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Marine Pollution Bulletin

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Microplastic fragment and fiber contamination of beach sediments from selected sites in Virginia and North Carolina, USA



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ARTICLE INFO

Keywords: Microplastic Microfiber Coastal sediment Microplastic identification Microplastic particle extraction

ABSTRACT

Microplastic particles (<5 mm) constitute a growing pollution problem within coastal environments. This study investigated the microplastic presence of estuarine and barrier island beaches in the states of Virginia and North Carolina, USA. Seventeen sediment cores were collected at four study sites and initially tested for microplastic presence by pyrolysis-gas chromatography–mass spectrometry. For the extraction, microplastic particles were first separated from the sediment using a high-density cesium chloride solution (1.88 g/mL). In a second step, an oil extraction collected the remaining microplastic particles of higher densities. Under the light microscope, the extracted microplastic particles were classified based on their morphologies into fragments and fibers. Raman microspectroscopy chemically identified a subset of microplastic particles as polypropylene, polyethylene terephthalate, poly(4-vinylbiphenyl), polystyrene, polyethylene, and nylon. The results show a concentration of microplastic particles (1410 \pm 810 per kg of dry sediment) even in protected and ostensibly unpolluted estuarine and beach sediments of Virginia and North Carolina.

1. Introduction

Microplastic fragments and fibers of < 5 mm in diameter have become anthropogenic pollutants of concern worldwide (Barnes et al. 2009; Mathalon and Hill, 2014; Yu et al., 2016; Crichton et al., 2017). Chemical compositions of microplastic particles found in sediments commonly include: polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC) (Browne et al., 2010; Andrady, 2011; Vianello et al., 2013; Wessel et al., 2016; Yu et al., 2016; 2018; Abidli et al., 2017).

Microplastic particles and adsorbed contaminants such as persistent organic pollutants and polycyclic aromatic hydrocarbons are deposited in all marine environments, from beaches to deep oceans (Browne et al., 2010, 2011; Van Cauwenberghe et al., 2013; Wessel et al., 2016; Fischer and Scholz-Böttcher, 2017; Besley et al., 2017; Yu et al., 2018). The abundance of microplastic particles tends to increase with proximity to highly populated urban areas (Yonkos et al., 2014).

The Chesapeake Bay is the largest estuary in the USA. Eighteen million people live in the Chesapeake Bay watershed, which covers 64,000 mile² and has 11,684 miles of shoreline. This watershed extends from the village of Cooperstown, New York, down south to the city of

Norfolk, Virginia, and over 51 billion gallons of water enter the Bay each day (The Chesapeake Bay Foundation, 2019).

The northern two study sites of this investigation are located along the Chesapeake Bay. There are three metropolitan statistical areas (MSA): (i), the largest is Greater Washington (including Washington, DC, Arlington, and Alexandria) with a population of > 6.2 million; (ii), Hampton Roads comprises the cities of Virginia Beach, Norfolk, Newport News, Portsmouth, Chesapeake and Suffolk with a population of over 1.7 million, and, (iii), Greater Richmond, including Richmond and Petersburg is populated by over 1.3 million people (U.S. Census Bureau, 2018). These metropolitan statistical areas are within 200 miles of one another.

The southern two study sites are influenced by the Albemarle-Pamlico Sound, which is the second largest estuary in the USA. The Albemarle-Pamlico watershed is approximately 28,000 mile² in size. It incorporates almost 10,000 miles of rivers and streams in northeastern North Carolina and southeastern Virginia (Albemarle-Pamlico National Estuary Partnership, 2012; Matthews and Sinha, 2016). Inflow of water into the sound is approximately 6 billion gallons of water from the rivers and streams each day (Harned and Davenport, 1990). Hampton Roads and Raleigh, (North Carolina MSA), influence this region and

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together have a population of over 3 million people (U.S. Census Bureau, 2018).

One source of microplastic fibers in the environment is from clothing. The Chesapeake Bay region is home to 472 municipal and industrial wastewater treatment plants (Chesapeake Bay Program, 2019), the Albemarle-Pamlico watershed has over 60 wastewater treatment plants (Water Environment Federation, 2018). This wastewater is released into rivers and other receiving waterbodies, and eventually into the estuaries. Browne et al., 2011 sampled washing machine effluent from household washing machines and found that a single item of clothing can produce over 1900 fibers per wash. It is important to note that to ~80% of microplastic fibers found in beach sediments worldwide are directly linked to such washing machine effluent (Browne et al., 2011). Therefore, it is important to determine the amount of microplastic fibers that make their way into the sediment, in proximity to metropolitan areas.

Microplastic particle contamination is a significant disturbance and causes ecological damage to the marine life in coastal regions worldwide. Due to the long residence time and chemical stability of microplastic particles, they are being transferred through marine food webs and eventually become buried in sediments. Microplastic particles are ingested by aquatic organisms (Andrady, 2011). These particles may negatively impact the physiology and ecology of marine and aquatic organisms (Claessens et al., 2011; Mathalon and Hill, 2014; Barboza and Gimenez, 2015; Van Cauwenberghe et al., 2015; Anderson et al., 2016; Welden and Lusherz, 2017). For example, stomach ulcers, clogged gills, and intestinal obstructions are all physical effects which can lead to a decrease in the size of organisms and infections (Andrady, 2011; Jang et al., 2018). Previous studies have found that microplastic particles may also act as transport vectors for toxins. It was shown that the adsorption of contaminants such as metal ions and polychlorinated biphenyls to microplastic particles cause harm to organism's endocrine systems (Andrady, 2011; Syberg et al., 2015).

