Glaser, L., **Escher, B.I.**, Engelhardt, M., Liu, Y., **Krauss, M.**, **König, M.**, **Schlichting, R.**, Zarfl, C., Spahr, S. (2023):
Particle-associated organic contaminant and cytotoxicity transport in a river during storm events. *J. Hydrol.* **621** , art. 129600

https://doi.org/10.1016/j.jhydrol.2023.129600

Particle-associated organic contaminant and cytotoxicity transport in a river during storm events

Clarissa Glaser*[,1;+], Beate I. Escher[1,2], Michelle Engelhardt[1], Yuyuan Liu[1], Martin Krauss[3], Maria König[2], Rita Schlichting[2], Christiane Zarfl[1], Stephanie Spahr[4,1]

*Corresponding author: cglaser@uni-bonn.de

[1]Department of Geosciences, University of Tübingen, Schnarrenbergstr. 94-96, 72076 Tübingen, Germany

[2]Department of Cell Toxicology, Helmholtz Centre for Environmental Research – UFZ, Permoserstr. 15, 04318 Leipzig, Germany

[3]Department of Effect-Directed Analysis, Helmholtz Centre for Environmental Research - UFZ, Permoserstr. 15, 04318 Leipzig, Germany

[4]Leibniz Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany

[+]present address: Department of Geography, University of Bonn, Meckenheimer Allee 172, 53115 Bonn

Abstract

Storm events lead to mobilization of particles as well as organic contaminants that can pose a risk to river ecosystems. Assessment of total loads of particle-bound and dissolved contaminants in rivers is challenging, especially during rain events since multiple, often unknown contamination sources may be triggered, among them tributaries, combined sewer overflows, direct soil and road runoff, and the riverbed sediment. We investigated whether cytotoxicity (i.e., reduction of the cell viability of human cell lines) could be used as a proxy for the total chemical mixture to assess the transport of organic contaminant mixtures either in particle-associated form or in the aqueous phase. Time-proportional composite samples were collected in the Ammer River (Southwestern Germany) during two storm events in June 2021. All samples were screened for 171 organic contaminants and cytotoxicity in the water and particle phase separately. In total, 56 organic compounds were found, of which 12 and 34 were detected only in the water and particle extracts, respectively, and 10 compounds were present in both phases. Thermodynamic equilibrium between the aqueous phase and suspended solids was observed for four compounds namely triphenyl phosphate, octocrylene, galaxolide, and *p*-chlorocresol. Substances that were not in equilibrium were mainly constituents of plastics and tire rubber, and their high concentrations in the particle phase were probably due to extractions from the particles themselves. Linear correlations between TSS and total compound concentrations were found for ten PAHs and two musk fragrances, suggesting stable particle loadings and aqueous concentrations over the course of the rain event, most likely due to particle delivery ~~of~~ from a particle source with a constant degree of contamination. The total cytotoxicity of water plus particles in the river correlated linearly with TSS during storm events. Inflowing water and particles from all sources appeared to be integrated, such that the total cytotoxicity of all contaminants per mass of particles and volume of water in the river was stable throughout storm events. From this relationship, a threshold of 0.5 g L^{-1} TSS was derived above which particle-facilitated transport exceeded dissolved cumulative contaminant transport in the Ammer River, indicating the relevance of particle-facilitated contaminant transport.

Keywords

Water quality, river, suspended solids, organic contaminants, cytotoxicity

1. Introduction

River pollution is a major concern worldwide as an increasing number of organic contaminants enters surface waters (Luo et al. 2014; Schwarzenbach et al. 2006). Organic contaminants in fluvial systems partition between water, suspended particles, and the riverbed sediment. Suspended solids are an important transport medium for many compounds, as particles carry or contain legacy and emerging pollutants (Esser et al. 2020; Hagemann et al. 2020; Müller et al. 2021; Niu et al. 2021). Hydrophobic contaminants are transported mainly associated to particles, while hydrophilic and ionizable compounds are rather transported in their dissolved forms (Niu et al. 2020). During storm events, suspended solid concentrations in rivers are high due to mobilization of particles from riverbeds or riverbanks (Liu et al. 2018). Moreover, stormwater runoff and combined sewer overflows (CSOs) contribute to the particle and contaminant input into rivers (Furén et al. 2022; Spahr et al. 2020; Pamuru et al. 2022; Zgheib et al. 2011; Zgheib et al. 2012). Particles can also be introduced by runoff from agricultural areas with potential pesticide contamination (Knauer et al. 2017), by street runoff containing, e.g., tire wear (Fuchte et al. 2022; Rauert et al. 2022), or by inflow from urban or industrial areas carrying, e.g., polycyclic aromatic hydrocarbons (PAHs) (Zhang et al. 2009). Storm-induced mobilization of particles causes contaminants to be widely dispersed in river networks and be transported further downstream (Ciszewski and Grygar 2016; Salomons et al. 1987). Suspended particles in rivers consequently represent an integral signal of a wide range of particles from different sources carrying different mixtures of pollutants (Walling 2005).

The monitoring of particle-associated and dissolved contaminant fluxes in catchments during storm events is time-consuming and labor-intensive. Moreover, the dissolved fraction of hydrophobic compounds is often difficult to quantify because concentrations are close to detection limits. Easy to measure proxies are required to overcome these obstacles and allow determination of contaminant fluxes in different catchments. Turbid water samples contain particle-associated and dissolved organic contaminants according to the mass balance (eq. (1)):

$$C_{i,total} = C_{i,particle} \cdot TSS + C_{i,dissolved} \tag{1}$$

Where $C_{i,total}$ is the total concentration of a compound *i* in a sample [e.g., in µg L^{-1}], $C_{i,particle}$ is the concentration of the compound per mass of particles [e.g., in µg g^{-1}], TSS is the total suspended solids concentration [e.g., in g L^{-1}], and $C_{i,dissolved}$ is the dissolved concentration of the compound [e.g., in µg L^{-1}]. If total concentrations of the compound *i* are plotted versus TSS, a linear relation can be obtained, where $C_{i,particle}$ corresponds to the slope, and $C_{i,dissolved}$ to the y-intercept. This correlation provides information on particle contaminant loads integrative for the upstream catchment, as well as the dissolved fraction of the compound based on contaminant bulk concentration, i.e., the sum of dissolved and particle-associated contaminant concentrations, and TSS measurements. TSS may be replaced by turbidity measurements if relationships have been established between the two variables in the respective river (Rügner et al. 2013). Linear contaminant-TSS correlations have already been identified for PAHs (Chiffre et al. 2016; Schwientek et al. 2013a), PCBs (Rügner et al. 2019), and metals (Nasrabadi et al. 2018). These correlations were shown to be catchment-specific and reflect the urban pressure, geological background, and sediment quality of the catchments (Rügner et al. 2014; Schwientek et al. 2013a; Schwientek et al. 2017). In contrast, sediment samples from the river bed only display particles at a specific spot with a considerable heterogeneity within the river network (Chiffre et al. 2016). TSS-contaminant relationships are valid if the dissolved and particle-associated concentrations remain stable over time (e.g., during storm events), and the ratio of $C_{i,particle}/C_{i,dissolved}$ may be interpreted as the partition coefficient (K_d) [e.g., in $L\ kg^{-1}$] of the contaminant i between solids and the aqueous phase (Rügner et al. 2019). Partitioning of nonionic organic compounds between different phases depends on various factors such as physicochemical properties of the chemicals, characteristics of the particles, and the hydrological conditions. The latter, in particular, are highly dynamic during storm events and various non-point sources can contribute to the water and particle-associated contaminant flux in rivers. During storm events, the partitioning of compounds between particles and water has rarely been studied in rivers (Müller et al. 2021).

