Distribution of microplastics in freshwater systems in an urbanized region: a case study in Flanders (Belgium)

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Abstract:

Microplastics (MPs) are an emerging pollutant of concern in all known aquatic ecosystems. However, studies at a regional scale on MP pollution in freshwater systems and the necessary risk assessments are limited. Therefore, in this study, we examined microplastic concentrations, size distributions, and polymer types in surface waters and sediments in the geographic region Flanders (Belgium), as a case study for a densely populated region and one of the most developed parts of Europe. Samples have been taken on nine different locations, of which five

were repeated in a different weather condition. In total 43 aqueous and nine sediment samples have been collected. The quantity and identity of the microplastics in the samples were determined with μFTIR spectroscopy in the range of 25 - 1,000 μm. The MPs' abundances in surface waters and sediments ranged from 0 to 4.8 MP L^{-1} (average = 0.48 MP L^{-1}) and from 0 to 9,558 MP kg^{-1} dry weight (average = 2,774.57 ± 2,317.93 MP kg^{-1} DW), respectively. Polystyrene and polypropylene were the most common polymer compositions found. No correlations were observed between microplastic concentrations in the sediment/the surface water samples and the measured environmental variables rainfall, conductivity, pH, dissolved oxygen content, waterway flow rate and width, and surrounding land use. Risk assessment results for the measured surface water concentrations through the risk quotient (RQ) method and the probabilistic risk assessment framework suggest that most of the sampled sites in Flanders posed negligible risks to freshwater biota, while this was not the case for some of the sediment concentrations. Our results illustrate the need to urgently develop analytical methods that can routinely measure the full size range of MP in environmental samples to adequately assess risks for the environment.

Keywords: fourier-transform infrared spectroscopy; freshwater; river; surface water; sediment; microplastics; plastic pollution; probabilistic risk assessment

Introduction

Despite the fact that freshwater habitats cover less than 1% of Earth's surface, they house an extraordinary biodiversity (i.e., approximately one-third of vertebrate species and 10% of all species) (Strayer and Dudgeon 2010; Tickner et al., 2020). The Millennium Ecosystem Assessment MEA (2005) assessed that rivers, streams, lakes, and inland wetlands provide a range of crucial provisioning, regulating and maintaining ecosystem services to billions of people due to water mobility across different countries, landscapes, habitats, and ecosystems or

the energy, nutrients, organisms and sediment it contains. Hence, it is crucial to monitor and assess the degree of pollution in surface waters, including contaminants of emerging concern such as plastics. The current lack of adequate (monitoring) data impedes a reliable ecological risk assessment, but also hinders spatial and temporal comparisons between different publications (Duis and Coors, 2016; Löder and Gerdts, 2015; Rodrigues et al., 2018). While surface water quality in Europe has improved over the years, as necessitated by the European Water Framework Directive (WFD) (European Commission, 2000), there is no profound knowledge on water quality in terms of plastic contamination in freshwater bodies. In 2019, 368 million tons of plastic were produced globally (of which 57.9 million tonnes in Europe), ten years ago this was 245 million tons and in 1950s, at the start of the '*Plastic Age',* only 1.5 million tons was produced (Plastics Europe, 2020, 2009, Wagner et al., 2014). In Europe current waste management systems are insufficient to either recycle or dispose produced plastic waste, which ultimately results in the accumulation of plastic pollution into the environment (Ivleva et al., 2017; Lau et al., 2020).

Microplastics (MPs) are usually defined as plastic particles that range between 1 μm to 5 mm in size. These particles have been identified as an emerging pollutant in aquatic and terrestrial environments, but as well in the air (Aliko et al., 2022; Browne et al., 2008; Gasperi et al., 2019; Lu et al., 2021; Wong et al., 2020). Most microplastic particles found in the environment are created by fragmentation and weathering of plastic litter of any size (the so called 'secondary' microplastics), however 'primary' plastic particles such as microbeads and nurdles are also part of the total microlitter fraction (Leslie et al., 2017). Several studies have illustrated that they are widespread, as they have been found at both the highest (e.g., Mount Everest) and deepest points on Earth, namely the deep sea (Abel et al., 2021; Napper et al., 2020; Van Cauwenberghe et al., 2013). At the start of this study in 2018, efforts from the scientific community primarily focused on MP distribution in the marine environment, and less than 4% of microplastics-

related studies were conducted in freshwater systems (Lambert and Wagner, 2018). Recently, MP research has slowly shifted its focus away from the marine-centric viewpoint, resulting in a better understanding of MP pollution in freshwater and terrestrial systems (Lu et al., 2021). For most countries, like Belgium (Europe), no peer reviewed studies are available on MP pollution in aquatic systems or they are limited to marine systems (e.g., Claessens et al., 2011; Van Cauwenberghe, et al., 2015a). One recent study (Semmouri et al., 2022) has analysed drinking water originating from surface waters in Flanders and found on average 0.02 ± 0.02 MPs/L, indicating that microplastics are present in the Flemish waterways.

Freshwater systems are often in the proximity of MP point sources and they are often located in urbanised areas with high population densities, possibly resulting in higher MP concentrations in the environment (Szymańska and Obolewski, 2020). Moreover, freshwater matrices are often regarded as one of the main sources and a critical pathway for contaminants, including the MPs, to enter the marine environment (Dris et al., 2015; Drummond et al., 2022). Lebreton et al. (2017) estimated that, annually, rivers transport around 1.15 to 2.41 million tonnes of plastic into the ocean. However, a profound empirical assessment of different patterns of MP contamination or different fluvial processes governing its distribution is still missing (Hurley et al., 2018). Key intrinsic properties of MPs such as density, shape and size of microplastics likely affect transportation and distribution patterns (Eerkes-Medrano et al., 2015). Where microplastic particles with a high density, such as PET or PVC, tend to sink, low-density particles (such as PP or PE) tend to float on the water surface or in the water column. However, low-density plastics can reach the bottom of the water column through density modification (e.g., biomass accumulation due to biofouling, Van Cauwenberghe et al., 2015b; Van Melkebeke et al., 2020). So far, published research has shown that freshwater sediments can contain a much higher contamination compared to surface waters and that these sediments

are significant accumulation areas for MPs in fresh water ecosystems (e.g., Leslie et al., 2017; Rodrigues et al., 2018; Su et al., 2016, 2018).

Despite exponential growth of research on plastic pollution in the past decade, an adequate understanding of holistic effects of (micro)plastics on aquatic environments and, ultimately, their impact on ecosystem services and human wellbeing, is still lacking (Beaumont et al., 2019). Studies are usually only locally based or small scale which limits their transferability or scaling up (Beaumont et al., 2019; Ten Brink et al., 2016). The objective of this study was to identify, quantify and investigate the presence of microplastic particles in multiple surface waters across Flanders. This geographic region represents a densely populated (Belgium, Europe, 487 inhabitants/km^2) and highly developed area in western Europe (Human Development Index of Belgium was 0.931 in 2019, with rank 14/189; United Nations Development Programme, 2020). To improve our understanding of the fate of microplastics in freshwater ecosystems, we investigated MP particles occurrence with sizes bigger than 25 µm in both surface water and sediments. We also studied whether significant relationships existed between properties of polluting particles (e.g., polymer type, size fraction) and abiotic parameters (e.g., river flow, water depth, pH and level of urbanisation). Moreover, we also aimed to understand whether microplastic concentrations can fluctuate substantially in response to temporal differences in rainfall. Finally, for the sampled locations we also investigated whether the obtained microplastic concentrations pose a risk for the residing biota using probabilistic risk assessment framework, by comparing exposure data from this study data to published effect data in peer reviewed literature.

