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# Ingestion of microplastics by meiobenthic communities in small-scale microcosm experiments

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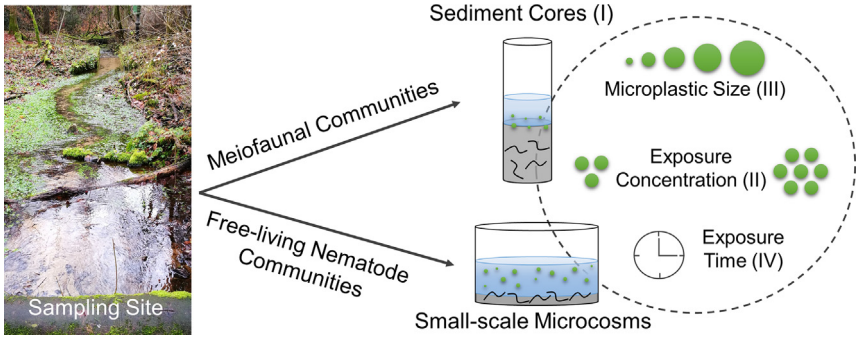
## HIGHLIGHTS

- Indigenous chironomids, copepods and nematodes readily ingested PS beads (0.5–6 μm).
- Exposure time and concentration correlated positively with PS bead body burdens.
- Sediment reduced PS bead body burdens for all investigated meiobenthic organisms.
- >30% of exposed nematodes and 56% of species ingested 1.0-μm PS beads in <24 h.
- The feeding type of the nematodes influenced PS bead body burdens.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Microplastics have been detected in many different environments. Nematodes are a rife meiofaunal taxon and occupy an important trophic position in benthic food webs. Laboratory-based ingestion experiments have demonstrated the susceptibility of single nematode species to microplastic uptake. However, the determinants of ingestion by meiofaunal assemblages, especially those of nematodes, have yet to be fully examined. We therefore conducted a microcosm study in which field-collected freshwater sediment was spiked with fluorescent polystyrene (PS) beads (1.0, 3.0 and 6.0 μm) in concentrations of $10^3$ and $10^7$ PS beads $ml^{-1}$ and the ingestion by the most dominant indigenous meiofaunal taxa (nematodes, rotifers, chironomids, copepods) was investigated after 2, 4 and 8 days using fluorescence microscopy. In additional small-scale microcosms, PS bead ingestion by nematode assemblages was quantified as a function of feeding type, exposure time (1–10 days), concentration ($10^3$, $10^5$, $10^7$ PS beads $ml^{-1}$) and bead size (0.5, 1.0, 3.0, 6.0 μm). PS beads at $10^7$ beads $ml^{-1}$ were largely ingested by chironomids and copepods. Exposure time and concentration correlated positively with PS bead ingestion for all taxa. The most relevant size class for ingestion for the majority of meiofaunal taxa was PS beads of 1.0 μm. Nematode communities, especially deposit-feeding species, effectively ingested micropastics from sediment, as >30% of the exposed individuals and 56% of the species ingested 1.0-μm PS beads in <24 h. Ingestion rates were mainly influenced by PS bead size and nematode feeding type/habit, with the exception of a bead concentration of $10^3$ beads $ml^{-1}$, at which exposure time was also an important factor. Sediment particles reduced microplastic ingestion considerably for all investigated meiobenthic organisms. Our study demonstrates the ability of free-living nematodes communities to readily ingest PS beads of various sizes. If the feeding-type distribution is known, the potential exposure of nematode communities may be predicted.



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## 1. Introduction

Microplastics (polymer particles <5 mm; Arthur et al., 2009; European Commission, 2013) entering the environment can meanwhile be found worldwide, even in remote areas. The presence of microplastics in the water and sediment phases of marine and freshwater ecosystems has been the focus of several studies (e.g., Eerkes-Medrano et al., 2015; Peeken et al., 2018). Among their findings is the transport of the polymers to the sediments of water bodies (van Cauwenberghe et al., 2015), as their specific densities are higher than that of water (>1 g $cm^{-3}$) and biofouling enhances their sedimentation (e.g., Kaiser et al., 2017; Kooi et al., 2017; Harrison et al., 2018). Consequently, microplastic densities in sediments may be 10,000-fold (Wendt-Potthoff et al., 2014) or even 600,000-fold (Scherer et al., 2020) higher than in the water column indicating that riverine sediments are sinks for microplastics (Scherer et al., 2020). The number of microplastic particles reported in aquatic environments strongly depends on the detected particle size as the size distribution increased exponentially with decreasing particle size (Scherer et al., 2020) as well as on processing and identification methods (e.g., Imhof et al., 2013; Enders et al., 2015; Ivleva et al., 2017). The paucity of information on the sediment concentrations of microplastics <20 μm (Phuong et al., 2016; Ivleva et al., 2017; Adam et al., 2019) is in part due to technical limitations (Lenz et al., 2016; Triebskorn et al., 2019). Nonetheless, it is clear that with decreasing particle size, the risk for freshwater organisms increases (Triebskorn et al., 2019), since the smallest particles are presumably those that are most easily ingested (Dris et al., 2015) at all levels of the trophic chain (Cole et al., 2013; Imhof et al., 2013). Studies have shown that microplastics of various polymers, shapes and sizes are ingested by ciliates, flagellates, rotifers, annelids, crustaceans, molluscs and fishes (reviews by Scherer et al., 2018; Adam et al., 2019; Triebskorn et al., 2019). Diverse effects on benthic invertebrates have also been described (Haegerbaeumer et al., 2019).

Nematodes are rife benthic invertebrates and abundant in freshwater, marine and terrestrial environments (Heip et al., 1990; van den Hoogen et al., 2019). In fine sediments, for example, they account for up to 90% of meiobenthic organisms (e.g., Strayer, 1985; Traunspurger, 2000; Bergtold and Traunspurger, 2005; Traunspurger et al., 2012; Majdi et al., 2017). Nematodes also represent different trophic positions while covering the entire food spectrum, feeding on detritus, bacteria, algae, fungi, and higher plants but also including omnivorous and predatory species (Yeates et al., 1993; Traunspurger et al., 1997). Hence, by linking lower and higher trophic levels they fill a key position in benthic food webs (Schmid-Araya & Schmid, 2000; Majdi and Traunspurger, 2015; Weber and Traunspurger, 2015). The most thoroughly investigated nematode species in terms of microplastic ingestion is *Caenorhabditis elegans* (Boyd et al., 2003; Fang-Yen et al., 2009; Fueser et al., 2019; Kiyama et al., 2012; Zhao et al., 2017; Lei et al., 2018) that was very susceptible to microplastic ingestion, actively ingesting microspheres ≤3.4 μm (Boyd et al., 2003; Fang-Yen et al., 2009). However, this nematode species is less relevant in natural assemblages since it is only rarely found in freshwater habitats and has been isolated only from anthropogenic habitats (e.g., rotting plant material; Kiontke and Sudhaus, 2006).

