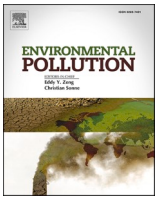
Environmental Pollution 322 (2023) 121198

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



An optimized acidic digestion for the isolation of microplastics from biota-rich samples and cellulose acetate matrices☆

Erin Tuttle [a,*], Aron Stubbins [a,b,c]

[a] Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA
[b] Department of Marine and Environmental Science, Northeastern University, Boston, MA, USA
[c] Department of Civil and Environmental Engineering, Northeastern University, Boston, MA, USA

ARTICLE INFO

Keywords:
Microplastic
Digestion
Cellulose acetate
Nitric acid
Organic matter

ABSTRACT

Plastic pollution is a growing concern. To analyze plastics in environmental samples, plastics need to be isolated. We present an acidic/oxidative method optimized to preserve plastics while digesting synthetic cellulose acetate and a range of organics encountered in environmental samples. Cellulose acetate was chosen for optimization as it can be purchased as a reference material, can co-occur with plastics in environmental samples and, if it can be completely digested, is a potential filter material for the collection of nano- and micro-plastics from natural waters. Other forms of particulate organic matter (POM) were chosen to provide a range of chemistries that might alter digestion efficiency and due to the interest in the community of isolating plastics from samples where these organics occur. For instance, microalgal POM occurs in lake and ocean waters, riverine POM in rivers, and inclusion of tuna provides a test for the suitability of the method for isolating plastics from animal tissues. The method is a one-pot overnight (16–18 h) digestion in 5 M nitric acid with 0.3 M sodium persulfate heated to 80 °C. The method provides quantitative removal of cellulose acetate (exceeding detection limits), near quantitative removal of microalgal POM and Albacore tuna tissue (>99%), but only 86% of urban river POM, all while retaining >99% by mass of C–C bonded polymers polyethylene, polypropylene, and polystyrene and >96% by mass of polyethylene terephthalate. Fourier transform infrared spectroscopy (FT-IR) and %-C content analysis confirmed plastic polymer stability during digestion. However, some additives in appear susceptible to digestion with FT-IR results indicating the loss of N,N′-ethylenebis(stearamide) from polyethylene. This method provides a simpler and more effective method than many in the literature. We present recommendations for the application of this method, as well as limitations and areas for future improvement.

1. Introduction

Global use of plastics began after World War II (Fisher 2013) and plastic production has grown exponentially in the decades that followed. By 2017 plastic production reached 348 million tons (Prata et al., 2019). Increasing production combined with mismanagement of plastic waste has led to an accumulation of plastic debris in the environment. Microplastic pollution in marine ecosystems was first reported in 1972 (Carpenter and Smith Jr, 1972) and plastic pollution now covers the globe (Stubbins et al., 2021). An estimated 30% of plastic produced is still in use, with ~60% accumulating in landfills, or entering the environment as mismanaged waste (Geyer et al., 2017). In 2016 an estimated 19 to 23 million metric tons entered aquatic systems, representing 11% of global plastic waste generated that year (Borrelle et al., 2020). Human exposure to microplastics in food (Chain 2016), drinking water (Koelmans et al., 2019), and air (Gasperi et al., 2018; Zhang et al., 2020) is also of concern, as the health risks are not well understood.

Numerous methods for the extraction, isolation, and cleaning of microplastics in environmental samples have been developed. Physical methods using density separation (Hidalgo-Ruz et al., 2012; Imhof et al., 2012), sieving (Foekema et al., 2013; Tiwari et al., 2019), and electrostatic separation (Felsing et al., 2018) can isolate plastic particles down to low μm sizes. Physical separations can be conducted in the field but have limitations on particles size and the degree of microplastic cleaning achieved. Chemical digestions are most prevalent in the literature, with

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.
* Corresponding author.
E-mail address: tuttle.er@northeastern.edu (E. Tuttle).

https://doi.org/10.1016/j.envpol.2023.121198
Received 5 December 2022; Received in revised form 13 January 2023; Accepted 31 January 2023
Available online 1 February 2023

the efficiency of organic material removal and stability of plastics varying widely for different digestions chemistries/conditions. Recently enzymatic digestions have been reported to digest organic material with minimal impact on microplastics (Courtene-Jones et al., 2017; von Friesen et al., 2019). Enzymatic digestion can remove complex organic matrices by modifying multi-step protocols according to the matrix composition (Loder et al., 2017). However, these complicated, multi-step methods can be expensive due to reagent costs. Thus, we chose to assess lower cost, simpler (i.e., one-step) chemical digestion methods.

A literature review to identify chemical digestion protocols with high microplastic stability indicates no clear advantage in terms of microplastic retention of using oxidative vs. alkaline digestion (see Table 1 and references therein). Oxidative digestions typically have high plastics recoveries when using 30–35% hydrogen peroxide. However, changes to the shape and size of polypropylene (PP) microplastics can occur when exposed to 35% hydrogen peroxide for 7 days at room temperature even with high recoveries (97.5%) (Nuelle et al., 2014) suggesting extended reaction times may affect microplastics. Optimized protocols typically result in greater than 95% recovery of plastics, with some exceptions. Polyethylene terephthalate (PET) is not stable under strongly alkaline conditions with mass recovery of 70–75% for 10 M sodium hydroxide digestions, but recovery increases when concentration is lowered to 1 M (Olsen et al. 2020; Hurley et al., 2018). A similar trend is observed under acidic conditions. Just 4% of polyethylene (PE) and polystyrene (PS) (4 ± 3%) was recovered from 22.5 M nitric acid after 12 h at room temperature followed by 30 min boiling (Avio et al., 2015). After exposure to boiling 15.7 M nitric acid for 2 h PE was found to be stable (95–100% recovery) and recovery of PS increased to ~49% (Schrank et al., 2022), suggesting reduced acid concentrations improve plastic stability. The optimized method developed here sought to lower concentrations of bases and acids to retain a higher percentage of plastic mass while still effectively removing other organics.

The organics used for testing included synthetic cellulose acetate, microalgal particle organic matter (POM), urban river POM, and fish tissue. These materials were chosen as they co-occur with plastics in environmental samples and have differing chemical characteristics that might impact digestion efficiency. Synthetic cellulose acetate was used for initial method optimization as it could be purchased in large quantities in consistent form, also allowing others to use it as a reference material in future work. Cellulose acetate was purchased as filters as we also wanted to determine if digestion methods could completely remove a filter material on to which plastics could be collected, allowing for isolation of nano- and micro-plastics down to the pore size of filters (i.e., ~0.2 μm) from natural waters and other solutions (e.g. tap water; wastewater). Finally, cellulose acetate and other cellulosic materials can co-occur with our target, synthetic plastics. Although cellulosic acetate is a synthetic material and some treatments to extract microplastics also recover cellulosic materials (Hermsen et al., 2017; Wesch et al., 2016; Yu et al., 2016; Dyachenko et al., 2017), we chose to develop a method that eliminates it from our analytical window as there is disagreement in the field of environmental plastic pollution concerning how best to define it and contrast it to other forms of microplastic pollution (Hartmann et al., 2019). For instance, trends in prevalence and degradation differ between cellulosic pollutants and petrochemical-derived plastic pollutants (Finnegan et al., 2022; Macieira et al., 2021), suggesting these classes of pollutants could be considered distinct. These differences in the chemistry and environmental behavior of cellulose acetate

Table 1
Summarized %-mass recovery of polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) microplastics and organic material following chemical digestion processes from the literature, rounded to whole numbers for comparisons. R.T. denotes room temperature as reported in the citation, where exact temperature is not provided.

