MASTERARBEIT / MASTER’S THESIS

Titel der Masterarbeit / Title of the Master‘s Thesis

Determination of phthalates and tire-derived compounds along the River Danube

verfasst von / submitted by

Valerie Wilkeit B.Sc.

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2023 / Vienna 2023

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 299
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Environmental Science
Betreut von / Supervisor:	Dr. Thorsten Hüffer, Privatdoz. MSc

Abstract

Plastic production has increased globally over the last decades. Chemical additives, such as phthalates or tire-derived compounds (e.g. antioxidants, vulcanising agents, crosslinking agents), are added to plastics to give them the properties intended. Road runoff, direct littering or atmospheric transport are pathways for plastics to enter the aquatic environment. These additives can leach, e.g., into the environment or food. Plastic additives (from PVC and tires) are ubiquitous in the aquatic environment. As the Danube is the second largest river in Europe and most of the compounds of interest are not monitored yet, it is essential to investigate phthalates and tire-derived compound concentrations throughout the Danube. Water samples were taken at 34 locations throughout the Danube during the CleanDanube project to quantify phthalates and tire-derived compounds. Each sample was filtered. The filtrates were extracted using solid phase extraction, while suspended particles on the filters were extracted in the ultra-sonication bath. Passive samplers were used to confirm the presence of the compounds of interest. Triple quadrupole liquid chromatography-mass spectrometry (LC-MS/MS) was used to quantify the compounds. The method's recovery to measure water and particle-bound concentrations was between 77 and 115 %. Heat maps of the compounds in the water phase and suspended particles throughout the Danube were created. Increasing concentrations starting in Slovakia were detected, connected to the decreasing wastewater treatment system complexity and the decreasing wastewater treatment plant density. The compounds Aniline, 1,3-Diphenylguanidine (DPG), 1,3-Di-o-tolylguanidine (DTG) and 2-mercaptobenzothiazole (2-S BTH) were present in elevated concentrations until Vienna due to increased highway density, higher speed limits, and population densities. The risk for aquatic organisms concerning each compound in the Danube was estimated based on a risk assessment. The concentrations of hexa(methoxymethyl)melamine (HMMM) and di(2-ethylhexyl) phthalate (DEHP) were determined to be at high ecotoxicological risk for aquatic organisms. For 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol (AO2246), phthalic anhydride (PAn) and N-(1,3-dimethylbutyl)-N‘-phenyl-1,4-phenylenediamine (6PPD), a medium ecotoxicological risk was calculated for aquatic organisms in the Danube. This study provides a first overview of the occurrence of additives from tires and plastics within an entire river. The sources of the compounds entering the Danube, as well as an overview of the ecotoxicological risks aquatic organisms are exposed to, are determined.

Kurzfassung

Die Kunststoffproduktion ist in den letzten Jahren rapide angestiegen. Zusatzstoffe wie Phthalate, sowie aus Reifenabrieb freigesetzte Zusatzstoffe (z. B. Antioxidantien, Vulkanisiermittel, Vernetzungsmittel) werden in Kunststoffen verwendet, um ihnen die gewünschten Eigenschaften zu erteilen. Diese Kunstoffe können über z.B. Straßenabfluss, direkten Abfalleintrag oder atmosphärischen Transport in Gewässer gelangen. Diese Zusatzstoffe können z.B. in die Umwelt oder in Essen gelangen. Daher ist es von äußerster Relevanz die Konzentrationen dieser Phthalate, sowie der aus Reifenabrieb freigesetzten Zusatzstoffe in der Donau, dem zweitgrößten Fluss Europas, genauer zu untersuchen. Zur

Quantifizierung der Konzentrationen von Phthalaten und aus Reifenabrieb freigesetzen Zusatzstoffen wurden an 34 verschiedenen Stellen der Donau Wasserproben genommen, welche gefiltert wurden. Die Filtrate wurden mittels Festphasenextraktion extrahiert, während die Schwebeteilchen auf den Filtern im Ultraschallbad extrahiert wurden. Um das Vorkommen der zu untersuchenden Verbindungen zu bestätigen kamen Passivsammler zum Einsatz. Für die Messung der Stoffe wurde die Triple-Quadrupol-Flüssigkeitschromatographie-Massenspektrometrie (LC-MS/MS) verwendet. Die Wiederfindungsraten der Methoden lagen zwischen 77 und 115 %. Zur Ergebnisdarstellung wurden Wärmekarten der Stoffe in der Wasserphase und der Schwebeteilchen in der Donau konzipiert. Für jede Verbindung wurde das Risiko für Wasserorganismen in der Donau bestimmt. Ein Trend sind die ab der Slowakei steigenden Konzentrationen, welche durch die sinkende Kläranlagendichte, sowie die sinkende Komplexität in den Klärsystemen erklärt werden kann. Die Verbindungen Anilin, 1,3-Diphenylguanidin (DPG), 1,3-Di-o-tolylguanidin (DTG) und 2-Mercaptobenzothiazol (2-S BTH) wurden bis Wien aufgrund der zunehmenden Autobahndichte, der höheren Geschwindigkeitsbegrenzungen und der Bevölkerungsdichte in erhöhten Konzentrationen gemessen. Das Risiko für aquatische Organismen wurde für jeden Stoff in der Donau auf der Grundlage einer Risikobewertung ermittelt. Die Konzentrationen von Hexa(methoxymethyl)melamin (HMMM) und Di(2-ethylhexyl) Phthalat (DEHP) erreichten eine Höhe, welche als ökotoxikologisches Risiko for aquatische Organismen eingestuft wird. Für 2,2'-Methylenbis[6-(1,1-dimethylethyl)-4-methyl-Phenol (AO2246), Phthalsäureanhydrid (Pan) und N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylendiamin (6PPD) wurde ein mittleres ökotoxikologisches Risiko für aquatische Organismen in der Donau berechnet. Diese Studie gibt einen Überblick über das Vorkommen von aus Reifen entschwindenden Zusatzstoffen und Kunststoffen in der gesamten Donau. Quellen für die in die Donau gelangenden Stoffe wurden bestimmt und das ökotoxikologische Risiko, dem aquatische Organismen ausgesetzt sind wurde ermittelt.

Table of Content

List of Figures

List of Tables

Abbreviations

TDC	Tire-derived compounds
TWP	Tire wear particles
WWTP	Wastewater treatment plant
PNEC	Predicted no-effect concentration
PEC	Predicted environmental concentration
LC_{50}	Median lethal concentration
EC_{50}	Median effect concentration
RQ	Risk quotient
C_w	Concentration in water
K_{ow}	octanol/water distribution coefficient
JDS	Joint Danube Survey
DEHP	Di(2-ethylhexyl) phthalate
BBP	Benzyl butyl phthalate
DBP	Dibutyl phthalate
DNOP	Di-n-octyl phthalate
DEP	Diethyl phthalate
DMP	Dimethyl phthalate
PA	Phthalic acid
PAn	Phthalic anhydride
MEHP	Mono-2-ethylhexyl phthalate
2-amino BTH	2-aminobenzothiazole
2-OH BTH	2-hydroxybenzothiazole
2-S BTH	2-mercaptobenzothiazole
2-Me BTH	2-methylbenzothiazole
BTH	Benzothiazole
5-Me BTH	5-methyl-1h-benzotriazole
6PPD	N-(1,3-dimethylbutyl)-N‘-phenyl-1,4-phenylenediamine
6PPD-Q	2-anilino-5-[(4-methylpentan-2-yl)amino]-cyclohexa-2,5-diene-1,4-dione
CBS	N-Cyclohexyl-2-benzothiazolesulfenamide
DPG	1,3-Diphenylguanidine
DPTU	N,N´-Diphenylthiourea
DTG	1,3-Di-o-tolylguanidine
HMMM	Hexa(methoxymethyl)melamine

Neozone	N-Phenyl-2-naphthylamine
PPD	p-Phenylenediamine
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
AO2246	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol
PVC	Polyvinyl chloride
SBR	Styrene butadiene rubber
PBD	Polybutadiene
NR	Natural rubber
PE	Polyethylene
PP	Polypropylene
PS	Polystyrene
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
TWA	Time-weighted average
SPM	Suspended particulate matter
SPE	Solid phase extraction
MRM	multiple reaction monitoring
GC-MS	Gas chromatography-mass spectrometry
LC-MS/MS	Triple quadrupole liquid chromatography-mass spectrometry

1. Introduction

Plastics

Due to growing plastic production over the last decades, plastics enter the aquatic environment through various pathways such as road runoff, direct littering or atmospheric transport (1–4). Plastics smaller than 5 mm have been defined as microplastics (5–8), while nano-plastics are defined as plastic particles smaller than 1 µm (6–8). They can enter the environment either as manufactured micro- and nano-plastics, such as microbeads from synthetic textiles or through the breakdown of larger items (7, 9).

To give the different plastic products the properties intended, chemical additives are added to the polymers (7, 10). Chemical additives can also catalyse the production process, e.g. for tires. These chemical additives can be present in plastics in high concentrations, e.g., plasticisers in polyvinyl chloride (PVC) (up to 70 % of weight) (7, 9, 11).

Additives can be categorised based on their critical functional properties, e.g. surface protection or improved processability, additives for processing, material protectants, physiochemical property augmenters or colourants (7). One of the subclasses of additives for processing is plasticisers, to which phthalates belong (7, 12).

Additives can leach from plastics into, e.g., the environment or food (13, 14). They can enter surface water through direct leaching from plastics present in water bodies via the effluent of wastewater treatment plants and atmospheric deposition (15). Especially street runoff is a source for tire-derived additives to enter aquatic environments (4, 16). Hence, additives are present in surface waters.

The Danube river

Originating in Germany and draining into the black sea, the Danube is with 2857 km Europe’s second-longest river (17, 18). It can be separated into three main sections, the Upper Basin, which is the section between the source and Devin Gate, the Middle Basin between Davin Gate and the Iron Gates and the Lower Basin, the part from the Iron Gates to the Danube Delta (18). The catchment area of the Danube is 801 463 km^{2,} with 83 million people living in the basin (17, 18). The Danube’s basin covers 20% of the area of Europe. With 19 countries sharing the river basin, the Danube is the most international river in Europe (17). Major cities along the Danube are Ulm (Germany), Ingolstadt (Germany), Regensburg (Germany), Linz (Austria), Vienna (Austria), Bratislava (Slovakia), Budapest (Hungary), Novi Sad (Serbia), Belgrade (Serbia), Drobeta-Turnu Severin (Romania), Ruse (Bulgaria), Brăila (Romania) and Galați (Romania). The water of the Danube is essential for drinking water production for domestic agricultural and industrial purposes, transportation and recreation throughout the total length of the Danube (18, 19).

There are various ways for contaminants to enter the Danube, such as untreated and partially treated wastewater, industry, agriculture, transport or waste disposal (18, 20). Surface waters and groundwaters in the European Union are regulated by the water framework directive (2000/60/EC), which states that all member states must achieve good status for all water bodies by 2027 (21). The current ecological state of the Danube is considered moderate (22), while its chemical status is in good status until Serbia and fails to reach good status from Serbia onwards (23). Measures for ecological water quality are based on biological quality, such as the composition and abundance of aquatic flora and benthic invertebrate fauna and the age structure, abundance and composition of fish fauna. These are supported by physicochemical elements, e.g. thermal conditions and salinity, as well as hydro morphological elements, such as hydrological regime and river continuity. The chemical status is based on priority substances (21). As the priority substances do not include substances such as tire-derived compounds or phthalates except for DEHP (21, 24), information on the contamination level of the Danube concerning these compounds is not available.

Plastics and released additives in the Danube river

Plastics degrade to microplastics and nano-plastics through pathways such as mechanical erosion, insolation and biological degradation by bacteria. Due to the mishandling of plastics or mishandling of plastic waste and low recovery rates from recycling, plastic pollution has become a global issue (25). Rivers are an essential pathway for plastics to enter the ocean (1). Due to the extent of plastics in the environment, it has become a topic of political, public and scientific interest (1, 2). Studies have shown that the black sea contains, on average, 90.5 items per km^2 of marine litter (26). The Danube is a contributor as it discharges to the black sea with an average discharge of 7320 m^3/s (27). The leaching of chemical additives from plastics is connected to their size. Due to the larger specific surface area, more additives can leach from micro- and nano-plastics compared to macroplastics (28).

The joint Danube surveys (JDS) are a collaboration between expert teams in the Danube basin to monitor the Danube’s water quality. JDS aims to investigate certain water quality elements by producing comparable data and information along the Danube (29). Throughout the Danube, the microplastic content in the suspended particulate matter and biota have been analysed within the report of the JDS4. Whilst the microplastic content in biota and SPM has been analysed throughout, the additives leaching from plastics have only partly been analysed in the scope of the JDS.

For the JDS4, industrial chemicals and plant protection products were analysed along the Danube (30). The compounds included benzotriazoles and benzothiazoles, which can also be TDC, as well as certain phthalates. Out of the compounds of interest, diethyl phthalate (DEP),di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), dimethyl phthalate (DMP), 2-hydroxybenzothiazole (2-OH BTH), 2-aminobenzothiazole (2-amino BTH) and 5-methyl-1h-benzotriazole (5-Me BTH) were analysed in sediments, wastewater treatment plants, biota and surface water and groundwater. In sediments and biota, the concentration of DEHP exceeded its predicted no-effect concentration (PNEC) (30). As not all of the compounds of interest have been investigated during the JDS4, it is necessary to measure the additive concentrations to determine their possible impact.

Using passive samplers to assess surface waters

Passive samplers are used to assess surface water chemically or toxicologically. Compounds can sorb to those samplers depending on the chemical properties of the compound and the sampler (31–33). Due to the difference in chemical potentials of the aqueous phase and the collecting phase of the passive sampler, the compounds of interest are collected as they diffuse through a diffusion barrier or a membrane (33).

The passive sampler can be used for the time-weighted average (TWA) concentrations of different compounds in aquatic environments (34). The passive sampler is used to confirm the detection and detect the different compounds below the detection limit of the grab samples throughout the Danube.

2. State of Knowledge

Phthalates

In synthetic polymer plasticisation, phthalates are typically added as they are low in cost and provide good flexibility (35–37). Of the globally produced plastic, 17 % is PVC (11). The fraction of phthalates in, e.g., PVC, can be up to 50% of the overall weight of PVC plastics. Phthalates are produced in high quantities. They leach from plastics such as PVC, and they are ubiquitously found in the environment (37). The drawback of using phthalates for plasticisation is that some phthalates have been shown to cause adverse health effects such as carcinogenicity, genetic mutation and developmental and reproductive disorders to laboratory animals as well as humans. Hence, phthalates can be considered toxic (35, 38, 39).

The six major phthalates (di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), diisononyl phthalate (DiNP), (dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), and diisodecyl phthalate (DiDP)) have been regulated by the European Union, European Protection Agency (EPA) and other regulatory institutions, but those as well as other phthalates are still detected in air, water, soil, sludge and sediment (38, 40). Phthalic acid (PA) is a transformation product from phthalates (41). The phthalates PA, phthalic anhydride (PAn), and mono-2-ethylhexyl phthalate (MEHP) are degradation products of DEHP. For the thesis, the concentrations of DEHP, MEHP, DNOP, diethyl phthalate (DEP), dimethyl phthalate (DMP), DBP, BBP, PA and PAn were analysed along the Danube.

Tire-derived compounds

In tires, a mixture of chemical additives is added during production, such as antioxidants, antiozonants, crosslinking agents, vulcanisation agents, curing agents, fillers and reinforcement agents (4, 7, 13, 42). Over the last years, the attention towards tire wear particles (TWP) as part of particulate organic contaminants increased. TWP emissions contribute 5-30% to the non-exhaust traffic emissions. The mass of TWP entering aquatic environments depends on the extent of road runoff collection and treatments (4). TWP can be found in environmental compartments such as air, water, soils, sediments, and biota (42). In Germany, it is estimated that tires emit 113 × 103 t of TWP in a year. In Europe, 1327 × 10^3 t of TWP is estimated to be emitted in a year (4). Tire-derived compounds (TDC) such as benzothiazoles are used as markers for TWP in the environment (42).

Tire rubber typically consists of a mixture of natural rubber (NR), styrene butadiene rubber (SBR) and polybutadiene (PBD). Tires are composed of carbon black or silica as a reinforcing agent, oils as softeners and extenders and vulcanising chemicals (4, 42).

It is known that TDC can have toxic effects on aquatic organisms (4, 42, 43). A study showed that tire additives and transformation products such as N-(1,3-dimethylbutyl)-N’-phenyl-p-phenylenediamine (6PPD) could be toxic to aquatic organisms, e.g. salmon (43).

Influence of separated and combined sewer systems on TDC entering surface waters

For 2-anilino-5-[(4-methylpentan-2-yl)amino]-cyclohexa-2,5-diene-1,4-dione (6PPD-Q), N-(1,3-dimethylbutyl)-N‘-phenyl-1,4-phenylenediamine (6PPD), Hexa(methoxymethyl)melamine (HMMM), 1,3-Diphenylguanidine (DPG) and benzothiazoles, stormwater concentrations, especially in urban areas, can be higher than their concentrations in surface waters reached (16, 44–46). Stormwater is one of the primary sources for TDC to enter surface waters (16, 44–46). Especially in urban areas, stormwater can be collected in sewer systems.

The type of sewer system can influence the concentrations of TDC in surface waters. Stormwater and municipal and industrial wastewater are collected in combined sewer systems (47–49). Traditionally combined sewer systems are used (47, 50). Thus, TDC is collected in combination with the wastewater. From the combined systems, the wastewater goes through a wastewater treatment plant (WWTP) before it is discharged to surface waters. Combined sewer systems can overflow during rain events, leading to TDC entering surface waters without prior treatment (47–49, 51). HMMM, benzothiazoles and DPG are known to be in the overflow of combined sewer systems (52).

In separated sewer systems, stormwater is collected separately from municipal and industrial wastewater (47, 48). Thus, the overflow events of combined sewer systems can be decreased by using separated sewer systems (50). The stormwater collected in separate sewer systems is directly discharged to surface water, which can lead to increased pollutants, including TDC, in surface waters (47, 50). It was determined that 47 % of rubber from tire wear reaches surface waters through these sewer systems (47). Thus, separate sewer systems are a path for TDC to enter surface waters.

Removal efficiencies of wastewater treatment systems for the compounds of interest

Wastewater discharges either result from industries or municipalities. The wastewater quality depends on physical, chemical and biological properties (53). The predicted environmental concentration for DEHP in European rivers downstream from WWTP discharges is 3.2 µg/L in the waters and 30 mg/kg dry weight in sediments (54). In WWTPs, phthalates can be removed by decantation (e.g. DEHP and DNOP), biodegradation (e.g. DMP and DEP) and volatilisation (e.g. DMP and DEP). Decantation by particle sorption is the primary mechanism. The removal of DEHP from wastewater has been referred to be 90% on average WWTPs (54), while TWP above 300 µm is 90 % and above removed (4). After the discharge of a WWTP, freely dissolved phthalate concentrations in rivers are typically higher due to the direct release of phthalates. Also, particle-bound phthalates are higher after WWTPs (54).

The removal efficiency for some of the compounds of interest in WWTPs was measured during the joint Danube survey 4 (30). While the removal of 2-OH BTH and DEP is classified as ‘efficient’ (above 80 %) based on influent and effluent concentrations WWTPs, the removal of 5-Me BTH and DBP is classified as ‘poor’ (below 80 %) (30).

The removal efficiencies for phthalates throughout the different wastewater treatment steps have been investigated in 2006 in a study in France (55). The phthalates DEP, DBP, BBP and DEHP are mainly removed during the primary treatment with removal efficiencies of 58.9 %, 61.4 %, 81.1 % and 52.7 %. For DMP, the primary removal takes place in the second wastewater treatment step, with a removal efficiency of 93.9 %. Even though the removal of those compounds mainly takes place during the primary and secondary treatment, especially for DEHP and DBP, the tertiary treatment is an essential step for removal, too (13.1 % and 18.5 %). The average removal efficiency for the phthalates in the WWTPs was 90.6 %. The DEHP concentration correlated with the suspended matter concentration (55).

