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Microplastics and Tire Wear Particles in South Carolina Coastal Waters: Sources, Pathways, and Toxicity

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Introduction

Since the first report of the presence of microscopic plastic particles in plankton tows from the North Atlantic in 1972 (Carpenter & Smith, 1972), numerous studies have revealed their widespread distribution in marine environments from coastal harbors (Zhang, 2017) and subtropical gyres (Poulain et al., 2018) to the deepest ocean trenches (Peng et al., 2018) and even arctic ice (Kanhai et al., 2020). These particles, now referred to as microplastics, represent a heterogeneous mixture of synthetic particles that vary in size (< 5 mm), shape, color, density, and polymer composition. The diverse nature of these particles reflects their multiple sources and infers complex environmental transport and fate in estuarine and marine systems. Some microplastics are manufactured to be microscopic for use in a variety of industrial and household applications including personal care products, pharmaceutical vectors, and pre-production pellets (i.e. nurdles) (Auta et al., 2017). These manufactured microplastics are referred to as primary microplastics. Secondary microplastics are those derived from larger plastic items, such as litter, as they fragment over time. Both primary and secondary microplastics can be transported into coastal waters through point and non-point pathways.

Owing to their small size and ubiquity in diverse marine habitats, microplastic exposure to biota is highly probable (Hermabessiere et al., 2017). Not surprisingly, the presence of these particles has been documented in a wide range of taxa including zooplankton, invertebrates, sea turtles, sea birds, and marine mammals (Miller et al., 2020), with consequences ranging from weight loss and reduction of energy reserves, decreased fitness, oxidative stress, and acute gut blockages (Avio et al., 2017). Because of their ubiquity in the environment, widespread bioavailability to biota, and potential to adversely impact environmental and human health (Browne et al., 2007; Sedlak, 2017; Sharma & Chatterjee, 2017), microplastics have been widely classified as contaminants of emerging concern. This short review will examine the extent of microplastic contamination in Charleston Harbor, SC and describe the efforts of my laboratory, in collaboration with others across the state, in characterizing the sources, pathways, and toxicity of these particles in South Carolina coastal waters.

Microplastic Surveys in South Carolina Coastal Waters

Our initial efforts to understand the extent of microplastic contamination along the South Carolina coast began in 2014 with a survey of intertidal sediments and surface water in Charleston Harbor and Winyah Bay. In intertidal sediments, average microplastic concentrations were similar between Charleston Harbor (414 ± 77 particles m^{-2}) (mean ± SE) and Winyah Bay (221 ± 26 particles m^{-2}) (Gray et al., 2018). However, in the uppermost layer of surface water, referred to as the sea surface microlayer, average microplastic concentrations were significantly higher in Winyah Bay (30.8 ± 12.1 particles L^{-1}) compared to Charleston Harbor (6.6 ± 1.3 particles L^{-1}) (Gray et al., 2018). These differences were attributed to the much larger drainage area of Winyah Bay's watershed (24,633 km^2), which is part of the Yadkin-Pee Dee River Basin, compared to that of the smaller watershed of Charleston Harbor (3,113 km^2), suggesting that inland sources of microplastics are important contributors to levels observed along the coast (Gray et al., 2018). Noteworthy among these findings was the observation that most particles in both estuaries were secondary microplastics, including fibers and fragments (Figure 1A and 1B). In fact, primary microplastics, such as microbeads, only comprised 1% of the total microplastics (Gray et al., 2018) (Figure 1C). Since these initial surveys, a spill of pre-production plastic pellets (nurdles) in 2019 alleged to be associated with an industrial facility in the Port of Charleston resulted in Charleston Harbor having the highest concentrations of plastic pellets along the East Coast (Charleston Post & Courier, 2019).

