Chemosphere xxx (xxxx) 136830

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Ingestion of microplastics and textile cellulose particles by some meiofaunal taxa of an urban stream

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ARTICLE INFO

Handling Editor: Michael Bank

Keywords:
Metazoan meiofauna
Functional traits
Nile red
Fluorescence microscopy
μFTIR
Focal plane array detectors

ABSTRACT

Microplastics (MPs) and textile cellulose are globally pervasive pollutants in freshwater. In-situ studies assessing the ingestion of MPs by freshwater meiofauna are few. Here, we evaluated MP and textile cellulose ingestion by some meiofaunal taxa and functional guilds of a first-order stream in the city of Florence (Italy) by using a tandem microscopy approach (fluorescence microscopy and μFTIR). The study targeted five taxa (nematodes, oligochaetes, copepods, ephemeropterans and chironomids), three feeding (scrapers, deposit-feeders, and predators), and three locomotion (crawlers, burrowers, and swimmers) guilds. Fluorescent particles related to both MPs and textile cellulose resulted in high numbers in all taxa and functional guilds. We found the highest number of particles in nematodes (5200 particles/ind.) and deposit-feeders (1693 particles/ind.). Oligochaetes and chironomids (burrowers) ingested the largest particles (medium length: 28 and 48 μm, respectively), whereas deposit-feeders ingested larger particles (medium length: 26 μm) than scrapers and predators. Pellets were abundant in all taxa, except for Chironomidae. Textile cellulose fibers were present in all taxa and functional guilds, while MP polymers (EVA, PET, PA, PE, PE-PP) differed among taxa and functional guilds. In detail: EVA and PET particles were found only in chironomids, PE particles occurred in chironomids, copepods and ephemeropterans, PA particles were found in all taxa except in nematodes, whereas particles made of PE-PP blend occurred in oligochaetes and copepods. Burrowers and deposit-feeders ingested EVA, PET, PA, PE and PE-PP, while crawlers and scrapers ingested PE and PA. Swimmers and predators ingested PE, PA and PE-PP. Our findings suggest a pervasive level of plastic and textile cellulose pollution consistent with an urban stream which propagates in the meiofaunal assemblage of the stream ecosystem.

1. Introduction

Microplastics (MPs), defined as solid polymer-containing particles ≤5 mm in size (Moore, 2008), have been tracked in almost all aquatic environments worldwide (Xu et al., 2020; Elizalde-Velázquez and Gómez-Oliván, 2021). MPs are regarded as globally pervasive pollutants in freshwater because, even with immediate and concerted action, 710 million metric tons of plastic waste are supposed to get into aquatic ecosystems in the coming years (Lau et al., 2020). Polyethylene, polypropylene, and polystyrene are the main constituents of MPs occurring in freshwater, each with a similar frequency of occurrence (Li et al., 2020). In urban streams, the occurrence of MPs is ascribed to multiple sources, encompassing primary industrial or domestic manufactured particles and secondary particles deriving from the breaking down of larger plastic debris through physical, biological, and chemical processes (Yang et al., 2021). Wastewater treatment plants are a major pathway for MPs in urban streams (Xu et al., 2019). Despite an average removal efficiency >95% (Ben-David et al., 2021), concentrations of

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https://doi.org/10.1016/j.chemosphere.2022.136830
Received 23 June 2022; Received in revised form 23 September 2022; Accepted 7 October 2022
0045-6535/

MPs in tertiary effluents can be as high as 500 particles/L (Simon et al., 2018). Since MP densities may be higher than water density, and biofouling enhances their sedimentation (e.g., Kooi et al., 2017), the number of MPs in river sediments is usually much higher than in the water column (Corcoran et al., 2020; Scherer et al., 2020). Artificial (man-made) particles obtained from cellulose, such as rayon/viscose, also occur in aquatic environments worldwide (e.g., Savoca et al., 2019). The primary source of cellulose fibers in urban streams is related to textiles during washing and toilet paper (e.g., Barrows et al., 2018; Suaria et al., 2020).

Aquatic metazoans ingest MPs and textile cellulose fibers, often showing a preferendum related to particle size, shape, and density (Franzellitti et al., 2019; Savoca et al., 2019; Morais et al., 2020; Macieira et al., 2021). For instance, low-density polymers (e.g., polypropylene and polyethylene), which float in the water column, are mainly ingested by filter-feeders (Scherer et al., 2017). High-density particles (e.g., polystyrene and polyvinyl chloride), which sink and accumulate on/in sediments, sometimes as plastispheres (Guasch et al., 2022), are available mainly to deposit-feeders (Scherer et al., 2017). The planktonic cladoceran *Daphnia magna* (filter-feeder) may consume up to 6200 particle/h exhibiting a higher feeding rate than benthic deposit-feeder taxa such as the dipteran *Chironomus riparius* and the oligochaete *Lumbriculus variegatus* (Scherer et al., 2017). Evidence of MP ingestion because of chemical cues (MP surface coverage by mixtures of organic and inorganic molecules) is reported for meiobenthic protists like ciliates (Dürichen et al., 2016) and cladocerans (Gerritsen and Porter, 1982). Shape and hardness can also influence the ingestion of MPs, as observed for the mysid *Praunus* spp., which mostly ingests small (100–200 μm) and spherical primary polystyrene microparticles (Lehtiniemi et al., 2018). MPs can also be ingested indirectly through trophic transfer, where contaminated prey is consumed by predators (Au et al., 2017; Chae et al., 2018; Fueser et al., 2020a). The ingestion of MPs by freshwater benthic invertebrates is known to alter aquatic ecosystem functioning by affecting bioturbation, predator–prey relationship and trophic status (e.g., Krause et al., 2021).

