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Development and Validation of an Efficient Method for Processing Microplastics in Biota Samples

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Abstract: The impacts of microplastics on aquatic ecosystems and biota are gaining attention globally. Although microplastics have been widely detected in biota, there currently are few standardized detection and identification methods. The present study developed a novel one-step digestion method which was evaluated with mussel and fish samples. This method employed nitric acid and hydrogen peroxide (HNO_3:H_2O_2 = 4:1 by volume) as digestion reagents, which completely digested biota samples <5 g weight within 30 min at 50 °C. A density separation step was subsequently used to remove organic residues as necessary. The efficiency and suitability of this method were tested by spiking microplastics of 7 different types and of various sizes (1000, 900, 675, 300, 250, and 150 μm) into mussel and gastrointestinal tracts of fish. The recoveries of microplastics ranged from 90 to 100%. No significant changes in weight, surface area, and particle size (*t* test, $p > 0.05$) were observed for all tested polymers. Fourier transform infrared spectral analyses demonstrated that the method did not degrade any of the polymers except for polyethylene terephthalate. The method was demonstrated with mussel and fish samples collected from the Pearl River delta, south China, and was able to recover microplastics effectively. Overall, the present method is time-saving and easy to operate, with low procedural cross-contamination. The properties of microplastics recovered by the present method remained largely intact, greatly benefiting subsequent qualitative and quantitative analyses. *EnvironToxicol Chem* 2019;00:1–9. © 2019 SETAC

INTRODUCTION

Approximately 335 million tons of plastics were produced worldwide in 2016 (PlasticsEurope 2017). As the global demand for plastics has consistently increased, the production of plastics was projected to reach 2 billion tons by 2050 if no action is taken to change the current trend (United Nations Environment Programme 2016). Obsolete plastic products enter the environment via losses from recycling and from landfilling, as well as incompletely collected incineration ash and illegal garbage dumping. Large plastic debris can be degraded into fragments in the environment through ultraviolet light exposure, mechanical abrasion, and other processes (Barnes et al. 2009; Lambert et al. 2014). Plastic fragments and/or synthetic polymers with diameter <5 mm are defined as microplastics (Anderson et al. 2016; Andrady 2017).

The heavy use and subsequent disposal of plastics have resulted in the widespread occurrence of microplastics in surface waters, freshwater, soil, sediment, and organisms around the world (Van Cauwenberghe and Janssen 2014; Klein et al. 2015; Baldwin et al. 2016; Lin et al. 2018; Scheurer and Bigalke 2018). Accumulated data have demonstrated the widespread occurrence of microplastics in aquatic organisms: mussels, fishes, and crabs among others (Davison and Asch 2011; Li J et al. 2018; Waite et al. 2018). A recent study demonstrated that microplastics were fragmented into nanoplastics by Antarctic krill (Dawson et al. 2018), suggesting the likelihood of fragmented microplastics crossing physical barriers and translocating into organisms (Farrell and Nelson 2013). Trophic transfer allows microplastics to accumulate in edible aquatic species (Farrell and Nelson 2013; Setälä et al. 2014), greatly increasing the chance of human exposure to microplastics (Catarino et al. 2017). To this end, efficient methods for detecting microplastics in aquatic organisms are desirable (Mai et al. 2018).

One of the major challenges in processing microplastics in biota samples is the lack of a standardized protocol for samples rich in organic material. Numerous methods have been

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developed in recent years to extract microplastics from organisms (Claessens et al. 2013; Foekema et al. 2013; Li et al. 2015; Roch and Brinker 2017). These methods generally include steps involving digestion, suspension, and filtration. In extraction, chemical reagents such as acids (Claessens et al. 2013; De Witte et al. 2014; Vandermeersch et al. 2015), alkalis (Foekema et al. 2013; Dehaut et al. 2016), oxidants (Mathalon and Hill 2014; Li et al. 2015), and enzymes (Cole et al. 2014) have been used solely or in combination (Collard et al. 2015; Roch and Brinker 2017). Enzymatic digestion is reliable for extracting microplastics from plankton samples (Cole et al. 2014; Dawson et al. 2018) but is cumbersome (Dawson et al. 2018) and not cost-effective (Avio et al. 2015). Digestion procedures using acids, alkalis, and oxidants generally involve multiple steps and are time-consuming (overnight to several weeks) and might require high temperatures (80 or 100 °C; Claessens et al. 2013; Desforges et al. 2015). Digesting samples with strong acids, alkalis, and oxidants at high temperatures may damage or alter microplastics and can be an issue with regard to laboratory safety. Currently available methods can be quite cumbersome once steps such as filtration, filter dissolution (Roch and Brinker 2017), and density separation, among others, are added to the procedures. There is therefore a need to develop a rapid, efficient, and easy-to-operate method to provide standardized analytical strategies (Avio et al. 2015). A previous study demonstrated that a 1:1 (v:v) HNO_3 and H_2O_2 mixture efficiently digested human hair and nail samples (Liu et al. 2015). Thus, it is hypothesized that a mixture of HNO_3 and H_2O_2 might be suitable for digesting fish gastrointestinal tract and other biota samples. In the present study, a previously published method (Liu et al. 2015) was modified to digest biota samples.

The primary aim of the present study was therefore to develop a reliable method to meet the following criteria. First, chemical reagents used can remove organic matter effectively at moderate temperatures. Second, digestion is easy to operate with minimal procedural contamination. Finally, the degradation of target microplastics during the process is within an acceptable range. The developed method was applied with mussel and fish purchased from markets and caught from rivers in the Pearl River delta, south China.

