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Food availability is crucial for effects of 1-μm polystyrene beads on the nematode *Caenorhabditis elegans* in freshwater sediments

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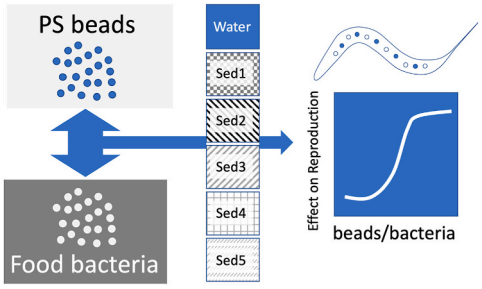
HIGHLIGHTS

- First study of the interaction between freshwater sediment, PS beads and nematodes.
- Neither PS bead ingestion, nor their effect on *C. elegans* were related to sediment type.
- Ratio of PS beads to food bacteria explains toxic effects matrix-independently.
- Food availability is a crucial mechanism for negative effects on reproduction.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater sediments represent a sink for microplastic (<5 mm) through various processes. Thus, benthic organisms can be exposed to relatively high concentrations of microplastics. Surprisingly, studies on benthic organisms are still underrepresented in the field of ecotoxicological effect assessment of microplastics. Therefore, we studied the effects of 1-μm polystyrene (PS) beads on the reproduction of the nematode *Caenorhabditis elegans* using a standardized protocol for toxicity testing in freshwater sediments (96 h; ISO 10872:2020), combined with ingestion experiments using fluorescent PS beads. To investigate the role of sediment properties (e.g., textures, organic contents) for ingestion and effects of PS beads, five different artificial and field-collected sediments were used.

Body burdens of 1-μm PS beads in the intestinal tract of the nematodes after 96 h differed between the sediments, however, differences were not significant over the whole course of the experiment. EC10 and EC50-values of 1-μm PS beads for *C. elegans*' reproduction in the various sediments ranged from 0.9 to 2.0 and 4.8 to 11.3 mg PS/g dry sediment, respectively. The ECx-values showed to be considerably higher than values reported for water exposure (EC10/50: 0.2 and 0.6 mg PS/ml, respectively), which was probably due to higher food densities in sediment compared to water exposure. Based on the PS beads/bacteria ratio, ECx-values were comparable between sediment and water exposure, suggesting that also in sediments microplastic reduces the food availability for *C. elegans* causing lower reproduction. This indirect effect mechanism was confirmed by experiments with varying food densities. Thus, the nutritious conditions might play a crucial role for the overall ecological risk of microplastics in benthic ecosystems.

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1. Introduction

Microplastics (<5 mm) are ubiquitous pollutants (e.g. Silva et al., 2018) whose risk for aquatic organisms is still under debate (Koelmans et al., 2017). Although there is evidence of toxic effects of microplastic particles on aquatic organisms (e.g. Triebskorn et al., 2019), a reliable risk assessment is hampered by inaccuracies (1) in the estimations of environmental microplastic concentrations, especially for the smallest fraction of microplastics (1–20 μm; e.g. Shim et al., 2017) and (2) in the transferability of ecotoxicity data derived from simplifying laboratory set ups to realistic field scenarios (De Ruijter et al., 2020).

Data on microplastics in freshwater sediments are scarce, both in terms of environmental concentrations (Yang et al., 2021) and ecological effects for benthic organism (Haegerbaeumer et al., 2019). Microplastic concentrations in sediments were shown to exceed those in the water phase by several orders of magnitude (e.g. Scherer et al., 2020). Sooner or later, microplastics entering the water phase of aquatic ecosystems settle, either due to a specific density >1 g/cm^3 of the polymer-type itself (e.g. polystyrene, PS; polyamide, PA) or after biofouling processes, which even force the settling of lighter polymer-types (e.g. polyethylene, PE; polypropylene, PP) (Leiser et al., 2020, 2021). Thus, it can be expected that sediment-dwelling (benthic) organisms are exposed to higher microplastic concentrations than pelagic organisms. However, toxicity data for benthic freshwater organisms are scarce, particularly studies exposing the organisms via a whole sediment matrix (Haegerbaeumer et al., 2019). Existing studies revealed a large range of effect thresholds for microplastics in sediments. For example, Redondo-Hasselerharm et al. (2018) exposed macrofauna species (amphipods, annelids, bivalves, isopods) for studying their response to PS fragments (20–500 μm) in sediment up to concentrations of 40% of the sediment (400 mg PS/g dry sediment) and observed almost no lethal and sub-lethal effects. Only the growth of *Gammerus pulex* was inhibited at high concentrations (effect concentration at which 10 or 50% of growth is inhibited = EC10/50: 1.07/3.57% of dry sediment; Redondo-Hasselerharm et al., 2018). However, other studies already observed sublethal effects on macrofauna at much lower concentrations (chironomids and PE < 54 μm: at 500 particles/kg sediment, Ziajahromi et al., 2018; bivalves and PE 125–500 μm: at 0.01 mg PE/g sediment, Bour et al., 2018).

Nematodes are important representatives of benthic ecosystems (Traunspurger, 2021), accounting for up to 90% of the meiobenthic organisms in freshwater sediments of lakes and rivers (Majdi et al., 2017; Strayer, 1985; Traunspurger, 2000; Traunspurger et al., 2012, 2020). Nematodes possess key positions in the benthic food web, on one hand, feeding on bacteria, algae, fungi, plants and other meiofaunal organisms, and on the other hand being food for microbenthic invertebrates and fish (Majdi and Traunspurger, 2015). Moreover, nematodes are suitable bioindicators for assessing the risk of environmental pollutants in benthic systems (Höss, 2021; Wilson and Kakouli-Duarte, 2009). Among nematodes, *Caenorhabditis elegans* is a commonly used model organism for assessing the toxicity of chemicals in water, sediments and soils (Haegerbaeumer et al., 2015; Höss et al., 2012; Leung et al., 2008) for which standardized test protocols exist (ASTM, 2014; ISO, 2020). The *C. elegans* toxicity test has been recommended for testing the toxicity of nanomaterials in the environment, especially in complex matrices, such as soil (Handy et al., 2012).

