al. (2019) calculated an EC_{50} of 1.348 mgL^{-1} for the exposure of fluorene to *D. magna*, which is about 7 times higher compared to the value of this study. However, in the study of Ha et al. (2019) the solubilizer DMSO was used, which does not represent an ecotoxicological relevant exposure scenario. An EC_{50} value of 0.12 mgL^{-1} was determined for the substance fluoranthene (Tani et al. 2021). Suedel and Rodgers Jr (1996) determined LC_{50} values for *D. magna* after 48 h and 10 d of exposure to fluoranthene (0.105 mgL^{-1} and 0.103 mgL^{-1}, respectively). These effect concentrations are in the same order of magnitude as in this study. In a study by Tani et al. (2021), a 48 h EC_{50} value of 0.33 mgL^{-1} was assessed for the exposure of phenanthrene to *D. magna*, which is about twice as high as in this study. This suggests that the nominal dosing approach applied by Tani et al. (2021) might underestimate the effect concentration and that a constant exposure concentration through passive dosing might better reflect the risk exposed to the daphnids. In addition, Niehus et al. (2018) could demonstrate that nominal spiking in the marine algae test resulted in enormous losses of the test substance and thereby in a striking underestimation of the toxic potential.

The comparison of the results of the passive dosing immobilization assay with *D. magna* and the passive dosing growth inhibition test with *R. subcapitata* (Annex 3) clearly shows that the alga *R. subcapitata* is the more sensitive species to the exposure of the PAHs acenaphthene, phenanthrene and fluoranthene (Table 1). For the substance fluorene, the EC_{50} value of the alga is about four times higher compared to *D. magna*, indicating that the daphnid is more susceptible.

Table 1: Effect concentration for PAHs at passive dosing for the water flea *Daphnia magna* and green alga *Raphidocelis subcapitata* (see Annex 3). EC_{50} values were calculated with a dose-response-inhibition model using GraphPad Prism (version 8.4.0, San Diego, USA).

Organism	PAH	EC_{50} (mg L^{-1})	95 % Confidence interval (mg L^{-1})
D. magna	Ace	0.382	0.2793 to 0.5218
	Fl	0.182	0.1516 to 0.2183
	Phen	0.186	0.1648 to 0.2090
	Fluo	0.115	0.0804 to 0.1634
R. subcapitata	Ace	0.23	0.20 to 0.27
	Fl	0.72	0.64 to 0.81
	Phen	0.12	0.10 to 0.14
	Fluo	0.02	0.019 to 0.021

Passive dosing with PAH mixtures

The second aim of this study was the application of realistic mixtures in the miniaturized *Daphnia* immobilization test using passive dosing. Therefore, the actual proportions of ten PAHs found in the real sediment samples of the North Sea stations were reproduced. The replication of a realistic mixture using passive dosing has already been successfully applied by Rojo-Nieto et al. (2012) and Niehus et al. (2018). In this study, the recreated mixtures were analyzed to whether the native C_{free} of the four North Sea stations (GB2, KSII, JB, W21) had an effect on the immobilization rate of the daphnids. The dose-response curves of all stations were not fully developed (Figure 3), for which reason the calculations of the mean effective concentrations (EC_{50}) depend on estimating maximum values for the increase. As a result, relatively small EC_{50} values with very wide 95 % confidence intervals (CI) were determined (Table 2). Since the measured C_{free} concentrations were below the limit of detection (LOD) of the HPLC method, it would be helpful to measure the concentrations in the O-ring before and after the exposure to determine the exposure concentrations. However, it must be ensured that the O-ring will not be depleted during the exposure time so that there is a constant concentration in the medium.

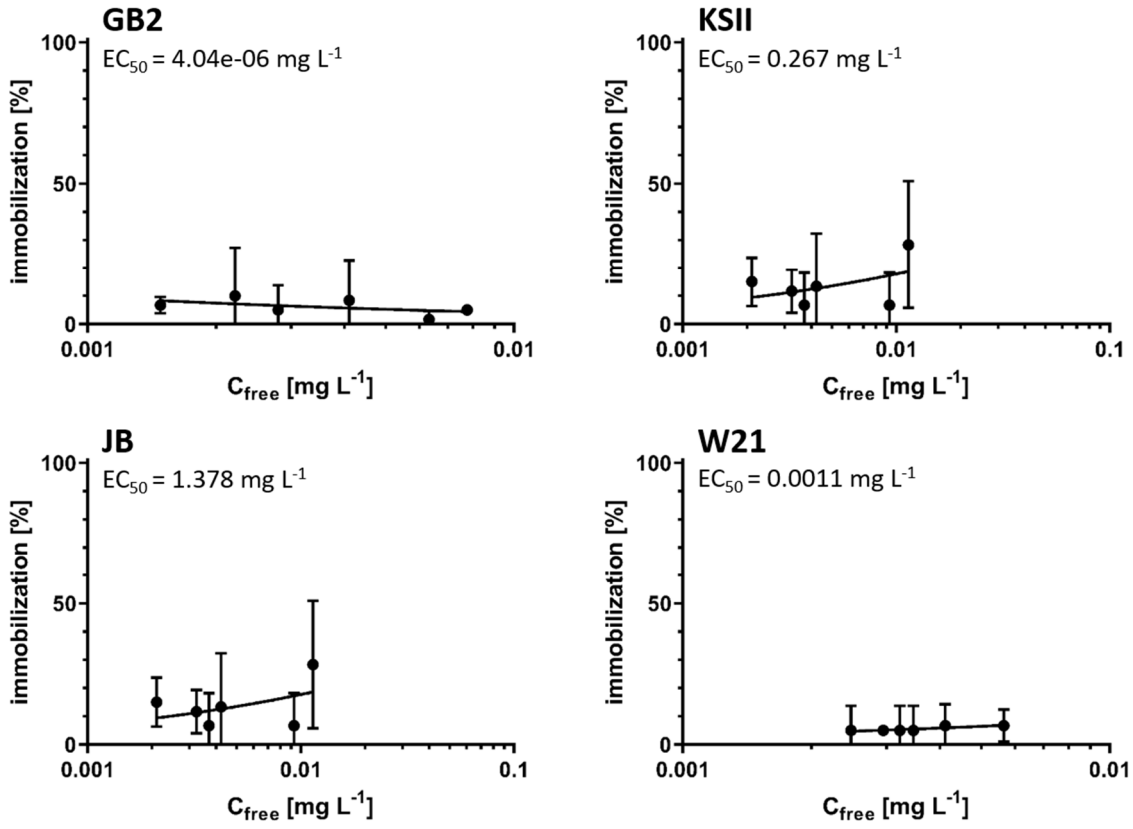


Figure 3: Immobilization assay with *D. magna* with recreated PAH mixtures (∑ 10 PAHs) of North Sea sediment samples using passive dosing. Plotted symbols are mean values (well replicates, n = 3) and their standard deviations

The results of this study show that the realistic mixtures of the North Sea stations did not have a major impact on the immobilization of *D. magna*. However, it can be concluded that the daphnids were not very susceptible to the environmentally relevant concentrations. The comparison of the *D. magna* mixture toxicity results to the green algae *R. subcapitata* (Annex 3) show that the realistic mixture caused a much stronger harmful effect on the algae.

Table 2: Effect concentration at 50 % immobilization for recreated PAH mixtures of the North Sea using passive dosing. EC_{50} values were calculated with a dose-response-inhibition model using GraphPad Prism (version 8.4.0, San Diego, USA).

station	EC_{50} (mg L^{-1})	95 % Confidence interval (mg L^{-1})
GB2	4.04e-06	6.952e-016 to 23493
KSII	0.267	5.534e-005 to 1288
JB	1.378	2.436e-012 to 780085896202
W21	0.0011	0.0001454 to 0.008188

Conclusion and Outlook

This study demonstrates that the toxicity of hydrophobic substances can be determined using the passive dosing approach. The use of the miniaturized format in 6-well microtiter plates facilitates a higher sample throughput, which increases the statistical power of the data obtained for the toxicity assessment as an increased number of test repetitions can be performed in a short time. The individual PAH exposures exhibited strong immobilization effects on the daphnids when exposed at the maximum exposure concentration. However, exposure to the natural recreated mixtures showed that the PAH mixture was less toxic to the daphnids than compared to the algae *R. subcapitata* (Annex 3).

Daphnia would probably only show clear effects in an event with greater contamination, as they did not respond sensitively enough in the immobilization assessment. The evaluation of the swimming behavior in a *Daphnia* toximeter could probably better indicate acute effects of toxic substances, as the swimming behavior of *Daphnia* is complex and multiparametric and is considered one of the most sensitive biomarkers of toxicity (Duquesne & Küster 2010). Furthermore, a reproduction test (21 d) could also be performed, which would reveal chronic effects. Therefore, to investigate the toxicity of the natural marine sediment load on organisms, it would be better to use the algae or a more sensitive endpoint of *Daphnia*.

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Supporting Information

Supplementary Table 1

Table S1: Ingredients of the dilution medium for biotests with *D. magna* according to OECD Guideline 202 (2004).

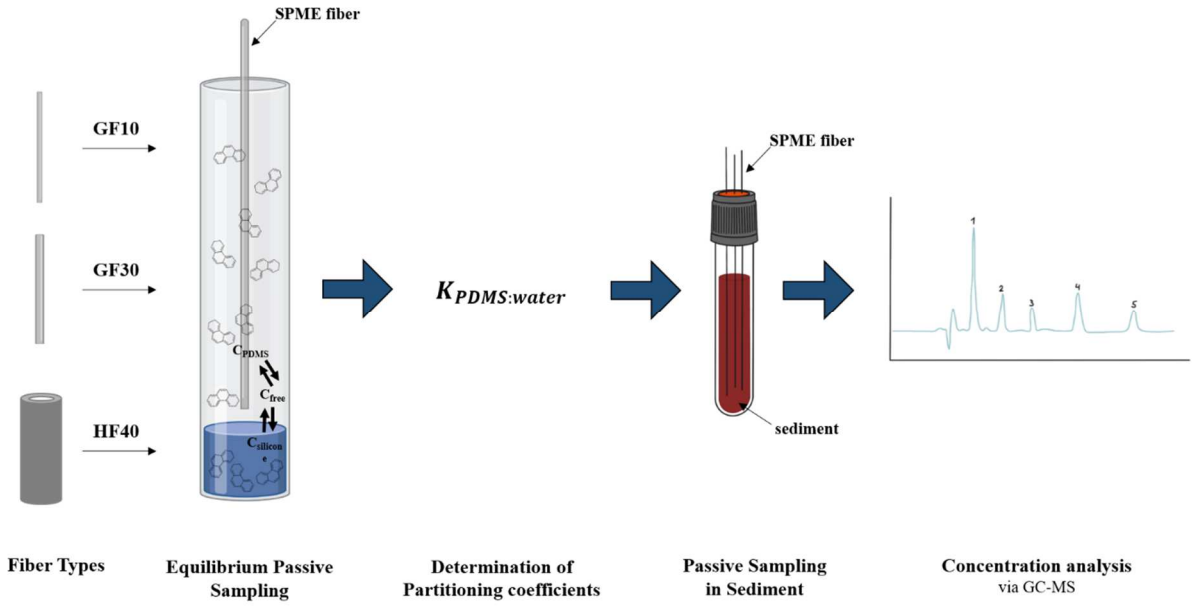
chemical	concentration [g L^{-1}]
calcium chloride ($CaCl_2 \cdot 2\ H_2O$)	11.76
magnesium sulphate ($MgSO_4 \cdot 7\ H_2O$)	4.93
sodium ($NaHCO_3$)	2.59
potassium chloride (KCl)	0.23

Supplementary Table 2

Table S2: HPLC-FD program for measuring of liquid samples diluted with methanol. Different flows and solvents were used. The oven temperature was set to 20 °C during measurement.

time [min]	acetonitrile [%]	ultrapure water [%]	flow [mL min^{-1}]
0.0	0.0	100.0	2.0
1.0	0.0	100.0	3.0
1.1	40.0	60.0	3.0
16.0	90.0	10.0	3.0
19.0	90.0	10.0	3.0
20.0	0.0	100.0	3.0
21.0	0.0	100.0	2.0
22.0	0.0	100.0	2.0

Development of a GC-MS method for equilibrium passive sampling of DDT in sediments



Development of a GC-MS method for equilibrium passive sampling of DDT in sediments

Anne Kreutzer[a,b], Moritz Kielmann[b] and Gesine Witt[b]

[a] Department Evolutionary Ecology and Environmental Toxicology, Institute of Ecology, Evolution and Diversity, Faculty Biological Sciences, Goethe University Frankfurt, Frankfurt 60438, Germany

[b] Department of Environmental Engineering, Faculty Life Sciences, Hamburg University of Applied Sciences, Hamburg 21033, Germany

Aims

In the North Sea, monitoring programs (e.g. MSFD, OSPAR and BSH) are currently not able to determine the crucial physicochemical parameters in high temporal and spatial resolution to comprehensively describe the transport, exchange and distribution processes of the most diverse chemical compounds. In Annex 1 a contribution to the monitoring programs was made to close this gap for the priority pollutant classes of PAHs and PCBs. A further contribution to a more holistic view in addition to the Annex 1 was raised in this study. Therefore, the bioavailability and the resulting risk of organochlorine pesticides, especially dichlorodiphenyltrichloroethane (DDT and its metabolites), in North Sea sediments were determined. The Stockholm Convention (2008) classifies DDT among the persistent organic pollutants (POPs), which are banned in western countries but can still be found in sediments in environmentally harmful concentrations due to their persistence. The hydrophobic compound is prone to accumulate in fish tissues (typically in fat) and is then biomagnified through food chains (Turusov et al. 2002). Since DDT continues to be intensively used in developing countries to combat malaria, the development of suitable indicators to assess the environmental risk of DDT and its metabolites is also of great international importance (Brockmeyer & Theobald 2016). The chemicals selected for this project are among the core indicators for the MSFD for OSPAR Commission (2010). Along with PAHs and PCBs, they are among the main contaminants in the sediment of the study area (Brockmeyer & Theobald 2016). However, there are currently no data on the contamination of the sediment pore water (C_{free}) by organochlorine pesticides, as the analytical methods are lacking. Therefore, the equilibrium passive sampling (EPSM) approach was applied by using solid-phase microextraction (SPME) with PDMS coated glass fibers to estimate C_{free} (see Annex 1 for SPME analysis of PAHs and PCBs) (Witt et al. 2010).

This study aims to develop an analytical method (GC-MS) to determine the total concentration of DDT in sediment. Furthermore, the method will be adapted to successfully apply SPME in sediments for DDT and its metabolites. To implement this approach, the polymer to water partition coefficients ($K_{PDMS:water}$) are required for translating polymer measurements into C_{free}. The determined $K_{PDMS:water}$ values were then applied to quantify equilibrium concentrations of DDT and its metabolites in pore water based on the analysis of fibers exposed ex-situ to field sediments.

Material and Methods

Test substances and PDMS Fibers

The following neat DDT and metabolite standards were used for the loading solution: o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT (Sigma Aldrich). The calibration of the GC-MS-system was performed using a liquid standard solution (Pesticide Mix 6, NEOCHEMA GmbH, 10 ng μL^{-1}). The equilibrium sampling experiments for DDT and its metabolites were conducted with three types of commercially available fibers: 1) GF10: 10 µm PDMS coated glass fibers SPC 210/230 (Fiberguide Industries, Stirling, NJ, USA); 2) GF30: 30 µm PDMS coated glass fibers (Polymicro Technologies Inc., Phoenix, USA); 3) HF40: 40 µm silicone tubing "Nagasep Hollow Fibre M-40" (Nagayanagi Co Ltd, Tokyo, Japan). The fiber properties are described in Table S1.

Dealing with DDT breakdown during GC-MS measurement

DDT is known to be fragile at high temperatures during GC measurements. The GC degradation is indicated by the formation of the degradation products DDD via dechlorination and/or DDE via dehydrochlorination (Foreman & Gates 1997, Gfrerer & Lankmayr 2005, Mirmigkou & de Boer 2015). The analysis of DDT in environmental samples requires monitoring for degradation of this pesticide in the GC injection system, where it occurs typically in the hot injection-port liner or on the front end of the capillary column (Foreman & Gates 1997). The breakdown is especially a concern for injection methods with an extended sample contact with the liner. Therefore, deactivated liners (deactivated CIS4 glass liners baffled; GERSTEL GmbH & Co. KG) were used to limit the breakdown level.

Cleaning SPME fibers

For cleaning the PDMS fibers, the fibers were cut in 10 cm pieces and placed in a pre-cleaned ASE cell with a glass fiber filter on the bottom of the cell. Subsequently, they were extracted three times with ethyl acetate using the ASE (ASE®350, DIONEX) (Table S2). After extraction, the cleaned fibers were transferred into an ASE vial and washed three times with ultrapure water in an ultrasonic bath for 15 minutes, while the water was renewed after every cleaning cycle. Afterwards, the cleaned fibers were stored in ultrapure water at 4 °C until their application.

Loading of Silicone Donor

The passive dosing vials were prepared based on previously published work (Birch et al. 2010, Mayer & Holmstrup 2008, Reininghaus et al. 2020). Therefore, medical-grade silicone was made with an elastomer kit (A-103 Platinum Silicone Elastomer, Factor II, Inc.). The two

components, the elastomer and the cross-linker were mixed according to the instructions given by the supplier. Each glass vial (60 mL, with PTFE screw caps) was coated by adding 1.5 ± 0.1 g silicone on the bottom, which resulted in an approximately 2500 µm thick layer. Afterwards, they were left in the refrigerator for at least 96 h to eliminate the bubbles in the silicone, at room temperature for at least 72 h followed by 48 h at 110 °C to complete the curing. According to Gouliarmou et al. (2012), the silicone layers were cleaned from impurities and oligomers by shaking three times for 72 h with ethanol and rinsing three times with ultrapure water to remove the ethanol.

The passive dosing vials were loaded by partitioning from an n-hexane suspension of the respective DDT and metabolites substances. To keep track of the possible DDT breakdown during the experiment, two different stock solutions were prepared. Therefore, a DDT stock solution included only o,p'-DDT (500 µg mL^{-1}) and p,p'-DDT (100 µg mL^{-1}), resulting in stock solution I. Furthermore, stock solution II contained the metabolites o,p'-DDE (1500 µg mL^{-1}), p,p'-DDE (1500 µg mL^{-1}), o,p'-DDD (150 µg mL^{-1}), p,p'-DDD (100 µg mL^{-1}). The target concentration in the silicone layer was achieved by adding 1 mL of either stock solution I or II to the corresponding vials. Afterwards, the hexane was slowly evaporated using a nitrogen flow. In the end, 60 mL of ultrapure water was added to each vial. The vials were placed on a shaker for 72 h to ensure chemical equilibrium between the silicone and water phase (Smith et al. 2010). The experiment was started by adding a total of 30 pre-cleaned fibers (10 GF10, 10 GF30, 10 HF40) to each vial. To avoid contact between the fibers and the passive dosing phase and the fibers themselves, a stainless steel holding fixture (pre-cleaned in 50 % / 50 % n-hexane/acetone) was used (Figure 1).

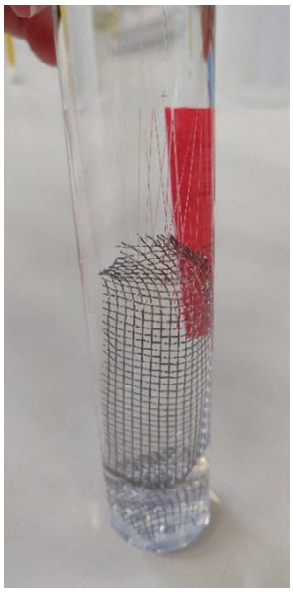


Figure 1: Ten fibers of each type (GF10, GF30, HF40) were placed in every vial using a stainless steel holding fixture.

The depletion of DDT and metabolites by the polymer needs to be negligible to prevent an artefactual reduction of the freely dissolved water concentration. This is fulfilled when the fibers do not reduce the concentration in the system by more than 5 % (Bartolomé et al. 2018). In order to meet this requirement, the fraction remaining in the dosing phase (e.g. silicone A-103) need to be > 95 %, while less than 5 % may partition into the water and sampling phase (e.g. fibers) (Lang et al. 2015, Mayer et al. 2003, Witt et al. 2013). The following relationship considering the partitioning coefficients between the different compartments has to be fulfilled:

$$K_{fib:sil} * (\frac{V_{fib}}{V_{sil}}) + \frac{V_{water}}{K_{sil:water} * V_{sil}} < 0.05 \quad (1)$$

where $K_{fib:sil}$ is the partition coefficient between fiber and A103-silicone, V_{fib} is the volume of the fiber PDMS (sampling phase), V_{sil} as volume of the A103-silicone (dosing phase), V_{water} as the volume of water and $K_{sil:water}$ as the partitioning coefficient between A103-silicone and water.

The $K_{sil:water}$ values were taken from Bao et al. (2013) as they applied 35 µm disposable PDMS fibers in a partitioning experiment in 35 ‰ seawater. In the present study, the depletion was calculated to reach a maximum of 2.72 % for p,p'-DDD with lower reductions expected for the other test substances at the following conditions: 39.84 µL fiber PDMS, 60 mL ultrapure water, 1530.6 µL A103-silicone, $K_{fib:sil}$ ~ 1.

Measurement of PDMS fibers, water and silicone donor

Over a time span of 49 days, the uptake kinetics of DDT and its metabolites into the different fiber types were measured. One fiber of each type (GF10, GF30, HF40) was removed from the system at 10 different time points (24, 48, 96, 168, 240, 336, 504, 696, 888 and 1176 h). Two replicates were sampled for every stock solution approach (I: DDT; II: DDD and DDE). Of each approach, two additional vials that had not been sampled during the experiment were used to provide two replicates for measurements at t = 1176 h (= 49 d). The fibers were not measured by thermodesorption to minimize the possible DDT breakdown as described above. Instead, the fibers were extracted in 1 mL hexane after measuring the exact length of the GF10 and GF30 fibers and the exact weight of the HF40 fibers. Afterwards, DDT, DDD and DDE concentrations were measured in the PDMS fiber coatings (C_{PDMS}) using a GC-MS system. Briefly, a gas chromatograph (GC 7890A; Agilent) and a quadrupole mass spectrometer (MS 5975C; Agilent, USA) equipped with an automated liner exchange system (ALEX; Gerstel,

Germany) was used. A sample volume of 1 µL was injected into the GC-MS system and the inlet PTV temperature was raised from 50 °C to 250 °C with 12 °C s^{-1} (hold for 15 min). The desorbed analytes were transferred splitless onto the column (HP-5MS; 325 °C: 30 m x 250 µm x 0.25 µm, J&W Scientific) and the GC oven temperature was programmed as follows: ramped from 100 °C to 250 °C with 15 °C min^{-1} (hold for 2 min) and ramped to 280 °C with 10 °C min^{-1} (hold for 1 min), whereby the transfer line temperature was kept constantly at 310 °C. The native compounds were measured in selected ion mode (SIM) (Table 1). Internal and external standard calibrations of DDT (Pesticide Mix 6, NEOCHEMA GmbH; 4,4´-DDT-d8, NEOCHEMA GmbH) were used to quantify target analytes desorbed from the fibers. The evaluation of the peaks was performed using the MassHunter software for quantitative analysis (version B.07.00/Build 7.0.457.0) by Agilent.