Digestion Conditions	Plastic Type				Organic Material (OM)			
	PE	PP	PS	PET	Cellulose Acetate	Type of OM	% Recovery	Citation
Alkaline								
1 M NaOH, 24 h at 60 °C	100 ± 0%	99 ± 0%	98 ± 2%	93 ± 8%		Sewage sludge	39 ± 6%	(Hurley et al., 2018)
10 M NaOH, 64 h at R.T.	100 ± 0%	100 ± 0%	100 ± 0%	75 ± 0%	94 ± 5%	Microalgae	39 ± 11%	(Olsen et al. 2020)
10 M NaOH, 24 h at 60 °C	100 ± 0%	100 ± 0%	100 ± 0%	72 ± 2%		Sewage sludge	33 ± 6%	(Hurley et al., 2018)
						Microalgae	9 ± 0%	(Cole et al., 2014)
10% KOH, 48 h at 40 °C	103%	104%	104%	97%		Organisms	99%	(Karami et al., 2017)
10% KOH, 1 h at 50 °C	100 ± 0%	100 ± 1%	100 ± 0%	99 2%	78 ± 1%	Microalgae	58 ± 6%	(Prata et al., 2019)
10% KOH, 24 h at 60 °C	100 ± 0%	99 ± 1%	112 ± 2%	99 ± 0%		Sewage sludge	43 ± 17%	(Hurley et al., 2018)
Oxidative								
30% H_2O_2, 24 h at 60 °C	100 ± 0%	100 ± 0%	100 ± 0%	100 ± 0%		Sewage sludge	20 ± 4%	(Hurley et al., 2018)
30% H_2O_2, 24 h at 70 °C	100 ± 0%	94 ± 9%	100 ± 0%	101 ± 1%		Sewage sludge	13 ± 13%	(Hurley et al., 2018)
35% H_2O_2, 7 days at R.T following flotation	98%	98%	95%	88%				(Nuelle et al., 2014)
30% H_2O_2/1% NaOH, 7 days at R.T following dissolution	100 ± 0%	100 ± 0%	100 ± 0%	99 ± 1%	3 ± 6%	Microalgae	2 ± 3%	(Olsen et al. 2020)
Fenton's reagent, 1 h at 50 °C	101 ± 1%	101 ± 1%	101 ± 5%	100 ± 3%	98 ± 1%			(Prata et al., 2019)
Acidic								
22.5 M HNO_3, 12 h at R.T. and 30 min at 100 °C	4 ± 3% (w/ PS)		4 ± 3% (w/ PE)					(Avio et al., 2015)
15.7 M HNO_3, boiled for 2 h	95–100%		49 ± 7%	0%				(Schrank et al., 2022)
H_2SO_5, 48 h at R.T.	95–100%	95–100%	95–100%	95–100%				(Schrank et al., 2022)
5 M HNO_3 and 0.3 M $Na_2S_2O_8$, 18 h at 80 °C	100 ± 0%	99 ± 1%	99 ± 1%	97 ± 3%	0 ± 0%	Microalgae	1 ± 0%	This paper

compared to common petrochemical-derived plastics such as PE, PP, PS, and PET, also impact the stability of cellulose acetate to digestion. Of relevance to method development to isolate plastics from other organics, a method designed to remove a wide range of natural POM from a sample will also likely remove cellulose acetate. For these reasons, we chose to develop a method that excludes cellulose acetate from the analytical window.

Of the other organics tested, Albacore tuna tissue was included to represent animal, specifically fish, tissue from which researchers may wish to isolate plastics. Albacore tuna tissue is composed of 21–27% proteins and 50–60% water, with the remaining mass comprised of lipids (Wheeler and Morrissey, 2003), suggesting the tissue will be relatively easy to digest compared to plastics. Two forms of POM that might be encountered in natural waters were also included. POM from unicellular algae was included to represent autochthonous POM found in lacustrine and marine settings. Algal POM is comprised of largely hydrolysable molecules, predominantly polysaccharides, proteins, and lipids (Baldock et al., 2004), suggesting it may also be relatively easy to digest. Urban river POM was used as a source of riverine organics and to provide a POM source that may prove harder to digest due to its chemistry. As well as autochthonous OC from microalgae, rivers also receive allochthonous inputs from the land composed of a greater variety of harder-to-degrade carbon forms such as wood chips, black carbon and anthropogenic materials like plastics or rubber debris (Chin et al., 1994; Jaffé et al., 2013; Onstad et al., 2000; Dimov et al., 2008).

Based upon assessment of the literature, one base was trialed but acidic digestions were prioritized as ether bonds present in cellulose acetate are susceptible to acid-catalyzed hydrolysis (Battista et al., 1956; Battista, 1950) and acids have been recommended for digestion of organic and biological materials (OSPAR, 2015). To develop and test an improved method a combination of standard and post-consumer plastics in concert with the organics noted above. The results are compared against the best performing existing methods summarized in Table 1. The optimized method is presented along with recommendations to improve standardization in future plastics studies, including further digestion method development.

2. Materials and methods

2.1. General labware and filter materials

47 mm 0.45 μm pore Whatman Cellulose Acetate Circle WCA Range filters were used as purchased. 25 mm Whatman GF/F glass microfiber filters (nominal pore size 0.7 μm) were combusted prior to use. 24 mL glass vials were used for digestions. A glass vacuum filtration setup was used to transfer particulates to filters. All glassware was cleaned before use to remove residual OC. Briefly, glassware was soaked overnight in a pH 2 hydrochloric acid bath, rinsed with Milli-Q ultrapure laboratory water, dried overnight at 60 °C, and combusted for 5 h at 550 °C. In this and subsequent method descriptions 'overnight' indicates a reaction time of 16–18 h.

2.2. Plastic particle preparation

Standard 3.5 mm PS granules (GoodFellow, ST316310/4), 2 mm PE granules (GoodFellow, ET306300/1), 3–5 mm PET granules (GoodFellow, ES306313/1), and 2 mm PP granules (Aldrich, 427861) were purchased. Additional post-consumer plastics were collected; PS (food tray), PE (Dasani bottle cap), PET (Dasani bottle), and PP (disposable spoon). Post-consumer plastics were soaked overnight with MilliQ water then dried and characterized via micro-Fourier transform infrared spectroscopy (FT-IR). Post-consumer plastics were cut into 3 mm by 3 mm pieces, with a thickness of ~1 mm.

2.3. Preparation of natural organic material

Unicellular algae, riverine particulate organic matter (POM), and store bought wild caught Albacore Tuna were selected as sources of natural organic material. Unicellular algae were obtained from a culture of mixed *Scenedesmus* sp. and *Nannochloropsis* sp. (Carolina Biological Supply). Algae were allowed to settle for 48 h in the dark. Excess water was then decanted producing a thick sludge which was oven dried at 60 °C. The dry algae were fumigated overnight in a sealed desiccator with concentrated (12 N) hydrochloric acid to remove inorganic carbon prior to analysis (Harris et al., 2001).

POM was collected from the Muddy River (42°20′ N −71°06' W; March 21st, 2022; Boston, MA, USA), a series of urban brooks, ponds, and culverts. Water from the river surface was collected in 20 L polyfluorinated carboys and returned to the laboratory for vacuum filtering. POM was operationally defined as carbon collected on a GF/F filter and present after inorganic carbon removal by fumigation (Turnewitsch et al., 2007). POM digestion was conducted by submerging the filter in digestion solution. The filter was removed and then rinsed into the digestion vial with Milli-Q water before re-filtering the digestion solution to capture remaining particulates.