Target chemical analyses cannot capture all chemicals present in rivers as some compounds occur in concentrations below analytical detection limits and there are numerous unknown chemicals and transformation products. *In vitro* bioassays can be used to quantify effects of all known and unknown compounds present in a sample (Escher et al., 2021a). Cytotoxicity is a measure of how a cell or organism responds to the exposure to all chemicals in environmental samples, with each chemical having a different contribution (potency) (Escher et al. 2021b). Effects and cytotoxicity of single chemicals in complex mixtures are typically additive (Escher et al. 2020). Therefore, cytotoxicity can be interpreted as an effect-scale measure of all organic contaminants in rivers. A combination of *in vitro* bioassays and chemical target analyses can be used to determine how much of the cytotoxicity can be explained by the measured contaminants in the sample (so-called iceberg modelling) (Neale et al. 2020; Niu et al. 2021). Different cytotoxicity measurements have been successfully applied in various environmental studies and matrices such as water, sediment, and suspended particles (Becouze-Lareure et al. 2016; Hollert et al. 2000; Milačič et al. 2017; Müller et al. 2021; Neale et al. 2020; Tuikka et al. 2011). Cytotoxicity can be easily compared between the water and particle phase of rivers, thus, providing insights into the importance of water versus particles as transport medium for all organic contaminants present in the river. A stable TSS-cytotoxicity relationship would enable a direct comparison of the relevance of water and particle fractions for the effect-based sum of all substances present in the river. We hypothesize that river discharge at the catchment outlet integrates various particle and contaminant, such that a relationship between particle concentrations and the sum of all contaminants (cytotoxicity) can be derived.

Therefore, the objectives of this study were 1) to analyze whether hydrophobic contaminants in a river are in thermodynamic equilibrium between the aqueous and particle fraction during storm events, 2) to correlate the total concentrations of hydrophobic contaminants with suspended particle concentrations and 3) to elucidate whether suspended particle concentrations are a suitable proxy for mixture effects expressed as cytotoxicity in a river during storm events. To this end, river samples were collected in southwestern Germany (Ammer River) during high discharge events. Target analysis of hydrophobic organic contaminants was performed by gas chromatography-mass spectrometry (GC-MS), and four human cell lines were used to measure the cytotoxicity of contaminant mixtures extracted separately from the water phase and suspended solids. Iceberg modeling was applied to quantify the contribution of the detected chemicals to the observed cytotoxicity.

2. Materials and methods

2.1 Sampling site

The Ammer River (4th order stream) is a tributary of the Neckar River located in southwestern Germany. The Ammer River flows over a distance of 22 km from the City of Herrenberg to the City of Tübingen (Figure 1A). The river is characterized by a mean annual discharge (Q) of 0.87 $m^3 s^{-1}$ measured at the gauging station “Pfäffingen”, which is located approximately 10 km upstream of the catchment outlet (Figure 1A). During baseflow conditions, the river water originates from karstic aquifers of the Upper Triassic Limestone and Middle Keuper gypsum formation (Villinger 1982; Glaser et al. 2020b). Landuse is dominated by agriculture (71%) and urban areas (17%) (Schwientek et al., 2013a). The gauged catchment (134 km^2) integrates water from various small tributaries and two wastewater treatment plants (WWTP) (Glaser et al. 2020c). The WWTP Gültstein (80,000 person equivalents) is located directly at the Ammer River and has a mean dry weather discharge of 0.10 – 0.12 $m^3 s^{-1}$. A second WWTP (9,000 person equivalents) discharges into the Kochhart creek, a tributary of the Ammer River, with a flow rate of 0.012 – 0.015 $m^3 s^{-1}$. Tübingen has a mean annual air temperature of approximately 8°C and the mean annual precipitation is 750 mm a^{-1}. Heavy precipitations occur mainly in summer. During heavy storm events, significant water inflow from urban areas and combined sewer overflows in the upper catchment dominate the water flow in the river resulting in distinct flood and turbidity peaks propagating through the catchment as described in previous studies at the Ammer River (Schwientek et al., 2013a; Schwientek et al., 2013b). The gauging station in Pfäffingen was selected for our investigations because it integrates the water and particle flux from the upper catchment (Figure 1A). Two rain events of different intensity were investigated in this study (Figure 1B).

2.2 Field instrumentation and sampling

In-situ probes (UIT GmbH, Dresden, Germany) were installed at the riverbank of the Pfäffingen gauging station (Figure 1A). The probes continuously recorded turbidity (with an optical backscatter sensor at a wavelength of 880 nm), water temperature, electrical conductivity, and water level. The latter was converted to discharge using pre-established rating curves (Glaser et al. 2020a). Using a daily weather forecast, two storm events of varying intensity were sampled in June 2021 (Figure 1B). In total, 24 river samples were collected during the high discharge events covering both the increasing and decreasing discharge limbs. Two autosamplers (ISCO 3700, Teledyne Isco, Inc., Lincoln, NE, USA) with eleven 950 mL transparent glass bottles were installed next to each other at the Pfäffingen gauging station and operated simultaneously. The Teflon tubing inlets were placed in close proximity to the in-situ probes to ensure comparability between the data obtained from the probes and the samples collected with the autosamplers. The autosamplers were programmed to collect 30-minute composite samples of 800 mL (400 mL every 15 minutes). All bottles were pre-cleaned with methanol and deionized water. Few hours after the peak discharge (maximum 12 hours), an additional grab sample was collected to capture the tailing of the recession discharge curve of each event. All samples were stored at 4°C in the dark until further analyses.

2.3 Laboratory Analysis

2.3.1 Total and dissolved organic carbon (TOC and DOC), and total suspended solids (TSS)

TOC concentrations were determined in unfiltered samples, which were homogenized with an Ultra Turrax T18 (IKA, Staufen, Germany) for at least 2 minutes (~6000 rpm) prior to the analysis. To quantify DOC concentrations, the samples were filtered through 0.45 µm PES filters (Minisart ® High Flow, Sartorius, Göttingen, Germany). DOC and TOC samples were acidified to pH 2 before being measured with a TOC analyzer (Elementar High TOC II Analyzer, Langenselbold, Germany). The samples were purged with nitrogen gas using a thermal oxidation at 680°C, which allows CO_2 quantification using an IR detector. For determination of TSS, glass microfiber filters (GFF, pore size 0.7 µm, 47 mm diameter, Whatmann, USA) were used. The particle-loaded filters were frozen overnight at -20°C, freeze dried (Alpha 1-4 LSCplus freeze drier, Martin Christ), and weighed.

2.3.2 Accelerated solvent extraction (ASE) of suspended particles

The particle-loaded glass microfiber filters were extracted using accelerated solvent extraction (ASE; DIONEX ASE 350, Thermo Fisher Scientific) using acetone (SupraSolv®, Merck) and ethyl acetate (Acros Organics, Thermo Fisher Scientific) (50:50, v/v) in 2 cycles, each with 5 min preheat, 10 min static time at 100°C and 100 bar, a flush volume of 60 %, and a purge time of 100 s. A pristine filter was run as control. The extracts were blown down to an approximate volume of 4 mL at 40°C under a gentle stream of N_2. The extracts were passed through clean-up columns (Chromabond Flash RS SiOH, 40 - 63 µm, Macherey-Nagel, Düren, Germany) using a binary pump (Agilent 1200). 0.3 mL of the ASE extract was loaded onto a column preconditioned with dichloromethane and was first eluted with 10 mL of dichloromethane, followed by 10 mL of methanol. The eluates were collected separately. Half of the dichloromethane extracts were evaporated to dryness and re-dissolved in 0.5 mL of ethyl acetate for GC-MS analysis.