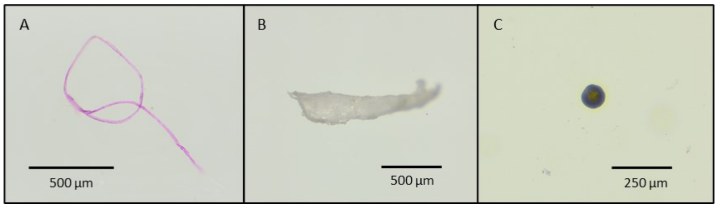

Figure 1. Light micrographs of common microplastic particles found in the environment. A=fiber, B-fragment, C=microbead. Photos courtesy of Sarah Kell.

Discovery of Microscopic Tire Particles in the Microplastic Litter

Although these initial surveys indicated that overall microplastic levels in Charleston Harbor and Winyah Bay were similar to that reported for other urbanized estuaries worldwide, both estuaries contained a high abundance of black fragments, many of which possessed a distinctive cigar-shaped morphology with a rubbery consistency (Gray et al., 2018; Leads, 2018) (Figure 2). For example, at Daniel Island in Charleston Harbor, these distinctive black fragments comprised >90% of the total microplastics in intertidal sediments, and harbor-wide, they comprised 73% of the total (Gray et al., 2018). Underscoring the uniqueness of these findings was that in a review of 68 microplastic studies by Hidalgo -Ruz et al. (2012), the most common color of microplastic fragments was white or related (e.g. discolored yellow, clear) with no studies reporting black fragments as the predominant particle type. Initial attempts at identifying the polymeric composition of these distinctive black fragments using Fourier transform infrared spectroscopy (FTIR) resulted in inconclusive results (Wertz, 2015).

To further investigate the identity and source of these distinctive black fragments, a follow-up survey was conducted in 2016 examining the abundance and composition of microplastics in the sediments of the three major tributaries of Charleston Harbor (Ashley, Cooper, and Wando Rivers). We initially suspected that the presence of these particles may have been related to industrial activity, in particular, point-source discharges in the Cooper River. We found microplastic concentrations in the intertidal and subtidal sediments in these three tributaries ranged from 0 to 652 microplastics m^{-2}, with blue fibers (26.2%) and the distinctive black fragments (17.1%) being the two most abundant particle types (Leads & Weinstein, 2019). Much to our surprise, significantly higher concentrations of total microplastics, as well as the distinctive black fragments, were found in the Ashley River, especially in close proximity to a high traffic boat landing and bridges (Leads & Weinstein, 2019). Further investigation into the association between these black fragments and their proximity to roads and bridges found very high levels (up to 1.3×10^7 particles m^{-2}) in the sediments of drainage ditches associated with the Ravenel Bridge (unpublished data), which has a high annual average daily traffic volume of 90,900 vehicles day^{-1} (SC Department of Transportation, 2022).

Using a weight of evidence approach based on their environmental distribution, particle morphology, and spectral characteristics from

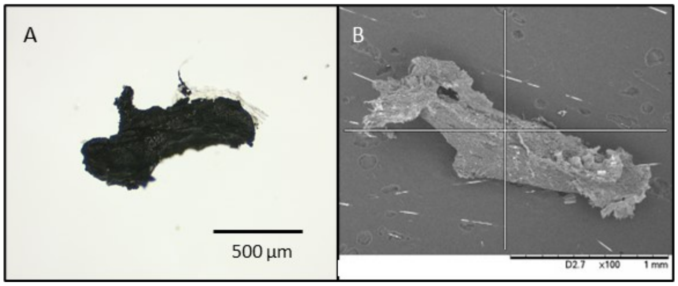

Figure 2. Light micrograph (A) and scanning electron micrograph (B) of tire wear particles. Note the distinctive cigar shape morphology of these particles. Photos courtesy of Sarah Kell.