Material & Methods

2.1. Collection of the samples

To investigate the microplastics distribution in freshwater environments, samples were collected from surface water and sediment in nine waterways in Flanders (Figure 1; S1). Sample locations were selected to include both navigable and non-navigable waterways, as well as rural and urbanized regions, covering the entire Flemish region (at least one site per province). Five sites out of nine, were selected for repeated sampling in wet weather conditions to investigate the microplastic load in waterways, experiencing temporal differences in rainfall. A wet period is defined as the day after a day with at least 5 mm precipitation per day, while a dry period is defined as starting on the 3rd day, after at least 2 days with less than 0.2 mm precipitation per day, of which the precipitation on the day itself is also less than 0.2 mm precipitation per day. Samples were collected in triplicates (3 replicates/ location). In total 43 aqueous samples were collected. At six selected locations, sediment was sampled as well (9 different samples in total; Figure 1). Table 1 reports the details of the selected sampling locations and dates.

Table 1: Summary of sample collections in the surface waters *and sediment in nine different waterways in Flanders. The number of technical replicates (subsamples) of the sediment samples is denoted between brackets.*

	Waterway	***Latitude N***	***Longitude E***	***Location***	***Time***	***Weather conditions***	***# Replicates***	
							Surface water	*Sediment*
1.	Oude kale	51.0832	3.6209	Lievegem	17/04/2019	Dry	3	4 (1,3,1,3)
2.	Watersportbaan	51.046115	3.704603	Gent	24/05/2019 8:45 – 9:30	Dry	3	/
3.	Grote Struisbeek	51.147541	4.381735	Aartselaar	18/09/2019 11:00 – 13:00	Dry	3	1 (1)
4.	Grote Nete	51.162914	5.23527	Balen	17/05/2019 10:35 – 11:00	Dry	3	/
5.	Tangebeek	50.945672	4.414038	Grimbergen	11/09/2019 11:15 – 12:20	Dry	4	1 (1)

6.	Dommel	51.074792	5.455907	Peer	17/05/2019 9:15 - 9:45	Dry	3	/
7.	Zenne	50.960401	4.455872	Eppegem	16/12/2019 10:30 - 10:45	Dry	3	/
8.	Heulebeek	50.843009	3.217122	Gullegem	27/06/2019 11:10 - 12:30	Dry	3	1 (1)
9.	Koevaardeken	50.984794	2.825817	Houthulst	4/12/2019 9:00 - 10:00	Dry	3	/
3b.	Grote Struisbeek	51.147541	4.381735	Aartselaar	2/10/2019 10:30 - 11:45	Wet	3	/
5b.	Tangebeek	50.945672	4.414038	Grimbergen	8/11/2019 12:00 - 12:40	Wet	3	/
7b.	Zenne	50.960401	4.455872	Eppegem	13/06/2019 10:50 - 12:20	Wet	3	1 (1)
8b.	Heulebeek	50.843009	3.217122	Gullegem	11/12/2019 10:45 - 11:15	Wet	3	/
9b.	Koevaardeken	50.984794	2.825817	Houthulst	28/05/2019 11:00 - 12:45	Wet	3	1 (3)

On each sampling location, approximately 10 sub-samples of about 1 L of surface water were used to fill a pre-rinsed 10 L bottle using a sampling cup, mounted on a telescopic arm (Acuradmin, 1 L, up to 3 m) and a stainless steel funnel. Samples were taken 1 to 2 meters from the bank and in the upper layer of the water body (water surface; top 0.5 m). For each location, 3 replicates (each 10 L) were taken over a period of 15 minutes, each 1 to 2 m further upstream of each other. Used material was rinsed with filtered water between consecutive samplings. Bottles were sealed with aluminium foil and transported to the lab. Samples were stored at 4°C until microplastic extraction. A Van Veen grab (Van Eijkelkamp, 2 L) was used to sample sediment, in which 5 to 10 separate Van Veen samples were integrated into 1 sample. Samples were collected in a stainless steel bucket and transferred to pre-rinsed 4 L glass jars untill the jar was completely filled. Jars were sealed with aluminium foil and transported to the lab to store at 4°C until further processing. The sediment, in contrast to the surface water (both in dry and wet weather periods), was sampled only once per location because of the integrated nature of the sample, already covering existing spatial variation in MP pollution. Yet, to confirm whether we captured this variation in such a sample, we collected four different integrated Van

Veen samples at one sampling location (Oude kale), each 1 to 2 m further upstream of each other. Hence, we obtain a better understanding of (the existence of) spatial variation of MP contamination in the sediment layer.

2.2. Sample treatment: microplastic extraction

For the aqueous samples, 50 g potassium hydroxide (KOH, AnalaR NORMAPUR) was added to each sample (±10 L) to digest organic matter present (60°C, 48 hours) (based on Thiele et al., 2019). After digestion, supernatant water was filtered through a cellulose nitrate filter (pore size 8.0 µm, ⌀ 47 mm, Whatman AE99). The sample residue (deposit) and rinse water were transferred to a pre-rinsed 200 mL polypropylene conical centrifuge tube (Thermo Scientific Nunc) and then centrifuged (megafuge 40R, Thermo Scientific; rotor radius of 195 mm) at 2,675 g (RCF), for 5 minutes. Supernatant water from the centrifuge tube was filtered again over the cellulose nitrate filter. The pellet was then diluted with 150 mL of sodium iodide (with a density of 1.6 g/cm^3; NaI, GPR RECTAPUR, VWR), solubilized and centrifuged again at the same settings (2,675 g, 5 min) to separate microplastics present in the sample from the denser, residual inorganic fraction, as done by Claessens et al., (2013) and Van Cauwenberghe et al., (2013). The supernatant was filtered using a sieve (mesh size of 1,000 µm) to remove large floating particles, and subsequently filtered again over a cellulose nitrate filter. Density separation with NaI was repeated two more times, but in the final centrifugation step the rotational speed was increased to 3,853 g (RCF). Cellulose filter(s) were transferred into in a glass beaker containing 10 % KOH (50 mL per filter) and were then left for digestion in a warm water bath (Memmert WTB) for one day (24 hours) at 60°C. Next, the obtained solution was brought over a polytetrafluoroethylene (PTFE) filter with a pore size of 10.0 µm (⌀ 25 mm, Omnipore Membrane filter, Merck). This filtration step was followed by a thorough rinse of both the measuring cup and the glass filtration system with filtered milliQ water (three times),

174 followed by a 0.1% Tween® 80 solution. Eventually, the filter was left to dry in a dust-free
175 environment at approximately 20 °C for 24 hours.

176 For the sediment samples, one subsample (± 20 g wet weight) per sample was collected after
177 homogenization (Table 1). First, the glass jar containing the entire sample was first shaken
178 thoroughly, after which the sample was mixed manually using a metal spoon to ensure
179 homogeneity as much as possible. For two locations (Oude kale and Koevaardeken), three
180 subsamples were collected. The use of these pseudo replicates (technical replicates) allows to
181 confirm whether subsamples are representative of the entire integrated sample and, thus, to
182 obtain a better insight in variability in the measurement of the MP concentrations.
183 Subsequently, about 20 grams of the homogeneous sample was transferred to a measuring cup
184 and 200 mL hydrogen peroxide H_2O_2 (30%, Ph.Eur, VWR) was added stepwise (per 25 mL)
185 for digestion of the organic material. First, the sample was kept at room temperature for 24
186 hours and then placed in a warm water bath (Memmert WTB) at 60°C for one day (24 hours)
187 to stimulate digestion (based on Claessens et al., 2011; Thiele et al., 2019; Van Cauwenberghe
188 et al., 2013, 2015a,b). After digestion, the supernatant water was filtered through a cellulose
189 nitrate filter (pore size: 8.0 µm, ⌀ 47 mm, Whatman AE99). An additional 25 mL of 69% nitric
190 acid HNO_3 (Supelco®, Merck) was added to the remaining sediment for one hour at room
191 temperature (based on Claessens et al., 2013; Nel et al., 2018). The acid sludge and rinse water
192 of the sample were then transferred with a glass funnel into a pre-rinsed 200 ml polypropylene
193 conical centrifuge tube (Thermo Scientific Nunc). Next, the subsequent density separation step
194 and the digestion step of the cellulose filters were performed as described above for the
195 treatment of surface water samples. The obtained solution was then sieved (pore size of 15 µm)
196 using a PVC sieve (based on Van Echelpoel, 2014). The fraction larger than 15 µm was diluted
197 with 40 mL NaI in a 50 mL conical centrifuge tube (Nerbeplus) and centrifuged again (2,675
198 g, 5 min). Finally, the supernatant was filtered over a PTFE filter as described above.

