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High Density of Microplastics in the Caddisfly Larvae Cases

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Abstract

This study aimed to assess the presence of microplastics (MPs) in an urban river (Gari, Lazio, Italy) using case-building caddisfly larvae as potential bioindicators. Results from the benthic faunal assemblage (STAR_ICMi = 0.797) revealed the presence of a rich and well-diversified macroinvertebrate community, thus reflecting a suitable ecological status. Of 279 caddisfly cases collected, 26% contained small plastic particles of various shapes and colours, while 542 MP items per m^2 were found in their substrate. Polyvinyl chloride (PVC) and Polyethylene terephthalate (PET) were the most abundant polymers identified by FT-IR analysis found in the Gari River, while the co-presence of lower-density polymers such as polystyrene (PS) and polyethylene (PE) or polypropylene (PP) reflects the contribution of multiple factors controlling MP deposition. The most abundant MPs were of secondary origin, as evidenced by the Carbonyl Index and the predominant shape. Despite the amounts of MPs found in the Gari River, their ecological and chemical status has been classified as "good" during the monitoring campaigns. These results highlight the need to further investigate the environmental impacts of MPs to implement water quality classification indices.

Keywords: benthic macroinvertebrate; water framework directive; microplastics; plastic pollution; caddisflies; bioindicators; Trichoptera

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1. Introduction

Microplastics (MPs) are considered an environmental contaminant of emerging concern, especially because the widespread use of plastic items in daily life has led to increasing rates of global plastic production. A great amount of plastic is not recycled; therefore, without effective changes in waste management, large quantities of plastic enter the environment. Subsequently, synthetic polymers exposed to environmental degradation (i.e., photochemical or mechanical degradation) cause first fragmentation of larger plastic objects and then the diffusion of micro- and nano-plastics in all ecosystems [1–4].

MPs having a size in the range of 0.1 µm to 5 mm [5] usually occur in different shapes (fragments, fibres, film, foam, and pellets), colour and chemical compositions because additives such as plasticizers, pigments, flame retardants, fillers, etc., are always added to polymers to optimise their mechanical, aesthetic, or chemical characteristics [6,7]. Some additives, such as phthalates and Bisphenol-A are considered dangerous to human health since they are classified as endocrine disruptors [8,9]. In addition, plastic particles can adsorb many pollutants such as heavy metals, polycyclic molecules, hydrocarbons, pathogens, and parasites, potentially causing ecotoxicological issues [10,11]. By now

organisms of all trophic levels encounter MPs in their habitats and may ingest them either accidentally or intentionally. Many studies have reported on the exposure of aquatic biota such as invertebrates, fish, birds, mammals, and plants to microplastics [4,12–15]. In addition to the adverse effects on the receiving organism, MPs can be accumulated and biomagnified within the food chain [16].

It was estimated that over 80% of MP pollution in the ocean comes from land and freshwater systems such as lakes and rivers, representing an important way for MPs to enter the sea [17–21]. Even groundwaters, used for the supply of drinking water, are affected by the phenomenon of MP contamination [22]. Despite this phenomenon, which can involve human health at risk, MPs in freshwater have been less studied than in the marine environment [23].

As pollution causes qualitative and quantitative changes in the biota communities, benthic macroinvertebrates can be considered as good indicators of local conditions due to their almost sedentary habits; they are sensitive to specific stresses with a wide range of tolerant/intolerant taxa and are easy to collect and identify [24]. For this reason, the biotic indices based on macroinvertebrate communities have been widely used in monitoring programmes and required by specific environmental policies, such as the Water Framework Directive (WFD) 2000/60/EC.

Among benthic macroinvertebrates, *Caddisflies* (Trichoptera) are an order of widespread insects occurring worldwide, with 16,266 described species distributed in 51 families [25]. Caddisfly larvae play important functions in the freshwater trophic level as they represent food for most aquatic organisms, and, moreover, they also decompose plant materials into fine organic particles through feeding [26] as well as using them for case construction. In most genera, the larvae produce and use silk to spin a net that can be used as a scaffold to construct a protective case employing various materials such as gravel, sand, shell pieces, pieces of wood and leaves, and other types of debris collected from the environment [27,28]. Remaining inside the case to survive predators, they catch food suspended in the water column. Among all aquatic taxa, *Caddisflies* are well suited for assessing ecological disturbance and water quality degradation due to their broad ecological and taxonomic diversity and general intolerance to environmental stressors [29–31]. The river sediments can be considered as a sink of microplastics, and their use to build by caddiesflies has been observed and confirmed also in laboratory studies [32–34]. It has also been observed that MPs can reduce the stability of trichopteran cases [33]. Recently, MPs were found in material collected from natural history collections; in particular, MPs were found in the casing of *Caddisflies* collected in 1971 [35]. MPs incorporated into tubiform bioconstructions were also documented in some species of marine polychaetes [36,37], representing an interesting analogy between marine and freshwater aquatic environments.

