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Suitability of aquatic mosses for biomonitoring micro/meso plastics in freshwater ecosystems

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Abstract

Background: Mesoplastics (5–25 mm) and microplastics (0.001–5 mm) are emerging pollutants of great concern. However, reliable methods of monitoring these types of plastic in river ecosystems have not yet been established. The goal of this work was to evaluate, for the first time, the suitability of *Fontinalis antipyretica* as a biomonitor of meso- and micro-plastics in rivers. With this aim, native samples of the moss and devitalized moss clones, held inside the bags, were compared for the uptake of fluorescent polystyrene particles under laboratory conditions, and for retention of plastic debris in the field, in sites close to wastewater treatment plants.

Results: In the laboratory experiment, the moss retained smaller microplastics, and a higher number of polystyrene meso and microplastics was counted in the moss bags than in the native moss.

In the field study, the moss retained plastic debris chiefly in the form of fibres regardless of the capacity and flow rate of the wastewater treatment plants affecting each sampling site. The uniform morphology of moss clone seems to affect the retention of this type of pollutant. The FTIR analysis confirmed the particles entrapped by the moss bags as plastic, specifically polyethylene and polyamide type 6, among the most common plastic polymers detected in rivers.

Conclusions: The study findings highlighted the value of using uniform material, as the clone exhibited a greater accumulation efficiency with respect to the native moss. The mesh bags could act as selective filters and/or prevent the loss of adhering plastics. In the field, the bags favour plastic fibres retention despite the river flow. Finally, although FTIR is useful for the identification of plastic type, it is not very sensitive when small quantities of ground samples are used.

Keywords: Bryophytes, Bioconcentration factor, *Fontinalis antipyretica*, FTIR, Microplastics

Background

In 2020 global plastic production amounted to about 370 million tonnes of which 58 million tonnes were produced in Europe [1]. Plastic waste generates environmental concern due to its high persistence and low rate of biodegradability in the environment and the high degree of degradation it undergoes. Fragmentation into particles of different shapes and sizes, including macroplastics (> 25 mm), mesoplastics (MEPs, 5–25 mm) and microplastics (MPs, 0.001–5 mm), was first reported several years ago in marine, freshwater and terrestrial ecosystems [2–5]. Although most studies have focused on the marine environment, rivers play a key role in transporting plastic debris to the sea [6].

Rivers receive plastics from land-based sources, through various pathways such as stormwater overflows, landslides, direct agricultural, urban and industrial dumping and discharges from wastewater

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treatment plants [7]. After reaching the river, different factors such as hydrological dynamics (water level, flow rate), and the value of pH, turbidity and temperature can affect the transport and fate of these types of pollutants [8, 9]. The plastics have been detected in organisms from different trophic levels, ranging from algae to fish [10–12] causing both physical damage and toxic effects associated with the presence of chemical additives, persistent organic pollutants and heavy metals adsorbed on the surface of MPs [10].

Aquatic vegetation (e.g. bryophytes, including mosses) is widely used in research involving active and/or passive biomonitoring of trace elements (heavy metals and metalloids) and organic compounds [13–15]. In addition to their usefulness as biomonitors, mosses are of interest as primary producers that substantially contribute to the energy balance of ecosystems [16], as well as to other ecosystem functions. However, aquatic mosses have not previously been used for biomonitoring plastic particles, although a first attempt to study the retention of plastic particles (polystyrene nanoparticles) in water by moss has been carried out with the peat moss *Sphagnum palustre* [17]. The moss *Fontinalis antipyretica* has been extensively used for biomonitoring rivers [13, 14]. To be used as biomonitor of MPs and MEPs, *F. antipyretica* firstly must be able to retain plastic particles from the environment without becoming saturated, and secondly a linear relationship between plastic concentration in water and moss must be established [18]. However, assessment of these criteria is not easy due to the analytical uncertainty that persists. Some problems regarding the quantification of MEPs or MPs in mosses remain unresolved because of the lack of a standardised biomonitoring methodology and of specific protocols, as most studies have focused on marine organisms such as shrimps, bivalves and fish [19]. The main approaches used to detect MEPs and MPs often employ oxidative, alkaline or enzymatic digestion (depending on the organisms studied), followed by filtration and finally characterization of the sample by spectroscopic methods [19, 20]. However, the method of digesting the moss matrix has not been optimised. On the other hand, visual methods that consider only the size, shape and colour of plastics can lead to underestimation of MPs, mainly due to the small size of the particles [21, 22]. Nevertheless, the use of fluorescent polystyrene granules has proven successful in laboratory tests with other species (algae) [23]. By this approach, since the exact concentrations of the plastics in the medium is known and the particles adsorbed on the moss surfaces could be quantified, it is possible to demonstrate both the absence of saturation and the linearity of the relationship.

