Entry and Effects of Particle-bound Pollutants in Urban Freshwater Bodies

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List of abbreviations

AChE	acetylcholinesterase
AEF	anthropogenic enrichment factor
CAT	catalase
EU	European Union
EC	effect concentration
IPCC	intergovernmental panel on climate change
MP	micropollutant
MT	metallothionein
OECD	organisation for economic co-operation and development
PAC	powdered activated carbon
PAC_{DCF}	diclofenac loaded powdered activated carbon
PAC_{native}	unloaded, native powdered activated carbon
PAC_{WWTP}	mp-loaded powdered activated carbon from a wwtp
PCP	pentachlorophenol
RRB	runoff retention basin
sp.	species
TOC	total organic carbon
US EPA	United State environmental protection agency
WFD	water framework directive
WWTP	wastewater treatment plant
NRW	North Rheine Westphalia
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyl

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Summary

Efforts to achieve a good status of most water bodies are continuously being hampered by poor sediment quality caused by the input of contaminated particles from anthropogenic sources. Most urban discharges such as stormwater and powdered activated carbon treated wastewater effluent contain particulate matter which harbours significant loads of contaminants. Entry of these particles in surface water bodies may result in chemical pollution of the sediment and adverse effects on benthic organisms. Therefore, the aim of the present thesis was to elucidate the fate of particle-bound contaminants in urban discharge on sediments of receiving freshwaters and the possible adverse effects on benthic biota. To this end, particles from stormwater discharges (separate sewer) and wastewater treatment plants (WWTPs) were investigated for their potential in degrading freshwater sediment and causing toxic effects on sediment-dwelling organisms. For the separate sewer pollution, two different catchments with stormwater basins were investigated (a predominant traffic catchment and a multipurpose land-use catchment). Studies were done with field-collected sediment and amphipod samples. Additionally, exposure experiments were performed in the laboratory with the endobenthic annelid *Lumbriculus variegatus*. Sediment and amphipod samples were analysed for their pollutant load followed by bioaccumulation and toxicity assessments in the laboratory with *L. variegatus*. Furthermore, the consensus-based sediment quality guideline (CBSQG) was applied to predict the toxic potential of sediments affected by stormwater discharges. For particles from WWTP, sediment toxicity experiments with *L. variegatus* were conducted with artificial sediment spiked with micropollutant loaded PAC from a WWTP. The present thesis recorded pollutants in sediments of stormwater receiving streams to occur above the natural background level. There was an increased accumulation of metals in sediment and biota downstream of the traffic stormwater basin outfall in the receiving stream while the multipurpose land-use catchment showed no increasing pattern downstream of the stormwater basin outfall in its studied stream. Bioaccumulation experiments revealed that *L. variegatus* took up stormwater pollutants from the sediment which in turn induced physiological responses in them. Although some particle-bound pollutants could escape retention in the stormwater basins and enter freshwater bodies, the investigated basins could still prevent receiving waters from a significant load of pollutants and the corresponding toxic effects. This was revealed by the high concentrations of pollutants and toxic potentials producing stronger physiological responses and lethal effects in the retention basin samples. For the studied WWTP micropollutant (MP) loaded particles, no effect on growth, reproduction and behaviour were found even with concentrations above environmental levels. However, there was evidence that these particles pose adverse effects on benthic dwellers if ingested after releasing the bound MP. Hence, the findings of this thesis further highlight the importance of upgrading prevention measures for anthropogenic-related particles that transport a considerable load of pollutants in freshwater bodies.

Zusammenfassung

Die Bemühungen, für die meisten Gewässer einen guten Zustand zu erreichen, werden immer wieder durch die schlechte Sedimentqualität dieser Gewässer behindert, die durch den Eintrag von Schadstoffpartikeln aus anthropogenen Quellen verursacht wird. Die meisten urbanen Einleitungen, wie Niederschlagswasserabflüsse und Kläranlagenabflüsse nach Aktivkohlebehandlung, enthalten Partikel, an denen hohe Mengen an Schadstoffen gebunden sind. Der Eintrag von Partikeln in Oberflächengewässer kann zu einer Schadstoffbelastung des Sediments und zu negativen Auswirkungen auf benthische Organismen führen. Daher war das Ziel der vorliegenden Arbeit, den Einfluss von partikelgebundenen Schadstoffen aus urbanen Einleitungen auf die Sedimente der Fließgewässer und die möglichen Auswirkungen auf die benthischen Organismen zu untersuchen. Zu diesem Zweck wurde untersucht, inwieweit Partikel aus Regenwassereinleitungen (Trennsystem) und Kläranlagen die obere Sedimentschicht belasten und toxische Auswirkungen auf sedimentlebende Organismen haben können. Für die Trennsystembelastung wurden zwei verschiedene Einzugsgebiete (ein Verkehrseinzugsgebiet und ein Mehrzweckeinzugsgebiet) mit Regenrückhaltebecken untersucht. Die Untersuchungen wurden mit Sedimenten und Amphipoden aus dem entsprechenden Fließgewässer durchgeführt. Zusätzlich wurden im Labor Expositionsversuche mit dem endobenthischen Ringelwurm *Lumbriculus variegatus* im Sediment durchgeführt. In Sediment- und Amphipodenproben wurden die Schadstoffbelastungen gemessen, gefolgt von Bioakkumulations- und Toxizitätsbewertungen im Labor mit *L. variegatus.* Darüber hinaus wurde die „Consensus-Based Sediment Quality Quideline“ (CBSQG) verwendet, um das Toxizitätspotenzial der Sedimente zu bewerten. Für Partikel aus der Kläranlage wurden Toxizitätsexperimente mit *L. variegatus* in einem Sediment-Wasser-Testsystem mit Pulveraktivkohle (PAK) aus der Kläranlage durchgeführt. Die Ergebnisse der vorliegenden Arbeit zeigen, dass mit dem Eintrag von Regenwasser die Metallkonzentrationen in den Sedimenten der Fließgewässer über dem natürlichen Hintergrundwert liegen. Die

Metallbelastung im Sediment und in den Biota-Proben war stromabwärts der Regenwassereinleitung des Verkehrsgebiets gegenüber der Referenzstelle erhöht. Für das Mehrzweckeinzugsgebiet war stromabwärts der Regenwassereinleitungen kein steigender Trend festzustellen. Bioakkumulationsexperimente zeigten, dass *L. variegatus* Schadstoffe aus dem Sediment aufnahm, was wiederum physiologische Auswirkungen auf die Organismen hatte. Obwohl einige partikelgebundene Schadstoffe dem Rückhalt in den Regenwasserbecken entgehen und damit in die Gewässerkörper gelangten konnten, verhinderten die untersuchten Rückhaltebecken einen erheblichen Schadstoffeintrag in die aufnehmenden Gewässer und entsprechende negative Auswirkungen. Dies wurde durch die hohen Schadstoffbelastung in den Sedimenten aus dem Rückhaltebecken und deren toxisches Potenzial, das sich im Labortest mit *L. variegatus* in Form von starken physiologischen Auswirkungen sowie Letalität zeigte, deutlich. Für die untersuchten Partikel aus Kläranlagen, die mit Mikroschadstoffen (MP) belastet waren, wurden keine Auswirkungen auf Wachstum, Reproduktion und Verhalten festgestellt, auch wenn die Konzentrationen über den in der Umwelt vorkommenden Werten lagen. Jedoch ergaben sich Hinweise darauf, dass diese Partikel negative Auswirkungen auf benthische Organismen haben, wenn sie nach der Freisetzung der gebundener MPs eingenommen werden. Die Ergebnisse dieser Arbeit unterstreichen, wie wichtig es ist, Vorsorgemaßnahmen für den Eintrag von Partikeln und deren gebundene Schadstoffe in urbane Gewässer zu optimieren, um erhöhte Schadstoffbelastungen in Oberflächengewässern zu vermeiden.

1. Introduction

Since the recognition of the danger posed by a variety of anthropogenic activities on our natural resources, environmental legislators have put forward several regulations to protect these resources from losing their core values. One important resource that is eminent to the survival of mankind is water. Growing industrialization, increasing urbanization and population growth are negatively impacting water resources all over the globe. Water according to the European Union (EU) is a “heritage that needs to be protected, defended and treated as such” (WFD, 2000). Typical examples of efforts around the globe to protect and defend the vital functions and services of aquatic ecosystems can be seen in the European Water Framework Directive (WFD, 2000) and the United States of America Clean Water Act (CWA). Both legislations have as the main objective to prevent further degradation of aquatic ecosystems and to restore already deteriorated waters to near natural and stressor-free systems. Achievement of a good status is important in re-establishing the vital functions and services freshwater bodies provide to mankind (WFD, 2000). Despite some significant improvement in the status of EU waters since the implementation of the WFD, growing pressures from climate change, increasing demand for water resources and other anthropogenic related alterations of the natural environment are posing substantial threads to most freshwater bodies.

Anthropogenic activities may result in three types of stressors on aquatic ecosystems: physicochemical, hydromorphological, and land-use-related stressors (Allan, 2004; Göbel et al., 2007; Kaushal and Belt, 2012; Liu et al., 2013). Of particular concern is the latter which has been identified as a major driver of aquatic ecosystem deterioration (Dahm et al., 2013). This is mainly due to the severe chemical burden, altered flow patterns, and hence hydrological changes that land-use related stressors cause on affected natural aquatic environments. Stressors from land-use activities may have negative impacts on aquatic community composition and species diversity due to the input of pollutants with ecotoxicological consequences on organisms as well as the destruction of habitats (Mackintosh et al., 2015; Meyer et al., 1999; Rule et al., 2006; Villeneuve et al., 2015). With two-thirds of the world's population predicted to live in cities by 2050 (UN, 2014), human pressures on urban natural resources owing to intensive use are expected to increase. As a result, land-use-related stressors would rise, putting more pressure on aquatic ecosystems due to exploitation and pollution events. Particularly important are the increasing urban discharges of harmful substances to aquatic environments. Urban discharges are serious threats to the ecological status of surface waters. The pollutant load of urban discharges significantly alters the chemical and hence biological conditions of receiving waters. Entry of pollutants into urban waters is possible via either point sources (industrial discharges, wastewater treatment plants, stormwater sewers) or non-point (diffuse) sources (including precipitation, soil erosion non-sewer stormwater discharges and atmospheric deposition) (Böhm et al., 2001; MKULNV NRW, 2014). To achieve the WFD goal of good ecological status of natural water bodies, measures to regulate and prevent the release of pollutants from their sources are continuously considered by EU member states. Accordingly, construction of sewer systems, development of additional treatment technologies for wastewater treatment plants (WWTPs) and construction of stormwater treatment and retention basins have seen an increase over the past years (Brombach and Dettmar, 2016; Weiß and Brombach, 2007). While these developments have improved the quality of water being discharged into aquatic ecosystems deterioration of urban freshwater bodies due to chemical stressors from anthropogenic pollutants is still a major issue.

The effects of climate change coupled with increasing impervious coverage and increasing use of pharmaceuticals and pesticides are among the many reasons for the continuous deterioration of urban water bodies. For example, due to climate change, heavy and long-lasting precipitation events are becoming more likely resulting in the generation of large volumes of stormwater. Based on the Intergovernmental Panel on Climate Change (IPCC) report (IPCC, 2014), the rate of sewer overflows due to overwhelming stormwater inflow is expected to increase because of the increasing frequencies and intensity of precipitation events. There are two main types of sewer overflows: stormwater overflow in separate sewer and combined sewer overflow (CSO) in combined sewer systems. The former results in the discharge of stormwater into receiving waters. On the other hand, CSO results in the input of a mixture of stormwater and untreated wastewater to receiving water. Both scenarios would result in the pollution of receiving water with foreign substances (Björklund et al., 2018; Burkhardt, 2018). For example, CSO and stormwater basin overflows in separate sewers were identified to release priority pollutants (metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and biocides) and emerging micropollutants to receiving waters (Hnaťuková, 2011; Launay et al., 2016; Petrie, 2021). In addition to sewer overflows, increasing direct inflow of surface runoff due to sealed surfaces and limited time for infiltration on porous surfaces is of equal concern (MKULNV NRW, 2014).

Stormwater-related pollution of the aquatic environment is a major cause of the deterioration of urban surface waters. For example, stormwater-related discharges alone are responsible for about 86 – 98% of the priority stormwater metals Cd, Cu, Pb and Zn emitted into freshwater bodies in North-Rhine Westphalia in Germany (MKULNV, 2014). The cleaning processes of urban paved surfaces by stormwater results in the transportation of a sheer number of pollutants. Contaminants in stormwater constitute metals, organic compounds (including emerging micropollutants), suspended solids, organic matter, pathogens and nutrients (Barbosa et al., 2012; Müller et al., 2020). They are released into the environment (due to wear of constructions, oil spills, illicit disposal of wastes and other sources) and are washed off by stormwater (De Toffol et al., 2007; Gasperi et al., 2012; Müller et al., 2019 and 2020). Priority pollutants in stormwater include metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and biocides. They are present in high concentrations in residential and traffic runoffs and may cause ecotoxicological effects on receiving water biota (Eriksson et al., 2007; Zgheib et al., 2012). Although second to residential runoff in terms of volume, traffic runoff contains high amounts of metals such as Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn), as well as PAHs emitted by cars and road surfaces due to wears, fluid leakages, exhaust emission and abrasion (Davis and Birch, 2010; Helmreich et al., 2010; Huber and Helmreich, 2016; Markiewicz et al., 2017; Müller et al., 2020, Ruchter and Sures 2015, Schertzinger et al., 2018). The concentration of traffic-related metals in runoffs usually exceed environmental quality thresholds. For example, Helmreich et al. (2010) recorded road runoff concentrations of traffic-related Cu, Ni, Pb and Zn to significantly exceed environmental quality values. Similarly, traffic-related metals and PAH were observed to be above environmental quality guidelines in road runoff in Sweden (Järlskog et al., 2021).

Pollutants in urban discharges exist predominantly in the particle-bound state. Owing to surface adsorption, fine particles in stormwater are preferentially enriched with metals and organic compounds (Beasley and Kneale, 2002; Loganathan et al., 2013). Following precipitation, it was observed that the highest concentrations of pollutants in stormwater are first recorded in the particle-bound fraction while the peaks for the water-soluble pollutants occurred at a later time (Launay et al., 2013). Zgheib et al. (2012) concluded that particle-bound pollutants account for a significant portion of pollutants entering freshwater systems through stormwater discharges. Concentrations of priority pollutants in stormwater particles were measured to range between 0.02–11, 11–135, 16–6000, 7–309, 9–675 and 51.3–4670 mg/kg for Cd, Cr, Cu, Ni, Pb and Zn respectively, PAH between 0.2 – 80 mg/kg and PCB between 0.0004 – 0.7 mg/kg (Jartun et al., 2008a). Similar values for metals, PAH and PCB were recorded in stormwater sediment in Paris (Zgheib et al., 2012). Depending on the land-use characteristics, particulate pollutants in stormwater may originate from traffic areas and parking lots (wears of tyres, brake parts and road surfaces, exhaust emission, fluid leakage on road dust particles etc.), residential origin (wears of roof and buildings, pet waste, pesticides on soil particles etc.) or agricultural soil erosion (Jartun et al., 2008a and b; Joshi and Balasubramanian, 2010; Keuken et al., 2010; Müller et al., 2019 and 2020; Winters et al., 2015). A review by Grant et al. (2003) elaborated on the important role particles plays in the fate, transport, and toxicity of pollutants in stormwater. Transport of pollutants in the environment is predominantly in the particle-bound state, and stormwater is primarily responsible for the mobilization to surface waters (Huber et al., 2016; Zgheib et al., 2012).

Another source of particle-bound pollutants to freshwater bodies, which was observed in recent years, are WWTPs using powder activated carbon (PAC) to remove micropollutants (MPs). Micropollutants are ubiquitous pollutants that are present in the environment in concentrations between µg/L to ng/L (Athing et al., 2018). MPs consist of substances predominantly of anthropogenic origin, including pesticides, pharmaceuticals, personal care products and transformation products (Schwarzenbach et al., 2006). PAC has been shown by many studies to significantly reduce MPs in WWTP effluents (Boehler et al., 2012; Mailler et al., 2015; Margot et al., 2013). However, the small particle size of PAC, which has the advantage of providing a large surface area for MP binding, makes it challenging for the complete recovery of PAC following application in the treatment of MP. Consequently, PAC loaded with MPs is released to freshwater with WWTPs effluent (Krahnstöver et al., 2016). In receiving water the PAC will most likely settle on the sediment compartment and may pose risk to sediment dwellers.

In the aquatic environment, pollutants bound to particles may dissociate and dissolve in the aqueous phase or remain bound. The fate depends predominantly on the physico-chemical properties of the water and the binding forces between pollutants and particles. In the bound state, pollutants may settle, becoming an integral part of the sediment. Beasley and Kneale (2002) observed that several potential toxic substances barely detected in the water column of freshwater bodies were found to accumulate in high amounts in sediments. Accordingly, sediment pollution of surface waters impacted by stormwater discharges have been reported in previous studies (Gasperi et al., 2009; Iannuzzi et al., 1997; Ruchter and Sures, 2015; Schertzinger et al., 2019a; Schertzinger et al., 2018). For example, Ruchter et al. (2015) recorded accumulation of traffic emitted metals (Cd, Cr, Cu, Ni, Pb, Pt, and Zn) in sediment downstream of the road runoff's outfall in the river Alb in Germany. Similar studies by Iannuzzi et al. (1997) and Schertzinger et al. (2018 and 2019a) recorded accumulation of priority pollutants (metals and organic compounds) in sediment downstream of CSOs. Concentrations of priority pollutants were also found to be above quality guideline values in settleable particles of a heavily urbanized transect of the Seine River (Gasperi et al., 2009). As a pollutant hotspot, sediment can also be a source of contamination in the water phase. This is accomplished by desorption and remobilization activities releasing contaminants to the water phase. Despite the role of sediments and the consequences of sediment pollution to the entire aquatic ecosystem, the WFD classification of the chemical status of water bodies is limited to compliance with environmental quality standards (EQS) of priority pollutants in the water matrix.

Sediments are important parts of the aquatic environment as they are providing habitat, feeding, spawning and rearing areas for many aquatic organisms. Hence the accumulation of particle-bound contaminants on streambeds can have detrimental effects on bio-integrity in affected waters. Pollutants in benthic habitats can bioaccumulate in benthic organisms (Brunson et al., 1998; Ruchter and Sures, 2015; Schertzinger et al., 2018; Zimmermann and Sures, 2004). For example, tissue accumulation of traffic-related metals was recorded in clams from stormwater contaminated sediment (Ruchter and Sures 2015). Accordingly, Schertzinger et al. (2018) observed pollutant accumulation in amphipods at different locations downstream of the CSO's outfall. Following bioaccumulation, pollutants may interact with cellular components inducing toxic effects, be detoxified by enzymes, be stored or excreted by the organisms (Gheorghe et al., 2017). Ecotoxicological effects resulting from pollutant uptake from contaminated sediment may range from molecular, cellular, organismic up to population, community and ecosystem level. Chronic exposures of organisms to contaminated sediment have been reported to have effects on standard ecotoxicological endpoints including development, survival, behaviour, growth, and reproduction on sediment-dwelling organisms (Feiler et al., 2013; Gheorghe et al., 2017; Tuikka et al., 2011; Vandegehuchte et al., 2013; Wolfram et al., 2012).

Biomarker response to toxic substances has been well established as important endpoints for exposure and effect studies of environmental pollutants. A biomarker is considered any biochemical, cellular or physiological variation that can be measured in tissue or body fluid samples that provides evidence of exposure to and/or effects of chemical pollutants (Depledge 1994). Frequently, biochemical endpoints in toxicological tests are biomarker protein synthesis (including enzymes) as well as inhibition of the activity of important enzymes. Biomarkers of interest include the protective proteins such as metallothioneins (MTs), enzymes involved in oxidative stress (e.g., catalase) or xenobiotic metabolism (e.g., glutathione-S-transferase) and the neurotransmitter hydrolyzing enzyme, acetylcholinesterase (Gheorghe et al., 2017; Huggett, 1992). For example, the antioxidant enzyme, catalase (CAT) is often produced in reactions of xenobiotic-mediated oxidative stress that results in the production of the reactive oxygen species hydrogen peroxide (H_2O_2). CAT facilitate the removal of H_2O_2 by decomposing it into water and oxygen. Contrary to CAT, inhibition of the neurotransmitter enzyme acetylcholinesterase (AChE) is an important indicator of xenobiotic exposure. AChE plays an important regulatory role in neurotransmission by breaking down acetylcholine. Many chemicals have irreversible inhibitory effects on AChE which have been explored for exposure studies of environmental pollutants such as pesticides, PCBs, PAHs and flame retardants (Fu et al., 2018, Kristoff et al., 2010). Last but not the least, the cysteine-rich metal-binding protein, MT known to play an important role in the regulation of essential Zn and Cu and detoxification mechanism of harmful metals is another important biomarker used for exposure studies of environmental pollutants. Binding of metals b the protein is possible through the cysteinyl residues which serve as ligands for metal chelating. Increased synthesis of MTs in living organisms is commonly induced by metal ions such as Cd, Cu, Zn, Hg, Co, Ni, Bi, and Ag. (Le et al., 2016, Hamilton and Mehrle, 1986; Samuel et al., 2021, Frank et al., 2013).

Given the importance of particle-bound toxic substances in the ecological and chemical status of surface waters and the pollutant mobilization characteristics of stormwater, there is still only a low number of studies relating stormwater discharges in urban catchments to pollutant concentrations in sediments of receiving waters and corresponding ecotoxicological consequences. In the case of CSO, previous studies by Schertzinger et al. (2018 and 2019a) gave a clear picture of the contaminant levels in sediments of affected streams including possible ecotoxicological effects on sediment-dwelling organisms. Even after performing an intensive literature search, no comprehensive study on the downstream impacts of stormwater retention basin discharges on sediment and sediment-dwelling organisms could be found. Additionally, there is meagre research on the possible adverse effects of PAC-bound micropollutant released by WWTP on typical sediment-dwelling organisms. It was already reported that activated carbon in sediment negatively affects benthic species by affecting their ingestion rate, lipid content, and growth as well as by destroying the benthic community structure, (Abel and Akkanen, 2018; Jonker et al., 2009; Samuelsson et al., 2017). However, the effects of PAC loaded with MP from WWTPs on sediment organisms are unknown.

There are several benthic organisms recommended for ecotoxicological studies of contaminated sediment, but the endobenthic oligochaete *Lumbriculus variegatus* is recognized to be one of the most suitable since its habitat preference, availability, biomass, toxicant sensitivity amongst others offer the possibility for assessment of all possible ecotoxicological endpoints (EPA, 2000; OECD, 2007). As an endobenthic organism, *L. variegatus* is exposed to toxicants in all matrixes including water column, pore water and sediment particles and can take up toxicants via all possible routes of exposure (Phipps et al., 1993).

Hence, this work aimed at exploring the entry and effects of the particle-bound contaminants through separate sewers and WWTP on sediment and sediment dwelling organisms of receiving urban freshwater bodies. Accordingly, the present study consists of three chapters:

I) The first chapter addresses the impact of highway runoffs on traffic-related metals in sediment and benthic biota of receiving water including possible ecotoxicological effects. The main objectives here were:

- to assess the accumulation pattern of traffic-related metals in sediment and benthic species downstream of a traffic stormwater basin outfall,
- to determine possible adverse effects of the traffic-related metals on the typical sediment dweller, *L. variegatus*.

To this end, sediments and amphipods were collected from a stream with an outfall of a stormwater retention basin. The basin receives runoffs from a highway road and overflows into the stream. The sampling protocol described in Schertzinger et al. (2018) was applied to determine the distribution of traffic-related metals in sediments downstream of the stormwater outfall. The concentrations of the traffic-related metals (Cd, Co, Cu, Mn, Mo, Ni, Pb, and Zn) were quantified in the field-collected sediment and biota samples. Ecotoxicity experiments with *L. variegatus* exposed to sediment samples from the studied stream were performed in the laboratory following the EPA guideline (EPA, 2000). Bioaccumulation of traffic-related metals and effects (on reproduction, growth and metallothionein induction) of the sediment samples on *L. variegatus* were assessed.

II) The second chapter aimed at assessing the sediment metal and organic pollutant distribution downstream of two stormwater retention basins' outfalls from a multipurpose land-use catchment. For metals (Ag, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb and Zn), a quantification analysis was done to determine their accumulation in sediment from the retention basins and downstream of the basins' outfalls. Similar to metals, semi-quantitative analysis was performed for different organic pollutant groups (polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), flame retardants and biocides) to determine their distribution in the sediment samples. Sediment toxicity experiments with *L. variegatus* were carried out to assess the possible adverse effect of the sediment contaminant on standardized endpoints (reproduction and growth) of the EPA guideline (EPA 2000) and non-standardized, but more sensitive physiological endpoints (catalase activity, acetylcholinesterase activity and metallothionein content).

III) The third chapter explores the possible ecotoxicological effects of particle-bound pollutants emitted by WWTPs using PAC for the removal of micropollutants. The rationale behind this experiment was based on the possibility that PAC released from WWTPs may settle to sediments of receiving water and may affect benthic dwellers. For this chapter, artificial sediment was formulated based on OECD 225 protocol (OECD, 2007) and spiked with different PAC treatments (MP-loaded PAC from WWTP, diclofenac-loaded PAC and unloaded PAC). Individuals of *L. variegatus* were then exposed to the spiked sediment and the endpoints reproduction and growth were evaluated for possible adverse effects.

The outcome of the three chapters is an important contribution to better assess (1) the impact of different urban emitted particle-bound pollutants on the contamination status of sediments of receiving waters and (2) the adverse effect this may have on typical sediment-dwelling organisms.