However, so far, there is no study that assessed the toxicity of nano- or microplastics on nematodes in sediments. Experiments showed that nematodes can rapidly ingest microplastic particles from water (Fueser et al., 2019) and sediments (Fueser et al., 2020a), and are also able to rapidly egest the ingested particles (Fueser et al., 2020b). However, the microplastic ingestion was dependent on the particle size, as well as on the feeding behavior and the size of the buccal cavity of the nematodes (Fueser et al., 2019). In aqueous medium, contrasting results are published on toxicity thresholds and mechanisms of nano- and microplastics for nematodes. PS beads of various sizes were shown to disturb the food consumption of *C. elegans* (Rauchschwalbe et al., 2021), leading to a 50% inhibition of the nematodes' reproduction over 96 h at concentrations ranging from 0.08 to 13 mg PS/ml, depending on the size of the PS beads (Mueller et al., 2020b). However, long-term microplastic exposure over several generations revealed a 100-fold higher sensitivity of *C. elegans* (approx. 5 μg PS/ml for 1-μm beads; Mueller et al., 2020a). Lei et al. (2018) found effects of microplastic particles on *C. elegans* at comparable concentrations (0.5 mg/cm^2 agar), suggesting oxidative stress and gut damage as effect mechanisms. PS nanoparticles (35 nm) induced transgenerational inhibition of brood size and gonad development of *C. elegans* already at very low concentrations (10–100 ng PS/ml; Sun et al., 2021). Only few studies addressed effects of microplastics on nematodes in soils, which might be more comparable to the sediment matrix than water. Kim et al. (2020) found inhibitory effects of PS nano- and microparticles (42 and 530 nm) on the reproduction of *C. elegans* already at 10 mg PS/kg dry soil. Prolonged exposure of *C. elegans* to tire-wear particles (average size: 125 μm) induced significant effects already at 1 mg/kg dry soil (Kim et al., 2021).

In the present study, the effects of 1-μm PS beads on *C. elegans'* reproduction after 96 h of whole sediment exposure was assessed using two formulated and three natural sediments as test matrix. In parallel, the ingestion of the PS beads by *C. elegans* was observed over a period of 96 h by exposing the nematodes to fluorescence-labeled PS beads in the respective sediments. To investigate, whether also in sediments a reduced food availability is a potential effect pathway, as shown for water exposure (Mueller et al., 2020b; Rauchschwalbe et al., 2021), in two sediments (silica sand and ISO control sediment) nematodes were exposed to 1 μm PS beads at various densities of food bacteria. With this experimental set up, we tried to answer the following questions: (1) At which sediment concentrations do 1-μm PS beads inhibit the reproduction of *C. elegans*? (2) Do ingestion and effects of 1-μm PS beads in sediment differ between the various sediments? (3) Are the effect thresholds comparable to water exposure? (4) Is food availability a potential effect pathway to explain adverse effects of PS beads on the reproduction of *C. elegans*?

2. Materials and methods

2.1. Sediments

Five different sediments were used to assess the effects of 1-μm PS beads on *C. elegans* in the sediment matrix (Table 1): two artificial sediments (Silica sand F36 [SS] supplied by Quarzwerke GmbH; Frechen, Germany; formulated sediment according to ISO 10872:2020; ISO, 2020) and three field-sampled sediments (Furlbach [FB]; Steekputbeek [SB] sampled and provided by University of Antwerp; Stockalper Channel [SC] provided by Ecotox Center, Lausanne, Switzerland; SC sediment was part of an ecotoxicological project, Beauvais et al., 2020). Sediments differed in terms of organic carbon content and grain size distribution (Table 1). All sediments showed no (formulated sediments: SS, ISO) or low chemical contamination. Regarding the concentrations of 33 priority toxicants (arsenic and metals [Cu, Cd, Cr, Hg, Ni, Pb, Zn], 16 PAHs, 7 PCBs, DDD/DDE) in the sediments and their toxic potential according to sediment quality guideline (threshold effect concentrations [TEC]; de Deckere et al., 2011) no toxic effects of the field-sampled sediments on invertebrates were expected (Table 1).

2.2. 1-μm PS bead stock suspensions

Stock suspensions of fluorescent and non-fluorescent polystyrene (PS) beads (diameter: 1 μm) were purchased from Polysciences Europe GmbH (Hirschberg, Germany; Fluoresbrite® yellow-green microspheres: cat.# 17154; Polybead® microspheres: cat.# 07310). Measured bead size (measured with ImageJ [Fiji, 1.50e] from image: 1000 × magnification; Zeiss Axio Scope.A1, Jena) and concentration in the stock suspension corresponded well with the values reported by the

manufacturer (diameter: 0.97 ± 0.003 μm; PS beads per volume: 4.1 × 10^{10} [± 9.1 × 10^{6}] PS beads/ml vs. reported: 4.55 × 10^{10} PS beads/ml; beads were counted in defined 1:1000 dilutions in a hemocytometer [Neubauer improved; BRAND GMBH + CO KG, Wertheim, Germany]; see Mueller et al., 2020b). The measured zeta potential was −82.18 (±2.16) and −76.24 (±0.87) mV at 10^{7} and -79.26 (±2.84) and −80.06 (±2.11) mV at 10^{9} PS beads/ml for fluorescent and non-fluorescent 1 μm PS beads, respectively, using a Zetasizer Nano ZS (Malvern Panalytical) (see Mueller et al., 2020b). The stock suspensions of the PS-beads contained residues of the tenside SDS (sodium dodecyl sulfate; max. 2%), which showed no toxic effects on the reproduction of *C. elegans* in toxicity test with the aqueous phase of the particle suspension (Mueller et al., 2020b). Also traces of the monomer styrene (<1 μg/ml) in the stock solution could be excluded as toxic agent in the test suspensions in a previous study (Mueller et al., 2020b).

