



Original Articles

Effectiveness of a methodology of microplastics isolation for environmental monitoring in freshwater systems

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ABSTRACT

The accumulation of plastics in aquatic systems constitutes an emerging scientific and societal concern, because of their ubiquity, high persistence and insufficient management by sewage and wastewater treatment processes. Microplastics (< 5 mm), a group of particles differing in physico-chemical properties (e.g. size, shape, colour, density and polymer type), are of particular concern as they can reach high densities and can interact with biotic and abiotic environment. Moreover, potential of bioaccumulation increases with decreasing of particle size. Although microplastics have been widely investigated in marine systems, very little attention is paid to freshwater systems. As the concern about microplastics started appearing recently, there is no unified method for microplastic isolation, which result in inconsistent data that differs in quality and resolution. Hence, this work aims to assess the effectiveness of distinct isolation methods as an attempt to identify and establish a unified method for environmental monitoring of aquatic systems. For that, artificial samples containing eleven plastics belonging to the most common types of polymers (e.g. low/high-density polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate) were prepared and subjected to different methods, including density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods with hydrogen peroxide (wet peroxide oxidation) and multienzymatic detergent (enzymatic digestion). The samples then underwent the detection, quantification, and identification of polymers using a stereomicroscope and Fourier transform infrared spectroscopy (FTIR). Several criteria were considered in order to achieve the aims of this work: efficiency of density separation and organic matter degradation, the total mass of recovered polymers, cost of each procedure, the time spent with each method, the simplicity, and the quality of recovered polymers. Based on this multi-criteria approach, this study concludes that the wet peroxide oxidation with addition of zinc chloride was the most effective method.

1. Introduction

Since the middle of the 20th century, the worldwide production of plastics has increased exponentially, reaching 322 million tonnes of plastics in 2015 (PlasticsEurope, 2016). Plastics possess a unique set of properties such as lightness, inexpensive, versatility, durability, resistance and strength (Thompson et al., 2009) that provide remarkable benefits (e.g. technological advances and energy savings) for many industries and almost every sector of our everyday life (Andrady, 2011; Andrady and Neal, 2009; Dris et al., 2015). According to PlasticsEurope (2016), the preference for more ecologically benign management options regarding plastics waste are increasing (e.g. recycling and energy

recovery). Notwithstanding, in many EU countries landfill is still the first option. The insufficient waste management coupled with high production, physical characteristics (chemical inertness and slow biodegradation), and improper waste disposal (e.g. industry, urban waste, sewage treatment plant – STP, agriculture, accidental) results in an accumulation of plastic debris in the environment, in particular in aquatic systems (Barnes et al., 2009; Dris et al., 2015; Eubeler et al., 2010; Thompson et al., 2004; Urgert, 2015). This contamination not only includes plastic debris with large size (macroplastics) but also microplastics (Dris et al., 2015). Currently, microplastics (MPs), usually defined as particles with less than 5 mm (Arthur et al., 2009), can differ in shape, colour, specific density and polymer type as well as in their

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origin (primary, if they are produced in a micro-size range for direct use or as precursors to other products; or secondary, if they result from the continuous fragmentation of macroplastics caused by a combination of abiotic and biotic mechanisms) (Barnes et al., 2009; Cole et al., 2011; Duis and Coors, 2016; Eubeler et al., 2010; Lucas et al., 2008; Mintenig, 2014).

MPs are considered contaminants of emerging scientific and societal concern (Wagner et al., 2014), as they can reach high densities (e.g. 6698,264 particles km⁻², according to McCormick et al. (2014)) and can interact with abiotic (e.g. Arthur and Baker, 2011; Simpson et al., 2005) and biotic environment (e.g. Blarer and Burkhardt-Holm, 2016; de Sá et al., 2015; Green et al., 2017; Huerta Lwanga et al., 2016; Neves et al., 2015; Oliveira et al., 2013; Rehse et al., 2016; Rochman et al., 2013; Silva-Cavalcanti et al., 2017; Tosetto et al., 2017). This interaction causes negative impacts in organisms such as physical impacts (e.g. blocked digestive tracts, debilitation, limited predator avoidance, or death/immobilisation) (de Sá et al., 2015; Eerkes-Medrano et al., 2015) and/or toxic impacts (e.g. liver stress response or inhibition of acetylcholinesterase activity) (Oliveira et al., 2013; Rochman et al., 2013) that can induce cascading effects with trophic and ecosystem consequences (Eerkes-Medrano et al., 2015; Lechner et al., 2014; Schindler and Scheuerell, 2002). The toxic impacts can be provoked by MPs and/or contaminants that adsorb to them (Oliveira et al., 2013; Rochman et al., 2013). Moreover, potential for bioaccumulation increases with decreasing of particle size.