2 Chapter 1: Ecotoxicological effects of traffic-related metal sediment pollution in *Lumbriculus variegatus* and *Gammarus sp.*

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Ecotoxicological effects of traffic-related metal sediment pollution in *Lumbriculus variegatus* and *Gammarus sp.*[☆]

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Toxicity

ABSTRACT

To reduce direct discharges of surface runoff to receiving waters, separate sewer systems have been implemented, with runoff retention basins (RRB) for pollutant pretreatment by sedimentation and infiltration. However, due to frequent and intense precipitation events, most RRBs are overwhelmed by runoff resulting in overflow into the receiving freshwater bodies. Hence, the present study evaluates the impact of traffic-related runoff overflow on metal concentrations in sediment and *Gammarus* sp. Downstream of the RRB outfall in the receiving stream. Samples were collected from the RRB, upstream (reference site) and at different distances downstream from the RRB outfall in the stream. The samples were analyzed for the presence and distribution of metals using ICP-MS. Furthermore, ecotoxicological effects of the overflow on benthic species were assessed using *Lumbriculus variegatus* exposed to the field sediments. Our findings reveal that overflow of the RRB results in elevated traffic-related metal concentrations in sediment and biota of the stream. Within the first 50 m downstream increased sediment metal concentrations were found. The gammarids downstream of the RRB outfall showed an increased accumulation of several metals. Similarly, the metals were found to be taken up by the endobenthic *L. variegatus* under laboratory conditions and the bioaccumulation pattern was related to the sediment concentrations. Bioaccumulation by both organisms is an indication that overflow of the RRB also leads to uptake of increased element amounts in organisms downstream. Laboratory-based studies addressing standard toxicity endpoints showed no clear toxic effects on growth and reproduction. However, elevated levels of metallothioneins were measured in the annelids during the test period. This indicates a physiological response induced by increased metal concentrations due to RRB overflow. Hence, the results of this study show that discharges by the RRB increase the metal concentration in the receiving stream with the possibility of adverse effects on organisms.

1. Introduction

Efforts to achieve a good ecological status in urban rivers are challenged by a still increasing discharge of untreated water mainly resulting from stormwater runoff and sewer overflows into freshwater bodies (Arnold and Gibbons 1996; MKULNV 2014; Tillinghast 2011). In recent years, increasing urbanization coupled with climate change scenarios have led to a huge volume of stormwater in rainwater channels, hence increasing their discharges to freshwater bodies. Rainwater runoff from paved urban surfaces like roads, parking lots and rooftops is a major contributor of pollutants (organic compounds, metals, pathogens and particulate matters) to aquatic ecosystems (Barbosa et al., 2012). Amongst the variety of pollutants in stormwater, toxic metals are of particular concern. Studies have shown that runoff from urban catchment surfaces significantly contributes to metal loads in the aquatic environment (Brombach et al., 2005; Fuchs et al., 2004; Huber et al., 2016; MKULNV 2014). Most important are particle-bound metals, which are transported via surface runoff to receiving water bodies (Davis and Birch 2010; Helmreich et al., 2010; Murphy et al., 2015; Zgheib et al., 2012). Wear of car parts (brake parts, tires, lubricants, paints), roofs, road construction materials and automobile exhaust emissions are the main contributors of particulate metal pollutants in surface runoff (Huber and Helmreich 2016; Keuken et al., 2010; Ruchter and Sures 2015; Winters et al., 2015).

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Particle-bound pollutants in stormwater will accumulate in the sediment of water bodies, become biologically available to sediment-dwelling organisms and may result in adverse effects on exposed benthic organisms (de Castro-Catala et al., 2016; Marshall et al., 2010; Schertzinger et al., 2019). For example, in a study of traffic-related pollution in the river Alb, discharges of road runoff contributed significantly to metal content in sediment and bivalve (*Corbicula* sp.) samples downstream of the stormwater inlet (Ruchter and Sures 2015). A similar study has also reported on sediment metal pollution by stormwater-related discharges and uptake by aquatic organisms (Schertzinger et al., 2018). However, bioaccumulation studies of particle-bound metals in natural samples are often reported for organisms directly exposed to pollutants in the water column such as bivalves. For these organisms, bioaccumulation is strongly influenced by factors that govern biological availability e.g. pH, oxygen concentration, conductivity, temperature (De Jonge et al., 2012; Superville et al., 2014). Unlike organisms in the water column, uptake of particle-bound pollutants by sediment-dwelling organisms is not that much influenced by changing water parameters. Sediment-dwelling organisms are exposed via all possible uptake routes including ingestion of sediment particles. Limited studies have reported comprehensively on the uptake and corresponding adverse effects of metal pollutants on sediment-dwelling organisms in naturally contaminated sediment samples (De Jonge et al., 2012).

Therefore, the present study was aimed at determining metal contamination of freshwater sediments along a transect following the discharge of surface runoff from a separate sewer system. Metal concentrations in sediments were compared to metal accumulation in amphipods and were tested for possible toxic effects using *Lumbriculus variegatus*, a sediment-dwelling annelid. The results of this study will exemplify sediment contamination due to traffic runoff and indicate its ecotoxicological implications on sediment-dwelling organisms.

2. Materials and methods

2.1. Sampling site

Samples were collected from the stream “Deininghauser Bach” in the city of Castrop-Rauxel, North-Rhine Westphalia (NRW), Germany (Fig. 1). The small lowland water body with loess and loam-dominated sediment layer (stream type 18 of the German stream classification index) is a restored tributary in the Emscher catchment and has the record of the longest and most comprehensive restoration stream in Europe (Schanze et al., 2004). It is approximately 9.5 km long and drains a catchment area of 17.2 km^2 in Castrop-Rauxel. This includes rainwater runoff from streets, the highway A42 and a combined sewer overflow. The studied stream stretch is connected to a retention basin that drains rainwater runoff from the heavily frequented highway A42 that runs from the city of Kamp-Linfort to Dortmund. During events of heavy or persistent rainfall, the basin gets overwhelmed with rainwater resulting in the direct flow of surface runoff into the Deininghauser Bach. In order to study the influence of the highway runoff on sediment and biota metal contents, transects were marked in the retention basin, upstream and downstream of the retention basin outfall into the stream for sampling (Fig. 1). The upstream transect (20 m from the outfall) was considered as a reference sampling site. Downstream of the outfall, sediment samples were collected at a distance of 1, 5, 10, 20, 50 and 100 m, respectively. In the retention basin, samples were taken at three different points where sedimentation is highest.

2.2. Sampling and preparation of sediments and amphipods

Sampling was done in July for sediment and August for amphipods. We sampled superficial (< 5 cm) sediment at three different points for each transect using a scoop. Samples were collected in polyethylene bags and stored at −20 °C for subsequent freeze-drying. To avoid large deviation due to site and sample heterogeneity, freeze-dried subsamples for each transect were properly homogenized by mixing in a porcelain bowl. The homogenous samples were divided into two parts: one for metal analyses and the other for bioaccumulation and effect experiments with *L. variegatus*. The samples for metal analyses were sieved into the grain size fractions; < 0.063 mm, 0.063−2 mm and > 2 mm and their weights recorded. On the other hand, samples for the bioaccumulation and effect experiment were sieved to < 2 mm. The > 2 mm fractions consisting mostly of gravels and large organic matter were discarded. Samples were stored at room temperature in glass beakers sealed with aluminium foil for subsequent uses.

Adult amphipods, i.e. *Gammarus pulex* and *G. fossarum*, were sampled in the retention basin, upstream (reference site) and downstream (approximately 70 m) of the runoff outfall. Due to the relatively low number of specimen and given their similarity in metal accumulation and seasonal variation (Lebrun et al., 2015) both species were combined to build a sample of sufficient weight (see below). Gammarids were not found in the first 50 m downstream of the runoff outfall. The samples were properly rinsed in reconstituted water in the lab and stored at −80 °C for subsequent freeze-drying and determination of metal content.

2.3. Exposure of L. variegatus to sediment samples

To study the effects of sediment-bound metals on benthic dwellers, an exposure experiment with the endobenthic annelid *L. variegatus* was conducted. The annelids were exposed to sediment samples from the different sampling sites of the Deininghauser stream to assess the uptake of toxic metals by sediment-dwelling organisms and to test for effects on standard toxicity endpoints including the induction of metallothioneins. For this purpose, freeze-dried and properly stored sediment samples (<2 mm particle size) were used.

2.3.1. Test organisms

The endobenthic annelids *L. variegatus* used in the toxicity experiment were cultured in the climatic chamber at the department of Aquatic Ecology, University of Duisburg-Essen, Germany. The worms were grown on prewashed quartz sand (Baumit GmbH, Germany) in 15 L rectangular glass chambers containing artificial fresh water according to the OECD 225 guideline (OECD 2007). The temperature of the climate chamber was maintained at 20 ± 2 °C and the light-dark circle was set at 16:8 h. Cultures were constantly aerated and worms were fed twice a week with Tetramin (Tetra, GmbH). In order to assess the suitability of the annelids for toxicity studies, their sensitivity to toxic substances was confirmed as recommended in OECD 225 using Pentachlorophenol (OECD 2007; Hickey and Martin, 1995).

2.3.2. Exposure experiment

Exposure of *L. variegatus* to sediment was done according to the EPA guideline (EPA 2000). First, a validation experiment was performed to determine whether the sediment samples are suitable for chronic exposure studies with *L. variegatus*. Adult worms of similar sizes were collected from the culture and conditioned in clean culture medium without food supply for 2 days followed by 24 h in reconstituted water. During this period, 100 ± 5 g sediment samples from different sampling points were weighed in 300 mL

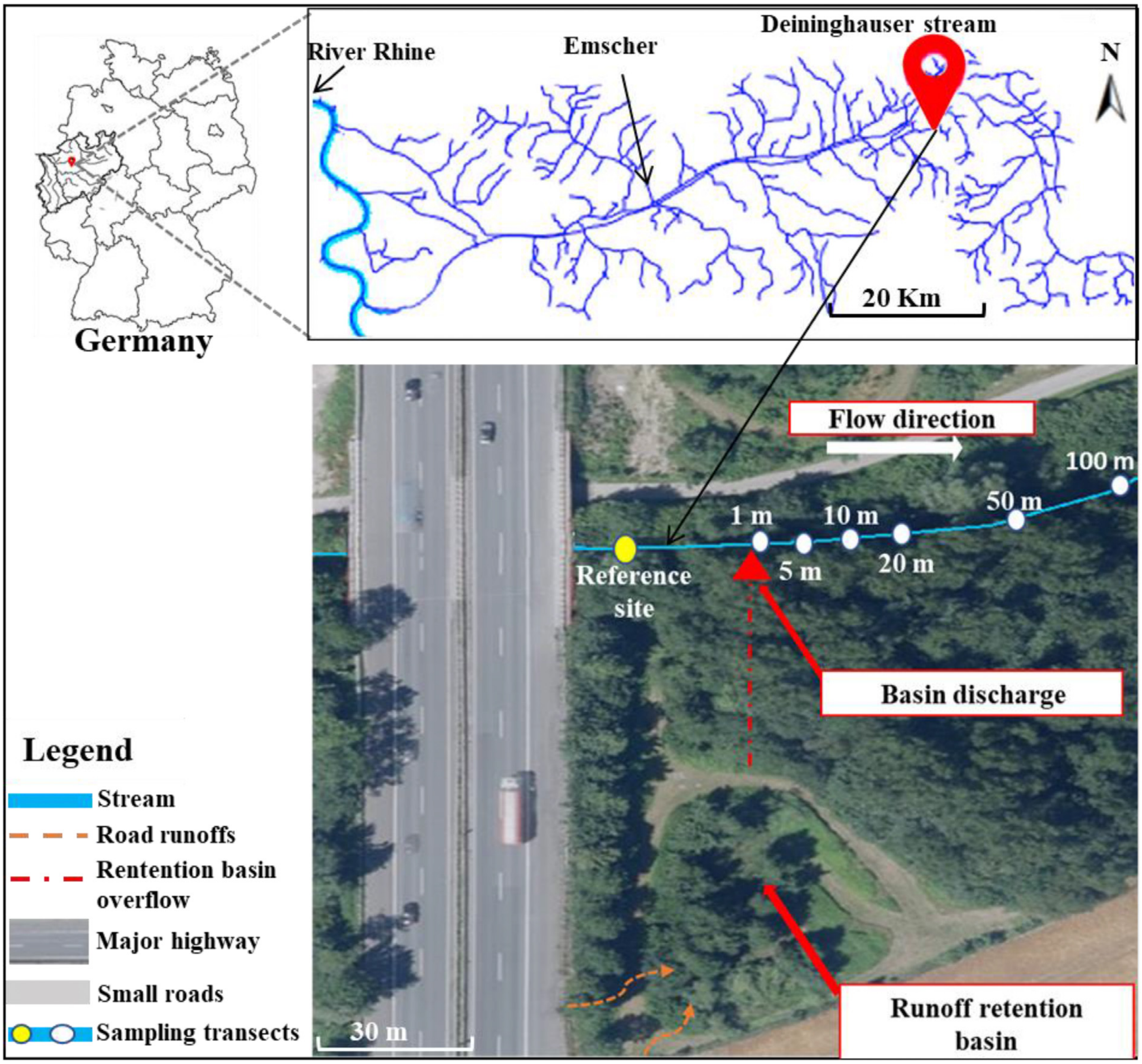


Fig. 1. Sampling location of the Deininghauser stream in the Emscher catchment, North-Rhine Westphalia, Germany.

beakers. Reconstituted water was carefully added into the beakers and the water level was adjusted to three times the sediment level. Water parameters were recorded, the setup aerated with the help of Pasteur pipettes and left to equilibrate for three days. On the last day of equilibration, water parameters were measured and 10 adult worms were added to each beaker. The experiment was run for 10 days during which the worms were being observed for their burrowing activity. At the end of the 10 days exposure, the worms were extracted by first carefully disturbing the sediment surface to bring the burrowed worms on the surface. This was followed by removal of the worms using a plastic Pasteur pipette with enlarged tip. To be sure that all the organisms were extracted, the sediment was rinsed with reconstituted water through a 100 μm sieve to recover worms that could be removed directly from the exposure beaker. Following extraction, the organisms were rinsed, counted and stored at −80 °C for further analysis.

The toxicity experiment was set up similarly to the validation experiment. The toxicity experiment was run for 28 days during which water parameters were recorded weekly and evaporated overlaying water was refilled with deionized water to prevent salt concentration. After 28 days worms were carefully extracted and analyzed for growth and reproduction. The latter was assessed by determining the increase in the number of worms after 28 days in the sediment while growth was determined by the change in dry biomass. For growth determination, worms were extracted from the sediment as described above and allowed for 2 h in clean artificial freshwater to excrete the intestinal content. This was followed by rinsing and freezing of the samples at −80 °C for subsequent lyophilization (Heto PowerDry LL3000, Thermo Fisher Scientific, USA) and determination of dry biomass (Labor AEG-220, Shimadzu, Japan). Additionally, metal bioaccumulation by the worms was determined. Samples for metal analysis were transferred into clean artificial freshwater for approximately 2 h to clear the gut for ingested sediment particles. Following gut depuration, samples were stored in Eppendorf tubes at −80 °C for subsequent processing for metal analysis.

2.3.3. *Metallothionein analysis*

The metallothionein (MT) induction by *L. variegatus* exposed to sediment samples was determined using the silver saturation method (Scheuhammer and Cherian 1986) with slight modification to compensate for the low sample masses (Frank et al., 2013). At least 10 worms per treatment were used. The fresh weight was determined followed by homogenization on ice in 150 µL of 0.25 M sucrose solution using a macro-pestle. The homogenate was saturated with 250 µL of 20 mg/L Ag solution (Bernd Kraft GmbH) and incubated for 20 min at room temperature to give enough time for Ag to bind MT. Excess Ag was removed by addition of 50 µL sheep red blood cells hemolysate (Sigma Aldrich) followed by heat treatment (100 °C for 10 min; HCL, DITABIS) and centrifugation (5000×g for 10 min; Centrifuge 5810R, Eppendorf AG). The process from the addition of hemolysate, to centrifugation, was repeated 3 times to ensure complete removal of unbound Ag. The final centrifugation was done at 16000×g for 15 min. The resulting supernatant was analyzed for Ag-MT by Graphite Furnace Atomic Absorption spectrometry (GF-AAS; AAnalyst − 600, PerkinElmer; see also Frank et al., 2013).

2.4. Metal extraction and analysis

Analysis of metals in sediment and biota samples was done following a suitable extraction method on three replicate samples per sampling sites. Sieved freeze-dried sediment samples (500 ± 10 mg) for each grain size fraction (0.063 mm and 0.063−2 mm) were digested in a microwave (MARS 5, CEM GmbH). The digestion was performed as described by Schertzinger et al. (2018) in a 1:3 aqua regia solution (2 mL 65% HNO_3 Sigma Aldrich Suprapur and 6 mL 35% HCl Sigma Aldrich Suprapur) in XP1500 plus vessels. Samples were heated to a maximum temperature of 160 °C, for 15 min and held for 20 min at this temperature. Following digestion, the contents in the vessels were allowed to cool to room temperature and the suspension was filtered (5 µm; Qualitative filter paper, 413, VWR) into 50 mL volumetric flasks. The vessels were rinsed repeatedly to collect all residual droplets into the 50 mL flask and the flask was brought to volume with Ultrapure water (Milli-Q, MiliPore).

Field collected gammarids (100 ± 10 mg; dry weight) were digested in 4 mL of 65% HNO_3 (Sigma Aldrich Suprapur) in a microwave oven (MARS 6, CEM GmbH). The samples were heated stepwise to 80 and 120 °C with a hold time of 2 min at each temperature, and finally to a maximum temperature of 180 °C for 30 min. The digested samples were transferred into a 5 mL volumetric flask and filled to the mark with ultrapure water. *L. variegatus* samples (14 ± 3 mg; dry weight) were digested in a mixture of 2.5 mL H_2O_2 (Merck KGaA Suprapur) and 1.3 mL 65% HNO_3 (Sigma Aldrich Suprapur) with the same device and program used for the gammarid samples. The resulting solutions in volumetric flasks were transferred into 15 mL falcon tubes and stored in the dark at room temperature for subsequent metal analysis. The concentrations of Cd, Co, Cu, Mn, Mo, Ni, Pb, and Zn were analyzed using inductively coupled mass spectrometry (PerkinElmer Sciex Elan 6100 ICP-MS System) after suitable dilution and a calibration procedure as described in Erasmus et al. (2020). Quantification of the metals of interest was done using the following stable mass lines: ^{111}Cd, ^{59}Co, ^{65}Cu, ^{55}Mn, ^{98}Mo, ^{60}Ni, ^{206}Pb, and ^{66}Zn. Estimation of the total metal concentration in the <2 mm particle size sediment fraction was made possible using equation (Eq.1).

Certified standard reference materials (SRM), SdAR-1 (modified river sediment, GeoPT31, International Association of Geoanalysts proficiency testing) and DOLT-5 (Dogfish liver Certified Reference Material for Trace Metals and other constituents, National Research Council, Canada) were used to validate the analytical procedure for sediment and biota samples respectively. Validation procedures were performed as described in Erasmus et al. (2020). Replicates of standard reference materials were digested following the same protocol for the sediment and biota samples from the investigated stream. The digested samples were diluted accordingly and metal concentrations were measured. The accuracy (recovery rate) of the analytical method was determined by dividing the average measured concentrations of a metal by its certified reference value for a given sample type. The results were expressed in percentages. The limit of detection and quantification (LOD and LOQ) were derived from blank digestion samples in accordance with DIN 32645 (DIN, 2008). The LOD and LOQ was taken as 3- and 9-times the standard deviation of element concentrations in the blank acid digestions, respectively.

$$C(<2\ mm) = \frac{(C{*}M)_{(<63\mu m)} + (C{*}M)_{(63\mu m \leq x < 2\ mm)}}{M_{(<63\mu m)} + M_{(63\mu m \leq x < 2\ mm)}} \tag{1}$$

where C = concentration (mg/kg); M = mass (mg).

2.5. Data and statistical analyses

Microsoft Excel (2019) and GraphPad Prism 5.0 (GraphPad Softwares) were used for data analyses and statistics. Metal concentrations in sediment and biota samples downstream of the rain runoff inlet were compared to their respective concentrations at the reference site (upstream). The relative values to reference site concentration was calculated to determine the impact of runoff inflow on the metal content in sediment and organisms downstream. The term runoff influencing factor (RIF) was coined for the relative concentration and it was calculated for all metals per sampling site using equation Eq.(2). The anthropogenic enrichment factors (AEF) were also estimated for metals at all sampling sites by dividing the measured concentration by the geogenic concentration of the given metal (Eq.(3)). This was only possible for the metals Cd, Cu, Pb and Zn with available background concentrations for the catchment area (Schertzinger et al., 2018). An AEF >1 indicates a human influence on the given metal in the sampling site. Kruskal-Wallis nonparametric statistics followed by Dunn's test post-hoc test were used to compare and test for significant differences between treatments for toxicological endpoints. Spearman correlation analysis was used for evaluation of the relationship between parameters.

$$RIF = \frac{Metal\ concentration\ at\ sampling\ site(mg/kg)}{Metal\ concentration\ in\ referencce\ site(mg/kg)} \tag{2}$$

$$AEF = \frac{Measured\,concentration\,in\,sediment(mg/kg)}{Background\,concentration\,of\,metal\,in\,the\,Emscher(mg/kg)} \tag{3}$$

3. Results

3.1. Metal concentrations in sediment, amphipods and L. variegatus

3.1.1. Sediment metal concentrations

Quality control data for the analytical procedures of sediment and tissue samples are summarized in Table S1 (supplementary datasheet). Limits of quantification (LOQ) were based on the blank method. The recovery rates range between 95% (Mo) to 115% (Cu) for sediment and 100% (Ni) to 120% (Cu) for the biota samples.

Total metal concentrations (mg/kg) in the <2 mm sediment fractions were determined for all sampling points (Table 1). The measured concentrations of the metals considered in this study ranged between 0.20 and 833 mg/kg sediment given the different

Table 1
Metal concentrations (mg/kg) in sediment and biota samples (field-collected amphipods and lab exposed *L. variegatus*). Mean ± SD, n = 3.

Sample type	Sample treatment	Cd	Co	Cu	Mn	Mo	Ni	Pb	Zn
Sediment	Ref.	0.27 ± 0.02	2.8 ± 0.2	7 ± 0	453 ± 55	0.24 ± 0.00	10.8 ± 0.3	10 ± 1	57 ± 2
	RRB	0.68 ± 0.02	5.3 ± 0.1	81 ± 1	440 ± 12	1.97 ± 0.04	24.3 ± 0.1	49 ± 2	364 ± 1
	1 m	0.33 ± 0.03	3.3 ± 0.3	17 ± 4	576 ± 11	0.46 ± 0.09	13.6 ± 1.2	14 ± 2	103 ± 16
	5 m	0.48 ± 0.04	4.3 ± 0.0	23 ± 0	833 ± 148	0.65 ± 0.07	18.3 ± 1.4	19 ± 1	137 ± 4
	10 m	0.45 ± 0.01	3.6 ± 1.2	25 ± 2	688 ± 131	0.69 ± 0.00	15.0 ± 4.2	18 ± 1	140 ± 9
	20 m	0.29 ± 0.05	3.3 ± 0.3	19 ± 2	402 ± 102	0.55 ± 0.07	14.0 ± 1.6	15 ± 2	92 ± 15
	50 m	0.38 ± 0.01	3.8 ± 0.4	23 ± 4	546 ± 138	0.64 ± 0.10	16.2 ± 1.4	18 ± 2	129 ± 18
	100 m	0.20 ± 0.01	2.5 ± 0.1	9 ± 1	254 ± 22	0.28 ± 0.05	9.9 ± 1.5	10 ± 1	64 ± 7
Gammarids	Ref.	0.20 ± 0.02	4.5 ± 0.9	62 ± 5	523.8 ± 72	0.60 ± 0.03	5.7 ± 0.2	0.5 ± 0.1	73 ± 6
	RRB	0.52 ± 0.07	5.8 ± 0.3	101 ± 9	923 ± 285	0.67 ± 0.09	8.8 ± 0.6	2.4 ± 0.5	116 ± 9
	Downstream	0.37 ± 0.03	4.4 ± 0.9	70 ± 7	630 ± 180	0.45 ± 0.06	5.3 ± 0.6	1.0 ± 0.3	82 ± 9
L. variegatus	Before exposure	0.17 ± 0.00	< LOQ	64 ± 0	325 ± 17	0.07 ± 0.01	0.3 ± 0.0	0.9 ± 0.8	73 ± 5
	Ref.	0.59 ± 0.09	6.1 ± 3.4	181 ± 38	2574 ± 641	0.47 ± 0.10	1.2 ± 0.3	3.1 ± 0.8	208 ± 43
	RRB	1.15 ± 0.14	7.4 ± 0.3	248 ± 18	3581	0.80 ± 0.09	5.8 ± 0.7	10.0 ± 0.5	284 ± 22
	1 m	0.75 ± 0.24	5.6 ± 1.3	186 ± 47	3092 ± 559	0.54 ± 0.16	1.9 ± 0.5	4.2 ± 0.9	216 ± 56
	5 m	0.49 ± 0.19	3.7 ± 0.7	144 ± 32	2136 ± 294	0.36 ± 0.09	1.2 ± 0.3	2.8 ± 0.7	167 ± 38
	10 m	0.99 ± 0.37	5.5 ± 0.8	207 ± 48	2987 ± 440	0.49 ± 0.03	2.0 ± 0.3	4.2 ± 0.4	239 ± 55
	20 m	1.03 ± 0.30	8.3 ± 2.0	208 ± 26	3869 ± 768	0.58 ± 0.07	2.2 ± 0.1	5.6 ± 1.2	241 ± 28
	50 m	0.94 ± 0.00	7.2 ± 1.1	209 ± 13	4128 ± 253	0.56 ± 0.06	2.3 ± 0.3	6.2 ± 0.6	240 ± 16
	100 m	0.81 ± 0.08	6.8 ± 1.8	184 ± 13	4127 ± 343	0.47 ± 0.02	1.8 ± 0.1	5.4 ± 0.5	212 ± 13

Sample treatment = sample location (m) from outfall; Ref. = reference site upstream from outfall; RRB = Runoff retention basin.

natural abundances of the metals. All metals (except Mn) recorded their highest concentrations in the retention basin compared to the reference sediment and downstream sediment. The Mn concentration in the RRB (440 mg/kg) was less than that of the reference site (452.6 mg/kg). The highest concentration of Mn was found at the 5 m sampling site (833 mg/kg). The other metals (Cd, Co, Cu, Mo, Pb an Zn) were recorded in higher concentrations in the first 50 m downstream compared to the reference site. The highest metal content downstream was detected in the 5 m, 10 m and 50 m sediment samples. At 100 m metal concentrations were similar or lower to those of the reference site.