2.3. Observation and characterisation of the microplastics

After drying, obtained PTFE filters could be analyzed by a µFTIR spectroscope (Nicolet iN10 FT-IR Microscope; Thermo Fisher Scientific, Madison, Wi, USA). The spectroscope scanned the entire surface of the filter and determined the spectrum of each recognized particle (settings: reflection mode, spectral range 1,300 - 4,000 cm^{-1}, 150 x 150 µm aperture, spectral resolution 16 cm^{-1}). Next, obtained spectra were compared with spectra from the reference library (Bibliotheque Particule, Thermo Fisher Scientific, France) and their identity was resolved based on correlation (Pearson correlation, threshold match 75%). Additionally, length and width of the particles were collected. The particles that could not be identified (either non-plastic particles or matches to reference plastics with a correlation < 75 %) were discarded.

2.4. Precautionary principles and quality control

In order to avoid contamination, good field and laboratory practices (GLP) were applied both *in situ* as in the laboratory (e.g., the wearing of a cotton lab coat; extractions under a laminar flow (Potteau, Heule), dust free storage and repeated pre-rinsing of lab material with deionized water). Moreover, to avoid air-borne contamination, we sealed all samples, bottles, and measuring cups with either aluminum foil or with watch glasses. We avoided use of plastic equipment (in favour of glass or stainless steel), but if deemed irreplaceable, potential contamination was investigated each time *a priori* using blanks (e.g., the 50 mL conical polypropylene centrifuge tubes used for density separation).

Additionally, to obtain an indication of the recovery rate of microplastics in our samples, we spiked six blanks (positive controls) with 47.33 ± 10.5 polyethylene particles L^{-1} (diameter of 90 - 106 µm, Cospheric) and we processed these like other samples. This preliminary work found a recovery rate of 82 ± 4% for the reference microplastics.

Finally, we also generated negative control samples in the field. These sample blanks (10 L of filtered deionized tap water) followed a similar processing and analysis compared to the other samples to evaluate the extent of additional contamination during either the sampling, transport or extraction process. As there are, currently, no standardized methods present to take these negative controls into account in the analyses (Brander et al., 2020), we decided to correct our data for possible contamination using both the limit of detection (LOD) and the limit of Quantitation (LOQ) (Armbruster and Pry, 2008; Uhl et al., 2018):

$$LOD = Average_{Blanc} + 1.645 \times Standard\ deviation_{Blanc} \quad \text{(Eq. 1)}$$

$$LOQ = Average_{Blanc} + 3 \times Standard\ deviation_{Blanc} \quad \text{(Eq. 2)}$$

These calculations were made for surface water samples per polymer type. All concentrations lower than LOQ for a specific polymer type were deemed unreliable to quantify, due to potential contamination, and, hence, we chose to not report these values (<LOQ). Obtained LOD and LOQ values are presented in Table S1 per polymer type. No blank correction was performed for sediment samples.

2.5. Statistical analyses

We tested statistical differences in terms of MP concentrations between sampled locations or environmental factors using either ANOVA or the non-parametric alternative, Kruskal-Wallis, followed by an appropriate post-hoc analysis (pairwise Wilcoxon test) to compare groups in pairs. P-values (corrected for multiple hypothesis testing) smaller than 0.05 were considered to indicate statistically significant differences. Similarly, we also investigated whether microplastic concentrations were significantly altered in river systems experiencing temporal differences in rainfall. For sediment samples, difference in microplastic concentrations between a wet and a dry period could not be analyzed on the basis of the collected data. We did not repeat collection of sediment samples in different weather conditions, as we assumed less

246 fluctuating microplastic concentrations in this matrix, and, hence, being less dependent on
247 weather or the flow rate of the watercourse.

248 To test for either normality or homogeneity of variances, we applied the Shapiro-Wilk test and
249 the Levene's test, respectively to all metric data. Finally, we calculated Spearman Rank
250 correlation ($p < 0.05$) to screen for potential correlations of the MP abundances with the
251 measured environmental factors. All these statistical tests were performed in R Studio (v4.1.1),
252 and graphs were created using the ggplot2 package (v4.0.3).

253 *2.6. Comparison of our findings with literature*

254 To compare our data with other studies, the available scientific (peer-reviewed) literature on
255 microplastics in freshwater environments was examined. In total, the results of 93 scientific
256 papers published between 2011 and 2021 were studied and analyzed. From these 93
257 publications, only those studies were selected for comparison that reported a comparable
258 minimal particle size (>25 µm) and that applied a spectroscopic technique for identification of
259 the polymer types (FTIR, Raman spectroscopy, or other variants). After this selection, a total
260 of 29 publications remained that describe the microplastics concentration in surface water (15
261 publications) and sediment (14 publications).

262 *2.7. Ecological risk assessment*

263 Untill recently, a major component largely ignored in microplastic research is considering the
264 occurrence of MPs in the environment as well as their (potential) effects in regard to the context
265 of risk. In this study, we applied the rationale of the deterministic risk assessment framework,
266 outlined by the European Chemicals Agency (2021). In this procedure a risk characterization
267 ratio (RCR) is calculated by dividing an environmental concentration by a safe threshold value,
268 a predicted-no-effect concentration (PNEC). The effect assessment for surface waters was
269 performed based on HC_5 (hazardous concentration for 5% of species) data extrapolated from

available published species sensitivity distributions (SSDs) in peer reviewed literature (Table 2). The HC_5 can be translated to the concentration at which 5% of the species in an ecosystem experience negative effects (Wheeler et al. 2002). It is a key regulatory parameter that is employed to derive legally binding environmental quality standards (Wheeler et al. 2002). The HC_5 value obtained from literature was converted into a $PNEC_{surface}$ by dividing it by an assessment factor of 5 (AF; European Chemicals Agency, 2008). Van Cauwenberghe et al. (2015a) determined a first $PNEC_{sediment}$ for marine sediment of 540 particles per kg sediment, as discussed below. Next, risk characterization ratios (RCRs) were obtained by dividing the measured environmental concentrations (MECs) by the available PNEC data ($PNEC_{surface}$), reported in Table 2.

Table 2. HC_5 and corresponding $PNEC_{surface}$ (= HC_5 /5) concentrations extrapolated from Species Sensitivity distributions of freshwater species exposed to microplastics, published in literature.