Despite the knowledge gained from single-species assays, experimental set-ups using entire communities and natural species assemblages are of higher ecological relevance. The few microcosm studies of microplastic exposure published thus far have focused on bacterial colonization and bacterial communities on microplastic biofilms (e.g. Harrison et al., 2014; Arias-Andres et al., 2018; Hossain et al., 2019), on heteroaggregation and microplastic density changes (Lagarde et al., 2016), on microplastic decay induced by earthworm gut bacteria (Huerta Lwanga et al., 2018), or on the impacts of polystyrene microplastics on the population growth of *Daphnia magna* (Aljaibachi et al., 2020). However, to the best of our knowledge, neither the community-level susceptibility of free-living nematodes to microplastic ingestion nor their factors constraining particle ingestion by natural nematode assemblages have been investigated.

Therefore, in this study, (1) the share of indigenous meiofaunal organisms able to ingest fluorescent polystyrene (PS) beads of 1.0, 3.0 and 6.0 μm in size at concentrations of $10^3$ and $10^7$ beads $ml^{-1}$ after 2, 4 and 8 days was determined in sediment-core microcosms using fluorescence microscopy. Moreover, since experiments with laboratory-cultured nematode species have shown that several species readily ingest microplastics when exposed in aqueous medium (Fueser et al., 2019), (2) the PS bead ingestion of free-living nematode communities in sediments was quantitatively determined in separate small-scale microcosms under exposure conditions (e.g. test concentration, test medium) comparable to those of the sediment-core microcosms. This second experiment allowed us to investigate the effects of feeding type/habit, microplastic size, exposure time and exposure concentration. Since dietary microplastic ingestion may also be guided by the general role of freshwater species in the food web (Scherer et al., 2017), we expected the same relationship for the taxon Nematoda, given the diverse functional roles and trophic positions of member species in the food web. We hypothesized that (1) the ingestion of PS beads is mainly related to the feeding type/habit of the nematodes but also to the size of the beads and that (2) the dietary uptake of PS beads by free-living nematode communities in small-scale microcosms would positively correlate with exposure time and PS bead concentration. Based on evidences supporting both hypotheses, we propose that the feeding-type composition of a nematode community determines the amount of dietary microplastic uptake.

## 2. Material and methods

### 2.1. Polystyrene bead suspensions

Microplastic suspensions were prepared by diluting stock suspensions of 0.5-μm (0.47 ± 0.01 μm), 1.0-μm (0.91 ± 0.01 μm), 3.0-μm (3.00 ± 0.15 μm), 6.0-μm (6.10 ± 0.24 μm) and 10.0-μm (10.10 ± 0.71 μm) PS Fluoresbrite® Yellow Green microspheres (excitation maximum: 441 nm, emission maximum: 485 nm; Polysciences Europe GmbH, Baden-Wuerttemberg, Germany) with natural stream water to achieve test concentrations of $10^7$ PS beads $ml^{-1}$ (0.5 μm: 0.69 mg $l^{-1}$; 1.0 μm: 5.49 mg $l^{-1}$; 3.0 μm: 148.81 mg $l^{-1}$; 6.0 μm: 1190.48 mg $l^{-1}$; 10.0 μm: 5494.51 mg $l^{-1}$), $10^5$ PS beads $ml^{-1}$ (1.0 μm: 0.05 mg $l^{-1}$) and $10^3$ PS beads $ml^{-1}$ (1.0 μm: $5.49 \times 10^{-4}$ mg $l^{-1}$; 3.0 μm: 0.01 mg $l^{-1}$; 6.0 μm: 0.12 mg $l^{-1}$). According to the results of preliminary ingestion experiments (Fig. S1), the final test concentration of $10^7$ PS beads $ml^{-1}$ ensured the ingestion and precise quantification of the beads. Lower concentrations were chosen to obtain more environmentally realistic exposure scenarios. Polystyrene beads were chosen since in riverine sediments, they are a common polymer and form of microplastics with high environmental concentrations at microplastic 'hot spot' sampling sites (Klein et al., 2015; Scherer et al., 2020). Test concentrations were confirmed using a hemocytometer to examine bead suspensions at 400× magnification with a fluorescence microscope (Zeiss Axio Scope.A1, Jena, Germany). The surface charge of the fluorescent PS beads was negative (zeta potential of 1.0-μm PS bead: −82.2 ± 2.2 mV; measured in 1% M9-medium at $10^7$ PS beads $ml^{-1}$; Zetasizer Nano ZS, Malvern Panalytical GmbH, Kassel, Germany). According to Hanna et al. (2018), beads do not heteroagglomerate with, e.g., negatively charged *Escherichia coli* cells.

### 2.2. Sampling and experimental setups

Field-collected freshwater sediment, including the indigenous meiofauna, and extracted nematode communities were exposed to PS beads in sediment-core and small-scale microcosm experiments, respectively, without additional food supply (Fig. S2). Note that the

egestion of the PS beads was not evaluated in this study. Inspected nematodes were morphologically identified to the species level following standard works (Andrassy, 1984; Loof, 2001; Eyualem et al., 2006) and classified into the following feeding types based on morphological characteristics: deposit feeders, epistrate feeders (mainly feeding on algae), suction feeders (omnivores or feeding on fungi, plants and roots) and chewers (omnivores or predators) (Traunspurger et al., 1997).