Dilutions of 1-μm PS bead suspensions were prepared with demineralized water for three different experiments to achieve the appropriate nominal concentrations: (1) Ingestion experiment; (2) Dose-response toxicity tests in five different sediments at constant densities of food bacteria; (3) Toxicity tests in two different sediments with varying densities of food bacteria.

For the ingestion experiment, the fluorescent PS beads were diluted to a concentration of 0.022 mg PS/ml (4 × 10^{7} beads/ml). After 1:1 dilution with a suspension of food bacteria (*Escherichia coli* strain OP50; 8000 FAU (turbidity unit) corresponding to approx. 1.6 × 10^{10} bacterial cells/ml) in M9-medium (Na_2HPO_4: 6 g/l; KH_2PO_4: 3 g/l; NaCl: 5 g/l; $MgSO_4$ $7H_2O$: 0.25 g/l; Brenner, 1974), the actual test suspension contained 0.011 mg PS/ml (2 × 10^{7} beads/ml), with a bacterial density of 4000 FAU (approx. 8 × 10^{9} bacterial cells/ml). This nominal PS bead concentration turned out to be suitable for ingestion studies with 1-μm PS beads and nematodes in water and sediments, as a reliable ingestion of PS beads and a precise quantification of bead body burdens can be ensured (Fueser et al., 2019, 2020a).

For the dose-response toxicity experiment, a concentration series was prepared for the PS beads: 1.37, 2.75, 5.50, 11.0, 22.0 mg PS/ml (corresponding to 2.5, 5, 10, 20, 40 × 10^{9} beads/ml). After 1:1 dilution with a suspension of food bacteria (*E. coli*; 8000 FAU corresponding to approx. 1.6 × 10^{10} bacterial cells/ml), the actual test suspensions contained nominal concentrations of 0.69, 1.37, 2.75, 5.50, 11.0 mg PS/ml (1.25, 2.5, 5, 10, 20 × 10^{9} beads/ml) with a bacterial density of 4000 FAU (approx. 8 × 10^{9} bacterial cells/ml).

For the toxicity experiment with varying food densities, two nominal concentration steps were prepared for the PS beads: 2.2 and 8.8 mg PS/ml (4 and 16 × 10^{9} beads/ml; corresponding to concentrations inducing medium to high effects on *C. elegans*' reproduction at standard food densities). After 1:1 dilution with a suspension of food bacteria with varying bacterial densities (2,000, 4,000, 6000 and 8000 FAU corresponding to approx. 4 × 10^{9}, 8 × 10^{9}, 1.2 × 10^{10}, 1.6 × 10^{10} bacterial cells/ml), the actual test suspensions contained nominal concentrations of 1.1 and 4.4 mg PS/ml (2 and 8 × 10^{9} beads/ml), with bacterial densities of 1,000, 2,000, 3000 and 4000 FAU (approx. 2, 4, 6 and 8 × 10^{9} bacterial cells/ml).

2.3. Sediment spiking with 1-μm PS beads

The sediments SS, ISO, SB and SC had to be moistened with M9-medium as they were available as dry substrates (SS, ISO) or as they had been air-dried for better storability (SB, SC). Moistening took place in the test vials (24- and 12-well multidishes for toxicity and ingestion experiments, respectively; Greiner Bio-One GmbH, Frickenhausen, Germany) by mixing 0.15 and 0.3 g dry sediment with 0.1 and 0.2 ml M9-medium for toxicity and ingestion experiments, respectively (40% water content; according to ISO 10872:2020; ISO, 2020). FB sediment had been freshly sampled and contained 27% water (73% dry weight). For FB, 0.25 and 0.5 g fresh sediment was weighed into the test vials for toxicity and ingestion experiments, respectively. Test vials with sediments were stored at 4–8 °C for one day before further use.

After thoroughly mixing PS beads and bacteria, 0.5 ml from the respective bead-bacteria-suspension (or water-bacteria suspensions for the controls) were transferred into the test vials containing 0.5 g of wet sediments, achieving final nominal sediment concentrations of 0.018 mg 1-μm PS beads/g dry sediment (FB: 0.015 mg 1-μm PS beads/g dry sediment) for the ingestion experiment. For the dose-response toxicity experiment, 0.25 ml from the respective bead-bacteria-suspension (or water-bacteria suspensions for the controls) were transferred into the test vials containing 0.25 g of wet sediments, achieving final nominal sediment concentrations of 1.1, 2.3, 4.6, 9.2 and 18.3 mg 1-μm PS beads/g dry sediment (FB: 0.9, 1.9, 3.8, 7.5, 15.1 mg 1-μm PS beads/g dry sediment) for the toxicity tests. For the toxicity tests with varying food densities (SS and ISO, only) final nominal sediment concentrations were 1.8 and 7.3 mg 1-μm PS beads/g dry sediment. Sediments, PS beads and bacteria were thoroughly mixed using a small spatula.

2.4. Culturing of test organisms

Stock cultures of the test organisms *Caenorhabditis elegans* (Maupas, 1900; wild-type, N2 strain) were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA).