3.1.2. *Anthropogenic enrichment factor (AEFs)*

Anthropogenic enrichment factors (Table 2) were calculated only for the runoff priority metals Cd, Cu, Pb and Zn. These were the only metals with available background concentrations for the catchment area (see Schertzinger et al., 2018). The AEFs for all metals at the reference site were below 1. Also, Cd showed AEF values below 1 at all sites, whereas the Pb value was greater than 1 only in the RRB. The AEF for Cu was greater than 1 at all sites receiving runoffs from the highway (except at the reference point where it was below 1). The highest value (10) was recorded in the retention basin and the lowest (1.26) at 100 m downstream from the inlet. The sites from 1 m to 50 m downstream of the inlet recorded AEFs of Cu between 2.24 and 3.3. Similarly, Zn recorded its highest AEF of 3.43 in the retention basin followed by values of 1.29, 1.31 and 1.22 at the 5 m, 10 m and 50 m, respectively.

Table 2
Anthropogenic enrichment factors (AEFs) based on sediment concentration for Cd, Cu, Pb and Zn.

Sample	Cd	Cu	Pb	Zn
Ref.	0.25	0.92	0.39	0.54
RRB	0.62	10.82	1.90	3.43
1 m	0.30	2.24	0.54	0.97
5 m	0.44	3.01	0.72	1.29
10 m	0.41	3.30	0.69	1.31
20 m	0.26	2.52	0.58	0.87
50 m	0.35	3.05	0.69	1.22
100 m	0.18	1.26	0.39	0.60

NB: AEFs were calculated only for metals with available background concentrations within the Emscher catchment.

3.1.3. *Bioaccumulation of metals in Gammarus sp.*

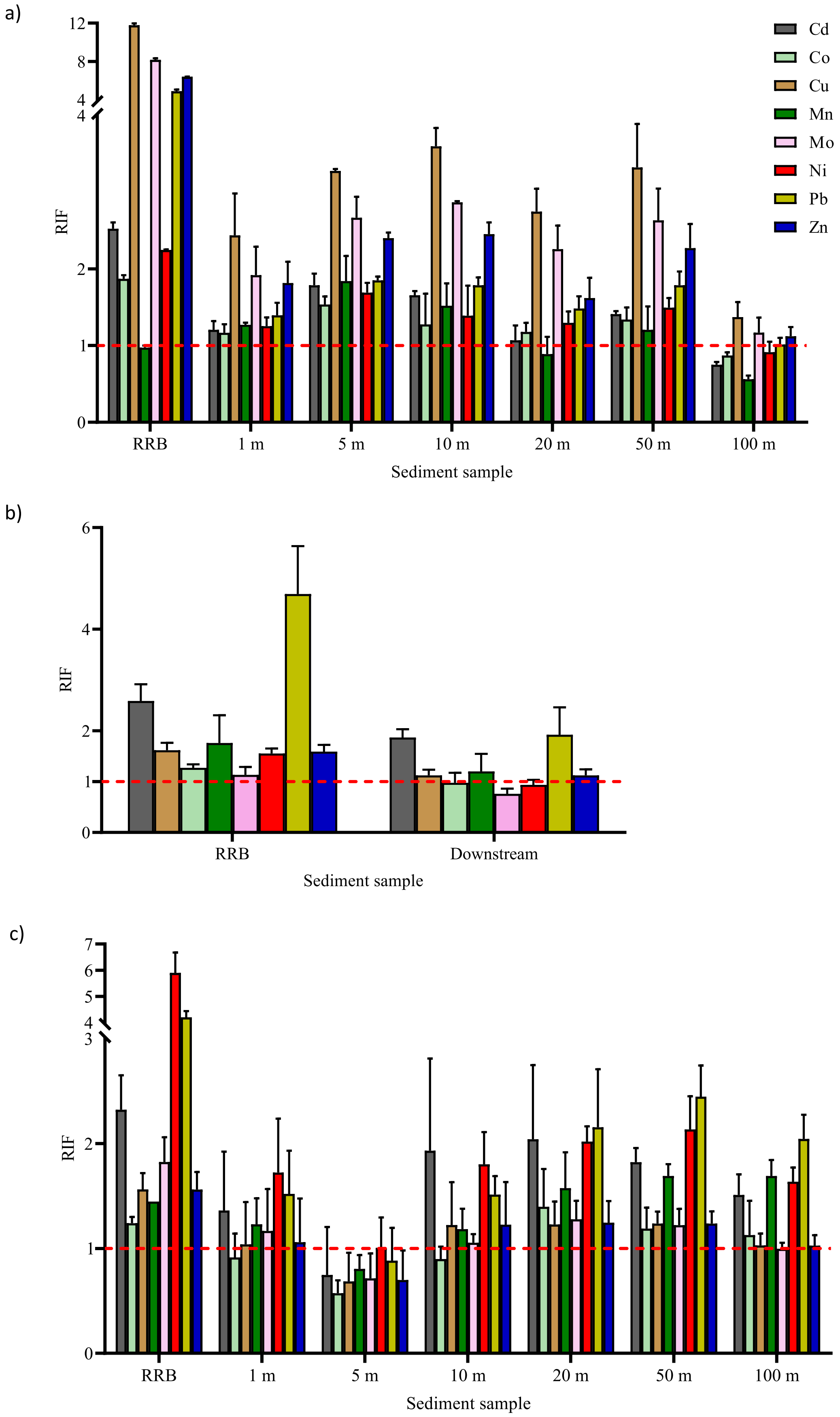
Analysis of metal concentrations in gammarids collected in the field could only be done for samples from the RRB, reference site and 70 m downstream of the inlet (Table 1). Amphipods were not found between the inlet and the following 50 m downstream. Similar to the sediment samples, the concentrations of most metals in the amphipods were higher in the RRB samples compared to samples from the reference site and downstream. The metals Cd, Cu, Pb and Zn were considerably higher in amphipods from the RRB compared to samples from the reference site. Unlike the sediment, the lowest Mn concentrations were found at the reference site and highest in the RRB. Amphipods from the reference site accumulated more Co, Mo and Ni than those from downstream sites. However, the highest accumulation was detected in the organisms from the retention basin.

3.2. *Bioaccumulation of metals in L. variegatus*

Concentrations of metals in *L. variegatus* exposed for 28 days to sediment samples in the laboratory are presented in Table 1. Element concentrations in worms before the exposure were extremely lower than the respective concentrations in the annelids after 28 days of sediment exposure. The concentration of Co in the annelids were below the LOQ before sediment exposure. The highest bioaccumulation of metals occurred in worms exposed to the sediment sample from the RRB. Contrary to measured concentrations in the sediment samples, metal concentrations in worms from the 5 m sediment-treatment were lower than at the references site. Bioaccumulation of Ni and Pb was considerably higher in the RRB sediment compared to the reference sediment. Correlation results between sediment metal concentration and bioaccumulation in *L. variegatus* gave p-values > 0.05.

3.3. *Runoff impact factors for sediment and biota metal concentrations*

Runoff impact factors (RIF) for each metal were calculated in all sampling locations to determine the impact of surface runoff on metal content in sediment and aquatic organisms. Graphs of RIF (Fig. 2a, b and c) clearly show the variation of metals in sediment and biota samples relative to the reference site from RRB to downstream of the runoff outfall. The RRB recorded the highest


a)
12
8
4
4
2
1
0
RIF
RRB
1 m
5 m
10 m
20 m
50 m
100 m
Sediment sample
Cd
Co
Cu
Mn
Mo
Ni
Pb
Zn
b)
6
4
2
1
0
RIF
RRB
Downstream
Sediment sample
c)
7
6
5
4
3
2
1
0
RIF
RRB
1 m
5 m
10 m
20 m
50 m
100 m
Sediment sample

RIFs for sediment metal content (see Fig. 2a). From the outfall up to 50 m downstream, RIF values above 1 were observed for most metals. At 100 m downstream, only Cu, Mo, Pb and Zn exhibited RIF values above 1, but still lower than those in the first 50 m. Manganese was the only metal with RIF < 1 in the RRB for sediment. On the other hand, there was no detectable impact of runoff inflow on the concentration of Co, Mo and Ni in amphipods downstream of the inlet (RIF < 1; Fig. 2b). RIF values for most metals in amphipods from the RRB were above 1. Based on bioaccumulation in *L. variegatus*, RIF was highest in the RRB for all metals (Fig. 2c). Contrary to the sediment, the annelids recorded RIFs below 1 for most metals at 5 m downstream. The other downstream sites had RIF for metals in *L. variegatus* above 1.

3.4. *Toxic responses of L. variegatus to sediment samples*

3.4.1. *Growth and reproduction of L. variegatus exposure to sediment samples*

Results of the sediment validation experiment for the standard toxicity assay with *L. variegatus* and water parameters during the respective experiments are summarized in the supplementary sheet (Tables S2 and S3). All parameters met the validity criteria for the toxicity and bioaccumulation experiment (EPA 2000).

Following 28 days of exposure to the sediments, worms were assessed for growth (dry biomass) and reproduction. In all treatments, there was a positive change in biomass (Fig. 3a). While the increase in biomass varies with sediment samples, there was no clear effect that could be attributed to the metal content in the sediment when comparing the different sites with the reference treatment. The 5 m sediment had the lowest increase in biomass but with high standard deviation. For the endpoint reproduction (Fig. 3b), the results were similar to growth. Most sediment treatments had a positive increase in the number of worms after 28 days. The only exception was the 5 m treatment which had on average 7.5% fewer worms.

3.5. *Metallothionein expression in L. variegatus*

Metallothionein content in worms was measured after 10 days and 28 days of sediment exposure (Fig. 4). After 10 days, there was an increase in MT in all treatments compared to the reference sediment. Worms from the sediment treatments 5 m, 10 m and RRB exhibited MT values of 12.14, 12.65 and 10.83 μg MT/mg wet weight, respectively. These values were significantly higher ($p < 0.05$) than those obtained for the reference site sediments. After 28 days, only RRB treatments recorded a considerable induction of 12.26 μg MT/mg compared to *L. variegatus* from the reference sediment with 4.9 μg MT/mg. The MT of 5 m and 10 m samples after 28 days dropped to 31% and 47% of their 10 days values while samples from the reference, 20 m, 50 m and 100 m sediments did not show clear differences in MT induction between 10 and 28 days. Also, correlation analyses between MT levels and the metal concentrations in sediment and *L. variegatus* usually yielded p-values > 0.05. However, merely the concentrations of Zn in sediments and *L. variegatus* showed positive correlations ($r = 0.81$ and 0.76; $p < 0.05$) with the MT levels in *L. variegatus* after 10 and 28 days, respectively.

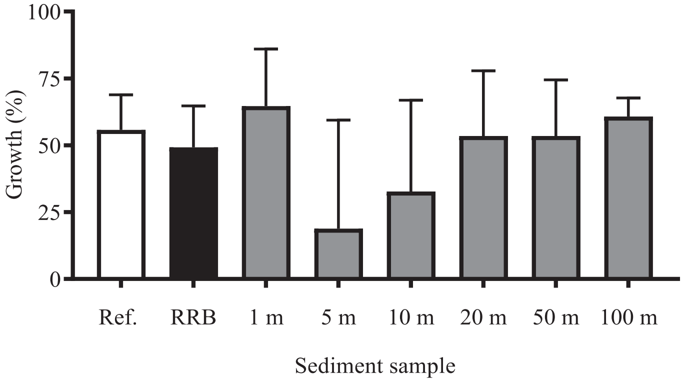
4. Discussion

In this paper, we studied the impact of surface runoff on metal concentrations in the Deininghauser stream, where an increased presence of traffic-related metals was observed (Schertzinger et al., 2018). The concentrations of all traffic associated metals were higher in the RRB compared to the reference and downstream location in the order RRB > downstream > reference site. Traffic road runoff is known to be one of the major sources of toxic metals such as Cd, Cu, Ni, Pb and Zn in receiving freshwater bodies (Barbosa et al., 2012; Brombach et al., 2005; Huber et al., 2016; Ruchter and Sures 2015; Schertzinger et al., 2018). For example, Ruchter and Sures (2015) observed high accumulation of traffic-related metals in sediments downstream of a road runoff outfall. Their observation is similar to that of the current study despite the presence of a RRB, which should normally prevent direct discharge of rain runoff into the stream. However, intensive runoff and overflow of the RRB might lead to high pollution levels in downstream waters due to resuspension and remobilization of sediment-bound pollutants (Allen et al., 2019; Fang et al., 2015; Grotehusmann et al., 2007). Therefore, the discharges of the basin would be the main reason for the elevated metal concentrations in the downstream sites compared to the reference site upstream. This could also explain the observed AEFs of Cu and Zn (which were greater than 1) in the downstream direction. In contrast, the AEF of all metals at the reference showed values less than 1, which therefore additionally supported this scenario. Elements such as Cu and Zn are known to be extensively emitted by tires and brake linings (Councell et al., 2004; Hjortenkrans et al., 2007) which is the likely reason for their high enrichment in the RRB and downstream respectively.

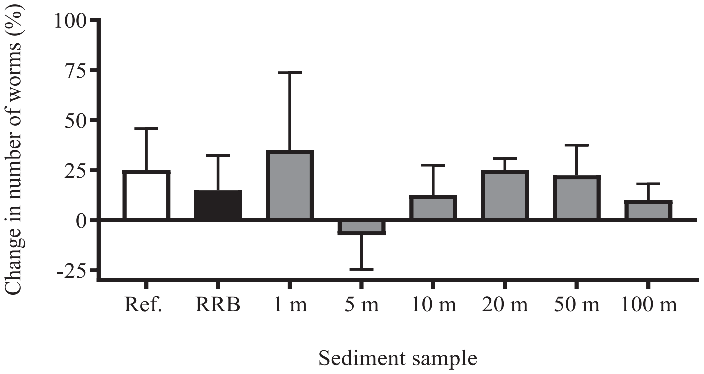
Bioaccumulation data for gammarids and oligochaetes indicate that the particle-bound elements are taken up by aquatic organisms and sediment dwellers in the stream. Similar to the sediment samples, the highest RIFs in the amphipods were found in the RRB while the gammarids downstream only enriched Cd, Cu, Mn, Pb and Zn. However, the biological availability of metals to aquatic organisms depends on many different factors such as water chemistry as well as biological and ecological traits of organisms (Erasmus et al., 2020; Goodyear and McNeill 1999). The enrichment of metals in amphipods from the stream could be due to an enhanced biological availability of metals through desorption and dissolution processes following sediment resuspension and changing water conditions (Schroeder et al., 2020; Superville et al., 2014). The latter is particularly important in the RRB with high sediment metal concentration and infrequent water flow leading to an extended time for bioaccumulation. Also, the feeding habits of the amphipods as suggested by Schertzinger et al. (2018) could enhance the uptake of metals. The authors detected enrichment of metals in amphipods downstream of combined sewer overflow inlets, a similar observation to the present study. However, given the limited data in the downstream sites, a detailed relationship with the metal concentrations in sediment could not be established.

On the other hand, uptake of metals by *L. variegatus* gave RIFs greater than 1 for all sediment samples except samples from 5 m downstream. The enrichment of metals in the annelids could be attributed to the different routes of pollutant uptake, i.e. across the body surface and through feeding. Ingestion of sediment by the worms increases the biological availability of sediment-bound

Fig. 2. Runoff impact factor on metal concentrations in RRB and downstream of outfall for sediment, amphipod and *L. variegatus* (mean, ±SD). a) RIF based on sediment metal concentrations; b) RIF based on metal bioaccumulation in field-collected amphipods and c) RIF based on metal bioaccumulation in lab exposed *L. variegatus*. NB: The horizontal dotted line at $y = 1$ represents the threshold above which the concentration of a given metal is higher than in the reference sample, indicating a possible influence by runoff inflow.



a: Growth as change in biomass of *L. variegatus* (%) exposed to sediment samples for 28 days (mean, ±SD)



b: Reproduction as change in the number of *L. variegatus* (%) after 28 days of exposure to sediment samples (mean, ±SD)

Fig. 3. a) Growth as change in biomass of *L. variegatus* (%) exposed to sediment samples for 28 days (mean, ±SD). **3b)** Reproduction as change in the number of *L. variegatus* (%) after 28 days of exposure to sediment samples (mean, ±SD).

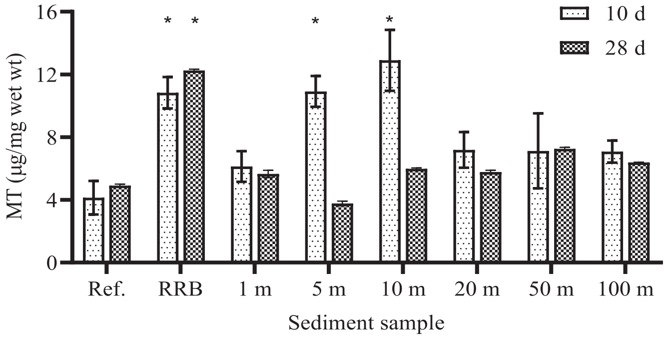


Fig. 4. Metallothionein concentration (µg/mg wet weight) in *L. variegatus* exposed to sediment samples for 10 and 28 days (mean, ±SD). * = significant difference from the reference site ($p < 0.05$).

metals due to enzymatic processes in the gut that facilitates solubility (Griscom et al., 2000). Also, the annelids can accumulate dissolved metals directly from the water phase through their body surface and during respiration. However, the bioaccumulation data of the annelids did not show a strong positive correlation with the element concentration in sediments. While the annelids can take up metals via different routes, their bioaccumulation potential is limited under certain conditions. For example, following asexual reproduction, uptake of pollutants by ingestion is not possible as the annelids do not feed (Brust et al., 2001). Additionally, the geochemical properties of the sediments and different solubility of metals may influence the biological availability resulting in varying tissue concentrations in the worms that do not reflect the levels in their environment. Nevertheless, the different bioaccumulation routes increase the metal accumulation potential of *L. variegatus* and the likelihood of toxic effects (De Jonge et al., 2012).

Toxic effects of metal-contaminated sediment on benthic dwelling organisms were already demonstrated with *Caenorhabditis elegans* (Schertzinger et al., 2019). The authors observed significant effects on growth and reproduction after exposing the nematode to sediment contaminated by combined sewer overflow (CSO). The results of the toxicity experiments exhibited positive correlations with the metal load in sediments downstream of a CSO basin. However, the results for growth and reproduction of *L. variegatus* in the present study did not reveal a clear impact of the downstream metal contamination on the annelids. The increase in biomass and number of worms after 28 days in the reference sediment was not significantly different from the other sediment samples. However, no sediment treatment had a 100% increase in biomass and number of worms. The absence of clear effects on these two endpoints is similar to findings of other studies (De Jonge et al., 2012; Feiler et al., 2013; West et al., 1993). For example, Feiler et al. (2013), did not observe effects on reproduction of *L. variegatus* in 20 out of 21 contaminated river sediments. Similarly, West et al. (1993) found no effect on survival and reproduction after exposing *L. variegatus* to metal-contaminated sediment. The tolerance of the annelids to toxic elements could partly be explained by the inability of the worms to feed after asexual reproduction resulting in limited exposure to toxic elements (Brust et al., 2001). Similarly, metal detoxifying mechanisms of the oligochaetes might also play an important role in preventing metal toxicity in the worms. The latter can be explained by their ability to produce the metal-binding protein metallothionein (Amiard et al., 2006; De Jonge et al., 2012; Liu et al., 2014). Accordingly, in the present study elevated levels of MT were detected in those *L. variegatus* showing high metal concentrations. The metal chelating and free radical scavenging ability of MT in the worms reduces the potential of toxicity of the bioaccumulated elements (Hall 2002; Liu et al., 2014). However, not all metals showed a significant positive correlation with the MT levels. While metals in general are known for their predominant role in the induction of MT in living organisms, the relationship between different metals and the detoxifying agent varies. For example, MT has been reported to be closely associated with Zn compared to other metals. Accordingly, MTs are known to serve as a reservoir for the metal and to play a vital role in its metabolism (Davis and Cousins 2000). This might explain the significant positive correlation between Zn and MT content in the present study. It should be noted that Zn is a relevant traffic-related metal mainly associated to tire abrasion and was detected in high concentrations in the RRB and downstream sites. However, other relevant traffic metals that were also measured in high concentrations did not correlate with the MT levels in the annelids. The induction of MT in aquatic invertebrates has been reported to be influenced by different confounding factors of environmental and physiological origin (Aly et al., 2014; Amiard et al., 2006; Langston et al., 2002, Le et al. 2016, Ritterhoff et al., 1996). The latter could explain why some metals did not agree strongly with the measured MT levels and the observed low induction in some downstream samples despite high metal concentrations. Nevertheless, the high MT level in worms from the RRB sediment and some downstream samples is an indication of a physiological response to metals because of the traffic runoff inflow. Also, the results of the MT levels show that this biomarker is more sensitive to metal contamination than the endpoints growth and reproduction.

5. Conclusion

According to the results of this study, the function of the RRB to prevent direct inflows of rainwater runoffs into the stream is being compromised by excess runoff that results in overflow of the basin. Consequently, we observed increased metal concentrations in sediment and biota in the downstream section of the overflow inlet. The highest metal concentrations in sediments were detected in the RRB followed by the sites downstream of the inlet. The bioaccumulation results in the amphipods and oligochaetes argue for the biological availability of the metals to aquatic organisms irrespective of their habitat preferences. Despite the high accumulation levels in *L. variegatus*, a clear toxic response based on the endpoint growth and reproduction could not be established. Nevertheless, the physiological response of MT in *L. variegatus* was an indication of the stress caused by metal contamination in the aquatic environment. Hence, in addition to the endpoint's growth and reproduction, physiological responses should be taken into consideration for effects related to metal contaminations. Also, optimization of RRBs to prevent overflow during heavy rainfall events is necessary for prevention of direct discharges into receiving waters.

Author contributions

J.K. and B.S. conceived the study. J.K. conducted the experiments, analyzed the data and wrote the manuscript. B.S. and M.N. oversaw the analyses, contributed to the draft version of the manuscript and critically revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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3 Chapter 2: Stream sediment pollution related to stormwater retention basins and corresponding ecotoxicological effects on *Lumbriculus variegatus*

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Abstract:

The overflow of stormwater retention basins during intense and prolonged precipitation events may result in the direct input of particulate pollutants and remobilization of already sedimented particle-bound pollutants to receiving freshwater bodies. Particle-bound pollutants may cause adverse effects on aquatic biota, particularly sediment dwellers. Therefore, we investigated the sediment pollution load of a stream connected to outfalls of two stormwater basins to determine the impact of the basins' overflow on the metal and organic pollutant load of the sediment. Also, the possible adverse effects of the pollutant load on benthic dwellers were evaluated in sediment toxicity tests with *Lumbriculus variegatus* and the effects on its growth, reproduction and the biomarkers catalase, acetylcholinesterase and metallothionein were analysed. The results showed that the retention basins contained the highest load of pollutants (metals and PAHs). The pollutant load in the stream did not show a clear pollution pattern from the inlets. However, metal enrichment ratios revealed contamination with Cu, Pb and Zn with Pb and Zn above threshold effect concentration in all sites. Ecotoxicity results showed that the retention basin samples were the most toxic compared to sediment from the stream. Exposure experiments with the stream sediment did not show considerable effects on reproduction, catalase activity and metallothionein concentration. However, modest inhibition of growth and activity of acetylcholinesterase were detected. Based on the observed results, it can be concluded that pollution sources other than discharge from stormwater basins seem to be responsible for the stream sediment pollutant load. Additionally, the high pollutant concentration in the stormwater basins that resulted in the death of worms is an indication of their importance in retaining particle-bound pollutants.

Introduction

Efforts to achieve the good ecological status of freshwater systems are continuously being challenged by the input of pollutants from anthropogenic origins. Given the increasing impervious surfaces in urban cities, the volume of runoff produced during precipitation events is overwhelming. Hence, increasing overflows of retention basins, combined sewer overflow and direct discharges in streams are common. Consequently, direct and indirect input of potentially harmful substances into freshwater bodies are inevitable. Stormwater from urban sealed surfaces is regarded as a major driver of pollution in freshwater ecosystems due to a variety of potentially toxic substances such as metals and organic compounds (Charters et al., 2021; Davis and Birch, 2010; Helmreich et al., 2010; Huber et al., 2016; Markiewicz et al., 2017; MKULNV, 2014).

The type and load of potentially toxic substances in runoffs is highly dependent on the land-use characteristics (Göbel et al., 2007; Huber et al., 2016; Joshi and Balasubramanian, 2010; Liu et al., 2013; Rule et al., 2006). However, irrespective of the source, most anthropogenic substances in stormwater occur in particulate or particle-bound forms (Baum et al., 2021; Zgheib et al., 2012). Their fate to stay in the particulate phase or dissolve in the water phase is governed mainly by the redox, pH and organic content conditions of the water body (Ball, 2002; Evans et al., 1990). Particle-bound in stormwater have a high sedimentation potential and will settle to the bottom of receiving water, changing the chemical status and thus the overall chemical quality of the water body. For example, road runoff was found to be responsible for the cumulative load of traffic-related metals in sediments downstream of stormwater outfall in the river Alb (Ruchter and Sures, 2015). The authors concluded that particulate and particle-bound traffic emitted substances on the road surface washed off by stormwater were responsible for the high concentration of these metals in the downstream sediment.

To reduce the direct release of runoffs to freshwater systems the inclusion of runoff retention and clarifying basins to pretreat the water by removal of particulate matters are being considered for most paved urban surfaces. However, the limited capacity of these basins and infrequent excavation of sedimented particles may result in their remobilization to receiving waters as a result of overflow during intense precipitation events. The consequence of these events may be the remobilization of previously sedimented, particle-bound pollutants to receiving waters. For example, in previous studies accumulation of pollutants was observed downstream of different retention basins (Kontchou et al., 2020; Schertzinger et al., 2019;

Schertzinger et al., 2018). Hence in addition to input by surface runoff, overflow during precipitation is also responsible for the cumulative load of particle-bound substances in the benthic zone.

