Citizens' awareness and education for tackling microplastic contamination in freshwater ecosystems

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Abstract Microplastics (MPs) are ubiquitous and persistent contaminants, particularly in river sediments reaching high abundances with potential to impair ecosystem functions and services of great importance to humankind. Thus, monitoring programmes and public awareness are pivotal to implement mitigation and remediation strategies. This study evaluated the abundance of MPs in freshwater sediments and in benthic macroinvertebrates collected at four sites with different anthropogenic pressures of Costa/Couros river (Guimarães - Portugal), in order to alert, raise awareness and educate local citizens for (micro)plastic pollution. Results showed higher levels of MPs in the sediments of sites with more anthopogenic preassure. High number of MPs was also observed inside macroinvertebrates' gut from all sites, particularly in low weight organisms indicating malnutrition and digestive disorders. Such results were incorporated into public awareness campaigns to increase the citizens' knowledge and understanding about MPs prevalence and threats to the environment, human health and the economy, as well as spread individual measures and actions that can reduce MP contamination in aquatic ecosystems. This educational study also promoted the artistic creation in public spaces to raise awareness about plastic pollution.

Keywords: Microplastic; Sediments; Aquatic organisms; Citizens' awareness; Environmental education

1. Introduction

The presence of microplastics (MPs, i.e., plastic fragments less than 5 mm in length) in the environment is a serious global issue because of its ubiquity, persistent nature, and potential threat imposed to ecosystems, organisms and human health (Li *et al.*, 2018; Veethaak and Legler, 2021), along with its socio-economic losses (GESAMP, 2015). Such polymeric small particles can result from the fragmentation of large plastics improperly discarded in the environment, by mechanical abrasion, biological degradation, or photodegradation (Browne *et al.*, 2007; Andrady and Neal, 2009; Cole *et al.*, 2011); or can be intentionally manufactured in microscale (Boucher and Friot, 2017). Achieving a micro-sized MP, these particles can be easily transported by wind and currents, reaching even the most remote areas (Thevenon *et al.*, 2014). Estimations suggest that 17 to 32% of the oceans plastic residues come from rivers annually (Lebreton *et al.*, 2017), and more than 60 billion MPs are flown from rivers into the sea daily, worldwide (GESAMP, 2015).

Several studies have shown that the abundance of MPs in freshwater environments can be higher than in marine ones (e.g., Peng *et al.*, 2017), with higher levels in the sediments (Hurley *et al.*, 2018). Contaminated sediments represent a serious threat to the health of aquatic ecosystems (Shumchenia *et al.*, 2018) that provide the habitat, substrate and food source to various organisms (Hurley *et al.*, 2018). It is estimated that sediments from freshwater ecosystems such as rivers, lakes or groundwater are the shelter to 100,000 benthic macroinvertebrate species, 10,000 algae species and more than 20,000 bacteria and protozoa (Palmer *et al.*, 1997, 2000). Recent studies have reported the ingestion of small-sized MPs (< 0.3 mm) by benthic macroinvertebrates of high ecological relevance (e.g., high biomass, crucial role on food-webs) and shown its negative effects at different levels of biological organisation (e.g., Scherer *et al.*, 2017; Ziajahromi *et al.*, 2018; Silva *et al.*, 2019, 2021a, b). Consequently, monitoring MPs contamination in freshwater ecosystems and raising awareness among the citizens are imperative for the successful implementation of mitigation and remediation strategies.

For this purpose, the presence and amount of MPs were assessed in freshwater sediments and in the gut of benthic macroinvertebrates collected from a watercourse within the municipality of Guimarães - Portugal, the Costa/Couros river. This monitoring study aimed to alert, raise awareness and educate the citizens of Guimarães about the sources and impacts of MPs in freshwater ecosystems by using a local case study.

2. Materials and Methods

2.1. Sampling sites and abundance of microplastics

Sediment and benthic macroinvertebrates were collected at four sampling sites in the Costa/Couros river during the summer of 2020. Costa/Couros river springs in Penha Mountain and runs for about 6.2 km until it flows into Selho river (Duarte *et. al*., 2016). Sampling sites represent different types of anthropogenic pressure, with C1 site located near the spring in Guimarães city park, C2 and C3 located in the urban centre of the city, and C4 located in Veiga de Creixomil, which is part of the National Agricultural and Ecological Reserves (Fig. 1).