HC_5 (particles L^{-1})	**PNEC (particles L^{-1})**	**Quantification method**	**Included size range (µm)**	**Most sensitive taxon**	**Reference**
740	148	5th percentile of the employed SSD, based on 53 values from 14 freshwater species	1-1,000	*Oryzias melastigma*; calculated mean NOEC of $3.90x10^3$ MP L^{-1}	Adam et al., 2019
1,015	203	5th percentile of the employed SSD, based on 21 values from 10 aquatic species	0.5-600	*Crassostrea gigas*; LOEC of $1.82x10^6$ MP L^{-1}	Besseling et al., 2018
3,500	700	5th percentile of the employed SSD, based on 9 values from 9 aquatic (marine and freshwater) species	10–5,000 µm	*Skeletonema costatum*; 1,368 MP/L	Burns and Boxall, 2018, 2019
251	50.2	5th percentile of the employed SSD, based on 54 values from 11 freshwater species	1-250	*Lemna minor*; Threshold	Koelmans et al., 2020

				concentration $EC_{X,env}$ of 28.4 MP/L	
75.6	15.12	5th percentile of the employed SSD, based on 54 values from 11 freshwater species, applicable for MP's in the same size range of 1–5,000 μm (rescaled and bioavailability corrected data)	1–5,000	*Lemna minor*; Threshold concentration $EC_{X,env}$ of 28.4 MP/L	Koelmans et al., 2020
71.6	14.32	5th percentile of the employed SSD, based on 63 studies covering 39-40 species	0.1-1,000	*Barbodes gonionotus*; calculated LOEC of 127.3 MP/L	VKM, 2019

Because of the scarcity of relevant and accurate ecotoxicity data on microplastics in freshwater, the order of magnitude of the published $PNEC_{surface}$ values varies between 14.32 and 12,000 particles L^{-1} (Table 2). Moreover, as environmental concentrations are highly variable, we can argue that it is desirable to assess this variability and uncertainty by using a probabilistic risk assessment. In this approach a probability distribution for environmental concentration data is compared to a probability distribution derived from toxicity values. A cumulative probability distribution of the reported $PNEC_{surface}$ values was fit using the ssdtools package in R (Thorley and Schwarz, 2018). The exposure assessment was based on building a cumulative exposure probability curve of the average measured environmental concentrations for each sampling point. The PNEC (predicted no effect concentration) probability distribution was then compared to that of the MECs. If exposure and ecotoxicity probability distributions overlap (or $RCR \geq 1$), a risk can be expected to occur toward the organisms living in the relevant compartment; no overlap ($RCR < 1$) indicates no immediate concern for risk. The probabilistic RCRs were obtained following the method of Verdonck et al. (2003): Risk : $P[EC > SS] = P[EC/SS > 1] = P(\log(EC/SS) > 0) = P(\log(EC) - \log(SS) > 0)$. The average proportion of

species exposed was calculated by the ssd_exposure() function of the ssdtools package (Thorley and Schwarz, 2018).

3. Results & discussion

Our study's objective was to quantify and investigate the presence of microplastic particles in multiple surface waters and their associated sediments across Flanders. All identified particles below the respective LOQ values were removed from the dataset. Hence, only those particles of which the quantification is considered reliable are reported below.

3.1 Microplastic contamination in Flemish surface waters

Microplastics (25 - 5,000 µm) were found in surface water of seven of the nine sampled waterways. Figure 1A provides an overview of measured average microplastic concentrations per sampled location (detailed information available in Table S2). No microplastics were found in the Watersportbaan in Ghent and the Grote Nete in Balen. On average (± SD) we found 0.48 ± 0.94 MP L^{-1} in Flemish surface waters. A maximum concentration of 4.8 MP L^{-1} was recorded in the Grote Struisbeek (Aartselaar). However, this observation was a one-off high concentration. Other measurements at the same location do not show systematically higher concentrations compared to other samples. This higher microplastic concentration therefore seems to have a time-bound component, which has also been described for other surface waters (Rodrigues et al., 2018; Stanton et al., 2020). No significant differences were observed in the measured microplastic concentration between the sampled sites (Kruskal-wallis test; $p = 0.36$).

In surface water of the sampled locations mainly PP (49%) and PS (49%) were found, and to a lesser extent PVC (2%). We also found several PET and PE particles, but due to relatively high concentrations of these polymer types in the blank samples, these concentrations of PET and PE in the samples were often smaller than the LOQ and were therefore omitted for further

analysis. The polymer composition of microplastics in the surface water varies among the locations (Figure 2A; Table S2).

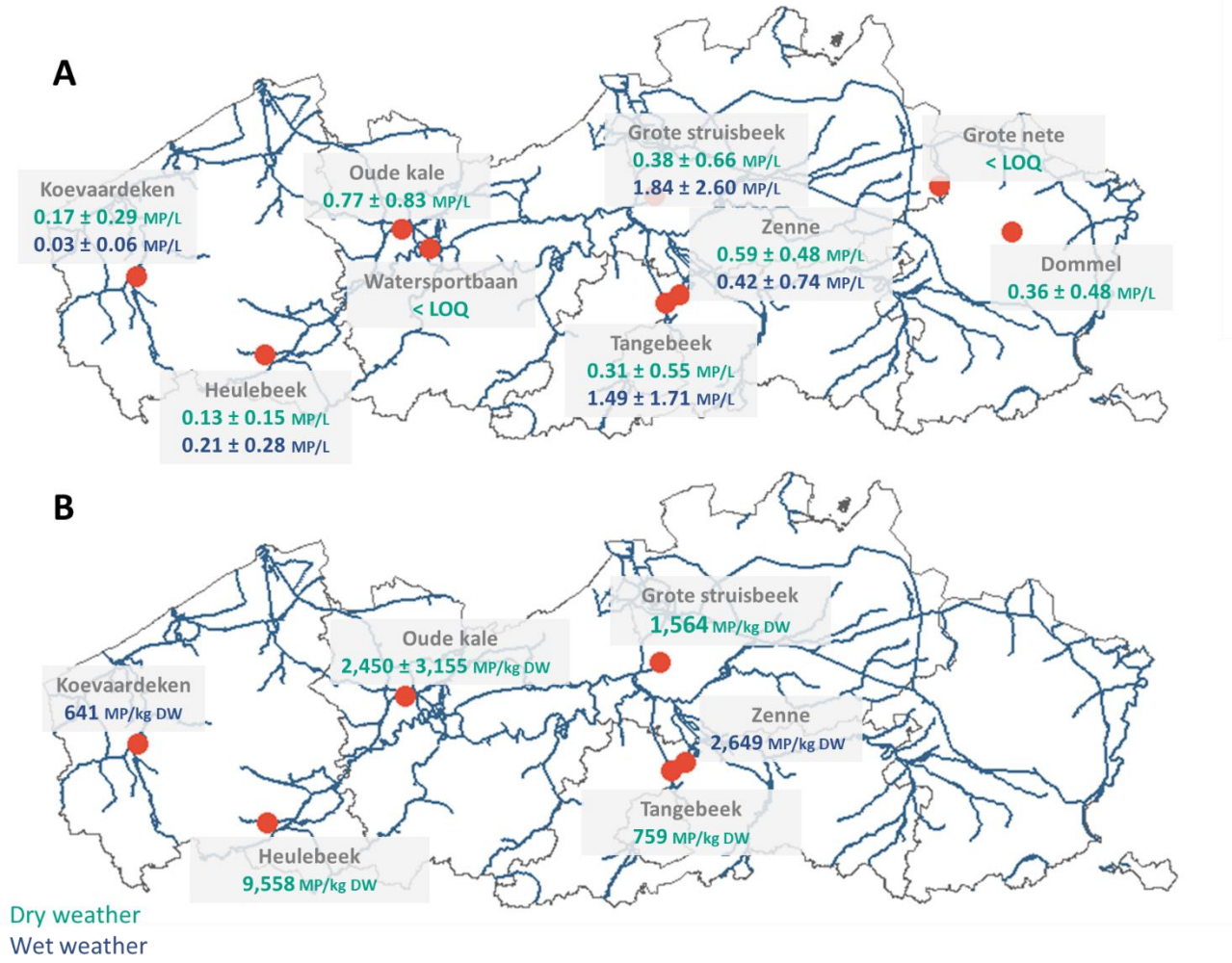

Figure 1. Concentration of average microplastics (± SD) found in Flemish surface waters (A) and sediments (B) during a dry (green) and a wet (blue) period. Note that the data of the water samples have been corrected for possible contamination during sampling and processing using the limit of detection (LOD) and the limit of Quantitation (LOQ) method.