ongoing FTIR analyses, we eventually identified these distinctive black fragments as microscopic tire particles, often referred to as tire wear particles or TWP (Leads & Weinstein, 2019) (Figure 2). These black fragments contained acrylonitrile/butadiene/styrene and carbon black, which are components of synthetic tire rubber (Leads & Weinstein, 2019). Tire wear particles are emitted to the environment following the abrasion of tires on roadways, especially during automobile acceleration and turning, and they are generally considered to be a subset of microplastics based on the presence of synthetic polymers. These findings provided the first evidence of relatively high abundances of TWP within the microplastic litter of a U.S. estuary. Prior to these findings, TWP were probably underreported in the U.S. because of challenges related to their identification (Leads & Weinstein, 2019). And, because tires, and the synthetic rubber of which they are composed, are part of the broader global plastic pollution problem, this research was highlighted in *National Geographic* magazine (Root, 2019). Since our initial reports, TWP as a major component of microplastic litter has been reported in San Francisco Bay (Werbowski et al., 2021) and freshwater ecosystems surrounding Portland, OR (Talbot et al., 2022).

Spatial and Temporal Variability of Microplastic Abundance in Sediments

During our initial sediment surveys in 2014, we found microplastic concentrations of 1195.7 ± 193.9 particles m^{-2} on the intertidal beaches of Daniel Island in Charleston Harbor (Gray et al., 2018). When we resampled those same beaches as part of our 2016 survey, we found considerably lower levels of microplastics (226.3 ± 45 particles m^{-2}; Leads & Weinstein, 2019). This suggests that levels of microplastics in intertidal sediments are variable and change over time. To better understand the spatiotemporal variability of microplastic abundance in these environments, we resampled the intertidal sediment from both the low and high intertidal zones of Daniel Island every 1 to 2 days at low tide over 17 days in March 2017 (12 sampling events; total n=72) (Leads et al., 2023). High temporal variability was observed as microplastic abundance ranged between 44 and 912 microplastics m^{-2} and differed significantly among sampling events ($p=0.00025$), as well as among some consecutive tidal cycles occurring within 12 h of one another ($p=0.007$). By contrast, low spatial variability was observed with no significant differences in microplastic abundance detected between the low and high intertidal zones ($p=0.76$) (Leads et al., 2023). Several environmental factors were investigated to determine how they influenced microplastic abundance on these intertidal beaches, and wind direction had the greatest effect on temporal microplastic variability (Leads et al., 2023). These findings demonstrated that there can be significant weather-influenced temporal variability of microplastic abundance in dynamic coastal environments, and one-time sampling of sediments in these habitats may not fully capture the variability in microplastic abundance.

Transport of Microplastics to Our Coastal Waters

Characterizing the pathways by which microplastics and TWP are transported to South Carolina coastal waters has been another area of active research. The role of municipal wastewater treatment plants in the transport of microplastics, mainly synthetic fibers from washing machines, to Charleston Harbor was studied by the laboratory of Dr. Barbara Beckingham at the College of Charleston. The three municipal wastewater treatment plants discharging to the harbor had high microplastic removal efficiencies ranging from 75 to 99% (Conley et al., 2019). On an annual basis, loadings of synthetic fibers to the harbor from treated municipal wastewater were estimated to be between 0.34 and 0.68 g microplastics per capita per year, which probably only represents <0.1% of the plastic debris input to Charleston Harbor's surface waters (Conley et al., 2019). We are currently investigating stormwater as a pathway, including stormwater detention ponds, in collaboration with Dr. Barbara Beckingham and Dr. Peter van den Hurk at Clemson University. It has been suggested that stormwater might be the dominant pathway by which terrestrial microplastics are exported into coastal waters (Zhang, 2017), and it is possible that stormwater detention ponds may act to capture these particles. In a survey of four Charleston-area stormwater detention ponds, microplastics, mostly composed of TWP (25-87%), were not only highly abundant in sediments of the ponds (49-8,812 microplastics kg^{-1} wet weight) but also in the sediments of their adjacent tidal creeks (4-4,835 microplastics kg^{-1} wet weight) (Kell, 2020). These adjacent tidal creeks, which serve as the natural receiving bodies for the discharge from these ponds, had sediment microplastic levels that were 29-146-fold higher than the Charleston Harbor-wide average. Collectively, these findings suggest that stormwater detention ponds retain some microplastic particles; however, given the relatively high levels of particles observed in adjacent tidal creeks, their capture efficiency is less than 100%.