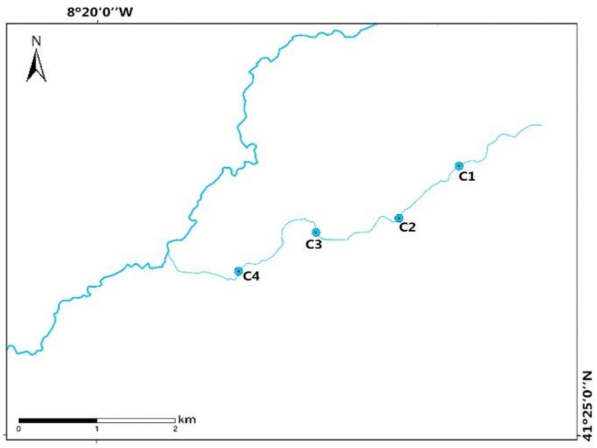

Figure 1. Location of the sampling sites in Costa/Couros river (C1 to C4), in the municipality of Guimarães.

Sediments were collected along a transept traced in a deposition zone (± 3 cm from the top layer; 5 replicates/site with a distance of 1 m between replicates) and placed in glass jars (1 L). Benthic macroinvertebrates (Oligochaeta, Lumbricid; 10 replicates/site) were collected using a hand net, following the national guidelines for the Water Framework Directive implementation (INAG, 2008), and preserved in 70% ethanol.
The extraction and quantification of MPs in sediments and in biological samples followed the protocols of Silva *et al.* (2019) and Prata *et al*. (2020). Briefly, the sediment samples were dried (60 ˚C; ± 72 h), sieved (5 mm, 1 mm and 0.5 mm) and weighed. Extraction and quantification of MPs from the sediments fraction > 0.5 mm in size were performed by hand under a binocular magnifying glass (Leica EZ4 HD). For the sediment fractions < 0.5 mm in size, MPs were extracted by density separation (NaCl 5M; 1:3 ratio). The supernatants containing MPs were vacuum filtered onto glass fibre membranes (Watman, Grade GF / F: 0.7 µm; diameter: 47 mm), treated to remove the organic matter ($H_2O_2 + FeO_4S7H_2O$) and stained with Nile Red dye (1 µg/mL of ethanol).
Organisms were weighed on a precision scale, digested (with 10% KOH; 60 ˚C ± 48 h), filtered and stained with Red Nile. Membranes containing MPs (from sediments or biological tissues, stored in glass petri dishes) were allowed to dry at room temperature. MPs were captured under an optical microscope (Leica DH750) in a darkroom, with an UV 470 nm light (Optimax ™ OFK-450A). Using an orange filter, particles presenting red fluorescence (with defined edges) were counted as MPs. Quantification and particles characterisation was performed using the free software ImageJ2 (Rueden *et al*., 2017). Contamination of the experiment was avoided by using cotton lab coats, by previously washing all the material with filtered distilled water, by avoiding plastic materials and by covering containers with aluminium foil, amongst other precautions.

2.2 Statistical analysis

The data sets for the organism and sediment analysis followed the D'Agostino & Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov normality tests to assess gaussian distribution. When normal distribution was verified, an ordinary one-way ANOVA, and Tukey's multiple comparison test were performed. When it was not verified, the data followed the non-parametric Kruskal-Wallis test and Dunn's multiple comparison test. In the analysis of the oligochaetes weight, definitive outliers were identified and removed according to the ROUT method (Q = 0.1%). All statistical analysis was done with GraphPad (GraphPad Prism version 9.0.0 (121) for Windows). A significance level of 0.05 was considered for all tests.

3. Results and Discussion

The results showed that MP contamination is widespread in Costa/Couros river, both in the sediments (Figs. 2A and 2B) and in the digestive tract of oligochaetes (Fig. 2C) from all the sampling sites. Significant differences between sites were found for the number of MPs existing in the digestive tract of oligochaetes, with lower abundance of MPs occurring in C3 compared to the other sites (H= 23.23; $p < 0.0001$; Fig. 2C). The size (in µm) of the plastic particles ingested by oligochaetes was significantly different between C3 and C4 sites ($F (3, 36) = 4.224$; $p = 0.0117$). This study also shows that oligochaetes are usually able to ingest particles of different sizes ranging from 20 to 90 µm (Table 1).
No significant differences were found between sampling sites for the amount of MPs with size < 0.5 mm (Fig. 2A) and size > 5 mm (Fig. 2B) present in the sediments ($F (3, 16) = 0.3851$; $p = 0.7652$; $F (3, 16) = 0.9467$; $p = 0.4414$). However, when comparing the different MPs sizes (> 0.5 mm VS < 0.5 mm) at each sampling site, there were significantly more MPs with size < 0.5 mm than MPs with size > 5 mm at all sampling sites (C1: t = 5.631, df = 4.000, $p = 0.0049$; C2: t = 5.243, df = 4.000, $p = 0.0063$; C3: t = 5.469, df = 4.000, $p = 0.0054$; C4: t = 7.182, df = 4.000, $p = 0.0020$). These results indicate that the MPs contaminating Costa/Couros river are mainly primary MPs (e.g., pellets, powders, and beads). It was also obvious that aquatic organisms ingested (un)intentionally MPs within the size range responsible for the contamination of all sampling sites. The presence of high number of MPs inside the gut of the organisms from all sites except C3, seems to be associated with low weight of the organisms (Table 2), since significant differences between sites were found for the weight of oligochaetes, with higher weights occurring in C3 compared to the other sites ($F (3, 46) = 36.88$; $P < 0.0001$). This might indicate malnutrition and digestive disorders (Litterbase, 2020). Significant levels of MPs were also found in the gut of other sediment-dwelling organisms such as chironomids and *Tubifex* sp. (Hurley *et al*., 2017; Nei *et al*., 2017)