Field-collected benthic organisms such as mollusks, crustaceans, and polychaetes have all been found to be contaminated with microplastic particles (Smith et al., 2018). These organisms live in estuarine and beach environments, which are productive ecosystems of economic and ecological importance (Barboza et al., 2018; Gray et al., 2018; Leads and Weinstein, 2019). Mollusks in the Pearl River Estuary in China contain an average of 4.2 to 7 microplastic particles per mollusk (Li et al., 2018). In a Florida estuary, the soft tissues of mollusks and crustaceans have been found to be contaminated with microplastic particles averaging 16.5 and 4.2, respectively (Waite et al., 2018). Microplastic fibers have been found in crustaceans and research indicates that these fibers reduce food consumption, thus less energy is available for growth (Watts et al., 2015).

The degradation of macroplastic also yields microplastic particles. Jang et al., 2018 found that polychaetes can produce microplastic particles by breaking down larger pieces of plastic and that these polychaetes then ingest the microplastic particles. These organisms are also found in the estuarine and beach environments of coastal Virginia and North Carolina.

The damage to marine life, namely stunted growth through the ingestion of microplastic particles and their adsorbed toxins is causing significant economic loss. The farm gate value of mollusks in Virginia was \$53.4 million during the year 2017 (Hudson, 2018). The price per pound of the crustacean (blue crab) harvest in the Chesapeake Bay was approximately \$78 million at dockside in 2009 (Chesapeake Bay Foundation, 2019). The farm gate value for mollusks in the Albemarle-Pamlico watershed was \$360,000 (Houtven et al., 2012). In 2011, the

crustacean harvest (blue crabs) in North Carolina had a dockside value of \$21 million (North Carolina Department of Environmental Quality, 2011). Over three quarters of commercial fishing is dependent on estuaries. The coastal communities along these estuarine shorelines account for over 43% of the Gross Domestic Product for the USA, and about \$531 billion in economic value (Restore America's Estuaries, 2019). Therefore, determining the concentration and distribution of microplastic particles in estuarine and barrier island habitats in Virginia and North Carolina is necessary for understanding the risk to these ecologically and economically important organisms.

Given the ecological and economic importance of coastal regions, the lack of data on microplastic particle contamination in estuarine and barrier island sites is inadequate (Yonkos et al., 2014; Wessel et al., 2016; Gray et al., 2018; Yu et al., 2018; Leads and Weinstein, 2019; Wiggin and Holland, 2019). In coastal, Virginia and North Carolina, only a few studies have investigated the abundance and distribution of microplastic particles. For example, one study conducted in the estuarine waters of the Chesapeake Bay by Yonkos et al. (2014) reported microplastic concentrations ranging from < 1.0 to > 560 g/km². In their study, the microplastic particle concentration was positively correlated with population density and urban development (Yonkos et al., 2014).

Microplastic particles have been categorized by size, shape, colour, or chemical composition globally and an overall standard of characterization is still in development (Hidalgo-Ruz et al., 2012; Vianello et al., 2013; Baztan et al., 2014; Barboza and Gimenez, 2015; Wessel et al., 2016; Fisner et al., 2017; Abidli et al., 2017; Bosker et al., 2018; Piperagkas et al., 2019). Standardization is still needed for comparison of microplastic particle studies in all environments. Our contribution employs the categorization of microplastic particles as fragments and fibers using an optical analysis (Gray et al., 2018; Yu et al., 2018; Leads and Weinstein, 2019). We further identify the chemical composition and positive identification of microplastic particles using chemical analysis.

Based on the significant danger of microplastic and microfiber contamination for the ecology and economy of the region, the objective of this study was threefold: (i), to assess the estuarine and barrier island sediments of coastal Virginia and North Carolina for microplastic particles, (ii), to determine the concentration of microplastic particles in the sediment at each study site, (iii), to identify main morphologies and, (iv), to analyze the chemical compositions of the microplastic particles. We hypothesized that microplastic concentrations would possibly differ among our study sites dependent on their proximity to metropolitan areas.

2. Methods & materials

2.1. Study sites

The four selected study sites were located on Fisherman Island (FI) and Back Bay National Wildlife Refuge (BB) in Virginia, and the Outer Banks (OB) and Cape Hatteras National Seashore (CH) in North Carolina (Fig. 1). FI is located at the mouth of the Chesapeake Bay. BB and FI are influenced by currents of the Chesapeake Bay and the Atlantic Ocean. OB is influenced by currents of the Albemarle Sound and the Atlantic Ocean. The currents of the Pamlico Sound and the Atlantic Ocean influenced CH. The samples were collected in February (FI and BB) and March (OB and CH) of 2018. Sampling took place during the winter, because human activity is limited. BB and FI are U.S. Fish and Wildlife Service bird conservation areas, and sampling permits

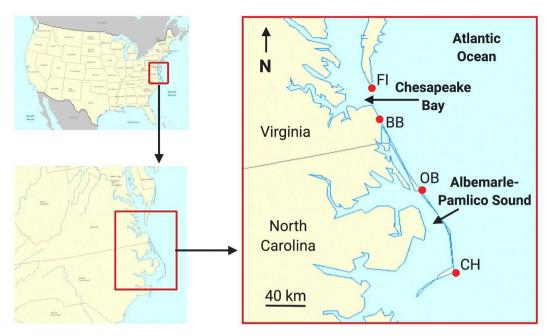


Fig. 1. Location of study area: Virginia and North Carolina, USA, with the four study sites (circles): Fisherman Island National Wildlife Refuge (FI) (37°518.28″ N 75°58′36.80″ W), Back Bay National Wildlife Refuge (BB) (36°40′2.20″ N 75°54′32.95″ W), the Outer Banks of North Carolina (OB) (37°51′27.03″ N 75°34′5.38″ W), and Cape Hatteras National Seashore (CH) (35°13′59.28″ N 75°31′37.93″ W). (Created with Biorender.com).

had to be requested. The sample sites were selected because they had not previously been sampled for microplastic particles. This was necessary for establishing baseline data in this region. Dredging was not occurring at any of the four locations and the potential for cofounding effects from dredge-associated sediment re-suspension was eliminated.