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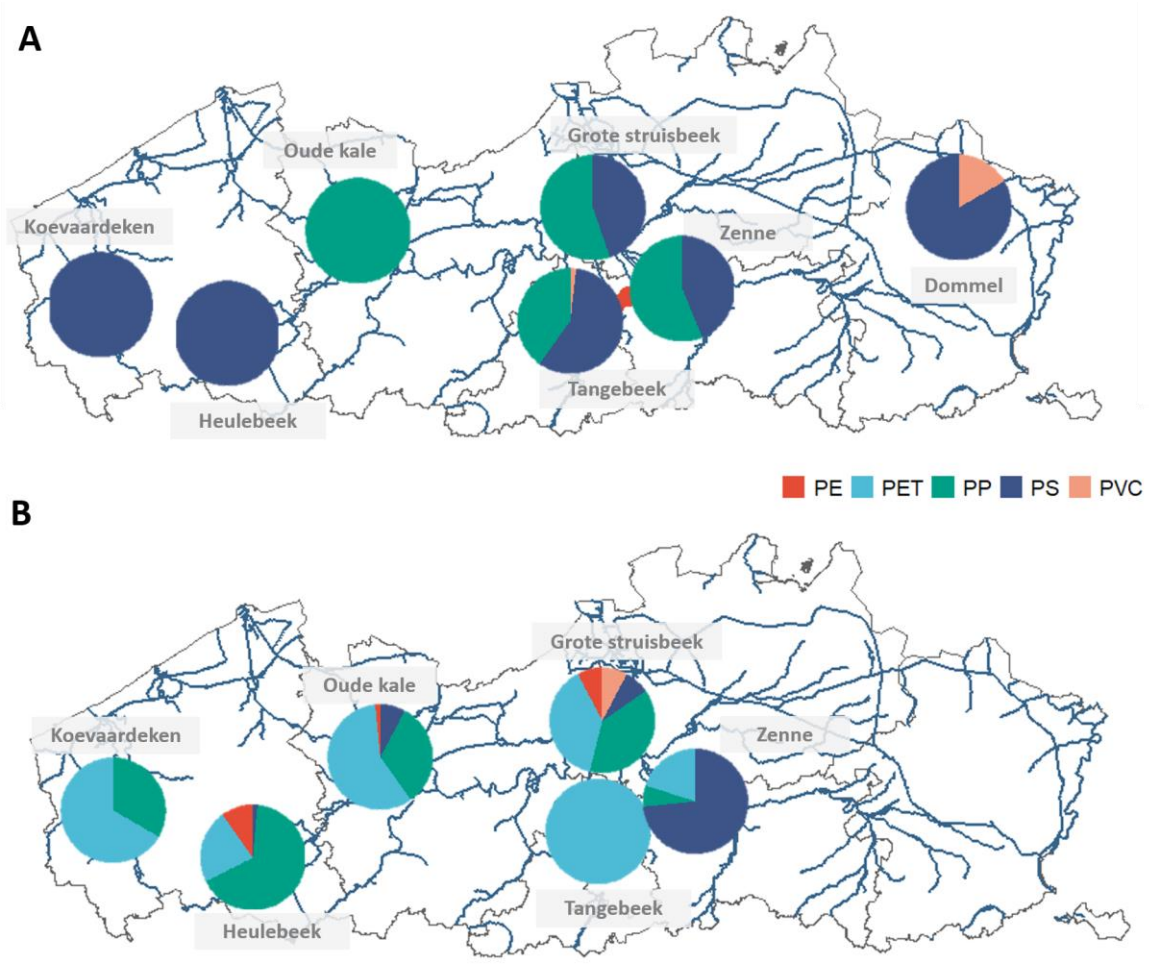

329

330 *Figure 2: Pie charts showing the distribution of microplastic polymers in (A) Flemish surface waters for each*

331 *sampled location and (B) in the corresponding receiving sediments.*

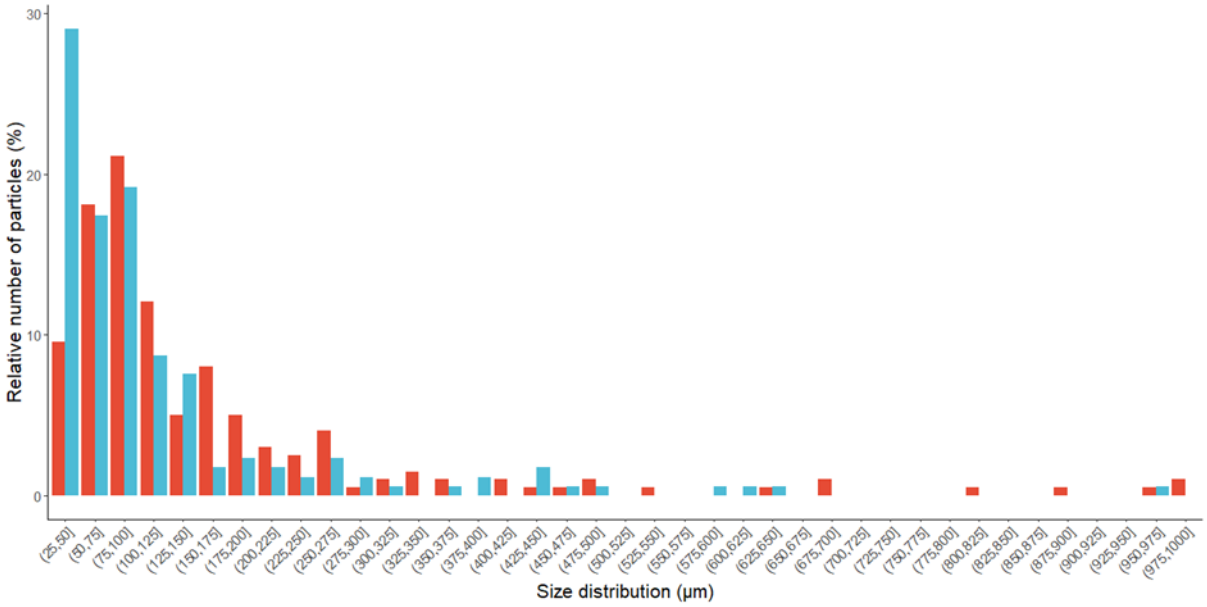

Figure 1: Size distribution of observed microplastic particles in all nine sampled waterways for both surface water (red) and sediment samples (blue).

The size distribution of observed microplastic particles in surface water shows an exponential increase with decreasing particle size (Figure 3), as expected from available literature (Scherer et al., 2020; Uurasjärvi et al., 2020).

3.2 Microplastic contamination in Flemish sediment

Except for two subsamples (one in the sediment of the Oude Kale (Lievegem) and one in the sediment of the Koevaardeke (Houthulst), microplastics were found in all other samples and replicates (Table S3). Overall, an average (± SD) of 2,774.57 ± 2,317.93 MP per kg dry weight (DW) was found in all sediment samples analyzed. A minimum (in addition to the 0 measurements mentioned above) of 641 MP kg^{-1} DW was found in the Koevaardeken (Houthulst) and a maximum 9,558 MP kg^{-1} DW in the Heulebeek (Gullegem). Figure 1B gives an overview of average microplastic concentrations in the sediment at different locations. Due to concentrations being relatively highly variable, no statistically significant differences are detected among the watercourses (Kruskal-wallis test; $p = 0.419$).