MATERIALS AND METHODS

Materials

Standard chemicals including HNO_3 (69%; Guangzhou Chemical Reagent Factory), H_2O_2 (30%; Chinasun Specialty Products), sodium hydroxide (NaOH at 1 mol L^{-1}; Guangdong Guanghua Sci-Tech), sodium chloride (NaCl; Damao Chemical Reagent Factory), and cellulose nitrate filters (47 mm diameter and 5-µm pore size; Whatman International) were obtained through Casmart. Seven microbead microplastics, including 1000-µm low-density polyethylene (LDPE), 900-µm polystyrene, 675-µm polytetrafluoroethylene (PTFE), 300-µm polyethylene terephthalate (PET), 250-µm unplasticized polyvinyl chloride (UPVC), 250-µm polyvinyl chloride (PVC), and 150-µm polyethylene, were purchased from Goodfellow Cambridge. Milli-Q water (18 MΩ cm) was used as generated (Millipore).

Optimization of digestion method

To minimize procedural contamination, all experiments were performed in a fume hood. Laboratory coats, masks, goggles, and gloves were worn throughout the procedure. Glassware was rinsed 3 times with Milli-Q water before use. Mussel and fish gastrointestinal tract samples were stored at –20 °C until analysis. Organisms were digested in glass beakers covered with aluminum foil to prevent contamination.

A 1:1 (v:v) HNO_3 and H_2O_2 mixture was demonstrated to digest hair and nail samples completely at 60 °C in 2 h (Liu et al. 2015). In the present study, the same method was adopted initially to see if biota samples could be completely digested. If not, a series of HNO_3 and H_2O_2 mixtures was employed to digest tissues at 50 °C to optimize the combination of HNO_3 and H_2O_2. For safety considerations, digestion was done at a relatively lower temperature (50 °C). Approximately 3 g of gastrointestinal tract samples were weighed into 250-mL glass beakers, and 50 mL of the HNO_3 and H_2O_2 mixture was added. The digestion reagents were heated to 50 °C on heating panels while stirring until the tissue was completely digested. All solutions were cooled to room temperature before further processing.

Afterward, the performance of selected digestion reagents under different temperatures was evaluated. The aim was to optimize at a relatively lower and safer working temperature.

Density separation

The generated digestion solution was directly filtered onto 47-mm cellulose nitrate filters if the solutions were homogeneous and clear. Otherwise, a density separation with saturated NaCl solution was employed to remove mineral residues to improve filtration efficiency. In this case, the solution was diluted with 150 mL Milli-Q water and transferred into a separatory funnel. After the addition of an appropriate amount of solid NaCl ashed at 450 °C for 4 h to remove organic materials (Supplemental Data, Table S1), the separatory funnels were shaken for 15 min. The funnels were then allowed to stand for 10 min, and the bottom cloudy layer was discarded. The supernatants were slowly filtered through 47-mm cellulose nitrate filters. The separatory funnels were then rinsed with Milli-Q water, which was then filtered through the same filters. This step was repeated 3 times to minimize procedural losses of microplastics. The filters were dried at room temperature before further processing.

Testing recovery rates with the developed digestion method

Soft tissues of mussels and gastrointestinal tract of fishes were mixed with 6 different sizes and shapes of commercial plastic particles, 1000-µm LDPE (white; 30 particles), 900-µm

polystyrene (white; 30 particles), 675-µm PTFE (white; 30 particles), 300-µm PET (white; 30 particles), 250-µm UPVC (white; 30 particles), and 150-µm polyethylene (white; 30 particles). The physical recovery rates of PVC particles were not tested separately because their sizes were similar to that of UPVC particles. Six replicates of organisms were processed for each type of plastic particle and each type of biota sample. Mussels (Unionidae) obtained in October 2017 from the Pearl River delta, south China, were dissected to obtain their soft tissues. Tilapias (*Oreochromis* spp.) cultivated in our laboratory were dissected to obtain the gastrointestinal tract samples. Soft tissues and fish gastrointestinal tract samples were rinsed with Milli-Q water before use. The length and fresh weight (mean ± standard deviation) were, respectively, 3.8 ± 0.4 cm and 4.4 ± 0.4 g for mussels and 14 ± 0.92 cm and 38 ± 6.2 g for tilapia. The described method was good for samples <5 g. If the sample weight was >5 g, the amounts of chemical reagents used should be increased proportionally (Supplemental Data, Table S1). Recovered particles were counted under a microscope (Leica DM500, ICC50 W).

Effects on the properties of tested common polymers

A suitable method should not degrade tested polymers unacceptably. The developed digestion method was tested accordingly. The suitability of the one-step digestion method was evaluated with 6 replicates of the 7 commercial microplastics mentioned in *Materials*. The weight, size, surface area, appearance, and spectroscopic properties of the tested particles were measured before and after treatment. The sizes and surface areas of particles were measured using Image J (Ver 2.1.4.5; National Institutes of Health). Two particles were randomly selected from each replicate sample (i.e., 12 particles total for each type of microplastic material) and photographed with a scanning electron microscope (LEO153VP; Carl Zeiss) under ×50 to ×800 magnification. To analyze changes in spectral properties, Fourier transform infrared spectroscopy (FTIR) spectra of each polymer type before and after digestion were recorded with an FTIR spectrometer (EQUINOX 55; Bruker) from 400 to 4000 cm^{-1}. The spectra of LDPE, polystyrene, and PTFE were directly measured by attenuated total reflection FTIR, given their size, and were the combination of 16 digital scans. Those of PET, UPVC, PVC, and polyethylene were tableted with potassium bromide and measured by FTIR and were the combination of 32 digital scans. The spectra of each plastic type before and after treatment were compared using SigmaPlot (Ver 10.0; Systat Software).