Besides changing benthic chemistry, particle-bound substances in sediment may also be taken up by benthic organisms. Given the concentrations and toxic potential of these pollutants, uptake by benthic organisms may result in ecotoxicological consequences. For example, De Jonge et al. (2012) and Kontchou et al. (2020) observed a significant induction of the metal-binding metallothioneins and a high accumulation of metals in *Lumbriculus variegatus* exposed to metal-contaminated sediment. Effects on standard toxicity endpoints such as growth, biomass and mortality have also been reported in similar studies to elaborate on the adverse effects of sediment pollution on aquatic organisms (Galluba et al., 2012; Wolfram et al., 2012). While some previous studies have reported on sediment pollution load and corresponding ecotoxicological effects on benthic dwellers, it remains important to address this issue in water bodies with different sources of pollution, such as runoff from residential or industrial areas. Additionally, it would be important to study the effectiveness of some stormwater retention basins in preventing particle-bound substances from entering streams and rivers and address the need for optimization. Therefore, the present study is aimed at analyzing the sediment pollutant content of a freshwater body and two stormwater basins and to determine any possible influence by the basin overflows on downstream sediment using chemical and ecotoxicological studies. For the former, whole sediment toxicity tests with the annelid *Lumbriculus variegatus* will be used to assess the possible effects of sediment contaminants on benthic species. This will be achieved by evaluating the relationship between sediment pollution load (main focus on metals and PAHs) and toxic effects on standard endpoints (reproduction and biomass) as well as physiological responses (biomarker analyses) of *L. variegatus*.

Materials and Methods

Sampling site

Sediment samples were collected from the Anger stream in Ratingen, North-Rhine Westphalia. This small, sand dominated lowland tributary (type 14 of the German stream classification index) of the Rhine is 35.8 km long and runs through the town of Ratingen (Fig. 1) with a flow velocity of 0.34 to 8.3 m^3/s and a depth between 30 to 140 cm. During sampling, the temperature was 18.6 $^{\circ}C$, pH 8.1 and conductivity was 411 µS/cm. The stretch of the stream that was studied receives runoffs from different sources, including residential and industrial surfaces, major and minor traffic roads and parking lots. Two runoff retention basins (RRB1 and RRB2) connected to the stream via overflow outlet were considered for this study (Fig. 1). RRB2 is equipped with a pump station to discharge stormwater into the stream during or after precipitation events. Their outfalls into the stream are located about 300 m from each other. The RRB1 is a small basin that drains an area of about 2.25 ha, dominated by residential surfaces and minor motorable roads. The other one RRB2 is a large retention basin that drains a catchment area of about 90 ha (ELWAS-Web, 2021). The basin receives stormwater runoffs from major traffic surfaces, parking lots, industrial and residential surfaces. Transects for sample collection were marked in the retention basins and at different locations along the stream from upstream of the first runoff inlet to 100 m downstream of the second inlet (see Fig. 1). Two transects upstream of the first runoff discharge points (Ref.1 and Ref.2) located 1.5 km apart were used as references for the downstream samples to assess the impact of runoff inflow on the downstream samples. The transect (Ref.2) is located 50 m from the first inlet. It should be noted that the chosen reference sites are not free of anthropogenic influence and are expected to be polluted by diffuse and non-diffuse sources further upstream. Nevertheless, they are suitable to compare the effect of "background pollution" to the additional effect of contamination from retention basins and other sources downstream.

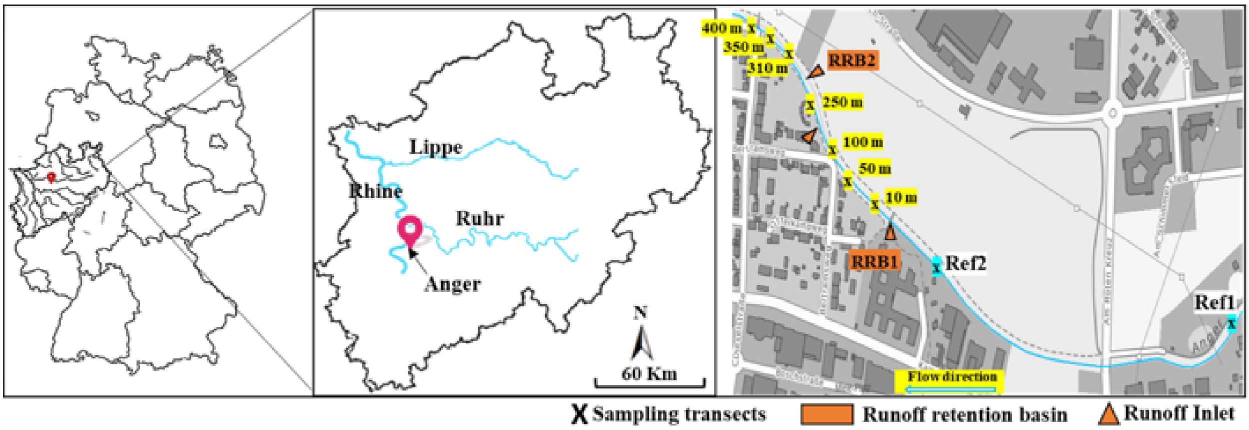

Figure 1: Sampling locations at the Anger stream in Ratingen, North Rhine-Westphalia, Germany. Samples were collected from the marked transects (x) located up- and downstream of the discharge points, and in the retention basins. Two locations upstream of the discharge points were considered as reference points for the downstream samples. The distances (10 - 400 m) are referred to the inlet of the first retention basin RRB1.

Sampling and preparation of sediments samples

Sediment sampling and sample preparation were done following a previous study by Kontchou et al. (2020). Briefly, superficial sediment samples (maximum depth of approximately 3 cm) were collected from at least three different locations in each transect with the help of a hand shovel. Samples were collected in polyethylene bags and stored at -20 °C for subsequent freeze-drying. Following freeze-drying, samples were properly homogenized by mixing in a porcelain bowl. The homogenous samples were divided into two parts: one for pollutant analyses and the other for exposure experiments with *L. variegatus.* All samples were sieved to a grain size of < 2 mm for analysis of metals, organic compounds and toxicity experiments. The samples for pollutant analyses were further sieved into grain size fractions of < 0.063 mm and 0.063 - 2 mm. The > 2 mm fractions that consisted predominantly of gravels and large organic matter were discarded. Samples were stored at room temperature in glass beakers sealed with aluminium foil for subsequent uses.

Extraction of metals and organic compounds

Metal extraction from sediment and *L. variegatus* samples were performed as reported in Kontchou et al. (2020). Freeze-dried sediment samples (500 ± 10 mg) for each grain size fraction (< 0.063 mm and 0.063 - 2 mm) were digested in a microwave oven (MARS 5, CEM GmbH, Germany). The digestion was performed in a 1:3 aqua regia solution (2 mL 65% HNO_3 Sigma Aldrich Suprapur and 6 mL 35% HCl Sigma Aldrich Suprapur, Germany) in XP1500 plus vessels. Following digestion, the contents in the vessels were brought to room temperature and filtered into 50 mL volumetric flasks. The vessels were rinsed repeatedly to collect all residual droplets and the flask was brought to volume with ultrapure water (Milli-Q, MiliPore, Germany). For validation of the analytical procedure for metals, replicates of certified standard reference materials (SRM), SdAR-1 (modified river sediment, GeoPT31, International Association of Geoanalysts proficiency testing) was also digested during microwave digestion of samples.

Extraction of organic compounds for qualitative screening experiment was done by accelerated solvent extraction (ASE 200, Dionex Corporation, USA) with 10 g of freeze-dried sediment using 1:1 hexane/acetone as solvent. The extraction was done for 10 min at a pressure of 150 bar. The settings of the ASE used for extraction are summarized in supplementary section. Following extraction, the samples were evaporated at 65 °C under a gentle nitrogen stream to < 1 mL. Finally, the extracts were filled up to 1 mL with hexane and stored at < 8 °C for further analyses. For quantification of EPA priority PAHs, the extraction, cleaning and quantification were performed according to DIN EN 15527. A mass of 10 g freeze-dried sediment per sample was used. An extraction blank without sediment, a sediment blank using reference sand (Ottawa Sand, CAS: 14808-60-7, Fisher Scientific, UK), and a quality control sample containing the reference sand spiked with 100 ng of each PAH were used. As internal standards, 1000 ng of naphthaline-d_8 and benzo(a)pyrene-d_{12} were spiked in each sample, blank, and the quality control. The samples were sonicated with acetone and cyclohexane. The acetone was removed and the extracts were dried using anhydrous sodium sulfate. A cleanup of the extracts was performed using silica gel and cyclohexane for conditioning and elution. The extracts were evaporated at 75 °C under a gentle nitrogen stream to 1 mL and stored at < 8 ˚C for quantification.

Detection of metals and organic compounds

Metals were quantified using inductively coupled mass spectrometry (Perkin Elmer Sciex Elan 6100 ICP-MS System, USA) after suitable dilution and a calibration procedure. Multi-element calibration solutions constituting all metals of interest were prepared in a suitable internal standard solution. Samples were diluted 10 times in internal standard solution and measured after the calibration solutions. The metals of interest were quantified using the isotopes ^{107}Ag, ^{111}Cd, ^{59}Co, ^{65}Cu, ^{57}Fe, ^{55}Mn, ^{98}Mo, ^{60}Ni, ^{206}Pb, and ^{66}Zn.

Screening analysis for organic compounds was done using GC-MS/MS (Shimadzu GC-MS-TQ8040, Germany). A total of 111 organic compounds including flame retardants, biocides, PAHs and PCBs were screened. Detailed information of the GC-MS/MS settings for the screening of organic compounds is presented in Tables S3 – S6. PAH quantification was done on GC-MS. The measurement was done following DIN EN 15527. The conditions of the GC-MS measurements and internal standard, as well as reference ion retention time, are presented in the supplementary section.

Sediment risk assessment for metal based on the Consensus-Based Sediment Quality Guideline (CBSQG)

To assess the potential of toxic effects of sediment samples based on the quantified metal concentrations, the consensus-based sediment quality guideline (CBSQG, MacDonald et al., 2000) was applied. Probable effect concentration quotients (PEC-Q) per site were calculated for metals and PAHs by dividing their measured concentrations by the respective predicted effect concentration givens in the CBSQG. The mean PEC-Q was then computed for all substances and the likelihood of toxic effect of the sediment to benthic organisms was computed using the equation (Eq.1) developed by MacDonald et al. (2000) and recommended by the Wisconsin Department of Natural Resources, (2003) for estimation of the probability of toxicity at any PEC-Q value. The results of the risk assessment based on the CBSQG were then compared to the toxic response of *Lumbriculus variegatus* following exposure to the sediment samples.

Exposure of *Lumbriculus variegatus* to sediment samples

$$\text{Probability of toxicity} = 101.48 * (1 - 0.36^{PEC-Q}) \qquad \text{Eq.}_1$$

Exposure experiment

For investigating the possible toxic potential of the sediment samples, a standard toxicological experiment with the sediment-dwelling oligochaete *Lumbriculus variegatus* was conducted. Detailed information on the source and handling of the test organisms can be found in Kontchou et al. (2020). The test organisms were synchronized 14 days before the experiment. This was done by dividing adult worms in the median body into two pieces. The anterior pieces were returned to the laboratory culture while the posterior ends were cultured in a separate beaker for 14 days for head development. They were kept under the same conditions as the normal culture but without renewal of overlying water. During culturing of the posterior pieces, food (mixture of Tetramin fish flakes (Tetra GmbH Germany) and nettle powder (Heinrich Klenk GmbH & Co. KG, Germany) in the ratio of 2:1 was added after 7 to 8 days after head development was evident in more than 90% of the worms. Head development was determined by observing the burrowing activities of the worms. After 14 days of growth, the worms were assumed to be of equal developmental stage and ready for exposure.

Preparation of test sediment and exposure worms

Sediment samples were prepared during the synchronization period and equilibrated for 24 h to the test conditions before exposure of synchronized worms. Artificial control sediment was prepared following OECD 225 guidelines as described by Woermann et al., (2021). The artificial sediment contained quartz sand (size 0-1 mm, Baumit GmbH, Germany), kaolinite clay (VWR International, Belgium), peat (Floragard GmbH, Germany) and food (the same used in the synchronization process) at a ratio of 75, 20, 5 and 0.5% dry weight, respectively. Freeze-dried sediment samples from the stream were properly mixed in bulk with the food source for the organisms. The resulting sediment (100 mg) containing food was measured in 300 mL glass beakers and reconstituted water according to OECD standard (OECD, 2007) was carefully added. The beakers were slowly aerated with the help of a Pasteur pipette and the setup was left to settle overnight before the addition of test organisms. Following overnight settling and equilibration, ten synchronized worms of approximately equal sizes (based on visual observation) were added to each beaker. The setup was run for the duration of the test (10 and 28 days) with constant aeration. Water parameters (temperature, pH and conductivity) were measured weekly and evaporated overlying water was replaced with deionized water. At the end of the exposure period, worms were carefully extracted from the sediment, rinsed, and transferred into Eppendorf tubes for further analyses. The number of complete and incomplete (fragmented) worms were noted for survival and reproduction analysis. Specimens for biomarker experiments were transferred into pre-cooled Eppendorf tubes directly from the exposure systems. All samples were stored at -80 ^{o}C. The biomass of the annelids per replicate was determined by taking the dry weight of the organisms following freeze-drying.

Biomarker measurements

To evaluate the physiological responses of *L. variegatus* to pollutants in the sediment samples, the activities of the enzymes acetylcholinesterase (AChE), catalase (CAT) and the relative amount of metallothioneins in the worms were analyzed after 10 days of exposure to sediment samples. For analysis of CAT and AChE, *L. variegatus* samples were thawed and homogenized on ice in 0.2 mL IP lysis buffer (25 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Igepal®-CA630 (Sigma-Aldrich), 5% glycerin (Sigma-Aldrich), Germany), pH 7.4 containing 1% protease inhibitor (Sigma-Aldrich, Germany). The homogenization was done using a micropestle. Samples for metallothionein analysis were homogenized with a similar procedure in 200 µL of 0.25 M sucrose solution. Following homogenization, the samples were centrifuged at 14,000 x g for 10 min at 4 ^{o}C and the resulting supernatants were distributed into labelled tubes for total protein measurement and analysis of enzyme activities (CAT and AChE). For metallothionein analysis, aliquots for total protein quantification were taken directly from the homogenates and centrifuged while the remaining homogenate was stored for the biomarker analysis. All homogenates and supernatants were stored at -80 ^{o}C for subsequent use. For diluting samples during biomarker analysis, the dilution buffer (25 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 5% glycerin), pH 7.4 was used. Protein quantification was done using the Pierce™ BCA Protein Assay following the manufacturer's protocol on a microplate reader (Tecan infinite M200, Switzerland).

Acetylcholinesterase activity analysis

Acetylcholinesterase (AChE) activity was analyzed according to the colourimetric method of Ellmann et al., (1961). A stock solution of Ellmann’s reagent was prepared by dissolving 790 mg of 5,5’-dithio-bis (2-nitrobenzoic acid) (Sigma-Aldrich, Germany) and 750 mg sodium bicarbonate in 100 mL 0.1 M phosphate buffer (57.7 mM Na_2HPO_4 and 42.3 mM NaH_2PO_4), pH 7. A volume of 5 µL of 5-fold diluted sample supernatants was dispensed into a 96-welled plate followed by the addition of 140 µL of Ellmann reagent. A volume of 10 µL, 20 mM acetylthiocholine iodide substrate (Sigma-

Aldrich, Germany) was added to the wells. The content in the wells was properly mixed using a multichannel pipette and the absorbance at 405 nm, 25 ˚C was measured every 30 s for 10 min.

Catalase activity analysis

Catalase activity was determined by measuring the rate of breakdown of hydrogen peroxide (H_2O_2) by the enzyme (Beers and Sizer, 1952) as described in Woermann et al., (2020). For this purpose, the sample supernatants were diluted in dilution buffer and 210 µL of the resulting product was carefully mixed with 90 µL of H_2O_2 (100 mM) in a 96-well plate (Greiner Bio-one UV-star). The plate was immediately inserted into the reader and the absorbance at 240 nm, 25 ˚C of H_2O_2 was measured every 15 s for 4 min.

Metallothionein measurement

Measurement of metallothionein (MT) in *L. variegatus* exposed to sediment samples was done using the silver saturation method (Scheuhammer and Cherian, 1986) as described by Kontchou et al. (2020). Samples were homogenized on ice in 150 µL of 0.25 M sucrose solution using a micro-pestle. Sample homogenates were saturated with 250 µL of 20 mg/L Ag solution (Bernd Kraft GmbH) and incubated for 20 min at room temperature. Excess Ag was removed by addition of 50 µL sheep red blood cell hemolysate (Sigma Aldrich) followed by heat treatment (100 ºC for 10 min; HCL, DITABIS) and centrifugation (5,000 x g for 10 min; Centrifuge 5810R, Eppendorf AG). The process from the addition of hemolysate, to centrifugation, was repeated 3 times to ensure complete removal of unbound Ag. The final centrifugation was done at 16,000 x g for 15 min. The resulting supernatant was analyzed for Ag which is proportional to MT content by graphite furnace atomic absorption spectrometry (GF-AAS; AAnalyst – 600, Perkin Elmer; see also Frank et al., (2013). Metallothionein content is expressed as extinction coefficient (E) per mg protein.

Data and Statistical analyses

All data analyses were done using Microsoft excel (2019) and GraphPad Prism 5.0 (GraphPad Softwares). Evaluation of the results for suspect screening was done using the Software LabSolutions Insight (Shimadzu Europe GmbH, Duisburg, Germany). Calculation of the total metal concentration in the < 2 mm particle size sediment fraction was made possible using equation (Eq. 2). To study the metal accumulation pattern along the stream, mean relative concentrations of all metals were calculated in each sampling site. The relative value for metal and PAHs along the stream were based on the site with the highest concentration (100%) of the respective substance. Anthropogenic enrichment factor was computed for Cd, Cu, Pb and Zn using the background concentration of these metals in the Emscher catchment. Although it should be noted that the Anger stream is not part of the Emscher catchment it is located in the same geographical region as the Emscher, justifying the use of the background concentration. Semi-quantitative results of organic compound classes were expressed in peak area/g sediment. The relative peak area/g sediment for each organic compound class was also calculated using the same method described for metals. The highest signal for the respective detected organic compounds was set at 100% from which the relative peak area/g sediment for each sampling site was calculated. Source diagnostic tools for PAHs was applied by calculating source apportionment ratios for different PAHs, see Table S13 (Budzinski et al., 1997; Crane and Hennes, 2016, Yunker et al., 2002). This was done to aid discussion on possible sources of pollution in the stream.

AChE and CAT activity was calculated and expressed in U mg total protein^{-1} (µmol min^{-1} mg protein^{-1}). Inhibition in AChE activity was presented in percentages. MT content was evaluated and expressed as extinction coefficient (E) per mg protein. Spearman rank correlation coefficient was used to assess the relationship between different variables while Kruskal-Wallis statistic (with correction for multiple comparisons) was used for significant difference evaluation between sampling sites for toxicity responses.

$$C\,(< 2\,mm) = \frac{(C * M)_{(<63\mu m)} + (C * M)_{(63\mu m \leq x < 2\,mm)}}{M_{(<63\mu m)} + M_{(63\mu m \leq x < 2\,mm)}} \qquad \text{Eq.2}$$

Where C = concentration (mg/Kg); M = mass (mg)

Results

Pollutant content and concentrations in sediment

Metal concentrations in sediment, anthropogenic enrichment and risk assessment

Quality data of the analytical method for metals are presented in Table S7. The recovery values were between 81 to 101% and the variation was between 2 to 6% for all metals. Results of metal concentrations recorded values above the limit of quantification (LOQ) for all elements. The metal concentrations in sediment showed variation in the different grain sizes and along the stream (Table 1). The highest accumulation of metals was detected in the < 63 µm particle fraction in most sites. For the total metal concentration in the sediment (grain sizes < 2 mm), the highest values were found in RRB2 (for Ag, Cd, Co, Cu, Fe, Mo, Ni and Zn) while Mn and Pb showed the highest concentration at 50 m and 310 m respectively. The concentrations of Ag, Co, Cu, Mo, Ni, and Zn in RRB2 were significantly higher than in all other sampling sites (< 5%). To compare metal load in the sediment samples, mean relative concentrations (%) were calculated for the < 2 mm fraction (Figure 3). Like the individual concentrations, the highest relative metal load was in the retention basin RRB2 with 97 ± 8%. The other sites showed relative metal loads between 35 – 23% which are < 50% of RRB2 values. The results of the anthropogenic enrichment factor (AEF, Fig. 2) recorded an enrichment of Cu, Pb and Zn in all sampling sites while no anthropogenic enrichment for Cd was recorded. The highest AEF of Cu and Zn occur in RRB2 while Pb is most enriched within the first 50 m downstream of RRB2. The site immediately upstream of the outfall of RRB2 recorded lower AEFs for all four metals compared to the downstream sediment.

CBSQG evaluation (based on the total metal in the < 2 mm particle size) showed that the concentrations of Pb and Zn in all sites were above threshold effect concentration (TEC). In addition, in RRB2 concentrations of Cd, Cu, Mn and Ni were above TEC with the values of Cu, Ni and Zn being greater than the probable effect concentration (PEC) threshold. The probability of toxicity based on the measured metal concentrations in the < 2 mm particle size class showed values between 18 – 70% (Table 1). The highest value was recorded in the retention basin RRB2 and the lowest at 50 m upstream of RRB2 outfall (250 m sampling site). The reference sites Ref.1 and Ref.2 upstream of the first runoff outfall showed toxic potentials of 19% and 22% respectively, while the values at the downstream sites were between 20 – 27%.

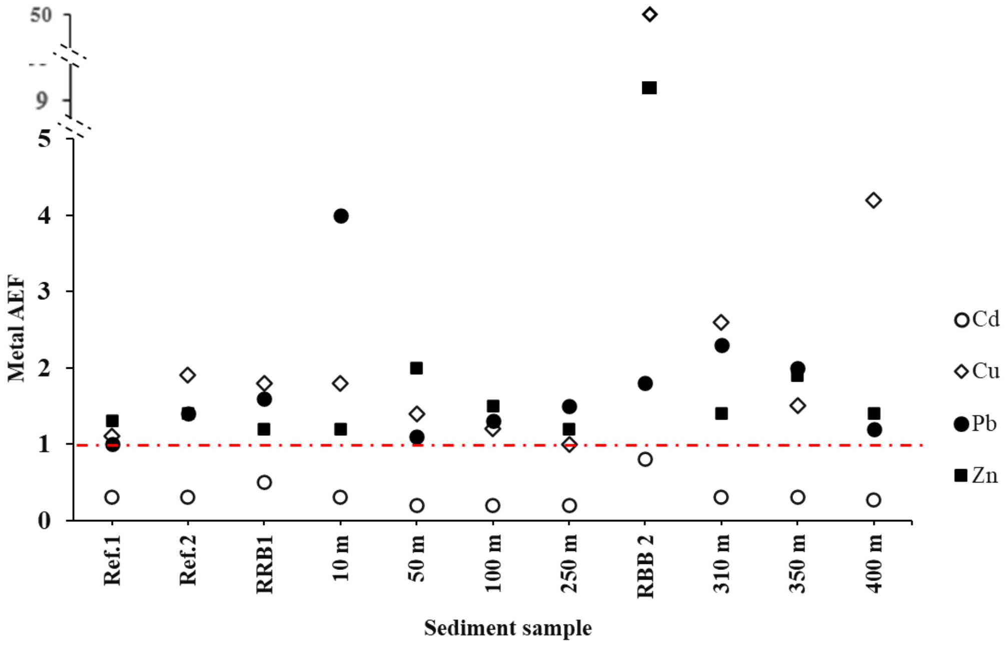

Figure 2: Anthropogenic enrichment factors (AEFs) of Cd, Cu, Pb and Zn in sediment

Table 1: Concentration of metals (mg/kg) in different grain sizes of superficial sediment (mean ±SD, n=5)