However, the uptake capability may vary depending on the type of moss used. At least for terrestrial mosses, the differences between laboratory-grown moss clones and samples of moss from natural populations have been verified for biomonitoring heavy metals [24]. For this reason, the choice of the most appropriate material for biomonitoring purposes should be determined by testing both native and cloned moss samples. In this respect, the availability of clones of *F. antipyretica* [25] represents an important advantage for verifying whether these differences also exist for MEPs and MPs. On the other hand, if laboratory tests of the biomonitoring capacity of this riverine moss species produce satisfactory results, the capacity to retain MEPs and MPs should also be tested in field studies, which to date has not been done for any moss species. In this context, wastewater treatment plants emerge as a unique field testing opportunity, because they enhance release of MEPs and MPs to the aquatic environment as a result of the low efficiency of operation and also the large volumes of treated water discharged [26]. For all these reasons, the aim of the present study was to determine the uptake response of the aquatic moss *F. antipyretica* (in terms of linearity and saturation) to increasing concentration of MEPs and MPs by comparing native and cloned moss samples ("moss bags"). In addition, field research was carried out for qualitative assessment of the capacity of native moss and moss bags exposed in rivers close to wastewater treatment plants to retain MEPs and MPs.

Materials and methods

Preparation of the experimental material: native moss and moss bags

Samples of *Fontinalis antipyretica* Hedw. were collected from a stream (− 8.627°W 42.816°N) at the end of June 2021. The mosses were sampled by hand, rinsed in situ by dipping them in river water 2 or 3 times and transported to the laboratory in a glass container. Apical portions of the samples were stored for 24 h in distilled water until the start of the laboratory assay.

Aliquots of previously stored devitalized clones of the moss *F. antipyretica* (0.4 g d.w.), obtained as reported by [25], were placed in each bag (7 × 7 cm; 2 mm mesh size).

Laboratory assay

MEPs and MPs particles (we shall call them 'items') were produced by grinding granules of green, fluorescent polystyrene (3 mm, UV-Granulate, Magic Pyramid Bruecher & Partner G, Frechen, Germany), at a vibrational frequency of 1200 min^{-1} for 10 min in a tangential mill (RETSCH MM 400). The ground material was then passed through 4 sieves (mesh sizes 0.05, 0.20, 0.50 and 1.25 mm) to yield two different sizes of MPs (Size

1: 0.05–0.20 mm; Size 2: 0.20–0.50 mm) and one size of MEPs (Size 3: 0.50–1.25 mm). The particles were stored in labelled glass vials until their use.

Five samples of the native moss apices (each of 3 g f.w.) were incubated in distilled water in Erlenmeyer flasks (500 mL) with three different concentrations for each of three different sizes of plastic particles, on an orbital shaker (Platform shaker INNOVA 2300), at 200 rpm for 48 h. The concentrations of plastic particles were expressed as weight/volume percentage: low, 0.055%; intermediate, 0.11%; and high, 0.22% (see Table 1). These concentrations were chosen to test a possible saturation of moss to retain plastic particles. For each particle size, to estimate the number of plastic particles added at each concentration, the number of particles in 0.5 g samples was counted through a stereomicroscope (OLYMPUS SZ2-ILST). Five bags, each filled with 0.4 g d.w. of devitalized *F. antipyretica* moss clone (corresponding to 0.3 g f.w. of native moss) were treated in the same way as the native moss samples. A total of 46 samples (5 replicates × 3 sizes × 3 concentrations + 1 without plastic particles, used as a control) of both native moss and moss bags were processed. The videos of the experiment are available as Additional files 2, 3.

At the end of the incubation period, the native moss samples were gently rinsed (10 s) to remove any weakly attached material. Successively, ten apical portions of each native moss sample were randomly selected and placed in previously labelled glass Petri dishes for microscopic analysis (see "Microscopic examination and image analysis" Section). Similarly, all the moss clone apices were extracted from the bags and treated in the same way as the native moss samples.