Manta trawls are the main sampling tool for microplastic sampling from surface and column water, whereas other sediment sampling items are used. There is a need to develop methods for reducing time and effort to detect MPs, at least to verify the presence/absence. Since microplastic contamination is a growing concern in freshwater ecosystems, the aim of this study was to assess and characterise the presence of MPs in an urban river and to test the role of *Caddisflies* as potential bioindicators of MPs in rivers.

2. Materials and Methods

2.1. Study Area

The sampling location was identified along the Gari River, a small urban river in the Lazio region (Italy), because it is included in the monitoring framework of the Regional Environmental Protection Agency (ARPA) according to the EU Directive 2000/60/CE. The river originates from karst punctual springs with a mean total discharge of 18 m^3

s^{-1}. The sampling site (Lat. 41.488657°, Long. 13.827033°) is located about 1 km far from the river source and in an urbanised area in Cassino town (34,800 inhabitants), as shown in Figure 1. The flow rate was about 18 m^3 s^{-1} and the water velocity, measured by a portable electromagnetic current metre (OTT MFpro), was <0.9 m s^{-1} and therefore could be considered "laminar". The river (0.4–0.6 m depth in the sample point) is characterised by abundant aquatic vegetation consisting mainly of *Berula erecta*, arboreal (*Alnus glutinosa*), and herbaceous riparian vegetation. Sediments are composed of polygenic sandy gravel, constituted mainly by carbonate and subordinate pyroclastic elements from the Roccamonfina volcanic complex [38].

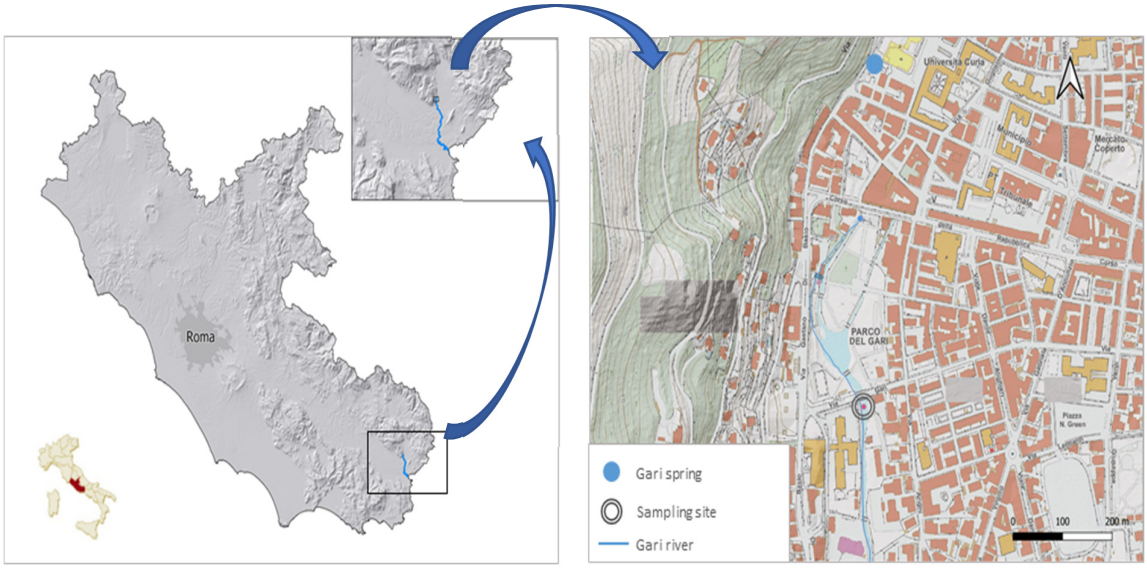

Figure 1. The study area and sampling site located in the urban area of Cassino, Lazio, Italy.

2.2. Sample Collection

Sediments, *Caddisflies*, and water were sampled during the baseflow conditions of the Gari River (June). Benthic macroinvertebrates were collected according to the multihabitat sampling methodology established within the WFD 2000/60/CE EU Directive to assess the ecological status of Italian rivers [39,40]. The sampling method for aquatic invertebrates and sediments involves the use of a Surber frame net with a 500 µm mesh size and a 0.05 m^2 sampling area capacity (frame of 22 cm × 23 cm). The larvae collected in the field were preserved using 70% C_2H_5OH. A total of 10 randomly colleceted amples were taken from the top few centimetres of the riverbed. Sediment samples from 10 random replicates were combined into a single sample representative of the habitat heterogeneity of the river [41]. Macroinvertebrates and their substrate were pooled and transported to the lab immediately after collection. MPs from surface waters were collected using a Manta-type net with a mouth opening of 60 cm × 25 cm and a net mesh of 330 µm. Water samples were also analysed, in particular dissolved oxygen, conductivity, pH and temperature were measured using a portable instrument (YSI Professional plus, YSI, Yellow Springs, OH, USA), while BOD, COD and nutrients were analyzed in the laboratory (details are in the Supplementary Data).