2.3.3 Solid-phase extraction (SPE) of water samples

Solid-phase extraction (SPE) was used to extract and enrich organic contaminants from the water samples. 500 mg Oasis HLB cartridges (Waters, Milford, MA, USA) were conditioned with 10 mL ethyl acetate (Acros Organics, Thermo Fisher Scientific), 10 mL methanol (Optima ®, Thermo Fisher Scientific), and 10 mL water (HPLC grade, Optima ®, Thermo Fisher Scientific). A volume of 800 mL aqueous, filtered sample was passed through the cartridges at a flow rate of 2.3 mL min^{-1}. Subsequently, the cartridges were washed with 10 mL water, dried, and eluted with 10 mL methanol and 10 mL ethyl acetate (combined in the same collection tube). The extracts were evaporated to dryness at 40°C under a gentle stream of N_2. The samples were re-dissolved in 1 mL of methanol and stored at -20°C. Process controls with HPLC-grade water were processed identically.

2.3.4 Chemical analysis

A total of 171 organic compounds were analyzed with gas chromatography high resolution mass spectrometry (GC-HRMS) as previously described by Neale et al. 2020. A Trace 130 gas chromatograph with a DB-5MS ultra inert column (30m x 0.25 mm, 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA) was coupled to a thermal desorption unit (TDU-2, Gerstel, Mülheim, Germany), a cooled injection system (Gerstel), and a QExactive instrument mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with electron ionization. The compound selection was based on previous studies on river quality and storm events (Neale et al. 2020, Niu et al. 2021) and included PAHs, personal care and household chemicals, pesticides, polymer additives, and other industrial chemicals (Table B8).

2.3.5 Cytotoxicity measurement

The extracts were dosed into 384-well plates that contained different human cell types. Four reporter cell lines derived from H4IIe, HEK293H, HEK293T or MCF7, respectively (Table B1), were used for the water extracts, while only one cell line (H4IIe) was deployed for extracts from the solids due to the limited number of extracted particles. The aim of this study was to assess the cytotoxicity from these different cell lines, though the applied cell lines are frequently applied to measure the activation of specific effects (Table B1). The percentage area covered by cells on the bottom of the wells of the 384-well plate defines the cell confluency, which can be used as a surrogate for the number of cells (cell viability). Confluency was measured in each plate immediately after dosing and after another 24 h of incubation (37°C, 5% CO_2) using an IncuCyte S3 live cell imaging system (Essen BioScience, Ann Arbor, Michigan, USA) as described in previous studies (Escher et al. 2019). The percent inhibition of cell viability was determined from the confluency of exposed cells in comparison to confluency of unexposed cells after 24 h of incubation. Inhibitory concentration of 10% reduction of cell viability (IC_{10}) describes the cytotoxicity and was obtained from evaluation of linear concentration-effect curves below 30% of inhibition. The IC_{10} was calculated based on the % inhibition of cell viability and the slope of the regression (eq. 2):

$$IC_{10} = \frac{10\%}{slope}$$

(2)

The concentrations were expressed as relative enrichment factors in units of L_{water} $L^{-1}_{bioassay}$ for water extracts and $g_{particle}$ $L^{-1}_{bioassay}$ for particle extracts. IC_{10} values of both extracts were blank corrected (Text A2) and transformed into toxic units TU ($TU_{dissolved}$ for water extracts and $TU_{particle}$ for particle extracts) for a better visualization as a high TU value corresponds to a high effect with eq. (3):

$$TU = \frac{1}{IC_{10}}$$

(3)

The standard error of the TU (SE_{TU}) was computed by error propagation according to eq. (4) ($L_{bioassay}$ L^{-1}_{water} for water extracts and $L_{bioassay}$ $g^{-1}_{particle}$ for the particle extracts):

$$SE_{TU} = \frac{1}{IC_{10}^2} \cdot SE_{IC_{10}}$$

(4)

2.4 Data evaluation

2.4.1 Thermodynamic equilibrium of contaminants between water and particles

Assuming that sorption of neutral organic compounds is mainly driven by the organic fraction of the solids, the K_d value can be described by the product of the organic carbon fraction of a sample f_{OC} [-] and the organic carbon to water partition coefficient K_{OC} [L kg^{-1}] (eq. (5)):

$$K_d = f_{OC} \cdot K_{OC}$$

(5)

The K_{oc} values were compiled from literature (see Table B2 and Niu et al. 2020). The mean f_{oc} value from all samples was used for the calculations and describes the fraction of the particulate organic carbon (difference of total organic carbon and dissolved organic carbon) in relation to the total suspended solids concentration (Table B2). The mean f_{oc} value of 3.56% (± 0.63%) is comparable to previous studies at this sampling site that found f_{oc} values of 2-4% during storm events (Glaser et al. 2020c; Schwientek et al. 2013a)).

The equilibrium contaminant concentration of substance *i* on the particles ($C_{i,particle,equilibrium}$, e.g., in mg kg^{-1}) was obtained by combining eq. (1) and (5) to yield eq. (6):

$$C_{i,particle,equilibrium} = \frac{C_{i,total}}{TSS \quad + \frac{1}{f_{OC} \cdot K_{OC,i}}} \tag{6}$$

To investigate the actual distribution of a compound *i* between water and particles during storm events, we compared the expected equilibrium contaminant concentration per mass particle (eq. (6)) with the measured contaminant concentration per mass particle ($C_{i,particle}$). Only substances that could be quantified in both phases (at least in one sample as dissolved concentration) during the second storm event (E2) were considered for evaluation.

2.4.2 Linear correlations of contaminants or cytotoxicity with TSS

Turbid water samples contain dissolved and particle-associated organic contaminants (eq. (1)). A linear relationship may be expected when the total concentrations of compound *i* are plotted against TSS (Schwientek et al. 2013a). For substances found only in the particle extracts and not in the water extracts, $C_{i,dissolved}$ was set to zero in order to calculate C_{total}. Only relationships with $R^2 > 0.75$ were considered a~~s~~ strong linear fit. Negative y-intercepts were forced through the origin due to their physical implausibility. Positive y-intercepts indicate $C_{i,dissolved}$ or $TU_{dissolved}$. Significance of differences ($p<0.05$) between the slopes of the correlations were determined by analysis of covariance using the statistic software GraphPad Prism 9.4.1. For interpretation of river-in processes, we only used the chemical data that was obtained from the sample set of the E2 storm event (12 samples).

We hypothesize that the conceptual model leading to a linear relationship between contaminants and TSS (eq. 1) is also valid for the mixture effect of all contaminants (cytotoxicity) in a sample (eq. 7):

$$TU_{total} = TU_{i,particle} \cdot TSS + \ TU_{dissolved} \tag{7}$$

As introduced earlier, cytotoxicity is expressed in toxic units TU, where TU_{total} is the total mixture cytotoxicity in a sample [L_{water} $L^{-1}_{bioassay}$], $TU_{particle}$ [$g_{particle}$ $L^{-1}_{bioassay}$] the cytotoxicity effect per mass particle, and $TU_{dissolved}$ the cytotoxicity in the dissolved phase [L_{water} $L^{-1}_{bioassay}$]. For

TU_{total}, the mean value of the four cell lines of each sample was used. The non-parametric Kruskal-Wallis test (GraphPad Prism 9.4.1) was applied to compare the median of the four different cell lines (H4IIe, HEK293H, HEK293T, MCF7).