Field experiment

The moss bags (5 replicates) containing the devitalized *F. antipyretica* clones were placed in each of 3 different sites, located at distances of between 100 and 400 m from the sewage discharge outlets of wastewater treatment plants in rivers of Galicia (NW Spain), at the beginning of October 2021 (Table 2). The moss bags were exposed attached with cords to concrete blocks placed at the bottom of the river, in areas of flowing water (free to move along 20–30 cm depth of water column), collected after 7 days and transported to the laboratory in a glass container. One moss bag filled with the clone was left in the laboratory as control.

At the same time, a composite sample of the native moss *F. antipyretica* was obtained from each of the three sampling sites (at a depth of about 30–40 cm), placed in a glass container and transported to the laboratory. Once in the laboratory, the native moss and the moss clones extracted from the bags were placed in an oven (40 °C) to remove residual water, and stored in dry glass Petri dishes for further analysis.

Microscopic examination and image analysis

All of the samples from both laboratory and field experiments were photographed in a stereoscopic microscope (Leica M205FA, equipped with a Leica DFC7000T CCD camera). A total of 1350 images (450 of native moss and 900 of moss bags) were obtained in brightfield (BF) and fluorescence mode using the ET GFP filter (ex: 470/40 nm, em: 525/50 nm). The images from the laboratory experiment were analysed with ImageJ 1.53 k software (Java 1.8.0_172) to count the number of plastic items of sizes 1, 2 and 3 per moss surface (items cm^{-2}) for each of the three different concentrations. For this purpose, the images were converted to 8 bits before the black and white threshold function was applied, and the sampled area was manually identified after pre-establishing the scale.

Identification of plastics from field samples

Each sample of the native mosses collected in the field and each of the moss bags exposed in the three rivers

Table 1 Number of plastic items of different sizes incubated with the native moss samples and moss bags at high, intermediate, and low concentrations

Number of plastic items			
Concentration in water	**Size 1**	**Size 2**	**Size 3**
	0.05–0.20 mm	**0.20–0.50 mm**	**0.5–1.25 mm**
Control (0%)	–	–	–
High (0.22%)	70,327	16,923	5220
Intermediate (0.11%)	35,163	8461	2610
Low (0.055%)	17,582	4230	1305

The concentration of polystyrene particles is expressed as weight/volume percentage

Table 2 Geographical location and characteristics of the wastewater treatment plants

Wastewater treatment plant	Geographical coordinates		Wastewater treatment plant characteristics	
	West	**North**	**Population equivalent**	**Mean flow rate**
WT1	− 8.599°	42.870°	220,100	74,625
WT2	− 8.648°	42.843°	13,000	2600
WT3	− 8.624°	42.821°	14,000	3857

Geographical coordinates (WGS84 coordinates), population equivalent (i.e. organic biodegradable load with a 5-day biochemical oxygen demand of 60 g of oxygen per day) and mean flow rate (m^3 day^{-1})

were divided into two subsamples of equal weight. One subsample, randomly selected, was ground in a tangential mill for 2 min at vibrational frequency of 1350 min^{-1} (RETSCH MM 400). This subsample was analysed by attenuated total reflectance FTIR spectroscopy (Agilent Technologies Cary 630) to identify the type of plastic debris. FTIR spectra were acquired at 4 cm^{-1} resolution in the mid-infrared (MIR) region 4000–400 cm^{-1}, by averaging 200 scans with a Gladi-ATR (Pike Technologies) spectrometer. Spectra were baseline corrected in order to prevent bias in the spectroscopic signal due to scattering, reflection, temperature, concentration or instrumental anomalies.

The second subsample was visually inspected, and where possible small plastic residues, trapped on the moss surface and visible to the naked eye, were separated from the moss surface, examined in a stereomicroscope and finally collected in glass Petri dishes. Visual examination was carried out to establish whether these particles fulfilled at least two of the criteria proposed by Windsor et al. [27]; (i) unnatural colour (blue, red, green, purple, black or grey) relative to other items/debris; (ii) homogeneous appearance and consistency without visible cellular structure; (iii) constant width throughout the entire length; (iv) absence of damage and brittleness if compressed, pulled or struck with fine tweezers; (v) shiny or glossy outward appearance, and consequently could be classified as MEPs or MPs. Moreover, the plastics were classified as fibres or films on the basis of their shape. Finally, the FTIR spectra were compared with reference spectra of the most common plastics to confirm the type.