Table 1
Description of sediments spiked with 1-μm PS beads; mean TEC-Q: mean quotients of measured sediment concentrations for priority chemicals by its respective sediment quality guidelines (n.c. = not calculated); information taken from following sources: SS: supplier information: https://www.quarzwerke.com; ISO: ISO (2020); FB: Brüchner-Hüttemann et al. (2021); SB: personal communication Raf Elst, Flanders Environment Agency, Alst, Belgium; SC: Beauvais et al. (2020).

Parameter		SS	ISO	FB[a]	SB	SC
		Silica sand	ISO 10872	Furlbach	Steekputbeek	Stockalper Channel
River Coordinates		artificial sediments		51.895223° N; 8.715505° E	50.723347° N; 4.284685° E	46.25627° N; 6.97728° E
Total organic carbon (TOC)	%	<0.1	2.10	<0.1	1.65[b]	2.72
Fine fraction (< 63 μm)	%	1	59	0.4	39.8	49.5
Clay (< 2 μm)	%		4.5		4.2	0.4
Silt (2–63 μm)	%		54.5		35.6	49.1
Sand (63–2000 μm)	%	99	41	99.5	60.2	50.5
fine (63–250 μm)	%	97	41[c]	26.0		43.1
medium (250–500 μm)	%	2		71.8		6.7
coarse (500–2000 μm)	%	0		1.6		0.7
mean TEC-Q[d]		n.c.	n.c.	0.21	1.2	1.1

[a] Deviating grain size classes: fine: <60 μm; sand: 60–2000 μm; fine sand: 60–200 μm; medium sand: 200–600 μm; coarse sand: 600–2000 μm.
[b] Measured as % organic matter (3.3%), assuming a 50% carbon content.
[c] Sand fraction consists of fine to medium sand (<400 μm).
[d] Based on sediment quality guidelines set up by De Deckere et al., (2011) (Consensus 1); formulated sediments were assumed to be not contaminated and thus not analyzed for toxic chemicals.

Caenorhabditis elegans was maintained as "dauer larvae" on nematode growth agar (17 g agar/l, 2.5 g peptone/l, 3 g NaCl/l supplemented with 1 ml of 1 M $CaCl_2$, 1 ml of 1 M $MgSO_4$, 25 ml of 1 M KH_2PO_4 buffer pH 6 and 1 ml of cholesterol solution (5 mg/ml in ethanol)) according to standard procedures (Stiernagle, 1999). Providing a culture of dauer larvae with food (by placing a piece of agar with "dauer larvae" on an agar plate with bacterial lawn of *E. coli* OP50), the larvae continue their development to adult hermaphrodites, producing sufficient offspring within 3 days that can be used as test organism (ISO 10872:2020; ISO, 2020).

2.5. Ingestion experiment

The nematodes were exposed to the PS bead-spiked sediments in 12-well multidishes (Greiner Bio-One GmbH; Frickenhausen, Germany). 50 *C. elegans* of the first juvenile stage (J1), taken from synchronized cultures (after filtration of age-mixed cultures through a 5-μm filter: 280–315 μm body length; n = 30), were transferred to each test well containing the spiked sediment using a micropipette. For each sediment 24 replicates have been set up, so that three replicates could be analyzed at eight time points (6, 12, 24, 36, 48, 60, 72, 96 h) and incubated at 20 °C in the dark. At each time point, three replicates of each sediment were removed from the incubator and nematodes were separated from the sediments using the silica-gel method according to ISO 10872:2020 (ISO, 2020) using Ludox TM50® (Sigma-Aldrich) adjusted with water to 1.13 g/ml. After separating the nematodes, at least 20 individuals of each replicate were manually transferred into a 2-ml vial containing a 4% formalin solution in order to kill and preserve the nematodes. For the fluorescence microscopic analysis of ingested PS beads, 7–20 individuals (on average 17 ± 4) were mounted on a microscopic slide and fluorescent PS beads in the intestinal tract of *C. elegans* were quantified at 400 × magnification using a fluorescence microscope (Zeiss Axio Scope.A1, Jena, Germany) according to Fueser et al. (2019). The beads in several gastro-intestinal tract regions (buccal cavity, oesophagus, terminal bulb, intestine and rectum) were quantified separately.

2.6. Dose-response toxicity test

The dose-response toxicity tests were carried out according to standard procedures (ISO 10872:2020; ISO, 2020). Briefly, the nematodes were exposed to the PS-spiked sediments in 24-well multidishes (see 2.3). Five J1-*C. elegans*, taken from synchronized cultures (after filtration of age-mixed cultures through a 5-μm filter: 300 ± 50 μm body length; n = 30), were transferred to each test well using a micropipette. During the test, *C. elegans* was fed with bacteria (*E. coli*, OP50; 1.10–1.33 × 10^{10} bacterial cells/g dry sediment). For each treatment (sediment, concentration step), four replicates were set up. After 96 h of exposure, the nematodes were heat-killed by placing the multidishes for 10 min in a drying oven (85 °C) after staining the test organisms with Rose Bengal for better recovery and easier counting. The multidishes were stored at 4–8 °C until further analysis.

Nematodes were separated from the sediments using the silica-gel method as described above (2.5). To determine nematode reproduction, the offspring produced by the test organisms during 96 h in each replicate was counted under a stereomicroscope at 40 × magnification (Leica L2) and divided by the number of introduced test organism (reproduction = offspring/5 test organism).