The metazoan meiofauna include various living forms, from rotifers to microturbellarians, gastrotrichs, tardigrades, nematodes, oligochaetes, microcrustaceans and even early larval stages of insects such as chironomids (Giere, 2009). Meiofauna are ubiquitous, thrive among sand grains, interstitial pores, biofilms, and detrital particles (Majdi et al., 2020) and play a complex and vital role in benthic food webs (e.g., Schmid-Araya et al., 2016; Cifoni et al., 2021). Evidence of MP ingestion by freshwater metazoan meiofauna is much more limited than for marine organisms and freshwater macroinvertebrates, both in the number of studies conducted and taxa investigated (Bellasi et al., 2020). The few available studies focused on the direct effects of MPs on single species (laboratory trials) and a narrow range of functional groups, mostly filter-feeders (Ockenden et al., 2021).

The aim of this study is to provide insights on the in-situ ingestion of MPs and textile cellulose fibers by some meiofaunal taxa of a stream in the highly urbanized area of Florence (Italy). To this end, we focused on the Mugnone creek, a representative case of an urban stream with poor ecological status. A recent study reported the presence of concerning pollution from synthetic and textile waste, with numbers of items per kg of sediments ranging from 500 to >1500 (Rimondi et al., 2022). We covered functional groups within three feeding (scrapers, deposit-feeders and predators) and three locomotion (crawlers, burrowers and swimmers) guilds. We hypothesized that the size, shape and type of ingested MPs and textile cellulose fibers would differ among taxa, feeding and locomotion guilds. The findings of this study may be helpful to better comprehend the pathways of these particles in freshwater ecosystems and tune indoor microcosm experiments.

2. Materials and methods

2.1. Study area

We investigated the ingestion of MPs by meiofaunal taxa of the Mugnone creek (catchment: 60 km^2; mean discharge: 0.046 m^3/s; ADAS - Autorità di bacino Distrettuale Appennino Settentrionale, 2021) where high loads of MPs in the water column and sediments were reported by previous studies (Rimondi et al., 2022). The small creek originates at 450 m above sea level (a.s.l.) and, at 390 m a.s.l., enters the city of Florence (Tuscany, Italy). We collected the meiofaunal sample in the city center, at the Ponte Rosso site (43° 47′ 09.6″ N; 11° 15′ 42.2″ E), where the streambed is heavily modified, on November 10th, 2020 (Fig. 1). Temperature, pH and dissolved oxygen (Table S1) were measured in the field by using a multiparametric probe (WTW Multi 3430 SET G). The Mugnone creek has been monitored by the environmental agency of the Tuscany Region at the Ponte Rosso site since 2000. In November 2020, diclofenac, Imidacloprid, glyphosate and total pesticides were detected at concentrations exceeding the legal limits denoting contamination by pharmaceutical compounds and pesticides (Table S1; ARPAT - Agenzia Regionale Protezione Ambiente e Territorio, 2020). The quality status of the Mugnone creek at Ponte Rosso was considered poor based on the Water Framework Directive 2000/60/EC (ADAS - Autorità di bacino Distrettuale Appennino Settentrionale, 2021).

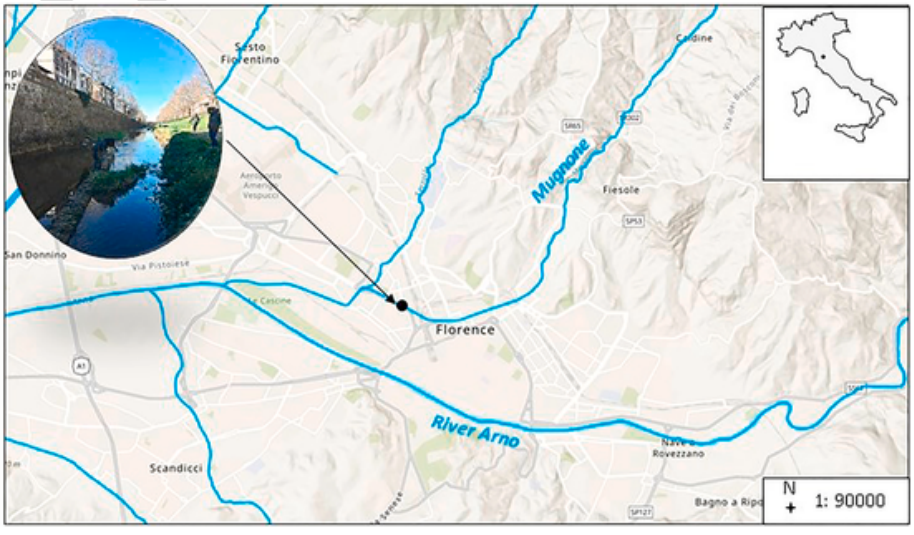

Fig. 1. The Mugnone creek and the Ponte Rosso (black dot) sampling site (43°47′09.6″N 11°15′42.2″E) in the city of Florence (Tuscany, Italy).