Wild caught Albacore tuna was purchased from Whole Foods. Tissue was dissected to remove bones and aliquoted into vials for digestion. Digestion efficacy was determined by massing tissue using a Sartorius analytical balance (Secura series) with 0.001 g precision pre-digestion, and by filtering the solution post-digestion onto a pre-weighed GF/F, drying, and reweighing.

2.4. Assessment of procedural blanks

A procedural blank was included for each digestion condition during optimization and in each batch of digestions during plastic stability and OM removal studies. Procedural blanks were filtered onto GF/F filters and analyzed via elemental analysis-isotope ratio mass spectrometry (EA-IRMS) to detect any particulate organic carbon (POC) contamination. POC present in procedural blanks always fell below the limit of quantification for the EA-IRMS.

2.5. Elemental analysis-isotope ratio mass spectrometry (EA-IRMS)

EA-IRMS was used to determine particulate C-mass (Zhu et al., 2020; Crable and Coggeshall, 1958). Plastic samples were homogenized into powder using a spice grinder prior to analysis. Powdered samples were packed into 3 × 5 mm tin capsules (Elemental Microanalysis, Marlton, NJ, USA) and samples filtered onto 25 mm GF/F filters were packed into 10 × 10 mm tin capsules. Samples were massed on a Sartorius analytical balance (Secura series) with 0.002 mg precision. The prepared capsules were combusted in an elemental analyzer (Flash 2000; Thermo Scientific) coupled to an isotope ratio mass spectrometer (Delta V *Plus*, Thermo Scientific). Peak area vs. C-mass was calibrated daily with pre-massed chitin standards stored in a desiccator. Verification of the applicability of chitin as a calibration material was conducted by evaluating linearity of a sample set consisting of chitin (n = 14), PE (n = 13), and cellulose acetate (n = 13). Linearity was observed for peak area to carbon mass for $^{44}CO_2$, $^{45}CO_2$, and $^{46}CO_2$ up to 450 μg-C with $R^2 > 0.989$ for each CO_2 isotope.

2.6. Fourier transform infrared (FT-IR) spectroscopy

Plastics were characterized via attenuated total reflection (ATR) μ-FT-IR using a Thermo Fisher Scientific Nicolet iN10 MX spectrometer equipped with a liquid nitrogen cooled detector. Two cleaned plastic pieces were randomly selected for characterization per plastic type. μ-FT-IR spectra (400–4000 cm^{-1}; resolution 8 cm^{-1}) were acquired from randomly selected locations on each plastic piece and compared against a library of reference polymer spectra (HR Hummel Polymers and

Additives) using Thermo Fisher's OMNIC search expert software (Zhu et al., 2020). Automatic baseline correction and atmospheric suppression was used.

2.7. Optical microscopy

Optical images of microplastics were collected using a Nikon SMZ18 digital microscope.

2.8. Detection limits

Assessment of limits of detection and quantification were critical in determining the percent removal of OC as recoveries of near 0% were typical. A Sartorius Secura series balance with 0.001 g precision was used for gravimetric analysis. EA-IRMS detection limits, calculated per analytical run during calibration, were consistently ~8 μg-C for limit of detection and ~21 μg-C for limit of quantification. Starting masses of samples were controlled to be no less than one hundredfold the limit of quantification, ensuring detection limits would not prevent determination of near quantitative removals of >99%. Larger starting masses were used where possible to improve the precision of percent removal calculations.

3. Results and discussion

3.1. Preliminary optimization of cellulose acetate digestion conditions

A review of chemical digestion methods for microplastic workup (Table 1) indicated widespread use of a limited suite of chemicals, including hydrogen peroxide, hydroxides, and acids. For the current work, one base (sodium hydroxide) and four acids (hydrochloric, sulfuric, nitric, and phosphoric acid) were trialed at 6 N with addition of sodium persulfate at 0.3 M. Persulfates efficiently oxidize organic material (Tsitonaki et al., 2010). In the current work, sodium persulfate was selected due to its aqueous solubility (Ma et al., 2017).

Initial optimization assessed the digestion of cellulose acetate filters in 24 h digestions at 70 °C. Digestions were conducted at 4.9 mg/mL cellulose acetate. No obvious digestion occurred in phosphoric acid and sodium hydroxide, and only partial digestion in sulfuric acid (Fig. S1). These digestants were not optimized further. Hydrochloric and nitric acid digestion left no visible fibers. Thus, further tests were conducted using both hydrochloric and nitric acid.

Preliminary microplastic stability was assessed for hydrochloric acid and nitric acid, each at 6 N with 0.3 M persulfate. Granules of PE, PP, PS, and PET were subjected to digestion and visually inspected for degradation (melting, discoloration, etc.). All plastics appeared physically unchanged after digestion in nitric acid, however PS and PET yellowed during digestion in hydrochloric acid. Therefore, nitric acid alone was selected for further optimization.

3.2. Temperature and concentration optimization

To determine optimal acid concentration and temperature for cellulose acetate digestion, digestion time (24 h) and persulfate concentrations (0.3 M) were maintained, while temperature (70°–90 °C) and nitric acid concentrations (1–6 N) were varied. Increasing temperature improved digestion efficacy (Table 2). However, at 90 °C evaporation of the digestion solution resulted in recrystallization of the persulfate. Digestion efficiency generally increased with increasing acid strength (Table 2). At 80 °C no cellulose acetate was detected at 5 or 6 N nitric acid. As high concentration nitric acid can degrade microplastics (Avio et al., 2015) and to minimize chemical use and hazards, 5 N nitric acid at 80 °C was selected as the mildest acidity capable of fully depolymerizing cellulose acetate.

Table 2
Percent recovery of cellulose acetate for tested nitric acid concentrations and temperatures, all with 0.3 M sodium persulfate and heated for 24 h. Limit of detection corresponded to 1% initial mass of cellulose acetate, below limit of detection noted n.d.

Nitric Acid Strength	Temperature		
	70 °C	80 °C	90 °C
1 N	48%	49%	n.d.
2 N	44%	46%	n.d.
3 N	40%	37%	n.d.
4 N	26%	23%	n.d.
5 N	37%	n.d.	n.d.
6 N	28%	n.d.	n.d.

3.3. Reaction time optimization

To optimize reaction time, nitric acid (5 N) and persulfate (0.3 M) concentration and temperature (80 °C) were maintained, while digestion time was varied from 0 to 24 h. Increased reaction time resulted in improved digestion up to an overnight digestion (16–18 h), with no detectable benefit in completing a full 24-h reaction (Fig. S3). The reaction began with a brief period of filter wetting (~30 min) followed by an increase in transparency and flexibility as depolymerization proceeded, then fragmentation (1–3 h), and eventual loss of filter integrity (>4 h; Fig. S3).

3.4. Optimized digestion of cellulose acetate

The optimized digestion method is defined as submersion in 5 N nitric acid with 0.3 M sodium persulfate, digested overnight (16–18 h) at 80 °C in a ventilated oven. Single cellulose acetate filters were digested at concentrations of 4.9 g L^{-1}. Vials were left uncapped to prevent pressure buildup during digestion. Cellulose acetate recovery was undetectable using gravimetric analysis and was below the limit of quantification for EA-IRMS (0.08% initial cellulose acetate C mass). Therefore, based upon our analytical limitations, we report that the optimized method removed >99.92% of cellulose acetate C.