2.7. Toxicity tests with varying densities of food bacteria

For two sediments (SS, ISO), toxicity experiments with only two concentrations of PS beads but with varying densities of food bacteria were carried out to see if a decrease of bacterial density at constant PS bead concentration levels has the same effect than an increase of PS bead concentrations at constant food levels. The test was carried out exactly as described above (2.6). Also here, for each treatment (sediment, concentration step, food density), four replicates were set up. However, suspension of food bacteria with varying bacterial densities had been prepared (2,000, 4,000, 6000 and 8000 FAU corresponding to approx. 4 × 10^9, 8 × 10^9, 1.2 × 10^{10}, 1.6 × 10^{10} bacterial cells/ml), so that during the test, *C. elegans* was exposed to 0.14, 0.28, 0.55 and 11.0 × 10^{10} bacterial cells/g dry sediment.

2.8. Data analysis

For each treatment, inhibition of reproduction expressed as a percentage (% IR) was calculated by using equation (1):

$$\%IR = 100 - R_X/R_C \times 100 \quad (1)$$

where R_X and R_C are the reproduction values for treatment X and the negative control C, respectively. To create dose-response curves for each sediment, the % IR per test sample were plotted against the corresponding concentrations from that sample, based on total PS bead sediment concentration (mg PS beads/g dry sediment), PS bead porewater concentrations (mg PS beads/ml porewater) and PS beads to bacteria ratios (number of PS beads/number of bacteria).

Dose-response curves were fitted to the effect data plotted against sediment concentration, porewater concentration and beads/bacteria ratios using a sigmoidal, 3-parameter logistic model (Sigma Plot; Systat Software Inc.). Based on the exported parameters of the fitted curves, effect concentrations were determined at test concentrations where 10 and 50% effect occurred (EC10 and EC50, respectively). Standard errors for ECx values were determined by Monte Carlo simulations (n = 1000) based on the standard errors of the parameters a, b, and x0.

A one-way ANOVA with suitable posthoc test for data with (Tukey) and without (Games-Howell) homogeneity of variances (Levene's Test) was performed to identify significant differences of 1-μm PS bead body burdens in the intestinal tract between the various sediments. A two-way ANOVA was used to test for significant difference of body burdens between the different sediments over the course of the experiment.

To test if two ECx-values were significantly different from each other, the Z-test was applied (Canada and Environment Canada, 2005) using equation (2):

$$Z = \frac{logECx_A - logECx_B}{\sqrt{\sigma^2_{logECx\_A} + \sigma^2_{logECx\_B}}} \quad (2)$$

where ECx_A and ECx_B are effect concentrations derived for sediment A and B, respectively, and σ represents the standard error. A difference can be regarded as significant if |Z| >1.645 (one-tailed test) or |Z| >1.96 (two-tailed test) (Canada and Environment Canada, 2005).

3. Results and discussion

3.1. Ingestion of 1-μm PS beads by C. elegans from sediments

After 12 h PS beads already sporadically occurred in the intestinal tract of *C. elegans* in all sediments (mean number <1), but a substantial ingestion of PS beads started between 12 h and 24 h (Fig. 1). After 12 h of exposure, probably not all of the juvenile nematodes had reached a sufficient size enabling them to ingest the 1-μm PS beads. Fueser et al. (2019) showed that the ingestion of PS beads is limited by the ratio of the size of the nematode's buccal cavity and the PS bead diameter, with no ingestion at ratios <1.3. As the buccal cavity of *C. elegans'* freshly hatched juveniles is 1.3 (±0.1) μm and only slowly grows to 1.7 (±0.2) μm after 24 h (Mueller et al., 2020b), the PS bead ingestion within the first 12 h was mainly related to nematodes growth in the various sediments. After 96 h of exposure to 1-μm PS beads in the various sediments, 15 (±5) to 117 (±18) PS beads have been detected in the intestinal tract of *C. elegans*, which agrees well with values reported for bacterial feeding freshwater nematodes that had been exposed to 1-μm PS beads in sediments of small-scale microcosms at a comparable exposure

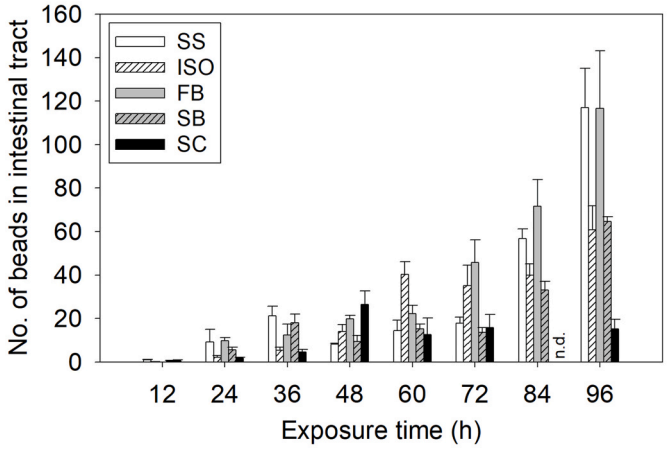

Fig. 1. Number (mean ± standard deviation; n = 3) of 1-µm beads counted in the intestinal tract of each individual of *C. elegans* (body burden) after 12–96 h of exposure in various sediments; n.d. = not determined; for sediment acronyms see Table 1.

concentration and time (43 ± 12 PS beads; Fueser et al., 2020a).