2.2. Sample collection and processing

The specimens were collected along a diagonal transect by adopting a validated method of intensive kick sampling: a 60 μm-mesh pond net was dragged behind the kicking leg in the opposite direction of flow to get the dislodged fauna (Bradley and Ormerod, 2002). The sample was immediately put in a 70% ethanol solution in the field, transported to the laboratory within an hour, and sorted under a LEICA M80 stereomicroscope at 16 × . The meiofaunal metazoans were picked up and identified. We analyzed the most abundant taxa, namely: Nematoda (4% of abundance), Annelida Oligochaeta Naididae (30%), Crustacea Copepoda (40%, represented by *Acanthocyclops robustus*), early larval stages of Insecta Ephemeroptera Baetidae (15%) and Insecta Diptera Chironomidae (10%). We discarded Tardigrada, Rotifera, and Ostracoda from further analyses due to their low abundances (≥1%). The body length (in mm) and biomass (μg of dry mass) of the individuals of each taxon were determined using the methods and size-biomass conversion equations in Reiss and Schmid-Araya (2008). The taxa were attributed to different feeding and locomotion guilds considering the mean trait profiles of their families in the biogeographic region (Neury-Ormanni et al., 2020; Di Lorenzo et al., 2021) and in-vivo observations performed in a preliminary survey. In detail: Nematoda were identified as swimmers (thrashers) and deposit-feeders (sRavizza and Zwick, 2006); Annelida Oligochaeta Naididae, all belonging to the genus *Pristina*, were burrowers and deposit-feeders (Glasby et al., 2021); the individuals of the cyclopoid *A. robustus* were swimmers and predators (Maier, 1990); the larvae of Diptera Chironomidae were burrowers and deposit-feeders (Kornijów et al., 2021) and, finally, the early larval stages of Ephemeroptera Baetidae were crawlers and scrapers (Jacobus et al., 2019).

2.3. MP processing

Three specimens for each taxon were analyzed under light microscopy to collect visual clues of MP ingestion (see Section 2.5.1). Then, we processed the remaining collected specimens by adapting the methodologies established for freshwater macroinvertebrates and marine plankton (Iannilli et al., 2019, 2020; Windsor et al., 2019; Alfonso et al., 2021). First, we rinsed the specimens with ultrafiltered MilliQ water to remove any exterior particles according to ISO Standard Method SS-EN ISO 6330 (ISO, 2021). Afterward, we loaded the specimens into 10-mL glass vials using a steel needle. Overall, we analyzed 99 specimens arranged in five pools (one for each taxon): 4 large nematodes, 50 individuals of *A. robustus*, 15 oligochaetes, 10 chironomids and 20 ephemeropterans. For each pool, we selected individuals similar in size (Table 1). The vials were filled with 10 mL H_2O_2 (30% SigmaAldrich, purchased from Merck, Darmstadt, Germany), topped with an aluminum foil, and placed at 60 °C for 72 h to digest the organic matter (Alfonso et al., 2021) before further microscopic analyses. The peroxide digestion at or below 60 °C proved effective for minimizing the loss of any constituent MPs in freshwater samples (water, sediments, and tissues; Munno et al., 2018). After 72 h, the digested suspensions were filtered on CHMLAB GROUP glass fiber discs, 0.2 μ in porosity, 47 mm in diameter. The filters were rinsed with ethanol (≥99.8% Sigma Aldrich, purchased from Merck, Darmstadt, Germany) before and after filtration, and then stored in a previously cleansed glass Petri dish and dried for 72 h under a fume hood until the analysis (Corami et al., 2020). Afterward, we inspected each filter without a priori knowledge about particle locations by acquiring an overview optical image of the entire filter by Focal Plane Array detectors equipped in the infrared microscope available for this study (see Section 2.5.2). We observed that distributions of the particles on the filters were homogeneous. Hence, we cut each filter in half. One half was stained with Nile Red (Iannilli et al., 2019 and references therein), air-dried under a fumehood for 24 h, and inspected for MPs through fluorescence microscopy (see Section 2.5.1). The other half was not stained (to avoid any fluorescent interference at the time of signal collection) and used for spectroscopic analyses (see Section 2.5.2).

2.4. Control for MP contamination from external sources

To minimize MP and textile cellulose contamination from external sources (e.g., solutions used in the animal processing, airborne particles, and worker clothing), glassware, steel ware and glass Petri dishes were rinsed with ultrafiltered MilliQ water, then with acetone (99.9%, SpS Romil, Cambridge, UK) and finally with ethanol (≥99.8% Sigma Aldrich, purchased from Merck, Darmstadt, Germany) under a laminar flow hood (Corami et al., 2020). In addition, white cotton laboratory coats and latex gloves were used at every stage of the MP processing (see Section 2.3). We assessed potential exogenous contamination because of processing procedures by using a blank control. In detail, we flushed 10 mL of H_2O_2 (30% SigmaAldrich, purchased from Merck, Darmstadt, Germany) through the 60-μm mesh net used to collect the animals and then poured it into a 10 mL glass vial. Afterward, we replicated every step of the procedures described in Sections 2.2 and 2.3 (filtering, staining etc.) as closely as possible to produce a blank control (Miller et al., 2021).

2.5. Microscopy and spectroscopy

A tandem microscopy technique was used to identify and count MPs and cellulose particles/fibers with size ≥1 μm and characterize those

Table 1
Mean (μ) and standard deviation (SD) of length (L; in mm), width (W; in mm) and biomass in dry weight (DW; in μg) of the meiofaunal individuals of the five taxonomic pools processed in this study and the individuals of the six feeding and locomotion guilds. *n* indicates the number of individuals in each pool/guild. The total biomass (TOT DW; in μg) and the number of particles per individual (n particles/ind.) and biomass unit (n particles/μg) of each pool/guild are also reported.