At all sampling sites, the sediments are predominantly composed of mature quartz sand. The sand grains ranged in size and were determined using the Udden–Wentworth scale (Wentworth, 1922). The sand grain sizes were very fine (0.0625 mm -0.125 mm), fine (0.125 mm -0.25 mm), medium (0.25 mm -0.50 mm), coarse (0.50 mm -1.0 mm), and very coarse (1.0 mm -2.0 mm). At FI and BB the sediments were mainly of fine grain size. At FI the sediment was composed of very fine (1%), fine (71%), and medium (26%) sand. BB had a similar composition of sandy sediment: very fine (4%), fine (61%), medium (28%), and coarse (8%). At OB and CH the sediment sizes ranged evenly from very fine sand to very coarse sand. OB was composed of very fine (1%), fine (21%), medium (33%), coarse (33%), and very coarse (11%) sand. At CH the sediment included, very fine (1%), fine (21%), medium (35%), coarse (32%), and very coarse (11%) sand.

2.2. Sampling in the field

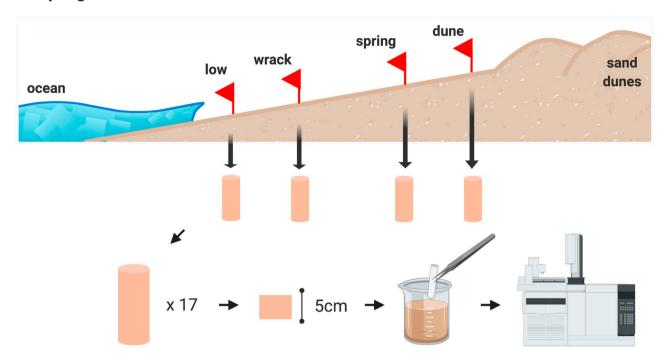
The samples were collected on February 10, 2018 (FI), February 27, 2018 (BB), and March 18, 2018 (OB and CH) in the time allotted by the sampling permits. At each sample site, a transect was established crossing the beach from the lowest swash line to the high water line (close to the dunes), (Fig. 2). The length of the transect at each sample site differed: FI 55.20 m, BB 24.08 m, OB 32.62 m, and CH 25.27 m. Along each transect, four to five samples were collected. One sample was taken each at the lowest swash line, the wrack line, the spring high water line, and in front of the primary dune belt, respectively (Fig. 2). The sediment samples were collected during low tide using a sediment

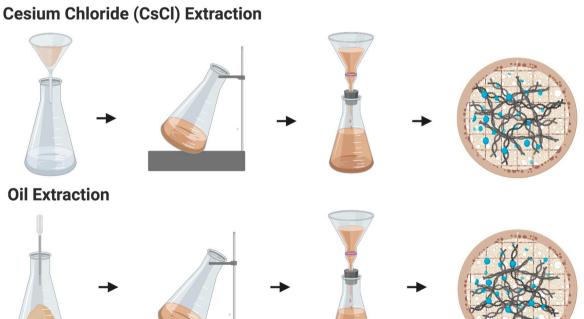
corer (AMS metal split hand auger) of $5.08~cm \times 15.24~cm$ in size. An AMS butyrate plastic liner was inserted into the corer to remove the cores with ease, placed in a box to be carried off of the beach and transported back to the lab. To account for the butyrate plastic liner, any butyrate detected in the chemical analysis results was not considered; however, butyrate was neither found nor identified in the analysis.

2.3. Sample preparation

Precautions were taken in order to limit contamination during the extraction process in the laboratory. Cotton clothes were worn by researchers at all times. Workspaces were sanitized using Clorox® wipes prior to opening petri dishes containing the samples. All glass containers were cleaned using a filtered Alcojet® solution and DI water before and after each use. The vacuum filtration apparatus used for extraction (Fig. 2) was cleaned and re-assembled for each of the samples. The chances for secondary microplastic contamination are estimated to be low if such precautions are taken (Löder and Gerdts, 2015). All of the solutions were mixed in a fume hood. Laboratory blanks of the deionized water (1000 mL), Alcojet® solution (300 mL), CsCl solution (500 mL), and oil (50 mL) were each filtered through a 0.45 μm membrane filter to quantify any possible procedural contamination. The laboratory blanks accounted for an overall percentage for the total volume of each liquid used during the extractions; deionized water (10%), Alcojet[®] solution (30%), CsCl solution (24%), and oil (15%). The laboratory blanks were optically tested under a Zeiss Stemi 305 compact microscope for traces of microplastic particle contamination. For deionized water, the contamination range (mean ± standard deviation) was a neglectable 6 ± 2 fibers; for the Alcojet® solution, it was 17 \pm 12 fibers; for the CsCl solution, it was 24 \pm 9 fibers; and for the oil, it was 4 \pm 2 fibers. No fragments were found in the laboratory blanks. All blanks accounted for < 5.3% of the total number of