Application to real samples

A total of 7 species of mussel and fish were used to test the applicability of the one-step digestion method in unspiked biota tissues. Mussel, tilapia, and grass carp (*Ctenopharyngodon idellus*) were collected from rivers and ponds in the Pearl River delta from July to December 2017. Silvery pomfret (*Pampus argenteus*), red coat (*Nemipterus virgatus*), yellow croaker (*Larimichthys*), and silver sillago (*Sillago sihama*) were purchased from aquatic product markets in July 2017. On sampling, samples were transported on ice to the laboratory and processed as described.

RESULTS AND DISCUSSION

Optimization of the digestion protocol

The 1:1 HNO_3:H_2O_2 mixture (Liu et al. 2015) produced dense foam while digesting biota samples, which hindered the subsequent filtration step and reduced the recovery rates of spiked microplastics. A previous study employing H_2O_2 solely to digest fish tissues yielded a relatively low recovery rate of 70% (Avio et al. 2015). The low recovery rate was partially ascribed to the dense foam produced during heating, likely from the use of H_2O_2, which affected subsequent filtration and caused extensive losses of materials (Claessens et al. 2013; Avio et al. 2015). In the present study, the HNO_3:H_2O_2 mixture was modified to reduce the percentage of H_2O_2. The digestion of fish gastrointestinal tract samples was tested with a series of HNO_3:H_2O_2 mixtures (v:v = 1:1, 2:1, 3:1, 4:1, and 5:1) at 50 °C. Fish gastrointestinal tract samples were completely digested by 4:1 HNO_3:H_2O_2 within 30 min, generating a homogeneous and clear solution without obvious bubbles (Supplemental Data, Figure S1A). Therefore, a 4:1 HNO_3:H_2O_2 mixture was selected as the digestion reagents in the present study.

Subsequently, fish gastrointestinal tract samples were digested with 4:1 HNO_3:H_2O_2 for 30 min at room temperature (~25 °C), 30, 40, 50, and 60 °C. Homogeneous and clear digestion solutions were generated (Supplemental Data, Figure S1B) at both 50 and 60 °C. For both efficiency and safety, 50 °C was selected as the working temperature (Dehaut et al. 2016; Karami et al. 2017; Budimir et al. 2018). In summary, the present study employed a 4:1 HNO_3:H_2O_2 mixture to digest biota samples at 50 °C for 30 min.

Performance of the digestion protocol

Soft tissues of mussel and fish gastrointestinal tract samples were completely digested by the one-step digestion method, which reduced the total digestion time to 30 min and the number of procedural steps compared to existing methods. Most currently available digestion methods take several hours to several days to digest biota samples fully (Table 1). For example, the complete digestion of organisms with proteinase-K needs more than 3 h (Cole et al. 2014). Complete digestion of mussel tissue with a mixture of HNO_3 and perchloric acid (4:1, v-v) is achieved overnight at room temperature (De Witte et al. 2014; Zhang and Liu 2018). Complete digestion of fish samples with NaOH alone requires 21 d at room temperature (Bellas et al. 2016).

A recent study developed a rapid and efficient 2-step digestion method, which completely digested fish gastrointestinal tract samples with 1 mol L^{-1} NaOH followed by 65% HNO_3 within 1 h at 80 °C (Roch and Brinker 2017). Although the digestion time

TABLE 1: Overview of present methods to digest soft tissue for enumeration of microplastics

Reagents used	Working temperature	Density separation	Filters dissolved	Duration	Procedural contamination (mean ± SD)	Reference
HNO_3 (65%):H_2O_2 (30%) = 4:1	50 °C	Yes NaCl (~1.2 g cm^{-3})	No	30 min + 15 min	Yes (1.7 ± 1.2)	Present study
NaOH (~4%), HNO_3 (~49%)	50 °C	Yes NaI (~1.6 g cm^{-3})	Yes	<1 h + 0.5 h	Yes (12.8 ± 6.3)	Roch and Brinker (2017)
Proteinase-K	50 °C	No	No	3 h	Yes (0)	Cole et al. (2014)
HNO_3 (15.9 M):HCl (12.1 M) = 1:1	RT, 80 °C	No	No	3 h	Yes (0)	Desforges et al. 2015
HNO_3 (69–71%)	90 °C	No	No	4 h	Yes	Scheurer and Bigalke 2018
HNO_3 (65%):NaClO (9%) = 1:10	RT	No	No	Overnight	No data	Collard et al. 2015
HNO_3 (95%)[a]	RT, 100 °C	No	No	Overnight + 2 h	No data	Claessens et al. (2013)
HNO_3 (65%):$HClO_4$ (68%) = 4:1	RT, 100 °C	No	No	Overnight + 40 min	Yes (1.5–4.7)	De Witte et al. 2014
$K_2S_2O_8$ (0.27 M), NaOH (0.24 M)	65 °C	No	No	24 h	No data	Dehaut et al. (2016)
KOH (10%)	60 °C	No	No	24 h	No data	Dehaut et al. (2016)
H_2O_2 (30%)	65 °C	Yes NaCl (~1.2 g cm^{-3})	No	24 h, 24–48 h	Yes (0.50 ± 0.55)	Li et al. (2015)
NaOH (1 M), SDS (5 g L^{-1}), HCl (2 M), H_2O_2 (30%)	50 °C	No	No	48 h	No data	Budimir et al. (2018)
KOH (10%)	40 °C	Yes NaI (~1.5 g cm^{-3})		48–72 h	No data	Karami et al. (2017)
Protease, cellulase, chitinase	50 °C	Yes	No	14–17 d	No data	Dawson et al. (2018)
SDS (5%), H_2O_2 (30%)	37 °C	$ZnCl_2$ (~1.7 g cm^{-3})				
NaOH (1 M)	RT	No	No	21 d	Yes (0)	Bellas et al. (2016)
KOH (10%)	RT	No	No	2–3 wk	No data	Foekema et al. (2013)