Within the first 36 h, nematodes in ISO and SC contained significantly lower numbers of beads than those in FB (24 h; $p = 0.012$; $F = 5.568$) and to SS and SB (36 h; $p = 0.001$; $F = 12.932$). Towards the end of exposure (84 and 96 h), a clear ranking of body burdens occurred (SS = FB > ISO = SB > SC; Fig. 1), with *C. elegans* showing significantly lower body burdens in SB compared to SS and FB (84 h; $p = 0.001$, $F = 16.815$), and SC containing significantly less beads than all other sediments (96 h; $p < 0.001$, $F = 23.720$). However, if the quantity of ingested beads is related to observed inhibitory effects on the nematodes' reproduction, might not be predicted from conditions at single time points during the exposure, but regarding the whole exposure period. A two-way ANOVA (fixed factor: sediment; random factor: time) revealed that time was a highly significant factor ($p < 0.001$; $F = 10.372$), but considering the whole course of the experiment no significant difference occurred between the sediments ($p = 0.100$; $F = 2.165$). However, the interaction of time and sediment was significant (time*-treatment: $p < 0.001$; $F = 17.244$), indicating a time-dependent sediment effect. At single sampling dates, significant differences of body burdens occurred between various sediments, however, the ranking fluctuated within the course of the experiment (Fig. 1). Therefore, we have no evidence that effects of the 1-µm PS beads on the reproduction of *C. elegans* were due to any differences of ingestion behavior in the various sediments.

The beads only occurred in the lumen of the intestinal tract of *C. elegans* and no beads could be observed in the nematodes' tissue. In all sediments and time points, the majority of beads occurred in the intestine (SS: 76–96%; ISO: 0–8%; FB: 2–13%; SB: 83–96%; SC: 78–91%), however, also in the oesophagus (SS: 3–17%; ISO: 89–98%; FB: 0–8%; SB: 3–12%; SC: 0–8%) and rectum (SS: 0–10%; ISO: 0–8%; FB: 85–98%; SB: 0–6%; SC: 2–11%). Thus, the translocation of 1–µm PS beads within the intestinal tract of *C. elegans* was similar in all sediment types, and well comparable to an ingestion study, where *C. elegans* was exposed to 1-µm beads in water (oesophagus: 4%; intestine: 86%; rectum: 10%; Fueser et al., 2019).

3.2. Effects of 1-µm PS beads on C. elegans' reproduction in sediments

In all sediments, the 1-µm PS beads caused dose-dependent inhibitory effects on the reproduction of *C. elegans* (Fig. 2). Significant dose-response curves could be fitted to the effect data that had been plotted against the sediment dry weight (mg PS/g; Fig. 2a) and the ratio of PS beads to bacteria (Fig. 2b). Based on sediment concentrations of the PS beads, ECx-values slightly differed between the various sediments, however, not more than by a factor of two between the minimum and the maximum value (Table 2; differences were not significant:$|Z| < 1.645$). Compared to water exposure (based on aqueous concentrations: mg PS/ml; Mueller et al., 2020b), nematodes responded to considerably higher concentrations when exposed to sediments (Fig. 2a), with significantly higher EC50-values in sediments compared to water (Table 2; $|Z| > 1.645$). For the EC10-value, the differences of sediment to water exposure were less pronounced, however still significant for FB and SB sediments (Table 2; $|Z| > 1.645$). Based on the ratio of PS beads to bacteria, the dose-response curves of the sediments moved very close to the curve of the water exposure, so that no difference occurred between the various EC10- and EC50-values derived after sediment (ratios: 0.12–0.30 and 0.83–1.50, respectively) and water exposure (ratio: 0.35 and 1.1; Table 2; $|Z| < 1.645$).

Existing sediment toxicity data for benthic invertebrates show a very large range of effect concentrations. Redondo-Hasselerharm et al. (2018) showed that several benthic invertebrates (*Asellus aquaticus, Sphaerium corneum, Hyalella azteca, Tubifex* spp., *Lumbriculus variegatus*) were not affected by 20–500 µm sized PS fragments at sediment concentrations up to 400 mg PS/g dry sediment. *Gammerus pulex*, however, responded with a 10 and 50% inhibited growth at 107 and 357 mg PS/g dry sediment (Redondo-Hasselerharm et al., 2018), respectively, which are about 100-fold higher ECx-values than observed for *C. elegans* in the present study. In contrast to these results, Ziajahromi et al. (2018) reported significant inhibitory effects of PE particles of various sizes (1–126 µm) on survival, growth and emergence of *Chironomus tepperi* already at 500 PE particles/kg sediment, with 10–27-µm sized PE particles being most effective. Assuming an average size of 18.5 µm, 500 PE particles have a total mass of 1.5 µg. Thus, the chironomids were affected at a sediment concentration of 1.5 ng PE particles/g (Ziajahromi et al., 2018), which is six orders of magnitude lower than observed for the nematodes in the present study. In turn, in another experimental set up with *Chironomus riparius* and PET fibers, the chironomids were not affected up to 50,000 fibers/kg sediment (Setyorini et al., 2021). Thus, it is obviously not possible to set up generally valid toxicity thresholds for microplastics in freshwater sediments. However, this is not surprising, due to the large heterogeneity of investigated microplastics in terms of size, polymer-type and shape. Unfortunately, still, too few studies on microplastic toxicity (especially those producing dose-respose curves) in freshwater sediments are available to apply more elaborate risk assessment approaches (Koelmans et al., 2020; Kooi and Koelmans, 2019).

Environmental concentrations of microplastics in sediments (125–5000 µm) were found to be several orders of magnitudes higher than water concentrations (e.g., on average 600,000-times for the river Elbe; Scherer et al., 2020). Fine sediments (20–125 µm fraction) collected from the river Elbe showed average and maximal concentrations of 119 and 482 g microplastics/m^3 sediment, respectively. Roughly assuming a water content of 50% and volume/mass conversion factor of 1, maximal concentrations of approximately 1 g microplastics/kg dry sediment can be expected, which matches quite well the low effect concentrations found for the nematodes in the present study (lowest EC10-value: 0.9 g/kg dry sediment). Although, the detected mass of sediment-resident microplastics was reported for larger plastic particles contributing more to the total mass of microplastics (125–5000 µm; Scherer et al., 2020) than used in the present study (1 µm), effects on benthic organism cannot be completely excluded under a worst-case scenario. However, it has to be kept in mind that even in sediments with low clay and organic contents (≤1%), 10-times higher concentrations of naturally occurring clay and organic particles can be expected. The fact that also the content of fine sediment microparticles can affect growth and reproduction of *C. elegans* (Franzen et al., 2011; Höss et al., 1999, 2010) should be carefully considered when evaluating the ecological risk of microplastics.