2.3. Laboratory Activities

According to the European multimetric STAR ICMi index (Standardisation of River classifications—Intercalibration Common Metric) developed to assess the ecological quality of rivers using macroinvertebrates [40], benthic fauna was manually sorted and identified up to the family level. Caddisfly cases were washed with ultrapure water to remove

adhered particles, dried at 40 °C for 4 h, and stored in individual glass Petri dishes. Caddisfly cases and their substrate (sediments, leaves, etc.) were visually inspected for MPs by using a stereomicroscope at 20–40x magnification (Olympus SZX12, Olympus Corporation, Tokyo, Japan). Considering that some MPs of the inner part of the case can be covered by sediment grains, the case disintegration was necessary and was performed using the Fenton-like reaction (0.1M Fe^{3+} + 30% H_2O_2 at pH = 3), commonly used for degrading contaminants in water, to remove the organic materials. Visual identification of plastics was carried out following Tibbetts et al. [42]. All the eligible particles found in the substrate (caddisfly cases, sediments, etc.) were handpicked using metal tweezers and placed onto Petri dishes and stored prior to analyse.

MPs were classified according to their size, shape (fragments, foam, fibres, etc.), and colour [43–45]. As for caddisfly cases, sediments also were treated using the Fenton-like reaction to remove the organic matter from the samples. The sediment grains were separated from the MPs using density (different salting solutions were used).

2.4. Polymer Characterisation

Plastic items from substrate samples and caddisfly cases were dried in a stove at 45 °C for 3 h before analyses. Fourier Transform Infrared spectroscopy (FT-IR) was applied for polymer identification of 130 plastic particles randomly selected among case and substrate samples, accounting for 30% of total items, by using a Thermo Fisher Scientific Nicolet iS5 6700 spectrophotometer (Waltham, MA, USA). The chosen samples had dimensions greater than 0.3 mm since these are the minimum dimensions of the samples that can be used by the FTIR. The measurements were carried out in attenuated total reflection (ATR) with the use of a single reflection ATR accessory (model Golden Gate Single Reflection ATR System). For each particle, the IR spectra were collected in the spectral range from 4000 cm^{-1} to 650 cm^{-1}, with a resolution of 4 cm^{-1} and 16 co-added spectra accumulations per scan. Polymers were identified by comparison with the spectra database in the reference library of the instrument using OMNIC-32 Specta Software (version 2.1.175), considering a match score $\geq$85% as a value of similarity among samples [23]. One of the most common methods to monitor polymer degradation is to observe the carbonyl (C=O) band variations by FTIR spectroscopy. Therefore, the polyethylene degradation was detected by means of the Carbonyl Index (CI = A (1720)/A (722)) that was calculated using the absorption band at 1720 cm^{-1}, stretching vibration of the carbonyl group (C=O), while the absorbance at 722 cm^{-1} is used as reference [3,46]. The CI is taken as the average of at least five PE fragments from random samples included in the cases.

2.5. Quality and Contamination Control

To prevent MP contamination, clean cotton laboratory coats and glass and stainless-steel equipment were used. All laboratory surfaces and equipment were cleaned using 70% ethanol and distilled water before starting [47]. During the lab activity (e.g., sediment treatment and separation), beakers filled with water were used to collect any MP pollution.

During the stereomicroscope observation, a clean Petri dish with a clean filter was kept open near to the operator to detect airborne MP deposition. Additionally, to prevent airborne MP contamination, all Petri dishes containing caddisfly larvae cases were immediately covered with aluminium foil after use. Furthermore, to prevent MP cross-contamination between caddisfly larvae cases, the tweezers between samples were rinsed thoroughly.

2.6. Statistical Analyses

Cluster analysis/dendrogram was used to quantify the similarity in terms of hierarchical relationship between data regarding the MPs diffusion in different sample structures.

Paired Group UPGMA (Unweighted Pair Group Method with Arithmetic mean) using the Bray-Curtis coefficient of similarity to group items. Software PAST 4.1 was utilized.

3. Results and Discussion

Research efforts to assess MP pollution in freshwater macroinvertebrate populations are increasing worldwide [48] therefore finding microplastics in the insects with aquatic larvae is not new. Despite this, there are still few studies documenting MP presence in different Trichoptera species; among them, only five regard the cases collected in the environment field (Table 1), and only one quantifies the MPs, whose number seems extremely variable depending on the site [47]. In addition, as far as we know, simultaneous control using multiple caddisfly families from riverine environments has never been achieved.

Table 1. MPs in caddisfly *larval* cases and tissue: a comparison with other authors. Data collection among peer reviewed scientific papers via Scopus and Google Scholar. LC = larval case, LT = larval tissue (ingestion). (n.d.) = undetermined.