Benzothiazoles and benzotriazoles are not efficiently removed during sedimentation (primary treatment) (4 %) (56). The primary removal for those compounds takes place in the bioreactors (secondary treatment) with a removal efficiency for benzotriazoles of about 60 % and benzothiazoles between 30 - 75 % (56, 57). During tertiary treatment, benzothiazoles are removed by 5 – 28% (58). Synthetic phenolic antioxidants such as BHT, BHA and AO2246 have removal efficiencies between 62 % and 93 % in WWTPs. Removing synthetic phenolic antioxidants is the most effective during the secondary treatment step, with efficiencies above 90 % (59).

In Serbia, 37.83 % of the population is not connected to a sewer system. Of the population's wastewater, 48.33 % is collected in sewer systems but not treated before discharge, while 1.25 % of the wastewater treatment plants only go to the level of primary treatment, 9.18 % secondary and 3.42 % tertiary (60). In Romania, 40.2 % of its population's wastewater is treated on a tertiary level, 6.2 % on the secondary level, 3.2 % on the primary level and 1.4 % is collected in the sewers and discharged without treatment. Thus, 49 % of the population is not connected to sewer systems. In Slovakia, secondary treatment systems are the most popular, with 63.2 %, 1.8 % tertiary, 2.2 % primary and 0.5 % collected and discharged without treatment, meaning that 32.3 % of the population is not connected to sewer systems. In Hungary, 72.08 % of the population's wastewater goes through tertiary treatment, 7.12 % through secondary, 0.7 % primary, and 2.18 % is collected and discharged without treatment. Hence 17.92 % of its population's wastewater is not collected in sewer systems. In Croatia, 54.6 % of its population's wastewater is collected and mainly secondarily treated, while 45.4 % is not collected in sewers. In Austria and Germany, over 95 % of the wastewater is collected and treated (mainly tertiary) (60).

Ecotoxicological effects of microplastics and released additives

Microplastics and their released additives can cause adverse ecotoxicological effects for aquatic and terrestrial biota, such as effects on populations, e.g., decreased fertility or transcriptional effects. These effects are associated with detoxification and immune system pathways (7, 61). Physical effects, such as the blocking of tissues in aquatic organisms, have been reported (7). Comparing the contaminant concentrations interacting with microplastic to the chemical additive contents, the concentration of additives is higher (7, 11). Therefore, ecotoxicological risks result from chemical additives (7). The effects of chemical additives can be neurotoxic, inflammatory or carcinogenic (7, 13).

Ecotoxicity has been characterised for some phthalates and tire-derived compounds (TDC). TDC can have toxic effects on aquatic organisms (4, 42–44). 6PPD is a tire-derived compound which can make up to 0.4 - 2 % of the mass in passenger and commercial tires (43). One transformation product is 6PPD-Q. Through road runoff, it can enter aquatic environments. A toxicity indication can be the median lethal concentration (LC_{50}), which indicates mortality for 50 % of the tested population. The LC_{50} of around 0.8 mg/L of 6PPD-Q has been the reason for acute mortality in coho salmon (43). The acute LC_{50} of DPG is known to be 17 mg/L for daphnia (62). In rainbow trout epithelial cell lines, benzothiazoles can cause cytotoxicity and a transient increase in reactive oxygen species. Phthalates and benzothiazoles are toxic for mussels causing sublethal effects (44).

Phthalates can cause endocrine disruption, oxidative stress, metabolic disruption and immunodeficiency in aquatic animals (63, 64). DEHP is mainly referred to as an endocrine disruptor. It has an LC_{50} of 0.5 mg/L for zebrafish and can lead to embryo mortality. For zebrafish, toxicity symptoms are necrosis or tail bending (63). Typical sources for DEHP to enter the environment are PVC material production or wastewater treatment plants, as well as agricultural mulch (64). DNOP has an LC_{50} of 1.45 mg/L for Daphnia carinata and a toxicity range of 1.45-1200 mg/L for aquatic organisms (65). DNOP is known to have a higher potency to cause endocrine disruption in zebrafish compared to DEHP (66).

Passive samplers as an indicator for compounds and to measure time-weighed average concentrations

Passive sampling can be used as an indicator for the compound concentrations by being exposed to the water containing the compounds for extended periods. The uptake rates for passive sampling depend on the water temperature and flow rate (67). Passive samplers are typically used to improve the limit of quantification or detection of a compound in aquatic environments by exposing the passive samplers to the compounds over a more extended period (68).

Using passive samplers, time-weighted average (TWA) concentrations for the compounds of interest can be calculated. Passive samplers are a recommended method by the European Commission for the chemical monitoring of surface waters as an addition to grab sampling to improve the reliability of the data. For hydrophobic compounds, passive samplers such as semi-permeable membrane devices, silicone rubber or low-density polyethylene strips are typically used for monitoring (68).

To determine the concentration of compounds in water, the concentration of the compound on the passive sampler can be used. Therefore, the passive sampler/water distribution coefficient, the diffusion coefficient, the octanol/water distribution coefficient of the compound or the compound-specific sampler uptake rate has to be determined (68, 69). The quantified concentration shows the level to which biota is exposed to the compound in the environment (68).

Methods to measure phthalates and TDC

As phthalates and TDC are diverse, looking at their hydrophobicity (**Appendix 2**), there is no standardised method to measure both phthalates and tire-derived compounds in aquatic environments simultaneously. Thus, an analytical method to measure both phthalates and TDC is needed.

Grab sampling is a targeted sampling method preferred if the compounds of interest are expected to be present in constant, equally distributed concentrations (67). It is one of the most used methods for the chemical monitoring of a water body (70). Grab sampling can be used to collect point data on compounds' concentrations. In moving waters such as rivers, fluctuations in the concentration of compounds are likely (71), which can lead to compound fluctuations from sample to sample. Thus, the combination of grab sampling and passive sampling can take those fluctuations into account (34, 71).

As phthalates and some of the TDC are hydrophobic with high octanol/water distribution coefficients (K_{ow}), aqueous concentrations can be low (37) (**Appendix 2**). Passive samplers can be a method to detect the compounds of interest at low concentrations and prove their presence along the Danube (34, 72). A potential passive sampler to measure hydrophobic and hydrophilic compounds can be an Empore® disk, typically used for compounds with a $\log K_{ow}$ between 1-6 (34, 73).

Passive sampling considers the dissolved concentration of the compounds of interest, not the concentration of a compound sorbed to particulate matter. It can be used in combination with grab sampling to see whether the compound is present (68).

For compounds such as benzothiazole, the water samples are filtered through a filter before further processing (74). Filtration of the water samples makes it possible to look at the dissolved concentration and the sediment-bound concentration of a compound separately (70). The typical filter size for environmental samples is 0.45 µm (70), while 0.2 µm filters are referred to in drinking water analyses for phthalates (75). Solid-phase extraction can be used to extract the compounds from the water samples (74, 76). Other methods, such as freeze-dried suspended matter sample analysis, were also referred to as a method to measure phthalates (77).

To analyse TDC from filters, energy dispersive X-ray spectroscopy is referred to with the disadvantage of not being selective enough for environmental samples. A spatially resolved analysis of filters from water samples can be used to analyse TDC (4). Using an LC-MS/MS to quantify TDC has also been referred to in literature (4, 52, 62, 78). For the quantification of phthalates in water samples, both gas chromatography-mass spectrometry (GC-MS) (79, 80) and LC-MS/MS can be used (80). Hence, the LC-MS/MS is suitable for TDC and phthalates.

Measured concentrations of phthalates and TDC in the Danube

The NORMAN project aims to create a network between laboratories and research centres to have a broader and faster exchange of data and information concerning emerging substances' environmental concentrations and effects (81, 82). The project has been ongoing since 2005 (83). Therefore, measured environmental concentrations of different emerging compounds at various locations, including the Danube, have been registered in the NORMAN database (84). PNEC values in surface waters according to the NORMAN database (84) for the compounds of interest are given in (**Appendix 1**). The PNEC values were either experimentally determined, based on quantitative structure-activity relationship model predictions or, in the case of DEHP, taken from external sources such as the European Commission (24, 84).

The overall plasticiser concentrations in suspended particulate matter in the German part of the Danube decreased by a factor of 2.5 (77). This decrease in plasticiser concentration is accompanied by the water volume of the Danube increasing by a factor of 10 in the area looked at (77).

Based on previous measurements for the JDS, DEHP is predicted to have environmental concentrations between 0.2 µg/L and 1 µg/L in surface waters in Europe (84, 85), which are lower than its PNEC of 1.3 µg/L (**Appendix 1**). Environmental concentrations for DEP are measured in the range between 0.04 µg/L and 1.64 µg/L, while higher concentrations are determined towards the lower Danube (84). For DMP, concentrations up to 0.04 µg/L are referred to in the NORMAN database. Previously measured concentrations in surface waters of benzothiazoles are up to 0.09 µg/L. 5-Me BTH is detected in the Danube at concentrations between 0.05 µg/L and 0.13 (84). The measured environmental concentrations of DPG are between 0.01 µg/L and 0.073 µg/L in surface waters. BHT can have concentrations of up to 4.4 µg/L in European surface waters. For HMMM, environmental concentrations between 0.024 µg/L and 0.42 µg/L are reported in surface waters (84, 85). The previously measured environmental concentrations for the JDS of BBP, DBP, DNOP, phthalic anhydride, 2-S BTH, 2-Me BTH, 6PPD and CBS are between either below the LOD or in between LOD and LOQ throughout the Danube (84).

Risk assessment as a tool to predict toxicity

Risk assessments can be used to estimate the adverse effects of a compound concerning its exposure to, e.g. specific organisms (61). This can be related to the likelihood of the event occurring (86). To predict the effect a compound can have on specific organisms, predicted no-effect concentrations (PNEC) can be related to the predicted environmental concentrations (PEC) (87). PNEC can be calculated from effect concentrations (65). Depending on the relation, it can be determined whether the risk is acceptable (87). An acceptable risk has no or minor adverse effects, e.g., for specific organisms. The risk can be accepted if the PNEC concentrations are higher than the PEC concentrations. The results of an assessment can indicate whether mitigation or monitoring strategies need to be taken (42, 87).

Priority substances

The list of priority substances was established in 2001 and is part of the priority substance directive (2008/105/EC). According to the European Commission, 33 substances and groups of substances are defined as priority substances (24, 88). Environmental quality standards were set for these substances, referred to in the priority substance directive (2008/105/EC). A compound qualifies as a priority substance if it is threatening the aquatic environment or through the aquatic environment (24, 88). A substance causes a threat through the aquatic environment if it, e.g. has adverse health effects for humans exposed to the aquatic environment the substance is in (24). DEHP is the most abundant phthalate, and its toxicity has been proven (63, 77). Since the implementation of the water framework directive (2000/60/EC) combined with the priority substance directive (2008/105/EC), DEHP has been regulated as a priority substance (21, 24). As the priority substances do not include substances such as TDC or phthalates except for DEHP (21, 24), frequent monitoring data is not available for those compounds. Thus, data on the contamination level of the Danube concerning these compounds is needed.

Aims and Hypotheses

The compounds of interest have not been quantified throughout the Danube. This thesis aims to quantify freely dissolved and particle-bound concentrations of phthalates and TDC in the Danube. Higher concentrations starting from Slovakia are hypothesised due to different wastewater treatment systems (89). We expect an increase in concentrations after the major cities due to higher concentrated direct street runoff (62, 90). The measured concentrations of each compound are related to their PNEC values in a risk assessment.

3. Methods

Sorbent, chemicals and standards

Methanol (≥99,8 %, AnalaR NORMAPUR® ACS) from VWR Chemical (Vienna, Austria) and ultra-pure water from a water purification system (Purlab® Chorus 1, ELGA LAB Water, Veolia Water Technologies, Celle, Germany) were used. Sodium azide (NaN_3) (99 %) from Thermo Fisher Scientific (Waltham, Massachusetts, US) was added to each Danube water sample to prevent bacterial growth. The passive sampler was an Empore^{TM} Solid Phase 47 mm Extraction Disk styrene divinyl benzene – reversed phase sulfonate (SDB-RPS) from IVA Analysentechnik GmbH & Co. KG (Meerbusch, Germany).

Nine compounds were considered phthalates (properties in **Appendix 2**). For the phthalate standard, the EPA phthalate esters MIX (2000 µg/mL each component in methanol) containing benzyl-butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-n-octyl phthalate (DNOP), diethyl phthalate (DEP) and dimethyl phthalate (DMP), mono-2-ethylhexyl phthalate (MEHP) (97 %), phthalic acid (PA) (analytical standard) and phthalic anhydride (PAn) (ACS reagent, ≥99 %) were purchased from Merck (Darmstadt, Germany). A mixed standard in methanol with a final concentration of 100 µg/mL of each compound was prepared and stored at 4 °C in a brown glass vial with Teflon septa in the fridge.

As TDC, 19 different compounds were considered (properties in **Appendix** 2). The TDC standard mix contained benzothiazole (96 %), 2-aminobenzothiazole (97 %), 2-Methylbenzothiazole (99 %), 2-Mercaptobenzothiazole (97 %), 2-Hydroxybenzothiazole (98 %), 5-methyl-1-H-benzothiazole (98 %), Aniline (ACS reagent, ≥ 99.5 %), N-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (6PPD) (Aldrich^{CPR}), p-phenylenediamine (PPD) (98 %), 1,3-Diphenylguanidine (DPG), 1,3-Di-o-tolyguadinine (DTG) (99 %), (98 %), N,N´-diphenylthiourea (DPTU) (98 %), N-phenyl-2-naphthylamine (Neozone) (97 %), 2,2-Methylenebis(6-tert-butyl-4-methylphenol) (AO2246), Butylated hydroxytoluene (BHT) (≥ 98.5 %), Butylated hydroxyanisole (BHA) (≥ 99 %, FCC, FG) purchased from Merck (Darmstadt, Germany) and N-Cycohexyl-2-benzothiazolesulfenamide (CBS) (98 %) from Combi-Blocks (San Diego, US), 2-anilino-5-[(4-methylpentan-2-yl)amino]-cyclohexa-2,5-diene-1,4-dione (6PPD-Q) from HPC Standards GmbH (Bordorf, Germany) and Hexa(methoxymethyl)melamine (HMMM) (> 98 % GC) from TCI Chemicals (Eschborn, Germany).

The standards used for quantification of the phthalates were deuterated di(2-ethylhexyl) phthalate (DEHP-d4) (analytical standard) from Merck (Darmstadt, Germany), deuterated diethyl phthalate (DEP-d4) (analytical standard) from Merck (Darmstadt, Germany) and deuterated phthalic acid (phthalic acid-d4) (≥ 98 atom % D, ≥ 98 % (CP)) from Merck (Darmstadt, Germany). For TDC, 6PPD-quinone-d5 from HPC Standards GmbH (Borsdorf, Germany) and benzothiazole-d4 from LGC Standards (Toronto, Canada) were used for quantification. The internal standard was prepared by adding the standards to methanol, achieving a concentration of 50 µg/mL for each phthalate standard and benzothiazole-d4, while 6PPD-quinone-d5 was at a concentration of 10 µg/mL. The standard was stored at 4°C in the fridge in a brown glass vial with a Teflon septa cap.

Preparation and sampling

To measure concentrations of each compound throughout the Danube, water samples were taken along the Danube (between Scheer, Germany (sample 1) and Galati, Romania (sample 34)) (Fehler! Verweisquelle konnte nicht gefunden werden.). The sampling locations are listed in **Table 1**. The samples were collected within the Clean Danube project. To identify the impact of contamination of major cities along the Danube, samples were taken before and after major cities. To avoid degradation of the TDC, the samples were picked up in three different locations along the Danube (Vienna, Austria, Kladovo, Serbia and Bucharest, Romania) and processed directly after arrival in the lab. During the sampling period, the pH, water temperature, electric conductivity, turbidity, oxygen, ammonium, nitrate and phosphorus were measured around every 50 km on the boat.

Figure 1 *- Map Sampling locations along the Danube. Each location a sample was taken is shown (red dots), while the source and end of the Danube are highlighted (purple dots). A close-up of the sampling locations in the major cities is displayed. The catchment area of the Danube is demonstrated (red line).*

***Table 1** - Sampling locations. The numbers are referring to the number of the sample, while the location of the sample was taken is on the right.*

Sample	Location
1	Scheer
3	Bertoldsheim
4	Ingolstadt
5	Kelheim
6	Donaustauf
7	Straubing
8	Haus am Strom
9	Linz 1
10	Linz 2
11	Krems
12	Wien 1
DK	Donaukanal
13	Wien 2
14	Bratislava 1
16	Komarno
17	Budapest 1
18	Budapest 2
19	Paks
20	Backi Monostor
21	Novi Sad 1
22	Novi Sad 2
23	Belgrade 1
24	Belgrade 2
25	Safanta Elena
26	Iron Gate 1 (Kladovo)
27	Iron Gate 2
28	Vidin
29	Oryahovo
30	Zimnicea
32	Ostrov
33	Ghindaresti
34	Galati

To avoid contamination, glass wear was put into the laboratory scale dishwasher (Miele Professional G7883) (Miele, Wals bei Salzburg, Austria), three times rinsed with ultra-pure water and then put into the muffle oven at 550 °C for five hours. All caps were rinsed with ultra-pure water. The caps with PTFE septa were additionally rinsed with acetone.

The water samples at each sampling location were taken in duplicates. The 1 L sampling bottles were filled up to its neck and taken in the direction of the river flow. Each sample was spiked with 1 mL of sodium azide (50 g/L in ultra-pure water) directly after sampling. The bottles were mixed by turning them three times upside down and stored in the dark at 8°C. After arrival in the lab, samples were filtered using 0.2 µm nylon membrane filters (diameter 47 mm) from Global Science Solutions Operations UK Ltd. (Amersham Place, UK).

Passive samplers were put on foot bands protected by a metal net, worn by Andreas Fath, who was swimming through the Danube. One foot band is placed on each foot. The passive samplers are stored in ultra-pure water overnight and changed after ~100 km of swimming to avoid the saturation of the sampling membrane (67).

Sample processing

Each sample was filtered using a vacuum filtration system. The samples were weighed before filtering each sample into the 1 L Schott bottles. Each filtrate was spiked with 3 µg of deuterated phthalates and 3 µg of benzothiazole-d4, and 0.6 µg 6PPD-quinone-d5 (60 µL of 50 µg/mL mixed INST phthalates and INST benzothiazole-d4 and 10 µg/mL 6PPD-quinone-d5 in MeOH). The filtrates were extracted via solid phase extraction (SPE) from Waters® (Milford, US). Each filtrate was poured from the Schott bottle with an aluminium disk through a funnel into 200 mg LP glass cartridges from Waters™ (Milford, US) under vacuum. The cartridges were extracted three times with 5 mL of methanol (LC-MS grade). The extraction speed was set to ~1drop/s. Each extract was concentrated to 1 mL using a gentle nitrogen stream at 40°C in the Barkey (Barkey vapotherm basic mobil I) (Leopoldshöhe, Germany). The concentrates were transferred into 1.5 mL vials and measured on the LC-MS/MS.

The filters were transferred into the 20 mL vials, which were weight empty and with the used dried filters to quantify the concentrations in the particulate matter. For each sample, the filter spiked with 3 µg of the deuterated phthalates (60 µL of 50 µg/mL mixed INST phthalates) and 3 µg of benzothiazole-d4 (50 µg/mL INST benzothiazole-d4) and 0.6 µg 6PPD-quinone-d5 (10 µg/mL 6PPD-quinone-d5 in MeOH). The filters were extracted one time with 5 mL methanol (LC-MS grade) in the ultra-sonication bath for 10 min. Each filter extract was filtered through a 0.45 µm filter (Altmann Analytik, München, Germany) using a 20 mL plastic syringe into a new 20 mL vial. A new plastic syringe and pre-filter were used for each filter extract to avoid contamination. The filtered extracts were concentrated in the Barkey to 1 mL and transferred to 1.5 mL vials. The concentrates were measured on the LC-MS/MS.