Table 1: Native and deuterated target compounds of DDT and metabolites with target masses and qualifiers for the detection with a mass selective detector.

compound	target mass	qualifier	retention time
o,p'-DDE	246.0	318.0	11.012
p,p'-DDE	246.0	318.0	11.421
o,p'-DDD	235.0	165.0	11.554
p,p'-DDD	235.0	165.0	12.056
o,p'-DDT	235.0	165.0	12.123
p,p'-DDT-d8	243.0	173.0	12.666
p,p'-DDT	235.0	165.0	12.711

After all fibers were removed at the last time point (t = 10), the DDT, DDD and DDE concentrations in the remaining water (about 60 mL) were determined by extracting three times with 40 mL n-hexane followed by 10 minutes of shaking in a 100 mL separating funnel. Afterwards, the extracts were combined and sodium sulfate (Na_2SO_4) was used as desiccant to remove water residues. Finally, the extract was evaporated under vacuum to a volume of 800 µL, using a rotary evaporator (Heidolph, Laborata 4000 eff.) and stored at 4 °C until analysis. Once the water was removed, the DDT, DDD and DDE concentrations of the silicone layers (dosing phase) were also quantified to confirm negligible depletion. Therefore, the silicone was extracted three times with 10 mL n-hexane for 24 h on a horizontal shaker and the n-hexane was then processed as previously described for the water samples.

Equilibrium confirmation

There are two different approaches to confirm the equilibrium state between PDMS and water. The first approach is built on the uptake profile for a passive sampling device, which operates in three regimes. The first one is the kinetic phase where increasing uptake into the PDMS fiber

takes place, followed by an intermediate one. The regime is completed by an equilibrium state resulting in a near-constant PDMS concentration. The uptake kinetics of DDT and its metabolites is best described by a first-order one-compartment model (Mayer et al. 2003):

$$C_{PDMS}(t) = C_{PDMS}(t_0) + (C_{PDMS\,max} - C_{PDMS}(t_0)) * (1 - e^{-K+t}) \quad (2)$$

$C_{PDMS}(t_0)$ = background concentration on fibre at test start [pg/µL$_{PDMS}$]
$C_{PDMS\,max}$ = maximum concentration on the fibre at equilibrium
K = desorption rate constant [h^{-1}]
t = time [h]

Reichenberg et al. (2008) described the second approach, which is based on parallel sampling with different PDMS fibers with multiple polymer coating thicknesses and the comparison of sampling rates to confirm the equilibrium state. Based on Endo et al. (2011) a time span of 49 days was chosen to provide sufficient time to achieve equilibrium between water and PDMS fibers. The calculation of the partitioning coefficients between PDMS and water is defined as:

$$K_{PDMS:water} = \frac{C_{PDMSeq}}{C_{water}} \quad (3)$$

where C_{PDMSeq} is the equilibrium concentration of the compound of interest in the PDMS fiber coating and C_{water} is the corresponding concentration in water (DiFilippo & Eganhouse 2010).

Total DDT concentration in sediment

The total concentration of DDT (C_{total}) in the North Sea sediment samples was determined by extracting freeze-dried sediment using accelerated solvent extraction (ASE 350®, Dionex). To eliminate interferences and avoid cross-contamination the 22 mL Dionex cell was cleaned with n-hexane and allowed to dry. Afterwards, a pre-cleaned glass wool filter and approximately 2 - 4 g dried sediment were introduced in the Dionex cell. The remaining volume of the cell was filled with diatomaceous earth. A cell containing only diatomaceous earth served as blank. Activated copper was added to the ASE vials for sulfur removal immediately after extraction. The sediment in each cell was extracted twice with about 80 mL of an acetone/hexane mixture (v/v = 40/60) using the methods described in Table S3.

Extracts of primary and secondary extraction were combined during the solid phase extraction (SPE) in a two-stage column using a Baker Bond (J.T. Baker) system with deactivated aluminum oxide (approx. 3 g, top) and silica (approx. 3 g, bottom). Teflon frits were added to

the bottom and top of each column to separate solid phases. Subsequently, the volume of the extract was reduced to about 500 µL using a rotary evaporator (Heidolph, Laborata 4000 eff.) and filled up with hexane to 800 µL. However, the sediment extracts were spiked with 100 ng of internal standard (4,4´-DDT-d8, Neochema GmbH) right before the GC-MS measurement to minimize the DDT breakdown process. Afterwards, the samples were measured using GC-MS as described above to determine the total sediment concentration (C_{total}).

Equilibrium sampling with SPME

Freely dissolved pore water concentrations (C_{free}) of DDT and its metabolites (DDD and DDE) were determined in sediment samples using SPME according to Witt et al. (2013). The ex-situ SPME experiments were conducted as described in Annex 1. Briefly, three PDMS coated glass fibers were placed in a chromacol vial containing 7-10 g of thawed and homogenized sediment. The vials were then shaken on an overhead shaker for 14 days in darkness until equilibrium between sediment pore water and fibers was reached (Lang et al. 2015). Afterwards, the cleaned fibers were stored at -20 °C until analysis. The DDT measurement in the fibers was performed using the GC-MS system as described above. The thermal desorption method for PDMS fibers was already performed in Annex 1, but other parameter settings were necessary for DDT analysis. Briefly, small glass beads (ø 0.75-1.0 mm; Carl Roth, Germany) were placed right above the notch in the glass liner (KAS4; Gerstel, Germany) to ensure the fiber stayed in position during thermal desorption. The fiber was placed in a cleaned glass liner, which was then transferred into the cooled injection system (CIS) for thermal desorption (50 °C raised to 100 °C at 15 °C s^{-1}, then held for 1 minute). The desorbed analytes were transferred in splitless mode onto the column (HP-5MS, 325 °C: 30 m x 250 µm x 0.25 µm; J&W Scientific, USA) and the GC oven temperature was programmed as follows: ramped from 100 °C to 250 °C with 15 °C min^{-1} (hold for 2 min) and ramped to 280 °C with 10 °C min-1 (hold for 1 min), whereby the transfer line temperature was kept constantly at 310 °C. The native compounds were measured in selected ion mode (SIM). External standard calibration of DDT and its metabolites (Pesticide Mix 6, NEOCHEMA GmbH) was used to quantify target analytes desorbed from the fibers. MassHunter software for quantitative analysis (version B.07.00; Agilent, USA) was used for peak evaluation.

Results and Discussion

Uptake Kinetics and Equilibrium Confirmation

The uptake profiles of DDT and its metabolites into the PDMS were generated through fitting a pseudo-first-order association model (Eq. 2) to the time series data of the 49 d passive dosing-passive sampling experiment (Figure 2).

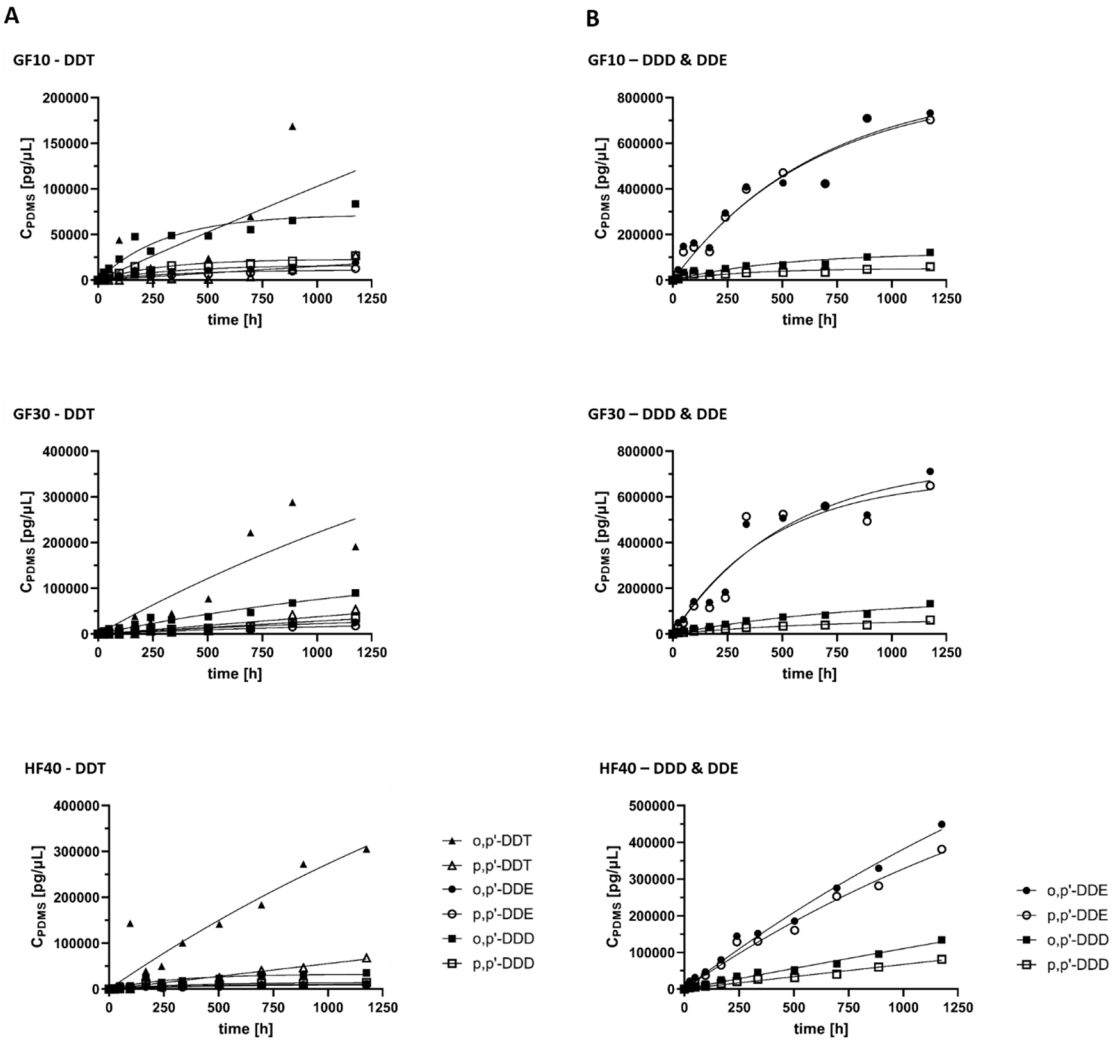


Figure 2: Uptake profiles of DDT and its metabolites DDE and DDD into the GF10, GF30 and HF40 PDMS fibers. **A:** Approach with only DDT in the silicone donor. **B:** Approach with DDD and DDE in the silicone donor.

The approach with only DDT in the passive dosing phase showed that the o,p'-DDT uptake in the PDMS fibers GF10 and GF30 was almost linear and decreased towards the end of the experiment. Furthermore, the absorption of p,p'-DDT into the PDMS was significantly lower than that of o,p'-DDT in all three fiber types. Since only DDT was introduced into the passive dosing phase at the beginning of the experiment, it can be clearly seen that both DDT isomers were partially degraded into their metabolites during the experiment (Figure 2A). Therefore, it is difficult to determine the exact equilibrium partition coefficients, as it is not possible to

determine the exact DDT uptake and state of equilibrium. The approach with DDD and DDE in the passive dosing phase showed that both DDE isomers accumulate exponentially in the PDMS of the fiber types GF10 and GF30 (Figure 2B). The uptake in the HF40 hollow fibers is more in line with a linear profile. Furthermore, the DDD isomers also showed a linear uptake profile in all fiber types and were about five times lower in their concentrations than the DDE isomers.

In all approaches, none of the substances reached a concentration plateau, which might denote that the state of equilibrium was not reached after 49 days. However, in the DDD and DDE approach no obvious concentration differences (pg/µL PDMS) between the GF10, GF30 and HF40 fibers were observed for most substances, indicating that equilibrium was reached in the fiber-water system for all PDMS fiber types (Figure 3B). Concentration ratios of 40 µm and 10 µm fibres (HF40/GF10) ranged between 0.77 (p,p'-DDE) and 1.40 (p,p'-DDD). Ratios for 30 µm and 10 µm fibres (GF30/GF10) ranged from 1.12 (p,p'-DDD) to 1.22 (o,p'-DDE) while 40 µm and 30 µm fibres (HF40/GF30) ranged from 0.66 (p,p'-DDE) to 1.25 (p,p'-DDD). The approach with only DDT in the passive dosing phase showed a clearly degradation of DDT during the experiment, but the o,p'-DDT uptake across all fiber types appeared to be more evenly distributed compared to p,p'-DDT (Figure 3A). The concentration ratios of HF40/GF10 ranged from 0.77 (o,p'-DDE) to 4.26 (p,p'-DDT). Ratios for GF30/GF10 were between 0.93 (o,p'-DDD) and 2.57 (p,p'-DDT) while HF40/GF30 ratios ranged from 0.71 (p,p-DDE) to 1.66 (p,p'-DDT).

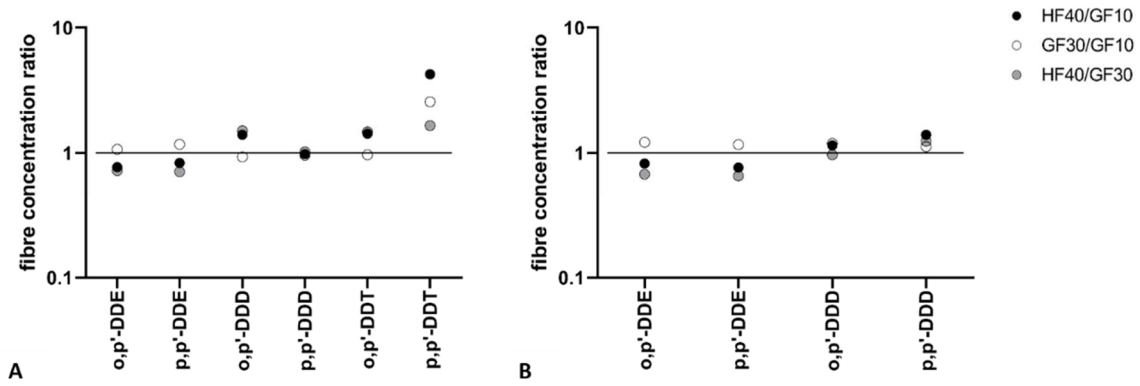


Figure 3: Relative fiber concentration ratios across coating thicknesses. Values were divided by each other (HF40/GF10, GF30/GF10 and HF40/GF30) and the resulting values are plotted. **A:** Approach with only DDT in the silicone donor. Higher differences for p,p'-DDT while remaining substances show higher similarities. **B:** Approach with DDD and DDE in the silicone donor.

Determination of partition coefficients

The PDMS water partition coefficients for the different fiber types and the silicone dosing layer were calculated from the water concentrations (C_{water}) determined for each vial (n = 4) at the end of the experiment and the respective fiber and silicone layer concentrations from the same vial (Table 2). The measured water concentrations between replicates showed moderate variations with a coefficient of variation (CV) of about 25 % for the DDT isomers (DDT approach) and about 15 % for DDD (DDD and DDE approach; Table S4). In contrast, DDE showed a much higher CV of about 49 %. (Table S5). The concentrations of DDD and DDE in the DDT approach were not taken into account, as they were side products of the DDT breakdown during the experiment. These high variations are probably due to the different handling of the vials, as vials 1 and 2 were repeatedly opened to remove fibres, while vials 3 and 4 remained untouched until the final measurement. In the DDT approach, the standard deviation between two identically treated replicates showed lower mean CVs of 26 % (untouched vial 3 and 4) and 42 % (vial 1 and 2), respectively. Whereas, in the DDD and DDE approach, the CVs of the untouched vials (vial 3 and 4) were higher (60 %) compared to vial 1 and 2 (6 %).

Table 2: Molecular weight, log K_{OW} and calculated log $K_{PDMS:water}$ for GF10, GF30, HF40 fibers and A-103 silicone coating for DDT determined in this study. Error in log $K_{PDMS:water}$ is calculated based on four replicates.

	MW [g mol^{-1}]	**log K_{OW}[a]**	**log $K_{PDMS:water}$ GF10**	**log $K_{PDMS:water}$ GF30**	**log $K_{PDMS:water}$ HF40**	**log $K_{A-103sil:water}$**
o,p'-DDE	318.03	6.96	6.23 ± 0.27	6.31 ± 0.33	6.11 ± 0.24	5.71 ± 0.38
p,p'-DDE	318.03	6.96	6.28 ± 0.31	6.35 ± 0.39	6.13 ± 0.28	5.76 ± 0.43
o,p'-DDD	320.04	6.22	5.76 ± 0.15	5.83 ± 0.18	5.82 ± 0.15	5.25 ± 0.19
p,p'-DDD	320.04	6.22	5.46 ± 0.15	5.50 ± 0.18	5.61 ± 0.15	5.13 ± 0.19
o,p'-DDT	354.49	6.91	4.95 ± 0.35	4.60 ± 1.10	5.47 ± 0.35	4.87 ± 0.24
p,p'-DDT	354.49	6.91	4.49 ± 0.44	5.32 ± 0.25	5.25 ± 0.36	4.85 ± 0.22

[a]: Mackay et al. (2006)

In this experiment, the log $K_{PDMS:water}$ values varied between the three fiber types GF10, GF30 and HF40, with the largest variation found for the substance p,p'-DDT (0.76 log units for HF40 vs. GF 10 and 0.83 log units for GF30 vs. GF10). The PDMS water partition coefficients for o,p'-DDT also varied considerably with 0.52 log units for HF 40 vs. GF10 and 0.87 log units for HF40 vs. GF30. However, the substances DDD and DDE accounted for more consistent log $K_{PDMS:water}$ values, with the highest variation between HF40 vs. GF10 for o,p'-DDE (0.12 log units) and p,p'-DDD (0.15 log units). For p,p'-DDE the largest deviation was found for HF40 vs. GF30 (0.22 log units), while for o,p'-DDD the highest variation was identified for

GF30 vs. GF10 with 0.07 log units. DiFilippo and Eganhouse (2010) described that most of the discrepancies in the published $K_{polymer:water}$ could be due to methodology, but may also be due to uncertainties in fiber coating thickness or fiber source. The coating of the A-103 silicone layer as well as the HF40 were determined by weighing and showed variation in $K_{polymer:water}$ values between 0.43 and 0.36 log units, respectively.

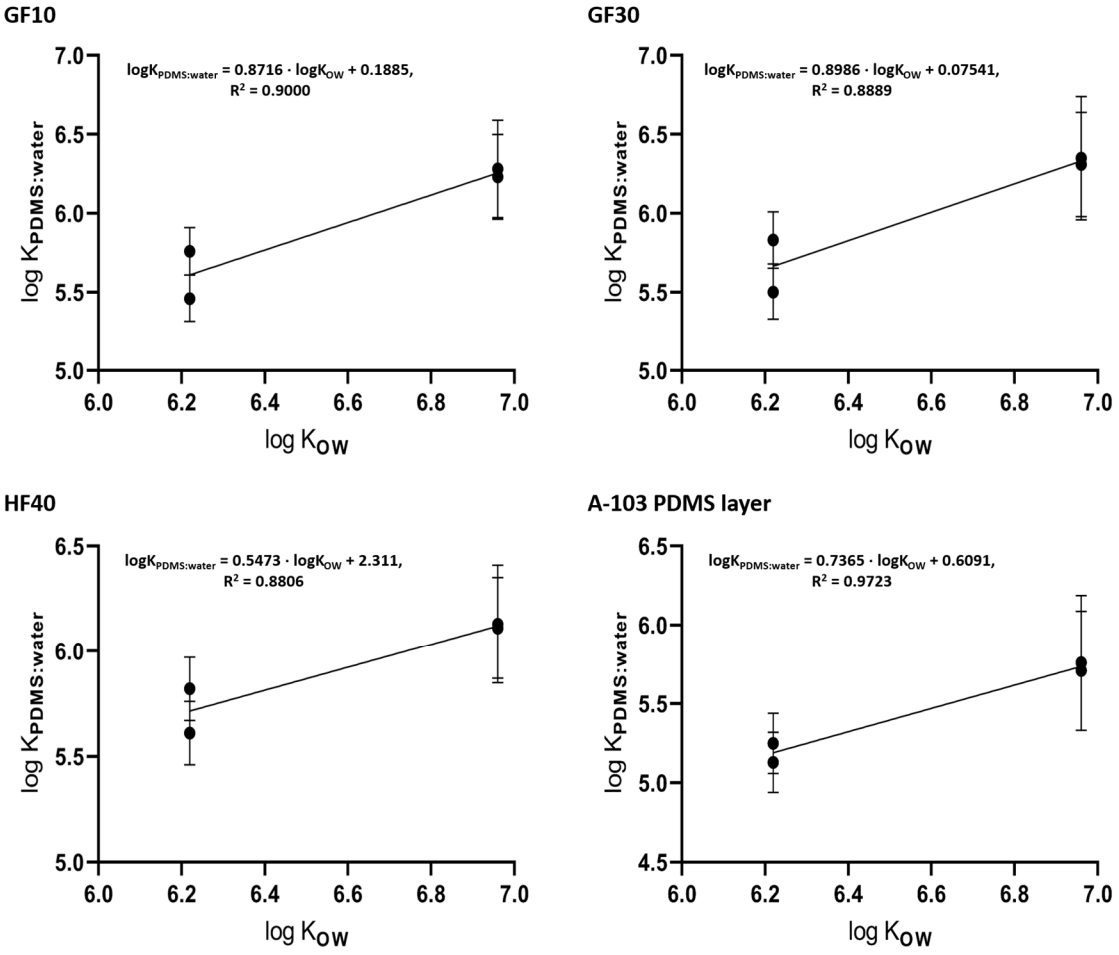


Figure 4: Relationship between log K_{OW} and log $K_{PDMS:water}$ of o,p'-DDE, p,p'-DDE, o,p'-DDD and p,p'-DDD. Data for GF10, GF30, HF40 and A-103 silicone layer (passive dosing phase) (each n = 4) with standard deviation as error bars.