[a]The concentration mentioned by Claessens et al. (2013) was 22.5 mol L^{-1} (= 95%), which was suspected to be an error (Roch and Brinker 2017).
SDS = sodium dodecyl sulfate.

was greatly shortened, subsequent processing steps could be further reduced. Reducing the number of processing steps is beneficial because it minimizes procedural contamination and losses of microplastics (Collard et al. 2015; Dawson et al. 2018). In comparison to the method developed in the present study, previous methods usually consisted of multiple-step digestion with one reagent after another (Table 1). For example, fish stomach content was digested overnight with 9% sodium hypochlorite solution (NaClO; Collard et al. 2015). The generated digestion solution was filtered through cellulose acetate and further rinsed with an HNO_3 and NaClO mixture (Collard et al. 2015). Soft tissues and small fish were digested with 1 M NaOH and 5 g L^{-1} sodium dodecyl sulfate (SDS) at 50 °C for 48 h, followed by 2 M hydrochloric acid and 30% H_2O_2 (Budimir et al. 2018). Biota samples were digested consecutively with SDS, protease, cellulase, H_2O_2, chitinase, and H_2O_2 again, followed by density separation to remove organic material (Dawson et al. 2018). Though more gentle to sensitive synthetic polymers (Klein et al. 2015), enzymatic digestion is generally comprised of multiple processing steps (Cole et al. 2014; Van Cauwenberghe et al. 2015), which can potentially increase losses of microplastics (Dawson et al. 2018).

One-step digestion methods have been developed previously (Claessens et al. 2013; Collard et al. 2015; Karami et al. 2017; Li et al. 2015). However, those methods require supplemental measures such as high working temperatures and need hours to days to digest biota samples fully (Table 1). The digestion efficiency with strong acids solely is generally low. Biota samples were digested overnight with 69% HNO_3 at room temperature, followed by 2 h of boiling and dilution with 80 °C filtered deionized water (Claessens et al. 2013; Van Cauwenberghe and Janssen 2014). Strong oxidants (H_2O_2) have been solely used as the digestion reagent and are less efficient with regard to processing time (Li et al. 2015). Bivalves were digested with 30% H_2O_2 in an oscillation incubator at 65 °C for 24 h and then at room temperature for another 24 to 48 h (Li et al. 2015). The digestion efficiency with strong alkaline (potassium hydroxide [KOH]) solely was lower than that with our method (Dehaut et al. 2016). For example, seafood was digested with 10% KOH at 60 °C for 24 h and at 40 °C for 48 h (Karami et al. 2017).

In summary, the one-step digestion method developed in the present study greatly reduced the overall processing time and minimized the processing steps while working at a relatively moderate temperature (50 °C).

Good recoveries (90–100%) of microplastics in each size class were achieved for both soft tissues of mussel and fish gastrointestinal tract samples (Figure 1). For soft tissues of mussel, the recoveries (average ± standard deviation) were 99 ± 1% for 1000-µm LDPE, 100% for 900-µm polystyrene, 97 ± 2% for 675-µm PTFE, 96% ± 4 for 300-µm PET, 96% ± 6 for 250-µm UPVC, and 98% ± 3 for 150-µm polyethylene. For fish gastrointestinal tract samples, the recoveries were 97 ± 4% for 1000-µm LDPE, 100% for 900-µm polystyrene, 97 ± 3% for 675-µm PTFE, 95% ± 5 for 300-µm PET, 93% ± 3 for 250-µm UPVC, and 93% ± 4 for 150-µm polyethylene. Compared with the larger particles (LDPE, polystyrene, and PTFE), the recoveries of PET, UPVC, and polyethylene were slightly lower but not statistically significantly ($p > 0.05$). The recovery rates in the present study (93–100%) were comparable to those (95–100%) achieved by Roch and Brinker (2017) and higher than those (78–98%) reported by Avio et al. (2015). The smallest polymer tested in the present study was 150-µm polyethylene. Further studies dealing with smaller particles and microplastic types other than microbeads are needed in the future.

Degradation of target polymers

The tested polymers processed with our developed digestion method were investigated for changes in color, weight, surface area, particle size, appearance, and FTIR spectra before and after digestion. No color change was observed for almost all polymer types except for polystyrene, which was white before digestion and became light yellow after digestion (Supplemental Data, Figure S2). None of the tested polymers showed statistically significant weight changes (*t* test, $p > 0.05$; Table 2). The surface areas and particle sizes of the tested polymers did not change significantly on digestion (*t* test, $p > 0.05$; Figure 2). Overall, almost none of the tested polymers were affected by the digestion method, with the exceptions of a slight color change (white to light yellow) and a slight surface corrosion (rough to smooth) of polystyrene particles (Supplemental Data, Figure S3). Morphological changes of plastic particles have been common problems of digestion methods using HNO_3 (Claessens et al. 2013; Roch and Brinker 2017). For example, digestion with HNO_3 resulted in dissolution and color changes for LDPE and polypropylene (Karami et al. 2017). Previous studies using 30% H_2O_2 as digestion reagent resulted in a color change for PET particles (Nuelle et al. 2014; Karami et al. 2017). In the present study, morphological changes of polystyrene particles were observed only for the 4:1 HNO_3:H_2O_2 mixture but not for the 1:1 HNO_3:H_2O_2 mixture (Supplemental Data, Figure S3). A higher proportion of HNO_3 might have resulted in a color change and a slight surface corrosion for polystyrene particles. Spectroscopic methods are useful to confirm structures of individual plastic polymers (Harrison et al. 2012; Löder and Gerdts 2015; Song et al. 2015). The FTIR spectra of polystyrene particles before and after treatment were similar to each other (Figure 3). Therefore, the changes in the surface morphology of polystyrene particles did not affect its identification. None of the tested polymers had significant changes in their FTIR spectra before or after treatment, except for PET for which the FTIR peak at 1382 cm^{-1} was enhanced on digestion (Figure 3). The change in peak intensity may be related to polymer degradation caused by structural rearrangements of surface chemical groups (Mazeikiene et al. 2006; Karami et al. 2017) or hydrolysis of PET (Yoshioka et al. 1998). This suggested that the present digestion method may have subjected PET to degradation.