To calculate the suspended particle concentrations in each sample, the weight of the particulate matter on each filter, as well as the volume of the water of each sample, was used. To measure the dissolved organic carbon (DOC) content in the Danube samples, 20 mL of each water sample was filtered through a 0.45 µm nylon filter into a 20 mL vial. The samples were acidified using 40 µL of 32% HCl from Carl Roth (Karlsruhe, Germany) and stored at 4 °C in the fridge until the DOC measurement with a Total Organic Carbon Analyser (TOC- LCPH/CPN, Shimadzu, Kyoto, Japan).

To avoid clogging the filters during the filtration process during sample processing, each bottle was weighed before and after the filtration process. This allowed to relate the concentration to the measured concentrations of the other samples. During the solid phase extraction, clogging of the cartridges could occur. Therefore, the internal standards were spiked before for quantification.

Processing of the passive samplers

Each passive sampler is pre-conditioned by placing it into 20 mL methanol for 30 min on a horizontal shaker at 100 rpm. Afterwards, the methanol is decanted. To remove the remaining methanol from the passive sampler, ultra-pure water is added and the vials are placed on the shaker again for 30 min. This step is repeated twice. The passive sampler is stored at 4 °C in ultra-pure water until further usage.

The passive samplers were stored in 20 mL methanol in 50 mL brown glass vials at 8 °C. Each passive sampler vial was spiked with 10 µL of the internal standard mix (0.5 µg deuterated phthalates and 0.5 µg of benzothiazole-d4, and 0.1 µg of 6PPD-quinone-d5) and extracted for 10 min in the ultra-sonication bath. The methanol was taken out from each vial with a 20 mL plastic syringe and filtered into a 20 mL vial through a 0.45 µm nylon pre-filter. Each vial is then concentrated at the Barkey. To the vials containing the passive sampler, 10 mL of methanol was added, and 10 µL of the internal standard mix was spiked. The extraction in the ultra-sonication bath for 10 min was repeated, and the extracts were filtered into the partly concentrated vials. Each vial was concentrated to 1 mL and transferred into a 1.5 mL measurement vial. The samples were measured with the LC-MS/MS.

As low concentrations of internal standards were detected in the first batch, for the two passive sampler batches collected in Kladovo and Bucharest, overall, 60µL of the internal standards (3 µg of deuterated phthalates and 3 µg of benzothiazole-d4 and 0.6 µg of 6PPD-quinone-d5) was spiked. 30 µL was spiked before the first ultra-sonication bath and 30 µL before the second.

Duplicates of passive samplers were stored in ultra-pure water instead of methanol on the boat until analysis. To consider these possible errors, for the second batch of passive samplers for each duplicate, one passive sampler was stored in ultra-pure water, and one was stored in methanol until the analysis.

Validation of method

A recovery experiment was conducted to validate the method for the analysis of the compounds of interest. Blanks were prepared for determining the lowest detection limit (LOD) and the lowest quantification limit (LOQ) and for analysing the Danube samples' matrix effects. Seven blanks, five with ultra-pure water and two with Danube water, and four samples, two with ultra-pure water and two with Danube water, were prepared. The Danube water was taken from the Danube in Vienna. For the recovery sample preparation, 1 µg of each compound (40 µL of 25 µg/mL phthalates and tire standard-mix) was spiked into 1 L of either Danube water or ultra-pure water. For mixing purposes, each sample was shaken for 1 minute before processing the sample. The samples were analysed according to the method described.

Quantification

The concentrations of the phthalates and tire additives were determined using a triple quadrupole liquid chromatography-mass spectrometry (LC-MS/MS). The LC-MS/MS is an ultra-high performance liquid chromatograph (UHPLC) (Agilent infinity 1290 II) (Agilent Technologies, Santa Clara, US), which is connected to a 6470 LC/TQ triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, US) running in multiple reaction monitoring (MRM) mode. A C18 column (Acquity HSS T3, 1.8 µm, 100 mm Waters) was used to achieve separation. The flow rate was set to 0.3 mL/min, and the column temperature was 40 °C. In mobile phase A, ultra-pure water was taken, while in phase B, methanol with 0.1 % formic acid was used. The eluent gradient was set to the following: 0 min, 95 % of phase A and 5 % phase B, which increased to 0 % of phase A and 100% of phase B at 25 min, where it was held for 5 min until it went back to 95 % of phase A and 5 % of phase B at minute 31. The post-time was set to 1.5 min to equilibrate the conditions. For quantification, a calibration curve with 8 standards (0.1 µg/L – 1000 µg/L in methanol) for each compound of interest and 1 µg of phthalate INST and 1 µg benzothiazole-d4 INST and 0.2 µg of 6PPD-quinone-d5 was used to calculate the different amounts of phthalates and TDC in each sample. The transitions from the precursor ion to each product ion are given in **Appendix 3**. The results of the LC-MS/MS were evaluated using the MassHunter software.

After data evaluation with MassHunter, the LOD and LOQ of the method were determined using the residual standard deviation of the calibration curve for the samples. The values for each compound are given in **Table 2**.

Table 2 *- LOD and LOQ of the method for each compound*

	LOD (µg/L)	LOQ (µg/L)
DEHP	0.027	0.081
DNOP	0.028	0.086
DBP	0.097	0.294
BBP	0.02	0.057
MEHP	0.028	0.085
DEP	0.037	0.112
DMP	0.056	0.17
PA	0.022	0.066
PAn	0.015	0.044
BTH	0.1	0.303
2-aminoBTH	0.0005	0.001
2-OH BTH	0.057	0.173
2-S BTH	0.039	0.117
2-Me BTH	0.068	0.205
5-Me BTH	0.0008	0.003
PPD	0.0028	0.009
6PPD	0.004	0.011
6PPD-Q	0.042	0.126
Aniline	0.0008	0.002
DPG	0.0003	0.001
DTG	0.0005	0.002
DPTU	0.007	0.02
HMMM	0.003	0.008
Neozone	0.0004	0.0013
CBS	0.037	0.11
AO2246	0.022	0.066
BHA	not detected	not detected
BHT	not detected	not detected

Risk Assessment

The risk assessment was based on the measured concentrations in the Danube and the PNEC values (**Table 1**). Using the PNEC values of the different compounds and the minimum, maximum and average concentrations in water (c_w) measured throughout the Danube, the minimum, maximum and average risk quotients (RQ) have been determined using the following equation:

$$RQ = \frac{Cw}{PNEC} \qquad \text{(Eq.1)}$$

With the risk quotients of each compound, the ecotoxicological risk concerning the compounds of interest is indicated. If the calculated risk quotient exceeds 1, the ecotoxicological risk is not acceptable or ‘high risk’ (87, 91). A risk quotient between 0.1 to 1 indicates ‘medium risk’, while values from 0.01 to 0.1 show ‘low risk’ (87).

The risk assessment uncertainty relates to the uncertainty within the PNEC values and the measured concentrations (92). Due to the differences in the determination of the PNEC values used in the NORMAN database (quantitative structure-activity relationship model, experimental or external sources) (84), the PNEC values for the compounds can be different in the environmental conditions leading to uncertainties in the RQ. Another uncertainty in the risk assessment is the variation in concentrations within the duplicates. Therefore, the standard deviation of the duplicates is taken into consideration.

4. Results and Discussion

The concentrations measured in the water samples were up to 9.8 µg/L (PA), while 47.96 mg/kg particulate matter (DEHP) was the filter's highest measured concentration. The passive sampler was exchanged in 9 different locations, so 18 passive samplers were analysed, detecting most of the compounds of interest.

Recovery of the method

The data from the recovery experiment showed recovery rates between 77 and 115 % for most of the compounds (**Table 3**). The compounds PPD, BHT, and BHA were not detected throughout the recovery experiment, which also have not been detected in the environmental samples or the passive sampler. Thus, the method seems unsuitable for PPD, BHT and BHA. As phthalic acid, phthalic anhydride, CBS, and Neozone were challenging to detect due to the matrix effects of Danube water, the recovery rate was calculated in ultra-pure water instead of Danube water. These matrix effects can be an impact of each Danube water component, leading to a change in ionisation efficiency (93). Matrix effects can cause alterations in the response of these compounds (93). For BTH, phthalic acid, phthalic anhydride, and 6PPD, it was only possible to recover up to 51 % (**Table 3**), which can be due to matrix effects (94).

Due to the lower sensitivity of the LC-MS/MS during the measurement of the recovery experiments, the INST could not always be detected, leading to a higher LOD and LOQ in comparison to the LOD and LOQ for the environmental samples. Thus, the recovery rates of the method can be lower than they would have been during the environmental sample processing. The method was validated as it worked for most substances with an average recovery of 83.19 %, which might have been higher during the actual sample.

Table 3 *- Recovery of each compound in Danube water. TDC = tire-derived compounds.*

Phthalates		TDC	
Coumpund	Recovery (%)	Coumpund	Recovery (%)
DEHP	96.36	BTH	47.14
DNOP	77.74	2-aminoBTH	106.25
DBP	121.11	2-OH BTH	101.89
BBP	106.55	2-S BTH	93.74
MEHP	105.71	2-MeBTH	5.01
DEP	89.36	5-MeBTH	91.39
DMP	86.01	PPD	0.00
PAn	15.90	6PPD	44.81
PA	16.33	6PPD-Q	114.18
		Aniline	50.43
		DPG	115.48
		DTG	114.29
		DPTU	43.79
		HMMM	103.71
		Neozone	109.51
		CBS	85.42
		AO2246	114.19
		BHA	0.00
		BHT	0.00

Water parameters throughout the Danube

The pH measured on the boat was around 8.3, with a standard deviation of 0.3 (**Appendix 4**) (95). The pH between 7.82 and 8.45, referred to in JDS3 (87), was similar to the measured pH. The temperature was 12.1 °C at the first sampling location, while it rose to 25.6 °C towards the Danube Delta as the sampling period was from April to June. The electric conductivity was higher at the beginning until Linz, Austria (416 – 618 µS/cm) due to an increase in water discharge and low electric conductivity (220 µS/cm (JDS4)) coming from the Inn (96, 97). Afterwards, the electric conductivity was constant between 333 and 401 µS/cm with an average of 404.92 µS/cm, which is a similar trend to the measurements in the JDS3 and 4 (566 µS/cm to 320 µS/cm (JDS3) 400 µS/cm to 275 µS/cm (JDS4)) (96, 97). The turbidity varied between 2.65 and 42.1 NTU. The oxygen content measured on the boat was constant at around 10.2 mg/L, with a standard deviation of 2.37 (95). On average, the SPM concentration in the JDS3 was 14.9 mg/L with a minimum of 2.7 and a maximum of 47.0 mg/L. The measured SPM concentration during the project in the upper and middle Danube varied between 10.47

mg/L and 57.84 mg/L and increased to between 89.49 mg/L and 137.98 mg/L in sampling locations 30 – 24 (**Appendix 5**). The water discharge measured JDS3 in 2013 is 1936.3 m^3/s with a standard deviation of 901.6 (96). The DOC we measured in our water samples was determined to be 1.10 mg/L with a standard deviation of 0.345. Combining the data measured on the boat, which can be accessed online (95), and the DOC measurements, the sampling conditions of the samples were stable despite the temperature settings (**Appendix 4**).

Samples along the Danube

The average water concentrations in µg/L of the different substances looked at along the Danube are shown in the heat map (Fehler! Verweisquelle konnte nicht gefunden werden.), with each sample b elonging to a specific sampling location (**Table 1**). The heat map is divided into three parts based on the point where each batch of samples was transferred to the lab and processed.

The concentrations of compounds such as DEHP, 5-Me BTH, Aniline and DPG appeared to be orders of magnitude lower. The concentration of DEHP decreased from around 0.2 µg/L to below the LOQ. The concentrations of 5-Me BTH Aniline and DPG decreased from around 0.01 µg/L to below the detection limit. For Aniline and 5-Me BTH, this can indicate the degradation of the compounds in the sample as both are degradable (98, 99). The half-life of Aniline was determined to be 11 hours in water bodies close to the surface in summer conditions and about one week in distilled water (98). 5-Me BTH was determined to be 87 % biologically degraded in activated sludge from WWTPs after 28 days at temperatures between 20.4 and 23 °C (99). As the samples were taken from a river, spatial and temporal variations among the samples have to be considered looking at degradation. Comparing the third data set to the first two, compounds found at concentrations above the LOQ in the first two were below the LOD in the third (**Figure 2; Figure 3**). As the trend of the third data set (locations 27 – 34; **Table 1**) is different compared to the data of the first two sets, the data of the third data set is not taken into account for further discussion. Due to the differences in the units of concentrations of the particulate concentrations (**Figure 3**) compared to the water concentrations (**Figure 2**) (water concentrations in µg/L up to 9.8 µg/L, particulate concentrations in µg/kg particulates up to 47955 µg/kg particulates), the heat maps are discussed separately.

For the first two sets, the samples were stored at 8 °C until the pick-up after the sample was taken. Due to logistical issues such as delayed pick-up of the samples and delivery delay, the third data set was stored for a month under undefined conditions. These issues are related to concentrations differing from the trend measured in the first two batches compared to those in the third batch (**Figure 2**; **Figure 3**).

Water concentrations of phthalates and TDC are connected to the complexity of WWTPs

An increasing trend in compound concentrations of the water samples occurs after Vienna (location 11) (Fehler! Verweisquelle konnte nicht gefunden werden.), which can be due to less treated (only primary o r primary and secondary treatment) and untreated (direct discharge) wastewater entering the Danube after Vienna (89, 100, 101). There, wastewater treatment plant density decreases (**Figure 4**). The connection to sewer systems is also decreased (60), which makes it more likely for untreated and partially treated wastewater to enter the Danube. WWTPs can influence the compound concentrations by directly discharging them to the river, leading to higher compound concentrations close to WWTPs (30, 54, 100, 102). They also influence the river flow due to the discharge of the WWTP (54). Organic matter can also be directly discharged from WWTPs (101), leading to more opportunities for the compounds to sorb to.

A hotspot for phthalates, especially DEHP, DNOP, DBP and phthalic acid, was Backi Monoster, Serbia (location 20) (**Figure 2**), which the discharges of WWTPs can explain in the area of the sampling location (**Figure 4**) (54). There, the WWTPs in Croatia are close to the sampling location between Croatia and Serbia and have either only wastewater collection or only treatment up to the secondary (**Figure 4**). As the removal efficiencies for phthalates depend on the tertiary treatment step, the measured increase can be explained. The predicted concentrations of DEHP were 0.2 – 1 µg/L (84) which is within the range of measured concentrations throughout the Danube (0.1 – 1.25 µg/L).

According to the SPARCS calculator, the solubility of phthalates increases with increasing temperatures. While the temperature was increased from the first to the last sample (**Appendix 4**) (95), the temperature conditions can influence the increasing phthalate concentrations after Vienna. In Novi Sad, DBP was detected, which was also reported to be detected in literature (103). Phthalic acid and phthalic anhydride concentrations are the highest in and after Belgrade, Serbia (location 23), which the difference in wastewater treatment in Serbia can explain. The wastewater is mainly collected without treatment which then is discharged to, e.g., the Danube in Novi Sad, where the sampling location 22 was very close to the direct discharge of the WWTP (60, 100, 101, 103). DEP was detected in Bertoldsheim, Germany (location 3), which relates to values (<LOD – 0.4 µg/L) measured in Germany in the NORMAN database (84). The two WWTPs close to the sampling location can be a source for the DEP concentrations (**Figure 4**), as the removal efficiency of DEP in WWTP is up to 90 % after tertiary treatment (55). Thus, DEP is still present in WWTP effluents. The increase in DEP concentration towards the lower Danube referred to in the NORMAN database measured for the JDS was not confirmed (84) as DEP was only detected in sampling location 3 (**Figure 2**).

BTH, 2-Me BTH, PPD, 6-PPD-Q, CBS, DPTU, BHA and BHT were not detected in the water samples throughout the Danube (**Figure 2; Figure 3**). In comparison to the LOQ of 0.0014 µg/L, 2-amino BTH was detected at low concentrations (0.001 – 0.006 µg/L) starting from Komarno, Slovakia (location 16), with the highest concentration detected in Paks, Hungary (location 19). The detection of 2-amino BTH relates to the predicted environmental concentration throughout the Danube benzothiazoles of around 0.09 µg/L (84). Neozone was only detected at low concentrations varying between detected but not quantifiable and 0.003 µg/L before Serbia. AO2246 was detected at the sampling points 19 and 20 (Paks, Hungary and Backi Monoster, Serbia) with the highest concentration in Backi Monoster (0.137 µg/L) (**Figure 2**). AO2246 can be present in wastewater treatment sludge and the effluent and river sections close to the discharge of these WWTPs (104). Untreated wastewater released to the Danube in Serbia can lead to increasing concentrations (101, 105, 106). For AO2246 and the benzothiazoles, it is known that secondary to tertiary treatment has the primary role in removing those compounds (56, 59), leading to the detected increase.

5-Me BTH was detected at concentrations varying between 0.017 – 0.087 µg/L throughout the Danube, as its removal in WWTPs is below 80 % (30). The measured concentrations are close to the predicted environmental concentration range of 0.05 – 0.13 µg/L (84). The concentration of 6PPD throughout the Danube was detected between 0.025 and 0.32 µg/L. The detection of 6PPD can come from WWTPs and direct street runoff, as 6PPD is known to be present in WWTP effluents and direct street runoff (43, 44, 52).

2-OH BTH was detected at not quantifiable concentrations along the Danube until Vienna, Austria (location 12) and after below the LOD. For HMMM, the concentrations in water were above 0.1 µg/L before Komarno, Slovakia and lower (around 0.05 µg/L) afterwards (**Figure 2**). Aniline, DPG and DTG were detected at higher concentrations until Vienna. Tire-derived compounds such as DPG and HMMM are typically found at high concentrations in direct street runoff (16, 44, 107). In Germany, combined and separated sewer systems are used (48), which can lead to elevated concentrations of those compounds. As the highway density decreases from Germany towards Romania (108), the concentration of these compounds is especially high where the highway density is high. Therefore, Germany's denser highway system and the higher speed limit also relate to the elevated concentrations (108, 109). As the river discharge in the area of the elevated concentrations of the tire-derived compounds discussed is lower than further downstream, it also contributes (96, 97, 110). Another contributor to the elevated compound concentrations in the Upper Danube is the population density, which is the highest in Germany (232 people/km^2), followed by Slovakia and Austria (111 and 106 people/km^2). The population density in Serbia, Romania, Croatia and Bulgaria is lower (90, 82, 73 and 64 people/km^2) (111). The population density could relate to the elevated concentrations as more vehicles are used, more waste is produced, and more wastewater has to be treated. The increase of surface runoff towards the lower Danube, with an average annual runoff volume of 12.11 m^3 in Germany, 58.12 m^3 in Austria, 64.51 m^3 in Slovakia, 72.26 m^3 in Hungary, 126.99 m^3 in Serbia, 183.44 m^3 in Romania and 180.05 m^3 in Bulgaria (110) can be connected to the decrease in concentrations towards the lower Danube.