*2.2.1. Sediment-core microcosms (I)*

To avoid disturbances of the meiobenthic community, samples were randomly taken from shallow, eutrophic Lake Obersee (0.15 $km^2$ surface area; coordinates: N52.058556, E8.557556, Bielefeld, Germany) with 36 sediment cores, where in over 3 years of monitoring a total of 152 nematode species have been identified (Michiels and Traunspurger, 2004). All samples were collected in April of 2019 from a muddy area of about 2 $m^2$ located next to macrophytes. The sediment cores, consisting of 2 cm of the uppermost layer of sediment (11.45 $cm^3$) and 1 cm of water, were placed in microcosms constructed from 50-ml centrifuge tubes (h = 11.50 cm, d = 2.70 cm) from which the conical end (h = 1.5 cm) was cut, and the screw cap was used as the bottom. The indigenous meiobenthic community was exposed to a mixture containing equal amounts of 1.0-, 3.0- and 6.0-μm PS beads to achieve test concentrations of $10^3$ and $10^7$ PS beads $ml^{-1}$ (Fig. S2). The microcosms were incubated for 2, 4 and 8 days (five replicates for each exposure time and concentration) in the dark at 20 °C without additional air ventilation. Six additional sediment-core microcosms without PS beads were used as controls to determine the initial meiobenthic abundances, and specifically nematode density and feeding type composition, as well as mortality during the experiment and to rule out pre-contamination of the sediment with fluorescent particles. After the water column had been spiked with PS beads, the uppermost 5 mm of the sediment was gently stirred with a glass rod to simulate natural sedimentation. All sediment-core microcosms were placed in a water bath to avoid external temperature fluctuations. The indigenous nematode community was fixed with 4% formaldehyde and extracted using a density-centrifugation procedure and Ludox® HS-40 colloidal silica (Sigma-Aldrich, Taufkirchen, Germany; the specific gravity of the solution was set at 1.14 g $cm^{-3}$ by adding deionized water), following the method of Pfannkuche and Thiel (1988). The density-centrifugation procedure did not provoke an egestion of the PS beads by nematodes and the fluorescent properties and shape of the beads remained unaffected. Nematodes retained on a 30-μm mesh were transferred to a fixative containing ethanol and anhydrous glycerol that gradually replaced the water in the nematodes and fixed them (Seinhorst, 1959). Ten nematodes each were transferred in a drop of glycerol on each microscopy slide, covered with a cover slip and sealed with nail polish. Ingested PS beads were localized and body burdens were quantified in meiofaunal organisms and nematode gastrointestinal tract regions (buccal cavity, oesophagus, intestine and rectum) at 400× magnification by fluorescence microscopy with a green fluorescence protein (GFP) filter set (Zeiss Axio Scope.A1, Jena, Germany) within 8 days of exposure, because thereafter nematode mortality increased drastically due to oxygen depletion. PS bead body burdens were evaluated in the most dominant meiofaunal taxa: rotifers (88 individuals), copepods (229 individuals), chironomids (249 individuals) and nematodes (1796 individuals with 46 species).

*2.2.2. Exposure concentration microcosms (II)*

Three wells of a cell culture plate (cell growth area of each well: 9.60 $cm^2$, VWR International GmbH, Darmstadt, Germany) were filled with 1.5 g of lime-free natural gravel (grain size: 0.1–0.9 mm) and 5 ml of spring water spiked with 1.0-μm PS beads in concentrations of $10^3$, $10^5$ and $10^7$ beads $ml^{-1}$ (Fig. S2). A field-sampled nematode community comprising 35–41 individuals collected from the headwater stream Furlbach (coordinates: N51.895049, E8.715357, Augustdorf, Germany) was added in each well. After 4 days of exposure in the dark, the nematodes were taken out, washed with K-medium (3.1 g NaCl $l^{-1}$, 2.4 g KCl $l^{-1}$) to remove the beads that had adhered to their cuticle, transferred to anhydrous glycerol and mounted on microscopic slides (see 2.2.1). The ingested PS beads of 115 nematodes (19 species) were localized and body burdens were quantified with fluorescence microscopy.

*2.2.3. Polystyrene bead size microcosms (III)*

Additional wells containing a field-sampled nematode community prepared using the same setup as in experiment II were spiked with a PS bead size mixture made up equal concentrations of 0.5, 1.0, 3.0, 6.0 and 10.0-μm beads to obtain a test concentration of $10^7$ PS beads $ml^{-1}$ (Fig. S2). After 4 days of exposure in the dark, nematodes were prepared as described above and the PS bead body burdens were quantified in 156 identified nematodes (21 species) with fluorescence microscopy.

*2.2.4. Exposure time microcosms (IV)*

The wells of a cell culture plate (cell growth area of each well: 3.85 $cm^2$, VWR International GmbH, Darmstadt, Germany) were filled with a 2-mm layer of natural sandy sediment containing a field-sampled nematode community – extracted from the Furlbach (see experiment II) and filtered onto a 100-μm mesh – and 1.8 ml of spring water spiked with 1.0-μm PS beads at a test concentration of $10^7$ PS beads $ml^{-1}$ (Fig. S2). After 1, 3, 6 and 10 days of exposure in the dark at 20 °C, the nematodes were mounted on slides as described in experiment I. The experiment was only run until day 10 to minimize the effects of nematode mortality on PS bead ingestion. PS bead body burdens were quantified in 412 identified nematodes (46 species).

*2.3. Data analysis*

All data were assessed for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) but were not transformed to improve normality. A significance level of $p \leq 0.05$ was used for all comparisons. For the sediment-core microcosms (I), significant differences in PS bead body burdens were detected for the factors exposure time and exposure concentration with a two-way ANOVA (post-hoc: Holm-Sidak) and separately with a one-way ANOVA on ranks (post-hoc: Tukey) for the factor PS bead size. For the exposure concentration microcosms (II) and PS bead size microcosms (III), significant differences in PS bead body burdens were detected with a one-way ANOVA on ranks (post-hoc: Dunn). Feeding types and exposure time were compared with a two-way ANOVA (post-hoc: Holm-Sidak) and temporal differences for deposit and suction feeders (omnivore) were detected with a one-way ANOVA on ranks. Additionally, Mann-Whitney *U* tests were performed to compare the proportion of nematodes with ingested PS beads in the sediment-cores (I) and in the small-scale microcosms. The number of ingested PS beads is reported as the mean and standard error. Statistical analyses were performed, and graphic representations were provided using SigmaPlot 12.0 (Systat Software Inc.). Three-dimensional surface plots were created with OriginPro, Version 2019b (OriginLab Corporation, Northampton, MA, USA).