Procedural contamination

Fibers are commonly found in background contamination (Davison and Asch 2011; Nuelle et al. 2014; Van

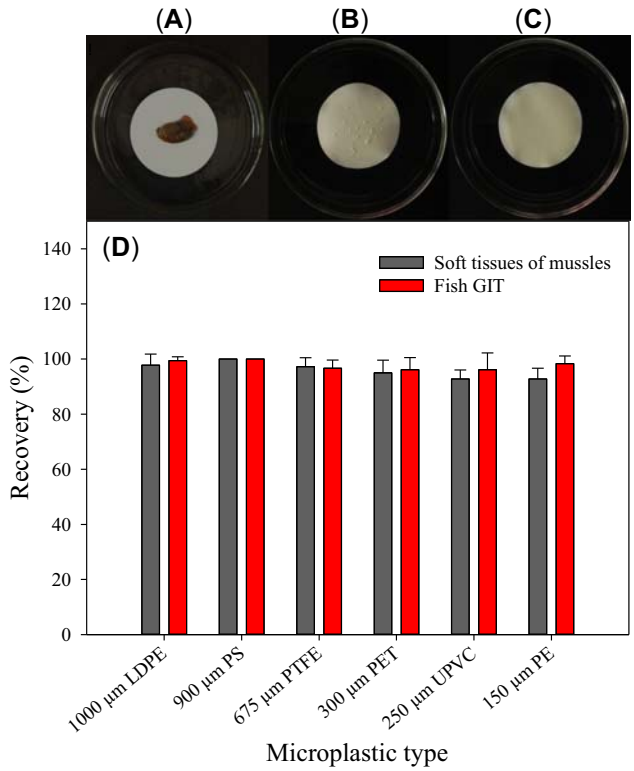

FIGURE 1: Recovery rates (average ± standard deviation) of spiked microplastic particles treated with the digestion method developed in the present study. (**A**) Gastrointestinal tract of tilapias before digestion. (**B**) Recovered 1000-μm low-density polyethylene. (**C**) Recovered 150-μm polyethylene. (**D**) Recovery rates of different types of tested polymers after the one-step digestion method (n = 6). GIT = gastrointestinal tract; LDPE = low-density polyethylene; PE = polyethylene; PET = polyethylene terephthalate; PS = polystyrene; PTFE = polytetrafluoroethylene; UPVC = unplasticized polyvinyl chloride.

Cauwenberghe and Janssen 2014) and are mostly derived from air circulation (Mathalon and Hill 2014) and clothing (Foekema et al. 2013). Thus, the presence of fibers in the background contamination could be the result of contamination during the processing of samples, a factor that needs to be minimized (Woodall et al. 2015). Many precautionary measures have been implemented to reduce procedural contamination, for example, operating in a clean fume hood; rinsing glassware with ultrapure water; covering samples with aluminum foil; and wearing laboratory coats, nitrile gloves, and masks (Mathalon and Hill 2014; Santana et al. 2016; Zhao et al. 2016). Mathalon and Hill (2014) observed high procedural contamination (~100 item filter^{-1}) with H_2O_2 as the digestion reagent. Another study using H_2O_2 as digestion reagent reported low procedural contamination, 0.50 ± 0.55 items filter^{-1} (Li et al. 2015). The magnitude of procedural contamination with a 2-step digestion method (Roch and Brinker 2017) was 12.8 ± 6.3 items filter^{-1} (Table 1). Because these methods with relatively higher procedural contaminations also followed careful precautions to avoid contamination, background levels need to be minimized. This can be done in part by minimizing the amount of time required to process samples and the number of processing steps needed, each of which can introduce contamination.

The present study detected 1.7 ± 1.2 items filter^{-1} in procedural blanks, with sizes ranging from 120 to 1600 μm. Most residual particles were fibers (Supplemental Data, Figure S4 A and B). Compared with other studies, the magnitude of procedural contamination in the present study was at the low end (Table 1), which was partially ascribed to adoption of strict precautionary measures throughout the procedure and the easy-to-operate and time-saving one-step digestion process. In addition to adopting precautionary measures suggested previously (Mathalon and Hill 2014; Santana et al. 2016; Zhao et al. 2016), NaCl was baked at 450 °C for 4 h before use in the present study to destroy any potential organic microplastics present so as to avoid potential contamination.

Application to field samples

The present method was applied for processing microplastics extant in field samples. A total of 55 biota samples together with procedural blanks were analyzed, including 6 wild mussels, 9 wild fish, and 40 market fish. Soft tissue of mussels and gastrointestinal tract of fish were weighed and digested as described (Supplemental Data, Table S1). Samples weighing <5 g were completely digested after 30 min. Heavier samples (>5 g; Table 3) required at least 1 h of digestion time, followed by a flotation step. Samples >10 g used a large amount of chemicals and water and typically required several cellulose acetate membranes and a great deal of time to filter.