Few methods are available for comparison of cellulose acetate digestion, or other cellulosic materials, that also analyze plastic stability. The method reported here exceeded previously reported cellulose removals. For cellulose acetate, previous studies report that digestion with 1 M potassium hydroxide at room temperature moved only 31% (Kühn et al., 2017) and increasing concentration to 2.5 M and temperature to 60 °C removed only ~50% (Dehaut et al., 2016). Considering other cellulosic materials, multi-step multi-day protocols using sodium hydroxide/urea/thiourea solutions can digest 97 ± 6% of cellulose from cotton and paper (Olsen et al. 2020) and ~98% of cellulose from sewage sludge (Egea-Corbacho et al., 2022) in methods with high plastic stability. To our knowledge, ours is the first acidic digestion method and one-pot, short-term (less than 24 h) method for cellulose acetate digestion targeting microplastic preservation, that matches or exceeds existing protocols for digestion efficiency.

3.5. Digestion of natural POM

To evaluate the efficacy of our method to remove unwanted OM from samples two sources of natural OM were selected: unicellular algae and POM from an urban river (Muddy River, Boston, MA). Unicellular algae were used to represent autochthonous marine and lacustrine sources of POM. Unicellular algae is comprised of largely hydrolysable molecules, predominantly polysaccharides, proteins, and lipids (Baldock et al., 2004). Riverine POM comprises a larger variety of OC sources. As well as autochthonous OC from microalgae, rivers also receive allochthonous inputs from the land composed of a greater variety of carbon forms such as humics, lignin phenols, and black carbon (Chin et al., 1994; Jaffé

et al., 2013; Onstad et al., 2000). Digestion of Muddy River POM removed 86.1 ± 4.5% of the OC with the remaining 13.9% of particulates bleached to grey or white (Fig. 1b). For unicellular algae, 99.18 ± 0.06% of OC was removed. The lower digestion efficiency for muddy River POM may be explained by recalcitrant organics resistant to nitric acid digestion including woody fragments containing lignin, products of fire such as black carbon, and anthropogenic materials like plastics or rubber debris (Dimov et al., 2008). The method presented here outperformed or equaled the best optimized microplastic work-up methods that tested microalgae digestion, which reported 91.3 ± 0.4% removed (alkaline) (Cole et al., 2014), 98 ± 3% removed (alkaline/oxidative) (Olsen et al. 2020) and >97% removed (enzymatic) (Cole et al., 2014).

3.6. Digestion of fish tissue

An additional application for chemical digestion protocols is the extraction of microplastics from the tissue (flesh) of higher organisms. In the current study, we assessed the digestion of Albacore tuna tissue. Albacore tissue is composed of 21–27% proteins and 50–60% moisture, with the remaining mass comprised of lipids (Wheeler and Morrissey, 2003). The chemical constituents of Albacore tuna more closely resemble that of algae than riverine POM and experienced similarly effective digestion. Less than 1% of albacore tuna tissue was recovered for tissue concentrations ranging from 5.5 g-fish L^{-1} to 54.8 g-fish L^{-1} (Fig. 1). Near complete digestion achieved using the method presented here is comparable to some previously reported methods for digestion of marine organisms. For instance, reported oxidative and acidic methods have high efficiency in removing marine biological tissues, however plastic recovery rates as low as 85% indicate methods were not ideal for microplastic sampling (Dyachenko et al., 2017; Majewsky et al., 2016). Therefore, our method is comparable to existing methods in its ability to digest biological tissue but as seen below, our method is superior in its ability to retain microplastics.

3.7. Stability of microplastics during digestion

Existing chemical digestion protocols for isolation of microplastics report plastic-mass recoveries of 95–100% for chemically stable, hard-to-hydrolyze plastics with C–C backbones such as PE, PP and PS (Table 1 and references therein). Our protocol also provided high mass recoveries for polymers with C–C backbones: PE (100.2 ± 0.4%), PP (99.0 ± 0.5%), and PS (99.3 ± 0.7%). Recovery of PET was lower (96.8 ± 2.8%). PET stability varies in reported digestion methods, with high stability typical for oxidative digestions, and complete removal in boiling 15.7 N nitric acid (Schrank et al., 2022). PET, and other plastics with heteroatoms in the backbone chain, have multiple pathways of chemical degradation such as hydrolysis of the ether bonds in PET (Gewert et al., 2015). Although hydrolysis can occur under acidic conditions and is also the hypothesized method of cellulose acetate depolymerization, the digestion conditions used here significantly improve PET stability compared to previously reported nitric acid methods (Table 1).

EA-IRMS was used to determine the %-C composition of the plastic standards pre- and post-digestion. PE and PP decreased in %-C content by 0.98% (independent *t*-test, $\alpha = 0.05$), while %-C content did not change for PET or PS (independent *t*-test, $\alpha = 0.05$). The potential for digestion conditions to inadvertently impact plastics is well known. Heteroatom (e.g., O or S) addition has been reported following exposure to oxidative conditions (Zhao et al., 2021), and heat-activated persulfate treatment has been reported to cause significant chemical changes in plastics (Liu et al., 2019). Heteroatom additions can offset plastic-mass loss, thus, reporting microplastic stability using only %-mass recovery can be misleading. %-C recovery reported alongside %-mass recovery or %-C by mass provides a more stringent assessment of the stability of the carbon filled backbone of the plastics. %-C recoveries for plastic standards using our method were: PE (98.1 ± 1.6%), PP (96.7 ± 1.5%), PET (97.3 ± 2.7%), and PS (98.2 ± 4.8%). As carbon recovery is not currently a common metric for plastic stability, comparison to other studies is not possible. However, the trend of slight reduction in carbon recovery relative to plastic mass recovery (Fig. 2a) highlights the importance of %-C recovery to detect minor changes in plastic polymer chemistry during digestion.

Post-consumer PE, PP, PET, and EPS plastics were also tested. Pre- and post-digestion imaging show high conservation of plastic morphology (Fig. 2b), although discoloration occurred for a fragment of the green PE bottle cap. Microplastic bleaching has been observed for some oxidative digestions (Nuelle et al., 2014), while other studies find no bleaching under similar conditions (Avio et al., 2015). These mixed results highlight the role of additives, specifically dyes, in the visual stability of plastics during digestion. The chemical structures of additives are expected to vary from that of the polymer and may be more or less reactive under digestion conditions.