Sampling





Analysis

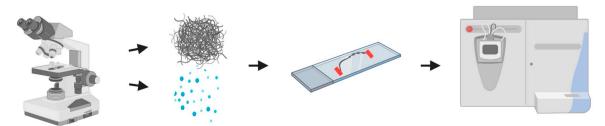


Fig. 2. Methods diagram depicting the field sampling and sample preparation (top row), cesium chloride extraction (second row), oil extraction (third row), and the analysis techniques (bottom row). In the top row, the sampling sites are located along a transect crossing the beach from the lowest swash line to the dune belt. From the total 17 cores derived from the 4 study sites, the center 5 cm of sediment was removed from each core and used for further analysis. Each sample was dried at 40° Celsius for 48 h. A sub-sample was extracted with a quartz tube and tweezers for pyrolysis-gas chromatography—mass spectrometry analysis. The analysis identified microplastic particles in the sediments. Second row: the dry sediment was added to a cesium chloride solution to float microplastic particles, and the solution and microplastic particles were left to rest at a tilt and then poured into the filtration apparatus. The filters from the extractions were stored in petri dishes for later analysis. Third row: oil was added to the remaining sediment to extract denser microplastic particles. Water was added to the sediment and the oil-coated microplastic particles, which were left to rest at a tilt. The top layer of oil was decanted into the filtration apparatus, and each of the filters was stored in separate petri dishes for later analysis. Fourth row: an optical analysis using a light microscope classified the microplastic particles into two morphologies (fragments and fibers), and selected microplastic particles were taped onto glass slides for Raman microspectroscopic analysis. Raman microspectroscopy identified the chemical composition of the microplastic particles. (Created with Biorender.com).

microplastic particles extracted from the field. Because of such low values, the reported data are not blank corrected.

The cores were removed from their containers and placed on aluminum foil. In order to consider only mechanically undisturbed sediment, a 5 cm-long portion of the core was sub-sampled from the middle of each core with a metal spatula and placed into a clean glass beaker. Each beaker was covered with aluminum foil to prevent contamination by possibly airborne particles. The subsamples were dried in a drying oven (Thermo Scientific Precision™ Compact Heating and Drying Oven) at 40 °C for 48 h. This drying method has been established to prevent potential damage to any microplastic particles in the subsamples (Crawford and Quinn, 2017).

2.4. Pyrolysis-gas chromatography-mass spectrometry analysis

Prior to the extraction process, a small portion (mean \pm standard deviation) of the dried subsamples (0.25 \pm 0.06 mg) from each location was collected in a 6.0 mm \times 0.20 mm size glass tube (Fig. 2) and tested with pyrolysis-gas chromatography–mass spectrometry (Py-GC/MS (Leco Pegasus II GC-TOF Mass Spectrometer)). The Py-GC/MS measurements were advantageous, as it gave an initial first insight into whether microplastic particles would be found in each sample, and what chemical compositions may be detected. Following the procedure outlined by Yu et al. (2016) and Fischer and Scholz-Böttcher (2017), we tested for the plastic types commonly found in beach sediments including polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC).

2.5. Extraction procedure

Effective methods for extraction of microplastic particles from sediment are discussed in Nuelle et al. (2014). Commonly established methods are density extractions using salt solutions (Hidalgo-Ruz et al., 2012; Nuelle et al., 2014; Sanchaz-Nieva et al., 2017; Piperagkas et al., 2019). A new and effective technique uses hydrophobic canola oil (Crichton et al., 2017). The oil adheres to the microplastic particles in the sediment and once water is added to the oiled sediment, the microplastic particles dispersed in the oil are then concentrated on the surface of the water, coated in oil. This is due to the canola oil having a lower density than the water and sediment grains. Crichton et al. had mean recovery rates of 99.0% for microplastics particles and 92.7% for microplastic fibers in sediment (Crichton et al., 2017). Sediment composition dictates which method of extraction is most effective. For sandy sediments, this oil method may have the potential to be more efficient than salt density extractions.

Here we used a two-step-extraction: (i), using an aqueous salt density (CsCl) solution, and, (ii), using canola oil (Crisco, The J.M. Smucker Co., Orrville, Ohio). Aqueous CsCl solution has a higher density (1.88 g/mL) compared to most plastic materials, but a lower

density than quartz sand (2.65 g/mL) which constitutes many coastal sediments (Nuelle et al., 2014; Crichton et al., 2017). Therefore, CsCl solution was used in the first extraction step. The second extraction step using canola oil ensured extraction of any microplastic particles that had a higher density than the CsCl solution (Crichton et al., 2017).

For the first extraction (i), each sediment subsample was introduced through a glass funnel into an Erlenmeyer flask containing 150 mL of CsCl and swirled for 1.5 min. In order for the sediment to deposit without additional resuspension effect, the flask was then positioned at a 65°tilt for 20 min. The supernatant fluid was decanted and filtered using a hydrovacuum filtration apparatus (after Crichton et al., 2017) through progressively finer membrane filters (Whatman® nitrocellulose membrane filters): $3.0~\mu m$, $0.45~\mu m$, and $0.22~\mu m$. Each filter was rinsed with deionized water to remove any remaining CsCl. The filters were each stored in a sealed petri dish for later analysis of the particles.