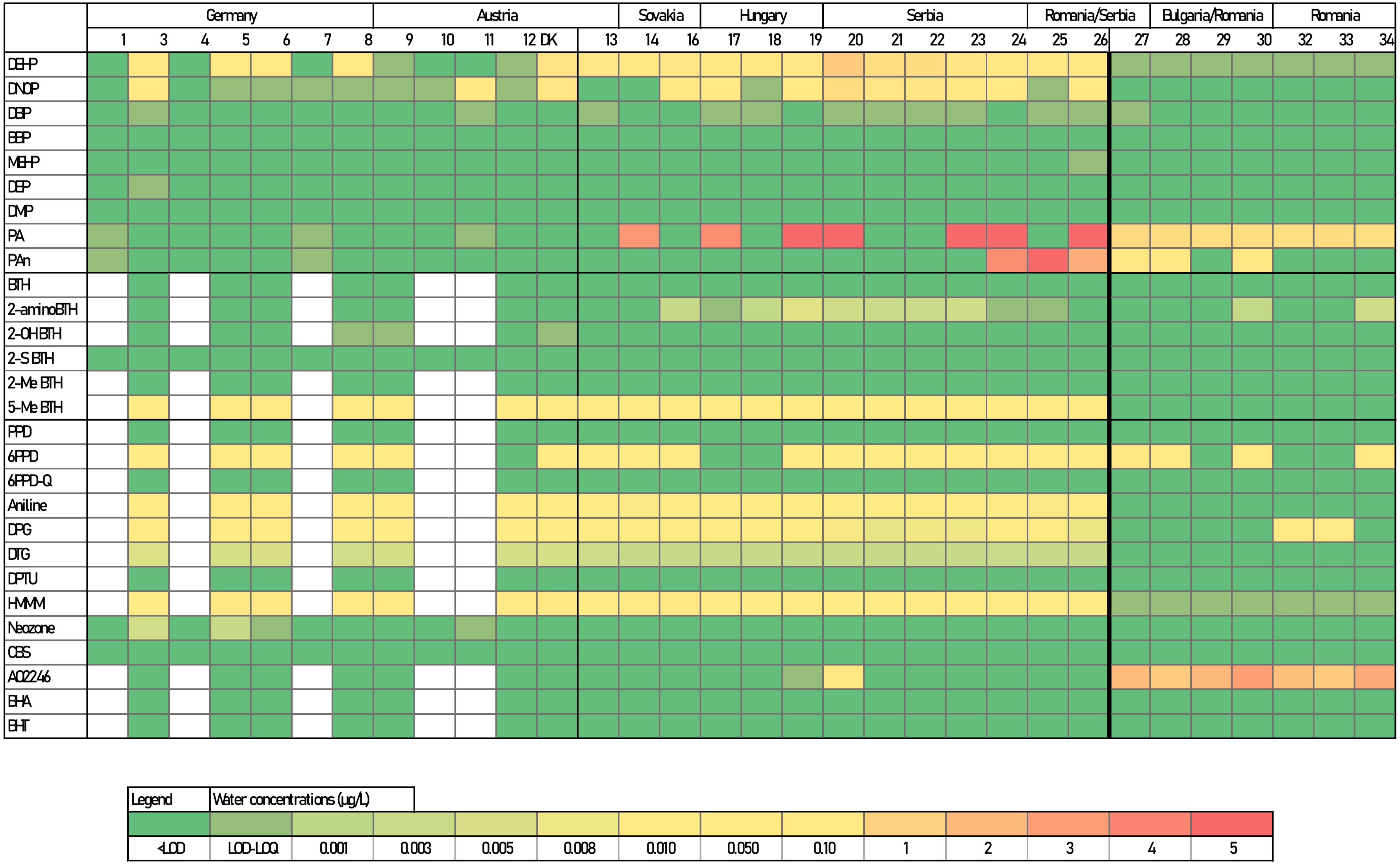


Figure 2 *– Heat map of water concentrations along the Danube. Dark green stands for concentrations below LOD, while red means concentrations were above 10 µg/L. The numbers stand for different sampling locations referred to in* ***Table 6****, while the different compounds are in the first column.*

Particle-bound concentrations can be connected to the complexity of WWTPs

Knowing that the compounds of interest are present in multiple environmental compartments (38, 42), the particle-bound concentrations of each compound are essential to understand their occurrence throughout the Danube. The heat map for particulate matter concentrations also shows an increase in contaminant concentrations, mainly after Vienna (**Figure 3**). The particle-bond concentrations of DEHP increased after Vienna, Austria (location 13) from below LOQ to 1.90 – 47.96 mg/kg particulate matter in Slovakia, Hungary, Serbia and Romania. The increase can be related to the decreasing complexity of WWTPs and the decreasing density of WWTP (**Figure 4**). The decreased population connected to sewer systems can also contribute to the increase (60). The SPM concentrations were, on average, 29 mg/L (**Appendix 5**), and the DEHP tends to sorb to particulate matter due to its hydrophobicity (logK_{ow} 7.95 (**Appendix 2**)). Therefore, the visible increase in particulate-bond concentrations of DEHP is not connected to the suspended particulate matter. DNOP, DBP and BBP were mainly detectable in Germany and Austria at concentrations below the LOQ. The surface runoff increase from 12.11 m^3 - 58.12 m^3 between Germany and Austria, to 126.99 m^3 in Serbia, 183.44 m^3 in Romania and 180.05 m^3 in Bulgaria (110) can be another explanation for those phthalates mainly being present in Germany and Austria due to dilution. MEHP, DEP and DMP were below the detection limit. Even though DEHP was restricted by the water framework directive and its environmental concentrations were decreasing between the mid-2000s and 2017 (77), it still is the most abundant phthalate measured throughout the Danube (24, 30, 77).

The benzothiazoles sorbed to particulates were mainly detected before and in Vienna, Austria (13) (**Figure 3**), with the highest concentrations for 2-S BTH in Linz, Austria (location 3) (3.21 mg/kg particulates). Aniline showed a peak in concentrations in Haus am Strom, Germany (location 8) and Linz, Austria (locations 9 and 10). The higher population density is a reason for the elevated concentrations until Slovakia (111). The sewer systems can also relate to the elevated concentrations as separated and combined sewer systems are used in Germany (48). Separated sewers can lead to increased contaminant concentrations due to the direct discharge of stormwater towards surface waters (49, 50). The elevated concentrations also relate to the higher speed limits in Germany and the elevated highway density in Germany (108, 109). The increasing surface runoff towards the lower Danube contributes to the lower concentrations (110). As 2-S BTH has the highest logK_{ow} among the benzothiazoles (1.99) (**Appendix 2**), it is also the most likely to be found sorbed to particulates. 2-amino BTH was mainly detected in the water phase and not sorb to particles due to its hydrophilicity with a logK_{ow} of -0.15 (**Appendix 2**).

5-Me BTH was present at quantifiable concentrations in the water and on the particulates due to its logK_{ow} of 0.77, too. The concentrations of 5-Me BTH, 6PPD, DPG, DTG, HMMM and Neozone increased after Vienna on particulates which can be related to the decreasing complexity of WWTP systems and the decreasing WWTP density leading to less and untreated wastewater entering the Danube (89, 100, 103). The connection to the sewer systems decreases towards the lower Danube, which can contribute to the increasing concentrations (60). As the $\log K_{ow}$ values of 6PPD, DPG, DTG and Neozone (5.18, 3.9, 4.68, 5.39) (**Appendix 2**) make them preferably sorb to particulate matter, the source was not connected to the suspended particulate concentrations. AO2246 was detected in Belgrade, Serbia (locations 23, 24) at concentrations of 1236 and 1086 µg/kg particulates, which the untreated wastewater discharged to the Danube can be the source for (101, 104). Due to the high $\log K_{ow}$ (7.15), AO2246 from the wastewater sorbs to the particulates. A decrease in water concentrations was determined for the compounds DPG, DTG and HMMM, while an increase in particulate concentration was measured. The decrease can be related to their $\log K_{ow}$ values of 0.61 – 4.68 (**Appendix 2**).

Relating the trend of the two heat maps (**Figure 2**; **Figure 3**), the wastewater treatment plant design and density (**Figure 4**) influence the compound concentrations in the Danube. Tertiary treatment, the most complex treatment, is mainly used in Germany, Austria and Hungary, explaining the lower concentrations of DEHP, DNOP, DBP, PA, PAn, 5-Me BTH, AO2246, 2-amino BTH, 6PPD and Neozone measured in the first batch of samples (samples 1 – 11) (**Figure 2**; **Figure 3**). The wastewater treatment plant density decreases in Slovakia, Croatia and Romania, and the complexity of the treatment plant changed to mainly secondary and primary treatment (**Figure 4**) (60, 89). The decreasing complexity and density can explain the higher concentrations measured throughout the Danube in Serbia, Romania, Hungary, Slovakia, and Croatia (**Figure 2**; **Figure 3**). This trend can additionally relate to the amount of wastewater collected per person in each country and the amount of wastewater treated by which system per person in a country. The wastewater collection rate in Serbia, Romania, Croatia and Slovakia is relatively low (51 – 67.7 %). Thus, more untreated wastewater enters the river systems (60). In Serbia, the wastewater is mainly collected without treatment, while in Croatia, secondary treatment systems are the most popular and in Austria and Germany, tertiary (60). Hence, the increase in concentration towards the lower Danube is connected to each country's wastewater collection and treatment systems.

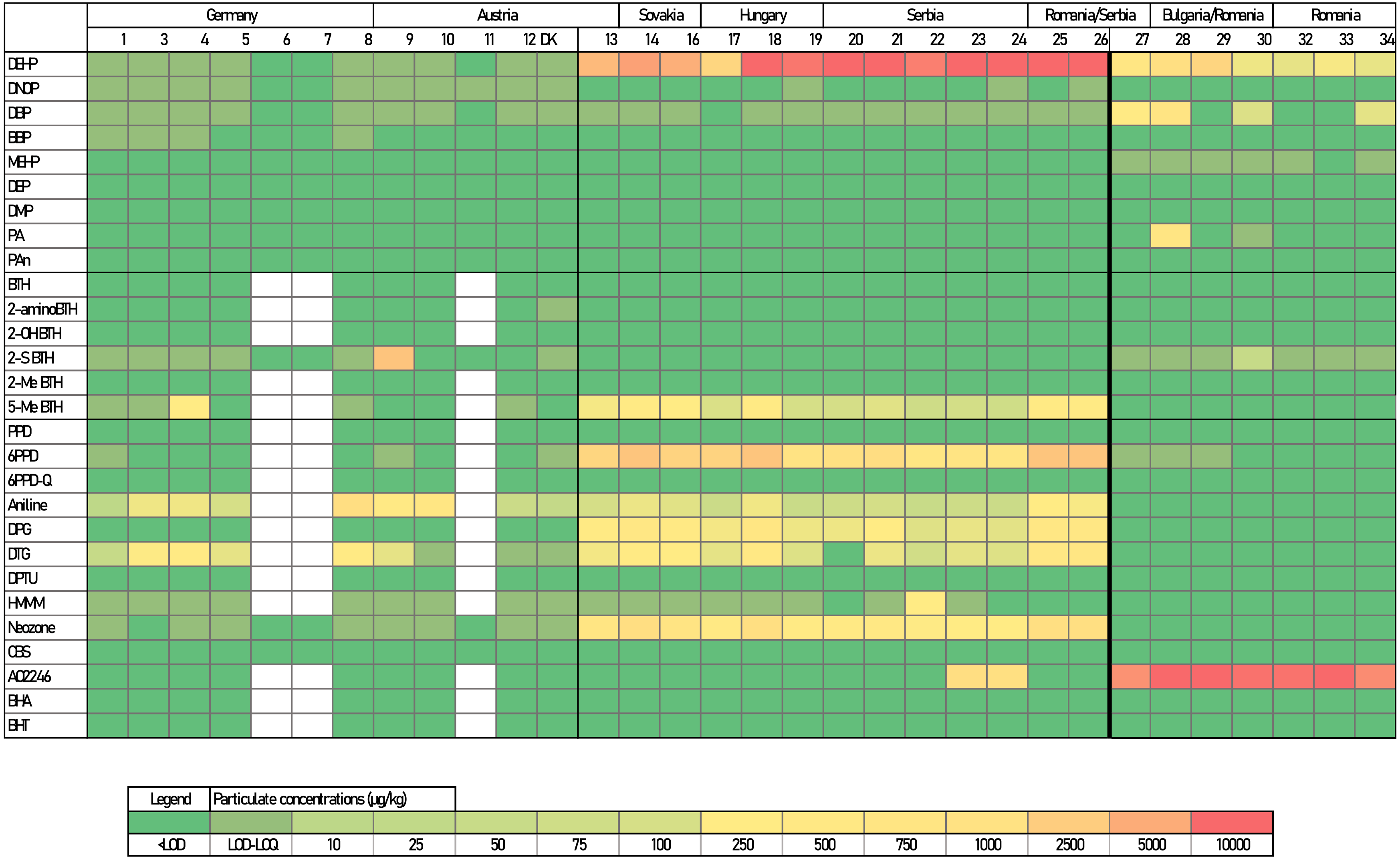


Figure 3 *– Heat map of particulate concentrations along the Danube. The darker the green, the lower the concentration measured. Dark green stands for concentrations below LOD, while red refers to concentrations above 10000 µg/kg particulate matter. The numbers are the different sampling locations referred to in* ***Table 6****, while the different compounds are in the first column.*

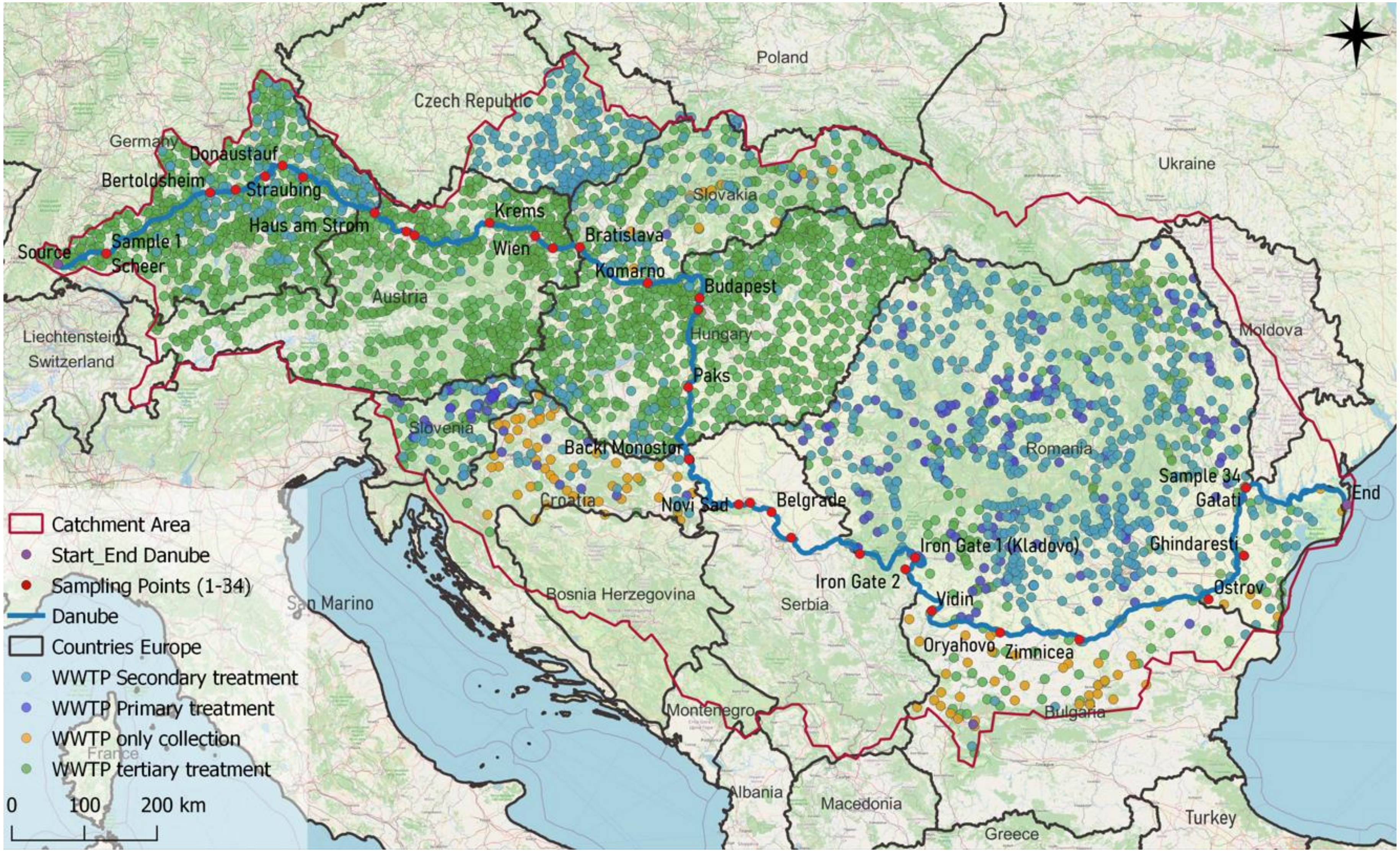


Figure 4 - *Wastewater Treatment plants in the catchment area; the wastewater treatment plant data are from the European Environment Agency (27). The red dots are the sampling locations, while the green dots show the WWTPs going up to tertiary treatment, the blue dots show WWTPs up to secondary treatment, the purple dots stand for primary treatment systems, while the orange dots stand for wastewater collection only. The red line refers to the catchment area of the Danube.*

Detection of the compounds on the passive sampler

The trends are similar, comparing the measured water concentrations (Figure 2) to the measured in the passive sampler (Figure 5). DEHP was constantly detected throughout the Danube, in the water samples, as well as on the passive sampler (**Figure 2**; **Figure 5**). Due to the high $\log K_{ow}$ (**Appendix 2**) of DEHP, it has a higher affinity to sorb to the passive sampler than staying in the water, explaining the high concentrations of DEHP sorbed to the particles and the detection on the sampler. DNOP was detected on the passive sampler throughout the Danube. It was also present at the sampling locations in Vienna 2 and Bratislava (locations 13 and 14), below the LOD in the water samples (**Figure 2**; **Figure 5**). The high $\log K_{ow}$ value of DNOP (8.6) (**Appendix 2**) can make the detection of DNOP in water more challenging, which is why the passive sampler can be used to prove the presence of DNOP in water, even at low concentrations. Despite the $\log K_{ow}$ of DNOP, it was not present at quantifiable concentrations in the particulate matter samples. PA and PAn were detected on the passive sampler throughout the Danube, showing their presence below the LOD in the water samples. DMP was also detected on all passive samplers, while it was not present in the water samples (**Figure 2; Figure 3; Figure 5**) as the $\log K_{ow}$ (1.36) (**Appendix 2**) is within the applicable range of the passive sampler (73). As the passive sampler shows the TWA concentrations (34), the concentration of DMP in water was lower than the detection limit, while its presence was proved with the passive sampler.

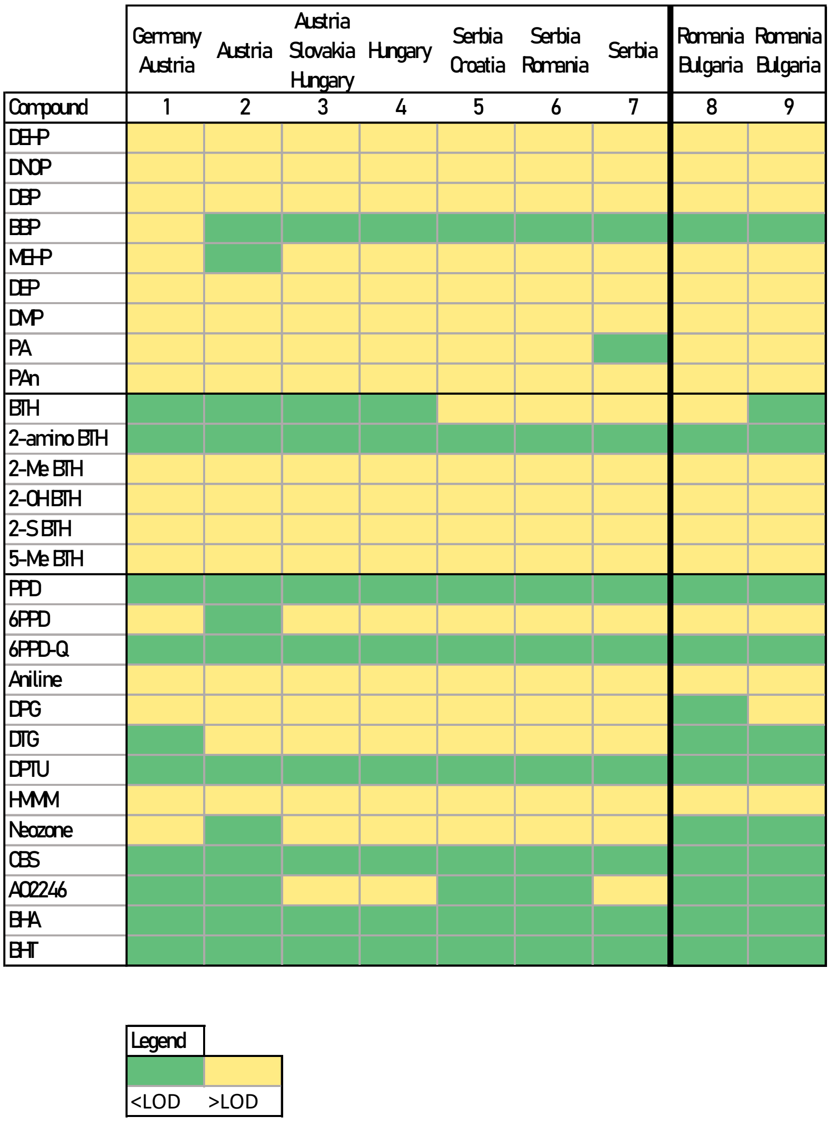
While BHT was not detected in the water samples, on the passive sampler, BTH could be measured starting from Serbia due to the untreated wastewater discharge (60, 100, 103). The $\log K_{ow}$ of BTH (1.87) is in the preferred range for the passive sampler, making it suitable for BTH. Measured BTH on the sampler is due to the TWA concentrations on the passive sampler (34). While the benzothiazoles were mainly not detected in the water samples **(Figure 2; Figure 3**), they were measured with the passive sampler. 2-amino BTH was detected from the passive sampler extracts at concentrations below the detection limit but at not quantifiable concentrations throughout the Danube (**Figure 2**; **Figure 3**; **Figure 5**). The passive sampler was not suitable for 2-amino BTH as the $\log K_{ow}$ (-0.15) was outside the preferred range for the passive sampler (73).

