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Novel methodology to isolate microplastics from vegetal-rich samples

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ABSTRACT

Microplastics are small plastic particles, globally distributed throughout the oceans. To properly study them, all the methodologies for their sampling, extraction, and measurement should be standardized. For heterogeneous samples containing sediments, animal tissues and zooplankton, several procedures have been described. However, definitive methodologies for samples, rich in algae and plant material, have not yet been developed. The aim of this study was to find the best extraction protocol for vegetal-rich samples by comparing the efficacies of five previously described digestion methods, and a novel density separation method. A protocol using 96% ethanol for density separation was better than the five digestion methods tested, even better than using H_2O_2 digestion. As it was the most efficient, simple, safe and inexpensive method for isolating microplastics from vegetal rich samples, we recommend it as a standard separation method.

1. Introduction

Plastics are synthetic organic polymers with features, such as durability and low price, that make them perfect for many applications. Unfortunately, the same characteristics that make plastic the perfect material cause it to become a serious pollution problem. Recent studies report that 4.8 to 12.7 million metric tons of plastic were disposed to the ocean in 2010 (Jambeck et al., 2015). At present, plastic marine pollution is one of the major concerns of the scientific community and organizations responsible for environmental policies at the global level (Andrady, 2010, 2011; European Parliament, 2008; Galgani et al., 2010, 2013; Scientific and Technical Advisory Panel, 2011).

Plastic particles smaller than 5 mm are classified as microplastics (Arthur et al., 2009). Secondary microplastics are the product of degradation and fragmentation of larger plastics, while primary microplastics are manufactured with size < 5 mm, mainly for use in cosmetics, cleaning products or as raw material for the production of plastic products (pre-production pellets). Due their small size, microplastics can impact marine organisms including zooplankton. They can be ingested directly or indirectly through the food web (Barnes et al., 2009; Setälä et al., 2014). Their consumption is likely to constitute a chemical, physical, and biological hazard (Browne et al., 2008; Setälä et al., 2014; Teuten et al., 2009; Von Moos et al., 2012; Wright et al., 2013; Zettler et al., 2013).

To obtain reliable and reproducible data on microplastic contamination and to investigate its effects on marine biota and the environment, it would be beneficial to first harmonize and standardize the sampling, extraction, and quantification methods that are being used by the scientific community (MSDF Technical Subgroup on Marine Litter, 2013; Rochman et al., 2017). Sampling techniques, and analytical techniques to isolate and quantify microplastic samples from different environments, have been reviewed extensively (Besley et al., 2017; Hanvey et al., 2017; Hidalgo-Ruz et al., 2012; Lusher et al., 2017; Miller et al., 2017; Van Cauwenberghe et al., 2015). For microplastics extraction, most techniques are based on density separation via flotation (Claessens et al., 2013; Cole et al., 2015; Coppock et al., 2017; Imhof et al., 2012; Thompson et al., 2004). Density separation requires highly dense solutions, such as sodium chloride (NaCl, 1.2 g/cm^3), sodium iodide (NaI, 1.6 g/cm^3) and zinc chloride ($ZnCl_2$, 1.6–1.7 g/cm^3) because the specific densities of the most common plastics in environmental samples range from 0.01 g/cm^3 to 1.60 g/cm^3 (Table 1). Other separation strategies for microplastics include evaporation, filtration, sieving, and visual sorting (Crawford and Quinn, 2017; Hidalgo-Ruz et al., 2012; Masura et al., 2015; Song et al., 2014; Yamashita and Tanimura, 2007). These techniques are useful for isolating microplastics from sediments, but isolating them from biological material requires a different treatment. The density of the biological material (leaves, seeds, wood, etc.) is, in most cases, lower than the density of the solutions used in the separation process, and therefore they float together with microplastics. Another problem is that microplastics are

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Table 1
Density ranges of common plastic polymers (modified from Crawford and Quinn (2017)) and 96% ethanol.

Plastic polymers	Abbreviation	Density in g/cm^3
Polystyrene (expanded foam)	EPS	0.01–0.05
Polystyrene (extruded foam)	XPS	0.03–0.05
Polypropylene	PP	0.88–0.91
Low-density polyethylene	LDPE	0.92–0.94
High-density polyethylene	HDPE	0.94–0.97
Nylon 6.6	PA	1.05–1.10
Polyvinyl chloride	PVC	1.45–1.70
Polyethylene terephthalate	PET	1.40–1.60
Polystyrene	PS	1.04–1.05
Polystyrene (30% glass fibers)	PS	1.40–1.50
Polyurethane	PUR	1.20–1.40
Polyurethane (foam)	PUR	0.03–0.80
Ethanol 96%	EtOH	0.805–0.812

imbedded in the organic material and cannot be isolated by density only.