2.4.3 Iceberg modelling

The mixture effect of the quantified chemicals, TU_{chem}, was predicted with the mixture model of concentration addition using the detected chemical concentrations (µg L^{-1} and µg g^{-1} for water and particle extracts, respectively) and the IC_{10} value of the detected chemical *i* according to eq.(8):

$$TU_{chem} = \sum_{i=1}^{n} \frac{c_i}{IC_{10(i)}} \tag{8}$$

Cytotoxicity data were available for 41 of the 56 measured compounds from own experiments and experimental data collected from the US EPA Tox21 database (Neale et al. 2020; Niu et al. 2021) (only presented for H4IIe and MCF7 cell line, Table B3).

We compared the measured cytotoxicity (TU_{bio}) with TU_{chem} to determine how much of the measured cytotoxicity can be explained by the detected chemicals in the water and particle extracts separately (eq. (9)):

$$Explained\ fraction\ of\ cytotoxicity\ = \frac{TU_{chem}}{TU_{bio}} \tag{9}$$

3 Results and discussion

3.1. Deviations from the thermodynamic equilibrium of contaminants between water and particles

During two storm events in June 2021, samples were collected at the gauging station in Pfäffingen with TSS values ranging from 0.01 to 1.4 g L^{-1} (Table B4). The water and particle extracts were both screened for 171 organic compounds out of which 56 were detected. 34 compounds were found in the particle extracts only, and 12 in the water extracts only (Table B2). Ten compounds were found in both phases with log K_{ow} values between 2.01 and 6.88. They included polymer and fluid additives (triphenyl phosphate, 2,2-dimethoxy-2-phenylacetophenone, benzothiazole, diphenylmethane, benzyl benzoate), ingredients of personal care and household products (octocrylene, benzophenone-3), pesticides/biocides (*p*-chlorocresol, 1-naphthol), and one musk fragrance (galaxolide).

Figure 2 shows the measured concentration of particle-associated compounds plotted against the theoretical equilibrium concentration on particles calculated from the water concentration and the K_d using eq. 6. Four substances including galaxolide, triphenyl phosphate, octocrylene, and *p*-chlorocresol appeared close to thermodynamic equilibrium between the particles and the water, as the measured particulate contaminant concentrations were close to estimated equilibrium concentrations (deviation is smaller than a factor 10, i.e., they fall between the 10:1 and 1:10 lines in Figure 2). For triphenyl phosphate (log K_{ow} of 4.38), equilibrium conditions were already described in previous investigations of storm events (Müller et al. 2021) as well as for baseflow conditions (Niu et al. 2021).

The measured $C_{particle}$ of five of the nine chemicals exceeded $C_{particle,equilibrium}$ (below the 1:10 line). Uncertainties in K_{oc} values could lead to an underestimation of $C_{particle,equilibrium}$. However, with the exception of 1-naphthol, all substances below the 1:10 line are fluid or polymer additives. This suggests that the high concentrations of fluid/polymer additives and, consequently, the higher measured $C_{particle}$ of these compounds could have resulted from ~~an~~ extraction of rubber particles or other microplastics with a particle size of >0.7 µm (our filter size). A higher measured particle-associated contaminant concentration compared to the expected concentration was previously determined for tris(1-chloro-2-propyl) phosphate (TCPP, log K_{ow} of 2.8) likely due to particle-associated TCPP input into the Ammer River (Müller et al. 2021). TCPP is used as flame retardant and polymer additive in, e.g., polyurethane foams and rubber (Cristale et al. 2013) and may, therefore, be-extracted directly from plastic particles

3.2. Relationship between contaminants and TSS

Total substance concentrations were determined according to eq. (1) using the measured TSS values, dissolved compound concentrations, and compound concentrations per mass of particles. Twelve substances showed a correlation between total concentration and TSS (Table A1; $R^2 > 0.75$). Of these twelve compounds, only galaxolide was detected in the water phase, while ten PAHs and the musk fragrance tonalide were detected only in the particle extracts. For the sum of the PAHs, a strong correlation with TSS ($R^2 = 0.955$) was obtained with a mean particle loading of 6.1 mg PAH kg^{-1} and an intercept near zero (subsequently forced through zero, Figure 3A), consistent with PAHs not being present in the water phase. This mean particle loading is in agreement with earlier studies at the Pfäffingen gauging station with a mean particle loading of 5.8 mg PAH kg^{-1} measured between 2011 and 2014 (Rügner et al. 2014; Schwientek et al. 2013a) and 5.7 mg PAH kg^{-1} in 2019 (Glaser et al. 2020c). Previous investigations at the Ammer River already revealed that water and particles (Schwientek and Selle 2016) as well as associated PAHs (Glaser et al. 2020c; Liu et al. 2019; Schwientek et al. 2017) were originally introduced into the river via urban areas in the upper catchment. This stable particle loading across storm events over the past decade indicates a stable particle source with consistent loading over the years. Schwientek et al. (2013a) demonstrated that stable PAH particle loadings obtained from regressions are in the same range as the median particle concentration of the Ammer riverbed sediment (6.9 mg PAH kg^{-1}), and that the slope of TSS-PAH regressions represents the average of spatially heterogenous contamination of sediments in river networks. This finding has also been confirmed for other catchments (Nasrabadi et al. 2018; Rügner et al. 2014). Therefore, the gradual mobilization of riverbed sediment during storms leads to stable linear relationship between TSS and PAHs.

The musk fragrances tonalide and galaxolide showed a strong correlation with TSS ($R^2 > 0.88$, Figures 3 B and C), which has not been described previously for these substances. The mean particle loading was 0.33 and 0.99 µg g^{-1} for tonalide and galaxolide, respectively. The correlation of galaxolide had an intercept of 0.09 ± 0.07 µg L^{-1}. This value is four times smaller than the measured galaxolide concentration of 0.38 ± 0.11 µg L^{-1} in the water phase. We probably overestimated the measured freely dissolved concentration of this very hydrophobic compound because it is likely bound to colloids and DOC, neither of which can be separated from the water phase by our filtration method (Niu et al. 2021). In contrast, tonalide was not detected in the water phase potentially due to the higher expected biodegradability of tonalide ($T_{1/2} = 7.4\ d^{-1}$, Table B2) compared to galaxolide ($T_{1/2} = 25\ d^{-1}$). A linear correlation between the total concentration of a compound and TSS indicates that the particle loading and, if applicable, the concentration in the water phase is constant throughout the quickly changing environmental parameters and conditions of a storm event. A correlation between contaminants and TSS might imply that musk fragrance-contaminated particles were mobilized from the riverbed during storm events. A constant delivery of particles with stable tonalide and galaxolide loadings via urban areas located in the upper catchment could also result in a constant particle loading during storm events. However, it is not possible to trace individual particles from specific sources along the stream as they are, in contrast to water, subject to fast particle-exchange processes with the riverbed sediment (Glaser et al. 2020c; Liu et al. 2018; Liu et al. 2019).

Triphenyl phosphate and octocrylene did not show a relationship with TSS, but concentrations were in thermodynamic equilibrium between water and particles. Notably, a correlation was obtained for both substances when only samples from the decreasing Q limb were considered ($R^2 \geq 0.75$). The categorization of samples from the same storm event with respect to increasing and decreasing discharge conditions can be found in Figure A1. This categorization has been made in previous studies and attributed to a higher shear stress dominating in the beginning of the event (Glaser et al. 2020c). This finding indicates that particle loadings as well as concentrations in the aqueous phase for these substances were not constant in the beginning of the event. An explanation could be a first flush event introducing stormwater with high concentrations into the river (Peter et al. 2020). At a later time point of event E2, the particle loadings and the concentrations of triphenyl phosphate and octocrylene in the aqueous phase were constant, resulting in a TSS-contaminant relationship as exemplarily shown in Figures 4A and B. The shear stress is smaller for decreasing discharge conditions, likely leading to less particle mobilization in close vicinity to the sampling site. Particles from the decreasing Q limb might, therefore, originate from further upstream in the catchment, either from contaminated sediment and/or additional inflow of more contaminated particles.