The effect concentrations of the PS beads derived in this study for the various sediments, in which the nematodes had been exposed, were not related to any of the measured sediment properties. This is in contrast to

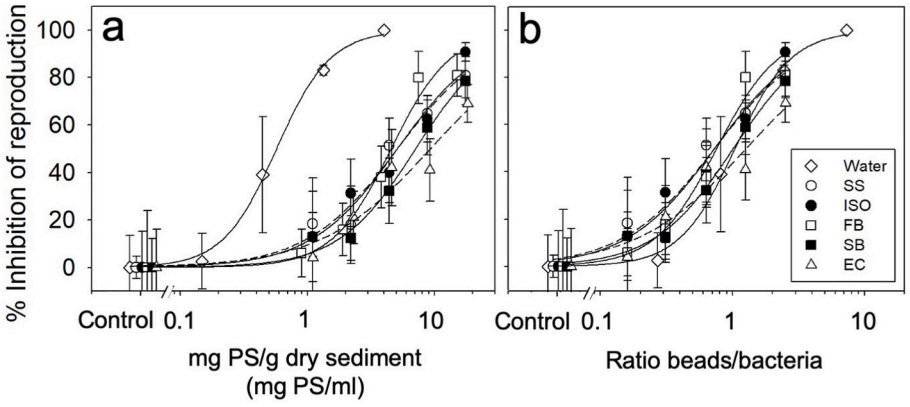

Fig. 2. % Inhibition of reproduction (mean ± standard deviation; n = 4) of *C. elegans* exposed to various sediments (for abbreviations see Table 1) and water spiked with 1-µm PS beads plotted against the PS bead concentrations based on (a) sediment dry weight and (b) the ratio of PS beads/bacteria; statistics for logistic functions (for a and b): water: p = 0.0025; F = 404.3; predicted residual error sum of squares (PRESS): 1019; r^2 = 0.998; SS: p = 0.0043; F = 54.8; PRESS: 654; r^2 = 0.973; ISO: p = 0.0048; F = 51.3; PRESS: 9802; r^2 = 0.972; FB: p = 0.0042; F = 56.0; PRESS: 25265; r^2 = 0.974; SB: p = 0.0013; F = 123.7; PRESS: 171; r^2 = 0.988; SC: p = 0.0148; F = 23.3; PRESS: 4574; r^2 = 0.940; data for water exposure were taken from Mueller et al. (2020b).

Table 2
Effect concentrations (EC10 and EC50-values; mean ± standard error) based on nominal sediment dry weight (mg PS/g dry sediment) or water concentrations (mg PS/ml) and the ratio of PS beads to bacteria for effects of 1-µm PS beads on the reproduction of *C. elegans* after 96 h exposure in various sediments (for abbreviations see Table 1) and water[a]; different capital letters indicate significant differences within each column (|Z| <1.645).

Sediment	EC10	EC50	EC10	EC50
	mg PS/g dry sed (mg PS/ml)		ratio beads/bacteria	
SS	0.87 (±0.72)^{AB}	6.0 (±3.8)^{A}	0.12 (±0.10)	0.83 (±0.54)
ISO	0.99 (±0.77)^{AB}	5.9 (±3.6)^{A}	0.13 (±0.11)	0.87 (±0.57)
FB	1.4 (±0.72)^{A}	4.8 (±1.7)^{A}	0.22 (±0.11)	0.79 (±0.27)
SB	1.6 (±0.84)^{A}	8.0 (±3.6)^{A}	0.22 (±0.11)	1.1 (±0.47)
SC	2.0 (±2.5)^{AB}	11.3 (±14.9)^{A}	0.30 (±0.39)	1.5 (±1.9)
Water[a]	0.19 (±0.04)^{B}	0.57 (±0.05)^{B}	0.35 (±0.06)	1.1 (±0.10)

[a] Unit: mg PS/ml; data taken from Mueller et al. (2020b).

a recent study on the effect of nano-plastics on *C. elegans* in soil which showed that the inhibitory effects of 530-nm PS beads on the nematodes' reproduction were enhanced at higher clay contents of the soils (Kim et al., 2020). However, in contrast to the sediments used in the present study, which contained maximally 4.5% clay, the soils used in Kim et al. (2020) contained up to 75% clay and, even without nano-PS beads, reproduction was negatively related to the clay content (Kim et al., 2020). It is also known for freshwater sediments that the growth of *C. elegans* is negatively related to the content of clay particles (Höss et al., 1999) that show a similar size range as synthetic micro- and nano-beads (<2 µm).

3.3. Food availability as possible effect mechanism

In order to reveal possible effect mechanisms, we wanted to compare the *C. elegans* sediment toxicity data with existing data derived from water exposure using the same test protocol (Mueller et al., 2020b). ECx-values were significantly higher in sediments compared to water exposure (Table 2; Fig. 2a). However, for sediment exposure, the test guideline (ISO 10872; ISO, 2020) prescribes a considerably higher density of bacteria in the food medium than for water exposure. As higher bacterial densities, and thus lower beads/bacteria ratios, are known to reduce inhibitory effects of PS beads on *C. elegans*' reproduction (Mueller et al., 2020b), also higher ECx-values based were expected in sediments.