Data analysis

The normality and the homoscedasticity of the data (number of plastic items of size 1, 2 and 3 per moss surface) was tested by the Shapiro–Wilk and Levene's test, respectively. Because most of the variables did not meet these assumptions, the non-parametric Scheirer–Ray–Hare test ($p < 0.01$) was used to compare the number of items/cm^2 of moss (both in native and in moss bags), with sizes and concentrations as factors. A post hoc multiple comparison procedure (Dunn's test, with $\alpha = 0.01$) was conducted for direct paired comparison of samples.

In addition, the number of plastic items on the moss surface was expressed in terms of moss weight by using the specific leaf area (SLA) reported by [28] for this species. This number was then used to calculate the bioconcentration factor (BCF), i.e. the ratio between plastic concentrations in moss and water, taking into account the density of water. These data were subjected to the same statistical procedure as described above. All analyses were performed using XLSTAT software (v. 2014.5.03; Addinsoft, Andernach).

Results

Laboratory experiment

The 1350 photographs of native moss and moss bags were examined and the numbers of plastic items for each size were counted (see e.g. Fig 1 for size 1; Fig. S1, S2 for size 2 and size 3, respectively, in Additional file 1).

The moss bags contained a higher median number of items (per moss surface unit) than the native moss samples, although the variability (expressed as a percentage

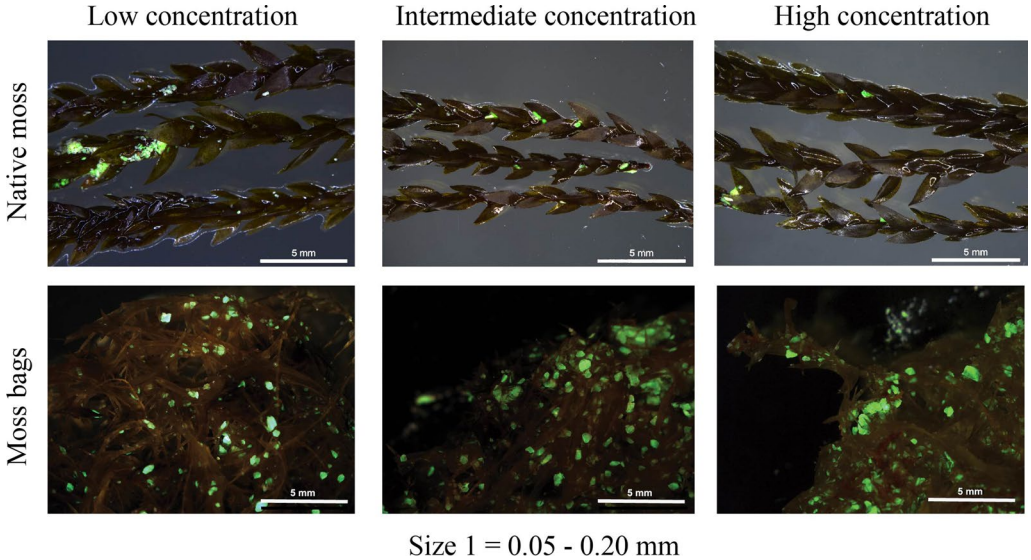

Fig. 1 Photographs of native moss (upper) and moss bags (lower) incubated with MPs of size 1 (0.05–0.20 mm) at three different concentrations expressed as percentage weight/volume (low, 0.055%; intermediate, 0.11%; high, 0.22%)

of median absolute deviation/median) was higher in the native moss (Fig. 2). In the native moss samples, the variability was higher than 100%, with minimum and maximum values of 43 (size 1 at low concentration) and 245% (size 1 at high concentration). The mean variability in the number of particles in the moss bags was close to 80%, ranging from 37 (size 2 at high concentration) to 114% (size 3 at intermediate concentration). Analysis of the control samples did not reveal any polystyrene particles.

According to the results of the Scheirer–Ray–Hare test (Table 3), the native moss, but not the moss bags, were affected by an interaction between the two factors (i.e. size x concentration). A post hoc test was therefore applied, showing that the median number of counted items per moss surface was lower at low concentrations (106 items) than at either intermediate or high concentrations (185 and 221 items, respectively), although the differences were not statistically significant (Fig. 2). For size 2 particles, the median number of items was significantly lower at the low concentration than at the intermediate and high concentrations, with the median numbers of particles at low, intermediate and high concentrations. For size 3 particles, the median number of items was lower at the low (32 items) than at the intermediate (44 items) and high (68 items) concentrations, although the differences were not statistically significant. However, in the moss bags the median number of particles was size 1 > size 2 > size 3 at each concentration, with the numbers of particles increasing with the concentration.