The presence of 6PPD, Aniline, DPG, DTG, HMMM and Neozone was proved by the passive sampler, and AO2246 was also detected on the passive sampler in the areas where it was detected in either the water samples or the particulate concentrations relating to the sample (**Figure 2**; **Figure 3**; **Figure 5**). PPD, 6PPD-Q, DPTU, CBS, BHA and BHT were neither detected in the Danube water samples nor the passive sampler extracts, showing that they were not present at quantifiable concentrations throughout the Danube (**Figure 2**; **Figure 3**; **Figure 5**). Combining the results of the water samples, filters and the passive sampler it becomes clear that the concentrations of a compound are higher if the complexity of a wastewater treatment system is lower.

In the scope of a study, a new passive sampler was developed, which is suitable for hydrophobic and hydrophilic substances. It has been tested for compounds in the range of $\log K_{ow}$ 1.44 – 9.49. The passive sampler is called a hydrophilic-lipophilic sorbent-embedded cellulose acetate membrane (HECAM), which can be used for measuring the TWA concentrations of hydrophobic and hydrophilic organic compounds (112). The partitioning coefficient of that passive sampler and water ranges between $\log K_{sw}$ 2.75 – 6. The passive sampler was tested for various compounds and followed the first-order kinetics model (112, 113). The sampling period of the HECAM is as for the Empore® disk five days (67, 112, 113). Looking at the distribution of the different K_{ow} values of the compounds of interest and the HECAM passive sampler, it might be suitable for especially the more hydrophobic substances. Still, it could have difficulties with 2-amino BTH as it has not been tested for compounds with a $\log K_{ow}$ below 1.

A simple teabag equilibrium passive sampler (STEPS) in combination with a hydrophilic divinylbenzene (h-DVB) sorbent could be an alternative option as a passive sampler. It has been tested for compounds with a polarity range of $\log K_{ow}$ -0.1 – 9.9 (114), which means it could be more suitable for 2-amino BTH. In contrast to the HECAM, the passive sampler was already tested for phthalates (112–114). The STEPS needs 1 – 2 weeks to equilibrate (114), which means it can mainly be used for stationary measurements in aquatic environments. It has partitioning coefficients varying between 4.1 – 6.5 L/kg.

Looking at the three passive samplers, the Empore® disk, HECAM and STEPS, they all have advantages and disadvantages in sampling the compounds of interest. While the preferred range of the Empore® disk is $\log K_{ow}$ 1-6 (73, 115), it has been used for compounds in the range of -1 to 6 (32). As the preferred range for the HECAM and STEPS is not defined, similar challenges to the ones with the Empore® disk could occur. Due to the timespan for the STEPS to equilibrate, the Empore® disk and HECAM seem the most promising to use as a passive sampler when swimming through a river.



	Germany Austria	Austria	Austria Slovakia Hungary	Hungary	Serbia Croatia	Serbia Romania	Serbia	Romania Bulgaria	Romania Bulgaria
Compound	1	2	3	4	5	6	7	8	9
DEHP	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DNOP	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DBP	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
BBP	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
MEHP	🟨	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DEP	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DMP	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
PA	🟨	🟨	🟨	🟨	🟨	🟨	🟩	🟨	🟨
PAn	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
BTH	🟩	🟩	🟩	🟩	🟨	🟨	🟨	🟨	🟩
2-amino BTH	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
2-Me BTH	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
2-OH BTH	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
2-S BTH	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
5-Me BTH	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
PPD	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
6PPD	🟨	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟨
6PPD-Q	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Aniline	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
DPG	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟩	🟨
DTG	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟩	🟩
DPTU	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
HMMM	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Neozone	🟨	🟩	🟨	🟨	🟨	🟨	🟨	🟩	🟩
CBS	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
AO2246	🟩	🟩	🟨	🟨	🟩	🟩	🟨	🟩	🟩
BHA	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
BHT	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩

Legend	
🟩	🟨
<LOD	>LOD

Figure 5 *– Detection passive sampler. The yellow fields mean the compound was detected above the LOD, while the green ones mean they were below the LOD (**Table 2**). The different numbers are duplicates of passive samplers used.*

Impact of cities on the compound concentrations

Due to higher concentrations in urban street runoff, cities can influence compound concentrations in surface waters (49, 90). The concentration of the phthalates DEHP and DNOP were between the LOD and the LOQ before Vienna. DNOP was below the LOD after Vienna, and DEHP increased to 0.205 µg/L from below the LOQ of 0.081g/L (**Table 2**). For 6PPD and DPG, an increase got visible after Vienna, while 5-Me BTH, Aniline and HMMM decreased (**Figure 6**). The concentration of DTG stayed almost the same. Comparing the water concentrations to the particulate concentrations, 6PPD, DPG, 5-Me BTH and Aniline were increasing in the particulate matter, differing from the decrease of 5-Me BTH and Aniline in water (**Figure 6**; **Appendix 6**, **Figure 1**). As 6PPD and DPG in Vienna increased, which might be connected to highly concentrated urban street runoff (44, 45, 49, 116). In Vienna, a combined and separated sewer system is used (117). There, the discharge can contribute to the increase. The decrease of 5-Me BTH, Aniline and HMMM in water concentrations can be due to the floodplain forests around Vienna (118, 119). Floodplain forests can decrease compound concentrations by biological and physical absorption (120). It has been determined that lettuce can take up TDC, such as HMMM (118). Thus, the floodplain forests might have caused the decrease. The increase of 5-Me BTH, 6PPD, DPG, DTG, Neozone, Aniline and DEHP in particle concentrations (**Appendix 6, Figure 1**) could be connected to a decrease in suspended particles. In Vienna, an increase in SPM was measured (from 20.99 to 31.41 mg/L; **Appendix 5**), and the standard deviation for the value measured after Vienna was high (20.92). In one of the duplicates, the suspended particles increased, while in the other, they decreased (52.33 mg/L and 10.48 mg/L), explaining why in Vienna, suspended particles cannot be connected to the increasing particle-bond concentrations. As the $\log K_{ow}$ of 5-Me BTH is 0.77, and the one of Aniline 1.16, the increase in the suspended particulate matter could lead to increasing concentrations in particulate matter and decreasing concentrations in water.

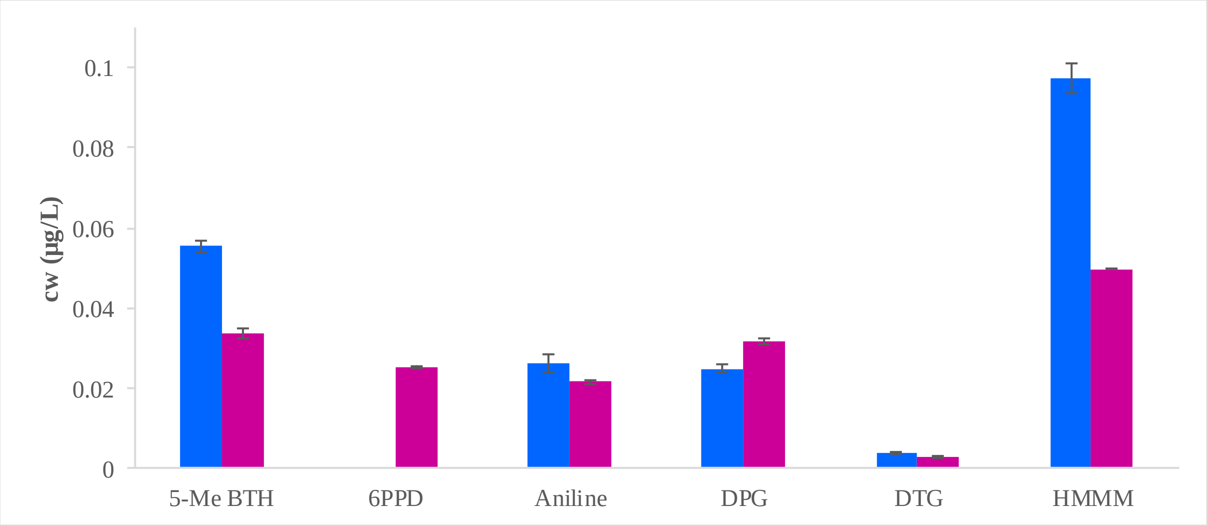


Figure 6 *– Quantifiable water concentrations measured before and after Vienna. Blue columns are the concentrations measured before Vienna, while the pink columns are measured after Vienna. Cw = concentration measured in water.*

In Budapest, the concentrations mainly stayed constant (**Figure 7**). The concentrations of 5-Me BTH and 2-amino BTH increased, which can be related to the removal efficiency of 5-Me BTH and benzothiazoles in WWTPs. The removal efficiency for 5-Me BTH in WWTPs is below 80 % (30). The increase can also be connected to highly concentrated urban street runoff (44, 45, 49, 116). The possible decrease in the concentrations of Aniline and DPG can be related to the standard deviation. Thus the concentrations might not have decreased in Budapest. DEHP, 6PPD, Aniline, DPG, DTG, 5-ME BTH and Neozone concentrations increased in the particles (**Appendix 6**, **Figure 2**). The increase in concentration can be related to the decrease in SPM from 32.08 to 10.56 mg/L (**Appendix 5**). Hence, Budapest appeared to be a source for particle-bond concentrations.

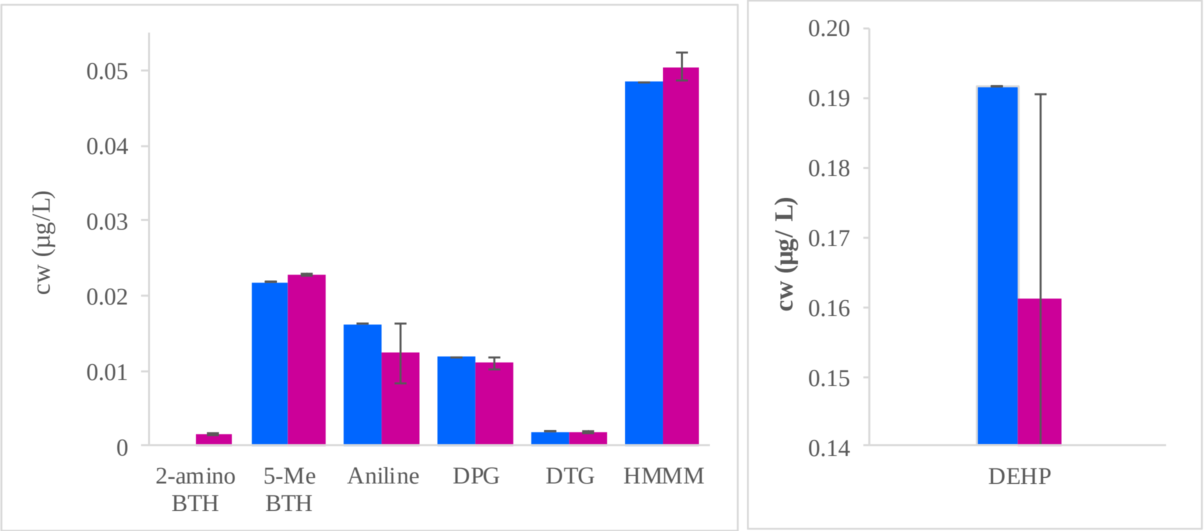


***Figure 7** – Quantifiable water concentrations measured before and after Budapest. The columns in blue are the concentrations measured before Budapest, while the pink ones are the concentrations measured after Budapest. Cw = concentrations measured in water.*

In Novi Sad, most of the compounds' concentrations remained constant (**Figure 8**). DPG and Aniline concentrations increased, which can be explained by the untreated wastewater entering the Danube before the sampling location (54, 100, 103). The concentration of DPG in particulates decreased, while DPG in water increased, which also relates to the increase in suspended particulate matter from 26.39 mg/L to 52.62 mg/L (**Appendix 2**). As DPG, DTG, Neozone and 6PPD have a $\mathrm{logK_{ow}}$ of 3.9 and higher (**Table 2**), the particulate concentrations decreased due to the dilution of the increasing suspended particulate matter concentrations (**Appendix 5**; **Appendix 6**, **Figure 3**). Hence, Novi Sad might be a sink for particle-bond concentrations.

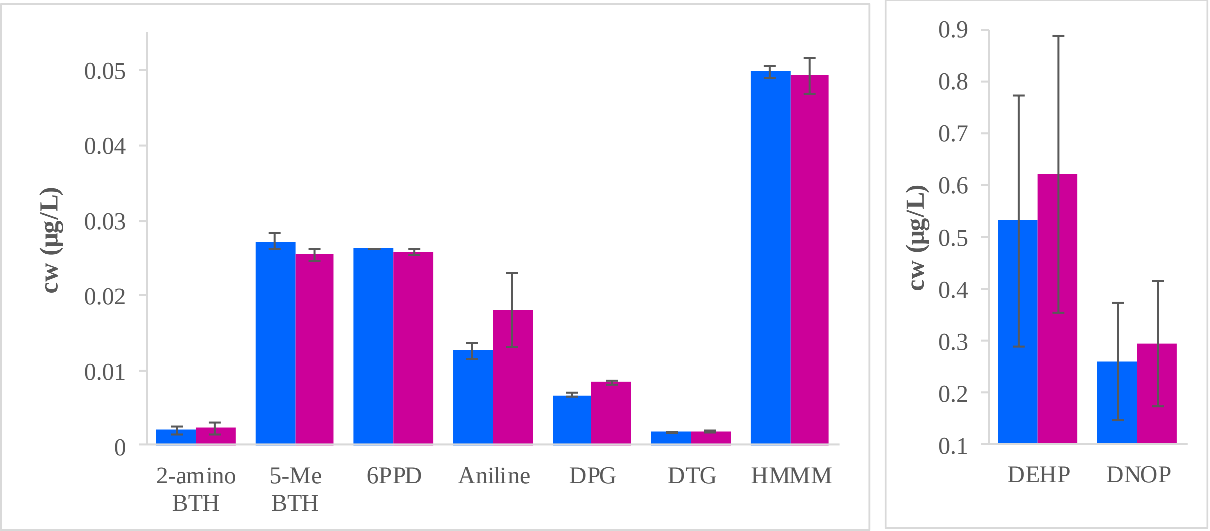


***Figure 8** – Quantifiable water concentrations measured before and after Novi Sad. The pink columns represent the concentrations measured after Novi Sad, while the blue was measured before Novi Sad. Cw = concentration of a compound in water.*

The DEHP and DNOP concentrations decreased in Belgrade, while 5-Me BTH, 6PPD and HMMM concentrations remained the same before and after Belgrade (**Figure 9**). The DPG, PAn and PA concentrations increased, which can be related to the untreated wastewater discharge in Belgrade (101). For DPG, the urban street runoff can also contribute to the increase (44, 45, 49, 116). The decreasing concentrations can be due to the increasing discharge (110). The decrease in water concentrations of DEHP and DNOP can be connected to their hydrophobicity (**Appendix 2**), as the suspended particle concentrations increase (42.04 mg/L to 57.84 mg/L) (**Appendix 5**), DEHP and DNOP sorb to particles. The difference in the size of the cities Novi Sad and Belgrade, while there is untreated wastewater released in both areas (100, 101, 103), can explain that there are more compounds detected in Belgrade compared to Novi Sad (**Figure 8**; **Figure 9**). In Belgrade, the particle-bound concentrations of 5-Me BTH, 6PPD, Neozone and AO2246 might have decreased (**Appendix 6, Figure 5**) due to the increasing suspended particles (**Appendix 5**). A trend of increasing compounds detected downstream of the Danube is visible (**Figure 6 - 9**; **Appendix 6**, **Figure 1 - 5**). Comparing the data of each compound before and after each city, concentrations of compound PA, PAn, 6PPD, DPG and DTG in water either remained constant or their concentrations increased. The particle-bond concentrations around cities depend on their logK$_{ow}$ values, and the suspended matriculate matter concentrations.

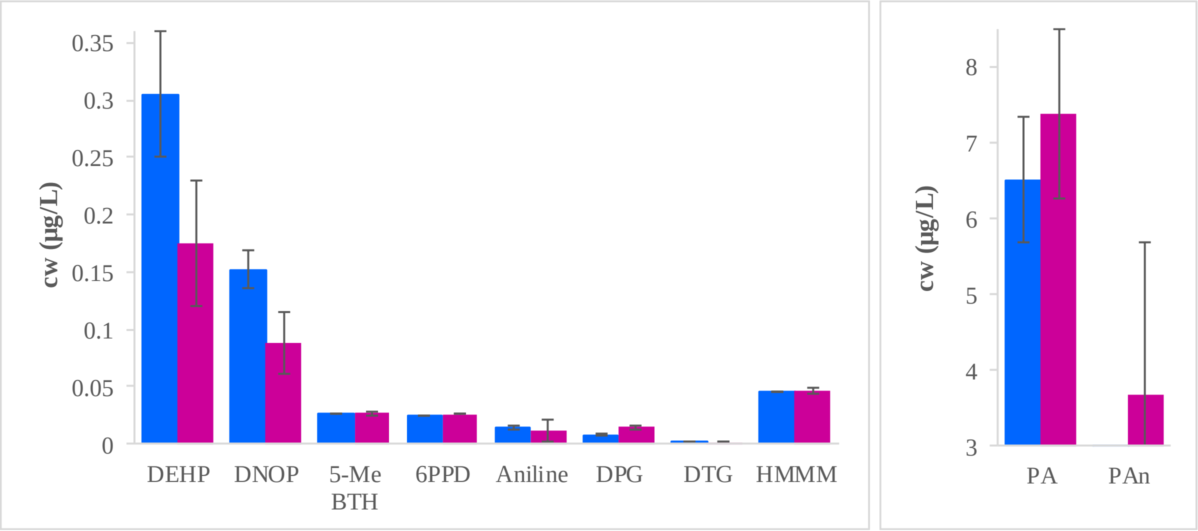


Figure 9 – *Quantifiable water concentrations measured before and after Belgrade. The blue columns are the concentrations before Belgrade, and the pink columns are measured after. Cw = the water concentration of the compounds.*

Evaluation of the risk assessment

Looking at the maximum risk quotients of each compound, for 5 compounds, the risk quotient is close to 1 or exceeds 1 (**Table 4**). For DNOP and HMMM, the average risk quotient exceeds 1, which means that a high risk from those compounds exists for aquatic organisms in the Danube (87, 91). High risk implies that adverse health effects can occur for aquatic organisms. The average risk quotient for 6PPD and DEHP show medium risk as their risk quotients are between 0.1 and 1. Thus, the probability of adverse health effects for aquatic organisms is lower compared to the high-risk compounds but still present. For most of the compounds of interest, the risk quotient according to the water concentrations shows low ecological risk, which means no adverse health effects for aquatic organisms are expected for those compounds at the determined concentrations. The maximum risk quotient for DEHP, DNOP and HMMM, is either almost 1 (DEHP 0.96) or exceeding 1 (DNOP 99.68, HMMM 2.97), stating a high risk for aquatic organisms. Concerning the maximum risk quotients, AO2246 (0.83), 6PPD (0.72) and PAn (0.2) are in specific locations present in concentrations posing a medium risk for aquatic organisms. Looking at the minimum average risk quotients, only HMMM is at medium risk concentrations for aquatic organisms, while the others are at low risk (**Table 4**).