## 3. Results and discussion

*3.1. Sediment-core microcosms (I)*

Nematodes (50.8%) were the most abundant meiobenthic taxon of the indigenous fauna in the sampled sediment cores (Fig. S3), followed by rotifers (33.5%), crustaceans (copepods respectively; 10.2%) and chironomids (1.3%). All of these groups effectively ingested PS beads during 8 days of exposure. In general, at exposure concentrations of $10^7$ PS beads $ml^{-1}$ chironomids were very susceptible to microplastics (96% of the individuals ingested PS beads) as were copepods (85%) and to lesser extents rotifers (41%) and nematodes (4%) (Fig. 1). At

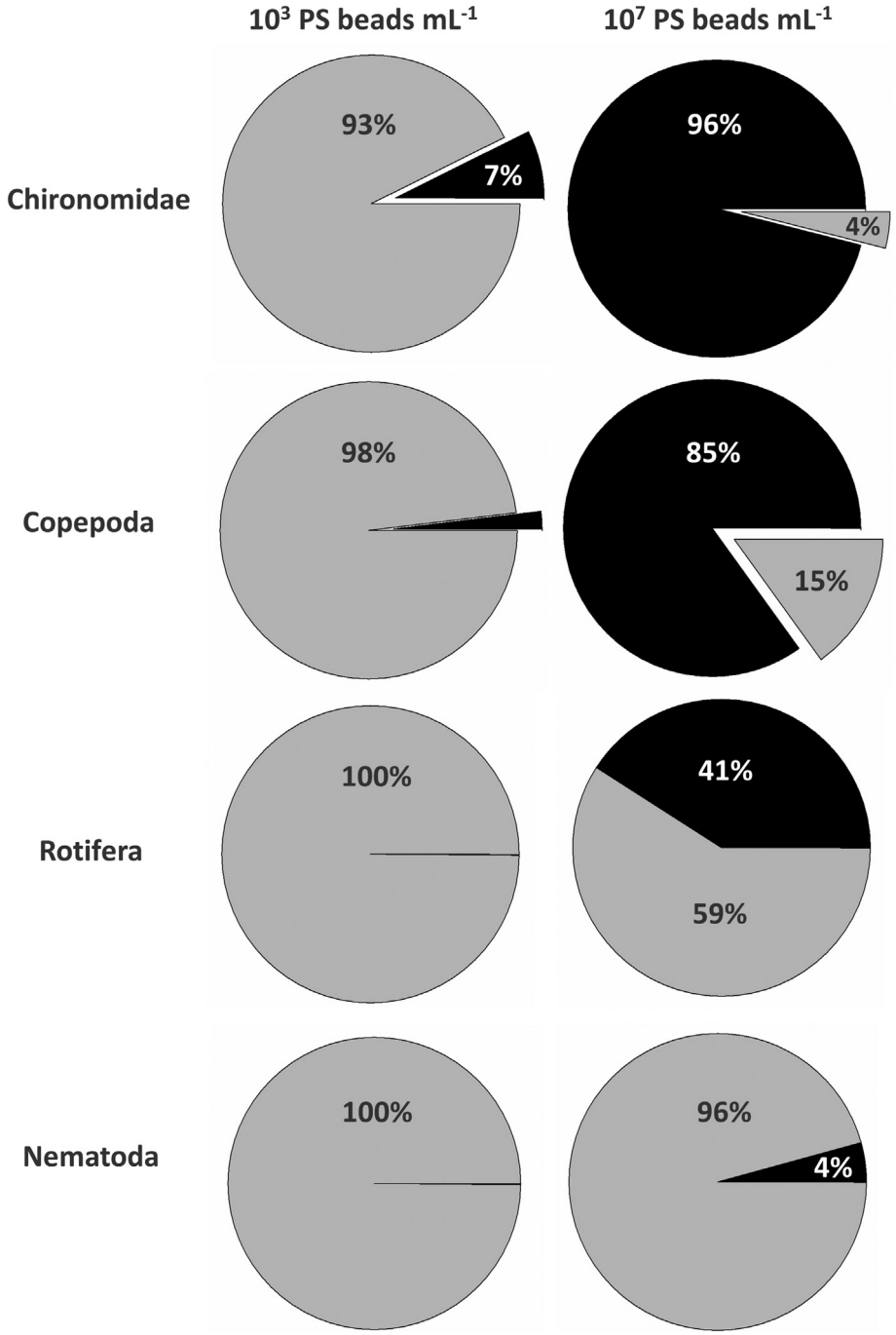


**Fig. 1.** Polystyrene (PS) bead body burdens by the four most abundant meiofaunal taxa found in the sediment-core microcosms within 8 days of exposure at two exposure concentrations ($10^3$ and $10^7$ PS beads $ml^{-1}$). PS bead body burdens were examined in 249 chironomids, 229 crustacea (mainly copepods), 88 rotifers and 1796 nematodes. Ingestion (black), no ingestion (grey). SigmaPlot 12.0 (Systat Software Inc.).

$10^3$ PS beads $ml^{-1}$ ingestion was low for all taxa. Although chironomids were the least abundant meiobenthic group in the samples (Fig. S3), 7% of the population ingested PS beads even at the lowest bead concentration (Fig. 1). The high ingestion rates of PS beads by chironomids can be explained by the generalist and therefore less selective feeding behavior of these organisms (Berg, 1995; Schmid-Araya & Schmid, 2000). Since chironomids constitute a major trophic link in aquatic systems, by serving as prey for invertebrates and fishes, the ingested PS beads may be quickly transferred to the next trophic level. The exposure time correlated positively with PS bead ingestion by chironomids, rotifers and nematodes ($F = 4.906$; $p < 0.008$), since in those taxa PS bead body burdens were highest after 8 days of exposure. At concentrations of $10^7$ PS beads $ml^{-1}$, 1.0-μm PS beads were the most frequently ingested size ($H = 35.437$; $p < 0.001$), as this size class was not limited by the size of the meiofaunal buccal cavity or head capsule. Thus, for the majority of meiofaunal individuals microplastics particles 1.0-μm in size are likely to pose the greatest potential ingestion risk.