The PDMS partition coefficients of DDD and DDE were plotted against the respective octanol-water coefficient (Burgess et al. 2013). The high coefficients of determination (R^2) for the PDMS fibers and silicone indicate that the regression predictions fit the data. (GF10: 0.90, GF30: 0.89, HF40: 0.88, A103-PDMS: 0.97; Figure 4). Figure 5 shows the well-fitting relationship ($R^2 = 0.8937$) between the log K_{OW} and log $K_{PDMS:water}$ for pooled data generated across the different fiber types for the substances DDD and DDE. However, it should be taken into account that DDT was excluded from the correlation due to its degradation and only four values (DDD and DDE isomers) were considered for the calculation of the model.

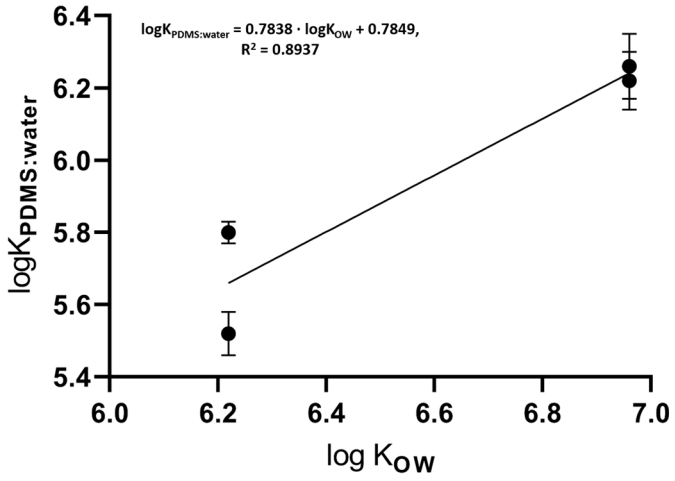


Figure 5: Relationship between log K_{OW} and log $K_{PDMS:water}$ of o,p'-DDE, p,p'-DDE, o,p'-DDD and p,p'-DDD. Pooled data from GF10, GF30 and HF40 (each n = 6) with standard deviation as error bars.

The obtained log $K_{PDMS:water}$ values (mean of GF10, GF30 and HF40 fibers) were compared with literature data (Table 3). Although the fiber coating thickness used in the literature experiments was different (100 µm or 7 µm coated PDMS fibers), it can be seen that the values are all in the same order of magnitude. The partition coefficients of DiFilippo and Eganhouse (2010) and Xing et al. (2009) fit the best with the data of this study. The partition coefficients for the study of DiFilippo and Eganhouse (2010) vary between 0.14 (p,p'-DDE) and 0.70 (o,p'-DDT) log units compared to this study. In contrast, the variations of the study by Xing et al. (2009) are higher, as the values range from 0.24 to 0.72 log units for p,p'-DDE and o,p'-DDT, respectively.

Table 3: Mean value of the distribution coefficients of the three fiber types (log $K_{PDMS:water}$) compared with literature data. The log $K_{PDMS:water}$ values of each fiber type are displayed in Table2.

	mean fibers	**study 1[a]**	**study 2[b]**	**study 3[c]**	**study 4[d]**	**study 5[e]**
o,p'-DDE	6.22	6.05	5.94			
p,p'-DDE	6.26	6.12	6.02	5.26	4.00	5.39
o,p'-DDD	5.80	5.30	5.09			
p,p'-DDD	5.52	5.13	4.89	4.55	4.32	4.45
o,p'-DDT	5.01	5.71	5.73			
p,p'-DDT	5.02	5.39	5.40		4.36	

[a] DiFilippo and Eganhouse (2010): 100 µm PDMS fibers (supplier Supelco Analytical, Sigma-Aldrich Corp., St. Louis, MO)
[b] Xing et al. (2009): 100 µm PDMS fibers, unknown coating thickness (supplier unknown)
[c] Paschke and Popp (2003): 100 µm PDMS fibers (supplier Supelco, Germany)
[d] Magdic and Pawliszyn (1996): 100 µm PDMS fibers (supplier Supelco, Canada)
[e] Paschke and Popp (2003): 7 µm PDMS fibers (supplier Supelco, Germany)

Total sediment concentrations

The total sediment concentrations (C_{total}) of DDT and its degradation products were determined at four sampling stations in the North Sea (Annex 1, Figure 1). The chemical load of all investigated substances (sum of DDT, DDD and DDE) was highest at station KSII with an increase towards the mean sediment depth with a maximum at 15 cm sediment depth (∑ DDTs = 74 ng g^{-1} DW) (Figure 6). The concentration at station GB2 increased slightly with sediment depth but was only about half as high at station KSII (maximum at 5 cm depth, ∑ DDTs = 37 ng g^{-1} DW). The pollutant distributions at the stations JB and W21 showed constant concentrations (all below ∑ DDTs = 14 ng g^{-1} DW) along the depth gradient and were considerably lower than at the other two stations. The sediment layers of stations KSII and GB2 might be disturbed and highly contaminated since these stations are close to the dumping area of the Elbe sediment (see Annex 1). At station KSII in particular, it can be seen that DDT is still present in relatively high concentrations as a burden of the past, although the production and use of DDT have been banned in Germany since 1977. Brockmeyer and Theobald (2016) reported p,p'-DDE concentrations for Helgoland Bay sediment of up to 1.4 ng g^{-1} DW, as this metabolite was used as the lead substance for the analysis. In this study, p,p'-DDE concentrations of up to 6.4 ng g^{-1} DW (station GB2) were measured.

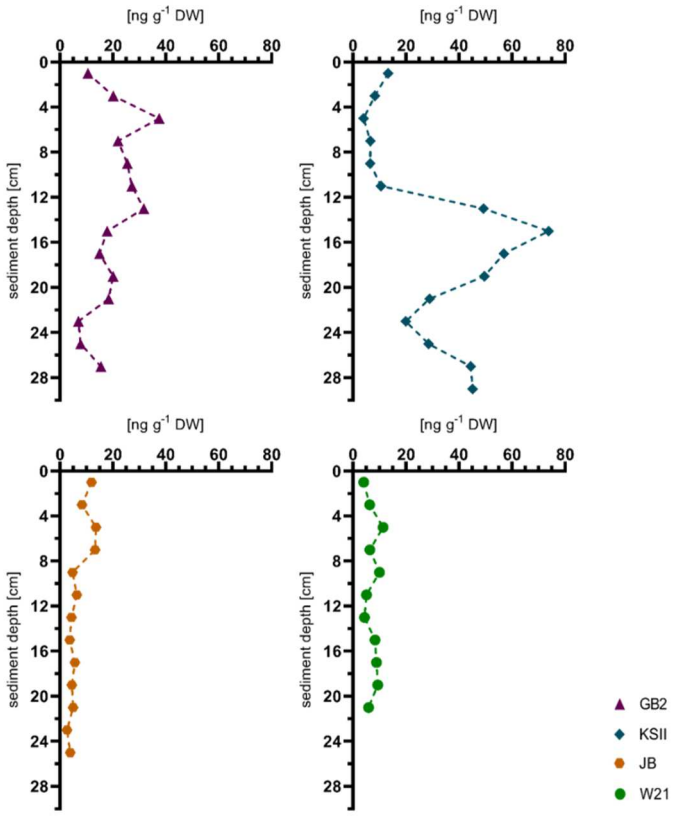


Figure 6: Depth profiles of the total sediment concentration of DDT, DDD and DDE of all four North Sea stations.

The analysis of the DDT composition of the surface sediment of the North Sea showed that p,p'-DDT dominated mainly at stations GB2 and KSII (Figure 7). At stations JB and W21, however, the degradation products o,p'-DDE and p,p'-DDE accounted for the largest share of the chemical load.

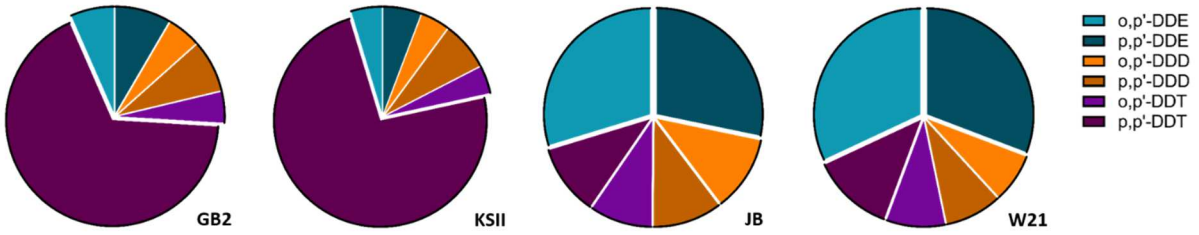


Figure 7: Percentage composition of C_{total} in North Sea surface sediment at all four sampling stations.

Pore water concentrations (C_{free})

The distribution of DDT and its metabolites was estimated by analyzing the surface sediment concentrations at four North Sea stations. The total sediment concentrations (C_{total}) of DDT and its degradation products has already been determined in nanogram range, which indicates that the freely dissolved pore water concentration (C_{free}) could once again be significantly lower. Therefore, the limits of detection (LOD) and limits of quantification (LOQ) for the applied GC-MS method were determined for all six substances (Table 4).

Table 4: Limit of detection (LOD) and limit of quantification (LOQ) for DDT and its metabolites.

	LOD [pg μL^{-1}]	LOQ [pg μL^{-1}]
o,p'-DDT	3.321	12.032
p,p'-DDT	10.115	35.612
o,p'-DDE	2.724	9.891
p,p'-DDE	2.283	8.304
o,p'-DDD	3.755	13.591
p,p'-DDD	8.759	30.695

However, the analysis of the thermal desorption method showed no distinct peaks for all six substances, indicating that all values were below the LOD and LOQ, respectively. Thus, the bioavailable environmental concentrations were not detectable in the surface sediments of the four North Sea stations.

Conclusion and Outlook

The determination of the DDT partition coefficients proved to be well feasible and the results were in the same order of magnitude as values already described in literature. However, the determination of the distribution coefficients should be repeated and DDT degradation should be monitored more closely. This will allow a better differentiation of the individual substance concentrations. The higher total concentration (C_{total}) of DDT and its metabolites in deeper sediment layers could be due to the fact that the ban on DDT only came into force in 1977. Nevertheless, the success of the restrictions on the insecticide DDT cannot be clearly demonstrated in the sediment depth profiles, as the sediment has not been dated. Furthermore, the sampling stations near the sediment dumping site do not show undisturbed sediment cores, as the dumping causes a continuous input and thus also mixing of the sediment layers. However, the bioavailable concentrations (C_{free}) of the surface sediment were below the limit of quantification. This demonstrates that the contamination of the surface sediment of the North Sea with DDT is low.

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Supporting Information

Supplementary Table 1

Table S1: Fiber parameters and fiber geometry calculated for 1 cm fiber length from Witt et al. (2013).

name	coating thickness[a] *s* [µm]	inner radius *r* [µm]	outer radius[b] *R* [µm]	PDMS-volume *V* [µL cm^{-1} fiber]	surface area *A* [cm^2]	*A*/*V* [cm^2 cm^{-3}]	V_{sed}/V_{fiber} [cm^3 cm^{-3}]
GF10	12.7	103.6	116.3	0.0877	0.0731	833.9	9118.2
GF30	26.7	53.6	80.3	0.1123	0.0505	449.2	7122.8
HF40	48.1	85.3	133.4	0.3305	0.0838	253.6	2420.7

[a]: Mean values for 12 replicates; [b]: R = r + s; GF: fiber with glass core and PDMS coating; HF40: PDMS tube/hollow fiber

Supplementary Table 2

Table S2: Extraction method for fiber cleaning using the ASE 350.

parameter	method
preheat [min]	0
heat [min]	7
static [min]	10
Flush % [vol]	90
purge [sec]	120
cycles	2
pressure [bar]	50
temperature [°C]	130
Sol A	other 100 %
Sol B	other 0 %
Sol C	other 0 %
Sol D	other 0 %

Supplementary Table 3

Table S3: Extraction method for sediment extraction using ASE.

parameter	method 1	method 2
preheat [min]	0	0
heat [min]	5	5
static [min]	10	5
Flush % [vol]	25	25
purge [sec]	120	300
cycles	2	4
pressure [bar]	140	140
temperature	100	100
Sol A	other 100 %	other 100 %
Sol B	other 0 %	other 0 %
Sol C	other 0 %	other 0 %
Sol D	other 0 %	other 0 %

Supplementary Table 4

Table S4: Measured water concentrations in four test vials of the DDT approach. Water concentrations (in µg/L) of each of the four test vials at the end of the kinetic experiment (1176 h). Vials 1 and 2 were opened repeatedly to remove fibers, whereas vials 3 and 4 were left untouched until the final measurement. The mean values of each set with standard deviation (SD) and coefficient of variation (CV, %) as well as for all four vials in total were calculated.

	Vial	o,p'-DDE	p,p'-DDE	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT
	1	0.0708	0.0738	0.0226	0.0114	0.5378	0.2634
	2	0.0074	0.0108	0.0166	0.0063	0.9921	0.4478
	3	0.0082	0.0085	0.0159	0.0049	2.0710	0.8372
	4	0.0091	0.0071	0.0106	0.0031	0.6743	0.2785
mean		0.0391	0.0423	0.0196	0.0089	0.7649	0.3556
SD	**1 & 2**	0.0317	0.0315	0.0030	0.0025	0.2272	0.0922
CV [%]		81.0399	74.3950	15.3392	28.4028	29.6968	25.9230
mean		0.0086	0.0078	0.0132	0.0040	1.3727	0.5579
SD	**3 & 4**	0.0005	0.0007	0.0026	0.0009	0.6984	0.2793
CV [%]		5.4482	8.9276	19.9680	22.6111	50.8765	50.0734
mean	**all**	0.0239	0.0251	0.0164	0.0064	1.0688	0.4567
SD	**vials**	0.0152	0.0173	0.0032	0.0024	0.3039	0.1011
CV [%]	**(1-4)**	63.8049	68.8420	19.4440	37.9477	28.4303	22.1434

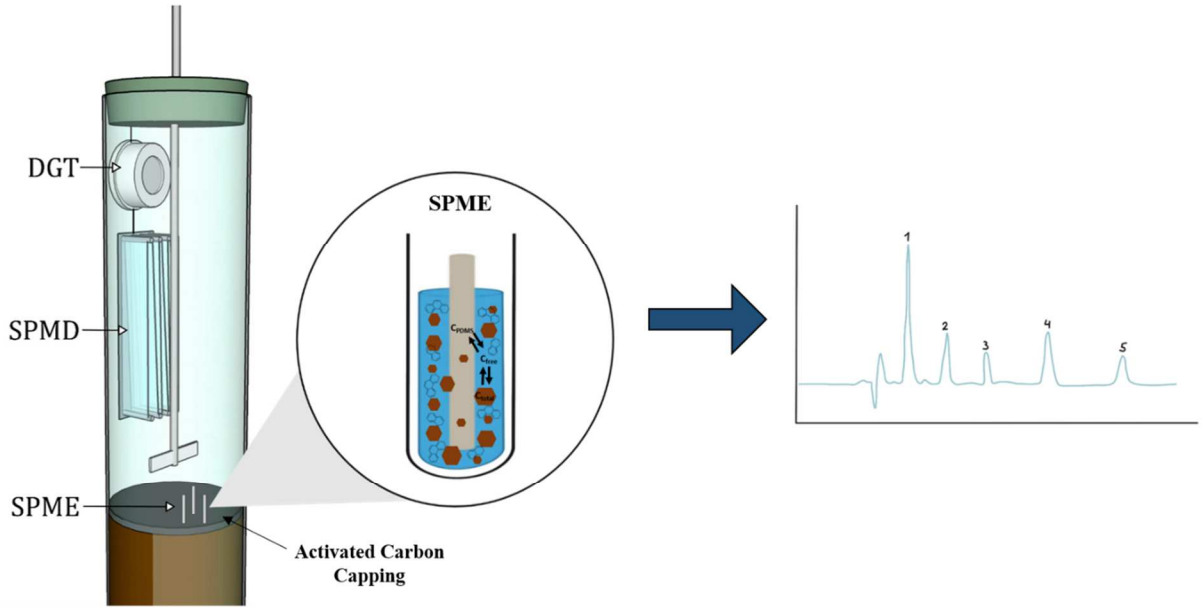
Supplementary Table 5

Table S5: Measured water concentrations in four test vials of the DDD and DDE approach. Water concentrations (in µg/L) of each of the four test vials at the end of the kinetic experiment (1176 h). Vials 1 and 2 were opened repeatedly to remove fibers, whereas vials 3 and 4 were left untouched until the final measurement. The mean values of each set with SD and CV (%) as well as for all four vials in total were calculated.

	Vial	o,p'-DDE	p,p'-DDE	o,p'-DDD	p,p'-DDD
	1	0.4277	0.3101	0.3301	0.3462
	2	0.4226	0.3420	0.2933	0.2740
	3	1.9705	2.0316	0.3384	0.2858
	4	0.2073	0.1672	0.1455	0.1445
mean		0.4251	0.3261	0.3117	0.3101
SD	**1 & 2**	0.0026	0.0160	0.0184	0.0361
CV [%]		0.6050	4.9011	5.9050	11.6322
mean		1.0889	1.0994	0.2419	0.2152
SD	**3 & 4**	0.8816	0.9322	0.0965	0.0706
CV [%]		80.9656	84.7960	39.8708	32.8239
mean	**all**	0.7570	0.7127	0.2768	0.2626
SD	**vials**	0.3319	0.3867	0.0349	0.0475
CV [%]	**(1-4)**	43.8401	54.2525	12.5992	18.0731

Annex 6

Sediment remediation using activated carbon: effects of sorbent particle size and resuspension on sequestration of metals and organic contaminants



Equilibrium Passive Sampling
with different sampling devices

Concentration analysis
via GC-MS

Sediment remediation using activated carbon: effects of sorbent particle size and resuspension on sequestration of metals and organic contaminants

Robert Rämö[a], Stefano Bonaglia[a], Inna Nybom[b], Anne Kreutzer[c], Gesine Witt[c], Anna Sobek[b] and Jonas S. Gunnarsson[a]

[a] Department of Ecology, Environment and Plant Sciences (DEEP), Stockholm University, 106 91 Stockholm, Sweden

[b] Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University, Stockholm, Sweden

[c] Department of Environmental Technology, Hamburg University of Applied Sciences (HAW Hamburg), Hamburg, Germany

Abstract

Thin-layer capping using activated carbon (AC) has been described as a cost-effective *in situ* sediment remediation method for organic contaminants. In this study, we compare the capping efficiency of powdered AC (PAC) against granular AC (GAC) using contaminated sediment from Oskarshamn harbor, Sweden. The effects of resuspension on contaminant retention and cap integrity were also studied. Intact sediment cores were collected from the outer harbor and brought to the laboratory. Three thin-layer caps, consisting of PAC or GAC mixed with clay, or clay only, were added to the sediment surface. Resuspension was created using a motor-driven paddle to simulate propeller wash from ship traffic. Passive samplers were placed in the sediment and in the water column to measure the sediment-to-water release of PAHs, PCBs, and metals. Our results show that a thin-layer cap with PAC reduced sediment-to-water fluxes of PCBs by 57 % under static conditions and 91 % under resuspension. Thin-layer capping with GAC was less effective than PAC, but reduced fluxes of high-molecular weight PAHs. Thin-layer capping with AC was less effective in retaining metals, except for Cd, which release was significantly reduced by PAC. Resuspension generally decreased water concentrations of dissolved cationic metals, perhaps due to sorption to suspended sediment particles. Sediment resuspension in treatments without capping increased fluxes of PCBs with $\log K_{ow} > 7$ and PAHs with $\log K_{ow}$ 5-6, but resuspension reduced PCB and PAH fluxes through the PAC thin-layer cap. Overall, PAC performed better than GAC, but adverse effects on the benthic community and transport of PAC to non-target areas are drawbacks that favor the use of GAC.

Introduction

Activated carbon (AC) amendment is a promising approach for *in situ* remediation of contaminated sediments, which offers a less intrusive and more cost-effective treatment than conventional methods such as dredging or isolation capping (Ghosh 2011; Patmont 2014; Kupryianchyk 2015). AC has a high sorption affinity for hydrophobic organic contaminants (HOCs) that readily accumulate in sediment, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Zimmerman 2004; Millward 2005; Chai 2012). AC amendment increases sorption and sequestration of HOCs in the sediment and reduces aqueous concentrations, resulting in lower bioavailability and bioaccumulation of HOCs in benthic organisms (Zimmerman 2004; Millward 2005; McLeod 2007; Kupryianchyk 2012). AC amendment has been primarily studied for sequestration of HOCs, but AC may also

increase the sequestration of metals in sediment. Netzer (1984) reported pH-dependent sorption of Pb, Cu, and Co to AC. Van Sprang (2001) reported sequestration of Cd and Ni and described AC as a weak cation exchanger. AC is also known to be an effective sorbent for Cd and Zn in wastewater treatment (Ku 1987).

Capping efficiency, i.e., reduction of freely dissolved contaminants and their bioavailability, has been found to depend both on the dose of AC and its particle size (Zimmerman 2005), where powdered AC (PAC, <300 µm) is more efficient than granular AC (GAC, 300 – 2000 µm). The higher sorption capacity of finer particles is due to a larger proportion of the total surface area of the particle being readily accessible from the outside of the particle, which allows for rapid sequestration (Zimmerman 2005; Sun 2007; Tomaszewski 2007). However, prolonged contact times allow for mass transport further into the particles, and GAC may over time achieve a similar capping efficiency to PAC (Werner 2006; Kupryianchyk 2013a; Choi 2014b).

Several techniques have been used to place AC in aquatic environments. Mixing AC into sediment results in a homogenous distribution of AC in the sediment with a high initial remediation effectiveness (Choi 2014a; Choi 2014b) but disturbs the contaminated sediment and its benthic community. *In situ* thin-layer capping, where AC is deposited on the sediment surface, often requires no specialized equipment and therefore is more cost-effective than mechanical mixing (Patmont 2014; Kupryianchyk 2015). Bioturbation is expected to distribute the AC from a thin-layer cap into the biologically active layer over time (Cornelissen 2011, 2012, Lin 2014).

The suitability of thin-layer capping depends on the capping materials, ongoing emission of contaminants and deposition of contaminated sediment, site conditions such as water currents and bathymetry, and erosional forces such as sediment resuspension (Lampert 2011, Cornelissen 2012, Graham 2013, Abel 2018). Resuspension may lead to dispersal of contaminated particles and facilitates contaminant desorption into the overlying water (Latimer 1999; Birdwell 2007; Kalnejais 2010). Because AC particles have near-neutral buoyancy, they may be transported from a site of application by storms, currents, and propeller wash, leading to erosion of the thin-layer cap over time. Various pelletized forms of AC and clay mixtures have been formulated to improve deployment and retention of AC thin-layer caps, including SediMite®, AquaGate®, and others (Menzie 2016, Chadwick 2017, Abel 2019), but these are not evaluated in the present study.

