Marine Pollution Bulletin 137 (2018) 91–95

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Pyr-GC/MS analysis of microplastics extracted from the stomach content of benthivore fish from the Texas Gulf Coast

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ARTICLE INFO

Keywords:
Microplastic
Polymer
Pyr-GC/MS
Fish
Ingestion
Texas

ABSTRACT

Fish ingestion of microplastic has been widely documented throughout freshwater, marine, and estuarine species. While numerous studies have quantified and characterized microplastic particles, analytical methods for polymer identification are limited. This study investigated the applicability of pyr-GC/MS for polymer identification of microplastics extracted from the stomach content of marine fish from the Texas Gulf Coast. A total of 43 microplastic particles were analyzed, inclusive of 30 fibers, 3 fragments, and 10 spheres. Polyvinyl chloride (PVC) and polyethylene terephthalate (PET) were the most commonly identified polymers (44.1%), followed by nylon (9.3%), silicone (2.3%), and epoxy resin (2.3%). Approximately 42% of samples could not be classified into a specific polymer class, due to a limited formation of pyrolytic products, low product abundance, or a lack of comparative standards. Diethyl phthalate, a known plasticizer, was found in 16.3% of the total sample, including PVC (14.3%), silicone (14.3%), nylon (14.3%), and sample unknowns (57.2%).

1. Introduction

Microplastics are major global contaminants, ubiquitous throughout freshwater and marine systems (Eriksen et al., 2013; Lattin et al., 2004; Moore et al., 2011; Ng and Obbard, 2006; Sadri and Thompson, 2014). Due to their small size (i.e. < 5 mm), it is difficult to predict particle transport following release into aquatic systems, however, microplastics have been discovered within waters from the near shore to open ocean (Eriksen et al., 2014; Kang et al., 2015), from the surface to benthos (Song et al., 2014; Woodall et al., 2014), and from subtropical to polar seas (Law et al., 2010; Obbard et al., 2014). While the environmental impact of these contaminants is not fully understood, microplastic ingestion has been identified within taxa spanning from invertebrates to large marine mammals (Hurley et al., 2017; Taylor et al., 2016).

Fish ingestion of microplastic has been confirmed within freshwater, marine, and estuarine species, ranging from a few percent to more than two-thirds of all fish examined (Lusher et al., 2013; Nadal et al., 2016; Peters and Bratton, 2016; Peters et al., 2017; Possatto et al., 2011; Romeo et al., 2015; Sanchez et al., 2014; Vendel et al., 2017). It is likely that variations in microplastic ingestion are the result of several factors, such as the species of examination, location of collection, methodologies employed for microplastic extraction, and the analytical analyses utilized for polymer identification. Due to the complex nature of these micro-contaminants, it is now becoming standard to employ two or more identification techniques for the confirmation of plastic. Initial identification routinely involves a physical characterization of the particle (e.g. size, morphology, and color), aided by microscopy, followed by a secondary identification via chemical characterization (e.g. spectroscopy) to identify the specific type of plastic polymer (Shim et al., 2017).

Polymer characterization often employs Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy (Lenz et al., 2015; Shim et al., 2017). Both techniques use electromagnetic radiation to profile samples, however, FTIR is a measure of the particle's covalent chemical bonds using the absorbance of their vibrational modes while Raman is a measure of the particle molecular structure using light scattering from key vibrational modes after excitation with a visible light source (Käppler et al., 2016; Löder and Gerdts, 2015). While both techniques explore molecular vibrations, the differences in their approach mean that different types of bonds are highlighted by each technique. FTIR spectra highlight polar covalent bonds, while Raman spectra highlight more purely covalent bonds such as carbon to carbon (C—C) or sulfur to sulfur (S—S). FTIR is coupled with varying modes of measure (e.g.

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https://doi.org/10.1016/j.marpolbul.2018.09.049
Received 7 June 2018; Received in revised form 28 September 2018; Accepted 29 September 2018

micro-FTIR, transmission, reflectance, and attenuated total reflectance), some of which can minimize method limitations such as the requirement of an extensive sample pretreatment and inhibited analysis of plastics which contain irregular surfaces, (Ng and Obbard, 2006; Song et al., 2014). While FTIR can analyze particles as small as 10 μm (the size of the IR beam aperture), particles of this size often require multiple analysis runs or produce unclear results, thus FTIR is most applicable for particles that are $>$ 50μm (Shim et al., 2017). Comparatively, the laser aperture utilized in Raman spectroscopy is smaller than that of FTIR, thus it can identify particles as small as a few μm in size. Raman has been found to be sensitive to additive and pigment chemicals, which are often incorporated during the production phase of plastics, resulting in the interference in polymer identification (Lenz et al., 2015). FTIR may also be challenged by additives and plastic copolymers; work by Hendrickson et al., 2018 indicates that ATR-FTIR may mask chemical constituents within heterogeneous particles that appear when the particle undergoes pyr-GC/MS analysis.

A third method of polymer identification is the coupling of thermal desorption or pyrolysis with gas chromatography–mass spectrometry or GC/MS (Dümichen et al., 2015; Frias et al., 2013). Pyrolysis-GC/MS (pyr-GC/MS) uses heat in an inert environment (i.e., no oxygen) to decompose polymeric material in a predictable fashion. The pieces of polymer generated can then be separated by gas chromatography on the basis of their size and polarity and analyzed by mass spectrometric detector at the outlet of the gas chromatography column. (Frias et al., 2013). This method yields a total chromatogram (abundance vs time) for the separated pyrolysis products and provides mass spectrometry data throughout the chromatogram as well. These can be compared against a known reference library to determine the specific class of polymer being analyzed. This method is beneficial over FTIR and Raman spectroscopy as it can characterize particles $<$ 10μg when measured in splitless mode, and the utilization of a thermal analysis combined with GC/MS, enables the separation and analysis of chemical additives as well as the polymer material (Hendrickson et al., 2018). However, pyr-GC/MS is destructive, resulting in the total loss of the particle and subsequently eliminating further particle analysis.