Taxonomic pool	μ_L	SD_L	μ_W	SD_W	μ_DW	SD_DW	TOT DW	n particles/ind.	n particles/μg
Nematoda (n = 4)	2.3	0.2	0.06	0.01	2	1	7	5200	2971
Oligochaeta (n = 15)	1.1	0.1	0.1	0.05	38	7	572	828	22
Copepoda (*Acanthocyclops robustus*) (n = 50)	1.3	0.2	0.2	0.02	75	11	3730	763	10
Chironomidae (n = 10)	1.3	0.2	0.3	0.02	24,304	6100	243 mg	1590	6/100 μg
Ephemeroptera (n = 20)	2.2	0.2	0.2	0.02	179,680	17,891	3593 mg	1012	6/1000 μg
Feeding guild									
Deposit-feeders (n = 29)	1.6	0.3	0.1	0.01	8115	120	243,622	1693	0.13
Predators (n = 50)	1.3	0.2	0.6	0.02	75	11	3730	763	10
Scrapers (n = 20)	2.2	0.2	0.7	0.02	179,680	17,891	3,593,599	1012	6/1000 μg
Locomotion guild									
Swimmers (n = 54)	1.8	0.2	0.1	0.01	38	7	3737	1092	15
Burrowers (n = 25)	1.2	0.3	0.2	0.01	12,171	8075	244 mg	1133	5/100 μg
Crawlers (n = 20)	2.2	0.2	0.7	0.02	179,680	17,891	3593 mg	1012	6/1000 μg

≥5 μm in the digested meiofaunal pools. In the first case, we adopted a protocol in Prata et al. (2019), which couples the digestion of all bioorganic matter with the successive staining of any remaining lipophilic matter with Nile Red, a lipophilic dye (Prata et al., 2019; Iannilli et al., 2020). Overall, even though it does not provide the chemical identification of the stained matter, this approach has been validated as an optimal protocol for the quantification of MPs in environmental and biological samples, allowing for the detection of smaller fragments (e.g., ~1 μm) if combined with microscopy (Prata et al., 2019). Particles ≥5 μm fell within the spatial resolution of Focal Plane Array (FPA) detectors equipped in the infrared microscope available for this study; these particles were thus fully characterized in terms of morphological features and chemical composition by using 2D Imaging Fourier Transform Infrared Spectroscopy (FTIR). Full details of the two analytical setups are provided in the following paragraphs.

2.5.1. Visible light and fluorescence microscopy

We used visible light microscopy (Nikon Eclipsse E600; 1000× magnification) in preliminary analyses with non-digested specimens (three individuals per taxon) to single out presumed MPs and textile cellulose fibers. Artificial/textile fibers were initially distinguished from natural sediments (algae, wood) based on their color (e.g., fully transparent, blue, and other colors ascribable to dyes) and shape (fiber-like shapes with no irregularities except from tears that might be due to aging in natural environments).

Afterward, we examined the stained half filters with an epifluorescence microscope (Nikon Ti–S) using a LED illuminator (CoolLED pe-300 Ultra) providing excitation light centered at 490 nm, and a filter set composed of an excitation filter with a bandpass of 455–495 nm and a dichroic mirror and an emission filter with a bandpass of 575–625 nm. Images of the fluorescent particles were acquired with a 20 × objective (Nikon Plan Apo; N.A 0,75) and recorded with a Hamamatsu ORCA-Flash4.0 V3 camera using the HCImage software (Hamamatsu) under a microscope in green fluorescence (ex: 450–490; em. 515–565 nm). Since the preliminary overview of the entire filters (see Section 2.3) highlighted a high number of particles, we applied a subsampling to speed up imaging and particle measurements. In detail, we examined six random 1-mm^2 spots in each half filter, following Imhof et al. (2016) and Oßmann et al. (2017). We cumulated the numbers of the fluorescent particles found in the six spots of each half filter and extrapolated the result to the entire filter. We subtracted the particles found in the blank control from the number of particles of each meiofaunal pool. Then, we analyzed the shape and dimensions (max length) of the fluorescent particles ≥ using the routine Analyze Particles of the ImageJ software (Schneider et al., 2012) with the following settings: size (1 μm – infinity), circularity (0.00–1.00). Since the sizes of the individuals in each pool were similar (Table 1), the results were expressed as number of particles per individual (or per μg of dry weight).

2.5.2. Spectroscopy

To characterize the composition of MPs and textile cellulose items ≥5 μm, we analyzed the non-stained dry half filters by 2D imaging FTIR, in reflectance mode (with no further sample preparation) by using a μFTIR-Microscope (Cary 62–670, Agilent Technologies) equipped with a 15 × Cassegrain objective and a Focal Plane Array (FPA) 128× 128 detector. We selected reflectance mode to avoid possible saturation of the signal by plastic/cellulose items ≥5 μm, or the fiberglass filter. This setup allows for the identification of MPs with a spatial resolution close to 5 μm, even on complex matrices (Cincinelli et al., 2021; Scopetani et al., 2021) using a "point-and-shoot" analysis. We recorded the spectra with an open aperture and a spectral resolution of 8 cm^{-1}, in the 3900–900 cm^{-1} range; 128 scans were acquired for each spectrum. A "single-tile" analysis produces a map of 700 × 700 μm^2 (128 × 128 pixels); each pixel has dimensions of 5.5 × 5.5 μm^2 and provides an independent spectrum. In each 2D map, the intensity of characteristic bands of the investigated polymers was imaged by using a chromatic scale of increasing absorbance as follows: blue < green < yellow < red. We analyzed the spectral profiles by using the Resolutions Pro software package by Agilent Technologies to identify diagnostic absorption bands, which were compared against reference libraries of MP materials. Only spectra with a match percentage (match %) ≥ 60% were accepted.