TABLE 2: Degradation effects on tested polymers with the one-step digestion method developed in the present study[a]

Polymer	No.	Color change (before/after)	Weight change (%)	p weight change	Spectra change	Other changes
LDPE	6	No	–0.10 ± 0.19	n.s	No	
PS	6	Yes (white/light yellow)	–0.02 ± 0.07	n.s	No	Smoother surface
PTFE	6	No	–0.13 ± 0.13	n.s	No	
PET	6	No	0.03 ± 0.04	n.s	Yes	
UPVC	6	No	–0.04 ± 0.06	n.s	No	
PVC	6	No	–0.20 ± 0.07	n.s	No	
PE	6	No	–0.17 ± 0.08	n.s	No	

[a]No. indicates number of replicate samples (each with 30–50 mg). The weight change of tested polymers is shown as a percentage (mean ± standard deviation). A t test was used to determine the significance of the weight change.
LDPE = low-density polyethylene; n.s. = not significant (α = 0.05); PE = polyethylene; PET = polyethylene terephthalate; PS = polystyrene; PTFE = polytetrafluoroethylene; PVC = polyvinyl chloride; UPVC = unplasticized polyvinyl chloride.

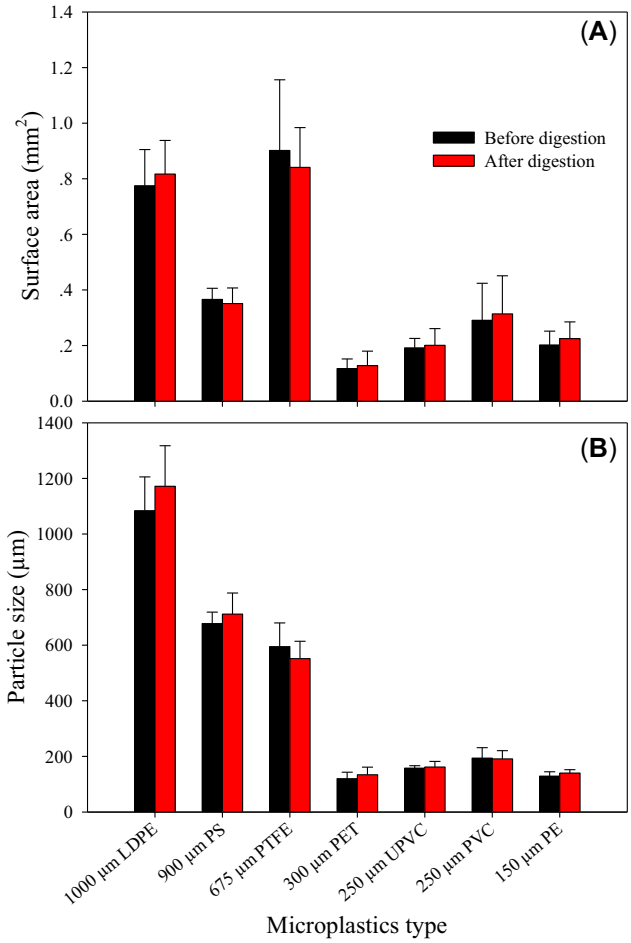

FIGURE 2: (**A**) Surface area and (**B**) particle size (average ± standard deviation) of tested polymers before and after treatment with the present method (*t* test, $p > 0.05$). LDPE = low-density polyethylene; PE = polyethylene; PET = polyethylene terephthalate; PS = polystyrene; PTFE = polytetrafluoroethylene; PVC = polyvinyl chloride; UPVC = unplasticized polyvinyl chloride.

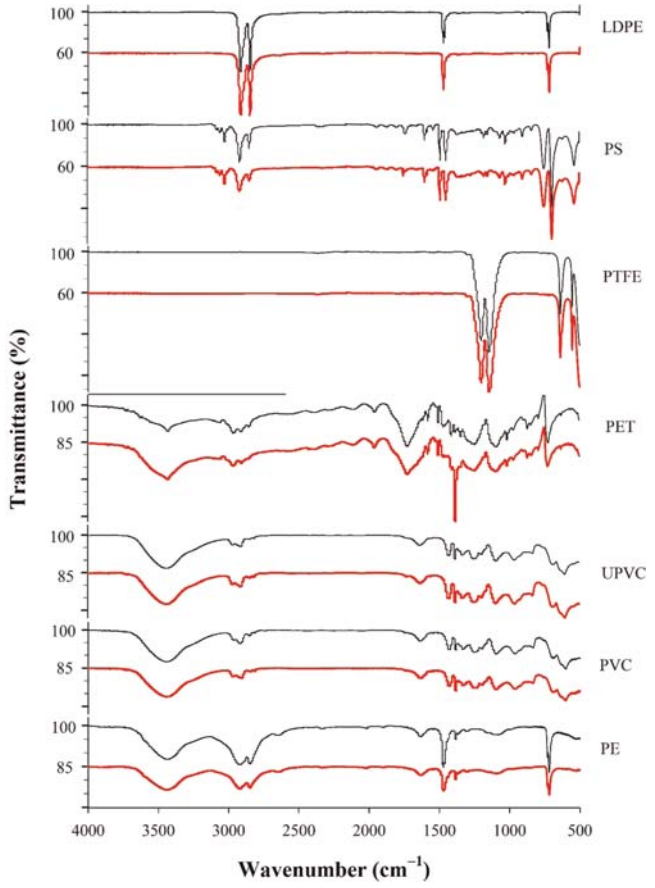

FIGURE 3: Fourier transform infrared spectroscopy spectra of tested polymers before (black line) and after (red line) digestion. Transmittance for low-density polyethylene, polystyrene, and polytetrafluoroethylene are all the same scale, whereas the rest are a different scale. LDPE = low-density polyethylene; PE = polyethylene; PET = polyethylene terephthalate; PS = polystyrene; PTFE = polytetrafluoroethylene; PVC = polyvinyl chloride; UPVC = unplasticized polyvinyl chloride.