Nematode species dominated the sediment microcosms numerically and accounted for more than half of the 1064 identified meiobenthic organisms (Fig. S3), with up to 66 nematodes per 10 $cm^3$ sediment. Nematodes were evenly distributed in the sediment columns (mean ± SD: 38 ± 13 nematodes $cm^{-3}$ sediment) and their numbers did not differ significantly between treatments and over time ($F = 0.201$, $p = 0.936$). Due to their high abundance and important ecological function,

this taxon was separately investigated for PS bead ingestion as a function of feeding type, exposure time, concentration and route in subsequent experiments (II-IV, Fig. S2).

In the sediment-core microcosms, the benthic nematode community was numerically dominated by suction feeders (50%; Fig. 2), due to the use of a 100-μm mesh for nematode extraction and the proximity of the sampling site to macrophytes, both of which favored the isolation of this feeding type. A complete list of all identified nematode species is provided in Tables S1–S5. With more than 95 individuals, *Dorylaimus stagnalis* (suction feeder, omnivore), *Mononchus tunbridgensis* (chewer, predator), *Tobrilus gracilis* (chewer, omnivore) and *Eumonhystera filiformis* (deposit feeder) were the most abundant species in the PS bead treatments. The highest PS bead body burdens were detected after 8 days in just 31 out of 1354 individuals, with a mean of 5 ± 1 PS beads per individual (maximum: 29 1.0-μm PS beads), and only in the treatments containing $10^7$ PS beads $ml^{-1}$ ($F = 7.740$; $p < 0.001$). Of the nematodes that ingested PS beads, >45% were deposit feeders and 48% were suction feeders (Fig. 2). Deposit feeders mainly ingested PS beads of 1.0–3.0 μm in size (Fig. 3A) and suction feeders almost exclusively 1.0-μm beads (Fig. 3B), but PS bead body burdens strongly depended on the exposure concentration. Without exception, the omnivorous suction-feeding species *D. stagnalis* accounted for all of the PS beads ingested by suction feeders, but only 2.5% of the *D. stagnalis* individuals ingested PS beads at all. Although previous reports concluded that suction-feeding nematodes are unable to ingest PS beads ≥0.5 μm (e.g. *Aphelenchoides* sp.; Fueser et al., 2019), PS bead ingestion by *D. stagnalis* probably occurs via its protruding odontostyle, which has an opening at the dorsal side of the anterior-most end. Using its odontostyle, the nematode can either puncture the target object or suck liquids from it (Peña-Santiago, 2006). The latter would allow the

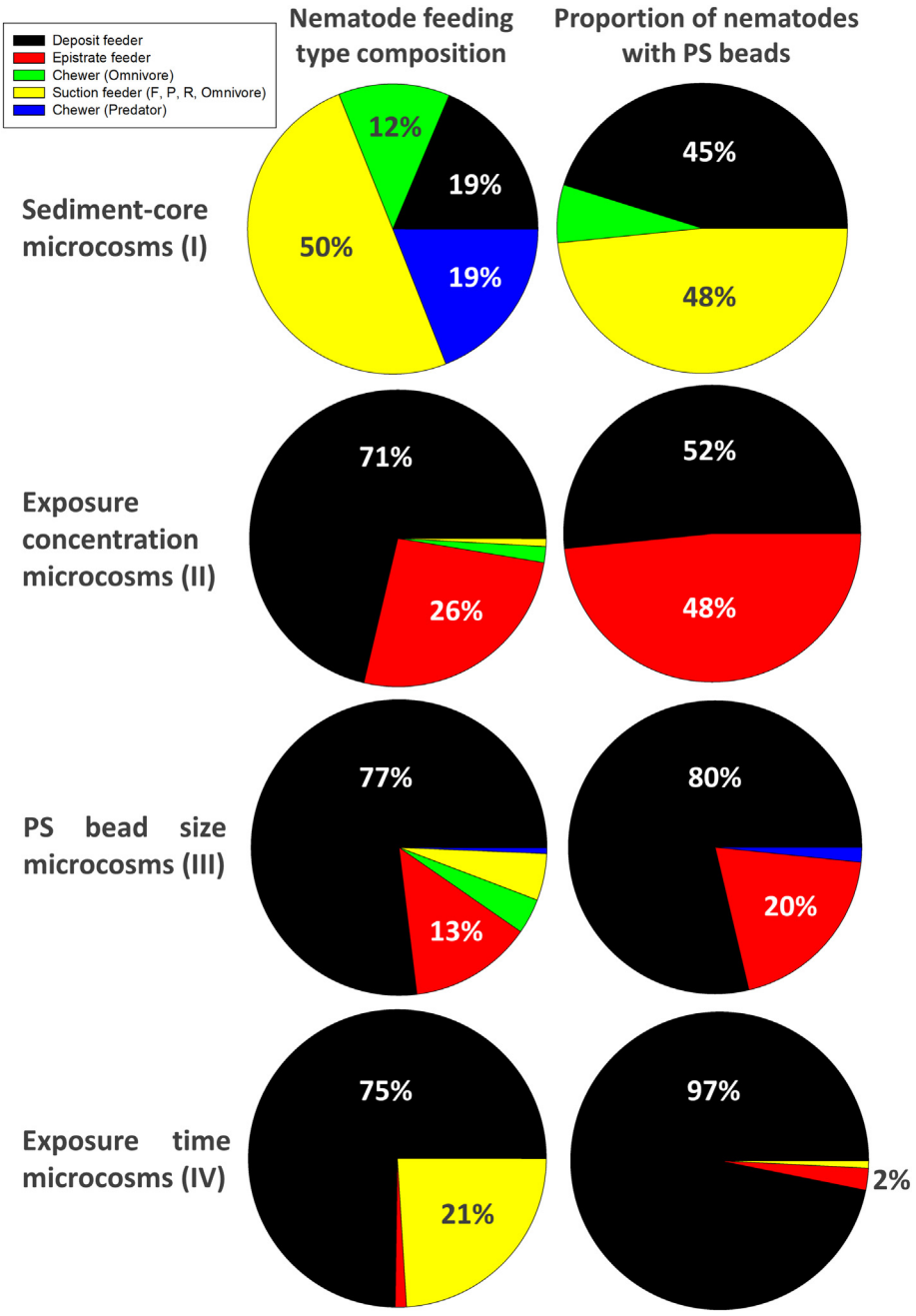


**Fig. 2.** Nematode feeding–type composition in the four microcosm experiments and polystyrene bead ingestion by the respective nematodes. F = fungi, P = plants, R = roots. SigmaPlot 12.0 (Systat Software Inc.).