FT-IR spectra for the post-consumer plastics were compared pre- and

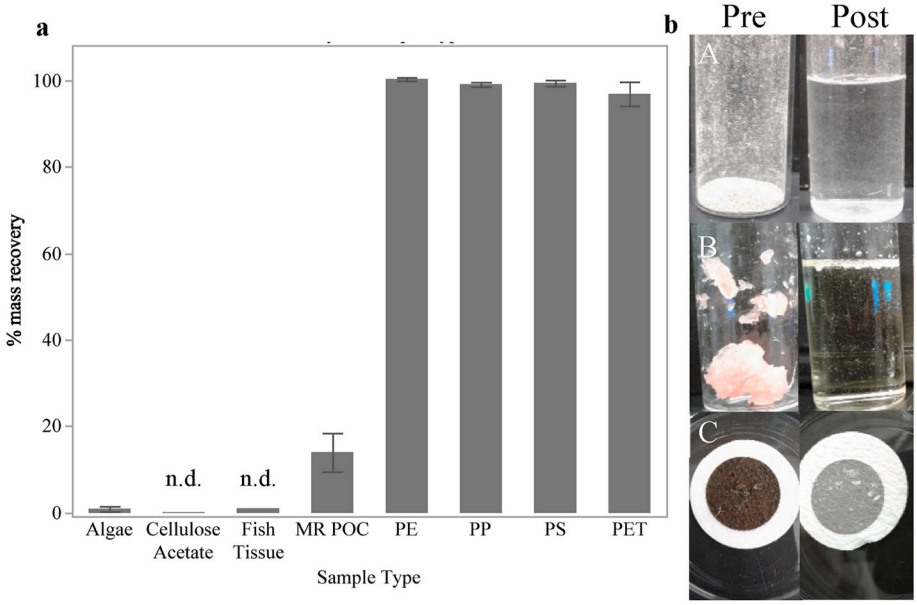
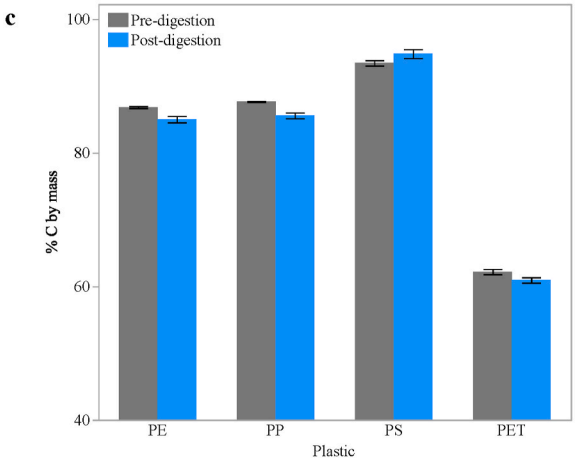


Fig. 1. Digestion efficacy of 5 N nitric acid with 0.3 M sodium persulfate at 80 °C. a) %-mass recovery post-digestion of organic matter and plastics, polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET). Not detected (n.d.) samples are indicated by the limit of quantification represented as %-mass recovery without error. b) Organic matter pre- (left) and post- (right) digestion in 5 N nitric acid with 0.3 M sodium persulfate at 80 °C overnight. A) Algae, B) Albacore tuna tissue, C) Muddy River particulate organic carbon (POC) on a GF/F filter.

a

Plastic	Percent mass recovery	Percent C recovery
PE	100.2 ± 0.4	98.1 ± 1.6
PP	99.0 ± 0.5	96.7 ± 1.5
PS	99.3 ± 0.7	98.2 ± 4.8
PET	96.8 ± 2.8	97.3 ± 2.7



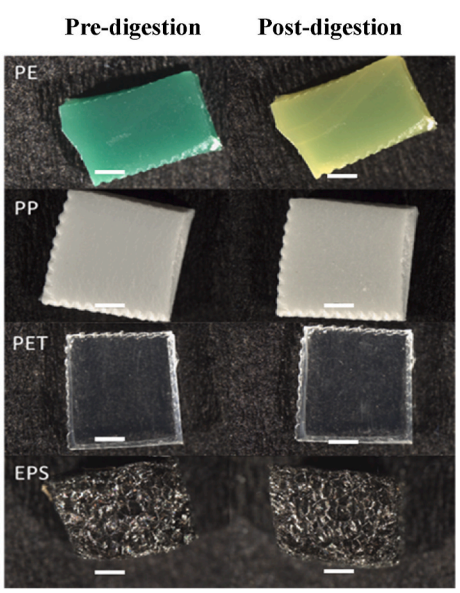


Fig. 2. Physical and chemical stability of plastics during digestions. a) Tabulated % mass and % C recovery. b) %-C content of plastics pre- and post-digestion. Data represents mean ± SEM (n = 3 per plastic type, one outlier removed from PET post-digestion set following one-tailed Grubb's test α = 0.05). c) Post-consumer plastics pre- and post-digestion, polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and expanded polystyrene (EPS). Imaged using a digital microscope. White scale bar = 1 mm.

post-digestion (Fig. 3). For all plastics the characteristic polymer peaks were unchanged during digestion. Potential oxidation was evaluated by analyzing the 1670 cm^{-1} to 1800 cm^{-1} region where peaks indicative of carboxyl groups appear (Rajakumar et al., 2009). Calculating oxidation metrics for PET is not recommended due to the ester groups in the polymer chain and their strong C═O peak circa 1714 cm^{-1}. Thus, we only calculated CI values for PE, PP, and EPS. Oxidation was evaluated using two methods: peak height carbonyl index (CI) (Miranda et al., 2021) and specific area under the band (SAUB) (Almond et al., 2020). The stable methylene peaks were selected as references for PE (2848 cm^{-1}) and PP (2844 cm^{-1}), and the C–H bending peak (697 cm^{-1}) was selected for EPS. CI indicated minor oxidation of the post-consumer plastics pre-digestion: PE (0.019), PP (0.049), and EPS (0.069). CI values increased for all three plastics during digestion: PE (0.036), PP (0.179), and EPS (0.112). Comparison of the PE CI values with values from rooftop weathering experiments suggests the post-consumer PE had the oxidation equivalent of 1.5 weeks of natural sunlight exposure before digestion, and 3 weeks of natural sunlight exposure post-digestion (Miranda et al., 2021). However, CI can be difficult for comparisons between studies as factors such as mode (transmission vs. ATR), reference peak selection, and oxidation peak width, impact CI values, and different methods to calculate CI yield differing results when applied to a single spectrum (Almond et al., 2020). Alternatively, Almond et al. suggest adoption of SAUB, though this has not yet been

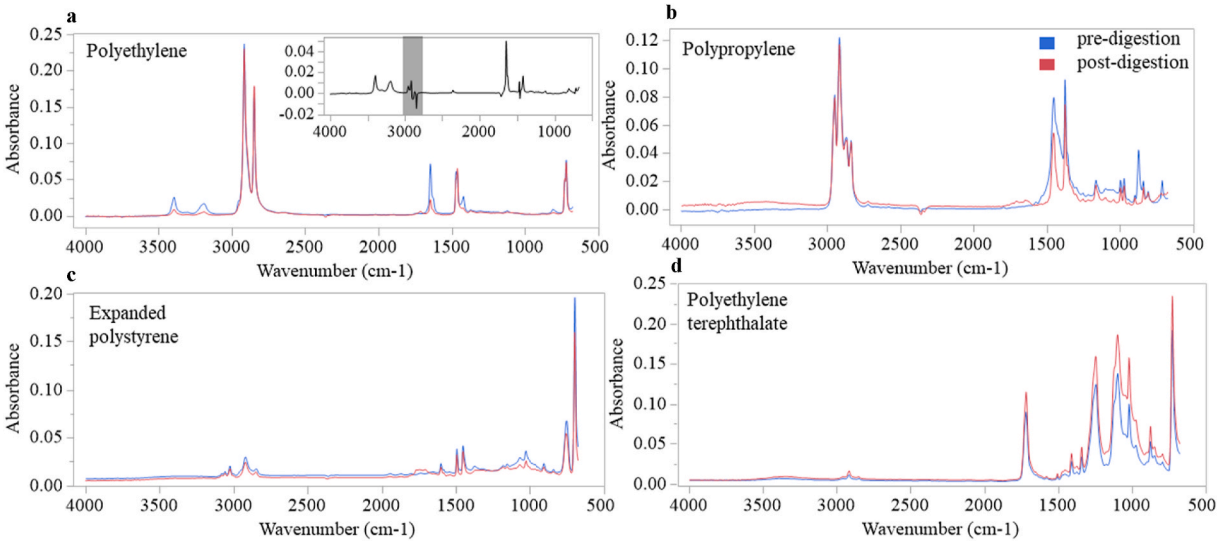


Fig. 3. Fourier transform infrared spectroscopy attenuated total reflectance spectra of microplastics subjected to chemical digestion, (red) pre-digestion and (blue) post-digestion. Polyethylene (A), polypropylene (B), expanded polystyrene (C), and polyethylene terephthalate (D). Inset in A: Polyethylene difference spectrum showing pre-digestion minus post-digestion, with region obscured by major polyethylene peaks complicating identification highlighted in grey. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

