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New techniques for the detection of microplastics in sediments and field collected organisms

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ABSTRACT

Microplastics have been reported in marine environments worldwide. Accurate assessment of quantity and type is therefore needed. Here, we propose new techniques for extracting microplastics from sediment and invertebrate tissue. The method developed for sediments involves a volume reduction of the sample by elutriation, followed by density separation using a high density NaI solution. Comparison of this methods' efficiency to that of a widely used technique indicated that the new method has a considerably higher extraction efficiency. For fibres and granules an increase of 23% and 39% was noted, extraction efficiency of PVC increased by 100%. The second method aimed at extracting microplastics from animal tissues based on chemical digestion. Extraction of microspheres yielded high efficiencies (94–98%). For fibres, efficiencies were highly variable (0–98%), depending on polymer type. The use of these two techniques will result in a more complete assessment of marine microplastic concentrations.

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1. Introduction

Plastic marine debris has been an environmental concern for decades (Derraik, 2002; Gregory, 2009; Hammer et al., 2012; Moore, 2008; Thompson et al., 2009). Despite the increased international attention, the build-up of these materials in the environment is considered problematic due to an increasing global plastic production and the continuing improper disposal of plastic waste. The impacts of plastic debris on marine species are widely reported (Derraik, 2002; Gregory, 2009). Up to now, over 660 marine species worldwide are known to be affected in by plastic waste one way or another (GEF, 2012). Relatively large items will, however, eventually undergo fragmentation under the influence of UV radiation, the oxidative properties of the atmosphere and hydrolytic properties of seawater (Andrady, 2005, 2011; Webb et al., 2013). Combined with the mechanical forces exerted by wave action, plastic items will break up into smaller particles (Barnes et al., 2009). Because of the large residence times of plastic debris in our seas and oceans, most plastic present in the marine environment fits in the smaller size classes. For instance, 72% of the plastics recovered from beaches in Portugal belonged to a size class $\leqslant$5 mm (Martins and Sobral, 2011). Similarly, plastic particles <1 mm accounted for 65% of total marine debris collected on beaches in the Tamar Estuary (UK) (Browne et al., 2010). These small items of plastic debris are commonly referred to as microplastics. Many authors have defined microplastics as particles smaller than 5 mm (e.g. Arthur et al., 2009) while other have set the upper size limit at 1 mm (e.g. Costa et al., 2010). While the value of 5 mm is more commonly used, 1 mm is a more intuitive value (i.e. 'micro' refers to the micrometer range). Moreover, once particles are smaller than 1 mm they can potentially be ingested by a range of aquatic invertebrates. Bivalves for instance will preferably ingest and process particles less than 40 μm, but larger particles (up to 600 μm) can be ingested and processed as well (Cefas, 2008).

Microplastics have been detected on beaches and in subtidal sediments worldwide (Table 1). The extraction method used by the majority of these authors was developed by Thompson et al. (2004). This technique, which is currently the most widely used (Hidalgo-Ruz et al., 2012), relies on the density of a concentrated NaCl solution (1.2 kg L^{-1}) to separate sediment from microplastic particles. Indeed, when this salt solution is added to the sediment sample, low density microparticles float to the surface. However, this method is only effective for polymers with a density lower than that of the saturated saline concentration, i.e. 1.2 g cm^{-3}, and not suitable for the extraction of high density polymers. Plastics such as polyvinylchloride (density 1.14–1.56 g cm^{-3}) or polyethylene terephthalate (density 1.32–1.41 g cm^{-3}) will not float in this concentrated NaCl solution. These two polymers, however, represent 18% of the European plastic demand (PlasticsEurope, 2012) and as such could represent an important proportion of the microplastics present in the marine environment. Especially in marine sediments, the proportion of these high density plastics

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Table 1
Maximum concentrations of microplastics found in sediments worldwide. All concentrations are expressed as either number of particles kg^{-1} dry sediment or mg kg^{-1} dry sediment.

Country	Location	Maximum concentration	Unit	Refs.
India	Ship-breaking yard	89	mg kg^{-1}	Reddy et al. (2006)
UK	Beach[a]	9	# kg^{-1}[b]	Thompson et al. (2004)
UK	Estuarine[a]	35	# kg^{-1}[b]	Thompson et al. (2004)
UK	Subtidal[a]	86	# kg^{-1}[b]	Thompson et al. (2004)
Singapore	Beach	16	# kg^{-1}	Ng and Obbard (2006)
UK	Sewage disposal site	15	# kg^{-1}[b]	Browne et al. (2011)
Belgium	Harbour	391	# kg^{-1}	Claessens et al. (2011)
Belgium	Continental shelf	116	# kg^{-1}	Claessens et al. (2011)
Belgium	Beach	156	# kg^{-1}	Claessens et al. (2011)

[a] Only fibre concentrations were reported.
[b] Original unit (# fibres 50 mL^{-1} sediment) converted using an average sediment density of 1600 kg m^{-3} (Fettweis et al., 2007) and 1.25 as average wet sediment/dry sediment ratio.

could be higher: because of their high density, these plastic types will tend to sink more easily than lighter plastics. Since the techniques currently used to extract microplastics from sediments are not efficient in extracting all types of plastics, the concentrations reported in these studies may be underestimates.