River, Lake (Country)	*Caddisflies*	MPs Pres.	Polymers	Abundance	Ref.
Tame (UK)	n.d.	LC	n.d.	n.d.	[42]
Saynbach (D)	Lepidostomatidae	LC	PP, PA, ABS, PU, VE, PE, PVC, PES	1.14 MPs/case	[47]
Gafos (E)	Lepidostomatidae Limnephilidae	LC	PVC, PET, PE, PP, PES, PSA	n.d.	[45]
Taff, Usk, Wye (UK)	Hydropsychidae	LT	PP	20–30 MPs/g	[49]
Kinnickinnic (USA)	Hydropsychidae	LT	n.d.	5–15 MPs/g	[50]
Lab. study	Lepidostomatidae	LC	PVC, PET	n.d.	[33]
Lab. study	*Odontocerum albicorne*	LC	ABS, PET, PP, PS, PVDF	17 MPs/case	[34]
Stour (UK)	Ord. Trichoptera	LT	PA, PS, PES, PE, PP	0.62 MPs/case	[51]
Vipacco (I)	Hydropsychidae Lepidostomatidae	LT	PES	0.003 MPs/case	[52]
Lab. study	*Agrynia* sp.	LC	PLA	n.d.	[53]
Lab. study	Hydropsychidae	LC, LT	PVC	n.d.	[54]
Sungai Chegeh and Sungai Galas (Malaysia)	n.d.	LT	CE	n.d.	[55]
Gafos River (Spain)	Leptoceridae Limnephilidae	LC	PE	n.d.	[45]
Baikal Lake (Russia)	*Baicalina thamastoides Hydatophylax nigrovittatus*	LC	PVA, PVC	n.d.	[56]
Lab. study	Limnephilidae	LC, LT	PET	n.d.	[57]
Lab. study	*Limnephilus hamifer*	LC	PET	16.4–28.7/case	[58]
Gari (I)	Limnephilidae Sericostomatidae Goeridae	LC	PP, PE, PA, PVC, PET, PVA, Nylon, PAN, CE	0–4 MPs/case	Present study

PP = Polypropylene, PE = Polyethylene, PVC = Polyvinyl chloride, PES = Polyester, ABS = Acrylonitrile-Butadiene-Styrene, PA = Polyamide, VE = Vinyl ester resin, PET = Polyethylene terephthalate, PLA = Polylactic acid, PVDF: polyvinylidene fluoride, PS = Polystyrene, PSA = Polystyrene acrylate, PVA = Polyvinyl alcohol, PAN = Polyacrylonitrile, PBT = polybutylene terephthalate, CE = cellophane.

The characterisation of the macrobenthic component sampled in the Gari River confirmed that caddisfly cases contain materials available in their habitats. Among these

materials, the microplastics especially where the water velocity is low because accumulation of MPs can be observed; indeed, a negative correlation within MP accumulation in the sediments and the water velocity was observed [42,59]. The flow rate at the time of sample collection was less than 0.9 ms^{-1}, and most polymers have a density >1 gr/cm^3; therefore, a consistent amount of MPs can be expected in the riverbed.

3.1. Water Quality

Concerning the biological characteristics of the Gari River (Table 2), the high Average Score Per Taxon (ASPT) score points out a "good" water quality, containing many high scoring taxa. The characterisation of benthic faunal assemblage, using the STAR_ICMi index (0.797), also revealed the presence of a rich and well-diversified macroinvertebrate community, thus reflecting a suitable ecological status as reported in Table 2. This assessment was also supported by water analyses performed by ARPA in the frame of the recurring freshwater monitoring activities. The analytical data indicated low COD (chemical oxygen demand), BOD (biochemical oxygen demand), and nutrient levels and proper oxygen saturation (86%) (Table S1, Supplementary Data). Considering the MP content on the water surface, 0.29 MPs/m^3 were found, probably due both to the proximity of the sampling point from the source (<1 km) and to the density of polymers; in fact, foams and fibres were mainly found, as reported in Table 3.

Table 2. Biological characterisation of the Gari River.

ASPT (*)	5.333	Shannon Index	2.555	EPT Families	6
Total families	18	log (SelEPTD+1)	1.898	Star_ICMi index 1-GOLD	0.797 0.736

(*) usually, ASPT values > 4 indicate clean water.

Table 3. Percentage of relative abundances of MPs in terms of shape and colour in sediment (A) and *Caddisflies* families Limnephilidae (B), Sericostomatidae (C), and water surface (D).