For alpha tocopherol acetate, which was only detected associated to particles and not in the water phase, a strong correlation ($R^2 > 0.98$) was also only observed for samples from the decreasing Q limb. This substance is a widely used pharmaceutical (vitamin e) that is well removed in conventional wastewater treatment plants (Mladenov et al. 2022). Alpha tocopherol acetate may, thus, serve as tracer for untreated wastewater and combined sewer overflows in the upper catchment of the Ammer River. Moreover, 11 of the 12 substances with TSS-contaminant relationship show higher particle loadings, $C_{particle}$, with decreasing discharge compared to increasing discharge conditions (Table A1). Riverbed sediment is known to be more contaminated closer to the inflow into rivers (Liang et al. 2016; Schwientek et al. 2017). Particles arriving at a later time during the decreasing discharge limb therefore likely represent the particle signal of the upper catchment. Our findings indicate that not all hydrophobic contaminants transported during storm events are correlated linearly with TSS.

Different contaminant sources contributing to the integrative (particle-associated) contaminant flux during storm events might be inferred from TSS-contaminant relationships.

3.3. Relationship between cytotoxicity and TSS

We hypothesize that the rivers' discharge at the catchment outlet integrates various particle and contaminant sources, as well as potential thermodynamic disequilibrium of individual substances, such that a relationship between TSS and the sum of all contaminants can be derived. Figure A3 (Appendix A) depicts the mean $TU_{dissolved}$ for all samples. No significant difference was found between the medians of the different cell lines after application of the Kruskal-Wallis test ($p < 0.05$). Thus, the $TU_{dissolved}$ values obtained from the different cell lines in the same samples are comparable within their error margin. Consequently, we can conclude that the mean of the cytotoxicity obtained from different cell lines yields a representative cytotoxicity value. To assess whether cytotoxicity constitutes a representative parameter for the detected substances, the mixture effect of the detected chemicals (TU_{chem}) was compared to the observed cytotoxicity (TU_{bio}) by applying iceberg modelling. Results are exemplarily discussed for the MCF7 and H4IIe cell lines and cover samples from both storm events. Measured IC_{10} values for the samples can be found in Table B5; TU_{bio}, predicted TU_{chem} and explained effect (%) for the cell lines H4IIe and MCF7 can be found in Table B6. For the MCF7 cell line (AREc32, see Table B1), 18 of the measured 56 compounds were cytotoxic in the water and particle extracts, and 11 of the 18 compounds were active in the water extracts. Less than 0.5% of the observed cytotoxicity (TU_{bio}) could be explained by the mixture effect of the detected chemicals (TU_{chem}). Nine of the detected compounds in the particle fraction led to cytotoxicity. The explained fraction was larger than for the water phase, but still only a maximum of 0.9% of TU_{chem} could explain the observed TU_{bio}. For the H4IIe cell line (AhR CALUX, see Table B1), 23 of the detected compounds were active, of which 11 were found in the water and 13 in the particle extracts. In the water phase, a maximum of 0.6% of TU_{chem} could explain TU_{bio}. For the particles, however, the fraction of TU_{chem} that could explain the TU_{bio} was significantly larger with up to 14.8%. The small fraction that could explain the observed effect fits very well to previous studies of surface water and particulate matter (Muz et al. 2020; Neale et al. 2020).

The contribution of individual detected compounds to TU_{chem} for the particles and water is displayed in Figure A4 and Figure A5, respectively, for all obtained samples. For the water, the main mixture effect drivers were *p*-chlorocresol and galaxolide. These two substances could explain up to 78.3% of the mixture effect for both cell lines (sample E2 P4, Figure A5 B and D, Appendix A). This finding differs from another study that evaluated rain events in 44 agricultural streams in Germany (Neale et al. 2020), where 2,4-dinitrophenol was identified as the main mixture effect driver for the aqueous cytotoxicity obtained from H4IIe and carbendazim for the MCF7 cell line. This is due to the fact that more compounds were detected (290) in their investigation, with a focus on hydrophilic compounds that are expected to be predominantly in the water phase. The higher number of detected compounds also resulted in a higher fraction of TU_{chem} that could explain TU_{bio} (on average 4.1% for AhR could be explained, Neale et al. 2020). The particle extracts of our study suggest that benz(a)anthracene, octocrylene, benzo(ghi)perylene, and benzo(a)pyrene were the main mixture effect drivers for the H4IIe cell line and dominated the mixture effect up to 92.8%. For the MCF7 cell line, no cytotoxicity data was available for benz(a)anthracene, benzo(ghi)perylene, and benzo(a)pyrene. Therefore, octocrylene, chrysene, and galaxolide were identified as main mixture effect drivers with up to 98.7%. The large contribution of the PAHs to the cytotoxicity is not surprising given the high PAH concentration in the river and their high hydrophobicity, which leads to high baseline toxicity. The UV filter octocrylene was found to be an important mixture effect driver in this study. This substance has also been previously detected in many German river sediments (Kaiser et al. 2012; Rodil and Moeder 2008) and identified as mixture effect driver for the activation of AREc32 in German and Australian river sediment (Muz et al. 2020). For the particle samples, the largest proportion of the detected mixture effect could not be explained (>85%, Figure A4 A and C), most likely due to many compounds that are not included in the target list.

TU_{total}, calculated with eq. 7 from $TU_{dissolved}$ and $TU_{particle}$, correlates linearly with TSS ($R^2 = 0.85$) in the river, just as $TU_{particle}$ multiplied with TSS ($TU_{particle,TSS}$; $R^2 = 0.81$) (Figure 5). $TU_{particle}$ obtained from the slope of the regression of 0.091 $L_{bioassay}\ g^{-1}{}_{particle}$ (SE 0.009) was very similar to the median $TU_{particle}$ of the measured samples (0.076 $L_{bioassay}\ g^{-1}{}_{particle}$, $\sigma=0.17$). Both particle loadings are in the same order of magnitude, which shows that the $TU_{particle}$ from the TU_{total} – TSS correlation is representative for all $TU_{particle}$ measurements. The mixture effect of all compounds associated to particles and in the water phase ($TU_{particle}$ and $TU_{dissolved}$) remained constant regardless of the hydrological conditions in the river. Niu et al. (2021) measured the cytotoxicity in the Ammer riverbed sediment at different sampling sites in the river network. The obtained values were between 0.01 and 503 $L_{bioassay}\ g^{-1}{}_{sediment}$ (Table B7). This large range shows that TU measurements of the riverbed sediment are highly heterogeneous in the river network. $TU_{particle}$ obtained from the correlation of TU_{total} with TSS, however, integrates the signal of all particle-releasing processes in the catchment, but the large variability of previous riverbed sediment mixture toxicity measurements restricts valid statements regarding the integrative measure of the slope for the riverbed sediment. The main mixture effect drivers in our study were PAHs (besides octocrylene), but the number of measured PAHs (21) is significantly smaller than the number of PAHs (>100) that exist in the environment. Therefore, the presented results might underestimate the contribution of this substance group to the measured cytotoxicity TU_{bio}. In general, cytotoxicity increases with hydrophobicity (Lee et al. 2021). Future research should focus on the role of hydrophobicity of detected compounds for the mixture effect. A systematic evaluation of a compounds' concentration and potency as a function of the measured mixture effect would allow us to determine if the total mixture effects represent mainly hydrophobic compounds, which typically are more potent, leading to a larger contribution to TU despite smaller concentrations. The extrapolation towards zero from the regression between TU_{total} and TSS leads to a y-intercept of 0.042 (SE 0.006) $L_{bioassay}\,L^{-1}{}_{water}$, which corresponds to $TU_{dissolved}$ as shown in Figure 5. This value fits the median of the cell line measurements (0.046 $L_{bioassay}\,L^{-1}{}_{water}$, σ=0.017).