To date, vertebrates have been the primary focus concerning plastic ingestion (e.g. Denuncio et al., 2011; do Sul et al., 2011; Laist, 1997; Lazar and Gračan, 2011; Poppi et al., 2012; van Franeker et al., 2011). However, as the plastic breaks down, it becomes available for ingestion by a much wider range of (smaller) organisms (Barnes et al., 2009; Betts, 2008). Recently, it has been shown that invertebrates, such as polychaete worms, barnacles, amphipods and sea cucumbers, can ingest microscopic plastics particles during laboratory trials (Graham and Thompson, 2009; Thompson et al., 2004). In these experiments, the presence of microplastics in the gut, and hence ingestion of these particles, was demonstrated using analysis of casts and dissection of the intestinal tract (Graham and Thompson, 2009; Thompson et al., 2004) as well as histological techniques (Browne et al., 2008). It has also been demonstrated that very small plastic particles (<10 μm) can translocate to the circulatory system of the bivalve *Mytilus edulis* (Browne et al., 2008). Although no significant adverse effects of ingestion and translocation of microplastics have been observed during these laboratory trials (Browne et al., 2008), the presence of microplastics in the environment still raises toxicity concerns, since plastics are known to contain and/or adsorb high concentrations of organic contaminants (Hirai et al., 2011; Mato et al., 2001; Rios et al., 2007; Teuten et al., 2007, 2009). The fate of these contaminants is, however, poorly understood, and recently Gouin et al. (2011) suggested that microplastics are "likely of limited importance" as vectors of the pollutants to marine organisms. Lack of supporting studies, identification of critical data-gaps (Gouin et al., 2011) and the lack of appropriate techniques to extract plastic particles from (soft) organic tissue justify the on-going interest in the presence of microplastics in marine organisms.

In this study, two new techniques to determine the presence and abundance of microplastics in natural samples are described. For sediments, data currently available on the concentrations of microplastics may be biased, since it is not possible to detect high density plastics using a saturated salt solution as frequently used (Hidalgo-Ruz et al., 2012) and initially described by Thompson et al. (2004). Using high density chemicals like sodium iodide (NaI) could resolve this. However, these are expensive to use: 1 kg of NaCl costs less than €1, while 1 kg of NaI costs approximately €70. In order to improve the cost efficiency of microplastics extraction from sediments, a new method using a fluidized sand-bath and a small volume of NaI is proposed. Also, whether or not organisms from natural populations contain microplastics is not known, as ingestion by invertebrates has only been demonstrated in laboratory trials. Determining the plastic body burden of resident, marine organisms is thus important for our understanding of the effects of microplastics. Therefore, a new technique was developed for detecting microplastics in tissue, involving a depuration phase followed by chemical digestion of the tissue.

2. Materials and methods

2.1. Extracting microplastics from sediments: elutriation and flotation

A device was developed to extract microplastics from sediment based on the principle of elutriation. Elutriation is a process that separates lighter particles from heavier ones using an upward stream of gas or liquid. This principle has, for example, been used extensively in marine biology for separating meiofauna from sand with an apparatus called "Barnett's fluidized sand-bath" (Southwood and Henderson, 2000).

Based on this design, a new apparatus was developed, represented schematically in Fig. 1. A PVC column (147 cm length with an internal diameter of 15 cm) is fitted with a 1 mm sieve on top and a 35 μm mesh screen (supported by a strong 1 mm mesh screen to support the weight of the sediment) at the bottom. A sediment sample of 500 mL is transferred into the column by washing it through the 1 mm sieve to remove all large debris. A sieve cover is used to prevent contamination with particles or fibres transported through the air. An upward water flow is then created by forcing tap water through the column from below. At this point the sediment becomes fluidized. At the bottom of the column, aeration is provided to ensure efficient separation of plastic and sediment particles. In order to avoid the creation of dead zones (without aeration) an aeration system using three large air stones ($50 \times 25 \times 25$ mm, Dohse Aquaristik) was constructed. The water flow, combined with the aeration, separates the lighter particles, including microplastics, from the heavier sand particles, and the rising water takes them to the top where they eventually flow over the edge and are retained on a 35 μm sieve (a smaller mesh size can be used if desired, or a series of sieves with decreasing mesh size to avoid clogging). The flow rate of the water is adjusted to achieve a maximum extraction efficiency and minimal contamination of the sample with sand: it was experimentally determined that this flow rate should be set at approximately 300 L h^{-1}, for 15 min. This rate was adequate to keep sand in the pipe while other material, including microplastics, flowed over the edge.

After this first clean-up step, the material collected on the 35 μm sieve subsequently undergoes a sodium iodide extraction (NaI-extraction). The solids are transferred to a 50 mL centrifuge tube, and 40 mL of a NaI-solution (3.3 M, with a density of approximately 1.6 g cm^{-3}), is added. This is followed by vigorous (manual) shaking and centrifugation for 5 min at 3500*g*. After centrifugation,

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