Site	Grain size (mm)	Ag	Cd	Co	Cu	Fe	Mn	Mo	Ni	Pb	Zn	Probability of toxicity
Ref. 1	**0.063**	0.15 ± 0.02	0.71 ± 0.04	4.6 ± 0.3	18 ± 1	12429 ± 993	401 ± 33	0.93 ± 0.06	20 ± 1.2	91 ± 7	253 ± 17	
	0.063 - 2	0.05 ± 0.02	0.29 ± 0.08	3.7 ± 1.6	8 ± 2	9625 ± 3958	230 ± 71	0.25 ± 0.08	14.4 ± 5.5	33 ± 11	135 ± 54	
	<2	0.06 ± 0.02	0.34 ± 0.08	4.0 ± 1.6	10 ± 2	10403 ± 3763	258 ± 66	0.33 ± 0.07	15.9 ± 4.9	39 ± 11	153 ± 52	19
Ref. 2	**0.063**	0.15 ± 0.01	0.62 ± 0.03	4 ± 0.2	16 ± 1	11043 ± 564	421 ± 22	0.5 ± 0.0	16.7 ± 1.5	72 ± 4	234 ± 9	
	0.063 - 2	0.05 ± 0.01	0.31 ± 0.03	4.4 ± 1.3	17 ± 10	12288 ± 3471	329 ± 64	0.28 ± 0.1	16.7 ± 3.7	50 ± 13	164 ± 61	
	<2	0.06 ± 0.01	0.33 ± 0.03	4.3 ± 1.3	17 ± 9	12212 ± 3238	335 ± 59	0.29 ± 0.1	16.7 ± 3.5	52 ± 12	169 ± 57	22
RRB 1	**0.063**	0.15 ± 0.02	0.69 ± 0.04	5.3 ± 0.3	18 ± 1	14375 ± 908	499 ± 29	0.66 ± 0.03	18.7 ± 1.6	81 ± 4	172 ± 10	
	0.063 - 2	0.11 ± 0.02	0.59 ± 0.02	4.3 ± 0.4	15 ± 0	12709 ± 698	408 ± 28	0.46 ± 0.02	13.7 ± 0.8	61 ± 6	144 ± 36	
	<2	0.11 ± 0.02	0.60 ± 0.02	4.3 ± 0.3	15 ± 0	12815 ± 664	413 ± 26	0.47 ± 0.02	14.1 ± 0.8	62 ± 5	146 ± 34	23
10 m	**0.063**	0.15 ± 0.03	0.7 ± 0.09	4 ± 0.5	15 ± 1	10766 ± 621	369 ± 21	0.38 ± 0.06	16 ± 1	66 ± 4	226 ± 14	
	0.063 - 2	0.14 ± 0.17	0.3 ± 0.05	3.2 ± 0.5	15 ± 11	8765 ± 581	268 ± 26	0.15 ± 0.02	12.7 ± 1.9	49 ± 21	126 ± 7	
	<2	0.09 ± 0.03	0.39 ± 0.02	3.4 ± 0.4	15 ± 9	9183 ± 563	289 ± 24	0.2 ± 0.02	13.3 ± 1.6	53 ± 17	147 ± 6	20
50 m	**0.063**	0.16 ± 0.04	0.71 ± 0.04	4.6 ± 0.2	19 ±	12555 ± 632	470 ± 19	0.85 ± 0.12	21 ± 1.4	101 ± 8	267 ± 9	
	0.063 - 2	0.07 ± 0.06	0.28 ± 0.1	4.8 ± 1.2	11 ± 4	11719 ± 3303	379 ± 111	0.16 ± 0.04	17.4 ± 4.7	36 ± 9	238 ± 32	
	<2	0.08 ± 0.06	0.28 ± 0.13	4.8 ± 1.1	12 ± 3	11802 ± 2989	543 ± 390	0.23 ± 0.03	17.7 ± 4.2	43 ± 8	241 ± 29	27
100 m	**0.063**	0.14 ± 0.03	0.62 ± 0.05	3.8 ± 0.1	15 ± 1	11063 ± 650	410 ± 20	0.42 ± 0.04	16.5 ± 3.5	69 ± 2	215 ± 10	
	0.063 - 2	0.2 ± 0.31	0.24 ± 0.03	4.5 ± 1.1	10 ± 4	11247 ± 2272	338 ± 67	0.2 ± 0.06	16.6 ± 3.7	47 ± 16	174 ± 84	

	<2	0.07 ± 0.03	0.25 ± 0.03	4.5 ± 1.1	11 ± 4	11241 ± 2215	341 ± 65	0.21 ± 0.06	16.6 ± 3.6	48 ± 16	175 ± 82	22
250 m	**0.063**	0.24 ± 0.09	0.58 ± 0.02	4.8 ± 0.2	19 ± 1	7922 ± 170	398 ± 13	0.7 ± 0.1	16.5 ± 0.8	76 ± 3	232 ± 9	
	0.063 - 2	0.05 ± 0.00	0.23 ± 0.02	4.0 ± 0.4	7 ± 1	5240 ± 694	261 ± 15	0.29 ± 0.08	10.7 ± 1.8	55 ± 7	134 ± 20	
	<2	0.07 ± 0.01	0.27 ± 0.02	4.1 ± 0.4	9 ± 1	5537 ± 614	276 ± 13	0.34 ± 0.07	11.4 ± 1.6	57 ± 7	145 ± 18	18
RRB 2	**0.063**	1.07 ± 0.34	1.38 ± 0.13	62.7 ± 4.6	1026 ± 28	21551 ± 940	658 ± 17	16.2 ± 1.5	160 ± 11	95 ± 1	1397 ± 97	
	0.063 - 2	0.45 ± 0.21	0.97 ± 0.33	29.4 ± 7.5	453 ± 145	17732 ± 6482	474 ± 126	7.73 ± 3.32	75 ± 21	66 ± 22	1148 ± 395	
	<2	0.49 ± 0.21	0.99 ± 0.32	31.5 ± 6.8	489 ± 135	17971 ± 6080	486 ± 118	8.26 ± 3.12	80 ± 19	68 ± 21	1164 ± 371	70
310 m	**0.063**	0.21 ± 0.02	0.6 ± 0.02	5.5 ± 0.3	22 ± 1.9	7970 ± 283	429 ± 11	0.81 ± 0.02	19.4 ± 2.8	76 ± 2	238 ± 5	
	0.063 - 2	0.09 ± 0.01	0.32 ± 0.02	5.6 ± 1.0	22 ± 13	7501 ± 993	348 ± 24	0.42 ± 0.06	14.4 ± 1.3	90 ± 35	163 ± 18	
	<2	0.10 ± 0.01	0.36 ± 0.02	5.6 ± 0.9	22 ± 12	7557 ± 815	358 ± 20	0.47 ± 0.05	15.0 ± 1.0	89 ± 30	172 ± 15	24
350 m	**0.063**	0.20 ± 0.02	0.56 ± 0.02	4.9 ± 0.4	19 ± 0.8	7232 ± 258	345 ± 12	0.74 ± 0.03	15.3 ± 1.0	70 ± 3	232 ± 6	
	0.063 - 2	0.09 ± 0.02	0.35 ± 0.06	6.0 ± 0.3	11 ± 1	8534 ± 484	387 ± 18	0.43 ± 0.04	18 ± 0.4	78 ± 22	227 ± 10	
	<2	0.11 ± 0.01	0.39 ± 0.05	5.8 ± 0.3	13 ± 1	8276 ± 378	379 ± 12	0.49 ± 0.03	17.2 ± 0.3	77 ± 17	228 ± 9	25
400 m	**0.063**	0.19 ± 0.03	0.85 ± 0.04	6.4 ± 0.4	31 ± 1.3	8583 ± 245	475 ± 17	1.8 ± 0.1	23.1 ± 1.4	102 ± 6	265 ± 10	
	0.063 - 2	0.07 ± 0.02	0.19 ± 0.02	5.1 ± 0.8	37 ± 20	8177 ± 1704	303 ± 51	0.38 ± 0.11	14.7 ± 2.6	45 ± 6	160 ± 26	
	<2	0.07 ± 0.02	0.21 ± 0.02	5.2 ± 0.8	37 ± 20	8185 ± 1673	307 ± 50	0.41 ± 0.11	14.9 ± 2.5	47 ± 6	162 ± 26	21
TEC		1.6	0.99	NA	32	20000	460	NA	23	36	120	
MEC		1.9	3	NA	91	30000	780	NA	36	83	290	
PEC		2.2	5	NA	150	40000	1100	NA	49	130	460	

Level 1	Level 2	Level 3	Level 4
≤ TEC	> TEC ≤ MEC	> MEC ≤ PEC	> PEC

TEC= Threshold effect concentration; MEC = Midpoint effect concentration; PEC = probable effect concentration; NA = Not available

Table 2: Concentration of 16 EPA PAHs (µg/kg) in superficial sediment normalized to 1% TOC

Site	Naphthalene	Acenaphthene	Acenaphthylene	Anthracene*	Benz(a)anthracene	Benzo(a)pyrene	Benzo(b)fluoranthene*	Benzo(g,h,i)perylene	Benzo(k)fluoranthene*	Chrysene	Dibenz(a,h)anthracene	Fluoranthene	Fluorene	Indeno(1,2,3-cd)pyrene	Phenanthrene*	Pyrene	Probability of toxicity
Ref. 1	8	7.1	6.9	71.3	122	151	148	124	111	122	20	250	17	97	158	184	8
Ref. 2	11	14.1	16.1	111.4	137	175	175	192	120	135	24	282	20	132	270	204	10
RRB1	13	16.2	11.5	128.1	512	550	591	434	374	551	69	629	32	326	312	549	23
10 m	4	4.9	6.3	79.9	118	135	133	115	102	128	19	229	13	92	175	166	7
50 m	2	1.8	3.3	35.4	70	73	79	53	63	75	9	121	5	43	59	89	4
100 m	3	3.9	4.0	44.9	73	92	96	79	71	78	14	148	9	62	117	106	5
250 m	3	4.0	10.2	53.8	215	316	291	258	200	215	48	278	9	213	138	229	12
RRB2	60	94.1	20.5	329.1	427	596	855	864	602	822	86	1724	53	478	979	1337	38
310 m	7	6.7	6.4	82.4	187	186	192	137	149	185	24	262	21	112	144	195	9
350 m	2	5.3	7.7	94.8	154	166	167	137	133	147	21	267	14	114	210	197	8
400 m	5	4.1	6.9	46.9	193	228	236	190	166	209	32	246	10	152	120	192	10
TEC	176	6.7	5.9	57.2	108	150	240	170	240	166	33	423	77.4	200	204	195	
MEC	369	48	67	452	579	800	6820	1685	6820	728	84	1327	307	1700	687	858	
PEC	561	89	128	845	1050	1450	13400	3200	13400	1290	135	2230	536	3200	1170	1520	

Level 1	Level 2	Level 3	Level 4
≤ TEC	> TEC ≤ MEC	> MEC ≤ PEC	> PEC

NB: 1 % TOC content was assumed for all samples for comparison with CBSQGs (Wisconsin Department of Natural Resources, 2003).
* = Not used for the calculated probability of toxicity because of poor separation of peaks
TEC= Threshold effect concentration; MEC = Midpoint effect concentration; PEC = probable effect concentration; NA = Not available

Presence of organic pollutants and PAHs concentrations in sediment

A total of 44 organic compounds of four main classes (PAHs, PCBs, flame retardant and biocides) were detected in the suspect screening (See table S15). PAHs made up 36% of the total organic compounds followed by biocides (30%), flame retardant (9%) and PCBs (5%). At least 94% of the 16 priority PAHs were detected in all the sites while the other organic compound classes did not occur in all sites from the screening analysis. The highest number of detected compounds was in RRB1 with 35 organic substances followed by RRB2 with 33. The number of detected organic compounds in the stream ranged between 19 to 27. The lowest number (19) was detected in Ref.1, 50 m, 250 m and 310 m transect while 27 compounds were detected in Ref.2.

We were able to quantify all 16 EPA PAHs in all sites. The quality data of the analytical techniques is shown in Table S9. The extraction method recorded recoveries in the range of 72 to 126 % and the LOQ was ≤ 10 ng/mL for all PAHs. Mean relative values to the maximum concentration were used for comparison of PAHs load at the different sites. The variation in PAH load was similar to that of metals at most sites (Fig. 3). The highest concentrations were measured in the RRB samples and the lowest at 50 and 100 m downstream of the first basin (RRB1). The reference sites upstream of RRB1 recorded PAH concentration similar to or higher than most downstream sites in the stream.

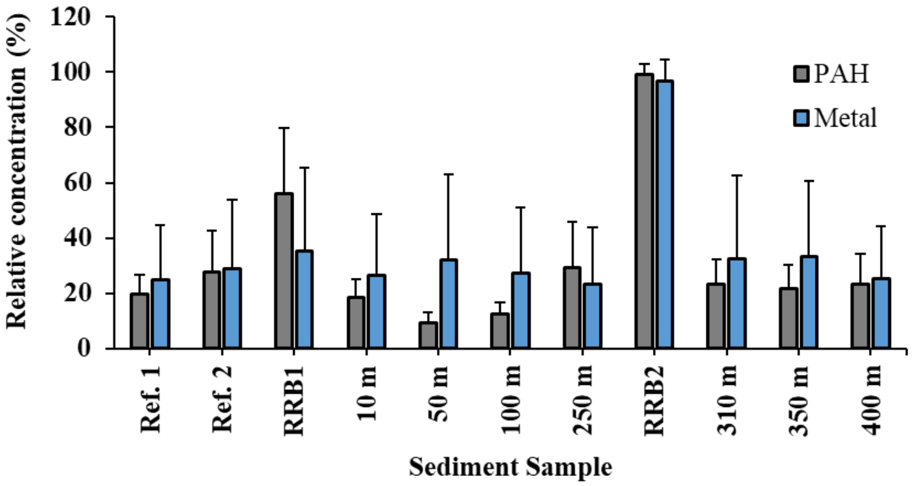

Figure 3: Mean relative substance load per mass of sediment samples for metals and PAHs in all sampling sites. The bars were generated using relative concentrations in µg/kg in sediment

Results of CBSQG analyses recorded the probability of toxicity in the range of 4 % (50 m) to 38 % (RRB2). The concentrations of naphthalene and fluorene were below TEC in all samples. The measured concentrations in the retention basins were > TEC for all other PAHs with acenaphthene > PEC while chrysene, dibenz(a,h)anthracene, fluoranthene and pyrene showed concentrations between midpoint effect concentration (MEC) and PEC in the RRB2 sample. The 50 and 100 m samples showed concentrations of all PAHs below TEC with toxic potentials of 4 and 5 % respectively.

Results of toxicity experiment

The water parameters for the exposure experiment are presented in the supplementary data section (Table S8). It should be noted that the results of biomass inhibition and biomarker responses in the retention basins were not indicated on the respective graphs due to the 100% mortality in all the RRB samples during the exposure experiment. For the reproduction endpoint, a 100% inhibition was calculated in the RRB samples.

Reproduction and biomass change after 28 days exposure to sediment sample

Results of reproduction and biomass inhibition in *L. variegatus* following 28 days in sediment samples are summarized in Figure 4. The results are presented as relative changes to the control sample. It should be noted that reproduction occurred in all samples except for the RRB sediment samples. In the latter group, 100% mortality (100% inhibition of reproduction as indicated in Fig. 4a) was recorded. The samples, Ref.2, 10, 50 and 310 m caused minimal inhibition in reproduction ranging between 1 – 10%. The rest of the sediment treatment did not induce inhibition of reproduction as some had slightly more or the same number of annelids as the control sediment at the end of the exposure period. However, the field sediment samples had more incomplete (fragmented) annelids at the end of the experiment in comparison to the control (see Table S9) which contributed to the total number used for the evaluation of the reproduction endpoint.

Unlike reproduction, optimum growth did not occur in all field sediments. All treatments recorded a reduction in biomass between 30 to 85% compared to the control sediment (Fig. 5b). The sediments from the reference sites Ref.2 and 250 m caused an inhibition in biomass of 30% while Ref.1 upstream of Ref.2 caused a biomass reduction of 54%. The latter was similar to the effects of sediment from the 50 and 100 m sites, downstream of RRB1. The highest inhibition of biomass was observed at 400 m downstream of RRB2. However, the Kruskal-Wallis test did not indicate any significant difference in biomass inhibition to control.

Also, the Spearman correlation analysis did not show any significant relation between the standard toxic endpoints and pollutant concentration in the sediment.

Biomarker response

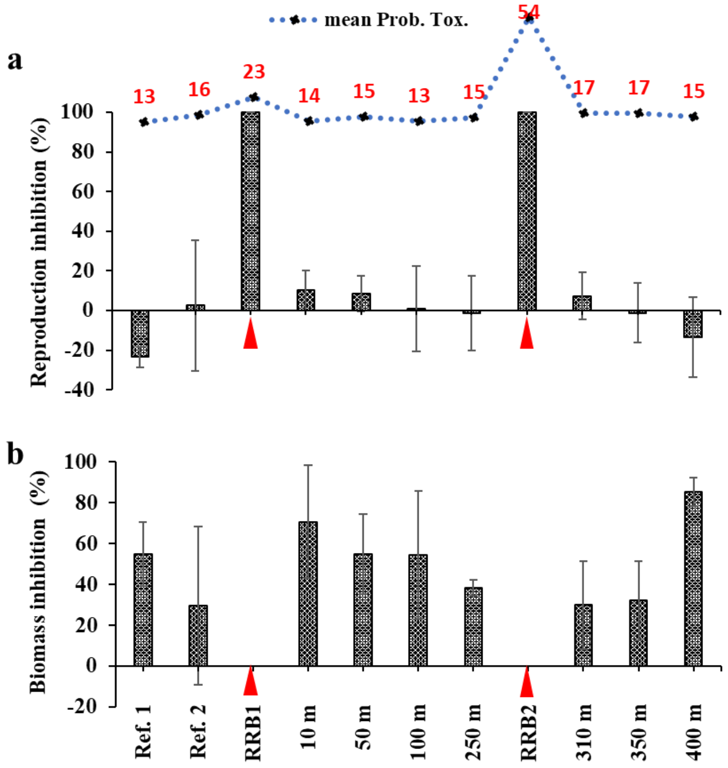

Figure 4: Reproduction as relative change (%) in the number of worms (a) and biomass inhibition (%) (b) of *L. variegatus* after 28 days in sediment sample relative to control (mean ±SD, n = 4). The mean probability of toxicity (mean Prob. Tox.,%) was calculated from the probability of toxicity of metals and PAHs (Table 1 & 2). The red triangles indicate the inlets of runoff retention basins. Since there was 100% mortality in the RRB samples, the inhibition of reproduction was calculated to be 100%.

A summary of the biomarker responses is presented in Figure 5. Except for 10 m downstream of RRB1, the activation of CAT was higher in all sediment treatments compared to the activity before exposure. The control sediment recorded CAT activity greater than or equal to the activity in sediment from the stream. The only exception was found for the sediments from the site 400 m downstream of RRB2 which induced the highest CAT activity of 40 U/mg total protein. The Kruskal-Wallis test did not show any significant difference in activity between the stream sediment and the control. The activity of the second biomarker AChE was inhibited in most sediment samples from the stream except for the Ref.1 treatment where AChE activity was about 30% higher than in the control sediment (Fig. 5b). The highest inhibitions in AChE activity were recorded in the 250 to 400 m samples and were about 48 to 53% less than in the control. However, statistical tests for differences between treatments did not indicate a significant difference between sediment treatments.

Lastly, the MT levels in *L. variegatus* which were exposed to the field sediment samples were higher in comparison to the initial content in most of the sediment treatments. The induction in the MT was most prominent in the sediment between Ref.1 to 250 m (Fig. 5c). Contrary to CAT and AChE, there was no clear induction of MT in worms exposed to sediment samples downstream of RRB2. The induction of MT in Ref.1 was similar to that of the control group, whereas no significant difference in MT level between the sediment treatments was observed. Moreover, the correlation analyses did not show any significant relationship between the biomarker response and pollutant load in the sediment.

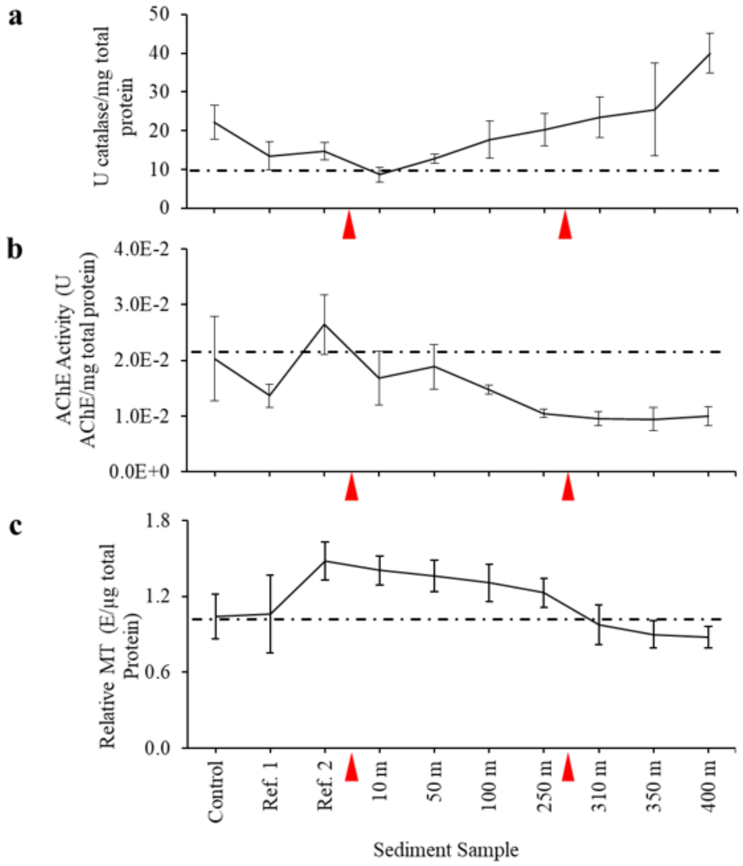

Figure 5: Biomarker responses in *L. variegatus* exposed to sediment samples (mean ±SD, n = 4). a) Catalase activity in U/mg total protein, b) Acetylcholinesterase activity in U/mg total protein, and c) Relative metallothionein induction in extinction coefficient (E) per mg total protein. The red triangles indicate the inlets of runoff retention basins. The dashed horizontal lines on the graphs represent pre-exposure values. Biomarker data for the RRB samples was not indicated due to the 100% mortality of *L. variegatus*

Discussion

The sediment is an important habitat in aquatic ecosystems, therefore, sediments are of high importance for the overall quality of a freshwater system. Alteration of sediment chemistry due to anthropogenic related pollution may result in adverse effects not only on benthic dwellers but the entire aquatic ecosystem. These may include bioaccumulation of potentially harmful substances in benthic organisms (Brunson et al., 1998; Kontchou et al., 2020; Ruchter and Sures, 2015), destruction of benthic communities (Berger et al., 2017; Desrosiers et al., 2019; Erasmus et al., 2021), secondary pollution of the water column (Colombo et al., 2016) and ecotoxicological effects on aquatic organisms (Galluba et al., 2012;

Schertzinger et al., 2019; Wolfram et al., 2012). This study attempted to shed more light on some aspects of sediment contamination related to rainwater retentions in basins. The high occurrence and concentration of the different pollutant classes in the retention basin support the importance of sedimentation basins to avoid direct discharges of runoffs into freshwater bodies. This was particularly true for site RRB2. Adsorption of metals to finer particles (< 63 µm) seems to be most important during transport and accumulation in the sediment. This agrees with the results of previous studies (Andral et al., 1999; Baum et al., 2021; Charlesworth and Lees, 1999; Díaz-Morales et al., 2021). For organic compounds such as PAHs, particle size plays no major role but rather the organic matter content of the sediment which has a higher affinity for these pollutants (Evans et al., 1990; Schorer, 1997). Additionally, it has been shown that the presence of metals bound to particles creates a hydrophobic environment around the particle which favours hydrophobic interaction with organic compounds (Liang et al., 2016). Therefore, in addition to the sedimentation rate in the RRB, the high organic matter content coupled with high metal concentration could also account for the high concentrations of PAHs and the occurrence of organic compound classes in the basins.

Similarly, the mechanisms governing binding of pollutants to sediment particles including differences in sedimentation rate of different particle sizes, flow velocity, depth, and diffuse pollution sources such as atmospheric deposition could account for the observed accumulation patterns of pollutants in the stream. In comparing metal and PAH concentrations up and downstream of the RRB inlet (Table 1, 2 & Fig. 3), it was evident that pollution by retention basin discharges was not the major influencing factor. The lack of a clear pollution profile from the RRB discharges could be due to their large storage capacity and low rate of overflow. For example, for the largest basin, (RRB2), with the highest load of all pollutants, the overflow frequency is 0.2 times per year. Hence it is likely that other factors such as diffuse sources including atmospheric deposition, runoffs not related to a stormwater basin and other sources further upstream could be responsible for the pollution load in the stream. The assumption on possible atmospheric pollution of the sediment was supported by the PAH diagnostic ratios at all sites (Table S13). These ratios indicate that these substances originate mostly from combustion of coal, wood and petroleum including road dust which are important sources in urban catchments (Yunker et al., 2002, Stout et al., 2004, Harris et al., 2011). Also, the presence of high loads of low molecular weight PAHs in the reference sites compared to most downstream sites suggest that in addition to atmospheric deposition from pyrogenic sources, petrogenic sources further upstream of the study transect also affect river sediments (McCready et al., 2000).

The anthropogenic pollution of sediment in the stream, especially downstream of RRB2 was obvious by the AER for Cu, Pb and Zn (Fig. 2). The enrichment of metals in the stream was also reflected in the risk assessment results for most stormwater priority metals. This was especially true for Cu, Pb and Zn which were mostly responsible for the overall related toxic probability of the sediment samples (≥ level 2 toxic level of concern). The occurrence of these metals in the sediment above the background and the environmental quality levels are frequently reported in studies related to stormwater pollution (Gasperi et al., 2012; Järlskog et al., 2021; Zgheib et al., 2012). For example, Zgheib et al., (2012) found concentrations of Cu, Pb and Zn in stormwater particles that exceeded guideline values. Upon entering aquatic environments, particles and their associated pollutants will at some point settle at the benthic zone and may result in adverse effects on sediment dwellers.

The mortality rate of 100% in the retention basin sediments could be explained by their pollutant load and toxic potential based on the calculated probability of toxicity. This concurs with a similar study by Galluba et al. (2012) where 100% mortality of *L. variegatus* was recorded in sediment samples with high metal and PAH content. Also, Sardo and Soares (2011) recorded more than 50% mortality at Pb concentrations similar to those of the present study. However, it was surprising to find equally high mortality in RRB1 that showed half the toxic potential of site RRB2. This might be partly explained by confounding factors that may affect toxicity in addition to the pollutant load. For example, factors such as the high amount of organic matter in RRB sediments may have contributed to the observed mortality rate due to oxygen depletion from increasing biological oxygen demand (BOD) for decomposition of the organic matter (Hyland et al., 2005).

The higher or similar reproduction rate in the field sediment compared to the control could be attributed to premature architomy from confounding factors such as heterogeneous particle sizes that might have induced mechanical injury resulting in fragmentation. Similar observations have been reported in previous studies where *L. variegatus* was subjected to sediments samples with different levels of contamination (Feiler et al., 2013; Höss et al., 2010). Although architomy is a natural and predominant mode of reproduction in *L. variegatus* (Christensen, 1984; Martinez et al., 2006), it is also used by the organism as an escape mechanism when trapped between sediment particles. Therefore, the lower fragmentation rate observed in the more homogenous control sediment compared to the field sediment (Table S12) could be explained by the fact that the worms always detached part of their segments when caught between the heterogeneous field sediment particles. Additionally, regeneration of a new head following fragmentation has been reported to be hindered by some pollutants (Martinez et al., 2006; Sardo et al., 2011). For example, Sardo et al., (2011) observed a reduction in segment regeneration ability of *L. variegatus* following exposure to Pb. Hence it can be argued that Pb which occurred at concentrations above TEC in all sites could have inhibited segment regeneration following fragmentation, therefore more incomplete worms.