The correlation between TSS and TU_{total} provides insights into the weighting of the bioactive compounds of the respective medium (water vs. particle) in comparison to the sum of bioactive compounds (TU_{total}) in the river. For this purpose, the TSS concentration at which $TU_{particle}$ is equal to $TU_{dissolved}$ (50% contribution of both fractions to TU_{total}) was determined. Using $TU_{dissolved}$ derived from the linear regression (0.042 $L_{bioassay}\,L^{-1}{}_{water}$) and rearranging equation (7) for toxic units leads to a TSS value of ~0.5 g L^{-1} for the Ammer River. Below a TSS of 0.5 g L^{-1}, more than 50% of TU_{total} can be explained by bioactive contaminants that dominate in the water fraction ($TU_{dissolved}$). Above a TSS of 0.5 g L^{-1}, more than 50% of TU_{total} can be explained by bioactive compounds attached to the particles. A particle concentration of 0.5 g L^{-1} does not require severe flooding conditions in the Ammer River. In fact, only smaller storm events leading to a river discharge of ~ 9 $m^3\,s^{-1}$ (data of this study, not shown), which is 9 times the mean discharge, are sufficient to reach this TSS value. This threshold is not very large considering a high discharge of 11.7 $m^3\,s^{-1}$ with a recurrence interval of two years (HQ_2) for the gauging station in Pfäffingen (https://www.hvz.baden-wuerttemberg.de/pegel.html?id=). Our results highlight the importance of particle-associated mixture effect transport during storm events, especially when cytotoxicity is placed in the context of risk assessment. For surface water quality monitoring, the so-called effect-based trigger (EBT) values are a tool to distinguish between poor and acceptable water quality using *in vitro* bioassays. Escher and Neale (2021) suggest that the EBT for cytotoxicity is defined as 1% of cytotoxicity in any original water sample to define this water as acceptable water quality. This value corresponds to an IC_{10} of 10 (concentration that causes 10% reduction of cell viability and growth) and is equivalent to $TU_{dissolved}$ = 0.1. According to this classification, the $TU_{dissolved}$ of the Ammer River (0.040 $L_{bioassay}\,L^{-1}{}_{water}$) can be classified as acceptable water quality. However, transferring the EBT to turbid water samples (TU_{total}) shows that water quality for TSS values larger than 0.65 g L^{-1} is already classified as poor. Consequently, particle-associated contaminant transport is highly relevant for water quality monitoring, especially in light of the expected increase in storm events due to climate change (IPCC, 2018).

4. Conclusions and implications

Suspended particles are an important transport pathway for organic contaminants during storm events. This study presents a novel approach to get insights into the importance of particle-associated contaminant transport during storm events on the catchment scale by measuring organic contaminants as well as the cytotoxicity as a proxy for the total chemical mixture. We found that PAHs and two musk fragrance compounds were in thermodynamic ~~GG~~ equilibrium between particles and water. These contaminants also showed a linear correlation with TSS concentrations, indicating a gradual release of hydrophobic, particle-associated organic contaminants from the riverbed sediment. Not all hydrophobic contaminants that were in thermodynamic equilibrium showed a linear TSS-contaminant relationship, because they may be freshly introduced into the river. The river discharge at the catchment outlet, thus, integrates various particle and contaminant sources.

Our study is the first to show that cytotoxicity correlated linearly with TSS during the studied storm events. This cytotoxicity – TSS correlation allows to derive information on the importance of the particle-associated mixture effect of all particle-bound contaminants present in a river, which is of great importance also from a risk assessment perspective. For the investigated storm events, the cytotoxicity in the water phase did not exceed the proposed effect-based trigger values (EBT). In contrast, particle-associated cytotoxicity exceeded the EBTs, indicating poor river water quality. While our cytotoxicity assessments do not allow to derive direct implications for the bioavailability and ecotoxicity of the particle-associated compounds, we use cytotoxicity as a sum parameter of all bioactive substances in a sample.

In the study presented here, we analyzed organic contaminants and cytotoxicity in the particulate and dissolved phases separately. Future investigations may focus on analyzing the total concentrations in turbid water samples to obtain information on the concentrations in the two phases from reliable correlations with TSS, thus avoiding time-consuming and labor-intensive separation of water and particulate phase. Moreover, the toxic potential of metals for cytotoxic effects should be considered. Furthermore, future investigations should address river monitoring over larger time spans to cover seasonal variability of stormwater quality. This study is based on data from two storm events of summer 2019. Repeated investigations at the same sampling site are needed, especially since stormwater runoff can be seasonally highly variable. Moreover, different catchments with different land use types should be compared to examine whether cytotoxicity in rivers correlates with agricultural or urban pressures. Information on the contaminant loads transported in the water versus attached to particles could be obtained from the cumulated effect-based contaminant flux, e.g., on an annual basis. From a management perspective, an assessment of annual loads for both transport media could enhance the development and implementation of control measures to protect aquatic ecosystems from pollution.

5. Acknowledgments

This work was financially supported by the Helmholtz Association within the framework of the Helmholtz POF IV program Topic 9 “Healthy Planet - towards a non-toxic environment” and the observation system MOSES (Modular Observation Solutions for Earth Systems). This work was partly funded by the Collaborative Research Center 1253 CAMPOS (Project P1: Rivers) of the German Research Foundation (DFG, Grant Agreement SFB 1253/1 2017). The authors thank Andrea E. Gärtner, Karolin Gogler, Nelly Wang, Mo Bacher, Jana Mayer, Carla Behringer, Raffaele Böhm, Bernice Nisch, Stephanie Nowak, Renate Seelig, Sara Cafisso, and Monika Hertel for support in sampling and assisting in the laboratory. In addition, we thank the MOSES consortium for fruitful discussions and weather forecasts relevant for sampling. The authors gratefully acknowledge access to the platform CITEPro (Chemicals in the Environment Profiler) funded by the Helmholtz Association.

6. Literature

Becouze-Lareure, C., Thiebaud, L., Bazin, C., Namour, P., Breil, P., Perrodin, Y. ,2016. Dynamics of toxicity within different compartments of a peri-urban river subject to combined sewer overflow discharges. *Sci Total Environ* 539, 503–514. 10.1016/j.scitotenv.2015.08.128.

Chiffre, A., Degiorgi, F., Morin-Crini, N., Bolard, A., Chanez, E., Badot, P.-M., 2016. How to Assess Temporal Changes of Point and Diffuse Contamination in a Rural Karstic Watershed? Relevance of Suspended Particulate Matter (SPM) for Efficient Monitoring. *Wat Air And Soil Poll* 227 (10). 10.1007/s11270-016-3044-3.

Ciszewski, D., Grygar, T. M., 2016. A Review of Flood-Related Storage and Remobilization of Heavy Metal Pollutants in River Systems. *Wat Air And Soil Poll* 227 (7), 239. 10.1007/s11270-016-2934-8.