Therefore, we recommend splitting a high-weight sample (>10 g) into several smaller portions (<5 g) and digesting them separately, after which the extracted particles can be recombined.

The field samples analyzed in the present study all contained microplastics, as visually identified, at different levels (Table 3), suggesting widespread occurrence of microplastics. A variety of microplastics, including lines, fragments, spheres, and films, were detected (Supplemental Data, Figure S4). A recent study also suggested that microplastics were widespread in oysters collected along the Pearl River estuary of south China (Li H et al. 2018). In the present study, tilapia (*Oreochromis* spp.) from the Dan'ao River at Huizhou was the most seriously contaminated, with each fish containing more than 200 microplastic particles. The remaining field mussel and

TABLE 3: Abundance of microplastics visually identified in mussels and fishes collected around the Pearl River delta region, south China

Location	Species	Sample no.	Weight extracted (g)	Visually identified microplastic (items individual^{-1})
Dan'ao River	*Oreochromis* spp.	2	12.4 ± 2.8	230 ± 31 (210–254)
Seagull Island	*Ctenopharyngodon idellus*	7	13.2 ± 6.2	8.9 ± 5.2 (2–19)
Nansha, Guangzhou	Unionidae	6	2.3 ± 1.6	3.5 ± 2.2 (1–7)
Yangjiang Market	*Pampus argenteus*	4	1.3 ± 0.13	2.5 ± 3.0 (1–7)
	Nemipterus virgatus	10	1.2 ± 0.25	1.4 ± 1.3 (0–4)
	Larimichthys	10	1.3 ± 0.39	0.4 ± 0.5 (0–1)
	Sillago sihama	10	1.1 ± 0.57	0.2 ± 0.4 (0–1)
Huizhou Market	*Pampus argenteus*	4	4.7 ± 1.5	1 ± 0.8 (0–2)
	Larimichthys	2	5.4 ± 2.2	1.0 (1)

[a]Abundance of MPs is shown as mean ± standard deviation (minimum to maximum).

fish samples each contained fewer than 10 microplastic particles (Table 3). However, because these samples were used for method application only, more detailed data such as the age, physiology, and ecology of the tested mussels and fishes were not taken into consideration. The types of plastics extracted were not further characterized. Therefore, no conclusion can be reached about the potential sources of the microplastic particles observed; instead, this application serves as proof of principle of the present method using real-world samples.

CONCLUSIONS

In the present study, we developed a one-step digestion method with 4:1 HNO_3:H_2O_2 as digestion reagent, which completely digested mussels and fish gastrointestinal tract samples within 30 min at 50 °C. Performance of this method was tested with 7 types of microplastics, including 1000-μm LDPE, 900-μm polystyrene, 675-μm PTFE, 300-μm PET, 250-μm UPVC, 250-μm PVC, and 150-μm polyethylene. No degradation effects were observed for all except for PET particles. The FTIR spectra of PET were slightly changed, with an enhanced peak at 1382 cm^{-1}. The developed method was successfully applied to the analyses of microplastics in field aquatic organisms. It is noteworthy that the smallest polymers tested in the present study were 150-μm polyethylene particles. This method needs to be verified for smaller particles of concern.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4416.

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Data Accessibility—Data and calculation tools are available by contacting the corresponding author (liuliangying@jnu.edu.cn or liuliangying3@126.com).

REFERENCES

Anderson JC, Park BJ, Palace VP. 2016. Microplastics in aquatic environments: Implications for Canadian ecosystems. *Environ Pollut* 218:269–280.

Andrady AL. 2017. The plastic in microplastics: A review. *Mar Pollut Bull* 119:12–22.

Avio CG, Gorbi S, Regoli F. 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from adriatic sea. *Mar Environ Res* 111:18–26.

Baldwin AK, Corsi SR, Mason SA. 2016. Plastic debris in 29 Great Lakes tributaries: Relations to watershed attributes and hydrology. *Environ Sci Technol* 50:10377–10385.

Barnes DKA, Galgani F, Thompson RC, Barlaz M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos Trans R Soc Lond B Biol Sci* 364:1985–1998.

Bellas J, Martinez-Armental J, Martinez-Camara A, Besada V, Martinez-Gomez C. 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. *Mar Pollut Bull* 109:55–60.

Budimir S, Setala O, Lehtiniemi M. 2018. Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. *Mar Pollut Bull* 127:586–592.

Catarino AI, Thompson R, Sanderson W, Henry TB. 2017. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. *Environ Toxicol Chem* 36:947–951.

Claessens M, Van Cauwenberghe L, Vandegehuchte MB, Janssen CR. 2013. New techniques for the detection of microplastics in sediments and field collected organisms. *Mar Pollut Bull* 70:227–233.

Cole M, Webb H, Lindeque PK, Fileman ES, Halsband C, Galloway TS. 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Sci Rep* 4:4528.

Collard F, Gilbert B, Eppe G, Parmentier E, Das K. 2015. Detection of anthropogenic particles in fish stomachs: An isolation method adapted toidentification by Raman spectroscopy. *Arch Environ Contam Toxicol* 69:331–339.

Davison P, Asch RG. 2011. Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. *Mar Ecol Prog Ser* 432:173–180.

Dawson AL, Kawaguchi S, King CK, Townsend KA, King R, Huston WM, Bengtson Nash SM. 2018. Turning microplastics into nanoplastics through digestive fragmentation by antarctic krill. *Nat Commun* 9:1001.

Dehaut A, Cassone A-L, Frere L, Hermabessiere L, Himber C, Rinnert E, Riviere G, Lambert C, Soudant P, Huvet A, Duflos G, Paul-Pont I. 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environ Pollut* 215:223–233.