Although micro-debris is not a new problem, only recently has substantial data on MPs pollution become available (Faure et al., 2012; GESAMP, 2015). The studies about MPs in aquatic ecosystems (between 2004 and 2017) are mainly focused on marine systems (85.03%), while freshwater systems have received very little attention (14.97%). In addition, for several authors rivers are being seen as an important carriage systems of MPs from terrestrial to marine environment (Eerkes-Medrano et al., 2015; Hidalgo-ruz et al., 2012; Klein et al., 2015; Wagner et al., 2014), acting as temporary sinks (Blair et al., 2017).

As the concern about MPs started appearing recently, there is no unified methods for MPs detection and monitoring (sample collection and preparation; MPs identification and quantification) in freshwater systems. This could result in inconsistent data that differs in quality and resolution, not allowing data comparison between different studies (large-scale spatial and temporal comparisons) (Duis and Coors, 2016; Löder and Gerdts, 2015). The development of a simple, low-cost and accurate method, as well as one that minimizes contamination is a main challenge of the scientific community (Eerkes-Medrano et al., 2015). Sample analysis is one of the most questionable procedures that commonly consists in size fraction sieving, organic matter removal, density separation, filtration, visual sorting and Fourier transform infrared spectroscopy (FTIR) identification (Cole et al., 2014; Hidalgo-ruz et al., 2012; Masura et al., 2015; Qiu et al., 2016; Tagg et al., 2015). Similar approaches to those implemented in marine environments have been used for freshwater systems (Blair et al., 2017; Hidalgo-ruz et al., 2012).

Hence, this work aims to identify and establish a cost-effective method to be applied in environmental monitoring programs, based on a multi-criteria approach, including: cost; density separation and organic matter degradation efficiency; total mass of recovered polymers; time spent with each method; simplicity; and quality of recovered polymers. For that, artificial freshwater samples containing eleven plastic products were prepared and subjected to six distinct methods, selected according to their common application and efficiency: density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods with hydrogen peroxide (wet peroxide oxidation) and multienzymatic detergent (enzymatic digestion).

Table 1

Identification and characterization of the 11 secondary MPs, according to the information obtained from package label/FTIR, visual inspection and literature. RIC – Resin Identification Code.

Polymer type	RIC	Source	Colour	Shape	Density (g cm ⁻³)
Low-density polyethylene (LDPE)	4	Supermarket bag	White Red	Film	0.89–0.93
Polyethylene (PE)		Milk box (Tetra Pak® packaging)	Blue Beige Silver	Fragment Fiber	0.89–0.98
		Toilet paper packaging	Clear Green	Fragment	
High-density polyethylene (HDPE)	2	Liquid yogurt	White	Fragment	0.94–0.98
Polypropylene (PP)	5	Pasta packaging	Clear White Yellow Green Black Red	Film Fragment	0.85–0.92
		Drinking straws	Orange Yellow		
Polystyrene (PS)	6	Rope	Clear		1.04–1.09
		Solid yogurt	Pink Green Yellow	Fragment	
Polyvinyl chloride (PVC)	3	Pipe	Grey	Fragment	1.16–1.58
Polyethylene terephthalate (PET)	1	Fabric Water bottles	Blue Blue	Fiber Fragment	1.37–1.45

2. Materials and methods

2.1. Microplastic preparation

Eleven different plastic products widely used in everyday life were cut into pieces before being further fragmented using the coffee grinder. These particles were passed through 5 mm sieve and material sized > 5 mm were discarded. Based on package label (e.g. Resin Identification Code (RIC) – recycling code (ASTM, 2013)), literature (Driedger et al., 2015; Kim et al., 2006; Mintenig, 2014; Wagner et al., 2014) and visual inspection, each MPs sample was characterized by its colour, shape, reference density and, in some cases, polymer type (see Table 1). Although of varying shape, these secondary MPs were mostly fragments (thick pieces with three size dimensions comparable). The samples were analyzed by FTIR to confirm the previous identification (see Supplementary Figs. S.1–S.11). These 11 plastics products included 5 of the most common types of polymers (PlasticsEurope, 2016) such as low/high-density polyethylene (LD/HDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET).

2.2. Samples preparation

In order to represent a realistic freshwater system with controlled contamination (only with MPs), artificial samples containing synthetic freshwater (75 mL), different types of MPs (see above; 0.05 g each), organic matter (cladocerans – 25 organisms, duckweed – 6 organisms and chestnut leaves – 0.05 g) and sediment (sand – 2 g) were prepared. The artificial samples were stirred for 5/10 min and allowed to stand overnight. Based on Smith et al. (2002), synthetic freshwater was prepared by adding magnesium chloride hexahydrate (MgCl₂·6H₂O), calcium chloride (CaCl₂), sodium sulfate (Na₂SO₄), potassium bicarbonate (KHCO₃) and sodium bicarbonate (NaHCO₃) to Milli-Q water, and stirring for 30 min.

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