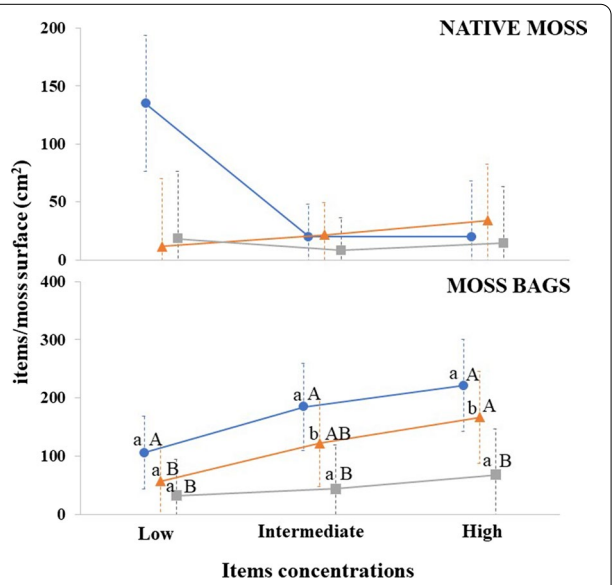

Fig. 2 Median number (± median absolute deviation) of plastic items (size 1: blue line, size 2: orange line; size 3: grey line) on the surface of native moss samples and moss bags after incubation at different concentrations of plastics items expressed as weight/volume percentage (low, 0.055%; intermediate, 0.11%; high, 0.22%). The same letters indicate statistically homogeneous subgroups (lower case letters for item concentration data and upper-case letters for item size data)

Table 3 Scheirer–Ray–Hare test results for native and clone moss bags for nº items cm^{-2} and bioconcentration factor

	SS	df	MS	H	*p*-value
Nº items/moss surface					
Native					
Size	1,270,836	2		60.2	< 0.0001
Concentration	66,023	2		3.13	0.210
Size x concentration	638,354	4		30.2	< 0.0001
Within	8,650,753	495			
Total	10,625,966	503	21,125		
Moss bags					
Size	14,988,170	2		161	< 0.0001
Concentration	4,732,893	2		50.7	< 0.0001
Size x concentration	77,273	4		0.830	0.935
Within	79,096,970	1052			
Total	98,895,307	1060	93,298		
Bioconcentration factor					
Native					
Size	972,291	2		65.4	< 0.0001
Concentration	1,795,681	2		121	< 0.0001
Size x concentration	92,720,83	4		6.23	0.182
Within	3,401,893	413			
Total	6,262,585	421	14,875		
Moss bags					
Size	21,638,966	2		348.25	< 0.0001
Concentration	29,128,936	2		46.9	< 0.0001
Size x concentration	1,670,926	4		26.9	< 0.0001
Within	27,338,447	854			
Total	53,561,232	862	62,136		

SS sum of square, *df* degrees of freedom, *MS* mean square, H: SS/MS_{tot}

Regarding the BCF (Fig. 3), in general, the median values for moss bags were higher than for native moss (except for size 1 at low concentration) and the bioconcentration of size 3 plastic particles (i.e. MEPs) was greater than for the other sizes. Furthermore, a decreasing trend in the median BCF values from low to high concentrations can be observed for each particle size for both native moss and moss bags. Differently from the number of particles per moss surface, BCFs did not show interaction between the two factors (i.e. size x concentration) for the native moss, while an interaction was observed for the moss bags; therefore, the post hoc test was only applied to BCF values of native moss (Table 3). Thus, at low concentration, the median BCFs for items of size 1 and size 3 were, respectively, 3- and 5-fold higher than for size 2 (i.e. 763), although no significant differences were found. At intermediate concentration, the