widely adopted. By the SAUB method the pre- (0.056) and post-digestion (0.170) PP had oxidation equivalent to less than 5 days of artificial accelerated aging (Almond et al., 2020). EPS also showed oxidation, with a greater SAUB increase from 0.087 to 0.548 post-digestion, with the stable 725 cm^{-1} to 810 cm^{-1} C–H bending peak as reference, although no literature comparison was available. The presence of an additive peak at 1463 cm^{-1} prevented calculation of accurate SAUB values for PE (Fig. 3A).

The largest changes in the spectra were the reduction in intensity of peaks not attributable to the polymers after digestion (Fig. 3). PE had four such peaks (3396 cm^{-1}, 3191 cm^{-1}, 1646 cm^{-1}, and 1421 cm^{-1}) present pre-digestion and that reduced in intensity post-digestion relative to the four main PE peaks (~2916 cm^{-1}, ~2848 cm^{-1}, ~1463 cm^{-1}, and ~719 cm^{-1}) (Gulmine et al., 2002). PP had one peak at 874 cm^{-1}. The difference spectrum calculated for PE as the pre-digestion spectrum minus the post-digestion spectrum (Fig. 3A inset) was run through a spectral library search (OMNIC search expert, HR Polymer Additives and Plasticizers) and resulted in a 74.1% match with N,N′-ethylenebis (stearamide), widely used in plastic manufacturing, suggesting a possible identification, although the match is lower than the commonly applied limit of 90% or greater for definitive identification. No significant matches were found for the difference spectra for PP or the other polymers. The chemical stability of the plastics, excluding peaks attributed to additives, demonstrate the suitability of this method for the tested plastics. Slight oxidation visible in FT-IR for PE and PP is comparable with the Egea-Corbacho et al. digestion optimized to remove cellulosic material. (PS not tested) (Egea-Corbacho et al., 2022). The correlation between post-digestion and reference spectra in this study is high (>94% for all plastics, calculated by OMNIC software) indicating the minor oxidation that occurs during digestion will not prevent accurate characterization. From FT-IR analysis and high mass and carbon recoveries we conclude that the digestion method reported here is suitable for the tested plastics (i.e. PE, PP, PET, and PS).

4. Conclusions

We present an optimized acidic digestion protocol suitable for the work-up of microplastics mixed with organic material including synthetic cellulose acetate filters. For this method as for all analytical methods, it is instructive to define the analytical window or operational definition. For any digestion method used to purify plastics in an environmental matrix, the operational definition is all non-digestible, measurable material, which is presumed representative of plastic content, and in this method will not include cellulose acetate. Depending on analytical methods of detection this may be measured in particle counts, mass, g-C, or some other metric. It is expected that non-plastic, non-digestible particulates will be present in complex environmental samples. We recommend the use of the method to extract microplastics for further analysis and to provide an estimate of total plastic mass and plastic-C in samples that may include nanoplastics.

We recommend the method be used as follows: complete submersion of samples in a solution of 5 N nitric acid with 0.3 M sodium persulfate heated uncovered at 80 °C overnight (16–18 h) in a ventilated oven. Heating uncovered or vented prevents pressure buildup during the reaction. The use of glass equipment is necessary as the digestion solution and fumes will react with metal. Our 5 N nitric acid and persulfate method digested >99% of cellulose acetate, albacore tuna tissue, cellulose acetate, and microalgae biomass, and is suitable for the tested plastics.

This method provided near-quantitative (>99%; Fig. 2) mass recoveries of PE, PP, and PS, and excellent recovery of PET (>96%; Fig. 2). These recoveries equal or exceed recoveries of microplastics after digestion to remove organic material reported previously (Table 1). The excellent recovery of PET is notable as PET is particularly susceptible to digestion by published methods (Hurley et al., 2018; Nuelle et al., 2014; Olsen et al. 2020). Nitric acid is effective at digesting organic material, including fish tissue (LamLeung et al., 1991) and cellulosic-rich rice husk (Rahman et al., 1997). Consequently, protocols including nitric acid were recommended for plastic extraction from marine organisms by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) (Dehaut et al., 2016). However, other studies report that nitric acid at high concentrations degrades plastics (Desforges et al., 2015; Lusher et al., 2020). However, as evidenced by the results here, a concentration of 5 N nitric acid results in high plastic stability (96–99%, Table 1). We conclude that concentration is a key factor for the use nitric acid for microplastics applications, and that 5 N nitric acid should be considered within the suitable range.

Samples collected from natural waters or sediments also require separation from other organics. As noted, our method was highly effective at digesting microalgae-derived OC. Current alkaline (91.3 ± 0.4%) (Cole et al., 2014) and oxidative (98 ± 3%) (Olsen et al. 2020) methods to digest microalgae are not as effective. Of the organic material sources trialed in this study the Muddy River POM was most resistant to digestion, with ~86% OC recovered. In chemical digestions it is necessary to balance plastic stability with organic removal, alongside safety and ease of use. The method reported here has several advantages, including quantitative cellulose acetate removal, high plastic stability, the ease of a one-pot digestion, and chemical safety easily ensured with the use of a fume hood. However, incomplete removal of riverine POC is a disadvantage for applications where this type of material is expected. Therefore, additional optimization is required for samples with high loads of terrestrial or anthropogenic particulate matter and other methods or modifications to the current method may be more suitable for those samples.

FT-IR spectroscopy is frequently used to analyze polymer oxidation. However, additives, plasticizers, and colorants can complicate FT-IR spectra. Although plastic mass, C and the polymer FT-IR signature were preserved during digestion, the FT-IR signatures of additives were degraded (Fig. 3). The chemistry of compounds associated with plastics, including additives, is critical to the environmental and human health impact of plastics (MacLeod et al., 2021). Thus, additional research is needed to assess how plastic sample preparation impacts additive chemistry and develop methods capable of preserving plastic additives when desired. Due to the observed instability of additives, this method is not recommended for studies of organic additives or their degradation products.

Despite decades of microplastic research, the methods used are not well standardized, making selecting appropriate protocols and comparison of the data obtained difficult (Hartmann et al., 2019; Li et al., 2020). At present, the source and composition of OM and the types of plastic polymers used to test digestion methods varies. To advance the field, a suite of internationally recognized OM and plastic reference materials for standardized testing of methods should be developed. Where possible, we also recommend organic matter losses and microplastic recovery be reported as both mass and carbon. Although standardized testing of our method against other methods would improve judgements of which method to use for a specific sample, based on the fact that our method achieves near quantitative removal of albacore tuna tissue, cellulose acetate, and microalgae biomass, while also offering near quantitative recovery of PE, PP, and PS, and >96% recovery of PET, we recommend our method for extraction of microplastics from a variety of organic and biological material.