We have also been investigating the role of nuisance flooding, also known as sunny day flooding, as a pathway by which microplastics are transported to coastal waters. Nuisance floodwater, for the most part, bypasses existing stormwater infrastructure (e.g., storm drainage systems, stormwater ponds, etc.) forming a direct connection by which street-derived contaminants, including microplastics and TWP, can be transported to adjacent tidal creeks. In a survey of three flood-prone areas of downtown Charleston, nuisance floodwater collected during 10 flood events contained high levels of microplastics (379 ± 297 microplastics L^{-1}), most of which were TWP (85.4%), but levels varied greatly between replicates, street locations, and flood events (Ertel et al., 2021). Adjacent tidal creek surface water did not contain more microplastics after these flood events, suggesting the possibility that most particles in the ebbing floodwater are captured by fringing marshes (Ertel et al., 2021).

Degradation of Plastics as a Source of Microplastics

Based on observations from our initial surveys that most microplastics in South Carolina coastal waters are derived from larger plastics (Gray et al., 2018), we performed a series of studies to better understand how degrading plastic litter contributes to microplastic loading. Previous surveys of Charleston Harbor estimated that there are 7.5 metric tons of plastic litter along its shoreline (Wertz, 2015), suggesting that degrading plastic litter may be an important source of microplastics. In one study, standardized plastic strips (15.2 cm x 2.5 cm) of three common plastic polymers (high density polyethylene [HDPE], polypropylene, and polystyrene) were deployed in a salt marsh and degradation was followed for 32-weeks (Weinstein et al., 2016). Degradation proceeded relatively quickly, with evidence of fragmentation and the release of microplastic particles from the surface of all three types of strips after as little as eight weeks of exposure. These results suggest that most plastic litter in the intertidal areas of our coastal waters, regardless of their age, are producing microplastics. This hypothesis was tested in a subsequent study in which eight plastic litter items of unknown age, ranging from drinking water bottles to plastic Solo® cups and food wrappers, were collected from a salt marsh and brought back to the laboratory. For six of the eight items, standardized strips of the collected plastic litter items emitted significantly more microplastic particles when placed in brackish water for 6 hours relative to strips from similar items not exposed to environmental conditions (Figure 3).

In a follow-up degradation study, bio-based (polylactic acid [PLA] cups, Mater-Bi® bags) and biodegradable plastics (biodegradable polystyrene plates, biodegradable HDPE bags) were compared to conventional plastics (recycled polyethylene terephthalate cups, HDPE bags, polystyrene plates) in a salt marsh over a 32-week period (Weinstein et al., 2020) (Figure 4). All plastics emitted particles beginning at 4 weeks,

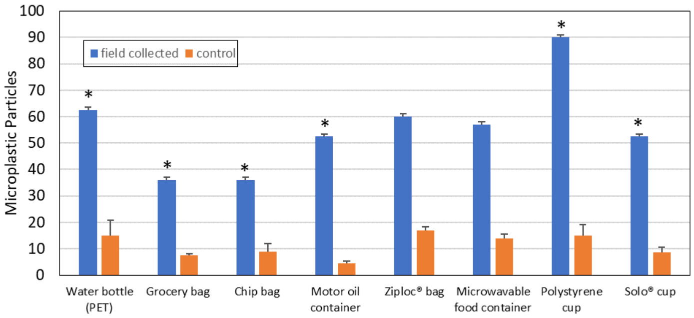

Figure 3. Number of microplastic particles produced from strips cut from various field collected plastic litter items and similar items not exposed to field conditions (=control) (15.2 cm x 2.5 cm; n=2) following exposure to filtered brackish water for 6 hours. Differences between field collected and control strips were determined using t-test with a Bonferroni correction. Significance is indicated by an asterisk ($p<0.05$).