Unlike reproduction, all sediments from the stream caused an inhibition in biomass increase compared to the control. The suboptimal biomass gain in the field sediment can be explained by the inability of fragmented worms to feed (Leppänen and Kukkonen, 1998). Growth retardation has been observed following fragmentation upon exposure to environmentally relevant concentrations of organic substances such as bisphenol (Vought and Wang 2018). Hence it is likely that the effect on biomass was as a result of the high fragmentation rate in the sediments. Another plausible explanation of the reduced biomass could have been the neurotoxic effect on AChE activity which may have resulted in more energy consumption due to high stress levels. However, due to the lack of a significant correlation between these endpoints and the pollutant loads, this assumption could not be validated.

At the sub-organism level, biomarkers have been recommended in *L. variegatus* as sensitive parameters that can quickly react to pollution and stress in their immediate environment (Contardo-Jara et al., 2009; Contardo-Jara and Wiegand, 2008; Wiegand et al., 2007). The lack of correlation between the biomarkers and the concentrations of xenobiotics in sediment was not in agreement with similar studies involving *L. variegatus* (Contardo-Jara and Wiegand, 2008; Wang et al., 2014). For example, Contardo-Jara and Wiegand (2008) observed a significant increase in catalase activity following 4 days of exposure to sediment samples with pollutant concentrations (for example Zn) about 10-fold greater than in the present study. Wang et al (2014) on the other hand used artificial sediment spiked with their investigated pollutant. The differences in pollutant concentrations, exposure time and experimental setup in the present study could be a possible explanation for the observed effect on the biomarkers. Additionally, it could also be possible that the responses of CAT and MT in our study were influenced by confounding factors or the bioaccumulated substances in the worms were not above threshold levels that can trigger a biochemical response greater than in the control sample. Contrary to CAT and MT, AChE showed a consistent but not significant inhibition relative to the control in all but one site (Ref.2). In addition to pesticides that inhibit AChE activity in *L. variegatus* (Contardo-Jara et al., 2009; Kristoff et al., 2006, 2008, 2010), PAH and metals have also been documented to possess neurotoxic potentials by inhibiting AChE activity (Frasco et al., 2005; Fu et al., 2018; Kais et al., 2015; Ricciardi et al., 2006). For example, Kais et al. (2015) recorded high AChE inhibition in PAH contaminated sediment. Similarly, metals were found to inhibit AChE activity in zebrafish (de Lima et al., 2013). Hence the presence of different potential neurotoxic substances in the sediment samples could be responsible for the observed effect on AChE activity especially from 100 m onward.

Conclusion

The results of this study showed that the investigated retention basins were not the major source of sediment pollution downstream of their respective outfalls in the stream but are important in retaining particle-bound pollutants. The detected pollutants in the sediment of the stream could not be attributed to discharges of the retention basin during intense rainfall events. Other pollution sources further upstream including atmospheric deposition seem to be more relevant. The latter, and the flow characteristics of the stream could explain why there was no clear difference in pollutant load between the site upstream of the inlets and the corresponding downstream sites. The anthropogenic enrichment of Cu, Pb and Zn, as well as the fact that Pb and Zn values above the impact threshold were found in all sediment samples, further support the argument that other sources of pollution predominate in the stream. Results of ecotoxicity experiments showed that the high concentration of pollutants in the RRBs led to the mortality of worms. Contrary to the observation in the RRBs sample, experiments with sediment from the stream did not result in the death of worms. Nevertheless, the endpoint growth and the activity of neurotransmitter enzyme AChE were sensitive to the stream sediment and associated pollutants Although, the responses of these endpoints did not correlate with the pollutant load in the sediment.

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4 Chapter 3: Micropollutant-loaded powdered activated carbon released from wastewater treatment plants – a risk for sediment-dwelling organisms?

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Micropollutant-loaded powdered activated carbon released from waste water treatment plants: a risk for sediment-dwelling organisms?

Marion Woermann[*†], Julios Armand Kontchou[†] and Bernd Sures

Abstract

Background: In order to protect aquatic environments and to reduce the presence of micropollutants in the global water cycle, wastewater treatment plants (WWTPs) often implement an additional treatment step. One of the most effective measures is the use of powdered activated carbon (PAC) as an adsorbent for micropollutants. This method provides sufficient elimination rates for several micropollutants and has been successfully employed in many WWTPs. Despite this success, there might be a drawback as the retention of the PAC in the WWTP can be challenging and losses of micropollutant-loaded PAC into the aquatic environment may occur. Upon emission, micropollutant-loaded PAC is expected to settle to the benthic zone of receiving waters, where sediment-dwelling organisms may ingest these particles. Therefore, the present study investigated possible adverse effects of micropollutant-loaded PAC from a WWTP as compared to unloaded (native) and diclofenac-loaded PAC on the sediment-dwelling annelid *Lumbriculus variegatus*.

Results: Native PAC induced the strongest effects on growth (measured as biomass) and reproduction of the annelids. The corresponding medium effective concentrations (EC_{50}) were 1.7 g/kg and 1.8 g/kg, respectively. Diclofenac-loaded PAC showed lower effects with an EC_{50} of 2.5 g/kg for growth and EC_{50} of 3.0 g/kg for reproduction. Although tested at the same concentrations, the micropollutant-loaded PAC from the WWTP did not lead to obvious negative effects on the endpoints investigated for *L. variegatus* and only a slight trend of a reduced growth was detected.

Conclusion: We did not detect harmful effects on *L. variegatus* caused by the presence of MP-loaded PAC from a WWTP which gives an auspicious perspective for PAC as an advanced treatment option.

Keywords: Oligochaete, Environmental impact, Advanced wastewater treatment, *Lumbriculus variegatus*, Sediment toxicity

Background

The occurrence of micropollutants (MPs) in aquatic environments is of growing concern worldwide due to their persistence and their potential harmful effects on aquatic organisms. Individually, as well as in mixtures, MPs exhibit a variety of toxic effects on organisms as well as on communities of freshwater biota [1–3]. MPs comprise mainly pharmaceuticals, personal care products, hormones, surfactants, industrial chemicals and pesticides [4] and are primarily discharged into surface waters by wastewater treatment plants (WWTPs) [5]. In order to reduce the release of these MPs into freshwater bodies, many WWTPs are currently upgrading their treatment protocols by applying additional treatment steps [6]. There are various possible options and the two techniques that are mainly applied are advanced oxidation processes (i.e., ozonation) or adsorption on activated

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carbon (AC) [7]. Application of ozonation is intended to mineralize MP and thereby to reduce their toxic potential. However, several MPs cannot be completely degraded which can result into undesired transformation products that can exhibit even a higher toxicity than the original MP [7–9]. The advantage in using adsorption techniques for MP elimination is the physical removal of MPs from the wastewater instead of their degradation [6, 10].

The use of powdered activated carbon (PAC) as an adsorbent has already been successfully implemented in several WWTPs [11–14]. PAC can be applied in a contact reactor after the biological treatment followed by its separation with sedimentation and filtration [10, 12, 15]. Moreover, it is possible to optimize the use of the PAC's binding capacity by recirculation of PAC within the WWTP [11, 16]. Several studies reported successful removal rates of 80% and more for many investigated MPs [11–14]. Furthermore, corresponding to the reduced MP concentrations in the effluent a significant reduction of toxicological effects in the form of improved invertebrate health, reduced genotoxicity and positive ecological changes were described by Peschke et al. [3], Stalter et al. [17] and Triebskorn et al. [14], respectively. Nevertheless, there is a drawback which is associated with the retention of PAC in the WWTP. The complete separation of PAC from the effluent appears to be challenging in order to prevent losses of MP-loaded PAC into the aquatic environment via the effluent [15, 18, 19]. Therefore, it is possible that adverse effects are associated with the introduction of MP-loaded PAC into the receiving waters with yet unknown ecological consequences. In previous studies, PAC from a WWTP did not cause negative effects to two filter-feeding invertebrates, the pelagic *Daphnia magna* [20] or benthic *Corbicula* sp. [21], which might be partly explained by sedimentation of PAC. It is expected that PAC from WWTP after entering receiving waters will mainly deposit in the sediment and therefore pose a risk to endo-benthic organisms rather than to pelagic or epi-benthic species. Accordingly, the question arises if sediment-dwelling organisms are negatively affected by the presence of MP-loaded PAC.

So far, only ecotoxicological studies on PAC in sediments in connection with sediment remediation by addition of unloaded activated carbon at contaminated sites are available. These studies showed that activated carbon can lead to a reduction in reproduction and growth in the sediment inhabiting annelid *Lumbriculus variegatus* [22–24]. Field application of PAC was shown to have a general negative effect on the benthic community structure with reduction in species abundance and biomass [25, 26]. Moreover, it was discovered that particles smaller than 100 µm are ingested by *L. variegatus* and that the powdered form of activated carbon was significantly more toxic than larger particles [27]. This underlines the need to assess possible adverse effects of MP-loaded PAC from WWTPs.

Therefore, the aim of the present study is to investigate possible harmful effects of MP-loaded PAC from WWTPs and to evaluate whether the release of MP-loaded PAC poses an ecological risk for sediment-dwelling organisms. For this approach, sediment–water toxicity experiments according to OECD guideline 225 [28] were conducted in which individuals of *L. variegatus* were exposed to micropollutant-loaded PAC from a WWTP as compared to unloaded (native) and diclofenac-loaded PAC. *L. variegatus* was chosen because it plays an important role in aquatic ecosystems and it is frequently used in eco-toxicity testing for risk assessment [29–31]. Moreover, by using annelids several uptake routes are covered, i.e., via the sediment (ingestion and direct contact) and via the pore and overlaying water [32].

Materials and methods

Test substances

In order to examine possible adverse effects to the sediment-dwelling annelid *L. variegatus*, sediments were spiked with different types of PAC as shown in Table 1. In order to have an intermediate test substance between the unloaded PAC and the MP-loaded PAC from the WWTP, PAC was loaded with the well-known micropollutant diclofenac.

The activated carbon used for the experiments, PAC Norit® SAE Super [average particle size of 15 µm, total surface area (B.E.T.) of 1150 m^2/g, Cabot Corporation, USA], was supplied by the wastewater treatment plant in Dülmen, North Rhine-Westphalia, Germany (capacity of 55,000 population equivalents, operated by the Lippeverband). Acquisition of the MP-loaded PAC (PAC_{WWTP}) as well as preparation of the other two PAC types is described in detail in Woermann and Sures [20]. Briefly, unloaded PAC was freeze-dried (Heto PowerDry LL3000, Thermo Electron Corporation) and either used as PAC_{native} in the exposure experiments or was loaded in the laboratory with diclofenac (DCF, Cayman Chemical Company, USA). For this approach, 1 g PAC was added

Table 1 Types of PAC used for exposure of *Lumbriculus variegatus*

PAC type	Group name
Micropollutant-loaded PAC from WWTP	PAC_{WWTP}
Native, unloaded PAC	PAC_{native}
Diclofenac-loaded PAC	PAC_{DCF}

to 200 mL of a 0.4 g/L DCF solution and agitated for 24 h which resulted in a loading of 80 mg DCF per g PAC. The MP-loaded PAC (PAC_{WWTP}) was collected from the sedimentation basin of the treatment plant in Dülmen during 2 weeks in April 2017. PAC_{DCF} and PAC_{WWTP} were freeze-dried as well to establish the same condition for each PAC type.

Test organism

The tests were conducted with the annelid *Lumbriculus variegatus* similar to the procedure described by Kontchou et al. [31]. Briefly, individuals of *L. variegatus* used to establish an own culture originally came from the Department of Aquatic Ecotoxicology at the Goethe University Frankfurt am Main, Germany. The culture was kept in a climate chamber at a temperature of 20 ± 1 °C with a 16:8 h light:dark cycle. The worms were grown in 15 L glass aquaria containing a small layer of prewashed quartz sand (Baumit GmbH, Germany) as sediment and reconstituted water as described in the OECD guideline 225 [28]. Aquaria were constantly aerated and worms were fed twice a week with ground TetraMin flakes (Tetra GmbH, Germany) following the renewal of overlying reconstituted water.

The sensitivity of the worms to harmful substances was validated using pentachlorophenol (PCP, 99% pure, Sigma-Aldrich, USA) as recommended by the OECD guideline. Briefly, adult worms were removed from the culture and kept in clean reconstituted water for 24 h. A stock solution of 5 mg/L PCP was prepared in reconstituted water and diluted to 2500, 1000, 500, 250, 100 and 50 μg/L, respectively. The pH of the dilutions was adjusted to a value between 6.4 and 7 with 0.1 M NaOH. Reconstituted water without PCP was used as the negative control. For each concentration, ten replicates were performed in 30 mL beakers comprising one adult worm. The experiment was run for 96 h under the same light and temperature conditions as the culture. After the termination of exposure, mortality of worms was recorded.

General test design

The exposure experiments were designed following the sediment–water toxicity test as described in the OECD guideline 225 [28]. For this approach, synchronized individuals of *L. variegatus* were exposed to the test substances spiked into the reconstituted sediment in a series of at seven concentrations along with a negative control. Each PAC compound was tested in concentrations of 0.5, 1, 1.5, 2, 3, 4 and 5 g/kg dry weight (dw). The control sediment was prepared without PAC addition. These test concentrations were chosen based on preliminary testing (data not shown) and in order to cover the EC_{50} as recommended by the OECD guideline. For each concentration and control, four replicates were prepared containing ten synchronized annelids resulting in 32 test beakers per exposure experiment.

Synchronization

Fourteen days prior to the start of the PAC exposure experiments, adult worms were synchronized as described in OECD 225 guideline [28]. After cutting the worms in two pieces, the posterior ends were used for the experiment while the anterior segments were returned to the culture. The posterior segments were cultured in a separate tank for 14 days for head development. They were kept under the same conditions as the normal culture, but without food supply and renewal of overlying water. Head development was evident after 7 to 8 days when worms burrowed into the sediment. After 14 days of growth, the worms were assumed to be of equal developmental stage and ready for exposure.

Test sediment preparation

Artificial sediment used in this study was prepared following OECD 225 guideline and contained quartz sand (size 0–1 mm, Baumit GmbH, Germany), kaolinite clay (VWR International, Belgium), peat (Floragard GmbH, Germany) and nettle powder (*Folia urticae*; Heinrich Klenk GmbH & Co. KG, Germany) at a ratio of 75%, 20%, 5% and 0.5% dry weight, respectively. Prior to use, the sand was properly washed with deionized water and dried at 70 °C. A dry mass of 20 g air-dried peat (< 0.5 mm particle size) was weighed into an 800 mL glass beaker followed by addition of 200 ± 20 mL deionized water (approximately 10 mL per gram of peat dry weight). The suspension was properly homogenized by stirring with a spatula and pH adjusted to between 5 and 5.5 using $CaCO_3$ (98%; Acros Organics, Belgium). The suspension was incubated for 48 h at room temperature on a shaker (Celltron, Infors HT, Switzerland) at a speed of 120 rounds per minute (rpm) in order to establish a stable microbial content in the peat suspension. After 48 h incubation, the pH of the suspension was measured and adjusted with $CaCO_3$ if required to a final value of 6 ± 0.5. The residual dry components, e.g., sand (300 g), clay (80 g) and nettle powder (2 g) were mixed together before adding the respective amounts of PAC_{native}, PAC_{DCF} and PAC_{WWTP}. The peat suspension was then added to the dry mixture and the sediment was thoroughly homogenized. Formulated sediment samples were used immediately and no storage was required. The treatments were distributed into 300 mL glass beakers and reconstituted water was added to achieve a 1:4 sediment:water ratio. The setup was aerated with the help of glass Pasteur pipettes and equilibrated for 24 h at 20 ± 1 °C with a light:dark circle of 16:8 h.

Sediment–water toxicity test

The test was carried out in order to obtain data for the endpoints reproduction (increase in number of worms) and growth (increase in dry biomass) and were conducted according to the OECD guideline 225 [28]. Following the equilibration phase, water parameters (temperature, oxygen, pH and electrical conductivity) were checked and ten worms from the synchronized batch were carefully added to each exposure vessel. In order to obtain the initially inserted biomass, a sample of reference worms from the synchronized batch were collected and stored at − 80 °C. The experiments were run for 28 days in a static exposure and water parameters were recorded every week. Moreover, the water level was refilled regularly with deionized water to compensate for losses due to evaporation and to keep the 1:4 sediment:water ratio. On the last day of exposure, water characteristics were recorded and the ammonium content was measured using the Aquamerck® Ammonium Test (Merck KGaA, Germany). Worms were carefully extracted, counted and frozen at − 80 °C for subsequent lyophilization (Heto PowerDry LL3000, Thermo Electron Corporation) and determination of dry weight (Labor AEG-220, Shimadzu, Japan). Additionally, at the end of the exposure photos were taken to visualize the moving activity of the test animals within the sediment.

Statistical analysis

The calculation and statistical analysis of the obtained data was performed with the program GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). The calculated effects of the endpoints reproduction and growth were plotted against the log-transformed exposure concentrations. A nonlinear regression analysis was carried out with a logistic four-parameter model with variable slope. In this analysis, the EC_{50} values (half maximum effective concentration) were calculated for reproduction and growth inhibition. Moreover, to investigate significant differences between control and exposed treatments Kruskal–Wallis test with the subsequent Dunn's multiple comparison test was applied. The significance level was set to $\alpha = 0.05$.

Results

Validity of the toxicity tests

The performed reference tests with pentachlorophenol (PCP) in a 96 h water only exposure resulted in LC_{50} value of 198.5 µg/L which was within the range of previous studies [33, 34]. The validity criteria according to OECD guideline 225 [28] for the tests were also met, as the average number of worms in the control increased by a factor of at least 1.8 except in the PAC_{DCF} test group where the number of worms only increased by a factor of 1.35. The dissolved oxygen concentration during the test was always above 30% of air saturation value at test temperature and the pH of the overlying water was between 6 and 9. Also, the measured ammonium concentration in the overlying water at the end of the test was below 0.5 mg/L in all treatments. The obtained TOC value was 1.8 ± 0.1% of the sediment dry weight.

PAC exposure

Following exposure to PAC_{native}, strong effects on the reproduction of the worms could be detected (Fig. 1). In the two highest tested concentrations the worms did not reproduce at all and only the ten initially inserted worms were recovered. The Kruskal–Wallis test resulted in a significant difference ($H = 26.2$, $p = 0.0005$) and the post hoc test revealed that the two highest concentrations were significantly different to the control group.

A similar trend was found in the experiments with PAC_{DCF}, where not even all ten worms could be recovered in the two highest concentrations at the end of the exposure (Fig. 2). The Kruskal–Wallis test displayed a significant difference to the control ($H = 20.2$, $p = 0.006$) which could not be confirmed by the post hoc test. In general, the worms reproduced less in the exposure with PAC_{DCF} including the negative control. Therefore, the validity criterion for this test was not met. It is also obvious that the

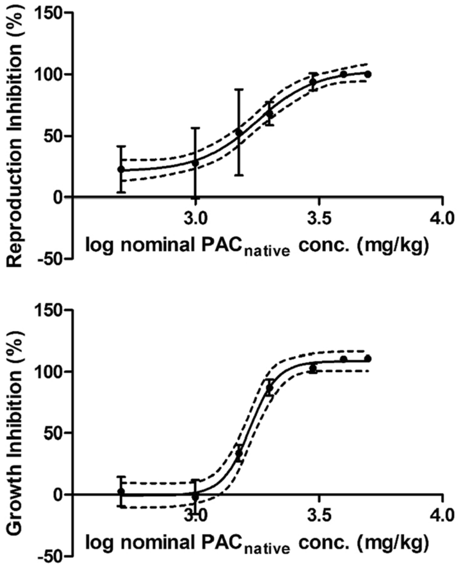

Fig. 1 Concentration–response curves for EC determination of reproduction (top) and growth (bottom) of *Lumbriculus variegatus* after 28 days of exposure with PAC_{native} using the nominal concentrations; circle symbols represent means with standard deviations of the four beakers; dashed lines show 95% confidence bands

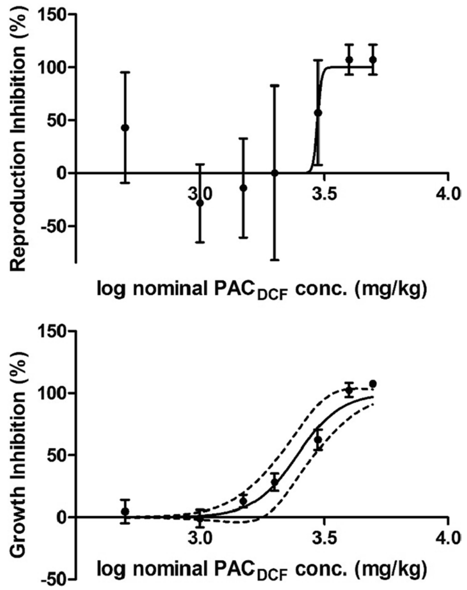

Fig. 2 Concentration–response curves for EC determination of reproduction (top) and growth (bottom) of *Lumbriculus variegatus* after 28 days of exposure with PAC_{DCF} using the nominal concentrations; circle symbols represent means with standard deviations of the four beakers; dashed lines show 95% confidence bands (could not be determined for the reproduction inhibition). This test was not valid according to the OECD guideline 225 regarding the reproduction in the control

standard deviations between the four replicates are comparatively high with regard to reproduction.

The worms that were exposed to PAC_{WWTP} did not show any negative effects with respect to reproduction (Fig. 3). The reproduction at the two highest concentrations was even higher than in the control, but not significantly ($H = 7.26$; $p = 0.402$). However, the standard deviations were found to be high as well.

The concentration–response curves for the growth inhibition (based on dry weight gain) give a similar picture. For PAC_{native} and PAC_{DCF} they show the typical sigmoid curve for concentration response relations (Figs. 1 and 2). The worms in these two groups show a very clear and significant effect concerning growth. According to the statistical analysis, significant differences to the control were detected for 4 and 5 g/kg PAC_{native} and for 5 g/kg PAC_{DCF} (PAC_{native}: $H = 28.97$, $p = 0.0002$; PAC_{DCF}: $H = 28.59$, $p = 0.0002$). In these highest concentrations they did not only gain less weight than the worms from the control, but instead they even lost weight over the exposure period of 28 days when compared to the initial dry body weight. The tested individuals from the PAC_{WWTP} experiment did not show significant differences ($H = 8.725$, $p = 0.2732$) compared to the control with regard to growth. Nevertheless, a trend is visible that the animals exposed to the higher concentrations grew less than the control (Fig. 3). It should also be noted that the standard deviations are particularly lower for the endpoint growth in contrast to the endpoint reproduction in all treatments. The corresponding EC_{50} values to all the graphs are given in Table 2.

The EC_{50} values confirm the patterns of the concentration response curves. PAC_{native} showed the highest adverse effects as the lower values of PAC_{native} in comparison to PAC_{DCF} indicate. Consequently, PAC_{DCF} is less harmful and PAC_{WWTP} was not found to have any effects at all during the experiments. In general, EC values for the endpoint growth are slightly lower than for reproduction indicating a slight tendency towards higher

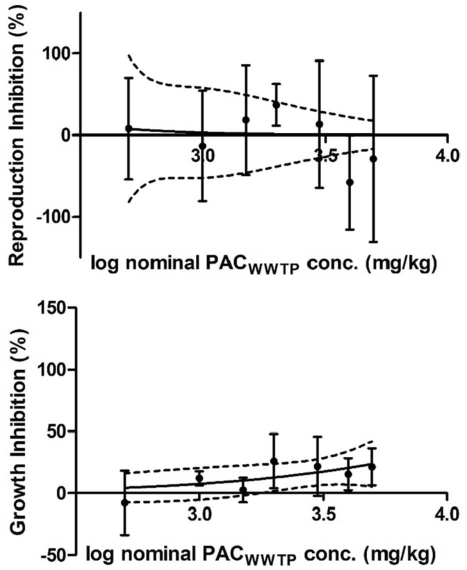

Fig. 3 Concentration–response curves for EC determination of reproduction (top) and growth (bottom) of *Lumbriculus variegatus* after 28 days of exposure with PAC_{WWTP} using the nominal concentrations; circle symbols represent means with standard deviations of the four beakers; dashed lines show 95% confidence bands

Table 2 Calculated EC_{50} values of reproduction and growth inhibition with corresponding 95% confidence intervals (in parentheses) from the tests with *Lumbriculus variegatus* (please note that the values are given in g/kg and not in mg/kg as usual)

Test substance	EC_{50} reproduction inhibition	EC_{50} growth inhibition
PAC_{native}	1.8 g/kg (1.6 to 2.0)	1.7 g/kg (1.6 to 1.8)
PAC_{DCF}	3.0 g/kg (n.d.)	2.5 g/kg (2.1 to 2.9)
PAC_{WWTP}	n.d.	n.d.

n.d. not determinable

sensitivity for the endpoint growth. Further to these evaluations, it was observed during the exposures with PAC_{native} and PAC_{DCF} that the worms in the two highest concentrations did not burrow as deep as the animals in the other concentrations and the control (Fig. 4a, b). These animals resided only in the top layer of the sediment, whereas all other test animals burrowed through the whole sediment. The worms in the PAC_{WWTP} exposures moved through the whole sediment even at the highest concentration of 5 g/kg and no avoidance behavior was observed (Fig. 4c).

Discussion

The aim of the present study was to investigate possible negative effects of MP-loaded PAC to *L. variegatus* as a representative for sediment-dwelling organisms. With the chosen test-setup, chronic effects on reproduction and growth could be observed. Generally, it was noticed that growth was the more sensitive endpoint than reproduction and that the variation was also lower. This can be explained by the type of reproduction of *L. variegatus* which only reproduce asexually by fragmentation under laboratory conditions. After termination, some test vessels contained many small worms, whereas in other replicates fewer but larger individuals were found that had not yet fragmented at that time. The biomass between the replicates was more equal which explains the lower standard deviation for the endpoint growth (gain of biomass). Therefore, growth was found not only to be the more sensitive, but also the more robust endpoint in the present study. Moreover, these results indicate that the exposure period of 28 days may not have been long enough for the asexual reproduction to have been completed in all test beakers. Therefore, it is possible that the presence of PAC may have delayed the reproduction.