Type	**A**	**B**	**C**	**D**	**Colours**	**A**	**B**	**C**	**D**
Fibres	-	6	-	37.5	White/yellow	8	31	34	-
Film	18	1		25.0	Blue	28	20	24	25.0
Fragment	78	93	100		Black/brown	8	7	-	12.5
Foam	-	-	-	37.5	Green	19	10	3	37.5
Pellet	1				Red	27	20	31	25.0
Sphere	2				Transparent	-	6	-	-
Grain	1				Others	9	6	7	-
TOTAL	352	55	18	8	TOTAL	352	55	18	8

3.2. MP Analysis in Caddisfly Cases

A total of 279 caddisfly cases were collected, of which 73 (26%) contained plastic particles of various shapes, dimensions, and colours. As a comparison, Tibbetts [42] found MPs in 7% of 30 caddisfly cases, while according to Ehlers et al. [47], out of the 29 Lepidostoma basale cases, 17 (59%) had MPs.

Taxonomic characterisation of sampled specimens leads to the identification of three families of casemaker *Caddisflies*: Limnephilidae, Sericostomatidae, and Goeridae. In Figure 2 the images of larval cases are illustrated. Among them, no MPs were observed in the 39 Goeridae cases collected, while one to four particles per case were found incorporated in the others. Out of 192 Limnephilidae cases collected, 55 (29%) showed MPs, while among 48 cases of Sericostomatidae, 18 (38%) had MPs fixed into their cases. Among the

caddisfly *larval* cases where MPs were found (73), the mean MP load was 1.45 ± 0.23 MPs per caddisfly *larval* case (range: 1–4 microplastics per case). The absence of MPs in the Goeridae cases suggests an active behaviour of the larvae that avoided plastic particles in the construction of their cases, using a flange of pebbles to stabilise them. In fact, the larvae of this family construct their cases completely with mineral grains. Some genera incorporate larger rock fragments laterally, serving a stabilising function [60,61].

Figure 2. Limnephilidae (S1), Sericostomatidae (S2), and Goeridae (S3) specimens and their larval cases. Red rows point out MPs particles. Scale bar = 1 mm.

Fragments were the most frequent particle shape found in the caddisfly cases, 93% and 100% in Limnephilidae and Sericostomatidae larvae, respectively. While filaments and films were found in Limnephilidae cases with values of 6% and 1% occurrence, respectively, as reported in Table 3. Spherical particles were found in the sediments, but contrary to what Ehlers et al. [47] found, they were not observed in the cases; this could be due to the size of the spheres found in the sediments (average size ≈ 1 mm).

The microplastic dimensions are shown in Figure 3. The size range of MPs observed in *Limnephilidae* larvae cases was between 0.1 and 5 mm with a mean value of 1.7 ± 1.2 mm, comparable with data from the river sediments (0.1–5 mm, mean 2.5 ± 1.3), while in Sericostomatidae larvae the particles showed a smaller range between 0.1 and 0.7 mm and a mean value of 0.3 ± 0.2 mm, more consistent with their case construction habits, which generally enclose particles in the sand size range. The size differences in the particles embedded in the cases reflect the peculiar abilities of the different Trichoptera families examined. The Limnephilidae group is well known for the diversity of case materials and architecture, usually composed of plant fibres, leaves, and mineral grains arranged in an irregular manner, with pieces of different sizes intermingled, making a rough case [60,61]. In our study, the cases of Limnephilidae had incorporated various MP shapes of different sizes, such as filament and film, in addition to the longer average length fragments. In contrast, the Sericostomatidae group, which builds cases of very fine sand grains of approximately uniform size, completely arranged in a smooth surface [60,61], incorporated only fragments while being smaller than average size.

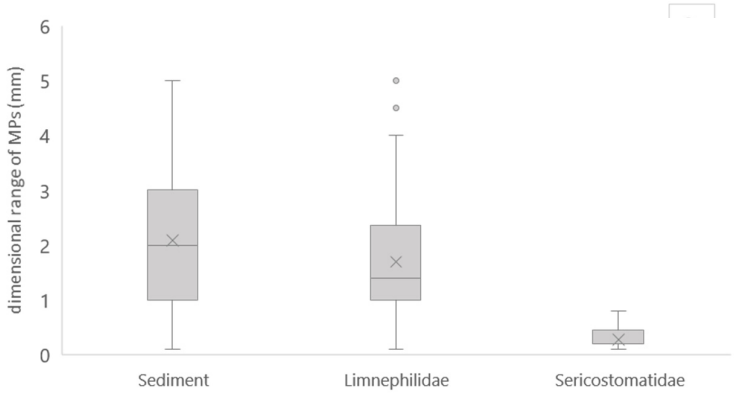

Figure 3. Dimensional range (mm) of MPs in substrate and *Caddisflies* families: Limnephilidae and Sericostomatidae.

In general, material selection for the building of the caddisfly larvae was observed; they showed a preference for the size of case construction materials, but there was no preference for colours [62,63].