Figure 4. Photograph of undergraduate student (Jack Dekle) collecting biodegradable plastic strips from field-deployed apparatus in a salt marsh.

with single-use bags producing the most microplastics over the 32-week period. Spectral analysis using FTIR indicated degradation occurred for all plastics except PLA, which ironically is a plant-based polymer. Results suggest that degradation rates of bio-based and biodegradable plastics vary widely, with Mater-Bi® bags and biodegradable polystryrene plates exhibiting the fastest rates of degradation, while PLA cups exhibited the least amount of degradation (Weinstein et al., 2020). Collectively, these results highlight how quickly degradation, and the subsequent production of microplastics, proceeds for most plastic litter in the intertidal areas of South Carolina coastal waters.

Toxicity of Microplastics to Estuarine Organisms

Characterizing the toxicity associated with acute microplastic and TWP exposures in estuarine organisms has been another area of active research. Our efforts have primarily focused on the daggerblade grass shrimp (*Palaemon pugio*) as a model organism because of their abundance, sensitivity, and ecological importance in southeastern U.S. estuaries. The usefulness of the grass shrimp in ecotoxicological testing, both in the laboratory and in the field, has been long recognized (reviewed by Key et al., 2006), and their transparency has proved quite advantageous for quantifying microplastic ingestion and gill ventilation. Our laboratory studies have demonstrated that adult grass shrimp can ingest and ventilate a variety of microplastic types, including microbeads, fibers, fragments, and crumb rubber (used as a surrogate in the laboratory for TWP), as well as a wide range of sizes from 30 to 165 µm (Gray & Weinstein, 2017; Leads et al., 2019; Kell, 2020) (Figure 5).

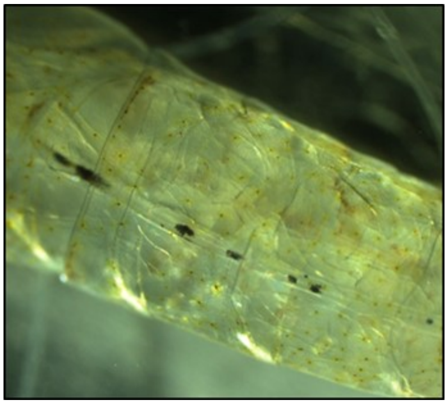

Figure 5. Light micrograph of tire wear particles passing through the intestine of a grass shrimp.

Many of the resulting toxicological manifestations are size-and shape dependent (Gray & Weinstein, 2017; Kell, 2020). For example, exposure to fibers (93 µm in length) resulted in mortality of 55%, whereas exposure to microbeads and fragments (<50 µm) were not acutely toxic (Gray & Weinstein, 2017). Microplastic residence times within the gut also varied, with the longest gut residence times associated with the 75 µm and 30 µm microbeads (75.9 ± 13.3 h and 60.6 ± 28.5 h, respectively), while the shortest residence time was associated with the 116 µm microbeads (27.6 ± 8.6 h) (Gray & Weinstein, 2017). However, it's important to note that the residence times of all tested microplastic particles were considerably longer than that of natural prey items (19.8 h; Kell, 2020). The consequences of prolonged residence times in the gut include decreased food consumption because of false satiation, which can ultimately lead to reduced growth and fitness.

Beyond acute exposures, research has also been directed at understanding the chronic and sublethal effects associated with microplastic exposure. Long-term (7-day) crumb rubber exposures in mummichogs (*Fundulus heteroclitus*) in the laboratory of Dr. Peter van den Hurk at Clemson University resulted in accumulation of crumb rubber within the intestinal tract of the fish, along with the leaching of polycyclic aromatic hydrocarbons associated with the rubber, as evidenced by bile fluorescence and induction of cytochrome P-450-1A (LaPlaca & van den Hurk, 2020). Longer, episodic crumb rubber exposures (20-day) in mummichogs elicited upregulation of CYP1A in gill, intestine, and liver; DNA damage; and increased liver glutathione levels, suggesting antioxidant systems were upregulated to deal with exogenous stressors associated with the crumb rubber particles (LaPlaca et al., 2022). Although previous studies have indicated that microplastic exposures can compromise invertebrate immune function (Paul-Pont et al., 2016; Pittura et al., 2018), research in my laboratory indicated that exposure to microbeads, fragments, fibers, and crumb rubber did not alter the susceptibility of grass shrimp to bacterial infection by *Vibrio campbelli* (Leads et al., 2019). We have also conducted chronic exposures of larval grass shrimp to microbeads. Microbead exposures (35 and 58 µm) increased mortality in larval shrimp; however, development time, as measured by the median time for transformation from the larval to the juvenile stage, was not delayed, and shrimp size did not vary among treatments (Gray et al., 2022). In fact, development time for the 35 µm microbeads was significantly faster (20.2 days) relative to that of control shrimp (20.8 days) (Gray et al., 2022). Although development was not delayed, the acute toxicity associated with microplastic exposures in larval grass shrimp is a concern due to their important ecological role of energy transfer within tidal creeks (Gray et al., 2022).