For the second step (ii) involving the oil extraction, 5 mL of canola oil (0.92 g/mL) was introduced into each glass Erlenmeyer flask with the sample and swirled for 3 min. Subsequently, 15 mL of deionized water was added to the flask, which then was swirled for another 1.5 min, in order to separate the higher density oil-coated plastic particles from the heavy sediment. The flask was again positioned at a 65° tilt for 20 min to allow the suspended sediment to deposit. The supernatant oil was decanted and filtered through a 3.0 µm and a 1.0 µm membrane filters (Crichton et al., 2017). The 0.45 µm, and 0.22 µm membrane filters were not used, because the oil would not move through the small mesh pores. Prior to removal of the sample from the filtration system, each filter was rinsed with a 0.5%-1% Alcojet® detergent solution to remove residual oil from the microplastic particles. Then, each filter with its sample was rinsed with deionized water to remove any remaining Alcojet® detergent. The extraction procedure was replicated for each subsample from each sediment core. The filters were then stored in a sealed petri dish for later analysis of the particles of higher density.

2.6. Light microscopic and RAMAN analyses

With Excelta Precision Tweezers with Offset Fine Tips (Tip specs 0.012 cm wide & 0.0031 cm in thickness), the extracted microplastic particles were sorted into fragments and fibers according to their morphologies. The fragments and fibers were then counted under a Zeiss Stemi 305 compact microscope at a $2.0\times$ magnification (Claessens et al., 2011; Vianello et al., 2013; Crichton et al., 2017). The lengths of the fragments and fibers were measured using a translucent millimeter ruler while in microscopic view. In order to mitigate common challenges, such as misidentification of plastic particles due to optical similarities between sediment and microplastic fragments and fibers, we chose to test for their chemical composition by Raman microspectroscopy. A total sample size of fifteen representative fragments and fibers were selected in the size range of 5.0 mm to 2.0 mm for easy

Table 1

Amount of microplastic particles at each sample site: Mass of dry sediment collected at each sample site (FI, BB, OB, CH) (left column); total number of microplastic particles extracted from the sub-sample of sediment (middle column); concentration of microplastic particles calculated per kg of dry sediment (right column).

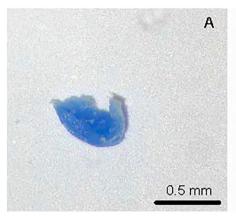
Sample site	Mass of dried sub-sampled sediment (kg)	Number of microplastic particles extracted	Amount of microplastic particles/kg of dry sediment
FI	0.733	971	1325
BB	0.761	454	596
OB	0.554	1231	2224
CH	0.687	928	1351

manipulation with the tweezers. The microplastic particles were mounted on a glass slide, secured with adhesive tape (Fig. 2) and subsequently analyzed using Raman microspectroscopy (California Department of Public Health, Environmental Health Laboratory). The Renishaw inVia dispersive Raman microscope was equipped with a 785 nm laser as suggested by Fischer and Scholz-Böttcher (2017). The obtained spectra were compared to GRAMS software, which is equipped with commercially available Raman spectral libraries.

3. Results

Each of the sites examined in this study were found to be contaminated with microplastic particles. The initial screening of the samples using Py-GC/MS prior to extraction revealed traces of polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC). Polyethylene (PE) was only found in the samples from North Carolina (OB and CH). Light microscopic analyses of the extracted plastic specimen showed that the concentrations and total numbers of particles were highly variable for all of the sample sites (Table 1). All concentrations are here reported as particles per kg dry weight of sediment. The highest concentration of microplastic estimated in beach sediment was found at OB (2224 particles/kg) while the lowest concentration was at BB (596 particles/kg). In terms of actual numbers, BB sediment appeared to have the lowest numerical amount of microplastic particles (454 particles), while OB had the highest number of microplastic particles (1231 particles; Table 1).

The material identified as microplastic had two morphologies which were classified into fragments and fibers Fig. 3. The fragments and fibers appear to be consistent with images in literature, observed at other estuarine areas of the world (Yonkos et al., 2014; Wessel et al., 2016; Gray et al., 2018; Yu et al., 2018; Leads and Weinstein, 2019). Notably, the microplastic fragments derived from FI display more rounded edges than those from other studies (Fig. 3).



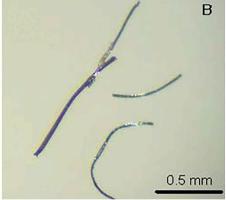


Fig. 3. Examples of the two microplastic morphologies (in light microscope): A: fragment, B: fibers. The fragments ranged in size from 0.5 mm to 4.5 mm and the fibers from 0.5 mm – 5.0 mm in length.

2.7. Statistical analyses

Following previous microplastic particle studies, one-way/single factor ANOVA analyses were conducted (Wessel et al., 2016; Yonkos et al., 2014). The differences in absolute numbers of microplastic particles in each sub-sample, from each the sample site were compared using analyses of variance (one-way/single factor ANOVA). The statistical analyses were conducted using Microsoft Office Excel. For all core sub-samples in total derived from each sample site, analyses were conducted (i), on the total number of fragments and fibers, (ii), the volumetric amount of microplastic particles per g/cm³ of dry sediment, and (iii), the numerical amount of microplastic particles per kg of dry sediment. In order to evaluate whether there were any significance differences in microplastic particle concentrations from the low to the high water lines, we compared the numerical amount of microplastic particles per g/cm³ of dry sediment of each sample from the lowest swash lines, the wrack lines, spring high water lines, and in front of the dune belt, respectively.

The microplastic particles typically measured between 5.0 and 0.5 mm. The size of the captured particles ranged from 5.0 mm to 0.5 mm (on the 3.0 μ m and the 1.0 μ m filters), 4.5 mm to 0.5 mm (on the 0.45 μ m filters), and 3.0 mm to 0.5 mm (on the 0.22 μ m filters). Both, the higher and lower density particles extracted using CsCl solution and oil extraction, respectively, contained both identified morphologies. However, fibers constituted the bulk of all identified microplastic types (93.91% \pm 4.74%; Fig. 4) at the four sample sites.