Desforges JP, Galbraith M, Ross PS. 2015. Ingestion of microplastics by zooplankton in the northeast pacific ocean. *Arch Environ Contam Toxicol* 69:320–330.

De Witte B, Devriese L, Bekaert K, Hoffman S, Vandermeersch G, Cooreman K, Robbens J. 2014. Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Mar Pollut Bull* 85:146–155.

Farrell P, Nelson K. 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environ Pollut* 177:1–3.

Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA. 2013. Plastic in North Sea fish. *Environ Sci Technol* 47:8818–8824.

Harrison JP, Ojeda JJ, Romero-Gonzalez ME. 2012. The applicability of reflectance micro-Fourier-transform infrared spectroscopy for the detection of synthetic microplastics in marine sediments. *Sci Total Environ* 416:455–463.

Karami A, Golieskardi A, Choo CK, Romano N, Ho YB, Salamatinia B. 2017. A high-performance protocol for extraction of microplastics in fish. *Sci Total Environ* 578:485–494.

Klein S, Worch E, Knepper TP. 2015. Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany. *Environ Sci Technol* 49:6070–6076.

Lambert S, Sinclair CJ, Boxall A. 2014. Occurrence, degradation, and effect of polymer-based materials in the environment. *Rev Environ Contam Toxicol* 227:1–53.

Li H, Ma L, Lin L, Ni Z, Xu X, Shi H, Yan Y, Zheng G, Rittschof D. 2018. Microplastics in oysters *Saccostrea cucullata* along the Pearl River estuary, China. *Environ Pollut* 236:619–625.

Li J, Green C, Reynolds A, Shi H, Rotchell JM. 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. *Environ Pollut* 241:35–44.

Li J, Yang D, Li L, Jabeen K, Shi H. 2015. Microplastics in commercial bivalves from China. *Environ Pollut* 207:190–195.

Lin L, Zuo LZ, Peng JP, Cai LQ, Fok L, Yan Y, Li HX, Xu XR. 2018. Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou City, China. *Sci Total Environ* 644:375–381.

Liu L, Salamova A, He K, Hites RA. 2015. Analysis of polybrominated diphenyl ethers and emerging halogenated and organophosphate flame retardants in human hair and nails. *J Chromatogr A* 1406:251–257.

Löder MGJ, Gerdts G. 2015. Methodology used for the detection and identification of microplastics—A critical appraisal. In Bergmann M,

Gutow L, Klages M, eds, *Marine Anthropogenic Litter*. Springer International, Cham, Switzerland. pp 201–227.

Mai L, Bao L-J, Shi L, Wong CS, Zeng EY. 2018. A review of methods for measuring microplastics in aquatic environments. *Environ Sci Pollut Res* 25:11319–11332.

Mathalon A, Hill P. 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Mar Pollut Bull* 81:69–79.

Mazeikiene R, Statino A, Kuodis Z, Niaura G, Malinauskas A. 2006. In situ Raman spectroelectrochemical study of self-doped polyaniline degradation kinetics. *Electrochem Commun* 8:1082–1086.

Nuelle MT, Dekiff JH, Remy D, Fries E. 2014. A new analytical approach for monitoring microplastics in marine sediments. *Environ Pollut* 184:161–169.

PlasticsEurope. 2017. Plastics—The facts 2017: An analysis of European plastics production, demand and waste data. Brussels, Belgium.

Roch S, Brinker A. 2017. Rapid and efficient method for the detection of microplastic in the gastrointestinal tract of fishes. *Environ Sci Technol* 51:4522–4530.

Santana MF, Ascer LG, Custodio MR, Moreira FT, Turra A. 2016. Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: Rapid evaluation through bioassessment. *Mar Pollut Bull* 106:183–189.

Scheurer M, Bigalke M. 2018. Microplastics in Swiss floodplain soils. *Environ Sci Technol* 52:3591–3598.

Setälä O, Fleming-Lehtinen V, Lehtiniemi M. 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environ Pollut* 185:77–83.

Song YK, Hong SH, Jang M, Han GM, Rani M, Lee J, Shim WJ. 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. *Mar Pollut Bull* 93:202–209.

United Nations Environment Programme. 2016. Marine plastic debris and microplastics: Global Lessons and Research to Inspire Action and Guide Policy Change. Nairobi, Kenya.

Van Cauwenberghe L, Devriese L, Galgani F, Robbens J, Janssen CR. 2015. Microplastics in sediments: A review of techniques, occurrence and effects. *Mar Environ Res* 111:5–17.

Van Cauwenberghe L, Janssen CR. 2014. Microplastics in bivalves cultured for human consumption. *Environ Pollut* 193:65–70.

Vandermeersch G, Van Cauwenberghe L, Janssen CR, Marques A, Granby K, Fait G, Kotterman MJJ, Diogene J, Bekaert K, Robbens J, Devriese L. 2015. A critical view on microplastic quantification in aquatic organisms. *Environ Res* 143:46–55.

Waite HR, Donnelly MJ, Walters LJ. 2018. Quantity and types of microplastics in the organic tissues of the eastern oyster *Crassostrea virginica* and Atlantic mud crab *Panopeus herbstii* from a Florida estuary. *Mar Pollut Bull* 129:179–185.

Woodall LC, Gwinnett C, Packer M, Thompson RC, Robinson LF, Paterson GL. 2015. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. *Mar Pollut Bull* 95:40–46.

Yoshioka T, Okayama N, Okuwaki A. 1998. Kinetics of hydrolysis of pet powder in nitric acid by a modified shrinking-core model. *Ind Eng Chem Res* 37:336–340.

Zhang GS, Liu YF. 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. *Sci Total Environ* 642:12–20.

Zhao S, Zhu L, Li D. 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers, *Sci Total Environ* 550, 1110–1115.