Fig. 3A and C shows for two different concentration levels of PS beads (1.8 and 7.3 mg PS beads/g dry sediment) that the inhibitory effect of the PS beads decreased with increasing available food supply in two sediments (SS, ISO). When plotting the effect data against the ratio of PS beads/bacteria, irrespective of the type of manipulation (constant or varying food densities), a significant dose-response curve could be fitted to the effect data of SS and ISO sediment (Fig. 3B and D), revealing EC10- and EC50-values not significantly different (|Z| < 1.645) from those shown in Table 2 (SS: EC10 = 0.18 ± 0.16 and EC50 = 1.1 ± 0.78; ISO: EC10 = 0.28 ± 0.19 and EC50: 1.33 ± 0.74). These results indicate that also in sediments food availability is a crucial factor for the microplastic effects on the nematodes' reproduction. Rauchschwalbe et al. (2021) could show that the food consumption of *C. elegans* is clearly disturbed in the presence of 1-µm PS beads due to the dilution of bacteria with non-nutritious PS beads. This more indirect, food-related effect mechanism is most likely also causing the inhibition of reproduction by PS beads in sediments. The experiments of the present study with varying food densities at constant PS bead concentrations are supporting this assumption. At a beads/bacteria ratio of approximately 1:1 in the sediments (SS, ISO), the reproduction was inhibited by 50%, irrespective of modifying food or PS bead abundance (Fig. 3), a phenomenon that was already found for water exposure (Mueller et al., 2020b). Also, for the marine lugworm *Arenicola marina*, feeding activity was inhibited by unplasticized polyvinylchloride (UPVC) and PS beads, however, at high concentrations (50 g UPVC/kg and 74 g PS beads/kg, respectively; Besseling et al., 2013; Wright et al., 2013), with negative impacts of UPVC beads on the energy reserves of *A. marina* already at 10 g UPVC beads/kg (Wright et al., 2013).

4. Conclusions

Also in sediments, 1-µm PS beads can inhibit the reproduction of *C. elegans*, although the PS beads only add minimally to the abundant natural mineral and organic microparticles in the sediments. Low effect concentrations (EC10-values) were in the range of highly polluted environmental sediment samples, thus effects on benthic organisms cannot be ruled out under a worst-case scenario. However, for interpreting the ecological risks of microplastics in sediments, inhibiting effects of simultaneously present natural microparticles should be considered. As the beads/bacteria ratio well explained the effects of the PS beads on the nematodes, irrespective of the exposure matrix, the nutritious conditions, i.e., amount of food, should be considered in the future when evaluating the risk of microplastics for aquatic ecosystems.

Credit author statement

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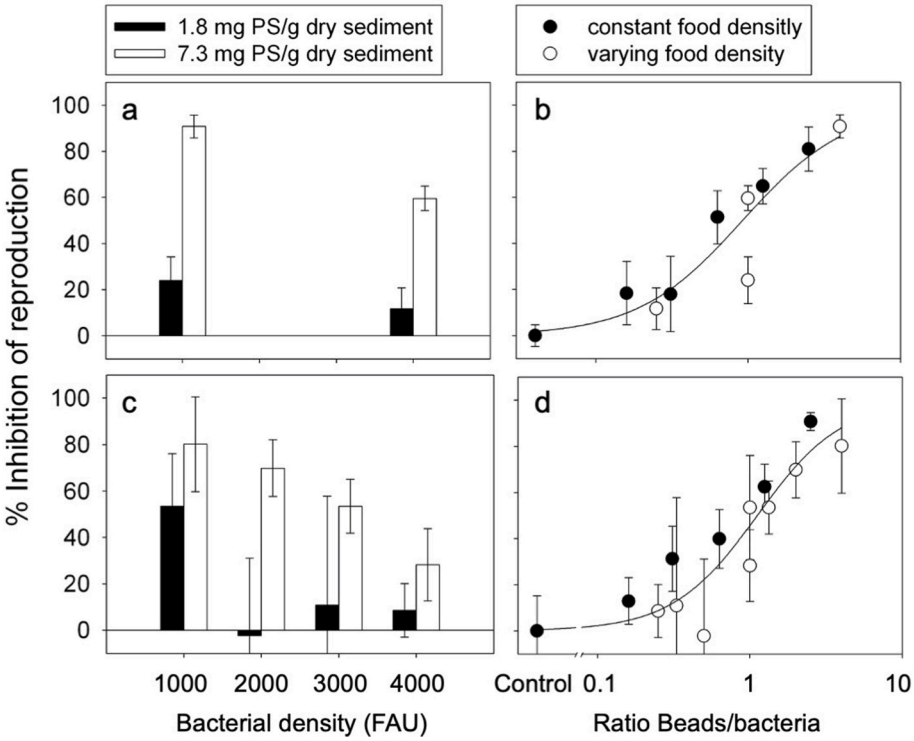

Fig. 3. % Inhibition of reproduction (mean ± standard deviation; n = 4) of *C. elegans* after 96 h of spiked-sediment exposure to 1-µm PS beads in SS (a, b) and ISO (c, d); (a, c): at different sediments concentrations and varying densities of food bacteria; (b, d): dose-response curve fitted to combined data of the experiments with constant food densities (black circles; compare to SS and ISO data in Fig. 2b) and with varying food densities (white circles) based on PS beads/bacteria ratios; ($p < 0.001$; SS: F = 22.7; predicted residual error sum of squares [PRESS]: 1953; $r^2 = 0.867$; ISO: F = 30.7; PRESS: 3588; $r^2 = 0.848$).

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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