Despite recent advancements in these analytical methods as applied to microplastics, the applicability and feasibility of each is somewhat incomplete due to the wide range of microplastic polymers, including weathered polymers, and additives found throughout the environment. This research serves as one of the first applications of pyr-GC/MS for microplastic polymer identification within a fish ingestion study, and specifically investigates the polymer distribution of microplastic recovered from the stomach content of six marine fish species from the Texas Gulf Coast. The pyr-GC/MS method utilized within this study has previously been applied to the identification of microplastic recovered from the waters of Western Lake Superior (Hendrickson et al., 2018). The use of this method here enabled a comparison of polymer results between freshwater and marine systems and an investigation of method applicability across sample matrixes.

2. Methods

Microplastics were collected from the stomach content of 1381 marine fish, inclusive of six species (i.e. southern kingfish (*Menticirrhus americanus*), Atlantic croaker (*Micropogonias undulates*), Atlantic spadefish (*Chaetodipterus faber*), sand trout (*Cynoscion arenarius*), pinfish (*Lagodon rhomboids*), and grunt (*Orthopristis chrysoptera*) from the Texas Gulf Coast (Peters et al., 2017). Fish collection took place from September 2014 to September 2015, and stomach content analysis followed the protocol of Peters and Bratton (2016). Following identification, microplastics were characterized via particle size, morphology, and color (Peters et al., 2017).

Approximately 5% of recovered microplastics were selected and transferred from Baylor to the University of Minnesota Duluth for pyr-GC/MS analysis. Particle mass was measured via a Mettler Toledo XP2U microbalance and particles $<$ 10 μg were measured in splitless introduction into the gas chromatograph, while particles $>$ 10 μg were introduced using a 1:100 split (Hendrickson et al., 2018). Samples were analyzed using an Agilent 7890B Gas Chromatograph with Agilent 5977A mass-selective detector (MSD) Mass Spectrometer and Gerstel Pyrolysis/Thermal Desorption Unit (Gerstel GmbH & Co. KG, Germany). All pyrolyzer and GC unit parameters adhered to the protocol of Hendrickson et al. (2018). The MSD utilized electron impact (EI^+, 70 eV) for the ionization source and scanned for ions from *m/z* 10–550 (Hendrickson et al., 2018).

Following analysis, ion chromatograms were assessed with the National Institute of Standards and Technology (NIST) mass spectra library (Version 2.0, 12/4/12, available through the mass spectrometer's software package) and the following standards: medium-density polyethylene (MDPE, catalog #: EV306010), polystyrene (PS, catalog #: ST316051), polyvinyl chloride (PVC, catalog #: CV316010), and polyethylene terephthalate (PET, catalog #: ES306030), all in powder form (250–350 μm) (Goodfellow, Inc.). Samples which yielded a low number of pyrolytic products ($<$ 4 total) or low pyrolytic product abundances were evaluated via Mass Hunter qualitative analysis software which was utilized to integrate total ion chromatogram peak areas and calculate a 3:1 signal-to-noise ratio (Hendrickson et al., 2018).

3. Results

A total of 43 microplastic samples were analyzed, inclusive of 30 fibers, 3 fragments, and 10 spheres (microbeads). Particles were identified into the following five polymer classes: PVC (Fig. 1; Table 1) and PET, constituting approximately 44.1% of the total sample, silicone (2.3%), epoxy resin (2.3%), and nylon (9.3%) (Fig. 2). Half of the nylon particles were further classified as Nylon 6 due to the high abundance of caprolactam within the pyrogram results (Lehrle et al., 2000). In addition to the five polymer classes, approximately 42% of particles were classified as sample unknowns, 21% of which displayed a similar chromatogram result inclusive of seven common pyrolytic products (i.e. Unknown Subsample A) (Fig. 3; Table 2).

PVC polymers were inclusive of microplastic fibers (73.3%), fragments (20.0%), and spheres (6.7%), while all PET, epoxy resin, and nylon polymers were in the form of microplastic fibers and the single particle identified as silicone was in the form of a microbead. Particles classified as “Unknown” contained fiber (55.6%) and sphere (44.4%) morphologies (Fig. 4) and particles further categorized as “Unknown Subsample A” contained sphere (55.6%) and fiber (45.4%) morphologies. Diethyl phthalate was found in 16.3% of all particles analyzed, including PVC (14.3%), Silicone (14.3%), Nylon (14.3%), Unknown (28.6%), and Unknown Subsample A (28.6%).

4. Discussion

Of the original marine microplastic data set (i.e. Peters et al., 2017), the most common particle morphologies were fibers (86.4%), followed by spheres (12.9%), and fragments ($<$ 1.0%), thus, samples chosen for

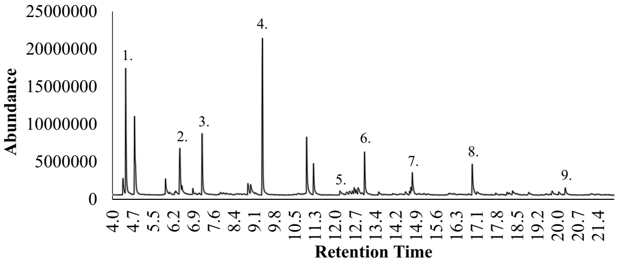

Fig. 1. Total chromatogram of PVC particle with pyrolytic products numerically labeled.