Furthermore, the MPs incorporated into the cases showed a wide spectrum of colours but white/yellow, blue, and red are the most prevalent in both groups, with relative abundance values between 20 and 34%, as reported in Table 3 [45,47].

The MPs characterisation confirmed the presence of a range of polymers in analogy with other studies conducted in freshwater [47,64]. The chemical composition of MP particles from all examined samples shows that the most abundant polymers are polyvinyl chloride (PVC), used to produce pipes and cables, and polyethylene terephthalate (PET), commonly used for beverage bottles. They were found in both families, Limnephilidae with 27% and 30% occurrence, respectively, and in Sericostomatidae with 23% and 13% (Figure 4).

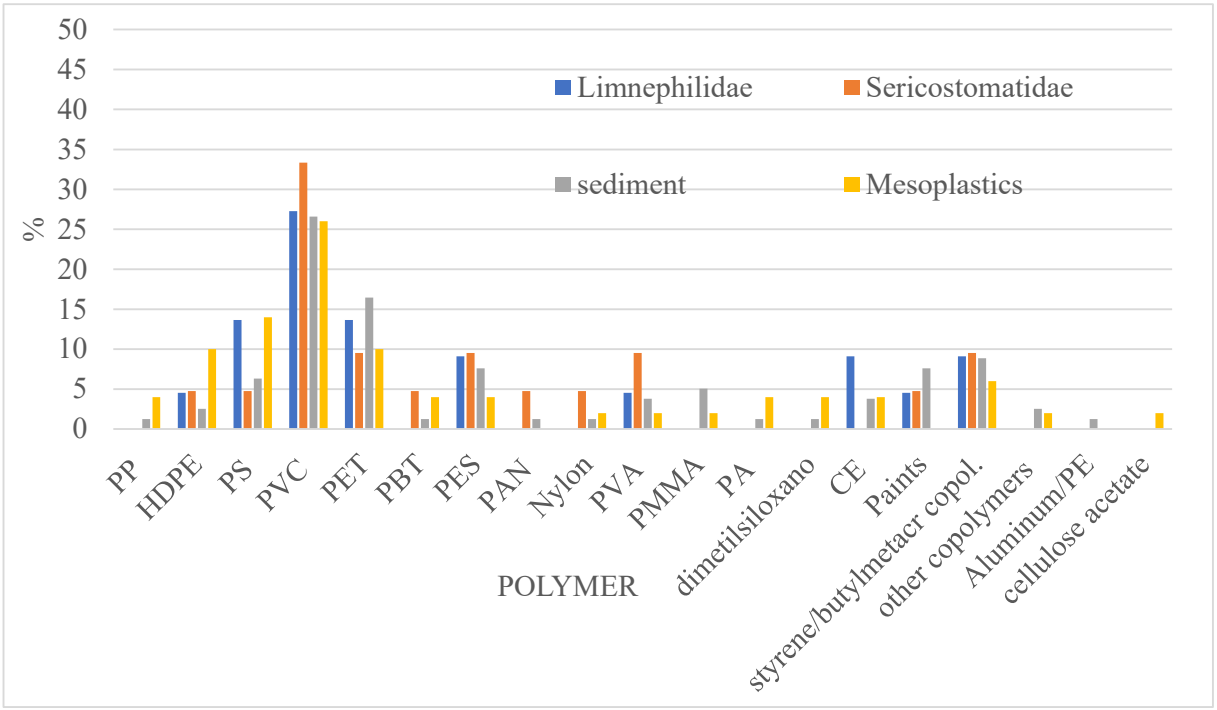

Figure 4. Percentage of relative abundances of polymers (acronyms in Table 1) found in caddisfly cases and river sediment.

Although in different and lower percentages between the two groups, common in both caddisfly families were other polymers such as polyethylene (PE), polyester, polystyrene (PS), and polyvinyl alcohol (PVA). Diverse distribution instead was observed for other polymers present in either group, such as cellophane (CE) and copolymer present only in Limnephilidae, while PE, nylon, polybutylene terephthalate (PBT), polyacrylonitrile (PAN), and polyester (PES) were found in Sericostomatidae only.

The degradation process due to thermo-oxidative processes, UV exposure, and mechanical abrasion modifies the surface of polymer materials; therefore, many of the MPs derive from the degradation of macroplastics [3,65]. The characterisation of the polyethylene fragments found in the caddisfly cases and river sediment makes it possible to evaluate polymer material degradation. As an example, in some polyethylene fragments the carbonyl index (CI) has reached rather high values (0.03–0.07), with the same order of magnitude found by other authors and justifiable only by considering a long stay in the environment. In Figure 5, the comparison of the IR spectrum regarding the PE fragment and standard polymer is shown. The standard PE spectrum has two intense peaks at 3100–2900 cm^{-1} characteristic of the stretching of the C-H bonds of the alkyl chains, as well as around 1400 cm^{-1} the bending motions of the same. In the PE sample spectrum, the presence of the peaks of the groups R-O-R (about 1100 cm^{-1}), C=O (about 1700 cm^{-1}), and OH (about 3300 cm^{-1}) represents clear degradation evidence.