Abel (2018) studied AC thin-layer capping in a shallow lake in Finland and reported high losses of PAC following storm events, as well as rapid burial of the thin-layer cap under freshly deposited contaminated sediment. Cornelissen (2011) studied thin-layer capping in a Norwegian harbor and reported 70 % loss of PAC from a pure thin-layer cap after one year, however, a blend of PAC and clay improved retention rates and reduced losses to 40 %. In a large field experiment in the Grenland fjords (Norway), a thin-layer cap of AC mixed with clay was found to persist 9 years post-treatment, and the thin-layer cap remained effective despite recontamination by sediment transported from surrounding areas (Schaanning 2021).

Generally, field experiments have favored PAC thin-layer capping rather than GAC due to the higher initial remediation efficiency, but it is clear that cap retention is sometimes an issue. Larger particles generally require more energy to resuspend than smaller ones (Graham 2013), which means that using GAC may improve retention of a thin-layer cap compared to PAC. However, the smaller AC particles have higher initial remediation efficiency, which means there is a trade-off to make between initial remediation efficiency and long-term retention of a thin-layer cap in sites with sediment resuspension.

The aims of this study were to assess the effects of AC thin-layer capping and resuspension on HOC and metal release from contaminated sediment. We simulated frequent resuspension by ship traffic in a two-month laboratory experiment using intact sediment cores from a contaminated harbor. We measured sediment-to-water release and porewater concentrations using a two-factorial design: static (non-resuspended) or resuspended condition, and four thin-layer capping treatments: 1) GAC mixed with clay; 2) PAC mixed with clay; 3) clay only; or 4) uncapped sediment.

To our knowledge, this is the first study that investigates the effects of resuspension on AC thin-layer capping under controlled laboratory conditions, and one of few studies to assess effects on both organic contaminants and metals. We expect that sediment resuspension increases the release of HOCs and metals to overlying water, and that AC thin-layer capping reduces contaminant release. We expect thin-layer capping with AC to be more effective than capping with clay only, and for thin-layer capping with PAC to be more effective than with GAC.

Material and Methods

Study area and field sampling

Sediment cores were collected from the outer basin of Oskarshamn harbor, Sweden (Figure 1). Oskarshamn is one of the most contaminated harbors in Sweden, owing to historic industrial emissions from a copper processing plant (in operation 1918–1969), a battery factory, and municipal wastewater, all of which were released into the inner harbor until more strict environmental regulations came into force in 1969. The harbor is an active freight port with an oil terminal and connects passenger ferries to the Baltic Sea and several Swedish islands.

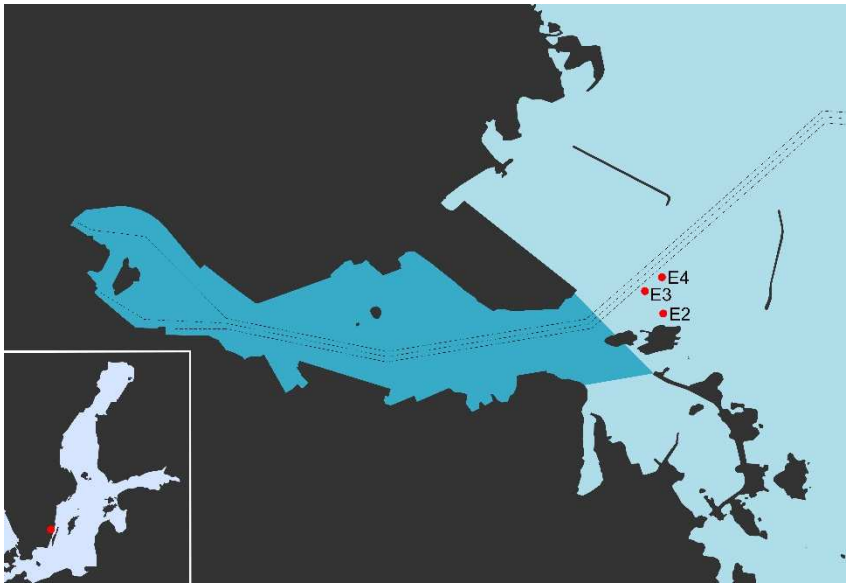


Figure 1: Map of Oskarshamn harbor (57°15'52"N, 16°29'4"E). The inner harbor (dark blue) has recently been remediated through dredging. The stations (E2, E3 and E4) selected in this study are located in the outer harbor basin. One grab per station was sampled for benthic community analysis and 32 sediment cores were sampled at E2 and brought to the lab to study the effect of thin-layer capping with AC and resuspension on contaminant sediment-to-water release fluxes. Location in the Baltic Sea is displayed in the lower left panel.

The Oskarshamn harbor sediment are known to contain PAHs, PCBs, PCDD/Fs (dioxins and furans), organotin compounds (*e.g.,* TBT), and metals (As, Cd, Cu, Hg, Pb, and Zn). Swedish environmental quality criteria show that concentrations of PAHs and PCBs are among the highest measured in Swedish sediment (exceeding 75th and 95th percentiles of Swedish sediment samples, respectively) and metals in the harbor very strongly deviate (exceed 95th percentile) from pre-industrial concentrations (Swedish EPA 2000; SGU 2017). Swedish guidelines and environmental quality criteria do not relate to toxicity risks, but sediment concentrations of $\sum PAH_{16}$, $\sum PCB_7$, As, Cd, Cu, Hg, Ni, Pb, and Zn in Oskarshamn harbor exceed Norwegian threshold values for ecological risks (Breedbeld 2018).

Due to the risk of contaminant spread from the harbor to the Baltic Sea, and to help reach national environmental objectives of a non-toxic environment, contaminated sediments were dredged from the inner harbor basin in 2016 – 2018 and deposited at a purpose-built land fill, which made it the largest state-funded sediment remediation project in Sweden (Swedish EPA 2012; Oskarshamn municipality 2019). The outer harbor basin was estimated to contain 25 % of the total contaminant load of the harbor, but it was not remediated. Greater water depth and higher sediment volume make dredging of the outer harbor basin challenging and expensive, however, storms and other erosional events, as well as resuspension from ship traffic, may pose risks of contaminants spreading from the harbor into other areas of the Baltic Sea.

In May 2017, we collected three sediment grab samples using a van Veen sampler to analyze the benthic community composition in the Oskarshamn outer harbor (at sites E2, E3 and E4, Figure 1). A CTD was used to measure sediment turbidity caused by ferry traffic over the sampling site (see Section SI-1 in the supplemental information). Finally, a GEMINI corer was used to collect 32 intact sediment cores at the E2 station (Figure 1). The cores were handled with care to avoid sediment resuspension, transported to the sediment laboratory at Stockholm University, and stored in a thermo-constant room at 6 °C for 8 weeks with constant gentle water aeration to acclimatize the benthic fauna in the cores and to allow sediment compaction. The temperature was gradually raised from 6 to 10 °C before experiment start to match summer field conditions.

Thin-layer capping

The two bituminous-coal-based AC (Jacobi Carbons, Kalmar, Sweden) used in this study were PAC with a 15 – 35 µm median particle size (AquaSorb BP2, size PAC-S) and GAC in the 0.425 – 0.850 mm range (AquaSorb 2000, size 20x50). The thin-layer capping treatments were 1) control (no cap), 2) cap with only clay, 3) cap with clay and GAC, and 4) cap with clay and PAC. The use of an AC-clay blend follows our previous work, where higher capping efficiency and fewer adverse effects on benthic macroinvertebrates were observed when AC was mixed with clay in comparison to applying AC only or covering AC with a layer of sand (Cornelissen 2011; Samuelsson 2015). Glacial clay collected from an offshore reference site at 112 m depth in the Baltic Sea (WGS84 57°24'00"N, 19°20'98"E) was sieved through 0.5 µm to remove any macrofauna and then used as capping-clay for the experiment.

Table 1: Doses of capping materials used. Shared letters indicate pairing of twin sediment cores. A dose of 600 g m^{-2} corresponded to a dose of 3.02 g dry weight per sediment core.

	Static				Resuspended			
	Control	**Clay**	**GAC**	**PAC**	**Control**	**Clay**	**GAC**	**PAC**
Sedimentary clay (g m^{-2})	0	600	600	600	0	600	600	600
Activated carbon (g m^{-2})	0	0	600	600	0	0	600	600
Replicates (n)	4	4	4	4	4	4	4	4
Treatment pairing	A	B	C	D	A	B	C	D

The dose of each capping material (600 g m^{-2}, Table 1) was chosen to reduce the release fluxes of contaminants but to be thin enough to allow for measurable contaminant release over the two-month experiment duration. The capping materials were mixed together with 50 mL of brackish seawater and a homogenous suspension was gently poured into the water column. The sediment cores were allowed to settle for 7 days before the experiment started. References to the sediment surface refer to the boundary between overlying water and solid material: thin-layer capping raised the position of the sediment surface by 3 - 5 mm above the natural sediment.

Simulating resuspension

The GEMINI core grabber retrieved two cores at a time, and each twin pair was amended with the same thin-layer capping treatment. The twin cores were then separated into the two resuspension treatments: one core was resuspended, and one core was kept in static condition with no resuspension (Table 1). The water column was maintained at 23 cm height (1.15 L water volume) and sediment resuspension was produced with a stainless-steel paddle (47x12 mm) mounted on a stainless-steel rod (8 mm) placed 2 cm above the sediment surface. The paddle rotation was controlled by an electric motor (RH158 30:1 12V, Micro Motors, Italy) set to 100 rpm, which was operated for 15 minutes twice daily. The setup is shown in Figure 2. The speed of the rotor paddle was adjusted to obtain similar turbidities in all cores. Suspended particles were measured during resuspension by retrieving water samples (25 mL) from the center of the water column of each sediment core. The water sample was then vacuum-suctioned onto a GF-C filter and dried at 60 °C overnight to determine suspended matter concentration (g DW mL^{-1}).

Passive samplers

Three types of passive samplers were used in the experiment. Detailed experimental and analytical methods are presented in the supplemental information (Section SI-2 to SI-4). A semipermeable membrane device (SPMD; 91.4 cm standard purity from Environmental Sampling Technologies, Inc., USA) was used to calculate sediment-to-water fluxes of dissolved HOCs (PCBs and PAHs were analyzed here). The SPMD was mounted on a stainless-steel holder and hung in the water column using stainless-steel wire. Before use, all stainless-steel materials were placed in 0.1 M HCl overnight and then washed in deionized water to minimize metal leaching. The SPMD functions as an infinite sink for freely dissolved HOCs.

PDMS-coated glass fibers (12.7 µm thickness on GF10 of Witt (2013); Fiberguide Industries SPC210/230, Stirling, NJ, USA) were used to quantify freely dissolved porewater concentrations of PAHs and PCBs. The fibers were pre-cleaned with a series of 15-minute supersonic baths in ethyl acetate then ultrapure water. Fibers were cut to 15 cm length and pushed into each sediment core in triplicate, leaving up to 2 cm of the fiber above the sediment surface. Matrix solid-phase microextraction (matrix SPME) utilizes the whole sediment matrix as a reservoir for equilibrium extraction of freely dissolved HOCs to the PDMS, and based on previous studies, equilibrium for PAHs is reached after 30 days (Witt 2013, Witt 2019).

A diffusive gradient in thin film (DGT) device was placed in the overlying water to collect dissolved metals from the water column. The DGT consists of a Chelex binding layer covered with a 0.8 mm agarose cross-linked polyacrylamide (APA) diffusion gel (LSNM type, DGT Research Ltd, Lancaster) placed in a plastic housing. The device maintains near-neutral buoyancy and was monitored during the experiment to prevent exposure to air. The DGT functions as an infinite sink and was used to measure the cationic metals Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, U and Zn.

Experimental setup

The experiment started with the placement of passive samplers in the sediment cores, and the twice-daily resuspension events began on the same day. Constant aeration was provided to each core by gentle bubbling of air through glass tubes inserted through the top stoppers (Figure 2). Monitoring consisted of observing temperature (10 °C) and maintaining water column height, resuspension, and passive sampler positions during the experiment.

After 60 days, the passive samplers were retrieved from the water column and the paddles were removed. Each SPMD was removed from its holder, wiped clean using lint-free tissue wetted

with ultrapure water, and stored in a glass container at -20 °C. The DGT was gently washed with a small volume of ultrapure water and then stored moist in a plastic bag at 4 °C.

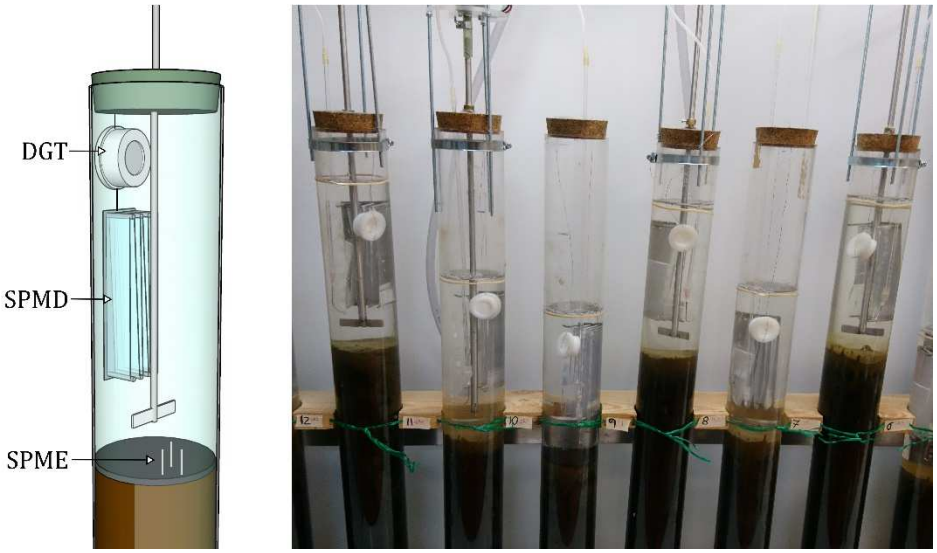


Figure 2: Sediment core experimental setup. The picture shows 7 sediment cores with fixed volume of overlying water. A paddle stirrer, placed 2 cm over the sediment surface, is visible in cores in the resuspended treatments. Two passive samplers are visible in the water column: a DGT for metals and a SPMD for HOCs. Three SPME passive samplers are submerged in the sediment to sample HOCs in the porewater. There were 32 cores, with 4 replicates per treatment (n = 4).

The overlying water was drained from the sediment core, and the sediment column was pushed to the top of the core liner to retrieve the SPME fibers. The length of the fibers protruding above the sediment surface was measured for each fiber, after which the fibers were retrieved and wiped clean of particles using wetted lint-free tissue, and stored in aluminum foil at -20 °C.

A sediment sample (5 mL) was collected from the sediment surface of each sediment core to determine dry weight (DW), organic matter (OM; loss-on-ignition), total carbon (TC), total organic carbon (TOC), and total nitrogen content (TN). Two sediment core replicates from each treatment were sieved through 1.0 mm mesh to recover macroinvertebrates, and the sieved sediment was discarded.

The sediment from the two remaining replicates of each treatment were sliced in sections (0 – 1 cm, 1 – 3 cm, 3 – 5 cm, 5 – 7 cm, and 7 – 10 cm depth) using a thin Teflon sheet. Each section was homogenized and then sampled for sediment DW and OM content. Additionally, vertical profiles of TC, TOC, TN, and sediment metal concentrations were determined by sampling each sediment section in the Control treatment.

Finally, sediment sections (surface sediment in all treatments, and each sediment depth from Controls) were sieved through 1.0 mm mesh using a small volume of ultrapure water to remove macroinvertebrates and allow for sediment HOC analyses. The sieved sediment was dried at

ambient temperature under laminar flow and stored at 4 °C for analyses of total sediment concentrations of PAHs and PCBs. The sediment samples retrieved from each treatment are summarized in Table SI-1.

Chemical analyses

PAHs and PCBs in overlying water

Analyses of PAHs and PCBs in SPMDs were conducted at the Department of Environmental Science at Stockholm University. SPMDs were twice extracted in *n*-hexane and the extracts were spiked following the methods of Mustajärvi (2017), then split 1:5 for PAH and PCB analyses, respectively. The PAH fraction was cleaned using the methods of Mandalakis (2004) as modified by Mustajärvi (2017), and the PCB fraction was cleaned following the methods of Zebühr (1993). All samples were analyzed by GC/MS and the targeted analytes were 15 PAHs (standard 16 PAHs excluding acenaphthylene) and the standard 7 PCBs. More detailed information on chemical analysis of SPMDs is provided in Section SI-2.

Method detection limits (MDL) were calculated as the mean plus three times the standard deviation of all blank values (n = 4). Data below MDL were removed from further analysis and analyte concentrations were corrected for the blank mean. MDLs were 0.02 – 1.2 ng $SPMD^{-1}$ for PAHs (except for naphthalene at 42 ng $SPMD^{-1}$) and MDLs for PCBs were 0.004 – 0.16 ng $SPMD^{-1}$ (except for PCB-53 at 0.73 ng $SPMD^{-1}$). Mean measured concentrations of PAHs were 1.5 – 168 times higher than MDLs, and PCBs were 1.8 – 172 times higher than MDL (excluding naphthalene and dibenz[a,h]anthracene which were not detected).

The total quantity of each analyte in the SPMD was assumed to correspond to the total sediment-to-water release of the analytes, and fluxes were calculated using the sediment surface area and the experimental duration (*flux = SPMD uptake / sediment surface area / sampling time* [g m^{-2} d^{-1}]).

PAHs and PCBs in sediment porewater

Analyses of freely dissolved concentrations of PAHs and PCBs in porewater were performed at Hamburg University of Applied Sciences (HAW). The PDMS-coated glass fibers were cut into 5 segments to produce a vertical profile of PAHs and PCBs in sediment. The first segment was 0 – 2 cm above the sediment surface, followed by 0 – 4 cm, 4 – 8 cm, 8 – 12 cm, and > 12 cm below sediment surface. Contaminant concentrations in the PDMS were determined by GC/MS. The PDMS fiber samples were placed in individual GC liners, and an automated liner exchange system (GERSTEL ALEX) was used for automated introduction (GERSTEL

Multi-Purpose Sampler [MPS]) and thermal desorption (CIS) of the fibers following the methods of Witt (2009). Analyte concentration in fibers were calculated using external standard and fiber geometry information from Witt (2013), and freely dissolved concentrations were calculated using PDMS-water partitioning coefficients from Witt (2009). Targeted analytes were the standard 16 PAHs and 7 PCBs (external standard calibration solutions: PAH-Mix 9 and PCB-Mix 3, Dr. Ehrenstorfer). Naphthalene C_{free} data from porewater was excluded from further analyses as the measured concentrations were inconsistent and highly variable and may have resulted from contamination of PDMS fibers in the lab or during transport.

MDLs and method quantification limits (MQLs) were calculated as the mean mass in blank fibers, plus three times the standard deviation for MDL, or plus 10 SD for MQL, and then converted to fiber coating concentrations. The MQL_{PDMS} were 3.6 to 8.9 ng mL^{-1} for PAHs and 3.2 to 7.2 ng mL^{-1} for PCBs. The MDL_{PDMS} were lower than 2.0 ng mL^{-1} for all target compounds. MQL based on freely dissolved concentrations (MQL_{free}) were lower and decreased with increasing PDMS-to-water partition coefficient (K_{PDMS}). Further details on analyses are provided in Section SI-3.

Total PAHs and PCBs in sediment

Sediment samples (2 – 4 g dry weight) were spiked with surrogate standards for PAHs and PCBs and analyzed at HAW. Sediment samples were extracted twice with acetone:hexane (40:60 by volume) using an accelerated solvent extractor (ASE 200, Dionex) at 100 °C and 140 bar, followed by clean-up and analyses in GC/MS. Target analytes were the standard 16 PAHs and 7 PCBs. The HPLC clean-up was tested with standard solutions (PAH-Mix 9, PAH-Mix 9 deuterated and PCB-Mix 3, PCB-Mix 3 deuterated, Dr. Ehrenstorfer) which gave final mean standard recoveries from the surrogate and external PAH and PCB target compounds of 95 % ± 8 % (n = 10). Further details on analytical methods are provided in Section SI-4.

The analytical quality control for the whole procedure was carried out with a certified reference material for PAHs and PCBs in marine sediments (QPH 058 and QPH 059, QUASIMEME, Laboratory Performance Studies, quasimeme.org). Surrogate solutions were added to each sample. The mean recovery values ranged between 71 % (naphthalene) and 102 % (n = 10). The measured concentrations for single compounds were in all cases in the range of certified values (± certified uncertainty). Relative standard deviations of the measured values for the single compounds (n = 10) ranged between 5 and 12 % and mean recovery rates of the surrogate solutions were calculated for all target compounds with 84 % ± 5 %. MQLs were estimated to be 0.3 – 5 μg kg^{-1} DW for PAHs and 0.5 – 1 μg kg^{-1} DW for PCBs.

Metals in sediment and overlying water

Analysis of metals in DGTs and sediment was conducted at ALS Scandinavia AB in Luleå, Sweden. Sediment concentrations of 16 elements were analyzed in sections down to 10 cm depth in the Control treatment and in surface sediment in the capping treatments. Targeted analytes in sediment were As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, P, Pb, Sr, V, and Zn. DGTs were extracted by drying the diffusive gel and binding layer at 50 °C and leaching the sample in 10 % HNO_3. Sediment samples were dried at 105 °C, grinded, and extracted through digestion in 10 % HNO_3. Metal quantification followed SS EN ISO 17294-1:2004 and 17294-2:2016 protocols and a modified EPA method 200.8 protocol. Targeted analytes in DGT samples were Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, U, and Zn. The output was the mean concentration of dissolved species in the overlying water over the experiment duration.

Statistical analyses

For statistical analyses, each metal was tested separately, PCBs were tested as $\sum PCB_7$, and PAHs were separated into two groups and tested due to clear dissimilarities in partitioning. Seven low molecular weight PAHs (PAH_L) and nine high molecular weight PAHs (PAH_H) were analyzed as independent groups. The classifications of USEPA (2007) were used: PAH_L consists of naphthalene, acenaphthene, acenaphthylene (not analyzed in all matrices), fluorene, phenanthrene, anthracene and fluoranthene, and PAH_H consists of pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-CD]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene.