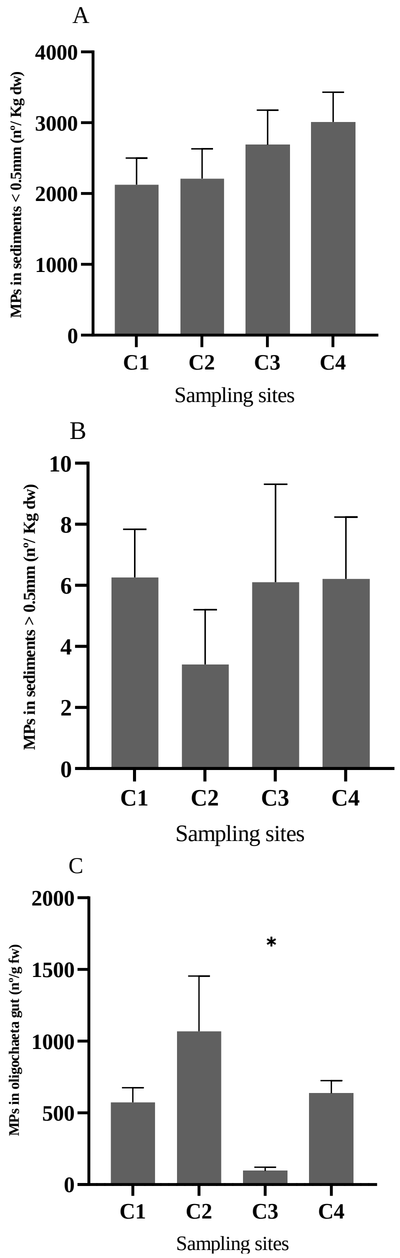

Figure 2. Number of MP extracted from sediments (A, fraction > 0.5 mm; B, fraction < 0.5 mm) and oligochaetes' gut (C). Data are presented as mean ± SEM. Asterisks indicate significant differences ($p < 0.05$) as indicated by the Kruskal-Wallis followed by Dunn's multiple comparison test.

Table 1. Minimum, maximum and mean ± SEM sizes of random samples of ten MPs existing in the digestive tract of oligochaetes collected from four sampling sites of Costa/Couros river (C1 to C4).

	C1	**C2**	**C3**	**C4**
Min. (µm)	20	30	40	20
Max. (µm)	70	70	90	40
Mean (µm)	44	47	53	31
± SEM	± 5.62	± 3.96	± 4.96	± 3.15

Table 2. Minimum, maximum and mean ± SEM weight of the oligochaetes collected from four sampling sites of Costa/Couros river (C1 to C4), analysed after the removal of outliers.

	C1	**C2**	**C3**	**C4**
Min. (g)	0.076	0.007	0.158	0.008
Max. (g)	0.134	0.520	0.744	0.019
Mean (g)	0.111	0.081	0.464	0.010
± SEM	± 0.005	± 0.046	± 0.045	± 0.001

Moreover, a number of MPs (> 500 items) ranging between 20-60 µm found in the gut of chironomids triggered an anti-inflammatory and immune response leading to an oxidative stress condition of larvae and impairment of development and reproduction (Silva *et al.*, 2020, 2021a,b). Most of these MPs might come from human activities related to agriculture and urbanized areas in the vicinity of the sampling sites. According to research works, the main entry routes of MPs in aquatic systems are road runoff (66%) and effluents from wastewater treatment plants (25%) (Boucher and Friot, 2017).

The results of this study results were integrated into public awareness campaigns to promote citizen science, thus bringing society (school community and green brigades in a first approach) closer to science in a participatory way. These campaigns aimed to increase the citizens' knowledge and understanding about MPs and their threats to the environment, human health and the economy, as well as to spread individual measures and actions that can reduce MP contamination in aquatic ecosystems. This educational study also promoted the artistic creation in public spaces to raise awareness about plastic pollution (some campaigns can be seen in Youtube Videos, e.g., 'Aqualastic: + water – plastic', 2021; 'Aqualastic documentary', 2021).

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