Bioavailability of Microplastics

One of the primary concerns associated with microplastics is their bioavailability to a wide variety of marine organisms having diverse feeding strategies. In field studies along the South Carolina coast, the presence of microplastics and TWP have been documented in zooplankton (Payton et al., 2020), fish across several different feeding guilds (Parker et al., 2020), and stranded bottlenose dolphins (*Tursiops* *truncatus*) (Battaglia et al., 2020). Bivalves are particularly vulnerable because their filter-feeding activity exposes them directly to any harmful material within the water column and can result in the accumulation of a variety of chemical and biological contaminants, including microplastics. Because of their vulnerability to microplastic uptake, as well as their consumption by humans as a food source, we assessed the levels of microplastics and TWP in eastern oysters (*Crassostrea virginica*) from nine state-managed shellfish grounds in South Carolina. Oyster microplastic abundances ranged from 13.5 ± 2.2 particles individual^{-1} from St. Helena Island to 34.6 ± 5.7 particles individual^{-1} from Folly River, with fibers (63%), TWP (18%), and fragments (11%) being the predominant types across all sampled sites (Blosser, 2022). Follow-up toxicokinetic studies demonstrated that accumulation and elimination of these particles are dependent upon particle size-and-shape, with crumb rubber (<140 µm) having both faster rates of uptake and slower rates of depuration than either fibers or fragments (Weinstein et al., 2022). The broader implications of these findings is that depuration, which is a common practice in the shellfish industry for reducing oyster bacterial loads for human consumption, is also an effective way to reduce microplastic loads; however, the U.S. FDA recommended time of 44 h (U.S. FDA, 2015) would only reduce microplastic loads by 55.5 to 67.6% (Weinstein et al., 2022).

Engaging Students in Research

Over the past eight years, much progress has been made toward a comprehensive understanding of the sources, pathways, and toxicity associated with microplastic contamination in South Carolina coastal waters. The global problem of plastics and microplastics is a research topic that has resonated with the current generation of students, and the student-led projects described above are examples of how a primarily undergraduate institution (PUI) has engaged undergraduate and master's level students in scientific research. This research topic has also resonated with grant funding agencies, including South Carolina Sea Grant Consortium and the National Institutes for Environmental Health Sciences, and has led to important collaborations across the state with Dr. Barbara Beckingham at the College of Charleston, Dr. Peter van den Hurk and the late Dr. Stephen Klaine at Clemson University, and Drs. Geoff Scott and Saurabh Chatterjee at the Center for Oceans and Human Health and Climate Change Interactions at the University of South Carolina. Central to these research efforts has been the concept of laboratory "cross-pollination" by which students working in my laboratory, both undergraduate and graduate, have the opportunity to travel and be trained in the laboratories of my collaborators (and vice versa). I wholeheartedly agree with my colleague, Dr. Dena Garner (Garner, 2020), that these collaborative efforts among faculty and students across PUIs and the R1 research institutions, with their very different educational missions, provide unique, and often times, transformative research experiences and mentoring opportunities, which in turn, supports both STEM workforce development within the state of South Carolina and scientific advancement on an important global issue.

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