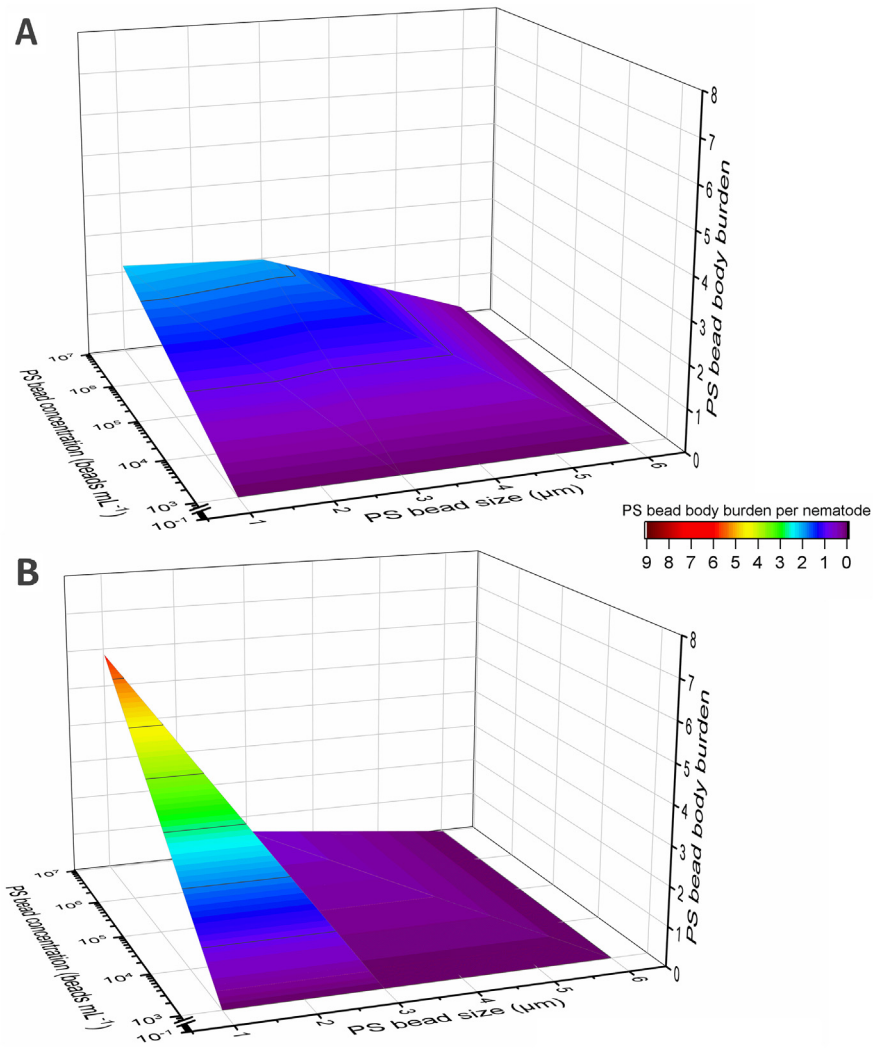


**Fig. 3.** Nematode ingestion of PS beads in the sediment-core microcosm experiment as a function of PS bead size, exposure concentration and time. PS bead ingestion of deposit–feeding (A) and suction–feeding (omnivore; B) nematode species. OriginPro, 2019b (OriginLab Corp.).

incidental ingestion of PS beads with the flow, since the diameter of the odontostyle of *D. stagnalis* is large enough to accommodate 3.0-µm PS beads. Suction-feeding (fungi, plant, roots) nematodes, e.g., *Aphelenchoides* sp., were unable to ingest PS beads, as their stomatostyle allows objects to be punctured but not the sucking of liquids.

In more realistic exposure scenarios, PS bead ingestion by nematodes is considerably constrained by natural sediment and by the microplastics exposure route, through the water and sediment column. Scherer et al. (2017) determined reduced rates of PS bead ingestion by *Chironomus riparius* and *Gammarus pulex* when sand or leaf material was included in the experimental setting. Fine sediments consist of suspended solids of small grain size that effectively reduce the encounter rate of nematodes with PS beads. Only 4% of the indigenous nematodes in the sediment cores had PS beads in the body since the exposure of nematodes below the uppermost 5 mm of sediment was presumably very low. In addition, ingestion occurred slowly since the largest number of PS beads was found in >90% of the nematodes not until day 8 of exposure ($t = 6.336$; $t = 6.784$; $p < 0.001$). For these reasons, PS beads were exclusively found in the $10^7$ PS beads ml$^{-1}$ treatment ($t = 6.503$; $p < 0.001$) of *D. stagnalis*, *Brevitobrillus stefanskii*, *T. gracilis*, *Monhystera paludicola*, *Monhystera stagnalis*, regardless of PS bead size and exposure time. In the absence of sediment, nematode species will be confronted with increased amounts of microplastics, the internal microplastic exposure will be longer, and egested microplastics will more likely be re-ingested (Scherer et al., 2017). After 8 days of PS bead exposure, in *D. stagnalis* mainly 1.0-µm beads whereas in *T. gracilis* and *M. paludicola* 3.0-µm beads but only in *B. stefanskii* 6.0-µm beads were detected. When the nematodes in small-scale microcosms with less sediment (experiments II–IV) were separately exposed to PS beads of the size class most frequently ingested in the sediment-core microcosms (1.0 µm; $H = 140.365$; $p < 0.001$), the proportion of PS bead-containing nematodes and the number of ingested PS beads per nematode were significantly higher at the same exposure concentrations ($U = 37{,}986.00$; $U = 37{,}734.50$, $p < 0.001$).