The concentrations of DNOP exceeded its' PNEC value of 0.006 µg/L (**Table 4**) whenever it was detected, as the LOD of DNOP is higher (0.028 µg/L) than the PNEC. As DNOP was detected throughout the whole Danube except locations 1, 4, 13 and 14, where it also might have exceeded the PNEC value, the high ecological risk for aquatic organisms from DNOP can be present in the Danube. As expected, with the predicted environmental concentrations of 0.024 – 0.42 (84), HMMM exceeds the PNEC value of 0.057 µg/L (**Table 4**) until Backi Monoster (location 20) (**Figure 2**). Afterwards, the concentrations remain close to the PNEC, which the medium risk quotient also refers to. The compounds DNOP, DEHP, and HMMM are present in the Danube at concentrations with a high ecological risk for aquatic organisms, while the compounds 6PPD, AO2246 and PAn show a medium ecological risk. Thus, close monitoring of the compounds throughout the Danube is needed.

The PNEC value of DNOP reported by the NORMAN database is a predicted value based on a novel quantitative structure-toxicity relationship model (84, 121). A study by Huan-yu et al. 2022 referred to an experimentally determined acute PNEC value of 0.23 mg/L and a chronic PNEC of 0.05 mg/L in surface waters for aquatic organisms (65). The PNEC values were based on LC_{50} and median effect concentrations (EC_{50}) values in algae, manga and fish. The maximum RQ based on the chronic PNEC would be 0.012, while the average RQ would be 0.003. Thus, the risk for aquatic organisms coming from DNOP is considered low risk.

Table 4 - Risk Assessment. The average (Av. Risk), minimum (Min. Risk) and maximum risk (Max. Risk) are given. Min Conc. stands for minimum measured concentration, Max. Conc. for the maximum measured concentration and Av. Conc. for the average measured concentration. Red means 'high risk', while orange and yellow present 'moderate risk' and green means 'low risk'. RQ = risk quotient

Risk Assessment							
Compound	PNEC (µg/L)	Min. Conc. (µg/L)	Max. Conc. (µg/L)	Av. Conc. (µg/L)	Min. Risk (µg/L)	Max. Risk (µg/L)	Av. Risk (µg/L)
DEHP	1.30	0.00	1.25	0.23	0.00	0.96	0.17
DNOP	0.006	0.00	0.60	0.14	0.00	99.68	23.44
DBP	10.0	0.00	0.00	0.00	0.00	0.00	0.00
BBP	5.20	0.00	0.00	0.00	0.00	0.00	0.00
MEHP	0.189	0.00	0.00	0.00	0.00	0.00	0.00
DEP	73.0	0.00	0.00	0.00	0.00	0.00	0.00
DMP	35.74	0.00	0.00	0.00	0.00	0.00	0.00
PA	108.1	0.00	9.82	2.02	0.00	0.09	0.02
PAn	36.72	0.00	7.18	0.58	0.00	0.20	0.02
BTH	240.0	0.00	0.00	0.00	0.00	0.00	0.00
2-AminoBTH	1.25	0.00	0.01	0.00	0.00	0.00	0.00
2-OH BTH	3.96	0.00	0.00	0.00	0.00	0.00	0.00
2-S BTH	0.760	0.00	0.00	0.00	0.00	0.00	0.00
2-Me BTH	1.92	0.00	0.00	0.00	0.00	0.00	0.00
5-Me BTH	5.90	0.02	0.09	0.04	0.00	0.01	0.01
PPD	16.42	0.00	0.00	0.00	0.00	0.00	0.00
6PPD	0.045	0.00	0.03	0.02	0.00	0.72	0.53
6PPD-Q	n.a.	0.00	0.00	0.00	-	-	-
Aniline	0.800	0.01	0.07	0.02	0.01	0.08	0.03
DPG	1.05	0.01	0.06	0.02	0.01	0.06	0.02
DTG	0.793	0.00	0.00	0.00	0.00	0.01	0.00
DPTU	0.632	0.00	0.00	0.00	0.00	0.00	0.00
HMMM	0.057	0.03	0.17	0.08	0.58	2.97	1.39
Neozone	0.064	0.00	0.00	0.00	0.00	0.05	0.00
CBS	0.270	0.00	0.00	0.00	0.00	0.00	0.00
AO2246	0.164	0.00	0.14	0.01	0.00	0.83	0.04
BHA	n.a.	0.00	0.00	0.00	-	-	-
BHT	0.382	0.00	0.00	0.00	0.00	0.00	0.00

5. Conclusion

In this study, TDC and phthalate concentrations throughout the Danube were determined. Water samples were measured to quantify each compound throughout the Danube, while the passive samplers showed whether a compound was present. The results state that most of the compounds of interest are present in the Danube.

For some compounds, detecting the compound in water is more challenging due to their low concentrations, which is why a passive sampler is needed to prove their presence (68). Using the passive sampler, it was possible to prove the presence of the compounds PA, PAn, DMP, BBP, MEHP, BTH, 2-OH BTH, 2-Me BTH and 2-S BTH, which were determined to be below the detection limit in some of the water samples. The passive sampler used in this study was not suitable for detecting 2-amino BTH as the $\log K_{ow}$ value was outside the preferred range of the passive sampler (73).

The risk assessment showed that the measured concentrations of HMMM, DNOP and DEHP are at high-risk concentrations for aquatic organisms (63). Taking a closer look at different PNEC values for DNOP, it was determined that DNOP poses a low risk for aquatic organisms in the Danube. The compounds AO2246, PAn and 6PPD were determined to be present in the Danube at medium-risk concentrations for aquatic organisms.

A connection can be made between WWTP systems and the compound concentrations of 6PPD, 2-amino BTH, 5-Me BTH, Neozone, AO2246, DBP, PA, PAn, DEHP and DNOP, but it is not the only factor influencing the compound concentrations. The connection of the populations towards sewer systems also influenced the concentrations (60). As expected, the concentrations increased after Vienna due to less treated wastewater entering the Danube (60, 89).

For Aniline, DPG, DTG and 2-S BTH, elevated concentrations were measured before Vienna due to increased highway density, higher speed limits, population densities and highly concentrated road runoff (44, 45, 108, 111, 116). DPG, DTG and HMMM increased in water after Vienna, while they decreased in particulate concentrations, which is connected to their $\log K_{ow}$ values of 0.61 – 4.68.

The compound concentrations of PA, PAn, 6PPD, DPG and DTG increase in urban areas due to the lower quality of surface runoff water (44, 45, 90, 116) and higher wastewater volumes discharged from WWTPs. The particulate concentrations after significant cities showed that the concentration of the compounds of interest in those areas strongly depends on suspended particle matter.

6. Outlook

The concentrations of TDC and phthalates in rivers are dependent on temperature. For phthalates, the solubility in water increases with increasing temperatures, while for BTH, the solubility decreases with increasing temperatures (122). The solubility of 2-ME BTH increases with increasing temperature (123). Concerning the Danube samples, it would be interesting to compare the influence of temperature in a specific sampling location to see how the temperature influences the concentrations of each compound, as the temperature influences the solubility of each compound (122, 123). Temperature can change the river discharge due to evaporation or precipitation (124). These factors can also relate to the suspended particulate matter of the river (124), which has been shown to influence the compound concentrations in particulate concentrations.

As extreme weather conditions, such as heavy rain events, have not been measured during the sampling period, determining the influence of those can be beneficial as they can influence the river flow. Hence, river discharge measurements can add to conclusions concerning some fluctuations in the concentrations measured.

Measurements of phthalate and TDC concentrations in WWTP effluents directly discharged to the Danube are required, similar to what has been done for the Seine in France for certain phthalates and TDCs (54). These measurements would allow further investigation of the connection between the WWTPs and the measured concentrations in the area.

For further use of the passive samplers for detecting the compounds of interest, the missing values, e.g., the distribution coefficient of the passive sampler and water (124) for quantifying the water concentration from the concentration of the water samples, would be needed. The sorption kinetics of the compounds to the passive sampler are also essential to determine. These parameters would make it possible to compare the absolute water concentrations measured to the ones on the passive sampler.

Close monitoring is needed to determine increasing concentrations quickly for the compounds posing a high or medium ecotoxicological risk to aquatic organisms. Hence, especially towards the lower Danube, frequent monitoring is needed. As DEHP, DNOP and HMMM are already present at concentrations having a potentially high ecotoxicological risk and AO2246, PAn, and 6PPD a medium risk, further governmental restrictions for the use of those compounds need to be released. Including these compounds on the substance priority list would lead to them being included in the water framework directive. Adding the compounds would mean that the compounds would be an index for the chemical water quality status of water bodies.

For the high-risk compounds, remediation in the high-concentration areas is necessary. For phthalates remediation, bimetallic catalysts have been shown to degrade up to 86% in marine sediments at pH 2 and 76 % at pH 6 (125, 126). To remove phthalates from sediments, degradation using catalysts could be used. Phytoremediation can also be a suitable remediation strategy for phthalates as it can be applied in situ (125).

7. Acknowledgements

This thesis was made possible because of the cooperation with the Clean Danube project and the University of Vienna. I would like to thank Charlotte Henkel and Ruoting Peng for supporting and supervising me throughout the project (both PhD students at the Department of Environmental Geosciences, Centre for Microbiology and Environmental System Science, University of Vienna). I would also like to thank Dr Thorsten Hüffer for his supervision during the thesis (Department of Environmental Geosciences, Centre for Microbiology and Environmental System Science, University of Vienna).

8. References

1. Asenova M, Bannick CG, Bednarz M, Kerndorff A, Obermaier N, et al. 2021. Joint Danube Survey 4: Chapter 42: Occurence of microplastics in the Danube River - A first screening
2. Crew A, Gregory-Eaves I, Ricciardi A. 2020. Distribution, abundance, and diversity of microplastics in the upper St. Lawrence River. *Environ. Pollut.* 260:113994
3. Lebreton LCM, van der Zwet J, Damsteeg J-W, Slat B, Andrady A, Reisser J. 2017. River plastic emissions to the world’s oceans. *Nat. Commun.* 8(1):15611
4. Wagner S, Hüffer T, Klöckner P, Wehrhahn M, Hofmann T, Reemtsma T. 2018. Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. *Water Res.* 139:83–100
5. Arthur C, Baker J, Bamford H. 2008. Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris
6. Frias JPGL, Nash R. 2019. Microplastics: Finding a consensus on the definition. *Mar. Pollut. Bull.* 138:145–47
7. Barrick A, Champeau O, Chatel A, Manier N, Northcott G, Tremblay LA. 2021. Plastic additives: challenges in ecotox hazard assessment. *PeerJ*. 9:e11300
8. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, et al. 2019. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* 53(3):1039–47
9. Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, et al. 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. *Chemosphere*. 182:781–93
10. Kutz M. 2015. *Mechanical Engineers’ Handbook, Volume 1: Materials and Engineering Mechanics*. John Wiley & Sons. 1040 pp.
11. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* 344:179–99

12. Ventrice P, Ventrice D, Russo E, De Sarro G. 2013. Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. *Environ. Toxicol. Pharmacol.* 36(1):88–96

13. Capolupo M, Sørensen L, Jayasena KDR, Booth AM, Fabbri E. 2020. Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. *Water Res.* 169:115270

14. Bridson JH, Gaugler EC, Smith DA, Northcott GL, Gaw S. 2021. Leaching and extraction of additives from plastic pollution to inform environmental risk: A multidisciplinary review of analytical approaches. *J. Hazard. Mater.* 414:125571

15. Schmidt N, Castro-Jiménez J, Fauvelle V, Ourgaud M, Sempéré R. 2020. Occurrence of organic plastic additives in surface waters of the Rhône River (France). *Environ. Pollut.* 257:113637

16. Johannessen C, Helm P, Metcalfe CD. 2022. Runoff of the Tire-Wear Compound, Hexamethoxymethyl-Melamine into Urban Watersheds. *Arch. Environ. Contam. Toxicol.* 82(2):162–70

17. Bisselink B, Bernhard J, Gelati E, Jacobs C, Adamovic M, et al. 2018. Impact of a changing climate, land use, and water usage on water resources in the Danube river basin

18. Schmedje U, Bachmann J, Behrendt H, Birk S, Biza P, et al. 2005. The Danube River Basin District – River Basin Characteristics, Impacts of Human Activities and Economic Analysis Required under Article 5, Annex II and Annex III, and Inventory of Protected Areas Required under Article 6, Annex IV of the EU Water Framework Directive (2000/60/EC) – Part a – Basin-wide Overview

19. Kirschner AKT, Kavka GG, Velimirov B, Mach RL, Sommer R, Farnleitner AH. 2009. Microbiological water quality along the Danube River: Integrating data from two whole-river surveys and a transnational monitoring network. *Water Res.* 43(15):3673–84

20. Lechner A. 2020. “Down by the River”: (Micro-) Plastic Pollution of Running Freshwaters with Special Emphasis on the Austrian Danube. In *Mare Plasticum - The Plastic Sea: Combatting Plastic Pollution Through Science and Art*, ed M Streit-Bianchi, M Cimadevila, W Trettnak, pp. 141–85. Cham: Springer International Publishing

21. *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy*. 2000

22. ICPDR. 2015. Map 21 Ecological Status and Ecological Potential of Surface Water Bodies. *Danube River Basin Manag. Plan*. Part A-Basin-wide overview:

23. ICPDR. 2015. Map 22a Chemical Status of Surface Water Bodies (Priority Substances in Water). *Danube River Basin Manag. Plan*. Part A-Basin-wide overview:

24. *Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council*. 2008

25. Raković M, Tomović J, Kolarević S, Paunović M, Stanković S, et al. 2021. Joint Danube Survey 4: Chapter 44: Microplastics in biota - Asian calm case. *Jt. Danube Surv.* 4:

26. Slobodnik J, Alexandrov B, Komorin V, Mikaelyan A, Guchmanidze A, et al. 2018. National Pilot Monitoring Studies and Joint Open Sea Surveys in Georgia, Russian Federation and Ukraine, 2017. *Jt. Black Sea Surv.*, p. 572

27. Jungwirth M, Haidvogl G, Hohensinner S, Waidbacher H, Zauner G. 2014. *Österreichs Donau: Landschaft – Fisch – Geschichte*. Institut für Hydrobiologie & Gewässermanagement (IHG), Universität für Bodenkultur Wien (BOKU). 422 pp.

28. Sharma VK, Ma X, Lichtfouse E, Robert D. 2022. Nanoplastics are potentially more dangerous than microplastics. *Environ. Chem. Lett.*

29. *Joint Danube Survey | ICPDR - International Commission for the Protection of the Danube River*. www.icpdr.org

30. Alygizakis N, Galani A, Nika M-C, Thomaidis NS, Kunkel U, et al. 2021. Joint Danube Survey 4: Chapter 29: Wide-scope target screening of industrial chemicals and plant protection products in wastewater, groundwater, river water, sediments and biota by liquid and gas chromatography coupled with high-resolution mass spectrometry. *Jt. Danube Surv.* 4:

31. Alvarez DA, Cranor WL, Perkins SD, Clark RC, Smith SB. 2008. Chemical and Toxicologic Assessment of Organic Contaminants in Surface Water Using Passive Samplers. *J. Environ. Qual.* 37(3):1024–33

32. Charriau A, Lissalde S, Poulier G, Mazzella N, Buzier R, Guibaud G. 2016. Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part A: Principles, calibration, preparation and analysis of the sampler. *Talanta*. 148:556–71

33. Górecki T, Namieśnik J. 2002. Passive sampling. *TrAC Trends Anal. Chem.* 21(4):276–91

34. Vrana B, Mills GA, Kotterman M, Leonards P, Booij K, Greenwood R. 2007. Modelling and field application of the Chemcatcher passive sampler calibration data for the monitoring of hydrophobic organic pollutants in water. *Environ. Pollut.* 145(3):895–904

35. Li M, Wu C-Q, Chen Y-H, Gong J, Li Y, et al. 2016. A Research for Reduction the Pollution in the Pretreatmentprocess of PAEs Inhuman Serum

36. Marcilla A, García S, García-Quesada JC. 2004. Study of the migration of PVC plasticizers. *J. Anal. Appl. Pyrolysis*. 71(2):457–63

37. Oehlmann J, Oetken M, Schulte-Oehlmann U. 2008. A critical evaluation of the environmental risk assessment for plasticizers in the freshwater environment in Europe, with special emphasis on bisphenol A and endocrine disruption. *Environ. Res.* 108(2):140–49

38. Lee Y-S, Lim J-E, Lee S, Moon H-B. 2020. Phthalates and non-phthalate plasticizers in sediment from Korean coastal waters: Occurrence, spatial distribution, and ecological risks. *Mar. Pollut. Bull.* 154:111119

39. Shinohara N, Mizukoshi A, Uchiyama M, Tanaka H. 2019. Emission characteristics of diethylhexyl phthalate (DEHP) from building materials determined using a passive flux sampler and micro-chamber. *PloS One*. 14(9):e0222557

40. U.S. Environmental Protection Agency. 2012. Phthalates ActionPlan - 2012-03-14. , p. 16

41. Bang DY, Lee IK, Lee B-M. 2011. Toxicological Characterization of Phthalic Acid. *Toxicol. Res.* 27(4):191–203

42. Wik A, Dave G. 2009. Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. *Environ. Pollut.* 157(1):1–11

43. Tian Z, Zhao H, Peter KT, Gonzalez M, Wetzel J, et al. 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. *Science*. 371(6525):185–89

44. Zhang H-Y, Huang Z, Liu Y-H, Hu L-X, He L-Y, et al. 2023. Occurrence and risks of 23 tire additives and their transformation products in an urban water system. *Environ. Int.* 171:107715

45. Rauert C, Charlton N, Okoffo ED, Stanton RS, Agua AR, et al. 2022. Concentrations of Tire Additive Chemicals and Tire Road Wear Particles in an Australian Urban Tributary. *Environ. Sci. Technol.* 56(4):2421–31

46. Neuwald IJ, Hübner D, Wiegand HL, Valkov V, Borchers U, et al. 2022. Occurrence, Distribution, and Environmental Behavior of Persistent, Mobile, and Toxic (PMT) and Very Persistent and Very Mobile (vPvM) Substances in the Sources of German Drinking Water. *Environ. Sci. Technol.* 56(15):10857–67

47. Sieber R, Kawecki D, Nowack B. 2020. Dynamic probabilistic material flow analysis of rubber release from tires into the environment. *Environ. Pollut.* 258:113573

48. Baensch-Baltruschat B, Kocher B, Stock F, Reifferscheid G. 2020. Tyre and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. *Sci. Total Environ.* 733:137823

49. Seiwert B, Klöckner P, Wagner S, Reemtsma T. 2020. Source-related smart suspect screening in the aqueous environment: search for tire-derived persistent and mobile trace organic contaminants in surface waters. *Anal. Bioanal. Chem.* 412(20):4909–19

50. Thorndahl S, Schaarup-Jensen K, Rasmussen MR. 2015. On hydraulic and pollution effects of converting combined sewer catchments to separate sewer catchments. *Urban Water J.* 12(2):120–30

51. Johannessen C, Metcalfe CD. 2022. The occurrence of tire wear compounds and their transformation products in municipal wastewater and drinking water treatment plants. *Environ. Monit. Assess.* 194(10):731

52. Seiwert B, Nihemaiti M, Troussier M, Weyrauch S, Reemtsma T. 2022. Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. *Water Res.* 212:118122