In sediment samples mainly PP (44 %) and PET (40 %) particles were found, but also PS (20 %), PE (5%) and PVC microplastics (0.5 %) in lower concentrations (Figure 2B). The same polymer types were also found in the corresponding surface waters. Comparing sediment samples of different locations, we found small variations in occurring polymer types: PS is the dominant polymer type in the Oude Kale (54 %), which was found in much smaller quantities in other locations (up to 25 %, Figure 2B, Table S3). For other locations PP is the most abundant polymer type (Table S3). In the Heulebeek and Koevaardeke, PET is present in 33 % and 36 % of the samples (Fig. 2B; Table S3).

As in surface water, the size distribution of observed microplastic particles shows an exponential increase with decreasing particle size in sediment (Figure 3). Sediment samples contain more particles with a size between 25 and 50 µm, as compared to water samples. The water samples contain relatively more particles between 75 and 125 µm and there is no spatial variation in size distribution given on the basis of the collected samples.

3.3 Correlation between microplastic pollution and environmental parameters

As we expected, we found no correlation between measured microplastic concentrations in surface water and its receiving sediment (Spearman Rank; cor = -0.28; p = 0.38). This is likely due to the different dynamics of each compartment: the more static sediment where an accumulation of plastics over a longer period of time is possible versus the dynamic water surface, where currents, wind and rainfall, among other parameters, affect the distribution and transport of microplastics (Lassen et al., 2015). The concentrations in the sediment were much higher compared to the surface water, albeit both are expressed in different units. These higher concentrations in sediment samples as compared to water surface have been frequently described in literature (e.g., by Scherer et al., 2020). Our findings have important implications for future research as they highlight that different locations in a river show different fluxes of

microplastic pollution and that water surface, sediment and perhaps even water column must be taken into account to obtain a detailed insight regarding microplastic pollution.

Measured environmental data for each sample are reported in Table S4. In the exploration of our dataset, no correlations were observed between an increased microplastic concentration in the sediment/the surface water samples and the measured environmental variables (Spearman rank correlation/Wilcoxon rank sum test; corresponding p-values in Table S5, S6). Only the presence of urbanized areas around the sampling location seems to indicate a higher microplastic concentration in the sediment (Wilcoxon rank sum test; $p = 0.09$; Table S6). In contrast, other studies did find a significant correlation between surrounding land use and concentration of microplastics (Barrows et al., 2018; He et al., 2020). To further investigate these potential correlations in Flanders, future studies should incorporate more data from strategically chosen locations.

For five locations, sampling of surface water was repeated during both a wet (≥ 5 mm precipitation day^{-1}) and a dry period. In dry weather ($n = 27$), an average of 0.30 ± 0.47 MP L^{-1} was found, while in rainy weather ($n = 15$), we observed 0.80 ± 1.43 MP particles per L. Concentrations in both weather types were statistically not significantly different from each other, despite a higher average concentration in wet periods of sampling (Wilcoxon test; $p = 0.44$).

3.4 Global comparison of microplastic contamination in freshwater surface waters and sediments

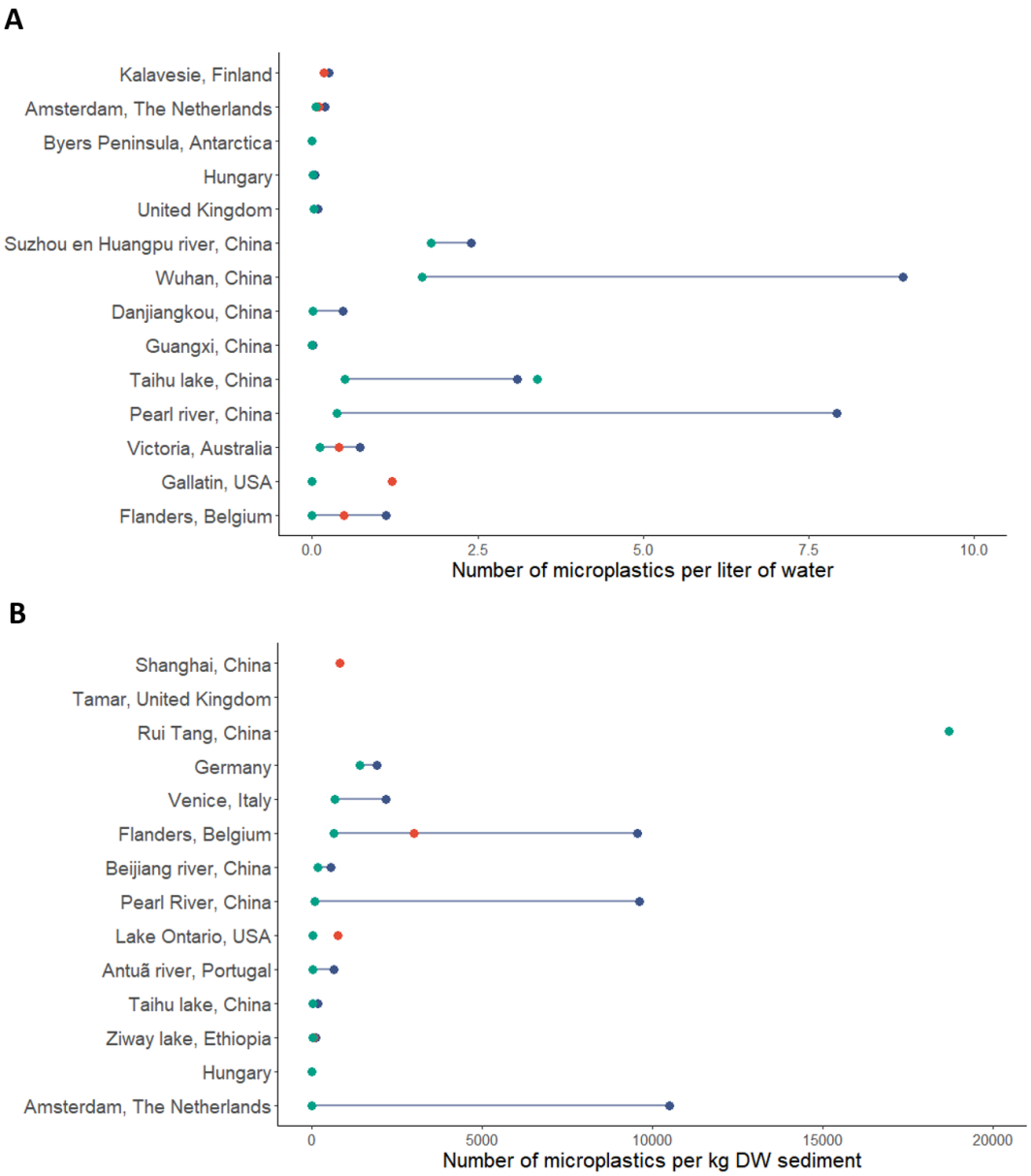

Figure 4: Comparison between reported concentrations (minimum = green; average = red; maximum = blue) in (A) surface waters and (B) sediment samples. References (from top to bottom) for surface water: Uurasjärvi et al., 2020; Leslie et al., 2017; González-Pleiter et al., 2020; Bordós et al., 2019; Stanton et al., 2020; Luo et al., 2019; Wang et al., 2017; Di et al., 2019; Zhang et al., 2021; Su et al., 2016; Lin et al., 2018; Nan et al., 2020; Barrows et al., 2018; current study. References (from top to bottom) for sediment: Peng et al., 2018; Browne et al., 2010; Wang et al., 2018; Leslie et al., 2017; Vianello et al., 2013; current study; Wang et al., 2017; Lin et al., 2018; Ballent et al., 2016; Rodrigues et al., 2018; Su et al., 2016; Merga et al., 2020; Bordós et al., 2019; Leslie et al., 2017.