2.6. Statistical analyses

Differences in the dimensions of the fluorescent particles detected in the five taxa and the blank control, as well as in the feeding and locomotion guilds, were assessed by unbalanced one-way permutational analyses of variance (Permanova; Anderson, 2001) based on a Euclidean distance-based matrix and followed by pairwise permutational *t*-tests (Anderson et al., 2008). We applied permutation of raw data and Type I sum of squares because they yield the best power and the most accurate type I error for unbalanced designs (Anderson, 2001). The significance of the main test, as well as those of the pairwise *t*-tests, were obtained under 999 permutations and set at $\alpha = 0.05$ since permutation tests do not demand α adjustments (Anderson, 2001). Although PERMANOVA does not rely on assumptions concerning data distribution, we accounted for the potential heterogeneity of the variances by a Levene's test through means of the PERMDISP routine (Anderson, 2001). Strong skewness in the distribution was treated by log (x + 1)-transformation, when necessary (Anderson, 2001). The analyses were performed with the PRIMER software (vs. 7; Anderson et al., 2008).

3. Results

3.1. Light and fluorescence microscopy

From the preliminary examinations by visible light microscopy (see Section 2.5.1), we gathered evidence of the possible presence of MPs in the fecal pellets of all taxa (Fig. 2a–f). The mean and standard deviations of length, width, and biomass of the five meiofaunal pools processed in this study are presented in Table 1. The highest number of fluorescent particles ≥1 μm per individual was detected in the nematodes' pool, followed by those of chironomids, ephemeropterans, oligochaetes, and copepods (Table 1). The blank control had smaller MP contamination than the taxonomic pools (Table 1). The ratios of fluorescent particles to taxonomic pool biomass (in μg) presented in Table 1 indicated a marked difference between nematodes and the remaining taxonomic pools. Concerning the feeding guilds, the highest number of particles per individual was detected in the deposit-feeders, followed by scrapers and predators, while the highest ratio of fluorescent particles to biomass was noted in the predators (Table 1). Finally, in regards to locomotion traits, the number of particles was comparable among the three guilds, whereas the swimmers showed the highest ratio of fluorescent particles per biomass unit (Table 1).

The mean and standard deviation of the length of the fluorescent particles, as well as their size range, are presented per each taxonomic pool in Table 2. The fluorescent particles in ephemeropterans were the smallest, followed by those in nematodes, copepods, oligochaetes, and chironomids (Table 2). The blank control had smaller MPs than the taxonomic pools (Table 2). Most of the fluorescent particles of each taxonomic pool were ≤30 μm (Fig. S1a in logarithmic scale). The difference in the particle lengths among the five taxa and blank control was significant (Pseudo-$F_{5,535} = 11.56$; p-value = 0.001). However, the results of the pairwise permutational *t*-tests revealed that the particle lengths were different among all pools except for Copepoda vs. Ephemeroptera, Control vs. Copepoda and Control vs. Ephemeroptera (Table S2).

The size range of the fluorescent particles per each feeding and locomotion guild is presented in Table 2. The PERMANOVA test highlighted a significant difference in the particle lengths among the three feeding

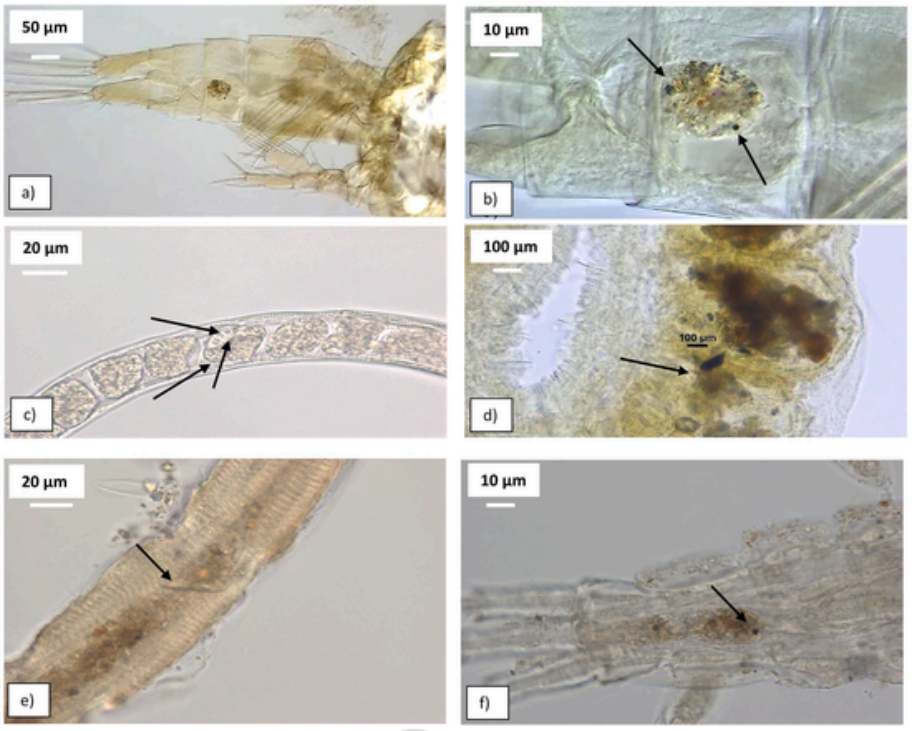

Fig. 2. Suspected microplastics in the fecal pellets of: (a, b) the cyclopoid *Acanthocyclops robustus* (fecal pellet: 43 µm; suspected MP pellets: 1–4 µm); c) nematodes (fecal pellets: 20 µm; suspected MP pellets: 1 µm); d) Annelida Naididae *Pristina* (fecal pellet: 350 µm; suspected MP fragment: 157 µm), e) chironomids (suspected MP fragment: 27 µm) and f) Ephemeroptera Baetididae (fecal pellet: 17 µm; suspected MP pellet: 2 µm). Benthic meiofaunal taxa of the Mugnone stream (Tuscany, Italy).