Regarding the toxicity of PAC_{native}, PAC_{DCF} and PAC_{WWTP}, results of the present study are in line with the results from previous studies where exposure experiments were conducted with *Daphnia magna* [20] and *Corbicula* sp. [21]. Similarly, PAC_{native} induced the strongest effects, whereas PAC_{WWTP} did not lead to any negative responses [20, 21]. The evaluation of toxic mechanism was not within the scope of this study, however, possible explanations will be discussed briefly based on the obtained results and current knowledge. The strong effects, especially on growth, caused by PAC_{native} indicate either direct toxic effects or reduced nutrient availability during the exposure treatments. In studies conducted to assess toxicity of activated carbon amendments in sediments of contaminated sites, similar effects

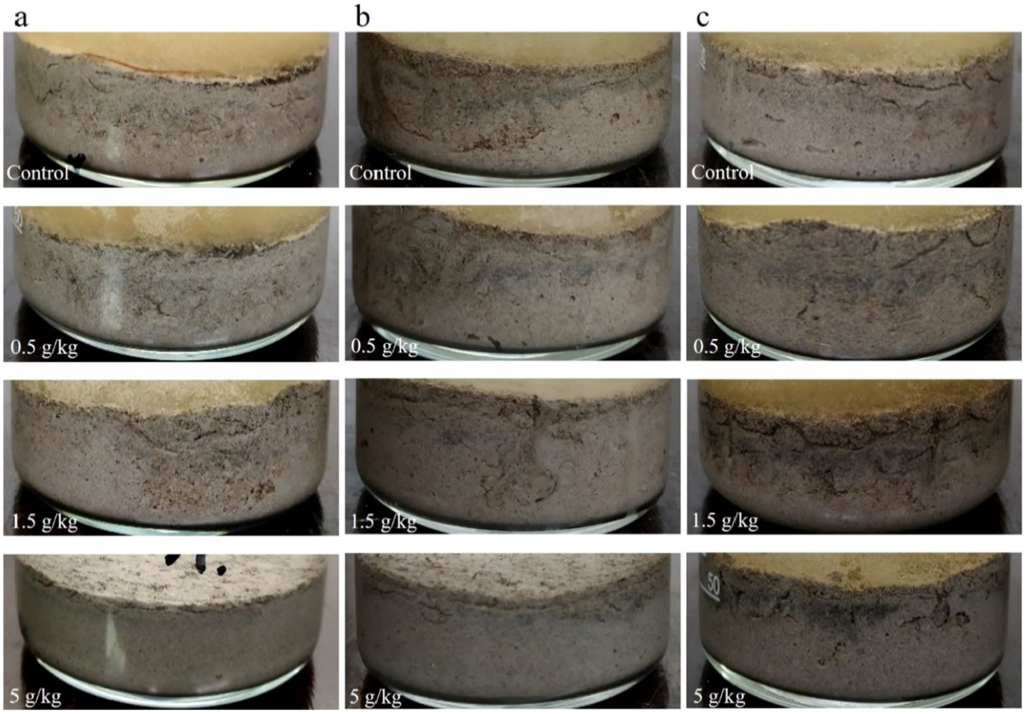

Fig. 4 Photo documentation of the test vessels from *Lumbriculus variegatus* exposure experiments after 28 days. Depicted are the test sediments of the negative control and three selected concentrations from exposures with **a** PAC_{native}, **b** PAC_{DCF} and **c** PAC_{WWTP} (the overlaying water phase is not shown due to reasons of space). Pictures were brightened in PowerPoint to increase visibility

on *L. variegatus* were found. For example, Nybom et al. [27] detected a significant decrease in biomass already at a concentration of 0.05% PAC in artificial sediment (dw). The EC_{50} value on biomass described by Nybom et al. [27] was 0.35% (corresponding to 3.5 g/kg) which is comparable to the EC_{50} values calculated for PAC_{DCF} and PAC_{native} in the present study (2.5 g/kg and 1.7 g/kg on biomass, respectively). Furthermore, the egestion rate of the worms was investigated which is directly related to sediment ingestion and it was observed that the worms tried to avoid the AC exposure by a reduced or even terminated ingestion of the sediment. The same trends were reported by Abel et al. [22], Nybom et al. [35], Jonker et al. [24] and Han et al. [23]. In the present study, egestion and ingestion were not directly measured, but it was observed that the worms in the higher concentrated PAC_{native} and PAC_{DCF} treatments were less active and stayed in the upper layer of the sediment (see Fig. 4a, b). Therefore, the reduced biomass can most likely be explained to a great extent by avoidance of sediment ingestion and an associated reduced food uptake. Conversely, it can be concluded that during the PAC_{WWTP} exposure, food uptake by the worms was not affected as they burrowed through the whole sediment and did not show avoidance behavior.

An explanation why PAC_{WWTP} did not lead to any negative effects regarding the reproduction and growth nor any avoidance behavior might be due to the application and processing of PAC during its residence in the WWTP. Its use could possibly have smoothed the surface of the PAC and led to less sharp particles. In studies of Abel et al. [22] and Nybom et al. [35], it was found that the exposure with AC led to a decreased microvilli layer inside the gut wall in *L. variegatus* which can possibly be explained by damage due to sharp AC particles. This mechanical damage may as well have played a role in the observed avoidance behavior in the higher concentrations of PAC_{native} and PAC_{DCF}. Moreover, in the previous study conducted by Woermann and Sures [20] it was observed that PAC_{native} and PAC_{DCF} did adhere to the cuticle of the daphnids, which led to obstruction of movements of *D. magna*. This could also be the case for *L. variegatus* and explain why the worms moved less in these treatments. Jonker et al. [24] suggested that AC could adsorb skin constituents (e.g., mucus) which could lead to the impairing effects.

Additionally, it is very likely that PAC adsorbs nutrients [36, 37] both externally from the sediment and also internally within the intestine of the annelids. Consequently, the high capacity of free binding sites of PAC_{native} would be responsible for the observed adverse effects. This would also explain why PAC_{WWTP} did not show any inhibitory influence as the PAC was probably efficiently loaded during its application in the WWTP. This hypothesis is underlined by the fact that PAC_{DCF} whose adsorption capacity has not been exploited was less harmful than PAC_{native}. The minor negative tendency on the biomass that was detected in the higher concentrations with PAC_{WWTP} may be explained by a reduced food intake with increasing amounts of PAC_{WWTP} in the sediment and that PAC_{WWTP} has no nutritional value. Generally, evaluation of detailed toxic mechanisms of the different PAC types applied was not the objective of this study, but should be addressed in future studies.

As a last point, it should be highlighted that the experimental setup was designed to test at a concentration range in which adverse effects will occur. Therefore, the tested concentrations are rather high and probably orders of magnitude higher than what would be expected to be reached in the aquatic environment. Still in the present study, no negative effects were induced by PAC_{WWTP} in *L. variegatus*. The exact amount of PAC being released from WWTPs is to date still unknown as is the environmental fate and both topics should be the subject of future research. Up to now, leakages are considered to be small [38]. However, in standard operation also small amounts of PAC that are continuously released may settle in waters with low disturbance and will accumulate over time in sediments. Therefore, it is important to keep losses as low as possible through optimization efforts. Overall, it should be emphasized that the results of this study strongly support that PAC emissions from WWTPs are not associated with negative consequences for sediment-dwelling organisms.

Conclusions

The present study investigated possible adverse effects of native PAC, DCF-loaded PAC and MP-loaded PAC from a WWTP in a sediment–water test system with *L. variegatus*. The annelids exposed to PAC_{native} and PAC_{DCF} showed a reduced gain in biomass and a reduced reproduction. Moreover, it was observed that *L. variegatus* avoided sediments spiked with high amounts of PAC. In contrast to that, *L. variegatus* did not show any negative effects when exposed to sediment spiked with PAC_{WWTP}, although very high concentrations were tested that would widely exceed expected values in the environment. Therefore, together with the results from previous studies with *D. magna* [20] and *Corbicula* sp. [21] the present study indicates that leakages from WWTPs are unlikely to pose a hazard for aquatic organisms. However, it is desirable to perform more research with more sensitive endpoints and longer exposure periods in order to ensure safe handling and avoid negative consequences for aquatic wildlife. In conclusion, the results of the present study are an important contribution for the risk assessment of PAC_{WWTP} and give a good perspective for PAC as a safe removal option for MPs in WWTPs.

Abbreviations

AC: Activated carbon; DCF: Diclofenac; dw: Dry weight; MPs: Micropollutants; PAC: Powdered activated carbon; PAC_{DCF}: Diclofenac-loaded PAC; PAC_{native}: Unloaded, native PAC; PAC_{WWTP}: MP-loaded PAC from a WWTP; PCP: Pentachlorophenol; WWTPs: Wastewater treatment plants.

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Authors' contributions

BS, JK and MW designed the experimental setup. The exposure experiment and data analyses were carried out by MW and JK to equal parts. MW and JK wrote the draft manuscript including the preparation of all pictures and tables. Final reviewing and editing of the manuscript was done by BS and MW amended the manuscript accordingly. All authors read and approved the final manuscript.

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Availability of data and materials

Material and data are described in the main article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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5. General discussion

The present thesis addresses the fate and impact of particle-bound pollutants on receiving waters following stormwater discharges in separate sewers and discharges of WWTP with advanced PAC treatment. Previous works on particle-bound pollutants have shown that particle-bound pollutants may play a vital role in the degradation of aquatic environments in terms of their chemical and biota status. However, the extent to which this is true depends on the pollutant load and types of particles entering surface water bodies which are influenced by the anthropogenic characteristics of the source. Therefore, the present thesis explores the possible adverse impacts of particle-bound pollutants on receiving water bodies from two potential sources: stormwater sewers (of highway road, chapter 1 and a multipurpose land-use catchment, chapter 2) and wastewater treatment plants (chapter 3). Accordingly, stormwater from the traffic area results in the accumulation of traffic-related metals in the retention basin followed by downstream from the inlet compared to the reference site upstream. Following the determination of traffic-related metal accumulation patterns in sediment, pollutant bioaccumulation studies in field-collected amphipods and *L. variegatus* revealed a similar pattern to the sediment concentrations. However, a clear toxic effect on the growth and reproduction of *L. variegatus* was not observed following the sediment toxicity experiment. Nonetheless, the more sensitive biomarker endpoint, metallothioneins (MT) recorded high induction in the annelids exposed to metal accumulation hotspots in the retention basin and downstream of the inlet compared to the upstream reference sample. Similar to chapter 1 sediment from the retention basins in chapter 2 recorded the highest load of pollutants when compared to all transect samples in the stream. The distribution of pollutants in sediment downstream of the stormwater outfalls suggested sources of pollution other than the retention basin discharges, e.g., diffuse sources or sources further upstream. This was further supported by the enrichment of these metals upstream of the first retention basin outfall. The highly polluted retention basin sediments resulted in lethal effects on *L. variegatus* with 100 % mortalities. Sediment samples from the stream did not show considerable effects on reproduction, catalase and metallothionein. However, there was modest inhibition of growth and acetylcholinesterase activity. In the third chapter, the toxicity of micropollutants loaded particles was investigated. This was to determine whether the emission of particulate micropollutants following activated carbon treatment of wastewater is detrimental to sediment-dwelling organisms. Here the toxicity test was conducted following the OECD 225 guideline (OECD 2007). Upon assessment of burrowing behaviour, growth, and reproduction, it was observed that particles loaded with micropollutants were less toxic to *L. variegatus* than native particles with active binding sites.

Based on the pollutant concentrations in sediment samples of chapters 1 and 2, it is obvious that sedimentation of particles from anthropogenic sources was the major reason for the accumulation of pollutants at the studied sites. It is most likely that the sources of the investigated substances in these chapters are the different land-use activities of the catchments. For example, the metals Cd, Co, Cu, Mn, Mo, Ni, Pb and Zn in chapter 1 are among the typical metals emitted by traffic activities on road surfaces (Müller et al., 2020, Hubber et al., 2016). These metals are released on road surfaces from the wear and tear of vehicles and road construction materials. For example, particulate Cd, Cu, Mn, Ni, Pb and Zn emission are associated with wears of tyres, brake pads, engine, asphalt surface and welded body parts (Ball 2002, Hjortenkrans et al., 2007, Mckenzie et al., 2009, Ward et al., 1990, Sörme et al., 2000, Loganathan et al., 2013). The use of Pb and Co in paint on vehicles and Mn in gasoline has been linked to their presence in traffic-related emissions (Geivanidis 2003, Huber et al. 2016, Kayhanian 2012). Given their high association to the particulate phase, sedimentation and accumulation of these metals in the sediment of aquatic environments are inevitable. Ruchter et al. (2015) recorded in river sediments downstream of a traffic runoff inlet in the river Alb, Germany, concentrations of Cd, Cu, Ni, Pb, and Zn in the respective range of 0.13 – 0.32, 9.6 -178, 9.8 – 33, 15 – 100 and 57 – 209 mg/kg which are in the similar orders of magnitude as those reported in the traffic affected stream in the present study.

For the catchment in chapter 2 with different land-use activities, particle-bound pollutants in stormwater may come from different sources including buildings, gardens, streets, and parking lots. Göbel et al. (2007) compared the influence of different road surfaces (motorways, main roads, pedestrian roads, cycleways and residential yards) on pollutant contribution in stormwater and concluded that the surfaces contribute differently to the distribution and load of stormwater pollutants. Additionally, materials used on roofs are important sources for metals like Cd, Cu, Ni, Pb, V and Zn as well as PAHs in stormwater (Andersson et al., 2015, Brown and Peake 2005, Gromaire 1999, McIntyre et al., 2019, Müller et al., 2019). The steel materials used in most urban structures are known to contribute to the concentrations of Fe, Mn and Ni in urban runoffs (Raffo et al., 2016). The use of biocides on buildings too has been reported to result in their release to stormwater hence pollution of surface (Gromaire et al., 2015, Bucheli et al., 1998, Jungnickel et al., 2008). Also, paints used on different infrastructures in urban catchment and facades are identified as important sources of PCB (Jartun et al., 2009, Andersson et al., 2004). Flame retardants found in almost all household and industrial products including building materials are often detected in freshwater sediment and urban runoff has been identified as a major contributor Jans 2016, La Guardia et al., 2013, van der Veen and de Boer 2012). Hence, several active sources may be responsible for the pollutant load in the sediment samples of a multipurpose land use catchment. In addition to stormwater, WWTP effluent has recently been considered an important source of particle-bound MP in freshwaters as a result of the application of PAC, a process that has the disadvantage of releasing MP loaded PAC to receiving water (Krahnstöver et al., 2016).

Following emission, the fate of pollutants depends for the most part on their interaction with particles. The association of pollutants with particulate matter in water can either be via sorption or complex formation (for hydrophilic pollutants) or adsorption/absorption (for hydrophobic pollutants). The smaller the particles size, the higher the sorption potential for metals and organic pollutants (Grant et al., 2003, Baum et al., 2021) since the surface-to-size ratio of a particle and thus the binding sites for pollutants increase with decreasing particle size. Although not covered in the present thesis, the particle size effect on pollutant binding could be observed in chapter 2 where the <63 µm sediment particle fraction contained higher metal concentrations compared to the larger sediment particles. Furthermore, the particle size effect on pollutant binding is a reason for the preferred use of PAC over granular activated carbon (GAC) for MP removal (Bonvin et al., 2016).

Given the role of particles in pollutant sorption and transport, retention measures such as sedimentation or filtration in storm- and wastewater treatment facilities are used to prevent their release to surface waters. For fine particles, complete retention in stormwater basins and WWTP is not possible (Andral et al., 1999, Baum et al., 2021, Grant et al., 2003, Charters et a., 2015). Also, it is important to note that the retention efficiency in stormwater basins decreases over time due to continuous deposition of sediment hence reduction of depth of the stormwater basin. The consequence of which is enhanced frequency of overflow and remobilization of particles by resuspension resulting in enhanced entry of particles into receiving freshwater bodies (Allen et al., 2019, Taylor and Owens 2009). Besides retention basin overflows, other sources such as atmospheric deposition, input from soil erosion, and input from stormwater sewer not connected to a retention basin are important for particle-bound pollutants in freshwater bodies. It is likely that these nonpoint sources were also contributing to particle-bound substances in the investigated streams. Even though the generated data in the present thesis showed differences in sediment pollution downstream of the investigated retention basins outfall, a comparison of their efficiencies in retaining stormwater pollutants from entering receiving stream is complicated. This is because the basins are different in terms of size, design, overflow frequencies and catchment areas. Also, the affected water bodies are of different stream types (stream types 18 and 14, based on the German stream classification index), have different flow velocities hence different sedimentation rates of particles.

A research question in the present thesis was the fate of particle-bound pollutants in aquatic systems. The enrichment of pollutants in the sediment of the studied stream (chapter 1 and 2) indicates possible sedimentation of stormwater particles from the urban surfaces. This also highlights the reservoir function of freshwater sediment in harbouring particle-bound pollutants. A similar observation was reported downstream of CSO (Scherzinger et al., 2018). In addition to acting as a sink of particle-bound pollutants, the sediment can also serve as an important source of pollution. Hence, accumulation hotspots upstream may serve as pollution sources to downstream sites and the water phase through disturbance of the benthic zone either from movement activities of benthic organisms (bioturbation) or hydrological alterations in the water column (Colombo et al., 2016, Superville et al., 2014, Schroeder et al. 2020).

An important concern of anthropogenic related particles in freshwater bodies is if sedimentation enhances the bioaccessibility of their bound pollutants to aquatic organisms occurring both in the water column and sediment. It is important to note that the concentration of substances measured by chemical analysis does not give any information on the bioaccessible and hence biological available fraction of pollutants in sediment. The biological availability of pollutants in sediment depends on different abiotic and biotic factors. Because of reasons such as strong binding, slow desorption, water chemistry, exposure route, biological and ecological traits of organisms, only a fraction of particle-bound pollutants in sediment are biologically available (Goodyear and McNeill 1999, Alexander 2000, Cornelissen et al., 1997, Kraaij et al., 2002). The biological availability of pollutants in the natural sediment was assessed in the present thesis through bioaccumulation data in the field (in amphipods) and laboratory exposed (*L. variegatus*) organisms in chapter 1. The bioaccumulation data for gammarids and oligochaetes provide experimental evidence on bioaccessibility and hence the biological availability of sediment pollutants to benthic organisms in the natural environment. The accumulation of metals in the field-collected amphipods may be explained by enhanced bioaccessibility and hence biological availability through desorption and dissolution processes following sediment resuspension and changing water conditions (Morrison et al., 1988, Schroeder et al., 2020; Superville et al., 2014). Also, uptake via diet by the species is not excluded since the organism feeds on decayed organic matter that may contain adsorbed pollutants that are released in the digestive system or on polluted organisms. Hence, the bioaccumulation of pollutants in the amphipod sampled in the field gives important insights into what may be happening in a natural setting.

For organisms that are in constant contact with sediment, knowledge of the biological availability of particle-bound pollutants in sediment is limited. Hence, it is important to consider filling this gap for better understanding and estimation of the risk to sediment-dwelling organisms. This was addressed through laboratory bioaccumulation experiments with *L. variegatus* using field-collected sediment. The advantages of considering laboratory bioaccumulation experiments for sediment bioaccumulation studies include greater control of the experimental conditions and higher replicability. It also makes it possible to investigate samples from sites that may not be easily accessible for field exposure. In contrast to field investigations, factors such as genetic variability, growth dilution, heterogenous food, habitat and biota compositions affecting the outcome of bioaccumulation investigations can be controlled in laboratory experiments. Another important advantage of considering laboratory bioaccumulation experiments is the availability of standard guidelines for a variety of suitable test organisms (Selck et al., 2011, Weisbrod et al., 2009, Hoke et al., 2016). Also, the lack of abundance of the test organisms at the sampling site in the field is an important limiting factor for spatial *in situ* bioaccumulation studies of sediment contaminants. For example, this was the case in the present field study with the amphipods. Unlike the field data with amphipods, the bioaccumulation results from the laboratory study with *L. variegatus* exposed to sediments from the investigated streams showed a clearer picture of the spatial distribution of biologically available pollutants in sediments to benthic species. The high enrichment of traffic-related metals in *L. variegatus* exposed to sediment downstream of the stormwater outfall compared to the site upstream is further indication that organisms (sediment dwellers) downstream of stormwater outfalls are particularly subjected to pollution stress due to input of particle-bound pollutants.

Advantages of using *L. variegatus* as a test organism for bioaccumulation studies are its habitat preference and feeding habitat as well as the availability of standard test guidelines. Additionally, for biological availability studies of sediment-bound pollutants, it is important to consider an organism that is in constant contact with sediment particles, may tolerate varying physical and chemical characteristics of the sediment and is readily available and easy to maintain in the laboratory (EPA 2000). The ability to ingest sediment particles during feeding by the oligochaete has been reported to increase uptake and bioaccumulation of sediment contaminants (Leppänen and Kukkonen 1998). Ingestion exposes sediment-bound pollutants to enzymes in the digestive system that solubilize them facilitating bioaccumulation in the organism (Weston and Mayer 1998, Weston et al., 2002, Sormunen et al., 2008,).

Analysis of the whole-body burden of pollutants through bioaccumulation gives a good estimate of the biological availability of pollutants which is an important component of toxicity assessment. However, it is important to note that organisms possess detoxifying mechanisms which can affect the bioaccumulated concentration of potentially toxic xenobiotics hence making them more tolerant to some pollutants. For metals, for example, accumulation in aquatic species is linked to many subcellular compartments divided into two main factions: the metal sensitive fraction and the biologically detoxified metal fraction. Metal concentrations in the metal sensitive fraction are predominantly responsible for metal toxicity in organisms (Wallace et al., 2003, Wang 2013). Therefore, the onset of toxic effects following uptake of metal pollutants in organisms is possible if accumulation in the sensitive fraction exceeds certain threshold values (Tsui and Wang 2006, Wang and Guan 2010). The bioaccumulation data in the present thesis (chapter 1) does not differentiate between the different subcellular fractions or intracellular forms (toxic and detoxified forms) of metal. Given the small size of the organism, proper differentiation of anatomical region for subcellular fractionation may be impossible but would be interesting to investigate in future studies. However, such a bioaccumulation study remains an important component in the bioavailability assessment and may provide a useful and more realistic prediction of possible toxic effects (Borgmann et al., 1998, 2001, Di Toro and McGrath, 2000). Thus, this aspect of subcellular fractioning following uptake of particle-associated pollutants should be addressed in future studies to better predict toxic effects on sediment-dwelling organisms.

An important objective of all three chapters was to study the possible risk of particle-bound pollutants from different sources to sediment-dwelling organisms. This was made possible by predicting sediment toxicity using the consensus-based sediment quality guideline (CBSQG) by MacDonald et al. (2000) and application of sediment toxicity test with the endobenthic *L. variegatus* (chapter 1, 2 and 3). The application of the CBSQG based on metal concentration in chapter 2 resulted in high toxic potentials of the stormwater basin samples that recorded mortality in the toxicity test. Although the application of quality guidelines like the CBSQG is recommended in risk assessment studies for the estimation of toxic levels of sediments, it is important to note that only a limited number of chemicals are used for the determination of the toxic potential of a sample. Concentrations of pollutants selected alone due to analytical limitations cannot assure accurate prediction of toxicity. Also, chemical analyses may underestimate the effect of some chemicals, especially organic compounds, that are already toxic at concentrations below the detection limit of the analytical method. Furthermore, the toxic threshold values in the guideline do not consider the chemical speciation of pollutants which is an important determinant for their uptake and toxicity in affected organisms. Additionally, substances like endocrine disruptors are difficult to identify by chemical analysis due to their complex chemical nature and origin and the endocrine-disrupting efficacy of numerous substances is unknown (Simpson and Batley 2016). Furthermore, metabolites of pollutants that may be more or less toxic are not considered in sediment quality guidelines. However, the CBSQG is still a useful tool to quickly estimate the toxic potential of sediment (Díaz-Morales et al., 2021).

In addition to the CBSQG, biotests with suitable test organisms are highly recommended (Ahlf et al., 2002, Hollert et al., 2003). Toxicity testing is one of the most important components for risk assessment of sediment contamination. It considers the pollutant "cocktail" of the sediment and integrates the toxic effects of all contaminants (known and unknown) present at their respective bioavailability while accounting for possible synergistic/antagonistic effects (Hollert et al., 2003, Connon et al., 2012). In this thesis, assessments of toxic effects of sediment contaminants were done on both standardized endpoints (growth and reproduction) and physiological responses in *L. variegatus*. Despite elevated concentrations of some potentially toxic pollutants in sediment samples and the respective exposed test organisms (chapters 1 and 2) with some pollutant concentrations above quality levels, assessment of growth and reproduction endpoints did not indicate toxicity to *L. variegatus* for most sediments. An important exception was the highly polluted retention basin sediment (in the multipurpose land use catchment) where mortality was significant. Also in chapter 3, PAC from WWTP loaded with a "cocktail" of micropollutants, which based on its chemical characteristic would be assumed toxic to organisms, was observed to be unlikely to cause adverse effects on aquatic organisms. (Woermann and Sures, 2020, Woermann et al., 2021 a and b). The results deviate from previous studies with activated carbon (Jonger et al., 2009, Abel et al., 2017) but only for the micropollutant loaded PAC from WWTP. This suggests that MPs on PAC may not be biologically available for uptake by *L. variegatus* to pose toxic effects on the organisms. Also, the fine nature of PAC is altered upon binding of MPs and this may alter their ingestibility and hence the ability to bind nutrients for sediment-dwelling organisms. Therefore, it is unlikely that the PAC from WWTP of the present study could have adverse effects on the sediment-dwelling organisms of the receiving water. However, it should be noted that the sensitivity of the test organism of choice and endpoints have major influences on the relevance, success, and interpretation of sediment toxicity tests (West et al., 1993).