53. Liu DHF, Liptak BG. 2020. *Wastewater Treatment*. CRC Press. 470 pp.

54. Tran BC, Teil MJ, Blanchard M, Alliot F, Chevreuil M. 2015. BPA and phthalate fate in a sewage network and an elementary river of France. Influence of hydroclimatic conditions. *Chemosphere*. 119:43–51

55. Dargnat C, Teil M-J, Chevreuil M, Blanchard M. 2009. Phthalate removal throughout wastewater treatment plant: Case study of Marne Aval station (France). *Sci. Total Environ.* 407(4):1235–44

56. Stasinakis AS, Thomaidis NS, Arvaniti OS, Asimakopoulos AG, Samaras VG, et al. 2013. Contribution of primary and secondary treatment on the removal of benzothiazoles, benzotriazoles, endocrine disruptors, pharmaceuticals and perfluorinated compounds in a sewage treatment plant. *Sci. Total Environ.* 463–464:1067–75

57. Gerba CP, Pepper IL. 2019. Chapter 22 - Municipal Wastewater Treatment. In *Environmental and Pollution Science (Third Edition)*, ed ML Brusseau, IL Pepper, CP Gerba, pp. 393–418. Academic Press

58. Kloepfer A, Jekel M, Reemtsma T. 2005. Occurrence, Sources, and Fate of Benzothiazoles in Municipal Wastewater Treatment Plants. *Environ. Sci. Technol.* 39(10):3792–98

59. Lu Z, Smyth SA, De Silva AO. 2019. Distribution and fate of synthetic phenolic antioxidants in various wastewater treatment processes in Canada. *Chemosphere*. 219:826–35

60. European Environment Agency. 2020. *Urban waste water treatment in Europe — European Environment Agency*. www.eea.europa.eu

61. Anbumani S, Kakkar P. 2018. Ecotoxicological effects of microplastics on biota: a review. *Environ. Sci. Pollut. Res.* 25(15):14373–96

62. Challis JK, Popick H, Prajapati S, Harder P, Giesy JP, et al. 2021. Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff. *Environ. Sci. Technol. Lett.* 8(11):961–67

63. Rowdhwal SSS, Chen J. 2018. Toxic Effects of Di-2-ethylhexyl Phthalate: An Overview. *BioMed Res. Int.* 2018:e1750368

64. Zhang Y, Jiao Y, Li Z, Tao Y, Yang Y. 2021. Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. *Sci. Total Environ.* 771:145418

65. Huan-yu T, Jiawei Z, Li bin, Hui G, Mengtao Z, et al. 2022. Development of ecological risk assessment for Diisobutyl phthalate and di-n-octyl phthalate in surface water of China based on species sensitivity distribution model. *Chemosphere*. 307:135932

66. Lee H, Lee J, Choi K, Kim K-T. 2019. Comparative analysis of endocrine disrupting effects of major phthalates in employed two cell lines (MVLN and H295R) and embryonic zebrafish assay. *Environ. Res.* 172:319–25

67. M. Vermeirssen EL, Asmin J, I. Escher B, Kwon J-H, Steimen I, Hollender J. 2008. The role of hydrodynamics, matrix and sampling duration in passive sampling of polar compounds with Empore^{TM} SDB-RPS disks. *J. Environ. Monit.* 10(1):119–28

68. Miège C, Mazzella N, Allan I, Dulio V, Smedes F, et al. 2015. Position paper on passive sampling techniques for the monitoring of contaminants in the aquatic environment – Achievements to date and perspectives. *Trends Environ. Anal. Chem.* 8:20–26

69. Gravell A, Fones GR, Greenwood R, Mills GA. 2020. Detection of pharmaceuticals in wastewater effluents—a comparison of the performance of Chemcatcher® and polar organic compound integrative sampler. *Environ. Sci. Pollut. Res.* 27(22):27995–5

70. Madrid Y, Zayas ZP. 2007. Water sampling: Traditional methods and new approaches in water sampling strategy. *TrAC Trends Anal. Chem.* 26(4):293–99

71. Valenzuela EF, Menezes HC, Cardeal ZL. 2020. Passive and grab sampling methods to assess pesticide residues in water. A review. *Environ. Chem. Lett.* 18(4):1019–48

72. Smedes F, Booij K. 2012. *Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers.* 1 p.

73. Vrana B, Allan IJ, Greenwood R, Mills GA, Dominiak E, et al. 2005. Passive sampling techniques for monitoring pollutants in water. *TrAC Trends Anal. Chem.* 24(10):845–68

74. Fries E, Gocht T, Klasmeier J. 2011. Occurrence and distribution of benzothiazole in the Schwarzbach watershed (Germany). *J. Environ. Monit.* 13(10):2838–43

75. Raharjo Y, Kartika A, Handajani U. 2016. Analysis of Di-(2-ethylhexyl)phthalate in Plastic Bottles of Drinking Water with Cone-shaped Membrane–Liquid Phase Microextraction. *Chem. Chem. Technol.* 10:247–50

76. Furtmann K. 1994. Phthalates in surface water — a method for routine trace level analysis. *Fresenius J. Anal. Chem.* 348(4):291–96

77. Nagorka R, Koschorreck J. 2020. Trends for plasticizers in German freshwater environments – Evidence for the substitution of DEHP with emerging phthalate and non-phthalate alternatives. *Environ. Pollut.* 262:114237

78. Klöckner P, Seiwert B, Wagner S, Reemtsma T. 2021. Organic Markers of Tire and Road Wear Particles in Sediments and Soils: Transformation Products of Major Antiozonants as Promising Candidates. *Environ. Sci. Technol.* 55(17):11723–32

79. Zaater MF, Tahboub YR, Al Sayyed AN. 2014. Determination of Phthalates in Jordanian Bottled Water using GC–MS and HPLC–UV: Environmental Study. *J. Chromatogr. Sci.* 52(5):447–52

80. Net S, Delmont A, Sempéré R, Paluselli A, Ouddane B. 2015. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review. *Sci. Total Environ.* 515–516:162–80

81. Brack W, Dulio V, Slobodnik J. 2012. The NORMAN Network and its activities on emerging environmental substances with a focus on effect-directed analysis of complex environmental contamination. *Environ. Sci. Eur.* 24(1):29

82. Dulio V. *WELCOME TO THE NORMAN NETWORK | NORMAN*. NORMAN Network. www.norman-network.net

83. Dulio V, Slobodnik J. 2009. NORMAN—network of reference laboratories, research centres and related organisations for monitoring of emerging substances. *Environ. Sci. Pollut. Res.* 16(1):132–35

84. Slobodnik J. *NORMAN Database System*. NORMAN. www.norman-network.com

85. Tamis JE, Koelmans AA, Dröge R, Kaag NHBM, Keur MC, et al. 2021. Environmental risks of car tire microplastic particles and other road runoff pollutants. *Microplastics Nanoplastics*. 1(1):10

86. Norton SB, Rodier DJ, van der Schalie WH, Wood WP, Slimak MW, Gentile JH. 1992. A framework for ecological risk assessment at the EPA. *Environ. Toxicol. Chem.* 11(12):1663–72

87. Hernando MD, Mezcua M, Fernández-Alba AR, Barceló D. 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*. 69(2):334–42

88. International. 2017. Priority Substances

89. European Environment Agency. 2022. *Waterbase - UWWTD: Urban Waste Water Treatment Directive – reported data — European Environment Agency*. www.eea.europa.eu

90. Li L, Yu Q, Gao L, Yu B, Lu Z. 2021. The Effect of Urban Land-Use Change on Runoff Water Quality: A Case Study in Hangzhou City. *Int. J. Environ. Res. Public. Health*. 18(20):10748

91. Amiard J-C, Amiard-Triquet C. 2015. Chapter 2 - Conventional Risk Assessment of Environmental Contaminants. In *Aquatic Ecotoxicology*, ed C Amiard-Triquet, J-C Amiard, C Mouneyrac, pp. 25–49. Academic Press

92. Ragas AM, Huijbregts MA, Henning-de Jong I, Leuven RS. 2009. Uncertainty in environmental risk assessment: implications for risk-based management of river basins. *Integr. Environ. Assess. Manag.* 5(1):27–37

93. Nasiri A, Jahani R, Mokhtari S, Yazdanpanah H, Daraei B, et al. 2021. Overview, consequences, and strategies for overcoming matrix effects in LC-MS analysis: a critical review. *Analyst*. 146(20):6049–63

94. Fries E. 2011. Determination of benzothiazole in untreated wastewater using polar-phase stir bar sorptive extraction and gas chromatography–mass spectrometry. *Anal. Chim. Acta*. 689(1):65–68

95. *cleandanube | swimming for a pure and plastic-free river*. 2022. Clean Danube. www.cleandanube.org

96. Hamchevici C, Dumitrache F, Sena F, Umlauf G, Postolache C, Udrea I. 2015. Joint Danube Survey 3: Chapter General physico-chemical determinands and nutrients. , p. 35

97. Hamchevici C, Postolache C, Pons M, Halder J. 2021. Joint Danube Survey 4: Chapter 22: General physico-chemical determinands and nutrients

98. ECHA. *Aniline*. Registration Dossier - ECHA. https://echa.europa.eu

99. ECHA. *6-methylbenzotriazole*. Registration Dossier - ECHA. https://echa.europa.eu

100. Hashmi MAK, Escher BI, Krauss M, Teodorovic I, Brack W. 2018. Effect-directed analysis (EDA) of Danube River water sample receiving untreated municipal wastewater from Novi Sad, Serbia. *Sci. Total Environ.* 624:1072–81

101. Kirschner A, Schachnerl I, Jakwerth S, Savio D, Toth E, et al. 2021. Joint Danube Survey 4: Chapter 19: Microbial faecal pollution and source tracking

102. Schmidt C, Kumar R, Yang S, Büttner O. 2020. Microplastic particle emission from wastewater treatment plant effluents into river networks in Germany: Loads, spatial patterns of concentrations and potential toxicity. *Sci. Total Environ.* 737:139544

103. Hrubik J, Glisic B, Tubic A, Ivancev-Tumbas I, Kovacevic R, et al. 2016. Toxicological and chemical investigation of untreated municipal wastewater: Fraction- and species-specific toxicity. *Ecotoxicol. Environ. Saf.* 127:153–62

104. Liu R, Ruan T, Song S, Lin Y, Jiang G. 2015. Determination of synthetic phenolic antioxidants and relative metabolites in sewage treatment plant and recipient river by high performance liquid chromatography–electrospray tandem mass spectrometry. *J. Chromatogr. A.* 1381:13–21

105. *Commission Regulation (EU) 2017/1151 of 1 June 2017 supplementing Regulation (EC) No 715/2007 of the European Parliament and of the Council on type-approval of motor vehicles with respect to emissions from light passenger and commercial vehicles (Euro 5 and Euro 6) and on access to vehicle repair and maintenance information, amending Directive 2007/46/EC of the European Parliament and of the Council, Commission Regulation (EC) No 692/2008 and Commission Regulation (EU) No 1230/2012 and repealing Commission Regulation (EC) No 692/2008 (Text with EEA relevance)*. 2017

106. Wastewater Management Plan in the Danube River Basin. 2021

107. Mooselu MG, Liltved H, Hindar A, Amiri H. 2022. Current European approaches in highway runoff management: A review. *Environ. Chall.* 7:100464

108. *The densest motorway networks across EU regions*. https://ec.europa.eu

109. Kim G, Lee S. 2018. Characteristics of Tire Wear Particles Generated by a Tire Simulator under Various Driving Conditions. *Environ. Sci. Technol.* 52(21):12153–61

110. Global Runoff Data Centre. 2022. *GRDC Data Portal*. https://portal.grdc.bafg.de/applications/public.html?publicuser=PublicUser#dataDownload

111. International centre for education and training. 2022. European Countries By Population Density

112. Gao X, Xu Y, Ma M, Rao K, Wang Z. 2019. Simultaneous passive sampling of hydrophilic and hydrophobic emerging organic contaminants in water. *Ecotoxicol. Environ. Saf.* 178:25–32

113. Gao X, Li J, Xu Y, Zhou Q, Ma M, Wang Z. 2022. Passive sampling hydrophilic and hydrophobic bisphenol analogues using hydrophilic-lipophilic balance sorbent-embedded cellulose acetate membrane in surface waters. *Sci. Total Environ.* 839:156239

114. Vanryckeghem F, Huysman S, Smedes F, Van Langenhove H, Vanhaecke L, Demeestere K. 2021. A Simple Teabag Equilibrium Passive Sampler using hydrophilic divinylbenzene sorbent for contaminants of emerging concern in the marine environment. *Sci. Total Environ.* 777:146055

115. Shaw M, Eaglesham G, Mueller JF. 2009. Uptake and release of polar compounds in SDB-RPS Empore^{TM} disks; implications for their use as passive samplers. *Chemosphere*. 75(1):1–7

116. Schulze S, Zahn D, Montes R, Rodil R, Quintana JB, et al. 2019. Occurrence of emerging persistent and mobile organic contaminants in European water samples. *Water Res.* 153:80–90

117. Magistrat der Stadt Wien. 2020. *KANIS - Kanalinformationssystem*. www.kanis.at

118. Castan S, Sherman A, Peng R, Zumstein MT, Wanek W, et al. 2023. Uptake, Metabolism, and Accumulation of Tire Wear Particle-Derived Compounds in Lettuce. *Environ. Sci. Technol.* 57(1):168–78

119. Hager H, Schume H. 2001. *The Floodplain Forests along the Austrian Danube*. Brill

120. Golladay SW, Battle J. 2002. Effects of Flooding and Drought on Water Quality in Gulf Coastal Plain Streams in Georgia. *J. Environ. Qual.* 31(4):1266–72

121. Aalizadeh R, Ohe PC von der, Thomaidis NS. 2017. Prediction of acute toxicity of emerging contaminants on the water flea Daphnia magna by Ant Colony Optimization–Support Vector Machine QSTR models. *Environ. Sci. Process. Impacts*. 19(3):438–48

122. PubChem. *Benzothiazole*. PubChem. https://pubchem.ncbi.nlm.nih.gov

123. PubChem. *2-Methylbenzothiazole*. PubChem. https://pubchem.ncbi.nlm.nih.gov

124. Dolman AJ, de Jeu R a. M. 2010. Evaporation in focus. *Nat. Geosci.* 3(5):296–296

125. Das MT, Kumar SS, Ghosh P, Shah G, Malyan SK, et al. 2021. Remediation strategies for mitigation of phthalate pollution: Challenges and future perspectives. *J. Hazard. Mater.* 409:124496

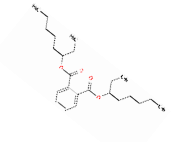
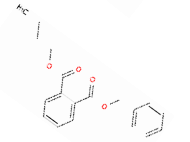
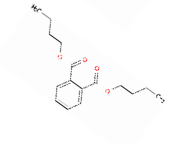
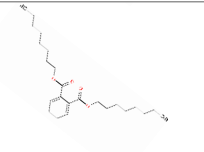
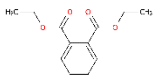
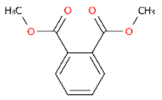
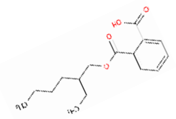
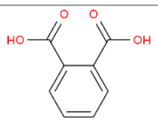
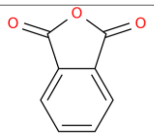
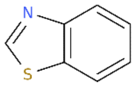
126. Dong C-D, Huang CP, Nguyen T-B, Hsiung C-F, Wu C-H, et al. 2019. The degradation of phthalate esters in marine sediments by persulfate over iron–cerium oxide catalyst. *Sci. Total Environ.* 696:133973

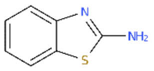
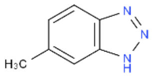
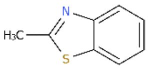
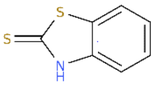
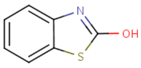
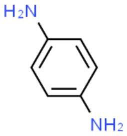
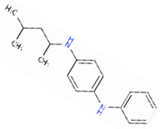
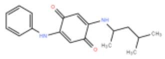
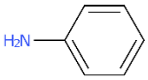
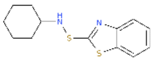
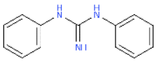
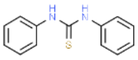
Appendices

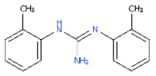
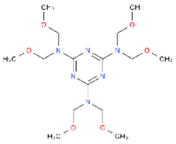
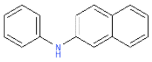
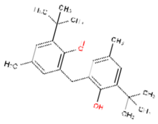
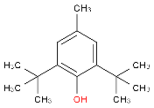
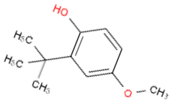
Appendix 1 – Predicted no-effect concentrations (PNEC) of the selected phthalates and tire-derived compounds according to the NORMAN database (84). For some compounds, the PNEC was not available (n.a.).

Compound	Name	PNEC (µg/L)
DEHP	Di(2-ethylhexyl) phthalate	1.30
BBP	Benzyl butyl phthalate	5.20
DBP	Dibutyl phthalate	10.00
DNOP	Di-n-octyl phthalate	0.01
DEP	Diethyl phthalate	73.00
DMP	Dimethyl phthalate	35.74
PA	Phthalic acid	108.1
PAn	Phthalic anhydride	36.72
MEHP	Mono-2-ethylhexyl phthalate	0.19
2-amino BTH	2-Aminobenzothiazole	1.25
2-OH BTH	2-Hydroxybenzothiazole	3.96
2-S BTH	2-Mercaptobenzothiazole	0.76
2-Me BTH	2-Methylbenzothiazole	1.92
BTH	Benzothiazole	240.0
5-Me BTH	5-methyl-1h benzotriazole	5.90
6PPD	N-(1,3-dimethylbutyl)-N‘-phenyl-1,4-phenylenediamine	0.05
6PPD-Q	2-anilino-5-[(4-methylpentan-2-yl)amino]-cyclohexa-2,5-diene-1,4-dione	n.a.
Aniline	Aniline	0.80
CBS	N-Cyclohexyl-2-benzothiazolesulfenamide	0.27
DPG	1,3-Diphenylguanidine	1.05
DPTU	N,N´-Diphenylthiourea	0.63
DTG	1,3-Di-o-tolylguanidine	0.79
HMMM	Hexa(methoxymethyl)melamine	0.06
Neozone	N-Phenyl-2-naphthylamine	0.06
PPD	p-Phenylenediamine	16.42
BHT	Butylated hydroxytoluene	0.38
BHA	Butylated hydroxyanisole	n.a.
AO2246	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	0.16

Appendix 2 - Properties of the compounds of interest at 15 °C calculated in SPARC. K_{ow} is the octanol-water distribution coefficient, and M is the molecular weight.