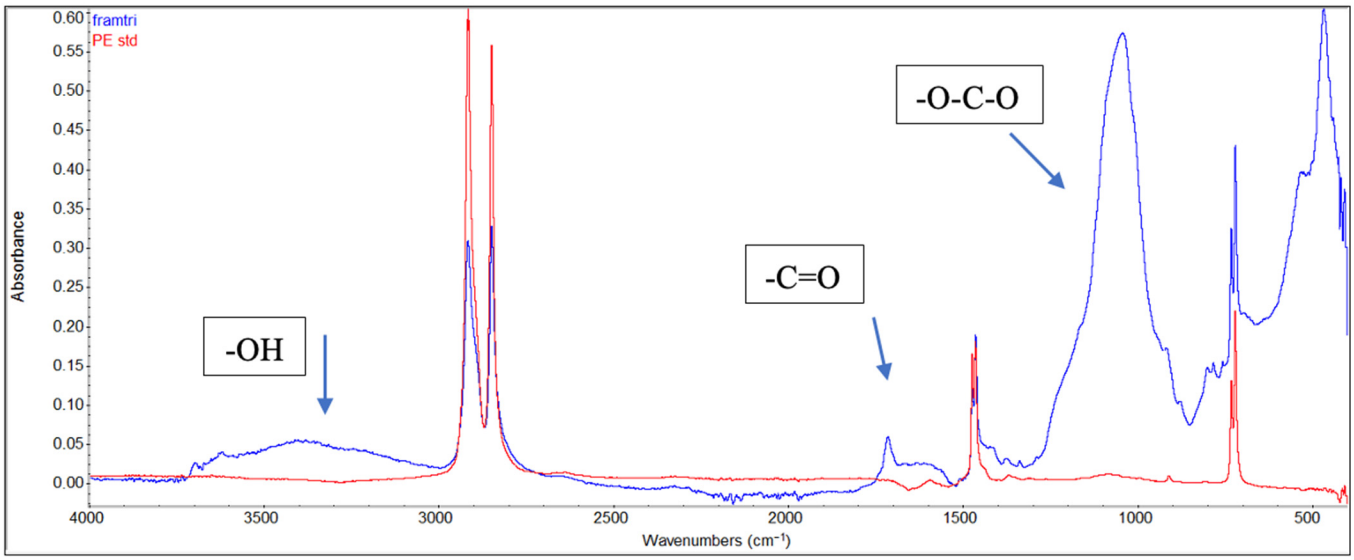

Figure 5. IR spectrum of the fragment (blue colour) and PE standard (red colour).

The predominance of secondary MPs and, in particular, fragments suggests that the main source of microplastic pollution in the Gari River is from degradation of larger plastic items from terrestrial sites [66]. The carbonyl index derived from the polyethylene IR spectra represents a confirmation of this sentence.

3.3. Sediment Analyses

The sampled substrate was also examined for the presence of MPs in relation to the macrobenthic component. Among the non-natural materials (576 items) found in the sediments, plastic accounts for 69% of the materials collected, and other materials, such as aluminium fragments, glass, and other materials, account for 23%, 6%, and 2%, respectively. Regarding the plastic fraction (352 items), microplastics represent 78%, while mesoplastics and macroplastics represent 18% and 4%, respectively. In particular, the data collected showed the presence of 542 plastic particles per square metre of riverbed surface area (corresponding to about 38 particles kg^{-1} considering a thickness of 1 cm and sand density

of 1400 kg m^{-3}), with micro-, meso-, and macroplastics ranging in size from 0.1 mm to 40 mm. As a comparison, sediments sampled in the urban tract of the Tame River (UK) were found to contain microplastics with an average abundance of 165 particles kg^{-1} [42].

In relation to the physical characteristics of the sampled particles, the collected microplastics showed a greater heterogeneity; they were mainly represented by fragments (78%), followed by films (18%), while spherical shapes represented 3% of particles. As a comparison, Ehlers et al. [47] found in 5 sediment samples taken from the Saynbach River, located in an urban park of Bedforf (D), fibres (86%) and films (14%), while polyethylene, acrylic, and polyester were the polymer materials.

As it was found for caddisfly cases, the most frequent colours were red and blue, and the polymer materials (Table 2) most represented in the sediment are PVC and PET, with 27% and 21%, respectively, as reported in Figure 4. These data corroborate the presence of the relationship between the MPs quantities and the presence in the caddisfly cases. Since FT-IR analysis revealed the presence of cellophane (density = 1.50–1.52 g cm^{-3}), a cellulose-based polymer widely used as cigarette and food wrappers, cellophane can be strongly related to urban pollution [67,68].