Credit author statement

Erin Tuttle: Methodology, Investigation, Writing – original draft **Aron Stubbins**: Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank Dr. Leila Deravi at Northeastern University for access to the Nikon SMZ18 digital microscope and Dr. Chris Nadeau for providing the *Scenedesmus* sp. and *Nannochloropsis* sp. culture. This work was funded by NSF EAGER 2127669 and NSF CBET 1910621.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121198.

References

Almond, Jasmine, Sugumaar, Piriya, Wenzel, Margot N., Hill, Gavin, Wallis, Christopher, 2020. 'Determination of the carbonyl index of polyethylene and polypropylene using specified area under band methodology with ATR-FTIR spectroscopy. E-Polymers 20, 369–381.
Avio, Carlo Giacomo, Gorbi, Stefania, Regoli, Francesco, 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.
Baldock, J.A., Masiello, C.A., Gelinas, Y., Hedges, J.I., 2004. 'Cycling and composition of organic matter in terrestrial and marine ecosystems. Mar. Chem. 92, 39–64.
Battista, Oo A., 1950. 'Hydrolysis and crystallization of cellulose. Ind. Eng. Chem. 42, 502–507.
Battista, O.A., Coppick, Sydney, Howsmon, J.A., Morehead, F.F., Sisson, Wayne A., 1956. 'Level-off degree of polymerization. Ind. Eng. Chem. 48, 333–335.
Borrelle, Stephanie B., Ringma, Jeremy, Law, Kara Lavender, Monnahan, Cole C., Lebreton, Laurent, McGivern, Alexis, Murphy, Erin, Jambeck, Jenna, , George H Leonard, Hilleary, Michelle A., 2020. 'Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369, 1515–1518.
Carpenter, Edward J., Smith Jr., K.L., 1972. 'Plastics on the sargasso sea surface. Science 175, 1240–1241.
Chain, EFSA Panel on Contaminants in the Food, 2016. 'Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J. 14, e04501.
Chin, Yu-Ping, George, Aiken, O'Loughlin, Edward, 1994. 'Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol. 28, 1853–1858.
Cole, Matthew, Webb, Hannah, , Pennie K Lindeque, Elaine S Fileman, Halsband, Claudia, Galloway, Tamara S., 2014. 'Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 1–8.
Courtene-Jones, Winnie, Quinn, Brian, Murphy, Fionn, Gary, Stefan F., E Narayanaswamy, Bhavani, 2017. 'Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. Anal. Methods 9, 1437–1445.
Crable, G.F., Coggeshall, N.D., 1958. 'Application of total ionization principles to mass spectrometric analysis. Anal. Chem. 30, 310–313.
Dehaut, Alexandre, , Anne-Laure Cassone, Frère, Laura, Hermabessiere, Ludovic, Himber, Charlotte, Rinnert, Emmanuel, Rivière, Gilles, Lambert, Christophe, Soudant, Philippe, Arnaud, Huvet, 2016. 'Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233.
Desforges, Jean-Pierre W., Galbraith, Moira, Ross, Peter S., 2015. Ingestion of microplastics by zooplankton in the northeast pacific ocean. Arch. Environ. Contam. Toxicol. 69, 320–330.
Dimov, M., Stoeva, S., Tsaikova, S., 2008. 'Interaction of nitric acid with rubber chunks derived from waste tires. Oxid. Commun. 31, 931–941.
Dyachenko, A., Mitchell, J., Arsem, N., 2017. 'Extraction and identification of microplastic particles from secondary wastewater treatment plant (WWTP) effluent. Anal. Methods 9, 1412–1418.
Egea-Corbacho, Agata, Martín-García, Ana Pilar, Franco, Ana Amelia, Albendín, Gemma, María arellano, Juana, Rodríguez, Rocío, María Quiroga, José, Dolores Coello, María, 2022. A method to remove cellulose from rich organic samples to analyse microplastics. J. Cleaner Prod. 334, 130248.
Felsing, Stefanie, Kochleus, Christian, Buchinger, Sebastian, Brennholt, Nicole, Stock, Friederike, Reifferscheid, Georg, 2018. A new approach in separating microplastics from environmental samples based on their electrostatic behavior. Environ. Pollut. 234, 20–28.
Finnegan, A.M.D., Süsserott, R.C., Gabbott, Sarah E., Gouramanis, Chris, 2022. Man-made Natural and Regenerated Cellulosic Fibres Greatly Outnumber Microplastic Fibres in the Atmosphere. Environ. Pollut., 119808
Foekema, Edwin M., De Gruijter, Corine, Mergia, Mekuria T., Jan van Franeker, Andries, AlberTinka, J Murk, Koelmans, Albert A., 2013. Plastic in north sea fish. Environ. Sci. Technol. 47, 8818–8824.
Gasperi, Johnny, Wright, Stephanie L., Dris, Rachid, Collard, France, Mandin, Corinne, Guerrouache, Mohamed, Langlois, Valérie, Kelly, Frank J., Bruno, Tassin, 2018. Microplastics in air: are we breathing it in? Curr. Opin. Environ. Sci. Health 1, 1–5.
Gewert, Berit, Plassmann, Merle M., MacLeod, Matthew, 2015. 'Pathways for degradation of plastic polymers floating in the marine environment. Environ. sci.: Process. Impacts 17, 1513–1521.
Geyer, Roland, , Jenna R Jambeck, Law, Kara Lavender, 2017. 'Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782.
Gulmine, J.V., Janissek, P.R., Heise, H.M., Akcelrud, L., 2002. 'Polyethylene characterization by FTIR. Polym. Test. 21, 557–563.
Harris, D., Horwath, W.R., van Kessel, C., 2001. 'Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J. 65, 1853–1856.
Hartmann, Nanna B., Huffer, Thorsten, Thompson, Richard C., Hassellov, Martin, Verschoor, Anja, , Anders E Daugaard, Rist, Sinja, Karlsson, Therese, Brennholt, Nicole, Cole, Matthew, 2019. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. ACS Publications.
Hermsen, Enya, Pompe, Renske, Besseling, Ellen, Koelmans, Albert A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Mar. Pollut. Bull. 122, 253–258.
Hidalgo-Ruz, Valeria, Gutow, Lars, Thompson, Richard C., Thiel, Martin, 2012. 'Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.
Hurley, Rachel R., Lusher, Amy L., Olsen, Marianne, Nizzetto, Luca, 2018. 'Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ. Sci. Technol. 52, 7409–7417.
Imhof, Hannes K., Schmid, Johannes, Niessner, Reinhard, P Ivleva, Natalia, Laforsch, Christian, 2012. 'A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol Oceanogr. Methods 10, 524–537.
Jaffé, Rudolf, Ding, Yan, Niggemann, Jutta, , Anssi V Vähätalo, Aron Stubbins, Spencer, Robert GM., Campbell, John, Dittmar, Thorsten, 2013. Global charcoal mobilization from soils via dissolution and riverine transport to the oceans. Science 340, 345–347.
Karami, Ali, Golieskardi, Abolfazl, , Cheng Keong Choo, Romano, Nicholas, Ho, Yu Bin, Salamatinia, Babak, 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494.
Olsen, L.M.B., Knutsen, Heidi, Mahat, Sabnam, Wade, Emma Jane, H Arp, Hans Peter, 2020. 'Facilitating microplastic quantification through the introduction of a cellulose dissolution step prior to oxidation: proof-of-concept and demonstration using diverse samples from the Inner Oslofjord, Norway. Mar. Environ. Res. 161, 105080.
Koelmans, Albert A., , Nur Hazimah Mohamed Nor, Hermsen, Enya, Kooi, Merel, Mintenig, Svenja M., De France, Jennifer, 2019. 'Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res. 155, 410–422.
Kühn, Susanne, Van Werven, Bernike, Albert Van Oyen, Meijboom, André, Rebolledo, Elisa L Bravo, Van Franeker, Jan A., 2017. 'The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90.
LamLeung, Suei Y., Cheng, Vincent KW., Lam, Yuet W., 1991. 'Application of a microwave oven for drying and nitric acid extraction of mercury and selenium from fish tissue. Analyst 116, 957–959.
Li, C.R., Busquets, R., Campos, L.C., 2020. 'Assessment of Microplastics in Freshwater Systems: A Review'. Science of the total environment, p. 707.
Liu, Peng, Qian, Li, Wang, Hanyu, Zhan, Xin, Lu, Kun, Gu, Cheng, Gao, Shixiang, 2019. New insights into the aging behavior of microplastics accelerated by advanced oxidation processes. Environ. Sci. Technol. 53, 3579–3588.
Loder, M.G.J., Imhof, H.K., Ladehoff, Maike, Loschel, Lena A., Lorenz, Claudia, Mintenig, Svenja, Piehl, Sarah, Primpke, Sebastian, Schrank, Isabella, Laforsch, Christian, 2017. Enzymatic purification of microplastics in environmental samples. Environ. Sci. Technol. 51, 14283–14292.
Lusher, A.L., Welden, N.A., Sobral, P., Cole, MJAoN., 2020. 'Sampling, isolating and identifying microplastics ingested by fish and invertebrates. In: Analysis of Nanoplastics and Microplastics in Food. CRC Press.
Ma, Jian, Yuan, Yuan, Zhou, Tingjin, Yuan, Dongxing, 2017. Determination of total phosphorus in natural waters with a simple neutral digestion method using sodium persulfate. Limnol Oceanogr. Methods 15, 372–380.
Macieira, Raphael M., , Leticia Aparecida Silva Oliveira, Cardozo-Ferreira, Gabriel C., Ribeiro Pimentel, Caio, Ryan, Andrades, , João Luiz Gasparini, Sarti, Francesco, Chelazzi, David, Cincinelli, Alessandra, Gomes, Levy Carvalho, 2021. 'Microplastic and artificial cellulose microfibers ingestion by reef fishes in the Guarapari Islands, southwestern Atlantic. Mar. Pollut. Bull. 167, 112371.
MacLeod, Matthew, Arp, Hans Peter H, Tekman, Mine B., Jahnke, Annika, 2021. The global threat from plastic pollution. Science 373, 61–65.
Majewsky, M., Bitter, H., Eiche, E., Horn, H., 2016. 'Determination of microplastic polyethylene (PE) and polypropylene (PP) in environmental samples using thermal analysis (TGA-DSC). Sci. Total Environ. 568, 507–511.
Miranda, Mariana N., J Sampaio, Maria, Tavares, Pedro B., Adrián, MT Silva, Pereira, M. Fernando R., 2021. 'Aging assessment of microplastics (LDPE, PET and uPVC) under urban environment stressors. Sci. Total Environ. 796, 148914.