Table 2
Mean (µ) and standard deviation (SD) of the length (measured as the distance between the two most distant points; in µm) and size range of the fluorescent particles >1 µm found in the blank control, the five taxonomic pools and the six functional guilds. n: number of particles in the six 1 mm^2 spots of each half filter for each pool/guild and control.

	µ	SD	max
Control (n = 15)	2	1	4
Taxonomic pool			
Nematoda (n = 98)	7	10	63
Oligochaeta (n = 67)	28	70	470
Copepoda (*Acanthocyclops robustus*) (n = 165)	9	38	328
Ephemeroptera (n = 99)	3	3	18
Chironomidae (n = 82)	48	89	504
Feeding guild			
Deposit-feeders (n = 247)	26	65	504
Predators (n = 165)	9	38	328
Scrapers (n = 99)	3	3	18
Locomotion guild			
Swimmers (n = 263)	8	31	328
Burrowers (n = 149)	39	81	504
Crawlers (n = 99)	3	3	18

guilds (Pseudo-$F_{2,510}$ = 55.38; p-value = 0.001). However, the pairwise permutational t-tests showed that the particle lengths were different between deposit-feeders and predators and deposit-feeders and scrapers but not between predators and scrapers (Table S2). Scrapers and predators ingested smaller particles than deposit-feeders (Table 2). The PERMANOVA also highlighted a significant difference in the particle lengths among the three locomotion guilds (Pseudo-$F_{2,510}$ = 101.44; p-value = 0.001). However, the pairwise permutational t-tests showed that the particle lengths were different between swimmers and burrowers and burrowers and crawlers but not between swimmers and crawlers (Table S2). Overall, swimmers and crawlers ingested smaller particles than burrowers (Table 2).

All the fluorescent particles in the blank control were pellets, i.e., "hard, rounded plastic particles", as defined by Free et al. (2014). In the pool of nematodes, most of the fluorescent particles were pellets (Table 3), while only a few (9%), the largest (max length: 63 µm), were fibers (i.e., "thin or fibrous, straight plastics" with length >3 × width; Free et al., 2014) and (8%) fragments (i.e., "hard, jagged plastic particles"; Free et al., 2014). Exemplary images of pellets, fibers and fragments are shown in Fig. S1b and Figs. S2a–f. Similarly, in the pool of copepods, represented by the sole species *A. robustus*, most fluorescent particles were pellets, while only 2% and 4% were fibers and fragments, respectively (Table 3). Similarly, in the pool of ephemeropterans, most fluorescent particles were pellets, while 5% were fibers and 4% were fragments (Table 3). In the pool of oligochaetes, 67% of the fluorescent par-

Table 3
Percentage of pellets (P), fibers (F) and fragments (Fr) found in the blank control, the five taxonomic pools and the six functional guilds. n: number of particles in the six 1 mm^2 spots of each half filter for each pool/guild and control.

	P	F	Fr
Control (n = 15)	100	0	0
Taxonomic pool			
Nematoda (n = 98)	83	9	8
Oligochaeta (n = 67)	67	12	21
Copepoda (*Acanthocyclops robustus*) (n = 165)	94	2	4
Ephemeroptera (n = 99)	91	5	4
Chironomidae (n = 82)	31	47	22
Feeding guild			
Deposit-feeders (n = 247)	60	23	17
Predators (n = 165)	94	2	4
Scrapers (n = 99)	91	5	4
Locomotion guild			
Swimmers (n = 263)	88	5	7
Burrowers (n = 149)	49	30	21
Crawlers (n = 99)	91	5	4

ticles were identified as pellets, whereas 12% were fibers and 21% were fragments. Finally, unlike what was observed in the other taxonomic pools, in the Chironomidae pool, 47% of the fluorescent particles were fibers, 31% were pellets and 22% were fragments. Concerning the feeding guilds, predators and scrapers showed >90% of pellets, whereas 60% of them were found in deposit-feeders being fibers and fragments equally represented (Table 3). Pellets were also dominant in swimmers and crawlers, while burrowers showed comparable percentages of pellets, fibers and fragments (Table 3).

3.2. 2D Imaging FTIR

The infrared spectra and 2D Imaging false color maps of MP and textile cellulose samples are shown in the Supplementary File (Figs. S2a–f). The reported analyses are representative of the items found in the IR analysis, i.e., larger fibers as well as small pellets and fragments whose size falls close to the spatial resolution of the FPA detector. 2D Imaging with the FPA detector allowed the simultaneous acquisition of independent spectra across the fibers/fragment surface, with large enough signal-to-noise ratio to read spectral features clearly. Typically, from tens to a few hundreds of spectra are collected on large items, while up to 5–6 are recorded on the smaller ones. For each sample, along with the visible light and IR map, a spectrum of the plastic or cellulose polymer is also included and compared with the spectrum of the fiberglass background filter. We previously showed that this approach allows the identification of diagnostic bands of MPs and cellulose, which are well distinguished from the filter absorptions (Pegado et al., 2021).