The presence of PVC, PET, PVA, PS, PE, PP, and CE polymers that are widely used in food and industrial packaging, household products, and disposable items appears to be quite common in other studies conducted in freshwater where the monitored area included an urbanised environment: the surface runoff contributes to the collection of washed-out waste that ends up in rivers [64,69]. Microplastics from tyre abrasion, although they are a significant source of environmental pollution, were not found. Probably this is due to both the proximity to the river source (<1 km) and the absence of very busy roads in the sampled site.

3.4. MPs Comparison

Dendrogram output for hierarchical clustering of polymer groups performed using the percentage of MPs found in the cases and in the sediments shows a great similarity (r = 0.9621), confirming that caddisfly larvae do not choose materials but take randomly what is present in their surroundings (Figure 6). These findings suggest a potential use of *Caddisflies* as potential bioindicators of MPs in freshwater habitats. Thus, in agreement with Ehlers et al. [47], case analysis can provide information on the diversity of MPs present in the caddisfly habitat.

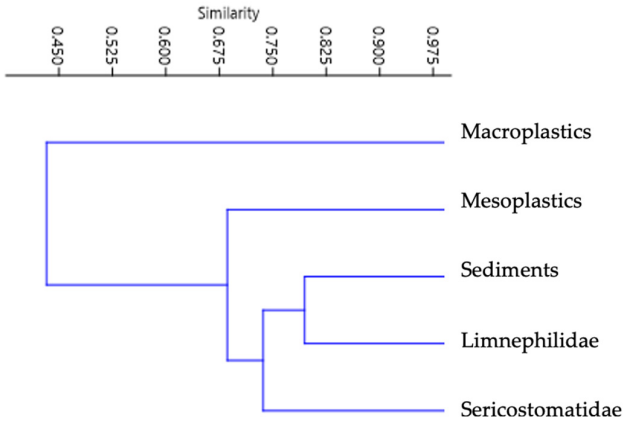

Figure 6. Dendrogram representing the hierarchical relationship between polymer groups. Paired Group UPGMA Bray–Curtis similarity r = 0.962, software PAST 4.1.

The most abundant MPs were of secondary origin, as evidenced by the predominant shape of the fragments present in both the substrate and the cases, confirming, together with the carbonyl indexes, that degradation processes of plastic items are strongly involved.

PVC and PET, according to their density (1.20–1.45 g cm^{-3} and 1.38–1.39 g cm^{-3}, respectively), as well as polyvinyl alcohol (PVA) (1.19–1.31 g cm^{-3}), although in lower percentages, were present in both matrices analysed. The co-presence of lighter polymers such as polystyrene (PS) and polyethylene (PE) (0.96–1.04 g cm^{-3} and 0.93–0.98 g cm^{-3}, respectively), found in both sediment and caddisfly, or polypropylene (PP) (0.89–0.93 g cm^{-3}), found only in sediment, reflects the contribution of multiple factors controlling MP deposition, such as different water flow velocities [70], riverine vegetation that might hold plastic debris in suspension, or an increase in MP density caused by a biofilm cover [71,72] could contribute to the sedimentation of low-density MPs, thus available to Trichoptera larvae [47].

4. Conclusions

MP particles were identified in water and sediments of a small river located in the south of Lazio; the composition, in terms of polymers, of the samples collected in the sediment is similar to the composition found in the cases; in particular, high portions of PVC and PET are used. In addition, this study, unlike the others, examines, by making a comparison for the first time, the cases of different Trichoptera families (Limnephilidae, Sericostomatidae, and Goeridae) in the same riverine habitats. Since the different caddisfly specimens have differentiated MP loading for case construction, if analysed together, they can better represent the MPs assortment present in their habitats [47]. Moreover, it has been confirmed that caddisfly larvae that construct cases in the Gavi River even from different families, have no rejection for MPs but actively incorporate them if they are available. The Goeridae family represents an interesting exception to those finding, as MPs were not found to be incorporated in their cases. The most abundant microplastics were of secondary origin, as evidenced by both the carbonyl index (an indicator of polymer degradation) and the predominant shape (fragments) present in both the substrate and the cases.

Despite the lack of a standardised procedure for monitoring microplastics in freshwater environments, this approach seems to be functional for monitoring the MP distribution in freshwaters and would be a cost-effective method for a systematic approach. Therefore, the use of bioindicator organisms to monitor the seasonal presence of MPs in freshwater systems can be a useful approach to obtain a comprehensive view of the problem [47,52]. Naturally, more research should be performed to develop a standard method to study MPs in the freshwater systems; our study could encourage the beginning of studies to be carried out in various freshwater ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments12100368/s1, Table S1: Chemical and physical parameters. In brackets, as a comparison, the limit values according to Italian legislation (D. Lgs 152/06): (a) discharge into surface water, (b) to produce drinking water.

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