### 3.2. Exposure concentration microcosms (II)

The nematode community in the exposure concentration microcosms was dominated by deposit (71%) and epistrate (26%) feeders (Fig. 2). When 1.0-µm PS beads were provided in three exposure concentrations, PS bead body burdens increased as the concentration gradient increased, since 2% (1 out of 14 species) of the individuals exposed to $10^3$ PS beads ml$^{-1}$, 23% (6 out of 10 species) of those exposed to $10^5$ PS beads ml$^{-1}$ and 56% (7 out of 8 species) of those exposed to $10^7$ PS beads ml$^{-1}$ had 1.0-µm PS beads in the intestine after 4 days (Fig. 4). The mean number of 1.0-µm PS beads found in the intestine of the nematodes increased dramatically regardless of the species ($H = 33.357$; $p < 0.001$), from $2 \pm 1$ ($10^5$ PS beads ml$^{-1}$) to $43 \pm 12$ ($10^7$ PS beads ml$^{-1}$), when the exposure concentration was increased

by 100-fold (Fig. 4). Nonetheless, the treatments were dominated by *Achromadora ruricola*, *Eumonhystera vulgaris* (only at $10^7$ PS beads $ml^{-1}$) and *Rhabdolaimus terristris*. Even though *R. terristris* generally showed a low ingestion in the small-scale microcosms (2 out of 46 individuals), exposure concentration constrains the ingestion considerably (Fueser et al., 2019) since the encounter rates between PS beads and nematodes increased with increasing exposure concentration.

### 3.3. Polystyrene bead size microcosms (III)

The field-sampled nematode communities of the PS bead size microcosms were also dominated by deposit (77%) and epistrate (13%) feeders (Fig. 2). In contrast to the sediment-core microcosms, suction feeders (omnivores, fungi, plants, roots) and chewers (omnivores) accounted for only 2–4%. *Mononchus* was the only predatory species, but the most abundant (>10 individuals) were *Plectus aquatilis*, *R. terrestris* (both deposit feeders) and *A. ruricola* (epistrate feeder). PS beads reached the nematode gastrointestinal tract in >38% of the exposed individuals and in 62% of the identified species. PS beads of any applied size were not ingested by any suction-feeding species but were readily ingested by deposit feeders (80%; e.g., *P. aquatilis*: 27 ± 10 PS beads after 4 days) and by epistrate feeders (20%; e.g., *A. ruricola*: 41 ± 16 PS beads after 4 days; Fig. 2). PS beads of 0.5 and 1.0 μm were mainly ingested by deposit-feeding species ($H = 111.126$; $p < 0.001$) such as *E. vulgaris* (40%) and *Plectus opisthocirculus* (80%), and 3.0-μm PS beads by epistrate feeders such as *A. ruricola* (63%). The species that numerically ingested the widest range of PS bead sizes was *P. aquatilis*, with a mean of 5 ± 3 of the 0.5-μm, 21 ± 12 of the 1.0-μm and 1 ± 1 of the 3.0-μm PS beads. PS beads of 10.0 μm were not ingested by any of the tested species but in the intestine of *Mononchus* four 6.0-μm PS beads (and eight 3.0-μm, two 1.0-μm and one 0.5-μm PS beads) were detected after 4 days of exposure. Predator nematodes can ingest larger PS beads because ingestion is not limited by the size of the buccal cavity (Fueser et al., 2019), which in these species is 5–6 times wider than that of most deposit feeders. According to Scherer et al. (2017), the general role of freshwater organisms in the food web (generalist vs. specialized feeders) may determine dietary microplastic uptake. A similar dependency can be proposed for feeding types in nematode communities. In our study, generalists or deposit feeders ingested more microplastics than other feeding types ($H = 140.365$; $p < 0.001$), whereas no beads were found in more specialized carnivorous feeders. However, when microplastics enter complex aquatic food webs at low trophic levels, their indirect ingestion via prey seems likely for predators (Lambert and Wagner, 2018).

### 3.4. Exposure time microcosms (IV)

The field-sampled nematode community of the exposure time microcosms was dominated by deposit feeders (75%) and suction feeders that feed on fungi, plants and roots (21%) (Fig. 2). Other suction feeders (omnivores) and epistrate feeders accounted for only 3% and 1%, respectively. The most abundant species were: *Plectus parvus*, *Ceratoplectus armatus*, *P. opisthocirculus*, *E. vulgaris*, *Eumonhystera simplex* (all deposit feeders) as well as *Aphelenchoides* sp. and a Tylenchidae species (both suction feeders on fungi, plants, roots). Chewers were not detected in the samples. The proportions of deposit and suction feeders (fungi, plants, roots) in the microcosms remained almost constant with exposure time (76–77% and 15–22%, respectively) except on day 3. Ingested PS beads of 1.0 μm diameter were detected in the nematode gastrointestinal tract in less than 24 h and, regardless of exposure time, in >30% of the exposed individuals and 56% of the species. More than 96% of the ingested PS beads were localized in the intestine, which is the most voluminous region of the nematode gastrointestinal tract. PS beads of 1.0 μm were not ingested by suction-feeding nematodes (fungi, plants, roots) but readily by deposit feeders (97%) and epistrate feeders (2%; Fig. 2). At every sampling date, about 40% of all the ingested PS beads were ingested by deposit feeders, with the mean number of ingested PS beads reaching a maximum after 3 days and then decreasing with exposure time (Fig. 5). Deposit feeders readily ingesting PS beads belonged to the Plectidae and Monhysteridae, two of the most common nematode families present in freshwater ecosystems (Traunspurger, 2000; Michiels and Traunspurger, 2004; Traunspurger et al., 2006). A comparison of the PS bead ingestion between experiments III and IV showed that 8% more individuals and 6% more nematode species ingested PS beads in the PS bead size experiment (III). This can be explained by the use of a variety of PS bead sizes such that more nematode species/feeding types, and especially epistrate feeders (+18% increase), were capable of PS bead ingestion. Since field-sampled microplastics differ in their sizes, a mix of PS bead sizes better reflects natural conditions. However, samplings limited to several days may result in a gross