Regarding microplastic pollution in surface waters, reported concentrations in Flanders are rather low compared to other countries/studies (Figure 4A). The measured microplastic concentrations in surface water are comparable to, among others, the Netherlands (Leslie et al., 2017), Finland (Uurasjärvi et al., 2020), and the United Kingdom (Stanton et al., 2020), but also to those measured in a river in Antarctica (González-Pleiter et al., 2019). For sediment, concentrations observed in this study tend to be in the higher range of internationally reported values (Figure 4B). Average microplastic concentrations in sediment are consistent with for example observations in the Netherlands (Leslie et al., 2017), Italy (Vianello et al., 2013) and Germany (Leslie et al., 2017).

3.5. Ecological risk assessment

For all surface water samples, reported concentrations are lower than the $PNECs_{water}$, indicating a low to negligible risk for the environment (Table S7). Additionally, to assess the risk that microplastics might pose in the world's freshwaters, a probability distribution of both the MECs and of the $PNEC_{surface}$ values were plotted (Figure 5). The large 95% confidence interval (95 % CI) of the cumulative distribution based upon the $PNEC_{surface}$ values illustrates the large variability emerging from combining different effect data (Figure 5). The probability distribution of exposure concentrations did not overlap with the PNEC probability distribution (Figure 5). The probabilistic risk characterisation revealed a small probabilistic risk, defined as the probability that a randomly selected environmental concentration exceeds a randomly selected PNEC, of 0.03. The average proportion of species at risk was calculated to be 0.83 % (Figure 5), as defined by Verdonck et al. (2003). Therefore, it can be concluded that current microplastic concentrations in surface waters represent a limited ecological risk in freshwater systems in Flanders. We recognize there are several limitations to the distribution we have presented in Figure 5. This is mainly due to the data (i.e., the HC_5/PNEC values) it is based on. The SSDs published by VKM (2019) and Besseling et al. (2018) included data for nanoplastics,

while Burns and Boxall (2018) included data from marine species, which make them less representative for a microplastic SSD for the freshwater environment. Koelmans et al. (2020) included more recent effect threshold data from studies published in 2018 and 2019. They provided two SSDs based on laboratory data for different types of particles, and based on the same data after corrections for bioavailability and rescaling to cover the size range of 1–5000 µm, resulting in two different HC_5 values of 251 and 75.6 MP L^{-1} (Table 2). Adam et al. (2019) also performed a preliminary probabilistic risk assessment using an exposure distribution based on 391 measured microplastic concentrations in Asia, Europe and North America. This distribution was divided by a PNEC distribution (cf. Table 2) to determine a risk quotient distribution for the three continents. No ecological risks were noted for European waters (based on data from France, Switzerland, Italy, Germany, Austria and the Netherlands). The obtained probability distributions showed that risks could not be excluded in Asia, where 0.4% of the risk quotients had a value above 1 (i.e., showed a risk). However, Adam et al. (2019) state that for Europe only microplastics larger than 80 µm were sampled, which may underestimate the risk. The detection limit in our study is 25 µm.

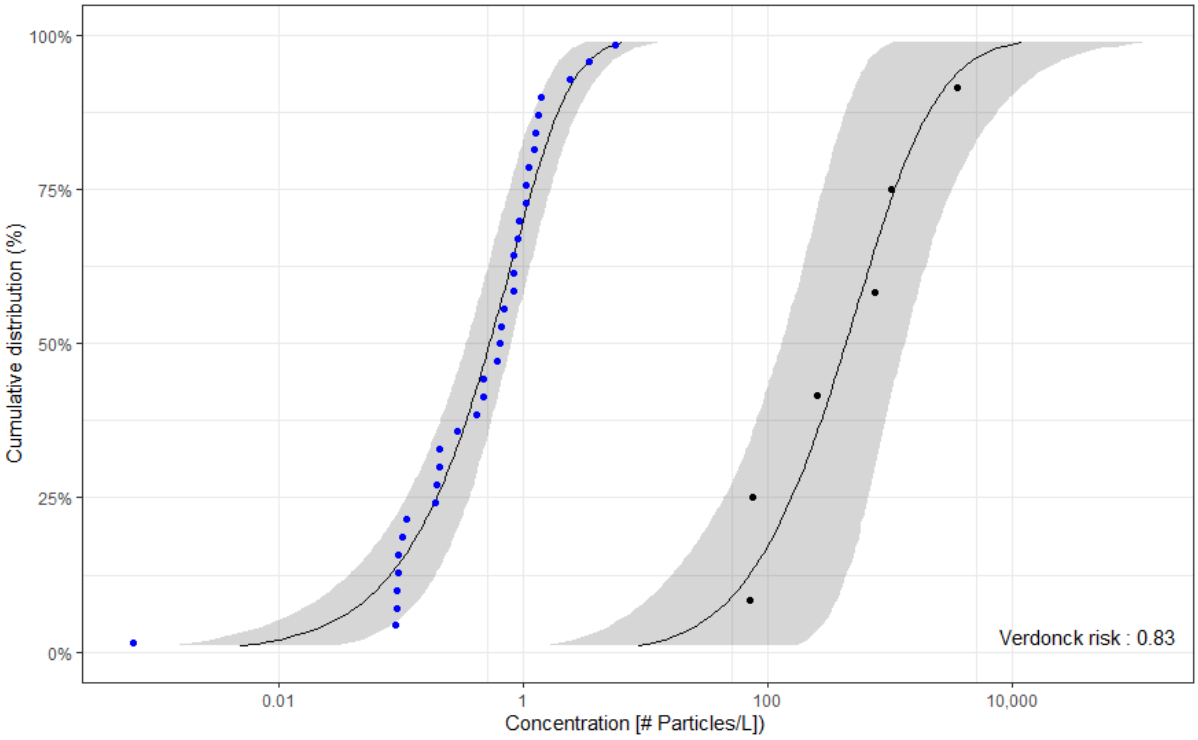

Figure 5. Cumulative distribution of PNEC data based on HC_5 data for floating microplastic in freshwater for log transformed microplastic concentration and an environmental concentration distribution (based on measured environmental microplastic concentrations (# MP.L^{-1}) in surface waters in this study. HC_5 and PNEC data are listed in Table 2. A log-normal distribution is fitted, and the labels indicate the PNEC data (black) and the environmental concentrations (blue). The actual cumulative distribution is depicted and is surrounded by a 95% confidence interval (shaded grey area) derived using 100 random parameter iterations of the lognormal distribution.

Sediment effect data are particularly scarce in the literature (for both marine and freshwater environments). This deficiency makes it impossible to determine a $PNEC_{sediment}$ through the construction of an SSD. In other words, no study is available that has determined a PNEC for the sediment using this approach (Besseling et al., 2019). Van Cauwenberghe et al. (2015a) determined a first $PNEC_{sediment}$ for marine sediment of 540 particles per kg sediment by dividing the no observed effect concentration (NOEC) value for the metabolic rate of the lugworm *Arenicola marina* (NOEC of 5.4×10^5 particles per kg) by an assessment factor of 1,000, in accordance with the ECHA guidelines (ECHA, 2003). The same $PNEC_{sediment}$ was later on

458 applied by Everaert et al. (2018) as well. However, up to now, a safe threshold value has not
459 yet been determined for freshwater sediment. Using the PNEC (540 particles kg^{-1}) determined
460 for marine sediment as a proxy for freshwater sediment, virtually every sampled location in the
461 current study (Figure 7) would be at risk for this environmental compartment, as the measured
462 microplastic concentrations are higher than the PNEC. However, according to ECHA
463 guidelines, an assessment factor of 100 is used for the freshwater environment (instead of 1,000
464 when using chronic ecotoxicity data in marine systems). This would mean that we could rescale
465 the $PNEC_{sediment}$ to 5,400 particles kg^{-1} sediment, hence the risk for each sampled location is
466 rather small, although in the Heulebeek, the RCR is close to 1 (Figure 7). Paradoxically, and in
467 contrast to most other toxicants, greatest uncertainty for microplastic research is related to the
468 availability of toxicity data for freshwater ecosystems (and therefore not the marine
469 environment, where microplastic research is much further advanced), which makes it arguable
470 that an AF of 1,000 is more appropriate here. In addition, the NOEC used to determine the
471 $PNEC_{sediment}$ is based on a marine organism. More relevant effect data is therefore urgently
472 needed for freshwater organisms to determine a relevant $PNEC_{sediment}$ for freshwater
473 ecosystems. Therefore, a risk for freshwater sediment cannot be excluded in this study.