Overall, the point-and-shoot analysis identified and characterized 64 particles in the range 5–400 μm, namely 29 in the chironomids, 14 in the oligochaetes, 15 in the copepods, 5 in the ephemeropterans, and 1 in the nematodes. The following polymers were identified through their characteristic absorption bands which are reported in the Supplementary File: i) polyamide (PA; Fig. S2a); ii) ethylene-vinyl acetate (EVA; Fig. S2b); iii) polyethylene (PE; Fig. S2c); iv) polyethylene terephthalate (PET; Fig. S2d); v) polyethylene-polypropylene blend (PE-PP; Fig. S2e); vi) non-plastic polymers identified as textile cellulose fibers (Fig. S2f). Cellulose textile fibers such as cotton, flax, and similar textiles, were distinguished from natural cellulose compounds (e.g., algae) based on their shape/color (as described before), and the absence of characteristic lipids or protein bands that are typically found in the spectra of algae (Jebsen et al., 2012).

Sixteen out of the 64 characterized particles were made of PA, 5 of EVA, 19 of PE, 2 of PET, 6 of PE-PP and 16 of textile cellulose. EVA and PET particles were found only in chironomids, PE particles occurred in chironomids, copepods and ephemeropterans, PA particles were found in all taxa except in nematodes, particles made of PE-PP blend occurred in oligochaetes and copepods, while textile cellulose fibers were present in all taxa. Burrowers ingested all the MP polymers and textile cellulose, whereas crawlers ingested PE, PA and cellulose and swimmers ingested PE, PA, PE-PP and cellulose. Deposit-feeders ingested all the MP polymers and textile cellulose, whereas predators ingested PE, PA, PE-PP and cellulose and scrapers ingested PE, PA, and cellulose. Due to their small size (max length: 4 μm), the particles found in the blank control were not characterized.

4. Discussion

MP number differed significantly among the meiofaunal taxa and functional guilds. We found that each taxon had ingested a high number of MPs and textile cellulose fibers, with nematodes being the most voracious organisms (5200 particles/individual). These findings are in line with laboratory-controlled experiments with *Caenorhabditis elegans* (Fueser et al., 2020a,b). In our study, we detected about 1500 particles/individual in the taxon Chironomidae. The result is in line with previous observations on *Chironomus riparius* (~2400 particles/individual; Silva et al., 2021). In line with our observations on oligochaetes, the MP intake of *Tubifex tubifex* is 129 ± 65.4 particles per tissue gram (Hurley et al., 2017). Marine copepods may ingest up to 1000 particles per individual per day in laboratory-controlled trials (Rodríguez-Torres et al., 2020), which is consistent with our findings. Finally, in our study, the number of particles found in ephemeropterans is consistent with other studies concerning benthic invertebrate communities and ecosystem functioning in artificial streams (e.g., Silva et al., 2022). In our study, the deposit-feeders, which feed on detritus and microbes associated with the sediment grains (Levin, 2013), ingested more particles than predators and scrapers. This result suggests that deposit-feeders discriminate the food items less than scrapers and predators (e.g., Schmid-Araya and Schmid, 2000). Scrapers are more selective because they primarily shear attached algae from surface particles, also grazing on interstitial biofilm, whereas predators feed by preying on living organisms (e.g., Gérino et al., 2003). In this study, the deposit-feeders and scrapers showed a higher number of fluorescent particles per individual than predators, as also observed in previous studies in marine habitats (e.g., Wesch et al., 2016). Our findings suggest that predators may be more selective in the number of MP ingestion than scrapers. Predators choose their prey intentionally so that the uptake of MPs is due to bioaccumulation rather than indiscriminate direct ingestion (Murray and Cowie, 2011). Finally, the particle abundance to biomass ratio computed in this study was significantly lower than that of macroinvertebrates from some urban streams in the UK (Windsor et al., 2019). This finding suggests that the MP and textile cellulose fibers ingested by meiofaunal metazoan taxa might be primarily egested rather than transferred through the freshwater food web, as also speculated by Fueser et al. (2020b).

We found that the size of the particles varied significantly among the taxa and functional guilds. The particles ingested by the deposit-feeders (nematodes, oligochaetes, and chironomids) were larger than those ingested by the scrapers (ephmeropterans) and predators (copepods). We found that the size range of MPs ingested by the predator cyclopoid *A. robustus* mirrored the dimension of its preferred preys, as typically observed for other predators (Cole et al., 2013; Setälä et al., 2014). Larger particles were found in burrowers (oligochaetes and chironomids) than in swimmers (copepods) and crawlers (ephemeropterans). This pattern is likely dictated by gravity since large, heavy particles quickly settle on/into the streambed sediments where burrowers can encounter them more easily than crawlers or trashing swimmers. Crawlers slowly move on the grain surface while swimmers move in the interstitial system voids and the water column (e.g., Heino, 2008). On the contrary, burrowers inhabit the interstitial habitats and may even construct burrows by ingesting their way through (e.g., Heino, 2008). They also likely contribute to MP sequestration through burial (Coppock et al., 2021).