Statistical analysis was based on null hypothesis significance testing. Pairwise *t*-tests were used to test for differences between static and resuspended treatments within a thin-layer capping treatment. This pairwise comparison assumes that our twin sediment cores are closely related. Tukey's HSD test was used to evaluate differences between thin-layer capping treatments sharing a resuspension condition (*i.e.*, static treatments were tested separately from resuspended treatments). Quantile-quantile and residual plots were evaluated to assess normality and heterogeneity, and an alpha of 0.05 was our criterion to reject the null hypothesis.

Results

Field and experimental conditions

Water quality parameters measured in bottom water at the site were temperature 8.7 °C, salinity 6.4 ‰, and dissolved oxygen 11.1 mg L^{-1}. Sediment cores had a water temperature of 10.3 ± 0.1 °C, salinity of 6.9 ± 0.3 ‰, and dissolved oxygen of 10.4 ± 0.03 mg L^{-1} at the end of the experiment.

Sediment resuspension in the cores caused a 15-fold increase in suspended matter content in overlying water, from mean 40 mg L^{-1} in static cores to mean 611 mg L^{-1} in resuspended cores (Table 2). Resuspension due to marine traffic has been visually observed in the inner harbor, and severe weather or large vessels may resuspend sediment in the outer harbor (Oskarshamn municipality 2019). However, turbidity measurement indicates that no sediment resuspension occurred in the outer harbor following ferry traffic (see Section SI-1). Resuspension may occur more intermittently or at a lower intensity in the outer harbor than in the present experiment.

Table 2: Sediment geochemical properties at the end of the experiment (mean of two samples), and suspended particles sampled during resuspension events (mean and standard deviation of three samples).

		Static				Resuspended			
Treatment	**Depth**	**Control**	**Clay**	**GAC**	**PAC**	**Control**	**Clay**	**GAC**	**PAC**
DW (%)	Surface	11.5	14.1	16.8	16.9*	11.6	13.1	17.9	15.7*
	Subsurface	17.7	17.7	17.5	17.9	17.6	17.9	17.9	18.3
LOI (%)	Surface	22.9	18.8	36.6	37.4*	22.6	18.8	38.4	33.7*
	Subsurface	18.5	17.8	17.9	17.7	18.3	17.5	17.9	17.3
TOC (%)	Surface	5.9	5.7	23.9	32.0	7.5	6.4	22.6	33.8
	Subsurface	7.7	-	-	-	6.9	-	-	-
TC (%)	Surface	7.8	6.1	22.9	34.0	7.7	6.6	22.8	31.6
	Subsurface	7.5	-	-	-	7.1	-	-	-
TN (%)	Surface	0.95	0.67	0.63	0.60	0.90	0.73	0.59	0.52
	Subsurface	0.89	-	-	-	0.84	-	-	-
TC/TN (ratio)	Surface	8.53	8.67	35.9	55.3	8.19	8.75	36.1	62.6
	Subsurface	8.10	-	-	-	8.10	-	-	-
Suspended particles (mg L^{-1})	Water	42.9 ±13.7	64.8 ±21.6	30.8 ±16.9	21.5 ±18.6	725 ±436	583 ±169	500 ±268	638 ±126

Surface indicates 0-1 cm depth and subsurface indicates the mean of samples from 1-3, 3-5, 5-7, and 7-10 cm depth.
*Measured in one replicate.

In the cores, the resuspended particles settled on the walls and other surfaces, including the surface of the SPMD passive sampler. This deposition consisted primarily of contaminated sediment in the Control treatment, glacial clay in the Clay and GAC treatments (as the granular AC was not resuspended), and a mix of AC and glacial clay in the PAC treatment. A photograph displaying particle deposition in resuspended cores is presented in Figure SI-1.

Sediment cores were visually examined to assess sediment conditions at the end of the experiment. The lower section of sediment was black, indicating euxinic (*i.e.*, anoxic and sulfidic) conditions. Above this was a section of brown sediment, which suggests non-euxinic conditions, perhaps partially oxygenated through bioturbation (see discussion below). The mean thickness of this section was 2.55 ± 0.55 cm. The sediment surface was a lighter brown

color and presumably fully oxygenated, with a mean thickness of 4.7 mm. Photographs of these sediment layers are presented in Figure SI-2.

Thin-layer capping affected sediment properties at 0 – 1 cm depth, but not deeper in the sediment (Table 2). Surface sediment dry weight slightly increased in the Clay treatment and further increased in GAC and PAC treatments. Clay somewhat reduced organic matter content, total carbon content, and organic carbon content in surface sediment, whereas GAC and PAC strongly increased them. This indicates that the AC fraction was captured in organic matter, total carbon, and organic carbon analyses. Total nitrogen content of surface sediments was reduced by all thin-layer capping treatments, a result that shows that the sediment contains more natural organic matter than the thin-layer caps amended with AC. The AC materials used here consists of ~90 % carbon and no nitrogen (Bonaglia 2019).

Benthic community

Macroinvertebrate burrows were formed in the sediment cores during the experiment. The most abundant species in both field samples and sediment cores were the gastropod genus *Hydrobia*, the bivalve *Limecola balthica* (previously in *Macoma*), aquatic larvae of the midge family Chironomidae, and the polychaete *Hediste diversicolor* (previously in *Nereis*). We identified 7 species in sieved sediment cores, and 10 species in field samples. These sampling efforts represent relatively low sediment surface areas, illustrated by presence of unique species in both samplings. We found the polychaete *Bylgides sarsi* in sediment cores but not in the field, and we found 4 species in the field that were not present in the sediment cores at the end of the experiment (see taxonomic data in Table SI-2).

In the present study, assessing effects on benthic organisms was not a primary goal. However, the high survival rate of benthic invertebrates in the experiment demonstrates that sediment parameters and environmental conditions remained tolerable for the observed species in thin-layer capping and resuspension throughout the experiment. The heterogeneity of the community made statistical analysis of differences in species composition between treatments and comparison to the initial benthic community unfeasible, but a simple comparison of organism abundances was made. This comparison reveals that GAC maintained the same organism abundance as Control, roughly twice as high as in the PAC and Clay treatments. Organism abundance was reduced by resuspension in all treatments except for GAC; resuspended GAC had higher abundance than the resuspended Control (Supplemental Information Table SI-2).

AC amendment may cause adverse biological effects on benthic invertebrates, and it has been observed that fine AC particles may have stronger adverse impact on benthic organisms than coarser particles (Janssen 2013). In this study, it also appears that PAC had an adverse effect on species abundance of macroinvertebrate, whereas GAC supported a higher organism abundance even during intense resuspension (see Table SI-2). Biological responses to thin-layer capping were recently reported to depend on AC particle size, where only ingestible AC cause adverse biological response in two deposit feeders (Rämö 2021), but further research would be necessary to assess the impact of resuspension on such responses.

Organic contaminants

Sediment concentrations

Sediment total concentrations (C_{total}) were measured in vertical profiles in the Control treatments, which show that $\sum PCB_7$ and $\sum PAH_H$ concentrations were relatively stable to 10 cm depth, whereas $\sum PAH_L$ was higher in sub-surface sediment than at the surface (Figure SI-3). Generally, Control in resuspended condition had lower C_{total} of individual PCBs and PAHs than Control in static condition (Table SI-4).

Surface sediment C_{total} was measured in all treatments to assess dilution effects of thin-layer capping. Mean $\sum PCB_7$ was reduced by 19 % in Clay, 43 % in GAC, and 52 % in PAC, as compared to surface sediment in uncapped Control (Figure 3, Table SI-3). This corresponds to the dilution expected from thin-layer capping: 600 g m^{-2} for Clay and 1200 g m^{-2} for the AC treatments. Resuspension had a small, but statistically significant, effect in reducing surface sediment C_{total} of $\sum PCB_7$ in the Clay treatment but not in the other treatments.

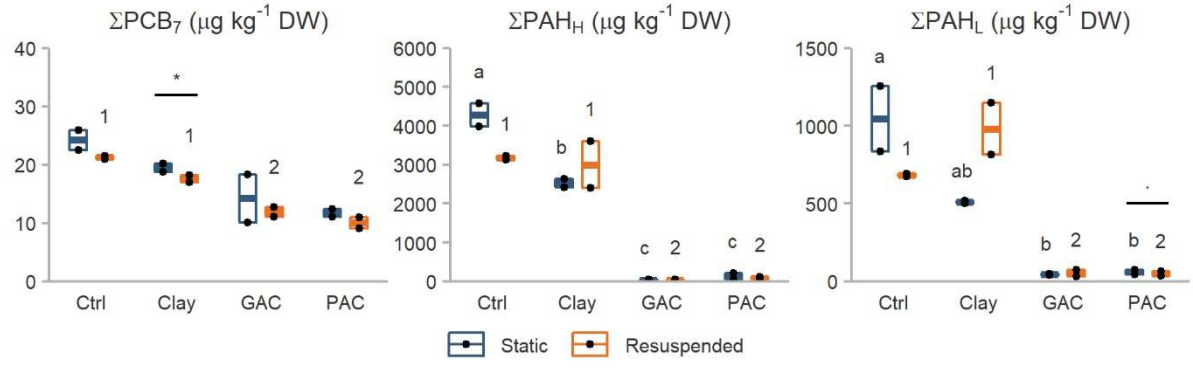


Figure 3: Sediment concentrations of $\sum PCB_7$, $\sum PAH_H$, and $\sum PAH_L$ at 0-1 cm depth (µg kg^{-1} dw). Crossbar boxes indicate mean and bootstrapped 95% confidence limits. Shared letter indicates similar treatments (Tukey HSD, 0.05 α). Asterisks denote a statistical difference between static and resuspended treatments (paired t-test, · $p < 0.1$, * $p < 0.05$).

Surface sediment C_{total} of PAHs was also studied. The GAC and PAC treatments reduced C_{total} of $\sum PAH_L$ by 96 and 88 %, respectively, and both AC treatments reduced $\sum PAH_H$ by 94 %. These reduced C_{total} of PAHs in the AC treatments far exceeded the effect of dilution observed

for PCBs, suggesting that reduced PAH concentrations cannot be attributed to dilution alone. Resuspension caused a small but significant reduction of $\sum PAH_L$ in PAC, but not in GAC. The Clay treatment reduced $\sum PAH_H$ by 51 % in the static condition only, and no significant effects were observed for $\sum PAH_L$ in Clay.

A possible explanation for the loss of PAH C_{total} in AC-amended sediment is reduced extraction due to strong PAH sorption onto AC. Jonker and Koelmans (2002a, 2002b) have previously reported poor extraction of PAHs from soot and sediment containing soot-like particles such as AC. Planar compounds, such as PAHs, have been observed to sorb more strongly to AC than non-planar compounds (Jonker and Koelmans 2002a, 2002b, Cornelissen 2005). Strong sorption was also observed in the 7 PCBs, where the mono-*ortho* coplanar PCB 28 and PCB 118 were reduced by 68 % on average in the AC treatments, while non-planar di-*ortho*-indicatory PCBs were reduced by 39 % on average. The effect of AC amendment on the total extractable fraction of PAHs and co-planar PCBs in sediment should be further investigated.

Porewater concentrations

Vertical profiles of freely dissolved concentrations (C_{free}) were measured to assess the influence of thin-layer capping on PAHs and PCBs in porewater at different depths. There was no general effect of sediment depth, but some treatments proved noticeable exceptions (Figure 4). Porewater concentrations of $\sum PCB_7$ were not different between treatments in the resuspended condition, but the static Control had elevated $\sum PCB_7$ concentrations relative to the thin-layer capped sediments. Depth profiles of individual compounds (Figure SI-4) reveal elevated concentrations of PCB 52, 101, 118, 138 and 153 in the static Control. There was an increase of $\sum PCB_7$ in the deepest (>12 cm depth) layer of the GAC treatment, which is attributable to PCB 28 and 52. An outlier in 0 – 4 cm porewater concentration of $\sum PCB_7$ (Figure 4) was attributed to PCB 118, 138, 153 and 180 (Figure SI-4).

Porewater concentrations of $\sum PAH_H$ were generally similar at the different depths, but the static Control and resuspended PAC had elevated $\sum PAH_H$ in at least one depth. In the static Control, high C_{free} were observed between 0 and 12 cm depth. This was caused by high concentrations of every PAH_H at 0-4 cm depth, whereas pyrene caused elevated concentrations at 4 – 12 cm depth (Supplemental Figure SI-4). In the resuspended PAC treatment, elevated C_{free} of $\sum PAH_H$ in the 0 – 4 cm depth was attributed to every PAH_H except pyrene.

Finally, porewater concentrations of $\sum PAH_L$ were higher in the static Control treatment than in any thin-layer capped treatment (Figure 4). This was attributed to every PAH_L occurring at

higher concentrations in the Control (Supplemental Information SI-4). No clear differences in PAH_L C_{free} in porewater were observed in the resuspended condition.

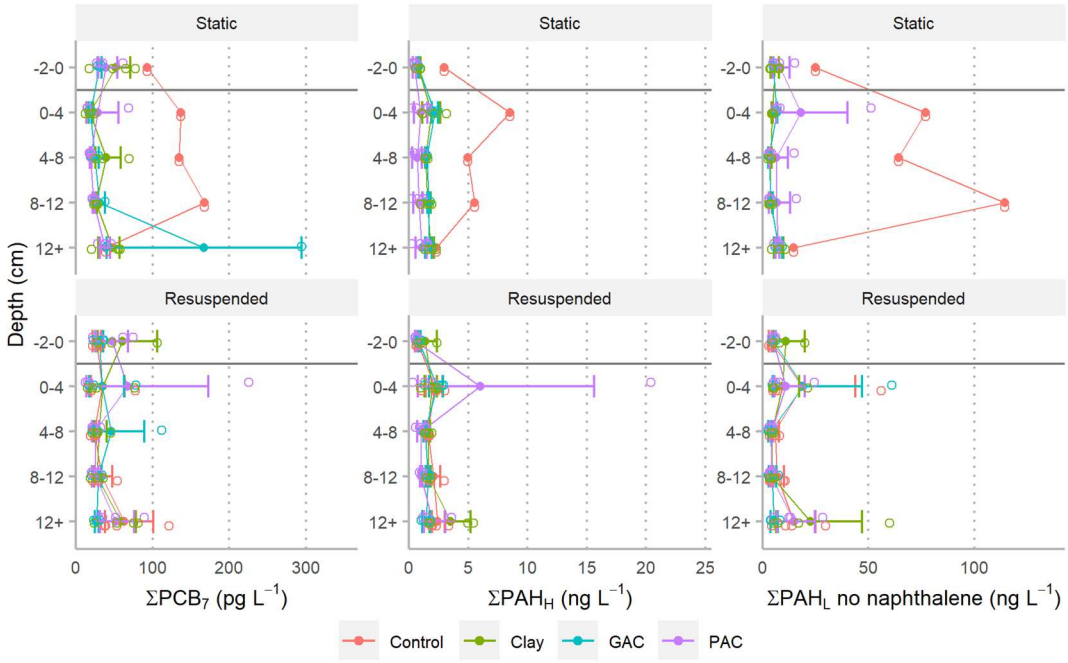


Figure 4: Vertical profiles of mean freely dissolved concentrations of $\sum PCB_7$, $\sum PAH_H$, and $\sum PAH_L$ in porewater (C_{free}) with 95 % confidence intervals. Absence of confidence interval represents $n = 1$. Horizontal line indicates the sediment surface. Open dots represent individual observations. Note the different units of $\sum PCB_7$ (pg L^{-1}) and the PAHs (ng L^{-1}).

The static Control appeared to have higher porewater C_{free} of PAHs and PCBs than thin-layer capped treatments, but PDMS fibers from the static Control were successfully retrieved and analyzed in one replicate only. On the other hand, the resuspended Control had multiple replicates and did not clearly differ from thin-layer capped treatments which may suggest that no clear effects of treatments were observed in PAH and PCB porewater concentrations.

Stringer (2014) used SPME passive sampling and reported that effects of AC amendment were measurable within only a few centimeters from the treatment. The present study analyzed porewater concentrations in 4-cm fiber segments, and a higher vertical resolution would have been necessary to capture the effects of thin-layer capping on freely dissolved porewater concentrations. The effects of AC thin-layer capping on porewater concentrations are also expected to increase over time as the AC is distributed into the bioactive sediment through bioturbation: AC mixed into sediment is effective in reducing porewater C_{free} of HOCs (Cornelissen 2006, Tomaszewski & Ghosh 2008, Beckingham & Ghosh 2013, Kupryianchyk 2013b).

Sediment-to-water fluxes

The flux of ∑PCB_7 from sediment to water was reduced by 57 % in static PAC and by 91 % in resuspended PAC compared to Control, whereas Clay and GAC treatments did not reduce ∑PCB_7 fluxes (Figure 5). In static condition, the sediment-to-water fluxes largely consisted of PCBs and PAHs with low log K_{ow} (Figure 6), which is to be expected as more hydrophobic substances are to a higher degree sorbed to particles. Most of the resuspended treatments had increased fluxes of PCBs with log K_{ow} > 7.0, likely due to desorption from sediment particles suspended in the water column. However, the PAC treatment further reduced fluxes of PCBs with high log K_{ow}, demonstrating strong sorption and higher capping efficiency of PAC relative to Clay and GAC treatments.

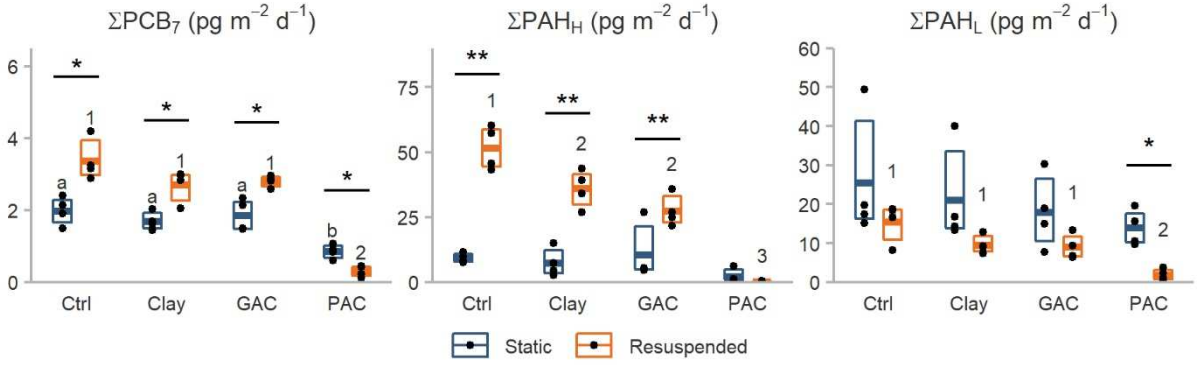


Figure 5: Sediment-to-water release fluxes of ∑PCB_7, ∑PAH_H, and ∑PAH_L (pg m^{-2} d^{-1}) measured using SPMDs. Crossbar boxes indicate mean and bootstrapped 95% confidence limits. Shared letter indicates similar treatments (Tukey HSD, 0.05 α). Asterisks denote a statistical difference between static and resuspended condition (paired t-test, · p < 0.1, * p < 0.05). Note that the lowest release fluxes of both PCBs and PAHs are found in the PAC treatment.

Release fluxes of ∑PAH_H were not significantly affected by capping in static condition, but resuspended Clay and GAC treatments reduced fluxes of ∑PAH_H by 30 % and 47 % respectively, and resuspended PAC reduced fluxes of ∑PAH_H by 99.4 %. Thin-layer capping had no effect on ∑PAH_L fluxes in the static condition, but the resuspended PAC treatment reduced the ∑PAH_L flux by 88 % compared to the Control. The resuspended Control, Clay and GAC treatments had higher ∑PAH_H fluxes than the static condition (Figure 5), which is caused by an increased release of PAHs with log K_{ow} around 5 - 6 (Figure 6). In contrast, resuspension of the PAC treatment instead further reduced fluxes of PAHs with a log K_{ow} around 4, resulting in very low PAH fluxes in resuspended PAC.

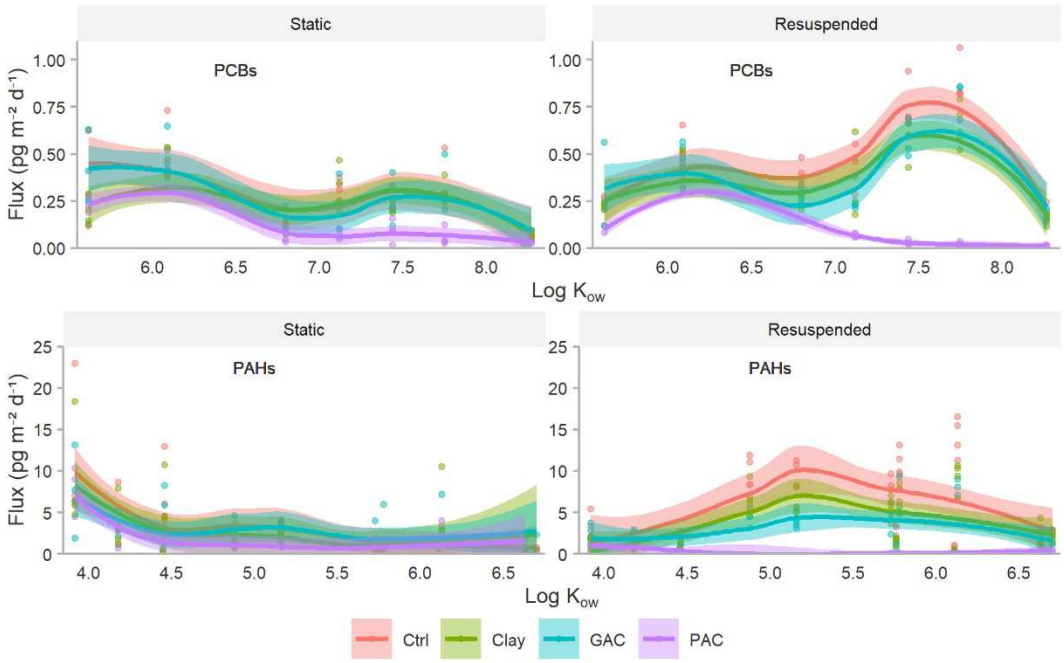


Figure 6: Fluxes of individual PCBs and PAHs as measured in SPMDs plotted against log K_{ow} in static and resuspended treatments, respectively. The R function ggplot2::geom_smooth() was used to fit a local polynomial regression with 95 % confidence intervals.