Cristale, J., García Vázquez, A., Barata, C., Lacorte, S., 2013. Priority and emerging flame retardants in rivers: occurrence in water and sediment, Daphnia magna toxicity and risk assessment. *Environ Int* 59, 232–243. 10.1016/j.envint.2013.06.011.

Escher, B. I., Braun, G., Zarfl, C., 2020. Exploring the Concepts of Concentration Addition and Independent Action Using a Linear Low-Effect Mixture Model. *Environ Toxicol Chem* 39 (12), 2552–2559. 10.1002/etc.4868.

Escher, B. I., Neale, P., Leusch, F., 2021a. Bioanalytical Tools in Water Quality Assessment. IWA Publishing.

Escher, B. I., Glauch, L., König, M., Mayer, P., Schlichting, R., 2019. Baseline Toxicity and Volatility Cutoff in Reporter Gene Assays Used for High-Throughput Screening. *Chem Res Toxicol* 32 (8), 1646–1655. 10.1021/acs.chemrestox.9b00182.

Escher, B. I., Neale, P. A., 2021b. Effect-Based Trigger Values for Mixtures of Chemicals in Surface Water Detected with In Vitro Bioassays. *Environ Toxicol Chem* 40 (2), 487–499. 10.1002/etc.4944.

Esser, V., Buchty-Lemke, M., Schulte, P., Podzun, L. S., Lehmkuhl, F., 2020. Signatures of recent pollution profiles in comparable central European rivers – Examples from the international River Basin District Meuse. *Catena* 193, 104646. 10.1016/j.catena.2020.104646.

Fuchte, H. E., Beck, N., Bieg, E., Bayer, V. J., Achten, C., Krauss, M. et al., 2022. A look down the drain: Identification of dissolved and particle bound organic pollutants in urban runoff waters and sediments. *Environ Pollut* 302, 119047. 10.1016/j.envpol.2022.119047.

Furén, R., Flanagan, K., Winston, R. J., Tirpak, R. A., Dorsey, J. D., Viklander, M., Blecken, G.-T., 2022. Occurrence, concentration, and distribution of 38 organic micropollutants in the filter material of 12 stormwater bioretention facilities. *Sci Total Environ* 846, 157372. 10.1016/j.scitotenv.2022.157372.

Glaser, C., Zarfl, C., Werneburg, M., Böckmann, M., Zwiener, C., Schwientek, M., 2020a. Temporal and spatial variable in-stream attenuation of selected pharmaceuticals. *Sci Total Environ* (741), 139514. 10.1016/j.scitotenv.2020.139514.

Glaser, C., Schwientek, M., Junginger, T., Gilfedder, B. S., Frei, S., Werneburg, M. et al., 2020b. Comparison of environmental tracers including organic micropollutants as groundwater exfiltration indicators into a small river of a karstic catchment. *Hydrol Process* 34 (24), 4712–4726. 10.1002/hyp.13909.

Glaser, C., Zarfl, C., Rügner, H., Lewis, A., Schwientek, M., 2020c. Analyzing Particle-Associated Pollutant Transport to Identify In-Stream Sediment Processes during a High Flow Event. *Water* 12 (6), 1794. 10.3390/w12061794.

Hagemann, L., Buchty-Lemke, M., Maaß, A.-L., Schüttrumpf, H., Lehmkuhl, F., Schwarzbauer, J., 2020. Potential hotspots of persistent organic pollutants in alluvial sediments of the meandering Wurm River, Germany. *J Soils Sediments* 20 (2), 1034–1045. 10.1007/s11368-019-02491-4.

Hollert, H., Dürr, M., Erdinger, L., Braunbeck, T., 2000. Cytotoxicity of settling particulate matter and sediments of the Neckar River (Germany) during a winter flood. *Environ Toxicol Chem* 19 (3), 528–534. 10.1002/etc.5620190302.

IPCC (2018). Summary for Policymakers. Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. (Masson-Delmotte, V., Zhai, P., Pörtner, H. O., Roberts, D., Skea, J., Shukla, P. R. et al., 2018.) IPCC.

Kaiser, D., Wappelhorst, O., Oetken, M., Oehlmann, J., 2012. Occurrence of widely used organic UV filters in lake and river sediments. *Environ Chem* 9 (2), 139. 10.1071/EN11076.

Knauer, K., Homazava, N., Junghans, M., Werner, I., 2017. The influence of particles on bioavailability and toxicity of pesticides in surface water. *Integr Environ Asses* 13 (4), 585–600. 10.1002/ieam.1867.

Lange, C., Kuch, B., Metzger, J. W., 2015. Occurrence and fate of synthetic musk fragrances in a small German river. *J Hazard Mater* 282, 34–40. 10.1016/j.jhazmat.2014.06.027.

Lee, J., Braun, G., Henneberger, L., König, M., Schlichting, R., Scholz, S., Escher, B. I., 2021. Critical Membrane Concentration and Mass-Balance Model to Identify Baseline Cytotoxicity of Hydrophobic and Ionizable Organic Chemicals in Mammalian Cell Lines. *Chem Res Toxicol* 34 (9), 2100–2109. 10.1021/acs.chemrestox.1c00182.

Liang, J., Fang, H., Wu, L., Zhang, T., Wang, X., 2016. Characterization, Distribution, and Source Analysis of Metals and Polycyclic Aromatic Hydrocarbons (PAHs) of Atmospheric Bulk Deposition in Shanghai, China. *Wat Air And Soil Poll* 227 (7), 1. 10.1007/s11270-016-2921-0.

Liu, Y., Zarfl, C., Basu, N. B., Cirpka, O. A., 2019. Turnover and legacy of sediment-associated PAH in a baseflow-dominated river. *Sci Total Environ* 671, 754–764. 10.1016/j.scitotenv.2019.03.236.

Liu, Y., Zarfl, C., Basu, N. B., Schwientek, M., Cirpka, O. A., 2018. Contributions of catchment and in-stream processes to suspended sediment transport in a dominantly groundwater-fed catchment. *Hydrol Earth Syst Sc* 22 (7), 3903–3921. 10.5194/hess-22-3903-2018.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J. et al., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ* 473-474, 619–641. 10.1016/j.scitotenv.2013.12.065.

Milačič, R. Zuliani, T., Vidmar, J., Oprčkal, P., Ščančar, J., 2017. Potentially toxic elements in water and sediments of the Sava River under extreme flow events. *Sci Total Environ* 605-606, 894–905. 10.1016/j.scitotenv.2017.06.260.

Mladenov, N., Dodder, N. G., Steinberg, L., Richardot, W., Johnson, J., Martincigh, B. S. et al., 2022. Persistence and removal of trace organic compounds in centralized and decentralized wastewater treatment systems. *Chemosphere* 286 (Pt 1), 131621. 10.1016/j.chemosphere.2021.131621.

Müller, M. E., Zwiener, C., Escher, B. I., 2021. Storm Event-Driven Occurrence and Transport of Dissolved and Sorbed Organic Micropollutants and Associated Effects in the Ammer River, Southwestern Germany. *Environ Toxicol Chem* 40 (1), 88–99. 10.1002/etc.4910.

Muz, M., Escher, B. I., Jahnke, A., 2020. Bioavailable Environmental Pollutant Patterns in Sediments from Passive Equilibrium Sampling. *Environ Sci Technol* 54 (24), 15861–15871. 10.1021/acs.est.0c05537.

Nasrabadi, T., Ruegner, H., Schwientek, M., Bennett, J., Fazel V. S., Grathwohl, P., 2018. Bulk metal concentrations versus total suspended solids in rivers. Time-invariant & catchment-specific relationships. *Plos One* 13 (1), e0191314. 10.1371/journal.pone.0191314.