Although the test organism was chosen based on its wide recommendation for ecotoxicological studies of sediment contamination (ASTM 1998, EPA 2000, OECD 2007, Chapman 2001), their sensitivity to sediment pollution has been questioned especially for the growth and reproduction endpoints (West et al., 1993, Brunson 1998, Phipps et al., 1994, ASTM 1998) Thus, also biomarkers were investigated in the present study.

The consideration of biomarkers in chapters 1 and 2 for toxicity assessment following sediment exposure was due to their sensitivity resulting in early responses to stressors from environmental pollutants as well as the fact that they are mostly affected only by biologically available pollutants and can integrate the effects of multiple stressors (Hugett 1992, US EPA 2002). The biomarkers considered in this thesis have been recognised for their response to environmental pollutants following exposure. They are among the frequently used ones to assess the exposure and toxicity of anthropogenic pollutants on aquatic organisms (Amiard et al., 2006, Huggett 1992, Lemos 2021). In the present thesis, it was interesting to observe different responses of the low molecular cysteine-rich metal-binding MT, the oxidative stress enzyme, CAT as well as the neurotransmitter hydrolyzing enzyme AChE. Induction of MT and CAT, as well as inhibition of AChE, are often reported in studies involving exposure of aquatic organisms to environmental toxicants (Contardo-Jara and Wiegand 2008, Frank et al., 2016, Frasco et al., 2005, Fu et al., 2018, Kais et al., 2015, Ricciardi et al., 2006, Le et al., 2016). However, an important point to note when employing some biomarkers for exposure assessment is that they may also demonstrate the same sensitivity to confounding variables making it difficult to distinguish between the response to anthropogenic pollutants from normal physiological or environmental conditions (Farcy et al., 2012, Gillis et al., 2014, Huggett 1992). Factors such as normal reproduction cycle, temperature, sediment grain size, algal bloom, presence of ammonia as well as hydrogen sulfide are examples of factors reported to have a significant influence on biomarker response (Farcy et al., 2012). For future recommendations it would be important to consider at least two of each biomarker classes, if possible, to have a better understanding of what may be happening in response to exposure to environmental pollutants. For example, by using CAT to assess the induction of oxidative stress by xenobiotics, it would be expected to have an increase in the enzyme. However, this may not be the case because the organism may be employing other enzymes to suppress oxidative stress instead of CAT (Lemos 2021).

Considering chemical analysis together with ecotoxicological lines of evidence such as in this thesis provides a comprehensive picture of the possible impact of anthropogenic pollutants on sediment quality in aquatic systems. However, for future studies in the laboratory, it would be important to consider improving some aspects of the sediment assessment approach used in this thesis to have a bigger and more realistic picture of the impact of sediment contamination on the entire aquatic system. For example, it may be important to consider using wet sediment and water collected from the investigated site as well as limiting the time between sampling and laboratory investigation. This would increase the closeness of the setup to natural conditions and limit variation in parameters that may alter the desorption, partitioning and speciation of pollutants in the sample as well as prevent loss of pollutants from sample preparation. Additionally, given that sediment is an important source of pollutants to the water column through diffusion processes it would be important to consider the toxicological effects of contaminated sediment on organisms in the water column to have an overall picture of the aquatic ecosystems. This is possible through the use of eluates (Díaz-Morales et al., 2021). Regarding the choice of test organisms, it is with no doubt that *L. variegatus* was appropriate for assessing the risk of the sediment samples. Using the oligochaete ensures that all exposure pathways are taken into consideration which reflects exposure conditions in the natural environment (Ingersoll et al., 1995, ASTM, 1999, US EPA 2000). However, to better identify the risk of sediment contaminants to aquatic organisms in the laboratory, future studies should consider test systems involving multiple species with varying degree of sensitivity to pollutants. For example, a test battery with multiple organisms from different trophic levels inhabiting different microhabitats in the sediment. Conducting multiple sediment tests with different organisms would increase the likelihood of detecting toxicity since different species may respond differently to contaminants in sediment. Also, it considers all possible exposure routes as well as increases the ecosystem relevance of the study (Burton, 1991, Feiler et al, 2005 and 2012, Höss et al., 2010). Another important recommendation for future laboratory experiments for sediment tests is to consider multiple sublethal endpoints such as movement behaviour, feeding habit, life table assessment (Sard and Soares 2011, Sheahan and Fisher 2012). Furthermore, it would be worthwhile to consider more biomarker endpoints such as specific biomarker of effect (e.g., imposex, EROD) in suitable test organisms in the future. Also, genomic and proteomic biomarkers should be considered to have a deeper understanding of the adverse effects of sediment pollution on organisms and ecosystem health.

As already mentioned above, chemical analysis of sediment alone does not provide reliable information about potentially toxic effects on organisms. Also, irrespective of all the advantages *L. variegatus* has for comprehensive sediment toxicity studies, the application of single-species tests on complex environmental samples as used in this thesis has limitations. Due to the considerable variation of the sensitivity of different species against pollutants, the representativeness of single-species tests for other potentially more vulnerable organisms in the natural environment is restricted. Also, single-species tests do not consider the interactions between species that organisms face in the natural environment. Hence, the application of ecotoxicological approaches with multiple organisms may be considered in future studies to provide data that is representative of organisms with pollutant sensitivity and trophic level (Connon et al., 2012). For example, a bioassay battery involving multiple organisms of different trophic levels may generate data more representative of a large group of sediment-dwelling organisms. For a more holistic ecological relevant picture, an integrated approach involving chemical analyses (target and nontarget), a suite of ecotoxicological tools, the study of population/community responses in the same water body and, preferably, different times of the year would be most relevant for the assessment of the ecotoxicological risk of sediment contamination (Connon et al., 2012). Such an approach could provide information along the route of the pollutant from exposure, via uptake to the possible adverse effects at the site of action not only on molecular up to individual level but also at population and community level.

The fact that the impairment of aquatic ecosystems is outpacing mankind's efforts to manage them, continuous awareness through research remains of utmost importance. Assessment of sediment pollution by anthropogenic related particles is very important to provide knowledge of sediment contaminant status for possible prevention or mitigation strategy for the protection of surface waters and their inhabitants. The possible sources and fate of particle-bound pollutants shown in the presented thesis highlight some important points on sediment pollution of aquatic environments. Firstly, the effects of particle-bound pollutants from anthropogenic origins on freshwater bodies emphasize the importance of limiting anthropogenic related particles contaminations in freshwater ecosystems. This was demonstrated through different lines of evidence including accumulation of pollutants in sediments and different animals, as well as toxic effects and biomarker responses induced by polluted sediments. Secondly, although assessment of sediment contamination is mostly focused on priority pollutants, it would be important to channel the same attention to conduct systematic studies on emerging pollutants (e.g., pharmaceuticals, industrial additive, personal care products and disinfection-by products) for better sediment risk assessment (Müller et al., 2021). However, it should be noted that sediment pollution by priority and emerging contaminants is just a fraction of anthropogenic stressors in freshwater bodies. This is because human pressures on freshwaters often alter more than one environmental factor generally resulting in a multiple stressor situation for the ecosystem. For example, urbanisation affects stormwater quantity and quality, freshwater quality, thermal regimes, habitat availability, the dispersal of invasive species etc. (Ormerod et al., 2010). Hence, research addressing multiple stressors on freshwater bodies and their biota are required for a more ecological relevance outcome and a better understanding of the necessities to achieve a good status of freshwaters. However, increasing complexity is generally combined with decreasing reproducibility, more difficulties to link effect to specific chemicals, increase response time and is time-consuming (Fent 2013). Nonetheless, focusing on one of the key anthropogenic stressors (i.e., pollution) in a complex, variable system, the present thesis provides an important piece of knowledge on the behaviour and possible effects of particle-bound pollutants in urban freshwater bodies, which is vital for better protection of surface waters in the future.

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7. Supplementary information

Chapter 1

Table S1: Quality data (Recovery, limit of detection and limit of quantification) of analytical procedures for sediment and tissue samples

Sample	Parameter	Cd	Co	Cu	Mn	Mo	Ni	Pb	Zn
Sediment	Recovery (%)	102	102	115	98	95	109	97	113
	LOD (µg/g)	0.00	0.00	0.72	0.31	0.01	0.46	0.04	1.23
	LOQ (µg/g)	0.01	0.01	2.17	0.92	0.04	1.38	0.12	3.68
Biota	Recovery (%)	105	119	120	119	105	100	115	110
L. variegatus	LOD (µg/g)	0.01	0.22	0.71	38	0.01	0.02	0.03	1.08
	LOQ (µg/g)	0.04	0.65	2.12	115	0.03	0.06	0.10	3.24
Gammarus sp.	LOD (µg/g)	0.00	0.03	0.11	6	0.00	0.00	0.01	0.17
	LOQ (µg/g)	0.01	0.10	0.33	18	0.00	0.01	0.02	0.50

Table S2: Result of 10 days sediment validation experiment as per EPA guideline (n = 4)

Treatment	Av. # after 10 d	% survival	pH	Conductivity (µs/cm)	Temp. (^{0}C)	NH_3 (mg/L)	Comment
Control (OECD)	12	100	7.6	999	21.0	0.041	Burrowed
Ref.	10	100	8.2	1016	20.8	0.036	Burrowed
RRB	10	100	7.9	1101	21.1	0.337	Burrowed
1 m	10	100	8.2	1107	20.9	0.106	Burrowed
5 m	9.75	97.5	8.4	1202	21.1	0.091	Burrowed
10 m	10	100	8.4	1231	21.1	0.076	Burrowed
20 m	10	100	8.2	1310	21.0	0.171	Burrowed
50 m	10	100	8.4	1099	20.9	0.387	Burrowed
100 m	10	100	7.9	1015	21.2	0.161	Burrowed

NB: Worms (10 per replicate) were exposed for 10 days in sediment under the same light, temperature and aeration condition for the 28 days experiment. Burrowing behaviour and survival were monitored in all treatment during the exposure period. Artificial control sediment was prepared following the OECD guideline (225) to validate the health of the worms. Water parameters were measured on the first and last days of exposure. Worms were counted at the end of the experiment for survival. The last burrowing was observed 3 days after of exposure.

Table S3: Water parameter for the 28 days exposure experiment

	Day 1			**Day 7**			**Day 14**			**Day 21**			**Day 28**			
Samples	pH	Temp (ºC)	Conductivity (µs/cm)	pH	Temp (ºC)	Conductivity (µs/cm)	pH	Temp (ºC)	Conductivity (µs/cm)	pH	Temp (ºC)	Conductivity (µs/cm)	pH	Temp (ºC)	Conductivity (µs/cm)	NH3 (mg/L)
Control (OECD)	7.3	20.9	671	7.8	20.4	834	8.0	21.0	922	8.4	20.1	991	8.2	20.2	1012	0.087
Ref.	7.3	21	860	8.2	20	1147	7.9	20.7	1156	8.2	20.2	1167	8.2	20.3	1159	0.044
RRB	7.9	20.5	784	8.2	20	1048	7.9	20.7	1044	8.2	20.7	968	7.9	20.2	979	0.473
1m	7.3	20.7	866	8.2	20.1	1267	8.2	20.4	1247	8.4	20.4	1184	8.4	19.9	1191	0.113
5m	7.3	20.7	899	8.4	19.9	1351	8.4	20.6	1292	8.4	20.1	1295	8.4	20.1	1165	0.124
10 m	7.6	20.8	917	8.4	19.6	1380	8.4	20.8	1358	8.6	20.2	1432	8.4	20	1225	0.119
20 m	7.6	20.8	924	8.2	20.1	1316	8.4	21.3	1325	8.4	20.3	1365	8.2	20.5	1174	0.172
50 m	7.6	20.8	922	8.4	20.1	1433	8.2	21.1	1328	8.4	20.4	1300	8.4	20.4	1253	0.490
100 m	7.6	20.8	833	8.2	19.9	1108	8.2	21.1	1075	8.2	20.5	1098	8.2	20	1074	0.291

NB: Evaporated water was replaced with deionized water following measurement of water parameters. Ammonia concentrations were measured only in the water samples collected on the last day of exposure. Oxygen concentrations in the water were not measured as there was a constant supply of oxygen during exposure.

Chapter 2

Table S1: Sediment particle size distribution and organic carbon content

	Weight (mg) of different fraction in subsample of bulk sediment				**% in < 2mm fraction**		
Sample	>2mm	< 2 mm	63 µm - 2 mm	< 63 µm	63 µm - 2 mm	< 63 µm	OC (%)
Ref.1	2.4	56.3	50.8	5.5	90	10	2.6
Ref.2	15.9	84.2	79.1	5.1	94	6	1.32
RBB1	3.2	97.3	91.2	6.2	94	6	13.34
10 m	4.1	96.2	76.1	20.1	79	21	2.65
50 m	9.9	89.0	80.1	8.9	90	10	2.67
100 m	10.1	90.0	87.1	2.9	97	3	1.31
250 m	3.7	95.9	85.3	10.6	89	11	2.31
RBB2	0.5	27.0	25.3	1.7	94	6	3.90
310 m	28.3	74.4	65.9	8.5	89	11	2.29
350 m	8.5	91.8	73.6	18.2	80	20	3.67
400 m	5.1	95.2	93.4	1.8	98	2	2.62

Parameter for extraction and screening of organic compounds

Table S2: ASE settings

Parameter	**Setting**
Preheat	5 min
Heat	5 min
Flush	50% vol
Purge	200 s
Cycles	1
Extraction Cells	33 mL
Solvent	Aceton/Hexan (1:1)
Temperature	80 °C
Pressure	150 bar
Extraction Time	10 min

Table S3: GC devices

Parameter	**Setting**
GC-MS/MS	Shimadzu GC-MS-TQ8040
GC Column	RESTEK-DB5-5% Diphenyl-95% Polisiloxan-Säule (Rxi®-5Sil MS)
Autoinjector	Shimadzu Autoinjector AOC 20 i
Autosampler	Shimadzu Autoinjector AOC 20 s

Table S4: GC settings

Parameter	**Setting**
Total Flow	16 mL/min
Column Flow	1,18 mL/min
Injection Temperature	270° C
Linear Velocity	40 cm/sec
Pressure	79.5 kPa
Injection Volume	1 µL
Injection Mode	Splitless
Purge Flow	3 mL/min

Table S5: Temperature program and the total time of the GC method

Rate (°C/min)	Temp. (°C)	Hold Time (min)
-	80	0
16°	200	3
8°	320	2
Total Time		27

Table S6: MS settings

Parameter	Setting
Ion Soure Temperature	250° C
Collision Gas	Argon
Interface Temperature	300° C
m/z	50 – 700
Scan Speed	2500
Time Event	0.3 sec

Parameters for quantification of 16 EPA PAHs with GC-MS according to DIN EN 15527

Table S7: Temperature program

Rate (°C/min)	**Temp**. (**°C**)	**Hold Time** (min)
-	75	1
25	250	0
3	310	7

Injector: 300 °C
Splitless injection: 1 µL
Column: OPTIMA 5 MS, 30 m x 0.25 mm, 0.25 µm, Macherey-Nagel (Düren)
Flow: 1 mL/min
Ion source: 230 °C
Interface: 280 °C

Table S8: PAH, internal standards, retention time, and reference ions

PAH	**Ret. Time**	**Ref.Ion 1 *(m/z)***	**Ref.Ion 2 *(m/z)***	**Ref.Ion 3 *(m/z)***
Naphthalene-d8	4.63	136	134	137
Naphthalene	4.65	128	127	129
Acenaphthylene	6.28	152	151	76
Acenaphthene	6.45	153	154	152
Fluorene	6.99	166	165	82.5
Phenanthrene	7.94	178	176	76
Anthracene	8.01	178	176	89
Fluoranthene	9.29	202	200	101
Pyrene	9.61	202	200	101
Benz[a]anthracene	11.98	228	226	229
Chrysene	12.08	228	226	229
Benzo[b]fluoranthene	15.29	252	250	126
Benzo[k]fluoranthene	15.36	252	250	126
Benzo[a]pyrene-d12	16.35	264	260	265
Benzo[a]pyrene	16.44	252	250	126
Indeno[1,2,3-cd]pyrene	20.89	276	138	277
Dibenz[a,h]anthracene	20.99	278	139	279
Benzo[ghi]perylene	21.91	276	138	137

Table S9: Quality data of analytical procedures for PAHs

	LOQ (ng/g)	Recovery (%)
Naphthalene	1.0	94
Acenaphthylene	1.0	102
Acenaphthene	1.0	126
Fluorene	1.0	115
Phenanthrene*	1.0	118
Anthracene*	1.0	100
Fluoranthene	1.0	72
Pyrene	1.0	77
Benz(a)anthracene	1.0	83
Chrysene	1.0	96
Benzo(b)fluoranthene*	1.0	93
Benzo(k)fluoranthene*	1.0	92
Benzo(a)pyrene	1.0	90
Indeno(1,2,3-cd)pyrene	1.0	86
Dibenz(a,h)anthracene	1.0	81
Benzo(g,h,i)perylene	1.0	93

Table S10: Quality data (Limit of detection, Limit of quantification, precision and accuracy) of analytical procedures for metals

	Ag	**Cd**	**Co**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Zn**
Recovery (%)	85	98	81	97	/	98	92	101	90	101
RSDV (%)	6	5	3	3	3	4	4	5	4	2
LOD (mg/kg)	0.003	0.002	0.003	0.4	10	0.3	0.01	0.5	0.1	5
LOQ (mg/kg)	0.008	0.007	0.008	1.3	29	0.8	0.04	1.4	0.2	15

Table S11: Water parameters during exposure experiment

	Day 1			**Day 10**			**Day 28**		
Samples	**pH**	**Temp (ºC)**	**Conductivity (μs/cm)**	**pH**	**Temp (ºC)**	**Conductivity (μs/cm)**	**pH**	**Temp (ºC)**	**Conductivity (μs/cm)**
Control	7	20	728	7.5	21.5	809	6.7	19.6	724
Ref.1	7	20	698	7.9	19.5	818	7.9	20	929
Ref.2	7.6	20.1	710	7.9	20.7	803	7.9	19.6	995
RBB1	7.4	20	699	8.1	20	881	-	-	-
10 m	7.3	20.5	724	7.8	20	803	8.2	19.6	811
50 m	7.3	20	711	7.9	19.9	864	7.9	19.5	850
100 m	7.3	20.1	711	7.5	20.1	893	7.9	19.2	916
250 m	7	19.9	685	7.9	19.2	796	7.9	20	929
RBB2	7.2	20	699	7.6	20	801	-	-	-
310 m	7	19.5	686	7.9	19.5	850	7.9	19.5	1048
350 m	7	19.6	975	7.9	19.3	966	8.2	20	928
400 m	7.3	19.6	781	8	19.5	853	7.9	20	929

Table S12: Percentage (%) incomplete worms after 28 days exposure.

Control	**Ref.1**	**Ref.2**	**RRB1**	**10 m**	**50 m**	**100 m**	**250**	**RRB2**	**310 m**	**350 m**	**400 m**
5	30	10	-	16	14	28	24	-	18	21	32

Table S13: Fluoranthene/pyrene ratio for determination of possible sources of the detected PAHs (Budzinski et al. 1997; Crane and Myre 2006)

Ratio	Ref. 1	Ref. 2	RBB1	10 m	50 m	100 m	250 m	RBB2	310 m	350 m	400 m
(F/P)	1.4	1.4	1.2	1.4	1.4	1.4	1.2	1.3	1.3	1.3	1.3
Fl/(Fl+Py)	0.6	0.6	0.5	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6
BaA/(BaA + Chry)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.3	0.5	0.5	0.5
IP/(IP+BghiP)	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.5	0.5	0.4

Phenanthrene/Anthracene (P/A), Fluoranthene/pyrene (F/P)-. A P/A ratio <10 and F/P ratio >1.0 are indicative of pyrogenic (i.e., combustion-derived) sources of PAHs (Budzinski et al. 1997; Crane and Myre 2006).

Table S14: Spearman correlation between substance load and toxicity endpoints ($p < 0.05$)

	Reproduction inhibition	Biomass inhibition	CAT	AChE	MT
%Tox. metal	0.52 (0.1)	0.12 (0.7)	0.25 (0.4)	0.01 (1)	-0.1 (0.7)
% Tox. PAH	-0,5 (0.2)	-0.33 (0.4)	.022 (0.6))	-0 28 (0.5)	-0.01 (0.8)

Table S15: Peak area of detected trace organic pollutants in sediment samples from screening analyses

	Ref. 1	Ref. 2	RRB1	10 m	50 m	100 m	250 m	RRB2	310 m	350 m	400 m
PAH											
Fluorene	4592	8481	4609	1343	22070	5586	2757	16846	5877	2211	9863
Naphthalene	7193	11269	7313	614	24669	7716	3143	72578	5638	4306	2641
Acenaphthylene			4050	101		798	371	18599			569
Acenaphthene	2500	5208	2156	629	11839	2900	1301	16562	3071	968	3008
Phenanthrene	37403	110828	93605	23757	184061	112153	51735	398507	47435	27989	161216
Anthracene	6089	21812	15564	5902	90301	15924	8425	38853	6726	4688	51035
Fluoranthene	76288	300758	292926	85889	300011	264249	118579	880147	101870	86049	372919
Pyrene	51316	209133	207410	54506	199428	164727	78474	645284	70189	55013	237583
Benz(a)anthracene	34621	172864	235533	46943	176133	123590	55154	298110	45352	45625	181733
Chrysene	40468	184678	323602	49593	216840	131994	64684	707864	51003	56898	186945
Benzo(b)fluoranthene	30399	142088	316029	34762	113603	92739	48447	328102	39211	40887	121856
Benzo(k)fluoranthene	7837	39882	76130	10607	31750	24474	11514		11188	11828	32791
Benzo(a)pyrene	14643	77548	178346	19148	63314	44802	24043	148218	20799	19714	63676
Indeno(1,2,3-cd)pyrene	7266	45150	82281	9265	28548	22988	13214	38686	11875	10777	30445
Dibenz(a,h)anthracene	2372	14623	25023	3484	10364	8464	4379	10269	3616	3769	10715
Benzo(g,h,i)perylene	6310	38577	72917	7364	25404	19225	11440	40679	10603	9340	24806
Biocide											
1,2,4-Trichlorbenzol		119	232					1308			
Dichlobenil								715			
Propazine	84										97
Propyzamide						109					
Methyltriclosan		208	2122					311		135	
p,p'-DDE		327	662	92						210	179
Oxadiazon											
o,p'-DDD		301	11922		117	106	110		251	539	
p,p'-DDD		233	583							208	149
Quinoxyfen				147							130
p,p'-DDT			2994								

Diflufenican		125	2292	134				1688	110	102	
Ethofumesate								246			
Pendimethalin			141					3563			
PCB											
2,2',4,5,5'-Pentachlorobiphenyl (#101)		151	372					1392			
3,3',4,4'-Tetrachlorobiphenyl (#77)			453					1003			
3,3',4,4',5-Pentachlorobiphenyl (#126)			97					254			
2,3,3',4,4',5-Hexachlorobiphenyl (#156)			352					134			
2,3,3',4,4'-Pentachlorobiphenyl (#105)			158					478			
2,4,4'-Trichlorobiphenyl (#28)								525			
Flame retardant											
2,4,6-Tribromphenol			281					1740			
Hexachlorobenzene			525					393			
TEHP (Tris(2-ethylhexyl)phosphat)	929	4437	266401	577	332	557	1758	1288010	2401	2993	347
Tri(2-chloroethyl) phosphate	96	1632	8922		137	155	184	57214	326	279	166
Tris(1,3-dichloroisopropyl)phosphate			2295								
2,2',4,4',6-Pentabromodiphenyl ether (#100)		108									
2,2',4,4',5-Pentabromodiphenyl ether (#99)		399									
Pentachlorobenzene	130	174	465		99			1703			
2,2',4,4'-Tetrabromodiphenyl ether (#47)								430			

8. Publications included in this thesis

The inclusion of the following publications in the dissertation does not infringe any copyrights.

Essen, date ____________________________ ____

Signature of the doctoral candidate

1. Kontchou A.J., Nachev M., Sures B., 2020. Ecotoxicological effects of traffic-related metal sediment pollution in *Lumbriculus variegatus* and *Gammarus sp*. Environmental Pollution, 268:115884, https://doi.org/10.1016/j.envpol.2020.115884
2. Woermann M., Kontchou A. J., Sures B., 2021. Micropollutant-loaded powdered activated carbon released from wastewater treatment plants: a risk for sediment-dwelling organisms? Environmental Sciences Europe 33(1), https://doi.org/10.1186/s12302-021-00531-w
3. Kontchou A. J., Bätz N., Grabner D., Nachev M., Türk J. and Sures B., 2022. Stream sediment pollution related to stormwater retention basins and corresponding ecotoxicological effects on *Lumbriculus variegatus*. (Submitted to Science of the Total Environment)

9. Curriculum vitae

Not included in the online version for data protection reasons.

10. Documentation of accreditation

Documentation of accreditation achieved during the qualifying phase in accordance with § 6a of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology of the University of Duisburg-Essen dated 05.08.2013 (Gazette Year 11, 2013 p. 955. /No. 121).

The accreditation during the qualifying phase has been achieved within the framework of the structured doctoral program “Future Water - Research College”.

The accreditation of 18 credit points during the qualifying phase has been achieved:

Julios Armand Kontchou Doctoral candidate	Prof. Dr. Bernd Sures Doctoral supervisor

Essen, date ______________________________

Signature of the doctoral candidate	Signature of supervisor

11. Declarations

In accordance with § 6 (para. 2, clause g) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I represent the field to which the topic “Entry and Effects of particle-bound pollutants in urban freshwater bodies” is assigned in research and teaching and that I support the application of Julios Armand Kontchou.

Essen, date ____________ Prof. Bernd Sures ____________
Name of supervisor Signature of supervisor

In accordance with § 7 (para. 2, clauses d and f) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I have written the herewith submitted dissertation independently using only the materials listed and have cited all sources taken over verbatim or in content as such.

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Signature of the doctoral candidate

In accordance with § 7 (para. 2, clauses e and g) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I have undertaken no previous attempts to attain a doctoral degree, that the current work has not been rejected by any other faculty, and that I am submitting the dissertation only in this procedure.

Essen, date ____________ ____________
Signature of the doctoral candidate