In detail for each site, at FI, the fragments were more abundant compared to all other study sites (Fig. 4). At OB, no fragments were represented in the low density group. At BB, OB, and CH, fibers of the high density group are more abundant, while fibers of the low density group are more abundant at FI. An increase in high density fibers occurred with decreasing latitude. The highest quantity of fibers in comparison to the fragments was found at OB (98.65%) and the lowest quantity at FI (89.17%); Fig. 4.

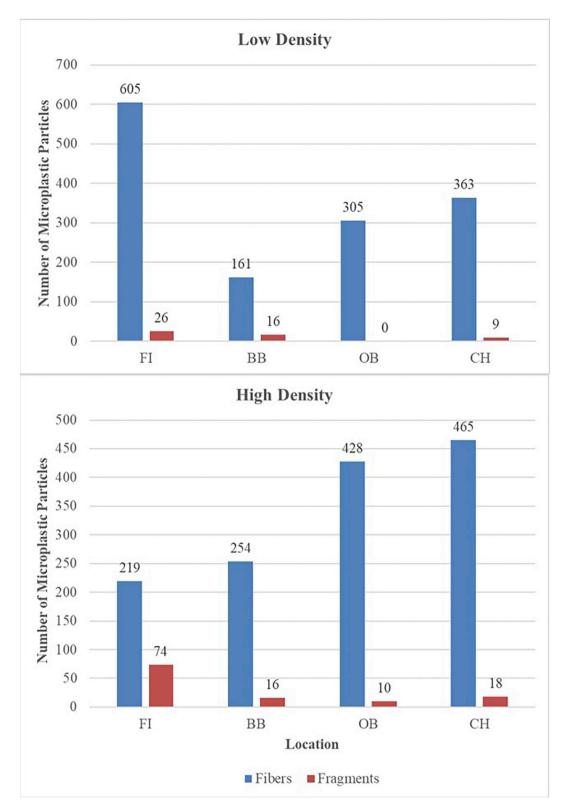


Fig. 4. Absolute numbers of microplastic particles extracted from beach sediment at the four sample locations (FI, BB, OB, CH). Low density particles (upper graph) and high density particles (lower graph) occurred. They were categorized by morphological type: fibers (blue), fragments (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In general, the distributions of the types of microplastic particles were variable at each location. Using a one-way ANOVA, it can be concluded that the concentration of fibers are significantly more abundant than fragments study wide (p = 0.0006, F critical = 5.987). The calculated volumetric amount of microplastic particles per g/cm³

of dry sediment (p=0.94) derived from the sample sites show no significant difference, nor did the concentrations of microplastic particles per kg of dry sediment (p=0.25). The samples collected at the swash lines (p=0.99), the wrack lines (p=0.95), spring high water lines (p=0.52), and in front of the dune belt (p=0.91), respectively,

did not have a significant difference in microplastic particles per g/cm³ of dry sediment either.

The identification of the particles as microplastics was further verified by randomly selecting (n=15) 2.0 mm–5.0 mm microplastics from each type for the exploration of chemical composition by Raman microspectroscopy. Fig. 5 shows examples of particles identified as polymers by this method. These microplastic fragments include polyethylene (PE). The fibers include polypropylene (PP), polyethylene (PE), poly(4-vinylbiphenyl) (PVB), polyethylene terephthalate (PET), poly (4-vinylbiphenyl) (PVB), polytetrafluoroethene (PTFE), polystyrene (PS), Ultem 1000 PEI (engineering thermoplastic), Halar ECTFE (engineering thermoplastic), polyisoprene, nylon, and teflon (Fig. 5).

4. Discussion

The objectives of this study were: (i), to test for microplastic fragment and fiber occurrence in beach sediments of estuarine and barrier island sites in Virginia and North Carolina; (ii), to determine the number of microplastic particles in the sediment at each study site, (iii), to identify their morphology and (iv), to analyze chemical composition.

4.1. Spatial distribution of microplastic particles in study area

The results confirm our hypothesis that an abundance of microplastic fragments and fibers contaminate the estuarine and barrier island sediment at all sample sites of the four study sites [Outer Banks (OB), Cape Hatteras (CH), Fisherman Island (FI), and Back Bay (BB)].

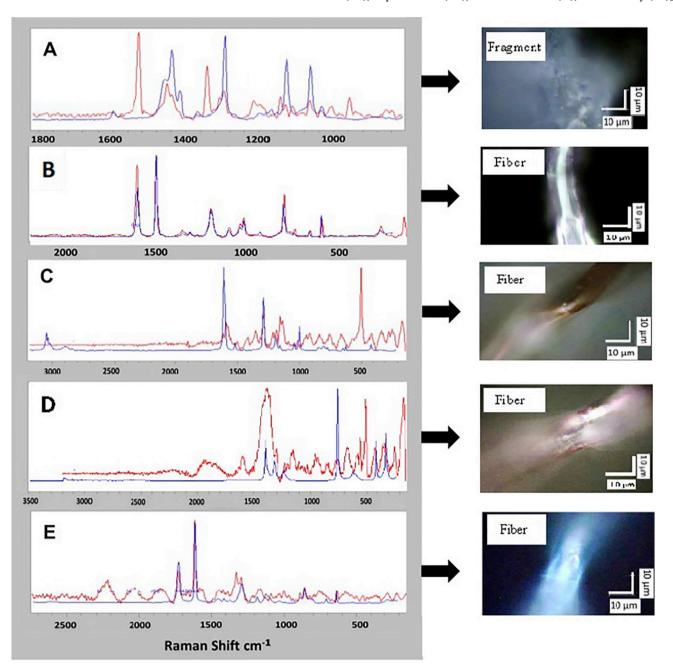


Fig. 5. Raman spectra (red curves) of randomly selected microplastic particles in comparison with the library spectra (blue curves). The measured particles are shown on the photos on the right. (A) fragment, (B–E) fibers. The spectra on the left represent: polyethylene (A), polypropylene (B), poly (4-vinylbiphenyl) (C), polytetrafluoroethene (D), and polyethylene terephthalate (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The distribution pattern of microplastic particles in our study area is characterized by the number of low-density fibers decreasing from north to south, whereas the number of high-density fibers increase. In contrast, both, low- and high-density fragments decrease from north to south