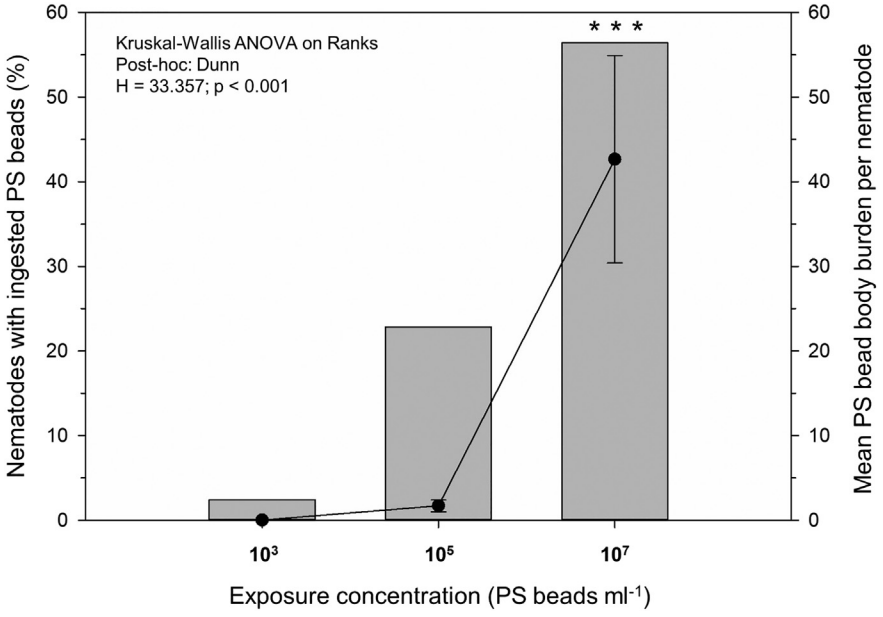


**Fig. 4.** Nematode body burdens of 1.0-μm PS beads as a function of exposure concentration. Relative and mean numbers of PS bead body burdens. Mean ± standard error. SigmaPlot 12.0 (Systat Software Inc.).

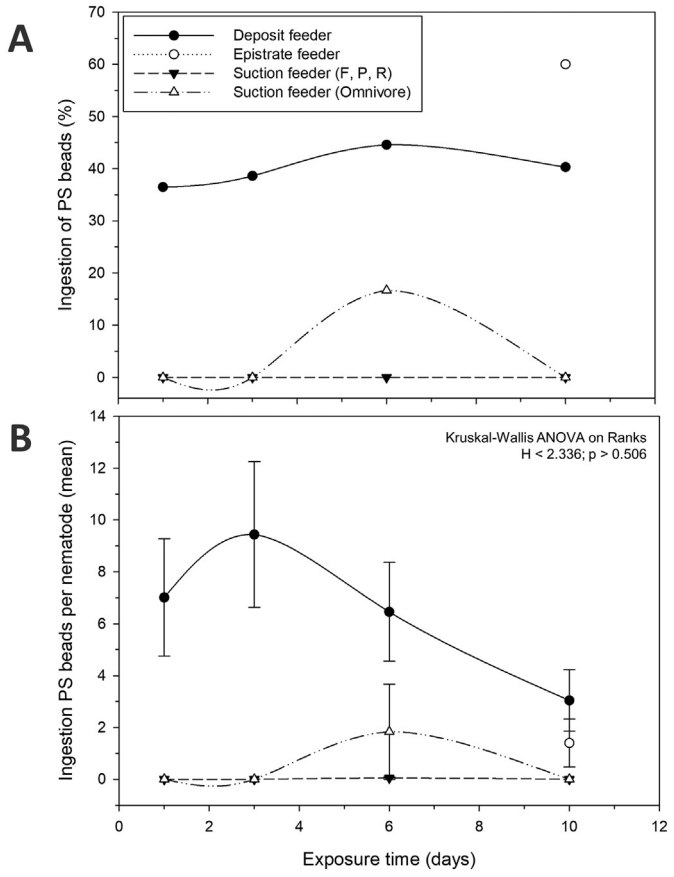


**Fig. 5.** Nematode body burdens of 1.0-μm PS beads by different feeding types as a function of exposure time. F = fungi, P = plants, R = roots. Mean ± standard error. SigmaPlot 12.0 (Systat Software Inc.).

underestimation of the real number of PS beads ingested and "digested" by meiobenthic organisms.

Our results demonstrate the capacity of free-living nematode communities to ingest PS beads of various sizes and in varying amounts within short periods of time. PS bead body burdens were detected in more than one-third of all examined nematode individuals and more than half of the nematode species, mainly deposit feeders ($H = 77.888$; $p < 0.001$), which are the dominant feeding type in most ecosystems. The quantity of PS beads in the gastrointestinal tract depended on the nematode feeding type ($F = 4.285$; $p = 0.005$), specifically, on the size and morphology of the buccal cavity, and on the availability of the microplastics. Decreasing exposure times and concentrations ($H = 33.357$; $p < 0.001$) as well as the presence of sediment particles generally reduce the microplastic ingestion whereas higher concentrations lead to a high encounter rate and thus an increased feeding rate (Scherer et al., 2017). A determination of microplastic body burdens in nematodes therefore may offer a method to assess polluted sediments. In this study, only uniformly shaped spheres were tested whereas the impact of irregularly shaped microplastic particles on feeding rates and on microplastic body burdens was not addressed. However, microplastics with different shapes (spheres, fragments, fibers, foils) will differ in their sedimentation, resulting in differences in their ingestion, feeding rate and egestion (Au et al., 2015; Ogonowski et al., 2016).

## 4. Conclusion

A prerequisite for determining the susceptibility of meiobenthic organisms to microplastic exposure in aquatic habitats is an understanding of the abiotic and biotic factors constraining the ingestion and internal exposure of microplastics. Our study showed that all dominant meiobenthic organism groups readily ingested PS beads applied in the water column during 8 days of exposure and therefore PS beads may be transferred to the next trophic level. PS bead body burdens by both laboratory-isolated and free-living nematode communities were governed by abiotic (PS bead size) as well as biotic (encounter rate, buccal cavity morphology, feeding type and habit) factors. Deposit-feeding species of nematodes (and presumably other meiobenthic taxa) readily ingested PS beads. It can therefore be assumed that the feeding-type composition of a community is an important determinant of the dietary uptake of microplastics.

## CRediT authorship contribution statement

**Hendrik Fueser:** Methodology, Supervision, Writing - original draft, Formal analysis, Writing - review & editing. **Marie-Theres Mueller:** Methodology, Supervision, Writing - original draft, Writing - review & editing. **Walter Traunspurger:** Methodology, Supervision, Resources, Writing - review & editing.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141276.

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