Compound	**Solubility (mg/L)**	**Henry's Constant** $\left(\frac{atm}{\frac{mol}{m^3}}\right)$	**LogK_{ow}**	**M** $(\frac{g}{mol})$	**Formula**	**Structure**
DEHP	0.00175	4.02E-8	7.95	390.6	$C_{24}H_{38}O_4$	
BBP	0.0614	1.55E-9	5.12	312.4	$C_{19}H_{20}O_4$	
DBP	4.49	3.9E-8	4.73	278.3	$C_{16}H_{22}O_4$	
DNOP	2.69E-4	4.79E-8	8.6	390.6	$C_{24}H_{38}O_4$	
DEP	578.4	1.18E-8	2.53	222.2	$C_{12}H_{14}O_4$	
DMP	5470.0	3.91E-9	1.36	194.2	$C_{10}H_{10}O_4$	
MEHP	0.44	2.19E-9	5.23	278.3	$C_{16}H_{22}O_4$	
PA	362.7	4.49E-12	0.8	166.1	$C_8H_6O_4$	
PAn	21000.0	5.3E-11	-0.76	148.1	$C_8H_4O_3$	
BTH	507.9	2.47E-7	1.87	135.2	C_7H_5NS	

2-amino BTH	3160.0	1.91E-15	-0.15	150.2	$C_7H_6N_2S$	
5-Me BTH	746.1	2.37E-13	0.77	133.2	$C_7H_7N_3$	
2-Me BTH	214.2	3.34E-7	2.45	149.2	C_8H_7NS	
2-S BTH	75.09	3.24E-10	1.99	167.3	$C_7H_5NS_2$	
2-OH BTH	1100.0	4.27E-10	1.04	151.2	C_7H_5NOS	
PPD	228000.0	5.36E-11	-0.82	108.1	$C_6H_8N_2$	
6PPD	2.59	1.38E-8	5.18	268.4	$C_{18}H_{24}N_2$	
6PPD-Q	73200.0	2.41E-16	1.71	298.4	$C_{18}H_{22}N_2O_2$	
Aniline	20400.0	1.74E-6	1.16	93.13	C_6H_7N	
CBS	8.96	1.63-10	3.89	264.4	$C_{13}H_{16}N_2S_2$	
DPG	3.54	2.63E-9	3.9	211.3	$C_{13}H_{13}N_3$	
DPTU	0.33	1.02E-9	4.74	228.3	$C_{13}H_{12}N_2S$	

DTG	0.34	2.63E-9	4.68	239.3	$C_{15}H_{17}N_3$	
HMMM	1.29E7	1.38E-16	0.61	390.4	$C_{15}H_{30}N_6O_6$	
Neozone	0.35	1.48E-7	5.39	219.3	$C_{16}H_{13}N$	
AO2246	0.00687	2.79E-9	7.15	340.5	$C_{23}H_{32}O_2$	
BHT	1.11	3.07E-5	5.26	220.4	$C_{15}H_{24}O$	
BHA	787.3	6.22E-8	3.25	180.2	$C_{11}H_{16}O_2$	

Appendix 3 - Transitions from precursor ions to product ions

Compound	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (V)	Cell accelerator voltage (V)	Retention time (min)
2-aminobenzothiazole	151	109	150	30	5	5.4
2-aminobenzothiazole	151	65	150	38	5	5.4
2-Hydroxybenzothiazole	152	124	140	22	4	11.7
2-Hydroxybenzothiazole	152	92	140	22	4	11.7
2-mercaptobenzothiazole	168	135	135	28	5	12.6
2-mercaptobenzothiazole	168	124	135	24	5	12.6
2-mercaptobenzothiazole	168	109	135	32	5	12.6
2-Methylbenzothiazole	150	109	150	30	5	14.3
2-Methylbenzothiazole	150	65	150	30	5	14.3
5-Methyl-1H-benzotriazole	134	106	150	22	5	10.5
5-Methyl-1H-benzotriazole	134	79	150	22	5	10.5
6PPD	269	184	150	37	5	15.6
6PPD	269	107	150	29	5	15.6
6PPD	269	93	150	41	5	15.6
6PPD-Quinone	299	241	150	38	5	20.4
6PPD-Quinone	299	215	150	22	5	20.4
6PPD-Quinone	299	187	150	26	5	20.4
6PPD-quinone d5	304.2	246.1	110	36	4	20.4
6PPD-quinone d5	304.2	220.1	110	28	4	20.4
6PPD-quinone d5	304.2	192.1	110	36	4	20.4
Aniline	94	77	100	23	5	1.9
Aniline	94	51	100	41	5	1.9
Aniline	94	50	100	53	5	1.9
AO2246	339.2	183	176	46	4	24.3
AO2246	339.2	163.1	176	30	4	24.3
BBP	313.2	149	100	13	4	21.3
BBP	313.2	91.1	100	34	4	21.3
Benzothiazole	136	109	150	23	5	13.8
Benzothiazole	136	77	150	23	5	13.8
Benzothiazole	136	65	150	38	5	13.8
Benzothiazole-d4	140	113	150	31	5	13.8

Benzothiazole-d4	140	81	150	19	5	13.8
Benzothiazole-d4	140	69	150	36	5	13.8
BHA	179.1	164	128	10	4	17.5
BHA	179.1	146.1	128	34	4	17.5
BHT	219.2	203.2	176	34	4	23.3
BHT	219.2	163.1	176	22	4	23.3
CBS	265	183	150	16	5	21.8
CBS	265	166	150	28	5	21.8
DBP	279.2	246.8	100	0	4	21.5
DBP	279.2	149	100	13	4	21.5
DEHP	391.3	149	100	13	4	25.7
DEHP	391.3	71.2	100	21	4	25.7
DEHP-d4	395	153	100	21	4	25.7
DEHP-d4	395	75	100	25	4	25.7
DEP	223.1	177	80	4	4	16.2
DEP	223.1	149	80	21	4	16.2
DEP-d4	227	181	80	8	4	16.2
DEP-d4	227	153	80	21	4	16.2
DMP	195.1	163	80	9	4	12.6
DMP	195.1	77.1	80	38	4	12.6
DNOP	391.3	149	100	13	4	26
DNOP	391.3	71.2	100	21	4	26
DPG	212	195	150	20	5	7.4
DPG	212	119	150	24	5	7.4
DPG	212	94	150	24	5	7.4
DPTU	229.1	136	110	22	4	13.8
DPTU	229.1	94.1	110	18	4	13.8
DPTU	229.1	77.1	110	50	4	13.8
DTG	240	133	150	25	5	7.5
DTG	240	116	150	33	5	7.5
HMMM	391	283	150	11	5	15.3
HMMM	391	253	150	23	5	15.3
HMMM	391	207	150	19	5	15.3
HMMM	391	177	150	27	5	15.3
MEHP	279.2	149	82	10	4	18.4
MEHP	149	65	82	26	4	18.4

Neozone	220.1	143.1	130	26	4	20.7
Neozone	220.1	116.1	130	50	4	20.7
Neozone	220.1	115.1	130	58	4	20.7
Phthalic acid	167	78	122	19	4	6
Phthalic acid	149	121	122	19	4	6
Phthalic acid	149	93.1	122	19	4	6
Phthalic acid	149	65.2	122	27	4	6
Phthalic acid_D4	171	127	122	11	4	6
Phthalic acid_D4	153	125	122	19	4	6
Phthalic acid_D4	153	97	122	23	4	6
Phthalic acid_D4	153	69	122	31	4	6
Phthalic anhydride	149	121	122	19	4	8.5
Phthalic anhydride	149	93.1	122	19	4	8.5
Phthalic anhydride	149	65.2	122	27	4	8.5
PPD	109	93	150	24	5	0.8
PPD	109	92	150	16	5	0.8

Appendix 4 – Parameters measured on the boat (95).

Number	Area	pH	Temp.	electric Conductivity	CSB	turbidity	O2	NH4	NO3	P
			°C	µS/cm	mg/L	NTU	mg/L	mg/L	mg/L	mg/L
1	Scheer	8.353	12.1	521	18.7	7.375	11.91		2.41	0.05
3	Bertoldsheim	8.225	12.2			6.55	10.33		2.2	0.05
4	Ingolstadt	8.301	12.1	557	21.7	4.1	10.64		2.14	0.05
5	Kelheim	8.315	12.1	618		5.65	10.4		2.44	0.05
6	Donaustauf	8.345	12.2	618		7.03	10.4		2.54	0.054
7	Straubing	8.233	12.09	530	26	2.65	10.31		2.48	0.063
8	Haus am Strom	8.407	13	422	7.48	6.3	10.55	0.04	1.59	0.062
9	Linz 1	8.419	12.9	416	6.46	10.85	11.86	0.07	1.63	0.087
10	Linz 2	8.245	12.4	396	6.08	8.125	11.49	0.09	1.43	0.072
11	Krems	8.531	13	395	5.97	13.35	12.53	0.06	1.36	0.05
12	Wien 1							0	1.31	0.05
DK	DK									
13	Wien 2	8.596	14.3	398	5.47	17.175	11.75	0.02	1.28	0.083
14	Bratislava 1	8.617	14.8	399	6.72	19.3	11.67	0.06	1.18	0.05
16	Komarno	8.52	17.7	401	8.57	17.425	11.07	0.08	0.98	0.058
17	Budapest 1	8.806	17.5	388	7.29	11.25	12.66	0.08	1.14	0.05
18	Budapest 2	8.853	18.5	386	8	42.1	13.29	0.12	0.992	0.05
19	Paks	8.662	19.3	366	9.02	29.55	13.91	0.15	0.852	0.05
20	Backi Monostor	8.532	19.9	353	6.61	14.15	13.47	0.05	767	0.051
21	Novi Sad 1	8.569	20.9	334	6.57	25.25	12.05	0.16	0.98	0.05
22	Novi Sad 2	8.597	21.5	333	6.61	21.675	11.88	0.14	1.06	0.051
23	Belgrade 1									
24	Belgrade 2	8.403	21.6	343	6.57	18.675	10.33	0.06	0.25	0.069
25	Safanta Elena	7.98	22.3	347	5.3	11.875	7.64	0.13	0.71	0.074
26	Iron Gate 1 (Kladovo)	7.8	21.4	352	6.53	8.025	6.14	0.03	0.838	0.09
27	Iron Gate 2	7.9	21.8	355	7.02	7.625	6.47	0.01	0.809	0.083
28	Vidin	7.71	22.2	351	8.99	8	5.65	0.02	0.895	0.09
29	Oryahovo	7.835	24	351	7.25	17.2	6.6	0.05	0.852	0.08

30	Zimnicea	8.16	25.6	347	7.74	12.125	8.84	0.1	0.639	0.081
32	Ostrov	8.056	24.5	350	7.93	13.55	7.88	0.02	0.653	0.112
33	Ghindaresti	7.883	24.6	352	24.4	17.25	7.08	0.03	0.895	0.096
34	Galati	7.995	24.1	359	22.7	31	7.05	0.03	0.98	0.09

Appendix 5 – Total suspended solids measured in each sample

	TSS	std. dev.
	mg/L	
1	94.10	0.00
3	21.10	10.61
4	26.32	15.72
5	26.37	5.24
6	36.79	26.36
7	42.00	0.11
8	10.47	0.09
9	15.67	5.17
10	15.83	5.24
11	20.91	10.42
12	20.99	0.09
DK	26.17	5.20
13	31.41	20.92
14	10.55	0.09
16	21.00	10.49
17	32.08	0.00
18	10.56	0.02
19	36.70	5.17
20	36.60	5.28
21	26.39	5.37
22	52.62	10.41
23	42.04	10.45
24	57.84	25.78
25	11.02	0.04
26	10.54	0.01
27	38.39	6.89
28	26.20	15.73
29	26.22	5.20
30	104.95	0.00
32	137.09	10.69
33	89.49	26.09
34	125.65	0.00

Appendix 6 – Particulate concentrations measured before and after major cities

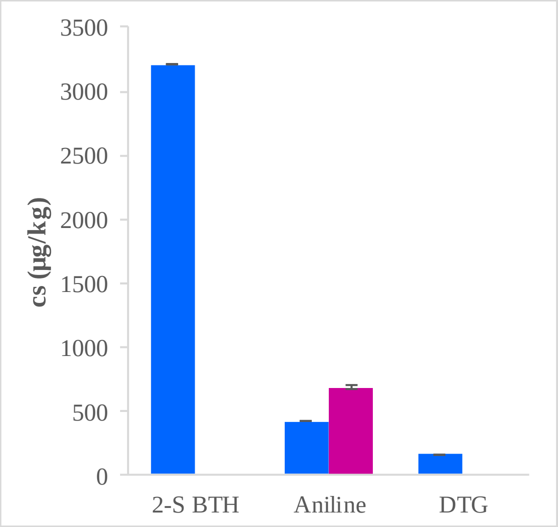


***Figure 1** – Particulate concentrations before and after Linz. The blue columns are the particulate concentrations measured before Linz, while pink is measured after. Cs = particle-bond concentrations.*

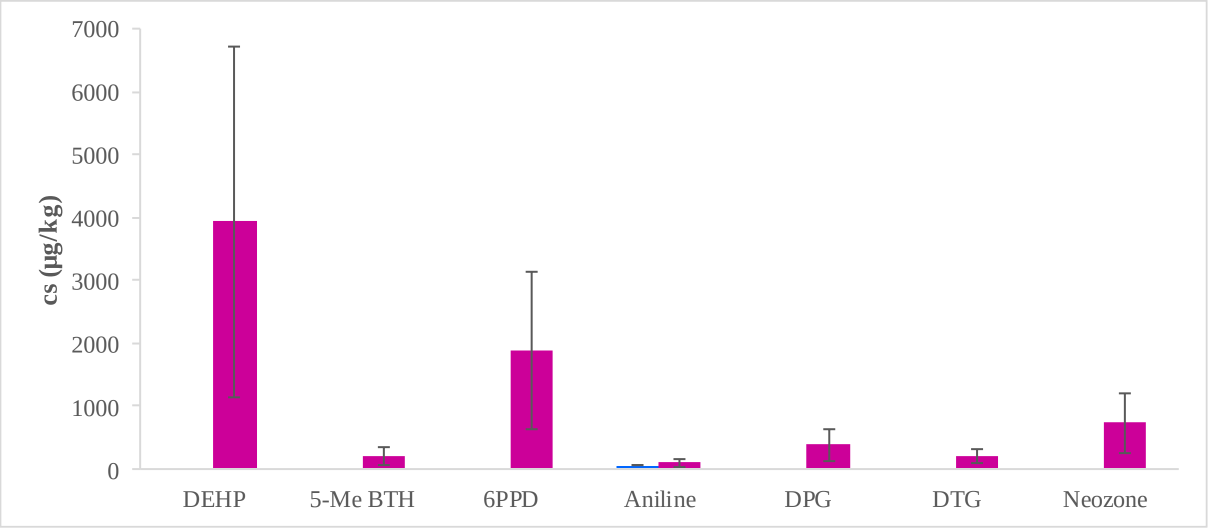


***Figure 2** – Particulate concentrations before and after Vienna. The pink columns represent the particulate concentrations measured after Vienna, while the blue columns are the concentrations measured before. Cs = particle-bond concentrations.*

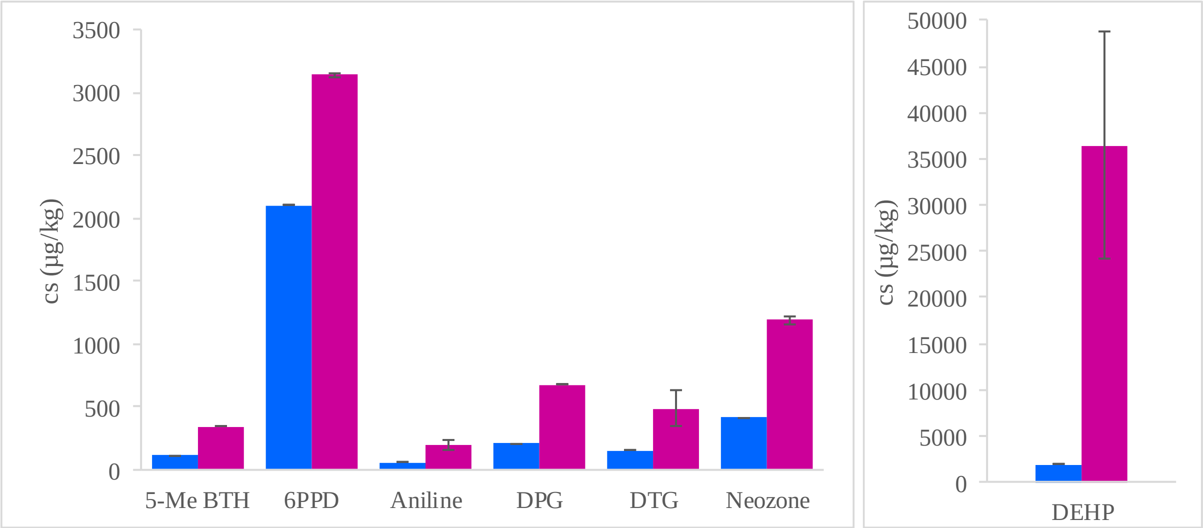


Figure 3 *– Particulate concentrations before and after Budapest. The blue columns are particulate concentrations measured before Budapest, while pink were measured after. Cs = particle-bond concentrations.*

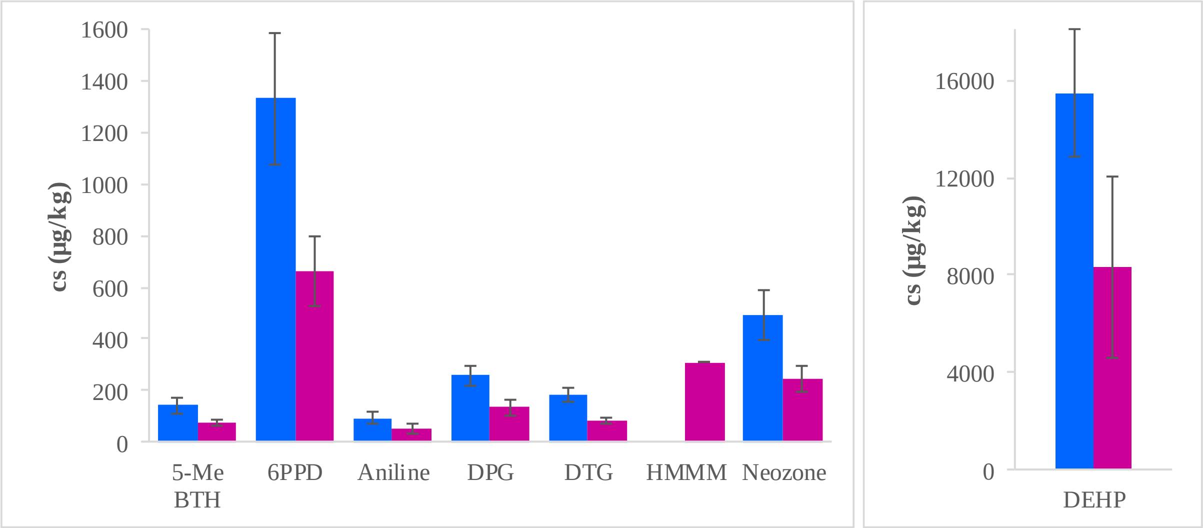


Figure 4 *– Particulate Concentrations before and after Novi Sad. The blue columns are the concentrations before Novi Sad, while the pink columns were measured after. Cs = particle-bond concentrations.*

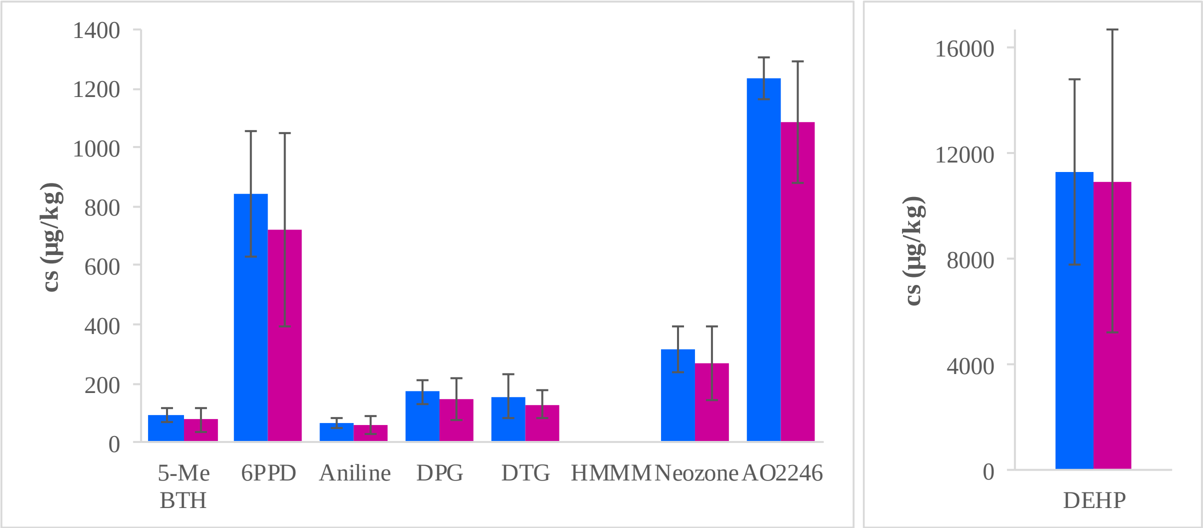


Figure 5 *– Particulate Concentrations before and after Belgrade. Blue columns represent particulate concentrations measured before Belgrade, while pink columns were measured after. Cs = particle-bond concentrations.*