Several digestion techniques for the removal of the organic material in microplastic samples have been described (Catarino et al., 2017; Claessens et al., 2013; Cole et al., 2014; Dehaut et al., 2016). Many of them were specifically designed to be effective in extracting microplastics from animal tissue or zooplankton. However, techniques for digesting the algal and plant component of sediment samples have not been developed (Hanvey et al., 2017). This type of biological material is abundant in beach samples, and can even retain microplastics on its surface (Gutow et al., 2015). Finding a way to separate microplastics from this vegetal material is thus important to assess the extent of microplastic pollution in the aquatic environment. A recent study suggested that dried algae and seagrasses, among other residues present in the microplastic samples, could be removed by visual sorting or sieving, using the naked eyed or a microscope (Crawford and Quinn, 2017; Hidalgo-Ruz et al., 2012). These procedures may be acceptable for the biggest fragments, for large pieces of algae and leaves, and for a small number of samples. However, for the smaller particles and for a large number of samples, these procedures are time consuming and are likely to lead to underestimating the extent of microplastics pollution.

The objective of the present work was to find an efficient method to remove algae and plant material from microplastics samples. In order to achieve this, five existing digestion protocols based on HCl, NaOH, KOH and H_2O_2 treatments, and a novel density separation procedure using 96% ethanol (EtOH), were tested, and their separation efficacies were calculated and compared. In addition, the integrity of six types of plastic polymers (polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET; polyester fibers), and polystyrene (PS)) subjected to the different methodologies was studied in order to confirm that these methods do not damage plastic particles.

2. Materials and methods

2.1. Sampling collection and preparation

A one-liter sample was collected along the high tide line near the dunes at Famara beach, Lanzarote, Spain (N 29°6.941, W 13°33.461), on January 29th, 2016 (Fig. 1a). The sample was placed in a 5 l plastic container and mixed for 1 min with 3 l of sea water from the same beach. The supernatant fluid was then filtered through a 1 mm aperture mesh. No measures to prevent contamination were taken during sampling, because we did not have to determine the exact concentration of microplastics, but only had to obtain a representative sample. After separation of the samples in the laboratory, measures were taken to avoid contamination. All the procedures were done inside a fume hood. All personnel wore cotton laboratory coats. In addition, all the materials used, as well as the workplace, were cleaned with ultrapure water. The sample was always well protected to avoid contamination in the laboratory. However, to evaluate contamination, should it occur, two clean filters were exposed during the digestion procedures and density separation. They were then examined immediately after each procedure under a microscope. No contamination was found on any of them.

The sample was composed of organic matter (mainly vegetal debris) at a concentration (w/w) of 1/6 and of microplastics, 5/6 (Fig. 1b). In order to avoid differences in the separation efficiencies due to the different amounts of organic material present in the samples, we homogenized the sub-samples. To accomplish this, the microplastics and organic matter were manually separated. Then, replicate sub-samples of 6 g each, composed of 1 g of biological material and 5 g of microplastics, were taken (Fig. 2). Before being subjected to each of the protocols, the sub-samples were oven-dried at 60 °C and weighed on a high precision balance (0.1 mg). When we were able to confirm that the treatment used was safe for plastics, we were certain that any "weight loss" was due to digestion or separation of organic matter.

2.2. Separation efficacy

Five existing protocols to digest organic matter were tested for vegetal rich samples: 3% HCl, 40% NaOH, 4% NaOH + SDS, 10% KOH and catalytic 30% H_2O_2 (chemical solutions information in Table 2). In addition, density separation by 96% EtOH (16.44 M) was tested (Table 2). Triplicates of sub-samples composed of 1 g of biological material and 5 g of microplastics were processed with each protocol.

Protocol 1

Protocol 1 corresponded to the acid digestion method tested by Cole et al. (2014). The sample was previously oven-dried at 60 °C, then 40 ml of 3% HCl (1 M) were added to sub-samples, they were stirred for a minute, and finally, maintained at room temperature (20 °C) for 24 h.

Protocol 2

Protocol 2 was based on the alkaline digestion method tested by Cole et al. (2014). As above, the sample was previously oven-dried at 60 °C, then 40 ml of 40% NaOH (10 M) were added to sub-samples, they were stirred for 1 min, and finally placed in an oven for 24 h, at 60 °C.

Protocol 3

Protocol 3 was adapted from Dehaut et al. (2016), and consisted of alkaline sample digestion. The sample was previously oven-dried at 60 °C, then 40 ml of 10% KOH (1.78 M) were added to the sub-samples, they were stirred for 1 min and maintained at 60 °C for 24 h in a drying oven.

Protocol 4

Protocol 4 is based on the work of Budimir et al. (2017), presented at MICRO 2016 International Congress. In this protocol, less concentrated NaOH was added to samples together with the detergent, SDS. Budimir describes an alkaline digestion procedure in which 10 ml of 4% NaOH (1 M) and 5 ml of SDS are added to the sub-samples, and in which only 2 h at 50 °C were enough to digest the biological material in the samples. The original protocol was modified in order to standardize all the procedures followed here. This was done by oven-drying the sample at 60 °C, adding 40 ml of NaOH and 20 ml of SDS, and maintaining it for 2 h in an oven at 50 °C. If no visual changes were observed in the sub-samples, they were maintained for 24 h at 60 °C.

Protocol 5

Protocol 5 was based on the Wet Peroxide Oxidation (WPO) method described by Masura et al. (2015). Here, only the WPO step was carried out despite Masura et al. (2015) describing several other steps for the analysis of microplastics on beach sediment samples. The sample was