A comparison of sediment-to-water fluxes to porewater concentrations of PCBs and PAHs shows slight correlation in the static but not in the resuspended condition (Figure 7). Linear regression models for PAHs and PCBs (fitted to all observations of respective contaminant class) had generally poor explanatory power in static conditions (mean $R^2 = 0.35$), and even lower explanatory power in resuspended conditions (mean $R^2 = 0.06$). The lack of correlation in the resuspended treatments is to be expected, as fluxes from sediment to water in these treatments is governed by additional variables, such as turbidity and sorption properties of suspended particles. Volatilization of low molecular weight compounds (PAH_L) from water to air may have taken place during the experiment, however, the observed high sediment C_{total} and porewater C_{free} were expected to have compensated for potential losses from water to air.

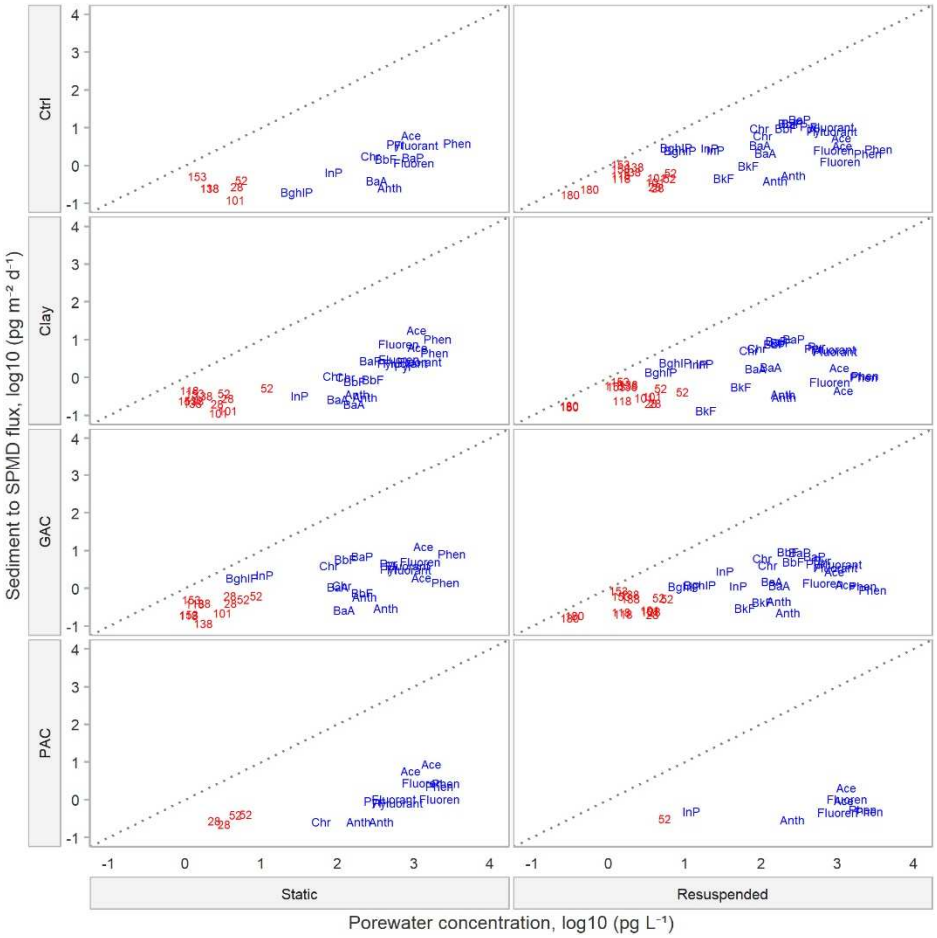


Figure 7: Comparison of porewater concentrations and fluxes to overlying water for individual PCBs and PAHs (log-log scale, n = 2). Red data indicates PCBs using congener numbers, blue data indicates PAHs using abbreviated names, with labels centered on the data point.

Sediment resuspension significantly increased the release fluxes of $\sum PCB_7$ and $\sum PAH_H$ in all treatments, except in the PAC treatment, where resuspension reduced fluxes of both $\sum PCB_7$ and $\sum PAH_L$ (Figure 5). The reversed effect on contaminant release in PAC can be explained by sorption of contaminants onto suspended AC particles. Nonetheless, the capping efficiency of PAC may be overstated in the present study as compared to the field, where resuspended particles are lost in advective transport from the site. In addition, resuspended PAC particles were observed coating surfaces of sediment cores and passive samplers (Figure SI-1), which may have increased sequestration of PCBs and PAHs from the water phase. The effect of the PAC-fouling on SPMDs has not be quantified in the present study. However, fouling by biofilm formation on samplers has been reported to reduce contaminant sampling rates by up to 50 % (Richardson 2002), and AC is known to be a stronger sorbent than natural organic matter.

Another point to consider is that in field conditions, resuspension and subsequent advection are likely to lead to off-site transport of PAC, reducing the effective dose of the PAC materials at the site of application. In comparison, we did not observe GAC resuspension in the present

study. The apparent densities of PAC and GAC are comparable (450 - 520 and 390 – 600 kg m^{-3}, respectively), whereas clay particles have higher density than AC and were still readily resuspended. Larger particles require higher fluid velocity (i.e., higher shear stress) to suspend (Graham 2013), implying that resuspension events achieved the required sheer forces to suspend PAC and clay particles, but did not achieve the forces required to suspend GAC particles. The clay component of the thin-layer caps was resuspended in both treatments, which meant that GAC was covered by clay particles over the course of the experiment. Although GAC was only effective in reducing fluxes of $\sum PAH_H$ in the present experiment, increased sorption efficiency over time has been reported in other studies (Werner 2006; Kupryianchyk 2013a; Choi 2014b), indicating that GAC may represent a better capping option for long-term remediation due to its higher retention rate relative to PAC. However, an increased dose of GAC would be necessary to obtain sorption efficiencies comparable to PAC in the short term.

Metals

Sediment concentrations

Total concentrations (C_{total}) of metals in Control were compared to Swedish environmental quality criteria (Swedish EPA, 2000). This places As, Cd, Cu, Pb, and Zn in Class 4 to Class 5, indicating that sediment concentrations far exceed pre-industrial levels.

Surface concentrations of As, Cd, Cu, Pb, and Zn also follow the dilution pattern that was observed for $\sum PCB_7$: the highest concentrations in Control, followed by reduced concentration in Clay, and the lowest concentrations in GAC and PAC treatments (Figure 8). An additional 11 metals were measured in sediment and conformed to this pattern of dilution, with the exception of Be which increased from 1.2 to 2.4 mg kg^{-1} DW in the GAC treatment (Figure SI-5). Resuspension did not significantly affect C_{total} of these 16 metals in surface sediment.

Vertical profiles of C_{total} in the Control treatments show that concentrations of most elements were constant or increased with depth, but 6 metals (As, Ba, Fe, Mn, P, and Sr) had elevated concentrations in surface sediment in both static and resuspended Control treatments (Figure SI-5). The cause of this is not well understood, as only small quantities could have leached from our pre-washed stainless-steel devices. A possible mechanism for the accumulation of certain metals near the sediment surface may be transport from deeper anoxic sediment to the oxygenated surface sediment.

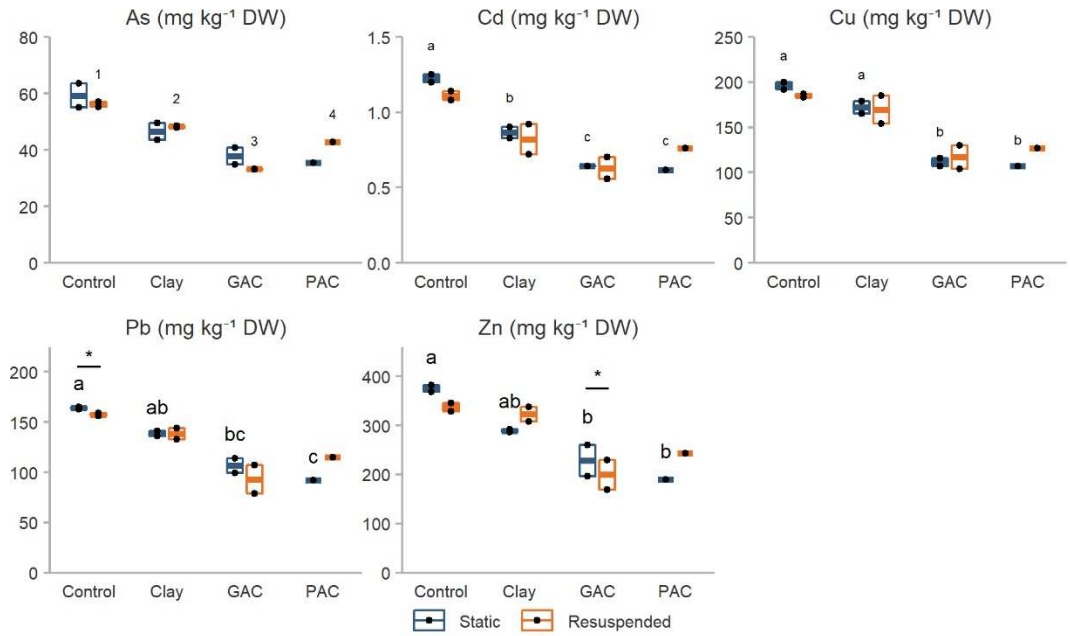


Figure 8: Sediment concentrations of As, Cd, Cu, Pb, and Zn at 0-1 cm depth (mg kg^{-1} dw). Crossbar boxes indicate mean and bootstrapped 95% confidence limits. Shared letter indicates similar treatments (Tukey HSD, 0.05 α). Asterisks denote a difference between static and resuspended condition (paired t-test, * $p<0.05$).

Water concentrations

The DGT passive samplers provide a time-weighted average of the freely dissolved concentrations (C_{free}) of metals. The metals of concern in Oskarshamn harbor showed varied responses to resuspension and thin-layer capping (Figure 9). Cd release was reduced by GAC and PAC treatments in static condition, and PAC reduced Cd release in resuspended condition. Resuspension reduced Cd release in Control, Clay, and GAC treatments, but not in PAC-treated sediment. Both AC treatments appeared to reduce Cu release compared to Control and Clay, but the effect could not be established statistically due to multiple outlier values. Resuspension appears to have had no effects on the release of Cu.

Water concentrations of Pb increased in GAC and was reduced in PAC, with resuspension causing only a small increase in Clay. However, the Pb measurements also contain outliers that interfered with statistical analysis. Zn concentrations were reduced by resuspension in Control and Clay, and PAC reduced Zn concentrations under static conditions, although outliers again interfered with statistical analysis. Results for additional metals are presented in the Supplemental Information (Figure SI-6 and Table SI-5).

In general, resuspension did not lead to a higher sediment-to-water release of dissolved metals. There were 11 metals quantified in DGTs, but Al and Cr were only detected in 1 and 2 sediment cores, respectively, and were not further assessed. Based on 9 metals and 8 treatments, there

were 72 possible metal-treatment combinations. Resuspension increased dissolved metal concentrations in 1 metal-treatment combination and reduced concentrations in 12 combinations (Figure 9, Figure SI-7), which suggests that resuspension had much lower effect on metal release from sediment to overlying water than we expected.

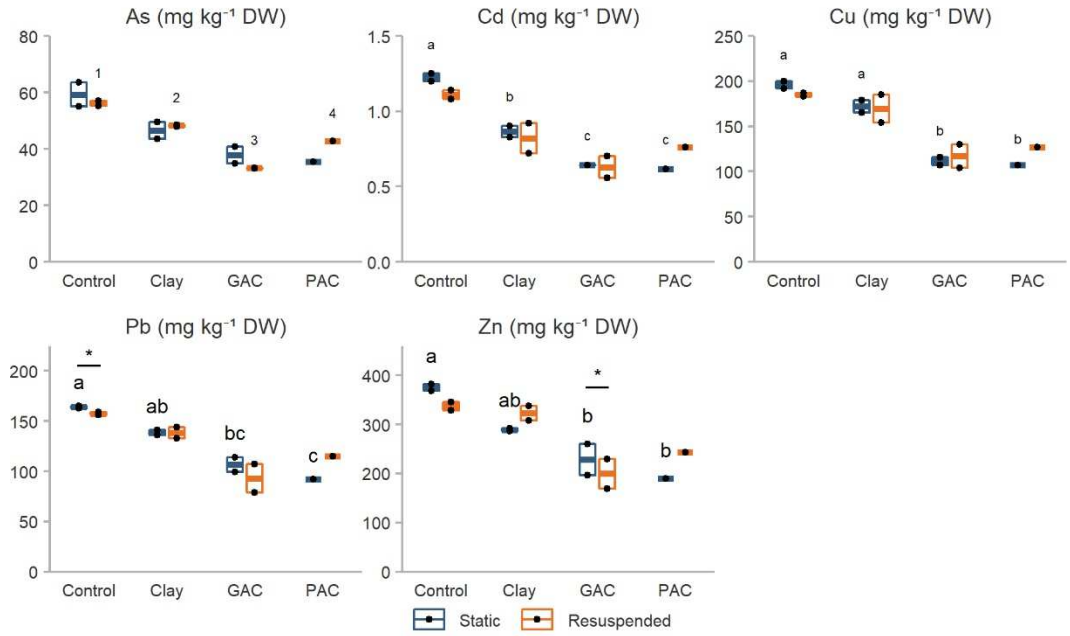


Figure 9: Water concentrations of As, Cd, Cu, Pb, and Zn as determined by DGT uptake (µg L^{-1}). Crossbar boxes indicate mean and bootstrapped 95% confidence limits. Shared letter indicates no statistical difference between treatments (Tukey HSD, 0.05 α). Asterisks denote differences between static and resuspended condition (paired t-test, · $p < 0.1$, * $p < 0.05$).

The overall response to resuspension may be due to interactions between dissolved metals and resuspended sediment or thin-layer capping materials. Apler (2019) reported that *in situ* resuspension of a contaminated sediment in the Baltic Sea increased particle-bound concentrations of metals in overlying water, but decreased their dissolved concentrations, and concluded that this may be due to sorption of dissolved metals onto resuspended particles (a particle scavenging effect).

Overall, AC treatments performed better than Clay in reducing overlying water concentrations of 5 metals of concern, and PAC was somewhat more effective than GAC. The precise mechanism of action of AC on metal sequestration is not known, but effects may be caused by non-specific binding of metals onto AC, dilution of the surface sediment, elevated pH in the AC thin-layer cap, or a shift of the oxygenated sediment zone into the thin-layer cap, as was reported by Bonaglia (2019). In addition, altered microbial processes following thin-layer capping with AC (Bonaglia 2020) may affect speciation of metals and their bioavailability and toxicity (Chapman 1998; Azeez 2006).

Table 3: Mean efficacy (%) of thin-layer caps in reducing contaminant release from sediment to overlying water, compared to untreated Control under static and resuspended conditions. Underlying data are provided in the Supplemental Information (Figure SI-10, SI-16). Positive values indicate a reduced release of contaminants.

	Static			Resuspended		
Contaminant	Clay	GAC	PAC	Clay	GAC	PAC
$\sum PCB_7$	15	6	57	20	17	91
$\sum PAH_H$	22	-13	76	30	47	99
$\sum PAH_L$	17	30	45	38	42	88
Cd	0	34	68	-13	4	53
Cu	-20	72	91	-2	42	96
Ni	81	71	90	-18	-2	34
Pb	10	-145	34	68	69	55
Zn	13	28	63	-58	-47	41

Conclusion

Thin-layer capping using a mixture of PAC and clay reduced sediment-to-water fluxes of PAHs and PCBs and its performance improved under resuspended conditions (Table 3). However, the capping efficiency of PAC may be overestimated, as advection of suspended PAC particles would reduce the effective dose and decrease the long-term efficacy of the treatment. Our results show that thin-layer capping with GAC, though less efficient in reducing contaminant fluxes in the present study, offers an alternative to PAC in turbulent waters as it is less prone to resuspension and is more likely to persist through frequent and repeated resuspension events. Benthic infauna abundance decreased in PAC treatments, whereas GAC maintained infauna abundance similar or higher than Control sediment. Additional studies with a wider range of particle sizes are necessary to identify optimal trade-offs between AC particle size, thin-layer cap persistence, and contaminant retention in resuspended sites.

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Supporting Information

Section SI-1. Sediment resuspension in the field

Sediment resuspension by marine vessels was measured in the outer harbor of Oskarshamn using depth profiles of temperature, conductivity, salinity, dissolved oxygen, light, and turbidity in the water column using a submersible CTD probe with oxygen and turbidity sensors operating in continuous mode (CTD90M, Sea & Sun Technology, Germany). Two to three CTD profiles were taken in the outer harbor shortly before and after passage of a large passenger ferry (SF1500, Destination Gotland). This vessel is the largest that traffics the harbor daily. Three sampling stations were used to measure turbidity: E2, E3, and E4. The water depth at these stations is approximately 14-15 meters. Profiles were collected within 15 minutes of the ferry passing through the station. Figure SI-1.1 displays pressure, oxygen content, salinity, and turbidity as they relate to water depth. Here, it is clear that turbidity was low before and after ship passage. An increase in turbidity was observed only near or at the bottom, once before and once after the ship passage, and was likely caused by the CTD contacting the bottom.

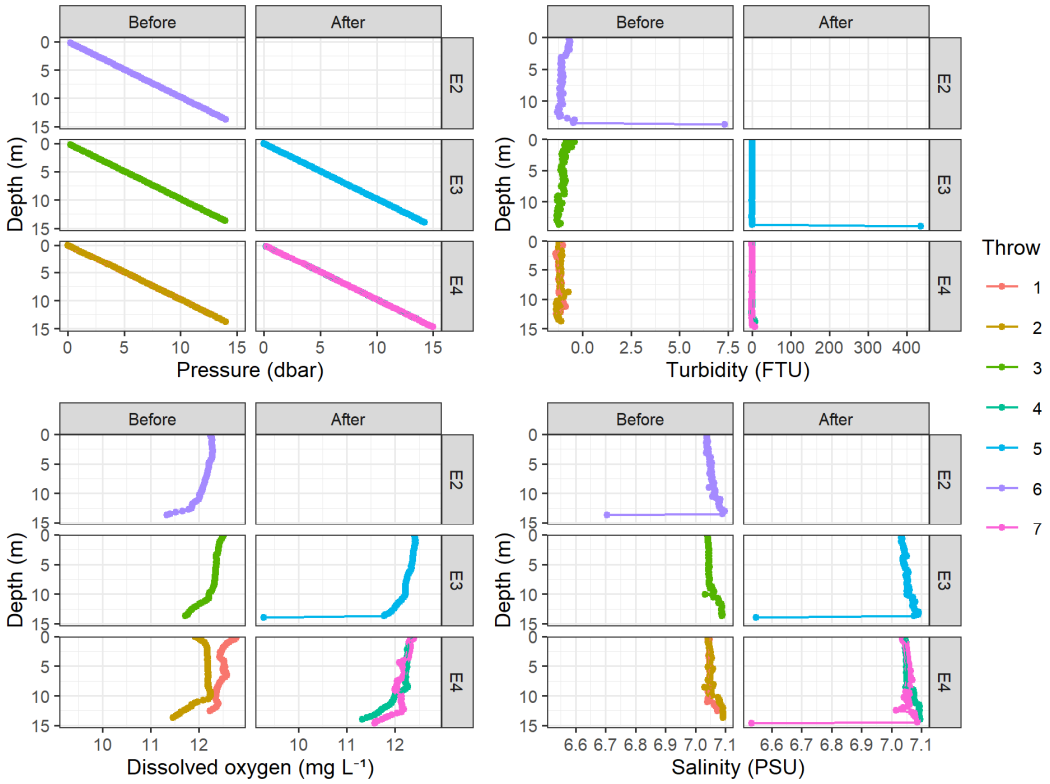

Figure SI-1.1. Pressure, turbidity, dissolved oxygen, and salinity measured in the overlying water before and after ship passages overhead at the stations E2, E3, and E4 in outer Oskarshamn harbor. A cast consists of a series of measurements taken during CTD descent.

Section SI-2. Quantification of PAHs and PCBs in SPMD

Extraction of the contaminants present in the semipermeable membrane devices (SPMD) were carried out following the procedure described by Mustajärvi et al. (2017). Each SPMD was soaked in 100 mL n-hexane for 24 h and this procedure was repeated twice. Extracts were spiked with a mixture of deuterated PAHs (naphthalene D8, fluorene D10, phenanthrene D10, anthracene D10, fluoranthene D10, pyrene D10, benzo[a]anthracene D12, chrysene D12, benzo[a]pyrene D12, dibenz[a,h]anthracene D14, benzo[g,h,i]perylene D12) and ^{13}C-labeled PCBs (PCB # 28, 52, 101, 118, 138, 180, 153). The SPMD extracts were split into two fractions (20 and 80 %) for PAH and PCB analyses, respectively. The PAH fraction was cleaned by the dimethyl formamide (DMF) method described by Mandalakis et al. (2004) and modified by Mustajärvi et al. (2017). Briefly, the extracts were first solved in n-pentane and then extracted twice with DMF (with 5 % ultrapure water [Milli-Q], v/v) and twice with n-hexane. The n-hexane extracts were combined, reduced in volume and run through a two-layer SiO_2-gel column (1 cm Ø) packed with 0.5 cm Na_2SO_4 on the top and 2.5 cm SiO_2 (10 % ultrapure water) on the bottom. The extracts were further cleaned with activated granular Cu to remove sulfur. The PCB fraction was reduced in volume and cleaned using a three-layer SiO_2-gel column (1 cm Ø) packed from the top to the bottom in 3 cm layers of SiO_2/H_2O (10 % ultrapure water w/w), SiO_2/KOH (36 % KOH w/w) and SiO_2/H_2SO_4 (40 % H_2SO_4 w/w) (Zebühr et al. 1993). The extracts were eluted with n-hexane. All samples were analyzed by gas chromatography/mass spectrometry (GC/MS, Thermo Scientific™ ISQ™) with a 30 m DB-5 column of 0.25 mm inner diameter and 0.25 µm film thickness, operating in electron impact mode (EI+,70 eV) and with single ion monitoring acquisition. Helium was used as carrier gas at 1.5 mL min^{-1}. Target compounds were the PAHs naphthalene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene; and the PCB # 28, 52, 101, 118, 138, 153, 180. Method detection limits (MDL) were calculated as the mean plus three times the standard deviation of all blank values (n=4). Data below MDL were removed from further analysis, and all analyte concentrations were corrected for the blank average. MDLs are expressed as quantity per passive sampler (ng SPMD^{-1}), and for PAHs were 42 ng SPMD^{-1} naphthalene, 0.9 ng SPMD^{-1} acenaphthene, 1.2 ng SPMD^{-1} fluorene, 0.2 ng SPMD^{-1} anthracene, 0.9 ng SPMD^{-1} phenanthrene, 0.7 ng SPMD^{-1} fluoranthene, 0.5 ng SPMD^{-1} pyrene, 0.02 ng SPMD^{-1} benzo[a]anthracene, 0.05 ng SPMD^{-1} chrysene, 1.2 ng SPMD^{-1} benzo[a]pyrene, 1.2 ng SPMD^{-1} dibenz[a,h]anthracene, 0.3 ng SPMD^{-1} benzo[b]fluoranthene,

0.3 ng $SPMD^{-1}$ benzo[k]fluoranthene, 0.6 ng $SPMD^{-1}$ indeno[1,2,3-cd]pyrene, 0.4 ng $SPMD^{-1}$ benzo[g,h,i]perylene. MDLs for PCBs were 0.08 ng $SPMD^{-1}$ PCB28, 0.73 ng $SPMD^{-1}$ PCB52, 0.08 ng $SPMD^{-1}$ PCB101, 0.16 ng $SPMD^{-1}$ PCB118, 0.006 ng $SPMD^{-1}$ PCB138, 0.06 ng $SPMD^{-1}$ PCB153, 0.004 ng $SPMD^{-1}$ PCB180.