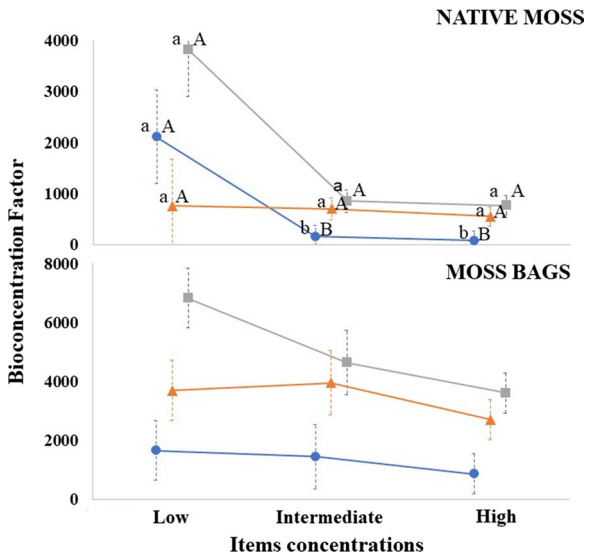

Fig. 3 Median values (± median absolute deviation) of the bioconcentration factor (size 1: blue line, size 2: orange line; size 3: grey line) in native moss and moss bags after incubation with different concentrations of plastics items expressed as weight/volume percentage (low 0.055%, intermediate 0.11%, high 0.22%). Same letters indicate statistically homogeneous subgroups (lower case letters for item concentration data, and upper-case letters for item size data)

median BCFs for both items of size 2 and size 3 were about fivefold higher than for size 1, although the difference was only significant between size 1 and size 2 (BCF: 157 and 703, respectively). For the high concentration, the median BCFs for items of size 3 and size 2 were, respectively, 7- and 10-fold higher than for size 1 (BCF: 78) and there were significant differences between size 1 and both size 2 and size 3. For size 2 particles only, the BCF values were similar among different concentrations, whereas for both size 1 and 3 particles at low concentrations, the BCFs were one order of magnitude higher than at intermediate and high concentrations.

Field experiment

FTIR analysis of the ground subsamples of both the native moss and moss bags from the three sampling sites did not yield plastic signals. By contrast, the fingerprint spectra of "suspected" plastic debris detected by naked eye on the surface of the unground subsamples and observed by a stereomicroscope confirmed them to be plastics, specifically polyethylene (PE) and polyamide type 6 (PA6) (Fig. 4).

As it is not possible to measure the moss surface area in the photographs, the numbers of plastic items isolated from the surface of unground subsamples of both native moss and moss bags were expressed in relation to the dry moss weight (in grammes). Specifically, according not only to the FTIR characterization, but also to the shape of the isolated plastic pieces, in WT1 the native moss and moss bags retained, respectively, 1 and 12 fibres of PA6 g^{-1} d.w. In WT2, the native moss yielded 1 and the moss bags 7 fibres of PA6 g^{-1} d.w. The greatest numbers of fibres of PA6 were detected in WT3: 1 in the native

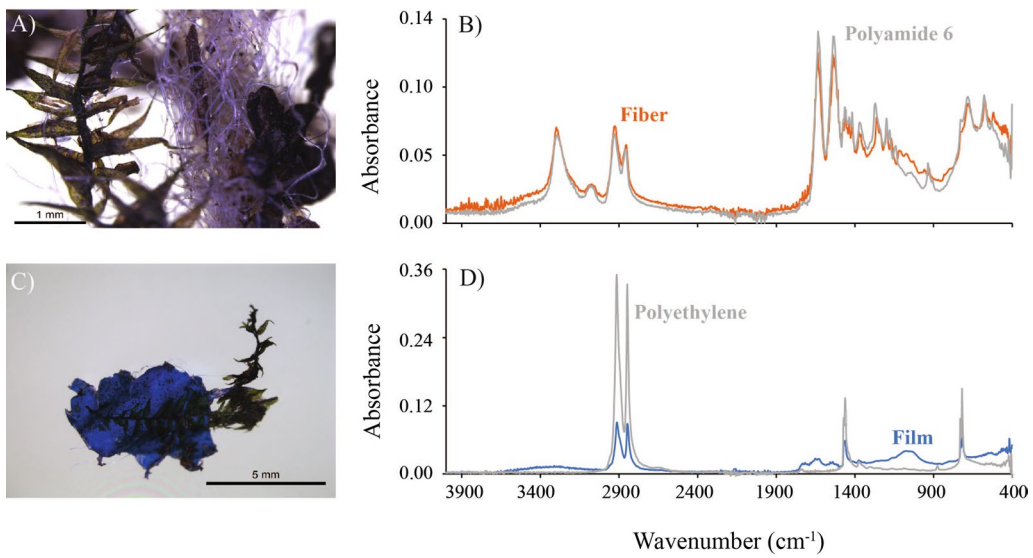

Fig. 4 **A** Examples of fibres observed on the surface of moss clone *F. antipyretica*; **B** FTIR spectrum of fibres (orange line) and polyamide type 6 reference spectrum (grey); **C** example of film observed on the surface of native moss *F. antipyretica*; **D** FTIR spectrum of film (orange line) and polyethylene reference spectrum (grey)

moss and 30 fibres g^{-1} d.w. in the moss bags, respectively. Finally, in the native moss collected in WT1 and WT3, only 1 film of PE was found, determined according to the shape of the pieces and FTIR spectra. The fibres of PA6 were of diameter 0.20 mm and variable length ranging between 1 and 8 mm, and they could therefore be classified as MPs and MEPs.