Greater Washington, Greater Richmond, Hampton Roads, and Raleigh are the most heavily populated metropolitan areas in the Chesapeake Bay and Albemarle-Pamlico Sound watersheds. These metropolitan statistical areas with > 1 million people each and over 500 wastewater treatments plants, are most likely the source locations of the microplastic material we detected (Fig. 1). The material is then transported from the respective sources by currents of the Chesapeake Bay and the Atlantic Ocean and finally deposited in sediment.

The highest concentration of fibers on the OB may be the result of these microplastic particles being carried from the Chesapeake Bay mouth southward by Atlantic longshore currents. In addition, polluted riverine water is being transported into the Albemarle-Pamlico Sound, located west of the OB. The riverine water may be washed over the barrier islands during storms and washed through tidal inlets connecting the Albemarle-Pamlico Sound and Atlantic Ocean.

The most southern study site is Cape Hatteras (CH), a geomorphological spit, which is the result of the southward Virginia longshore currents of the Atlantic Ocean and the northward Gulf Stream that converge here (Alperin et al., 2002; Thomas et al., 2002). This convergence of water bodies causes a deposition zone with a higher than average sediment accumulation rate (Alperin et al., 2002). It may be that high-density microplastic fibers are being transported by the Gulf Stream northward and deposited predominantly at this site.

Fisherman Island (FI) has the highest amount of low-density microplastic fibers. We assume that this is the result of its location at the Chesapeake Bay mouth. A high amount of wastewater and run-off may be transported from the Greater Washington, Greater Richmond, and Hampton Roads southeastwards into the Atlantic Ocean (Fig. 4). The apparent lack of high density contaminants on FI (Fig. 4) may also be explained by the fact that the barrier island is disconnected from the main land, so terrestrial run-off from local roads and neighborhoods (like possibly at BB, OB, and CH) cannot reach the beach.

In contrast to the fibers, both low- and high-density microplastic fragments show a north-southward decrease. This distribution pattern may point towards a northern source of this material, perhaps the large metropolitan areas in Virginia, Maryland, and New York. Southward transport is possible via longshore ocean currents. This hypothesis is supported by the fact that the fragments we found display rounded edges caused by erosion. Typically, only very angular fragments are known from other study sites (Piperagkas et al., 2019). Dissimilarly to our other three study sites, BB, being situated in a more natural setting (the Back Bay National Wildlife Refuge), shows the lowest concentration of microplastic particles in general.

The coastal sediments from our study sites are primarily quartz sand ranging from very fine to very coarse (0.0625 mm–2.0 mm) in size. It seems that sediment composition does not control microplastic distribution in our area. Also Browne et al., 2011; Yonkos et al., 2014; Wessel et al., 2016; Gray et al., 2018; Yu et al., 2018; Leads and Weinstein, 2019; Wiggin and Holland, 2019, found that US estuarine and barrier island beaches include microplastic fragment and fibers independently of the sediment type. Microplastic particles appear to occur even in sediments with a wide size range such as from mud to very coarse grain sizes (0.001 mm–2.0 mm) (Thompson et al., 2004; Browne et al., 2010, 2011; Claessens et al., 2011; Vianello et al., 2013; Yu et al., 2016, 2018; Bosker et al., 2018; Piperagkas et al., 2019).

4.2. Total numbers of microplastic particles in context of global data

Because microplastic contamination only recently was appreciated as a severe global issue, data on microplastic distribution world-wide is still limited (Browne et al., 2011; Yonkos et al., 2014). So, comparison

between different global areas is challenging. Also, as stated above in the context of classification differences, volumetric units to express microplastic presence vary in studies, making it more complicated to compare data. Examples are: microplastic particles/kg wet weight of sediment (Leads and Weinstein, 2019), microplastic particles/kg of sediment (Yu et al., 2018), microplastic particles/m² of sediment (Wessel et al., 2016; Gray et al., 2018), and grams of plastic in 1 liter of sediment (Baztan et al., 2014). Those differences are related to the specific methods necessary to collect sediment samples and extract microplastic particles. Our extraction method called for dry sediment, thus our results were given in the units microplastic particles/kg of dry sediment. Our results show that the lowest number of microplastic particles/kg of dry sediment was 596 particles (BB), while the greatest amount was 2224 particles (OB).