Section SI-3. Quantification of PAH and PCBs in sediment using SPME

The determination of the freely dissolved concentration (C_{free}) of PAHs and PCBs in the sediment pore water, was performed using a solid phase microextraction (SPME) according to Witt et al. (2009). For this, SPME fibers with a 10 µm PDMS coating (GF10) supplied by Fiberguide Industries (SPC210/230; Stirling, NJ, USA) were used. The calculation of the fiber concentration requires the knowledge of the fiber geometry, which was already analyzed by optical microscopy and published by Witt et al. (2013). Prior to use, the PDMS fibers were cut in 15 cm pieces, and cleaned in a glass cylinder filled with ethyl acetate and washed three times for 15 minutes in a sonic bath. After which, the fibers were rinsed three more times for 15 minutes with ultrapure water using a sonic bath. The fibers were then stored in ultrapure water at 4°C until they were inserted into the sediment in the experiment. At the end of the experiment, the fibers were taken out of the sediment and PAHs and PCBs were extracted and measurements using a gas chromatograph (GC 7890A) and a quadrupole mass spectrometer (MS 5975C) from Agilent. First, the length of fiber protruding above the sediment surface was cut off and analyzed separately. Thereafter, the fiber was cut into three segments of 4 cm and measured using a caliper. Then, the fiber segments were cut into pieces of about 2 cm and placed in glass liners. Prior to placing fibers in the liner, a few small glass beads were placed right above the notch to ensure the fiber stayed in position during thermal desorption. Additionally, the empty liner was washed three times with hexane and subsequently cleaned at 250°C for 19 minutes in the GC injector under helium flow. Since the measurement of only 4 cm glass fiber was below the detection limit, the top 4 cm of all three replicate fibers were measured together in one glass liner. For the actual measurement, the MPS transferred the loaded glass liner into the cooled injection system (CIS), where the thermal desorption took place (Figure SI-3.1). As soon as the liner entered the CIS the temperature was raised from 30 °C to 250°C at a rate of 12°C s^{-1} for 15 minutes. After 15 minutes, the desorbed analytes were transferred in splitless mode onto the column (HP-5MS, 325 °C: 30 m x 250 µm x 0.25 µm, J&W Scientific). Then the GC program was started immediately, and the split vent was set at 50 mL min^{-1}. The MS transfer line temperature was held at 310 °C, and the native compounds were measured in a selected ion mode. For each measurement, a fourteen-point external standard calibration curve of PAHs and PCBs (PAH Mix 9, PCB Mix 3, Dr. Ehrenstorfer) was analyzed in the same way as the samples.

Peaks were evaluated using the MassHunter software for quantitative analysis (version B.07.00/Build 7.0.457.0) by Agilent. Concentrations in the fibers (C_{PDMS}) were calculated with the external standards and related to the fiber length, which was determined before measurement. C_{free} was then calculated using the partitioning coefficients between PDMS and water obtained from Witt et al. (2009). For data quality control, a minimum of two fibers were pooled and analyzed together, and GC liners were checked for chemical purity. Cleaned and unexposed fibers served as analytical blanks (n=2). External standard solutions (PAH-Mix 9 and PCB-Mix 3, Dr. Ehrenstorfer) were used for quantification. Method detection and quantification limits (MDL and MQL) were calculated as the average mass in blank fibers plus 3 SD for MDL or plus 10 SD for MQL and converted to fiber coating concentrations. The MQL_{PDMS} were 3.6 to 8.9 ng mL^{-1} for PAHs and 3.2 to 7.2 ng mL^{-1} for PCBs. The MDL_{PDMS} were lower than 2.0 ng mL^{-1} for all target compounds. MQL based on freely dissolved concentrations (MQL_{free}) were lower and decreased with increasing PDMS-to-water partition coefficient (K_{PDMS}). Further details on oven temperature and hold times used during the GC are provided in the Table SI-3.1.

Figure SI-3.1. GC oven program for thermal desorption of PDMS fibers. Total run time 47 minutes.

Oven temperature ramp	Temperature (°C)	Rate (°C min^{-1})	Hold time (min)
Start	60	-	15
1	195	15	2
2	225	15	0
3	260	5	0
4	300	20	10

Section SI-4. Quantification of PAH and PCB total concentrations in sediment

For determining the total concentration of PAHs and PCBs (C_{total}) in sediments, dried sediments were extracted using accelerated solvent extraction (ASE®200, DIONEX). Approximately 2-4 g sediment dry weight (dw) was extracted twice under high pressure (140 bar) and temperature (100° C) with about 80 mL of an acetone/hexane mixture (v/v = 40/60). Prior to extraction, 100 ng of surrogate standards for PAHs and PCBs were added to each sample prior to extraction. These were PAH Mix 9 deuterated (Dr. Ehrenstorfer) containing acenaphthene D10, acenaphthylene D8, anthracene D10, benzo[a]pyrene D12, Benzo[b]fluoranthene D12, benzo[g,h,i]perylene D12, benzo[k]fluoranthene D12, benzo[a]anthracene D12, chrysene D12, dibenz[a,h]anthracene D14, fluoranthene D10, fluorene D10, indeno[(1,2,3-c,d]pyrene D12, naphthalene D8, phenanthrene D10, and pyrene D10, and MBP-D7 (Wellington Laboratories) containing the ^{13}C-labeled PCB congeners 28, 52, 101, 118, 138, 153, and 180. After extraction,

activated copper was added over night for sulfur removal, and subsequently, the extracts of both primary and secondary extraction were combined. The extract was solid phase extracted (SPE) with deactivated aluminum oxide (ca. 3 g, top) and silica (ca. 3 g, bottom) in a two-stage column using a Baker Bond (J.T. Baker) system. Teflon frits were added to the bottom and top of each column to separate solid phases. Subsequently, the volume of the extract was reduced to about 500 µL by roto-evaporation and filled-up with hexane to 800 µL. For the PAH and PCB measurements in the extracts, an aliquot of 1 µL sample was injected in a GC-MS (GC 7890A with a cooled injection system (CIS) and MS 5975C inert XL MSD with triple-axis detector, both from Agilent Technologies; equipped with an ALEX tray and multi-purpose sampler, MPS 2XL from Gerstel) system for analysis. The injector temperature was increased from 20 °C to 250 °C with a rate of 12 °C $s^{-1}$and held for 15 min. Then the sample was transferred splitless to the column and the injector purge flow to the split vent was set at 50 mL min^{-1}. For the chromatographic step, a GC device (7890A, Agilent Technologies) with a HP-5MS fused silica gel column (325 °C: 30 m x 250 µm x 0.25 µm, J&W Scientific) and helium as the carrier phase was used. The temperature of the GC oven was raised from 50 °C to 190 °C (ramp: 12 °C min^{-1}) and held for 3 min before further heating up to 300 °C (ramp: 5 °C min^{-1}) and holding this for 10 min (Table SI-4.1). The quadrupole MSD transfer line temperature was set at 310 °C and compounds were measured in selected ion mode (SIM) to assure a higher sensitivity in contrast to the scan acquisition mode. The total run time was 47 minutes. For quantification purposes by mass spectrometry, a three-point calibration curve with standard solutions of PAHs and PCBs was generated. Per compound of interest, the masses of two ions (target mass and qualifier 1) were monitored. The target mass was used for quantification, while the mass of qualifier 1 and the retention time were used for qualitative purposes. Peaks were evaluated using the MassHunter software for quantitative analysis (version B.07.00/Build 7.0.457.0) by Agilent. Recovery rates for analytes in each sample are provided in Table SI-4.2. For quality control and assurance purposes, the HPLC clean-up was tested with standard solutions (PAH-Mix 9, PAH-Mix 9 deuterated and PCB-Mix 3, PCB-Mix 3 deuterated, Dr. Ehrenstorfer) which gave final mean standard recoveries from the surrogate and external PAH and PCB target compounds of 95 % ± 8 % (n = 10). The analytical quality control for the whole procedure was carried out with a certified reference material for PAHs and PCBs in marine sediments (QPH 058 and QPH 059 (QUASIMEME, Laboratory Performance Studies, http://www.quasimeme.org/). Surrogate standard solutions were added to each sample. The mean recovery values ranged between 71 % (naphthalene) and 102 % (n=10). The measured concentrations for the single compounds were in all cases in the range of certified values (±

certified uncertainty). Relative standard deviations of the measured values for the single compounds (n =10) ranged between 5 and 12 % and mean recovery rates of the surrogate standard solutions were calculated for all target compounds with 84 % ± 5 %. Limits of quantification (LOQs) were estimated in the range of 0.3–5 μg kg^{-1} DW for PAHs and 0.5-1 μg kg^{-1} DW for PCBs.

Table SI-4.1. GC oven program for sediment extracts with a total run time of 47 minutes.

Oven temperature ramp	Temperature (°C)	Rate (°C min^{-1})	Hold time (min)
Start	50	-	0
1	190	12	3
2	300	5	10

Table SI-1. Number of replicates of each treatment sampled for various analyses. Bold values indicate samples where fewer replicates (than the maximum n=4) were taken due to operational constraints.

	Ctrl-S	Clay-S	GAC-S	PAC-S	Ctrl-R	Clay-R	GAC-R	PAC-R
Fluxes of PAHs and PCBs (SPMD)	4	4	4	4	4	4	4	4
Dissolved metals (DGT)	3	3	3	3	3	3	3	3
Dissolved PAHs and PCBs (SPME)	**1**	4	**2**	4	4	4	4	4
Benthic community	2	2	2	2	2	2	2	2
<u>Surface sediment samples (0-1 cm)</u>								
TOC, TC, TN	2	2	2	**1**	2	2	2	**1**
DW, OM	2	2	2	**1**	2	2	2	**1**
PAH, PCB	2	2	2	**1**	2	2	2	**1**
Metals	2	2	2	**1**	2	2	2	**1**
<u>Subsurface sediment samples</u>								
DW, OM	2	2	2	**1**	2	2	2	**1**
TOC, TC, TN	2	-	-	-	2	-	-	-
PAH, PCB	2	-	-	-	2	-	-	-
Metals	2	-	-	-	2	-	-	-

'Surface' are samples collected at 0-1 cm depth and 'subsurface' are samples collected at 1-3, 3-5, 5-7, and 7-10 cm depth.

Table SI-4.2 Recovery rates of PAHs and PCBs in sediment samples (1 is 100 % recovery rate). Depth in cm from sediment surface.

Treatment	Depth	Naph	Ace	Acy	Fluoren	Phen	Anth	Fluo	Pyr	BaA	Chr	BbF	BkF	BaP	InP	DBA	BghIP	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180
C1	0-1	1.06	1.39	1.68	1.54	1.73	2.36	2.50	2.48	4.20	2.41	4.03	3.66	5.35	5.33	3.77	3.57	1.71	1.73	1.82	1.82	2.41	2.29	2.69
C3	0-1	0.90	1.21	1.34	1.39	1.63	2.19	2.33	2.36	3.89	2.30	3.59	3.48	5.33	4.88	3.47	3.32	1.68	1.69	1.77	1.77	2.41	2.27	2.63
C3	1-3	0.93	1.29	1.52	1.42	1.57	2.16	2.31	2.23	3.94	2.16	3.58	3.33	5.12	4.88	3.72	3.28	1.56	1.58	1.64	1.64	2.15	2.03	2.42
C3	3-5	0.98	1.33	1.69	1.44	1.58	2.21	2.29	2.25	3.76	2.11	3.75	3.27	5.60	5.00	3.95	3.30	1.56	1.58	1.63	1.63	2.17	2.03	2.43
C3	5-7	0.79	1.23	1.58	1.38	1.55	2.17	2.24	2.23	4.02	2.13	3.72	3.27	5.68	5.07	3.52	3.20	1.49	1.53	1.58	1.58	2.10	1.96	2.32
C3	7-10	0.80	1.19	1.54	1.31	1.44	2.04	2.12	2.03	3.62	1.95	3.48	2.97	5.44	4.76	3.85	2.99	1.44	1.46	1.49	1.49	1.96	1.81	2.20
CR1	0-1	1.02	1.36	1.71	1.49	1.67	2.33	2.38	2.39	4.20	2.27	3.92	3.36	6.09	5.24	3.77	3.43	1.65	1.66	1.75	1.75	2.31	2.18	2.55
CR3	0-1	0.86	1.31	1.54	1.48	1.69	2.32	2.43	2.46	4.15	2.36	3.91	3.54	5.53	5.15	3.97	3.48	1.73	1.73	1.83	1.83	2.40	2.31	2.68
CR3	1-3	0.90	1.26	1.60	1.40	1.52	2.13	2.21	2.14	3.83	2.04	3.56	3.15	5.04	4.80	3.73	3.14	1.52	1.54	1.59	1.59	2.10	1.96	2.35
CR3	3-5	0.81	1.22	1.60	1.36	1.50	2.12	2.16	2.10	3.91	2.02	3.65	3.11	5.54	4.79	3.39	3.10	1.48	1.50	1.55	1.55	2.06	1.89	2.31
CR3	5-7	0.81	1.20	1.56	1.33	1.46	2.07	2.12	2.10	3.80	1.99	3.59	3.12	5.59	4.98	4.28	3.08	1.46	1.48	1.53	1.53	2.02	1.88	2.28
CR3	7-10	0.81	1.19	1.56	1.30	1.42	2.04	1.98	1.96	3.54	1.83	3.35	2.91	5.25	4.60	4.02	2.83	1.38	1.39	1.41	1.41	1.84	1.71	2.07
S2	0-1	0.94	1.34	1.64	1.48	1.67	2.27	2.40	2.39	3.99	2.27	3.82	3.37	5.57	5.01	4.58	3.39	1.70	1.71	1.80	1.80	2.37	2.25	2.63
S3	0-1	1.05	1.40	1.76	1.52	1.67	2.34	2.37	2.36	4.04	2.24	3.80	3.37	5.63	5.09	3.81	3.41	1.68	1.69	1.75	1.75	2.30	2.21	2.56
SR2	0-1	0.94	1.37	1.74	1.50	1.65	2.32	2.32	2.35	4.09	2.28	3.86	3.42	5.52	5.23	2.74	3.41	1.64	1.65	1.73	1.73	2.25	2.15	2.53
SR3	0-1	0.82	1.22	1.51	1.39	1.58	2.24	2.28	2.33	4.04	2.23	3.82	3.29	5.77	5.07	3.54	3.36	1.58	1.59	1.67	1.67	2.23	2.11	2.47
G1	0-1	0.37	0.64	0.65	0.56	0.48	0.58	0.59	0.55	0.63	0.35	0.54	0.36	0.60	0.42	0.29	0.30	0.87	1.08	1.07	1.07	1.41	1.34	1.51
G2	0-1	0.17	0.26	0.20	0.18	0.14	0.16	0.18	0.16	0.15	0.08	0.12	0.07	0.13	0.08	0.07	0.09	0.50	0.87	0.86	0.86	1.12	1.08	1.21
GR1	0-1	0.09	0.27	0.17	0.17	0.11	0.12	0.13	0.12	0.09	0.05	0.07	0.04	0.06	0.04	0.03	0.05	0.70	1.25	1.23	1.23	1.64	1.57	1.76
GR2	0-1	0.39	0.57	0.59	0.55	0.55	0.70	0.71	0.70	0.89	0.53	0.76	0.63	0.82	0.56	0.38	0.39	0.92	1.26	1.28	1.28	1.66	1.61	1.82
P2	0-1	0.09	0.14	0.08	0.06	0.04	0.04	0.08	0.07	0.04	0.02	0.04	0.04	0.04	0.03	0.02	0.04	0.38	1.25	1.25	1.25	1.61	1.56	1.77
P4	0-1	0.01	0.03	0.01	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.12	1.13	1.10	1.10	1.36	1.31	1.46
PR2	0-1	0.01	0.03	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.10	1.27	1.23	1.23	1.54	1.50	1.66
PR4	0-1	0.08	0.17	0.15	0.13	0.11	0.11	0.15	0.14	0.12	0.06	0.11	0.06	0.02	0.10	0.05	0.08	0.34	1.16	1.13	1.13	1.46	1.40	1.60
Blank 1	-	0.78	0.92	1.15	1.01	1.09	1.37	1.49	1.46	2.05	1.35	2.15	1.78	2.74	2.71	2.31	1.94	1.11	1.14	1.14	1.14	1.40	1.37	1.48
Blank 2	-	0.81	0.98	1.12	1.06	1.11	1.29	1.33	1.28	1.77	1.24	1.75	1.45	1.99	1.83	1.60	1.63	1.08	1.09	1.08	1.08	1.29	1.26	1.34
Blank 3	-	0.54	0.73	0.83	0.87	1.04	1.22	1.50	1.46	2.00	1.35	2.00	1.57	2.00	2.31	1.75	1.59	1.09	1.16	1.19	1.19	1.48	1.46	1.59

Figure SI-1. Photograph of three sediment cores. Left core is a resuspended PAC treatment, middle core is not resuspended, and the right core is resuspended without PAC. Note the black color of PAC on the walls and passive samplers to the left, the translucent SPMD in the static condition in the middle core, and the brown color of sediment or clay on the walls and passive samplers to the right.

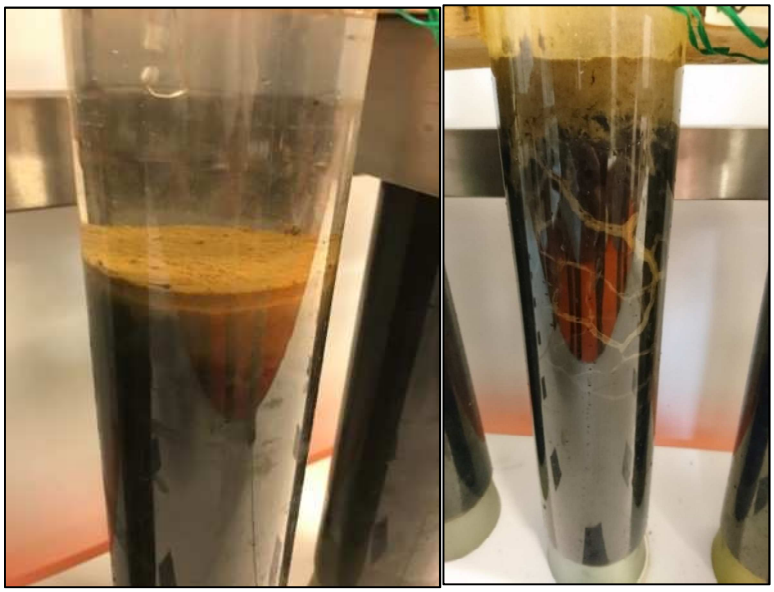

Figure SI-2. A sediment core displaying an oxygenated layer of sediment at the surface during the experiment (left) and a sediment core displaying oxygenated burrows of macroinvertebrates contrasting a black euxinic sediment at the end of the experiment (right).

Table SI-2. Benthic species composition of Oskarshamn harbor grab samples (Reference E2, E3, E4) collected during field retrieval of sediment cores, and benthic species composition of 2 sediment cores per treatment determined at experiment termination. Note that sediment cores have surface areas an order of magnitude lower than the reference samples.

		Oskarshamn references			Static condition				Resuspended condition			
Phylum	Species	E2	E3	E4	Ctrl-S	Clay-S	GAC-S	PAC-S	Ctrl-R	Clay-R	GAC-R	PAC-R
Annelida	*Hediste diversicolor*	4	6	4					1			
	Bylgides sarsi				1	1	1					
	Marenzelleria spp.		2	8	1	1			1		1	
	Pygospio elegans			13								
	Oligochaeta			7								
Arthropoda	Chironomidae	212	79	8	4	5	2		3		3	1
Mollusca	*Hydrobia spp.*	162	94	5	10	2	12	6	1	3	8	4
	Limecola balthica	36	7	4	2	2	3	5	2	4	5	2
	Mya arenaria	2	1	4					1			
	Mytilus edulis	3		3								
	Potamopyrgus antipodarum	3	1									
	Number of species in sample	7	7	9	5	5	4	2	5	3	4	3
	Sampled surface area (m^2)	0.1	0.1	0.1	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105
	Est. total abundance (per m^2)	4 220	1 900	560	2 280	1 393	2 280	1 393	1 267	887	2 153	887

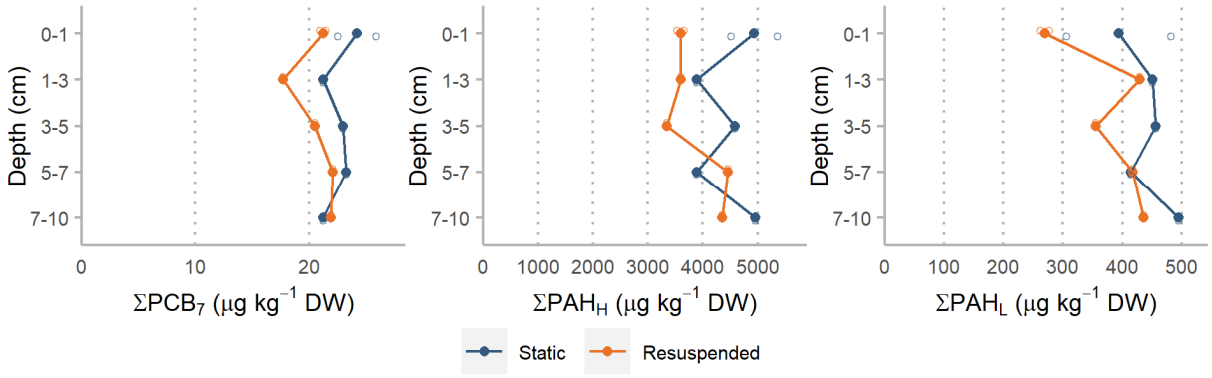

Figure SI-3. Depth profiles of $\sum PCB_7$, $\sum PAH_H$, and $\sum PAH_L$ total concentrations (C_{total}) in sediment (µg kg^{-1} dw) measured in the Control treatment. Average concentrations are represented by solid dots, whereas individual samples (for n = 2) are represented by open dots.