Discussion

The approach used in the present study involving the analysis of 1350 photographs was successful as it enabled the accurate quantification of ca. 140,000 MEPs and MPs of different sizes in the native moss and moss bags of *F. antipyretica* samples. The large volume of data allowed us to reliably assess the suitability of this moss species in MEPs and MPs retention. Bryophytes can accumulate large amounts of particles due to the high ratio between leaf area and leaf biomass and the presence of monostratified leaf laminae [17, 29]. In the case of *F. antipyretica*, it is known that the amount of particulate matter retained could be up to 40% of the total weight of the moss [30]. It was therefore expected that in the laboratory experiment both the native moss and moss bags would retain plastic items on their surfaces. It was also hypothesised that native moss would retain more items than moss bags, as the mesh bags could have excluded large particles (i.e. MEPs, size 3), and the arrangement of the moss inside the bags, with the apices closely packed together, could reduce water flow and plastic uptake. Surprisingly the results showed that the moss bags captured more items, especially of size 2 and 3, than the native moss (see Fig. 2).

Regarding the native moss, the high variability between replicates (see Fig. 2) may be due to the extremely variable morphology of the autochthonous native moss as a result of adaptation to different flow velocities [31]. In addition, the moss used in the laboratory experiment was a composite sample collected at different points in the river, which probably caused further morphological differences between the apices and thus their ability to trap particles. In addition to the high level of variability, the retention of plastic particles by the native moss was not linear at varying concentrations of polystyrene particles in water. The non-linear response was likely due to the different morphology, as explained above, as well as to the interaction between concentration and size, or even to the behaviour of the polystyrene particles. In fact, according to Tussellino et al. [32], polystyrene particles can form aggregates in the water, which would facilitate the retention on the moss surface of the smallest sizes of MPs at the lowest concentrations, unlike the larger MPs, which would tend to settle on the bottom of the incubation flask.

Conversely, the trend for increasing numbers of plastic particles captured by the moss bags as the concentration increased (size 1 and 2) was noteworthy, occurring despite the high between-replicate variability (Fig. 2). The moss bags retained more MPs of small sizes; this was not only affected by the higher concentration of size 1 particles than of size 2 and 3 particles, but was also accentuated by the meshes which, in this case, could have acted as a selective pre-filter, mainly excluding the larger particles.

On the other hand, the saturation of the moss surface cannot be ruled out due to the extremely high concentration of plastic particles tested in the laboratory experiment that are unlikely to be reached in rivers. Different studies, mostly conducted in China (see Table 2 in [33]), have reported MP concentrations in surface waters ranging from 293 to 8925 elements m^{-3} and therefore, for the MP concentrations common in rivers, *F. antipyretica* would show a linear response without reaching the saturation and thus act as a good biomonitor of these pollutants.

Regarding the BCF values, *F. antipyretica* generally bioconcentrated more MEPs than smaller MPs (size 3 > size2 > size1) regardless of the concentration in water (Fig. 3). As the number of smaller particles was higher, this could also lead to saturation of the moss surface due to the large number of small particles present in the water. Although these bioconcentration values may seem high, previously high BFCs values (i.e. 610,437 for Hg or 17,887 for Sb) have been reported for other pollutants for this moss species [34]. The comparison between the median number of items per moss surface in native moss and moss bags, and the bioconcentration factor demonstrated that using uniform material guarantees a greater efficiency in the accumulation of MEPs and MPs.

The wastewater treatment plants are one of the main sources of continuous discharge of MEPs and MPs into rivers [35]. Nevertheless, in the field experiment, despite the large volume of effluent discharged at each site (especially at WT1), the FTIR spectroscopy did not detect any plastic signal from the ground subsamples. The presence of different types of plastic items (PA6 and PE) was only confirmed in the unground subsamples from the same locations (Fig. 4). Rather than indicating the absence of plastic debris in the rivers under study, this discrepancy suggests that FTIR is not particularly sensitive when only a small amount of ground sample is used for analysis [20]. Small pieces of buoyant plastics, such as film of PE and textile fibres of PA6, are the most common type of plastic debris found in water bodies [36, 37]; their presence in native moss and moss bags, rather than other types of plastic, was expected and confirms that wastewater treatment plants do not generally retain textile fibres or