Notably, the abundance of microplastic particles/kg of sediment found in our study area is definitively higher than at other sites investigated along the southeastern USA (Wessel et al., 2016; Gray et al., 2018; Yu et al., 2018; Leads and Weinstein, 2019): in the intertidal sediments of the Gulf of Mexico, a maximum of 117 microplastic pieces occurred in a square meter (Wessel et al., 2016). Yu et al., 2018, found concentrations in National Park Service sites ranging from ~60 to 200 microplastic particle/kg of sediment. Piperagkas et al., 2019 took sediment samples from 37 National Park Service beaches and detected 21 to 221 microplastics particles/kg of sand. In the Charleston Harbor, SC, USA, intertidal and subtidal estuarine sediments contained 3-4375 microplastic particles/kg wet weight (Leads and Weinstein, 2019). Islands in the Atlantic Ocean had varying concentrations of microplastics. A maximum of 620 ± 96 microplastics/kg of dry sand, with an average of 261 \pm 6 were found in the beach sands of the Lesser Antilles (Bosker et al., 2018). In the intertidal zone of the Canary Islands, microplastics were concentrated at 109 g of plastic/1 L of sediment (Baztan et al., 2014). In Europe, Claessens et al., 2011 found 390 microplastic particles/kg of dry sediment in harbor sediments along the Belgian coast. Sites located along the Mediterranean Sea and the Lagoon Channel of Bizerte have microplastics particle concentrations in sediment that are higher than this study (Abidli et al., 2017). In the Lagoon of Venice, Italy, 672 to 2175 microplastic particles/kg dry weight of sediment occurred (Vianello et al., 2013). Abidli et al., 2017 found microplastic concentrations as high as 3000-18,000 particles/kg of dry sediment. Both sites were in close proximity to highly urbanized areas and industry.

4.3. Morphological types of microplastic particles

The dominant morphological type in the intertidal sediments of the Charleston Harbor, South Carolina, was fiber-like at our sites (Gray et al., 2018; Leads and Weinstein, 2019). Other regions include pellets and foams. Fisner et al., 2017 and Yu et al., 2018 identified pellets in beach sand. Lagoon sediments in Italy also identified pellets (Vianello et al., 2013). Foam microplastic particles were found in estuarine sediments from the Gulf of Mexico (Wessel et al., 2016) and Charleston Harbor, South Carolina (Gray et al., 2018; Leads and Weinstein, 2019). At other locations around the world an abundance of microplastic pellets is notable (Vianello et al., 2013; Piperagkas et al., 2019). However, our study did not find any pellets.

A 95% concentration of fibers in beach sediment samples was determined by Claessens et al., 2011, and Thompson et al., 2004. Their values are reflecting the 93.91% \pm 4.74% fiber concentration at all our study sites. Also, other studies show a pre-dominance of fibers over that of fragments: intertidal and subtidal sediments investigated by Leads and Weinstein, 2019, show that the most abundant microplastic morphological type were fibers (\sim 44–70%) with fragments following (\sim 30–48%). Fibers represent also the majority of microplastic particles found in intertidal sediments at two developed estuaries, Winyah Bay (77.5%) and Charleston Harbor (76.2%) in South Carolina USA (Gray et al., 2018). A study by Yu et al., 2018, measured the distribution of

microplastic particles in selected National Park Service (NPS) beaches. Microplastic fibers were the dominant morphological type also in the Caribbean beach sediments (Bosker et al., 2018).

4.4. Chemical composition of microplastic particles

With respect to the chemical composition of the microplastic particles in our area, polymers such as polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET) were identified. These polymers are also the most prevalent in USA estuaries. Along the Gulf of Mexico in Mobile, Alabama, PP and PE were the most abundant, yet polyester and polystyrene (PS) were also found in the sandy estuarine sediments (Wessel et al., 2016). Yu et al., 2018 investigated sandy National Park Service beach sites along the southeastern USA and found dominantly PET. Vianello et al., 2013 found PP and PE to also be the most commonly occurring polymers in the sediments of Venice Lagoon, Italy. Yu et al., 2016 found PE, PS to be the most commonly occurring polymers in beach sand of the Bohai Sea, China.

Polyester was the most commonly found microplastic particle type (< 55%) on over 18 sandy beaches that were tested worldwide (Browne et al., 2011). The Tamar Estuary in the UK included PVC, polyester and polyamide as the dominant types of polymers (Browne et al., 2010).

5. Conclusions

The beach sediments at each of the four study sites in Virginia and North Carolina contained a significant amount of microplastic particles. Two morphologies (fragments and fibers) could be differentiated. Fibers were predominantly found at each location (93.91% \pm 4.74%) with fragments compromising the remainder of the microplastic particles. The chemical composition of the particles included polypropylene (PP), polyethylene (PE), poly(4-vinylbiphenyl) (PVB), polyethylene terephthalate (PET), poly (4-vinylbiphenyl) (PVB), polytetrafluoroethene (PTFE), and polystyrene (PS), Ultem 1000 PEI (engineering thermoplastic), Halar ECTFE (engineering thermoplastic), polyisoprene, nylon, and teflon. The degree of contamination in our study area exceeds that of many comparable beach environments worldwide. Distribution pattern may reflect the influence of the transport via water currents in the Chesapeake Bay, the Albemarle-Pamlico-Sound, and the Atlantic Ocean. The proximity of metropolitan areas may increase the amount of microplastic particles in sediments.

CRediT authorship contribution statement

Gabrielle Z. Dodson: Writing - original draft, Project administration, Investigation, Conceptualization, Visualization. A. Katrina Shotorban: Writing - original draft, Investigation, Formal analysis, Validation, Conceptualization.Patrick G. Hatcher: Resources, Methodology. Derek C. Waggoner: Investigation. Sutapa Ghosal: Writing - review & editing, Visualization, Resources, Investigation. Nora Noffke: Writing - original draft, Supervision, Funding acquisition.

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.

Acknowledgements

This study was kindly supported by a grant provided by the Perry Honors College at Old Dominion University and the Department of Ocean, Earth & Atmospheric Sciences at Old Dominion University, Norfolk, Virginia, USA.

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