Table SI-3. Average concentrations of HOCs in surface sediment (0-1 cm, µg kg^{-1} DW), porewater (ng L^{-1} for PAHs, pg L^{-1} for PCBs) and fluxes from sediment to water (ng m^{-2} d^{-1}).

		Static				Resuspended				
	Class	Control	Clay	GAC	PAC	Control	Clay	GAC	PAC	Unit
Flux	$\sum PCB_7$	1.98	1.69	1.86	0.856	3.37	2.71	2.81	0.305	pg m^{-2} d^{-1}
	$\sum PAH_H$	9.37	7.34	10.6	2.29	51.6	36	27.3	0.290	pg m^{-2} d^{-1}
	$\sum PAH_L$	25.5	21.1	17.9	13.9	15.5	9.58	8.95	1.85	pg m^{-2} d^{-1}
Porewater	$\sum PCB_7$	20.8	18.2	19.0	14.9	20.2	19.0	17.8	16.6	ng L^{-1}
	$\sum PAH_H$	3430	1650	1336	954	1385	1209	1849	915	pg L^{-1}
	$\sum PAH_L$	27900	18100	18400	136000	19000	19900	32200	124000	pg L^{-1}
Sediment	$\sum PCB_7$	24.2	19.5	14.2	11.7	21.2	17.6	11.9	10.1	µg kg^{-1} DW
	$\sum PAH_H$	4290	2530	33.6	129	3180	3000	36.3	86.1	µg kg^{-1} DW
	$\sum PAH_L$	1050	509	43.0	59.2	684	982	52.5	49.9	µg kg^{-1} DW

Table SI-4. Mean total sediment concentration (C_{total}, n = 2) of PAHs and PCBs (µg kg^{-1} DW) in vertical profiles from surface to 10 cm depth in the static and resuspended Control treatments (concentration, n = 2).

	Static condition					Resuspended condition				
Compound	**0–1**	**1–3**	**3–5**	**5–7**	**7–10**	**0–1**	**1–3**	**3–5**	**5–7**	**7–10**
Naphthalene	42.4	217.1	216.5	187.0	177.1	37.3	203.4	168.9	190.3	187.4
Acenaphthene	13.7	11.7	9.9	10.1	12.4	10.1	10.2	8.7	10.5	12.4
Acenaphthylene	7.5	5.9	6.9	5.9	9.6	9.0	8.9	5.8	8.5	8.9
Fluorene	28.9	21.1	20.5	22.8	27.9	22.1	20.7	17.8	20.5	23.6
Phenanthrene	257.5	152.9	158.3	143.4	207.9	155.4	146.6	120.3	143.1	152.7
Anthracene	44.4	42.5	43.8	45.6	60.1	35.8	39.7	33.9	44.9	50.6
Fluoranthene	652.8	462.5	556.1	452.0	630.6	414.8	417.3	369.3	471.2	519.8
Pyrene	470.3	328.8	411.6	336.9	446.7	308.6	309.7	277.4	347.8	360.8
Benz[a]anthracene	369.1	307.6	405.8	308.5	399.8	265.9	289.2	246.6	335.9	373.8
Chrysene	321.0	235.1	280.7	213.4	266.1	205.0	201.7	184.2	232.7	265.8
Benzo[b]fluoranthene	528.1	428.1	511.3	452.4	581.6	413.8	416.0	408.3	529.1	528.2
Benzo[k]fluoranthene	424.8	338.4	369.6	324.4	382.3	323.4	288.6	273.1	370.5	341.9
Benzo[a]pyrene	667.1	547.2	644.2	509.4	629.0	448.8	479.0	461.7	609.4	543.9
Indeno[1,2,3-cd]pyrene	872.4	712.7	827.9	779.3	971.5	718.3	710.3	662.7	903.6	820.0
Dibenz[a,h]anthracene	117.6	97.6	117.7	103.8	137.5	100.6	91.5	91.2	143.3	131.8
Benzo[g,h,i]perylene	514.1	426.0	457.1	414.3	518.0	395.8	392.5	375.2	510.8	470.3
PCB28	1.3	0.6	0.5	0.5	0.5	1.0	0.4	0.5	0.6	0.7
PCB52	2.0	1.1	0.9	1.1	0.9	1.6	0.8	0.9	1.0	1.0
PCB101	2.6	1.9	2.1	2.3	2.2	2.1	1.6	1.9	2.0	2.4
PCB118	2.2	2.0	1.8	2.0	1.7	1.9	1.6	1.8	2.1	2.4
PCB138	7.0	6.4	7.3	7.0	6.4	6.4	5.2	6.0	6.6	6.2
PCB153	5.8	6.0	6.6	6.5	6.1	5.1	5.2	5.8	6.3	6.1
PCB180	3.4	3.2	3.8	3.9	3.4	3.2	2.8	3.5	3.7	3.2

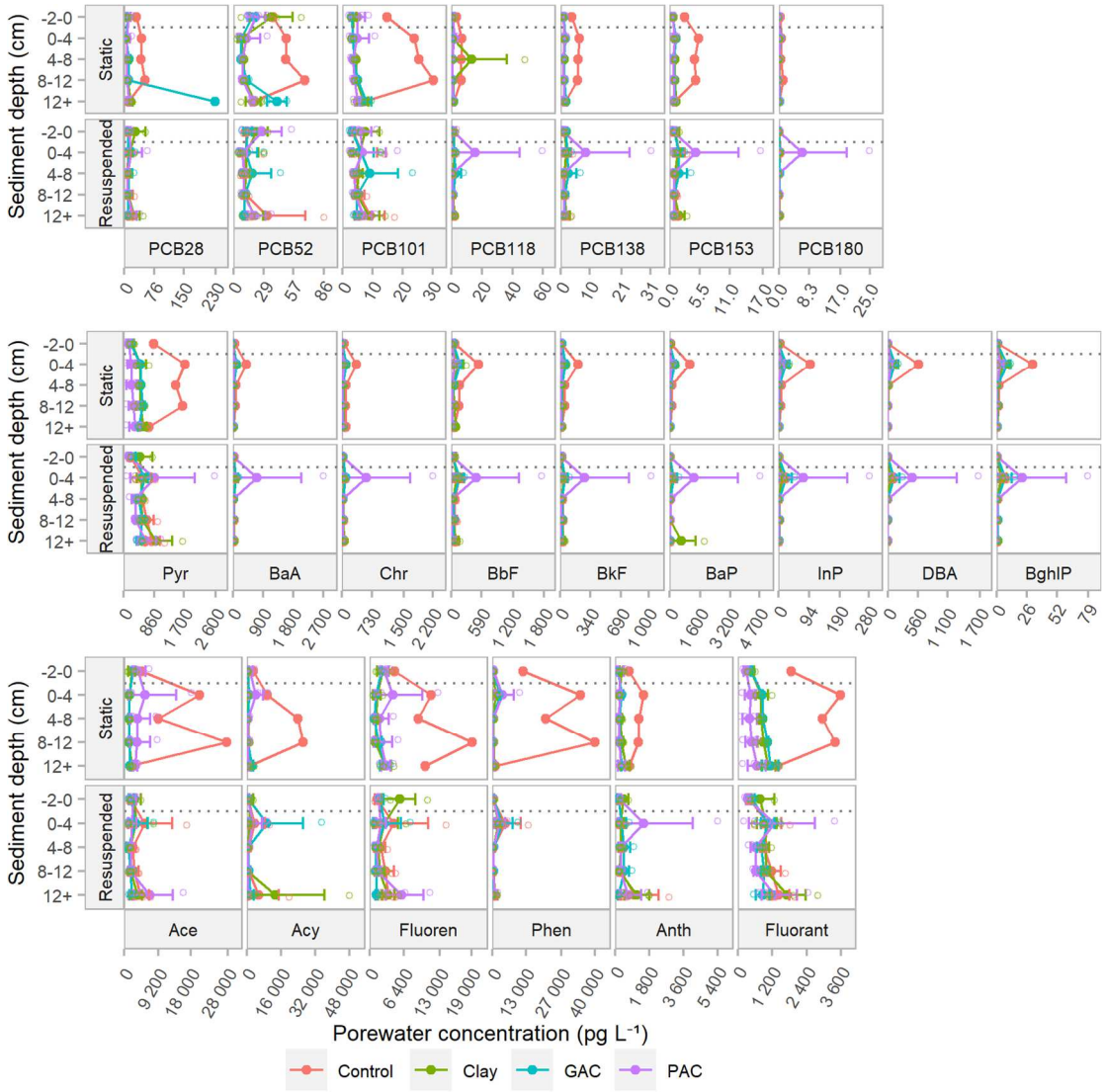

Figure SI-4. Vertical profiles of freely dissolved PCBs and PAHs in porewater (C_{free}, pg L^{-1}). Dashed horizontal line indicates the sediment surface. Top row shows PCBs, middle row high molecular weight PAHs (PAH_H), and bottom row shows low molecular weight PAHs (PAH_L).

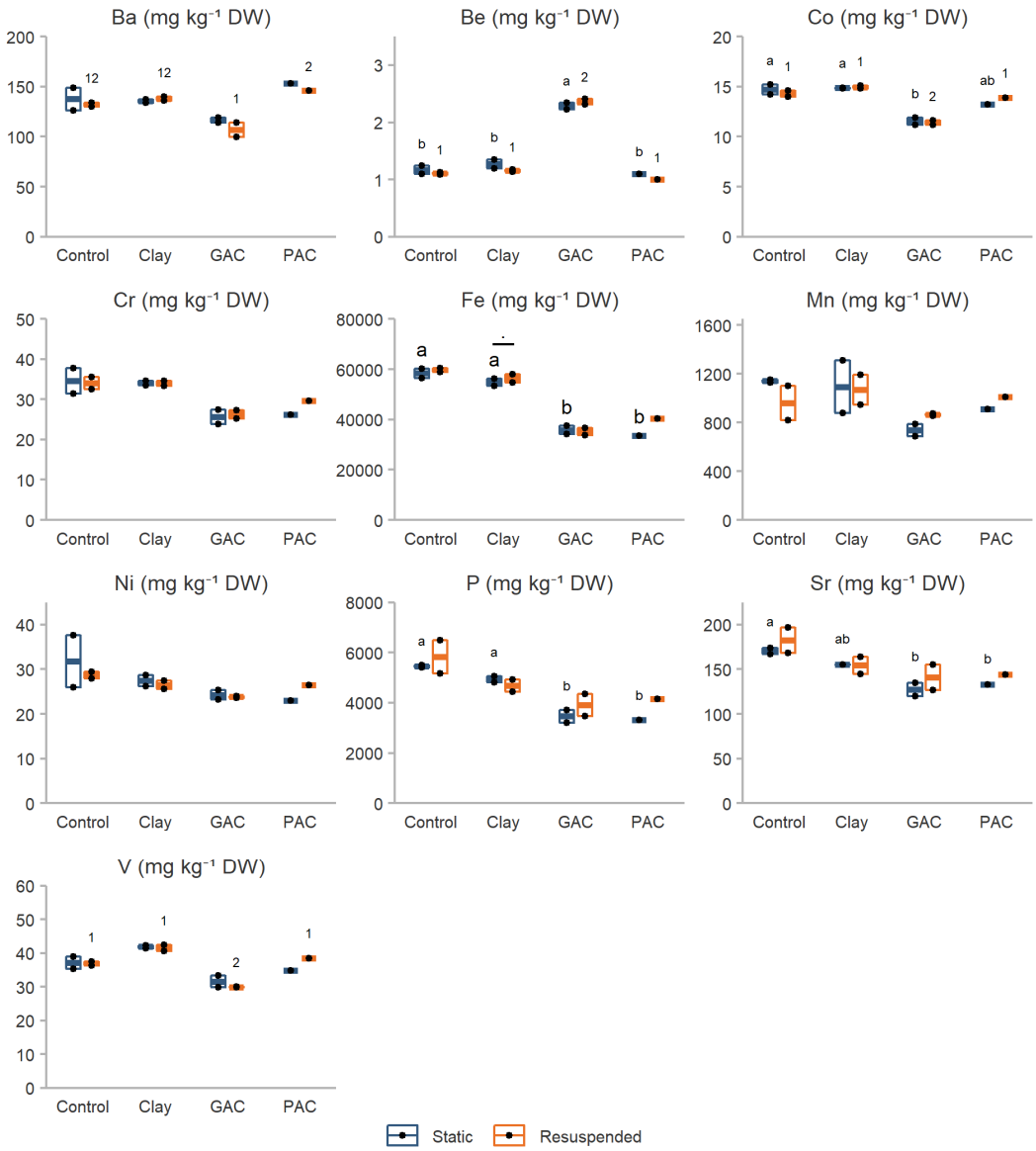

Figure SI-5. Sediment concentrations of 10 elements at 0-1 cm depth (mg kg^{-1} DW). Shared letter indicates no statistical difference between treatments (Post hoc Tukey HSD, 0.05 α). Asterisks denote statistically significant difference between static and resuspended condition (paired t-test, · p<0.1, * p<0.05, ** p<0.01, *** p<0.001). Only one replicate of each PAC treatment was profiled due to material loss in handling. Hg below limit of quantification (1 mg kg^{-1} DW).

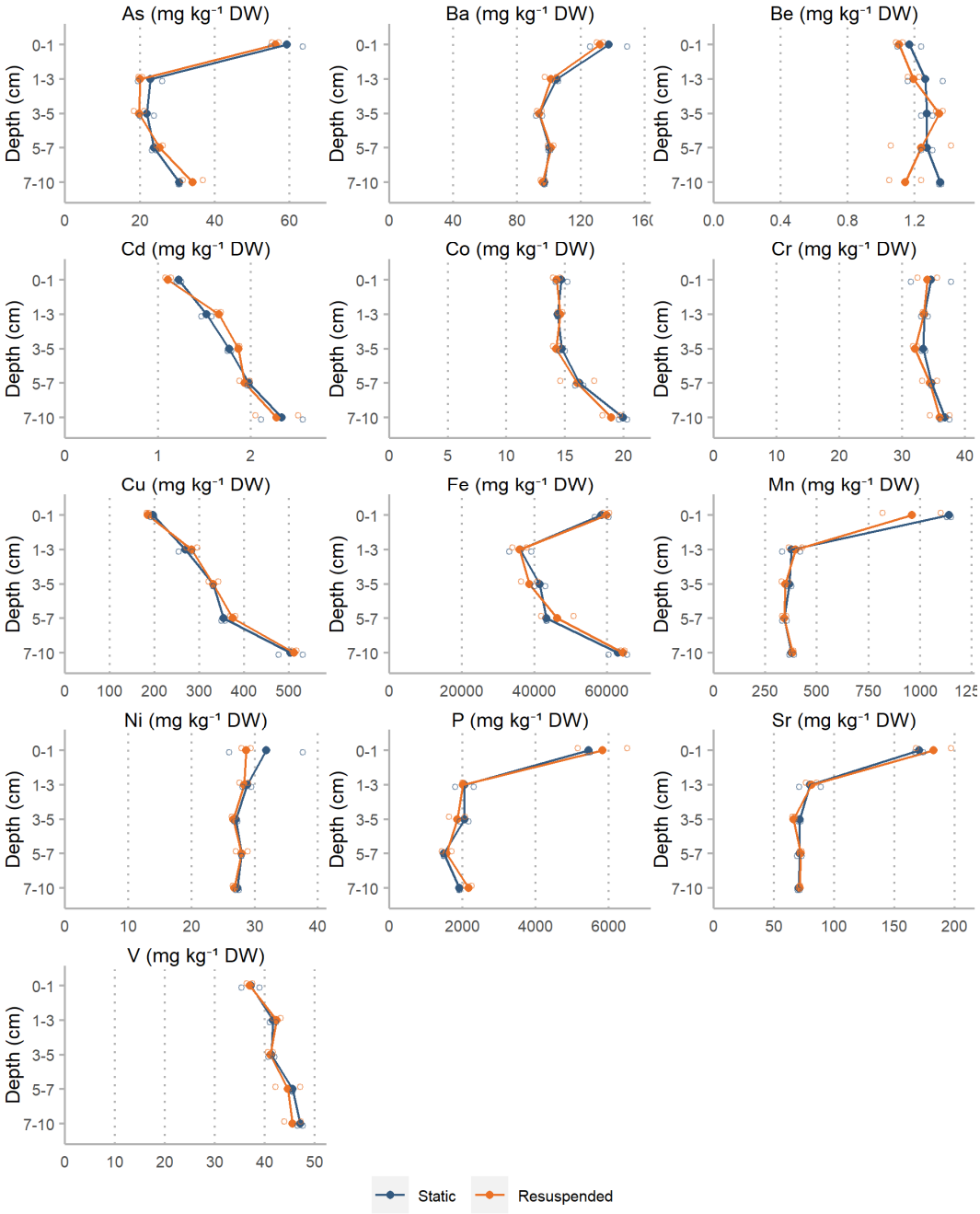

Figure SI-6. Depth profiles of 16 elements in sediment (mg kg^{-1} DW) in static and resuspended Control treatments. Filled dots represent averages or single values, open dots represent individual samples. Hg below limit of quantification (1 mg kg^{-1} DW).

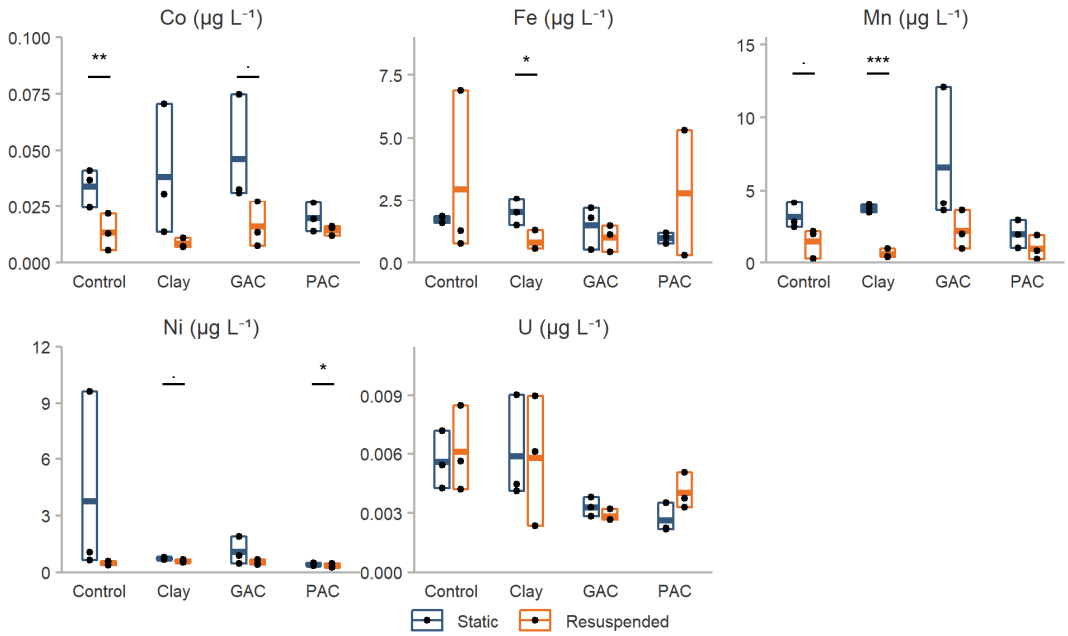

Figure SI-7. Water concentrations of Co, Fe, Mn, Ni, and U measured in DGT passive sampler (µg L^{-1}). Shared letter indicates no significant difference between treatments (Post hoc Tukey HSD, 0.05 α). Asterisks denote significant differences between static and resuspended condition (paired t-test, · p<0.1, * p<0.05, ** p<0.01, *** p<0.001). Al and Cr are not presented as they were detected in only 1 and 2 samples, respectively (LOQ: 0.3 µg Al L^{-1} and 0.03 µg Cr L^{-1}).

Table SI-5. Average concentrations of metals in water (measured using DGT, µg L^{-1}) and surface sediment (0-1 cm, mg kg^{-1} DW). Please refer to Figures 10 and 11 in the main text as well as Figures SI-13 and SI-15 for statistical test results.

		Static				Resuspended			
Medium	Element	Control	Clay	GAC	PAC	Control	Clay	GAC	PAC
Water	Al	nd	nd	nd	nd	0.553	nd	nd	2.79
	Cd	0.05	0.05	0.03	0.02	0.03	0.03	0.03	0.01
	Co	0.03	0.04	0.05	0.02	0.01	0.01	0.02	0.01
	Cr	0.03	nd	nd	nd	nd	nd	nd	nd
	Cu	2.21	2.66	0.63	0.19	2.81	2.87	1.62	0.12
	Fe	1.75	2.03	1.50	0.99	2.97	0.80	1.01	2.80
	Mn	3.14	3.73	6.61	1.95	1.45	0.59	2.17	0.96
	Ni	3.76	0.73	1.08	0.39	0.50	0.59	0.51	0.33
	Pb	0.00115	0.00104	0.00283	0.00076	0.01018	0.00327	0.00311	0.00455
	U	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00
	Zn	2.64	2.29	1.89	0.97	0.88	1.39	1.30	0.52
Sediment	As	59.3	46.5	37.8	35.4	56.2	48.3	33.2	42.8
	Ba	138	136	117	153	132	138	107	146
	Be	1.17	1.27	2.29	1.10	1.11	1.16	2.36	1.00
	Cd	1.23	0.87	0.64	0.62	1.11	0.82	0.63	0.76
	Co	14.7	14.9	11.6	13.2	14.3	15.0	11.4	13.9
	Cr	34.6	34.1	25.7	26.2	34.1	34.0	26.3	29.6
	Cu	196	172	112	107	185	170	117	127
	Fe	58400	54850	35850	33500	59750	56400	35200	40400
	Mn	1140	1094	738	908	959	1069	865	1010
	Ni	31.8	27.5	24.3	23.0	28.7	26.5	23.8	26.5
	P	5455	4940	3460	3320	5830	4690	3905	4160
	Pb	164	139	107	92	158	139	93	115
	Sr	171	155	128	133	183	155	141	144
	V	37.2	41.9	31.6	34.9	37.0	41.5	29.9	38.5
	Zn	375	289	229	190	338	323	200	243