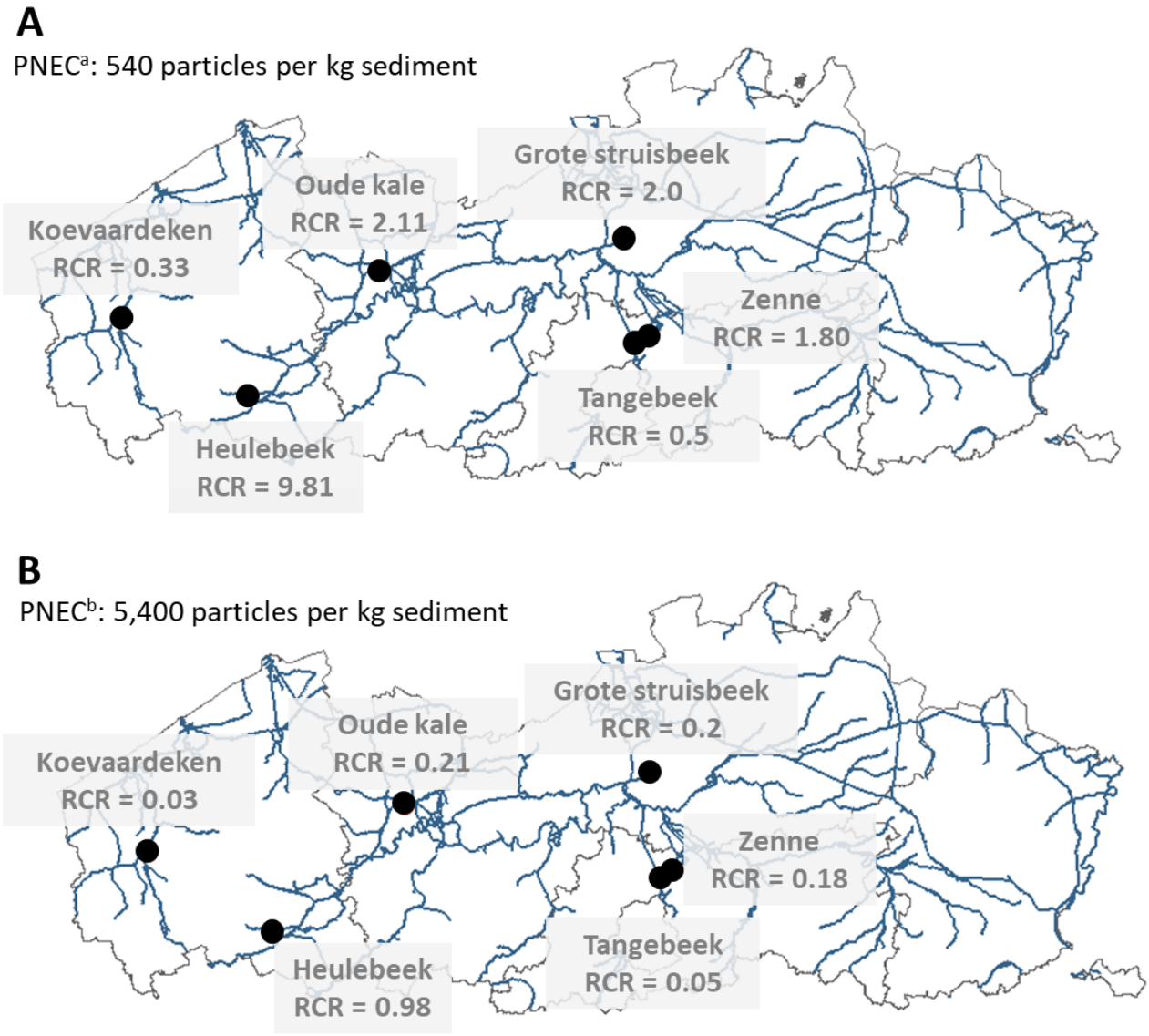

Figure 6: Calculated risks for freshwater sediment in Flanders, using A) the reported PNEC value of Van Cauwenberghe et al. (2015a) and B) the same PNEC value, but rescaled for freshwater based on ECHA guidelines. When the risk characterisation ratio (RCR) is smaller than 1, the risk is estimated to be low. When the RCR is greater than 1, there is a risk of negative effects.

Although the risk assessments in this study provide an initial indication of the state of the Flemish waterways, it is important to note that the current measurements (i.e., concentration determinations) in this study, as well as in all other studies, are limited by the detection limit of the measuring equipment used. Particles smaller than 25 µm are not/insufficiently detected in this study (due to the limitation of the available FTIR method), so our measurements are

484 underestimates of the real number of microplastics in the environment. In addition, accurate
485 PNEC values are also extremely important in order to draw up scientifically substantiated
486 quality standards that effectively protect the environment. The value of the standard stands or
487 falls with the reliability of the PNEC, as is discussed profoundly by Koelmans et al. (2017).
488 First, the types of particles used in effect tests are usually less diverse compared to those found
489 in the environment (Koelmans et al., 2020). Second, as there is a nonalignment of microplastic
490 particles (different tested sizes, shapes, polymer types, or combinations of these) used in effect
491 tests, it is challenging to compare these studies, and, ultimately, the SSDs that are based upon
492 them (cf. Table 2). Therefore, we have tried to overcome these issues by plotting a cumulative
493 distribution of the available PNEC values in literature aiming to encompasses all of these
494 differences. The current knowledge about effects of microplastics is insufficiently known to
495 make definitive statements and hence, more research is needed to define more accurate
496 threshold values (i.e., PNECs). However, by including these risk assessments in our manuscript,
497 we aim to steer the microplastic community from monitoring studies to incorporate risk
498 assessments in their future reports/studies and to focus on the effect assessment in particular.

499 ## ***Conclusion***

500 In conclusion, this study performed a broad investigation regarding the current state of
501 microplastic pollution in waterways in Flanders, a highly urbanized region in Europe. We have
502 detected relatively low microplastic concentrations (in the range of 25 - 5,000 µm) in surface
503 waters. Sediment was shown to be more heavily loaded with an average of 2,774.57
504 microplastics (25 -5,000 µm) per kg DW sediment. Nonetheless, the likely most prominent MP
505 fraction (1 - 25 µm) could not be taken into account using the applied FTIR spectroscopic
506 method. Hence, environmental concentrations can be highly underestimated, and in this study
507 nanoplastics have not even been considered yet. This also raises concerns regarding the applied
508 risk assessments. Based on the measured microplastic concentrations, the risks for negative

effects due to microplastic pollution are limited for surface water, while using limited data for sediment, risks could be identified in some of the locations. More data is urgently needed and required to increase reliability of the established PNEC values.

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Declaration of interest

No financial interests or personal relationships are known to the authors that could have influenced the work reported in this paper.

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