Nuelle, Marie-Theres, , Jens H Dekiff, Remy, Dominique, Fries, Elke, 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161–169.
Onstad, Gretchen D., Canfield, Donald E., Paul, D Quay, Hedges, John I., 2000. 'Sources of particulate organic matter in rivers from the continental USA: lignin phenol and stable carbon isotope compositions. Geochem. Cosmochim. Acta 64, 3539–3546.
Prata, Joana C., Costa, Joao P da, V Girão, Ana, Lopes, Isabel, Duarte, Armando C., Rocha-Santos, Teresa, 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Sci. Total Environ. 686, 131–139.
Rahman, I.A., Ismail, J., Osman, Hl, 1997. 'Effect of nitric acid digestion on organic materials and silica inrice husk. J. Mater. Chem. 7, 1505–1509.
Rajakumar, K., Sarasvathy, V., A Thamarai Chelvan, R Chitra, Vijayakumar, C.T., 2009. 'Natural weathering studies of polypropylene. J. Polym. Environ. 17, 191–202.
Schrank, Isabella, Möller, Julia N., , Hannes K Imhof, Oliver, Hauenstein, Zielke, Franziska, Agarwal, Seema, Löder, Martin GJ., Greiner, Andreas, Laforsch, Christian, 2022. 'Microplastic sample purification methods-Assessing detrimental effects of purification procedures on specific plastic types. Sci. Total Environ. 833, 154824.
Stubbins, Aron, Law, Kara Lavender, Muñoz, Samuel E., Bianchi, Thomas S., Zhu, Lixin, 2021. 'Plastics in the Earth system. Science 373, 51–55.
Tiwari, M., Rathod, T.D., Py Ajmal, Bhangare, R.C., Sahu, S.K., 2019. 'Distribution and characterization of microplastics in beach sand from three different Indian coastal environments. Mar. Pollut. Bull. 140, 262–273.
Tsitonaki, Aikaterini, Benjamin, Petri, Crimi, Michelle, Mosbaek, H.A.N.S., Siegrist, Robert L., Poul L Bjerg, 2010. In situ chemical oxidation of contaminated soil and groundwater using persulfate: a review. Crit. Rev. Environ. Sci. Technol. 40, 55–91.
von Friesen, Lisa, W., Granberg, Maria E., Hassellöv, Martin, Gabrielsen, Geir W., Magnusson, Kerstin, 2019. An efficient and gentle enzymatic digestion protocol for the extraction of microplastics from bivalve tissue. Mar. Pollut. Bull. 142, 129–134.
Wesch, Charlotte, , Anne-Kathrin Barthel, Braun, Ulrike, Klein, Roland, Paulus, Martin, 2016. 'No microplastics in benthic eelpout (Zoarces viviparus): an urgent need for spectroscopic analyses in microplastic detection. Environ. Res. 148, 36–38.
Wheeler, Sena C., Morrissey, Michael T., 2003. 'Quantification and distribution of lipid, moisture, and fatty acids of West Coast albacore tuna (Thunnus alalunga). J. Aquat. Food Prod. Technol. 12, 3–16.
Yu, Xubiao, Peng, Jinping, Wang, Jundong, Wang, Kan, Bao, Shaowu, 2016. 'Occurrence of microplastics in the beach sand of the Chinese inner sea: the Bohai Sea. Environ. Pollut. 214, 722–730.
Zhang, Qun, Xu, Elvis Genbo, Li, Jiana, Chen, Qiqing, Ma, Liping, Zeng, Eddy Y., Shi, Huahong, 2020. A review of microplastics in table salt, drinking water, and air: direct human exposure. Environ. Sci. Technol. 54, 3740–3751.
Zhao, Mengting, Zhang, Tong, Yang, Xinlin, Liu, Xinlei, Zhu, Dongqiang, Chen, Wei, 2021. 'Sulfide induces physical damages and chemical transformation of microplastics via radical oxidation and sulfide addition. Water Res. 197, 117100.
Zhu, Lixin, Zhao, Shiye, Bittar, Thais B., Stubbins, Aron, Li, Daoji, 2020. 'Photochemical dissolution of buoyant microplastics to dissolved organic carbon: rates and microbial impacts. J. Hazard Mater. 383, 121065.