Neale, P. A., Braun, G., Brack, W., Carmona, E., Gunold, R., König, M. et al., 2020. Assessing the Mixture Effects in In Vitro Bioassays of Chemicals Occurring in Small Agricultural Streams during Rain Events. *Environ Sci Technol* 54 (13), 8280–8290. 10.1021/acs.est.0c02235.

Niu, L., Ahlheim, J., Glaser, C., Gunold, R., Henneberger, L., König, M. et al., 2021. Suspended Particulate Matter-A Source or Sink for Chemical Mixtures of Organic Micropollutants in a Small River under Baseflow Conditions? *Environ Sci Technol* 55 (8), 5106–5116. 10.1021/acs.est.0c07772.

Niu, L., Carmona, E., König, M., Krauss, M., Muz, M., Xu, C. et al., 2020. Mixture Risk Drivers in Freshwater Sediments and Their Bioavailability Determined Using Passive Equilibrium Sampling. *Environ Sci Technol* 54 (20), 13197–13206. 10.1021/acs.est.0c05124.

Pamuru, S. T., Forgione, E., Croft, K., Kjellerup, B. V., Davis, A. P., 2022. Chemical characterization of urban stormwater: Traditional and emerging contaminants. *Sci Total Environ* 813, 151887. 10.1016/j.scitotenv.2021.151887.

Patrolecco, L., Ademollo, N., Capri, S., Pagnotta, R., Polesello, S., 2010. Occurrence of priority hazardous PAHs in water, suspended particulate matter, sediment and common eels (Anguilla anguilla) in the urban stretch of the River Tiber (Italy). *Chemosphere* 81 (11), 1386–1392. 10.1016/j.chemosphere.2010.09.027.

Peter, K. T., Hou, F., Tian, Z., Wu, C., Goehring, M., Liu, F., Kolodziej, E. P., 2020. More Than a First Flush: Urban Creek Storm Hydrographs Demonstrate Broad Contaminant Pollutographs. *Environ Sci Technol* 54 (10), 6152–6165. 10.1021/acs.est.0c00872.

Rauert, C., Vardy, S., Daniell, B., Charlton, N., Thomas, K. V., 2022. Tyre additive chemicals, tyre road wear particles and high production polymers in surface water at 5 urban centres in Queensland, Australia. *Sci Total Environ*, 158468. 10.1016/j.scitotenv.2022.158468.

Ricking, M., Schwarzbauer, J., Franke, S., 2003. Molecular markers of anthropogenic activity in sediments of the Havel and Spree Rivers (Germany). *Water Res* 37 (11), 2607–2617. 10.1016/S0043-1354(03)00078-2.

Rodil, R., Moeder, M., 2008. Development of a simultaneous pressurised-liquid extraction and clean-up procedure for the determination of UV filters in sediments. *Anal Chim Acta* 612 (2), 152–159. 10.1016/j.aca.2008.02.030.

Rügner, H., Schwientek, M., Beckingham, B., Kuch, B., Grathwohl, P., 2013. Turbidity as a proxy for total suspended solids (TSS) and particle facilitated pollutant transport in catchments. *Environ Earth Sci* 69 (2), 373–380. 10.1007/s12665-013-2307-1.

Rügner, H., Schwientek, M., Egner, M., Grathwohl, P., 2014. Monitoring of event-based mobilization of hydrophobic pollutants in rivers. Calibration of turbidity as a proxy for particle facilitated transport in field and laboratory. *Sci Total Environ* 490, 191–198. 10.1016/j.scitotenv.2014.04.110.

Rügner, H., Schwientek, M., Milačič, R., Zuliani, T., Vidmar, J., Paunović, M. et al., 2019. Particle bound pollutants in rivers. Results from suspended sediment sampling in Globaqua River Basins. *Sci Total Environ* 647, 645–652. 10.1016/j.scitotenv.2018.08.027.

Salomons, W., Rooij, N. M. de, Kerdijk, H., Bril, J., 1987. Sediments as a source for contaminants? *Hydrobiologia* 149 (1), 13–30. 10.1007/BF00048643.

Schwarzenbach, R. P., Escher, B. I., Fenner, K., Hofstetter, T. B., Johnson, C. A., von Gunten, U., Wehrli, B. 2006. The Challenge of Micropollutants in Aquatic Systems. *Science* 313 (5790), 1072–1077. 10.1126/science.1128845.

Schwientek, M., Rügner, H., Beckingham, B., Kuch, B., Grathwohl, P., 2013a. Integrated monitoring of particle associated transport of PAHs in contrasting catchments. *Environ Pollut* 172, 155–162. 10.1016/j.envpol.2012.09.004.

Schwientek, M., Osenbrück, K., Fleischer, M., 2013b. Investigating hydrological drivers of nitrate export dynamics in two agricultural catchments in Germany using high-frequency data series. Environ 69 (2), 381 – 393. 10.1007/s12665-013-2322-2.

Schwientek, M., Rügner, H., Scherer, U., Rode, M., Grathwohl, P., 2017. A parsimonious approach to estimate PAH concentrations in river sediments of anthropogenically impacted watersheds. *Sci Total Environ* 601-602, 636–645. 10.1016/j.scitotenv.2017.05.208.

Schwientek, M., Selle, B., 2016. Quantifying in-stream retention of nitrate at catchment scales using a practical mass balance approach. *Environ Monit Assess* 188 (2), 111. 10.1007/s10661-016-5097-6.

Spahr, S., Teizidó, M., Sedlak, D. L., Luthy, R.G., 2020. Hydrophilic trace organic contaminants in urban stormwater: occurrence, toxicological relevance, and the need to enhance green stormwater infrastructure. *Environ Sci Water Res Technol* 6 (1), 15 – 44. 10.1039/C9EW00674E.

Tuikka, A. I., Schmitt, C., Höss, S., Bandow, N., Ohe, P. C. von der, Zwart, D. de et al., 2011. Toxicity assessment of sediments from three European river basins using a sediment contact test battery. *Ecotox Environ Safe* 74 (1), 123–131. 10.1016/j.ecoenv.2010.08.038.

Villinger, E., 1982. Grundwasserbilanzen im Karstaquifer des Oberen Muschelkalks im Oberen Gäu (Baden-Württemberg). *Geologisches Jahrbuch* (Reihe C 32), 43–61.

Walling, D. E., 2005. Tracing suspended sediment sources in catchments and river systems. *Sci Total Environ* 344 (1-3), 159–184. 10.1016/j.scitotenv.2005.02.011.

Williams, Garnett P., 1989. Sediment concentration versus water discharge during single hydrologic events in rivers. *J Hydrol* 111 (1-4), 89–106. 10.1016/0022-1694(89)90254-0.

Zgheib, S., Moilleron, R., Chebbo, G., 2012. Priority pollutants in urban stormwater. Part 1 - case of separate storm sewers. *Water Res* 46 (20), 6683–6692. 10.1016/j.watres.2011.12.012.

Zgheib, S., Moilleron, R., Saad, M., Chebbo, G., 2011. Partition of pollution between dissolved and particulate phases: what about emerging substances in urban stormwater catchments?. *Water Res* 45 (2), S. 913–925. 10.1016/j.watres.2010.09.032.

Zhang, W., Keller, A. A., Wang, X, 2009. Analytical modeling of polycyclic aromatic hydrocarbon loading and transport via road runoff in an urban region of Beijing, China. *Water Resour Res* 45 (1). 10.1029/2008WR007004.