We found that the shape of the particles varied significantly among the taxa and functional guilds. The deposit-feeders (nematodes, oligochaetes and chironomids) ingested fewer pellets than predators (copepods) and scrapers (ephemeropterans). Burrowers (oligochaetes and chironomids) ingested a high percentage of fibers, whereas swimmers (copepods and nematodes) and crawlers (ephemeropterans) seemed to have ingested pellets almost exclusively, as already observed in other studies (Sidney et al., 2016; Bertoli et al., 2022). Flow dynamics might play a role in the interaction between MPs and freshwater meiofauna in the Mugnone creek, as observed by Dris et al. (2015b) in other urban rivers. Since the fibers were the largest particles detected in this study, the reason for the observed fiber selectivity by burrowers could be related to the size and the subsequent vertical distribution, as reported for aquatic oligochaetes and chironomids in several other urban rivers (e.g., Rodriguez et al., 2001; Hurley et al., 2017; Lin et al., 2021).

In our study, the variety of polymers ingested by the meiofaunal taxa and functional guilds indicated diverse potential sources of MPs typically occurring in urban streams (Dris et al., 2015a; Cutroneo et al., 2020). All taxa ingested textile cellulose fibers which are the main type of MPs in the Mugnone creek (Raimondi et al. 2022). The highest polymer diversity was found in deposit-feeders and burrowers (oligochaetes and chironomids), which feed on particles embedded in the sediments, easily ingesting MPs retained in the substratum while moving through the granules (Bertoli et al., 2022). Chironomids seemed to accumulate more diverse polymers than other taxa/functional guilds, as also observed in other studies (e.g., Nel et al., 2018; Windsor et al., 2019). The use of chironomids as MP bioindicators in freshwater systems has been strongly recommended because of this finding (e.g., Scherer et al., 2017; Kumar et al., 2021).

The implications of our study are numerous, and some of these are difficult to tackle. First, MPs may affect organism survival, energy budget, swimming speed, respiration, food clearance, growth, development, reproduction, body size and shape, and gene expression (e.g., Mueller et al., 2020; Xu et al., 2020; Gallitelli et al., 2021). It follows that biodiversity and essential ecosystem services, like organic carbon recycling, energy transfer efficiency across trophic levels, and burrowing (e.g., Guasch et al., 2022; Silva et al., 2022), may be impaired. Second, MPs ingested by freshwater meiofauna make the inherent and absorbed toxic chemicals pass into the food chain, also providing the pathway for secondary and/or enhanced chemical toxicity (Naqash et al., 2020; Wang et al., 2020). For this reason, the occurrence, in November 2020, of pharmaceutical compounds and pesticides in the Mugnone creek is a harbinger of potential toxicological interactions (e.g., Ateia et al., 2020; Bhagat et al., 2021) which should be further investigated. Since some meiofaunal taxa seek refugium in hyporheic zones during adverse environmental conditions (e.g., Stubbington, 2012; Di Lorenzo and Galassi, 2013; Di Lorenzo et al., 2021), they may release MPs through feces in the subsurface from where they can reach the underlying aquifers (Frei et al., 2019; Drummond et al., 2020). The risk of biomediated MP contamination in groundwater has important consequences since groundwater is the main source of drinking water in the world (UN - United Nations, 2003). The transport of MPs in groundwater by meiofauna has not yet been investigated. Microcosm studies might be helpful in this aspect. However, they present some limitations because experimental MP concentration and size do not always match those observed in the field (e.g., Phuong et al., 2016; O'Connor et al., 2020). From this point of view, our study provided important information by indicating the number, shape, and type of plastic polymers ingested in-situ.

5. Conclusions

The present study is the first to analyze the in-situ ingestion of MPs and textile cellulose fibers by some meiofaunal metazoans and multiple functional guilds in an urban stream. We found that the MP number, size, shape and type differed significantly among nematodes, oligochaetes, copepods, chironomids and ephemeropterans, as well as among deposit-feeders, predators and scrapers (feeding guilds) and swimmers, burrowers and crawlers (locomotion guilds). Since discharge rate can considerably alter MP dynamics in a first-order urban stream, the results presented here represent the premise for further investigations to link trait guilds to the sources and fluxes of MPs in the Mugnone creek. Further studies involving other meiofaunal taxa are desirable to assess MP transmission within the freshwater food webs of this urban watercourse. For instance, MP ingestion by meiofaunal metazoans <60 μm in size and protozoans should be considered in further studies to encompass the entire spectrum of meiobenthos. Finally, the potential combined effects of chemical cand MP contamination on the meiofaunal metazoans of the Mugnone creek should be examined to identify remediation measures based on a more complete biological risk assessment.

Author contributions

Conceptualization, T.D.L., S.C., A.C.; methodology, T.D.L., T.M., M.L., D.C., D.M.P.G., A.C.; validation, T.M., M.L., D.C., D.M.P.G., A.C.; formal analysis T.M., M.L., D.C., A.C.; investigation, T.D.L., S.C.; resources, T.D.L., A.C.; data curation, T.D.L., S.C., T.M., M.L., D.C., D.M.P.G., A.C.; writing-original draft preparation, T.D.L.; writing-review and editing, T.D.L., S.C., T.M., M.L., D.C., D.M.P.G., A.C.; project administration, T.D.L., A.C.; funding acquisition, T.D.L., A.C.

Funding sources

This research was granted by MUR-FOE-Project Capitale Naturale - Task Biodiversità.

Uncited references

Dahms et al., 2020, De Falco et al., 2019, Jung et al., 2018, Menéndez-Pedriza and Jaumot, 2020, Remy et al., 2015.

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.

Data availability

Data will be made available on request.

Acknowledgments

We thank Elisa Quidacciolu and the staff of the Department of Chemistry "Ugo Schiff" for their cooperation in the field work and laboratory analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136830.

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