components of synthetic products [38, 39]. However, the number of fibres retained by *F. antipyretica* differed greatly among the three sampling sites and moss types. Overall, at each site the native moss captured fewer plastic fibres than the moss bags, confirming the action of the bags as selective pre-filters, mainly excluding plastic film and preventing the release of fibres once trapped by the bags. The mesh bags could also reduce the impact of environmental conditions, such as that caused by the river flow velocity, which could detach weakly adhered plastics. This is important as the flow velocity is the main environmental parameters that can influence the horizontal transport of plastic debris within the freshwater ecosystems [9]. In addition, the characteristics of the plastic debris found on the moss surface, such as the small dimension and/or the high surface area-to-mass ratio, made them more strongly affected by the vertical transport along the water column, being more easily captured by the moss bags [9] which were able to fluctuate along the water column with respect to the native sessile mosses.

Moreover, the number of plastic pieces isolated from the surface of the native moss did not differ among the investigated sites, whereas differed for the moss bags (see "Field experiment" Section). The moss bags captured the smallest number of fibres at WT2, reflecting the low capacity and flow rate of the nearby wastewater treatment plant, the smallest such plant in the study area. Differences among the wastewater treatment plants in removal efficiency and hydraulic retention time may possibly explain the lack of consistency between the number of plastic pieces retained by the moss bags at WT1 and WT3. The possible interactions between plastics and other substances present in the water [40, 41] may also explain the lack of consistency of the results (or even the results obtained for native moss in rivers). Such interactions could cause the MPs to acquire electrical charges so that their uptake by the moss would be determined by physico-chemical as well as mechanical processes. However, further studies are required to investigate the mechanical and physico-chemical interactions between the moss surface and plastic particles, as these have not yet been established.

Conclusions

In the laboratory study, *F. antipyretica* mainly retained the smallest MPs and a higher retention capability was observed for the moss bags with respect to the native moss, as also confirmed by the field study where the moss bags exposed retained more MEPs and MPs than the native moss samples collected at the same site. The mesh bags could act as selective filters and/or prevent the loss of adhering plastics, suggesting active biomonitoring as more proficient than the passive one, with some practical implications in plastic monitoring campaigns in rivers. Not only the concentration and size of plastic particles, but also the moss morphology seems to affect the retention of this type of pollutant, being the number of MPs retained less variable in the clone than in the native moss, providing a further suggestion—the use of a uniform material—in the development of a river biomonitoring plan. However, the effects of environmental factors on plastic retention by moss should be deeply investigated in further studies with the help of an experimental river controlling several hydrodynamic parameters.

Lastly, regarding the method used to differentiate the type of plastics, although FTIR has been proved useful for identification of plastic type, when the retained plastic debris were isolated from the moss material, this method is not sensitive enough to detect clear signals in small amounts of ground moss sample. However, FTIR analyses confirmed that the particles (film and fibre) retained by moss bags exposed in the field belong to the plastic polymers most commonly found in rivers, that is PA6 and PE. Overall, the findings show the capability of *F. antipyretica,* used as autochthonous native moss and/or clone samples exposed in bags, to entrap plastic particles in water underlying the importance of this aquatic moss species in monitoring MEPS and MPs in rivers, and more generally in assessing freshwater ecosystem quality, having already been proven its ability to monitor a wide range of pollutants.

Abbreviations

MEPs: Mesoplastics; MPs: Microplastics; WT: Wastewater treatment; BF: Bright field; FTIR: Fourier-transform infrared spectroscopy; SLA: Specific leaf area; BCF: Bioconcentration factor; SS: Sum of square; df: Degrees of freedom; MS: Mean square; PE: Polyethylene; PA6: Polyamide type 6.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-022-00653-9.

Additional file 1: Figure 1SM and Figure 2SM. Photos of native moss and moss bags incubated with microplastics of size 2 and 3.

Additional file 2. Video of incubation of native moss.

Additional file 3. Video of incubation of moss bags.

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Author contributions

VC: formal analysis, investigation, writing—original draft, writing—review and editing. ZV: conceptualization, methodology, funding acquisition, writing—original draft, writing—review and editing. JRA: conceptualization, methodology, resources, supervision, writing—review and editing, FDN:

conceptualization, funding acquisition, methodology, resources, writing—review and editing. JAF: conceptualization, methodology, resources, supervision, writing—review and editing. All author read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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