MICROPLASTIC POLLUTION ON THE SURFACE WATERS OF THE SAN JUAN BAY ESTUARY, PUERTO RICO: FIRST OBSERVATIONS ON OCCURRENCE AND DISTRIBUTION

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Abstract – Urban areas have proven to be important sources of microplastic pollution. Since the San Juan Bay Estuary (SJBE) is the most diverse aquatic ecosystem located at the San Juan metropolitan area in northeastern Puerto Rico, it was subject of a surface water survey for microplastic pollution between April and July, 2016. Neuston samples were collected from three water bodies of the SJBE in areas characterized by industrial influence, varying population sizes as well as sites in proximity to natural reserves. All particles were classified according to microplastic type and analyzed with infrared spectroscopy for the characterization of synthetic polymers. La Torrecilla lagoon although located in an area with low population density and in close proximity to a natural reserve forest showed to be the most affected by microplastic debris over the more industrialized and urbanized sites San Juan Bay, and Los Corozos/San José lagoons, respectively. Secondary microplastics in the form of fragments, films and pellets were far more abundant than line/fibers and foam pieces. All of them seem to be product of anthropogenic activities which could represent a serious waste management problem. Analysis of the plastics showed a vast prevalence of polyethylene (75%), followed by polypropylene (24%) and polystyrene (1%), the only polymer types identified in the samples. This study provided and initial insight on the occurrence of surface water microplastic pollution in Puerto Rico given that, at the time of the sampling, no previous data was available.

Keywords: microplastics, FT-IR, polyethylene, polypropylene, polystyrene, estuary, San Juan Bay, Puerto Rico

Resumen – Las áreas urbanas han demostrado ser importantes fuentes de contaminación por microplásticos. Siendo el Estuario de la Bahía de San Juan (EBSJ) el ecosistema acuático más diverso en el área metropolitana de San Juan, al noreste de Puerto Rico, el mismo fue objeto de un estudio de contaminación por microplásticos en aguas superficiales entre abril y julio de 2016. Se recolectaron muestras de tres cuerpos de agua del EBSJ en áreas distinguidas por su actividad industrial, gran densidad poblacional y cercanía a reservas naturales. El material

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recolectado fue clasificado según el tipo de microplástico y analizado con espectroscopía infrarroja con el propósito de identificar polímeros sintéticos. La laguna La Torrecilla, aunque ubicada en un área de baja densidad poblacional y cercana a un bosque estatal, mostró ser la más afectada por microplásticos sobre áreas más industrializadas y urbanizadas como la Bahía de San Juan y las lagunas Los Corozos/San José, respectivamente. Microplásticos de tipo secundario compuestos de fragmentos, películas y gránulos fueron mucho más abundantes que las fibras y las partículas de espuma ("foam"). Todos ellos parecen ser producto de actividades humanas lo que puede representar un serio problema de manejo de desperdicios. El análisis de los plásticos mostró un predominio de polietileno (75%), seguido de polipropileno (24%) y poliestireno (1%), únicos polímeros identificados en las muestras. Este estudio proporcionó una mirada inicial de la presencia de microplásticos en aguas superficiales de Puerto Rico dado que, al momento del muestreo, no existían datos previos.

Palabras clave: microplásticos, FT-IR, polietileno, polipropileno, poliestireno, estuario, Bahía de San Juan, Puerto Rico

Introduction

Over the past 70 years, plastic has become a practical and valuable material due to its vast number of applications. Its uses are expected to increase given the continuous development of the plastics industry considering the world production of 359 million tons of plastic per year (PlasticsEurope, 2019). Unfortunately, the properties that add to its value also make it a problem at the time of its disposal, such as its longevity (1–500+ years), light weight and low cost. Although at one point it was only considered a matter of aesthetics (Fergusson, 1973), the impact of plastic debris on marine settings has been extensively investigated. Evidence of ocean pollution from plastics first appeared in the scientific literature in the early 1970s (Rothstein, 1973), and as of today, they have reached the ocean basins from both hemispheres (Eriksen et al., 2013a). However, the scientific community has awakened a renewed interest in microplastics: small granules, usually ≤ 5 mm, from personal care items, cosmetics, and airblast cleaning media, or derived from degraded macroplastics (> 25 mm).

The term *microplastic* was first introduced in 2004 referring to a type of contamination, not considered before, from small plastic particles, fibers, and granules (Thompson et al., 2004). Those produced as such are known as primary microplastics. These are the ones usually found in personal care products like toothpastes and exfoliating creams, replacing natural products like oatmeal, ground almonds or walnut husks (Fendall & Sewell, 2009). Similarly, microplastics can be the result of the breakdown of larger plastic waste due to overexposure to ultraviolet (UV) light emitted by the sun, abrasion, wave action and turbulence of the marine environment (Gregory & Andrady, 2003). This process is a continuous one so the fragments are reduced until they become microplastic in size, secondary microplastics. With almost half of the world's population living within 50 miles of the coast, microplastics have a high probability of reaching our beaches through rivers, sanitary systems, or simply through wind action. Extreme weather events such as flash floods or hurricanes can also exacerbate the movement of litter from land to sea, with the highest concentration of microplastics registered after significant rainfall events (Moore et al., 2002; Yonkos et al., 2014). The use of synthetic fabrics is another way by which microplastics reach aquatic environments. Experiments with wastewater samples from laundry demonstrate that a single garment can produce > 1900 fibers per wash (Browne et al., 2011). This suggests that a large number of microplastic fibers found in the ocean may be derived simply by washing our clothes. Additionally, coastal tourism, marine vessels, recreational and commercial fishing are all considered sources of plastic waste that can directly enter water bodies, putting fauna at risk in the form of macroplastics, and as secondary microplastics after long-term degradation.

Although the public interest has focused almost exclusively on marine plastic debris, microplastics have also been found in rivers, lakes, and even in ice, with hotspots near metropolitan areas (Bergmann et al., 2019; Eriksen et al., 2013b). Estuaries are a transition zone between river and marine environments. Therefore, they are subject to both, marine and riverine influences. The San Juan Bay Estuary (SJBE) is an urban aquatic ecosystem running all over the San Juan metropolitan area in northeastern Puerto Rico, composed of several bodies of water formed by lagoons, rivers, creeks and wetlands linked by channels to the Atlantic Ocean in a 251 km2 watershed (Figure 1). At the time of this study, no data was available on the levels of microplastic pollution present in Puerto Rico, including the SJBE, even though in the past sediment samplings have detected polychlorinated biphenyls (PCBs) in the estuary, a well known "plasticizer" used in paints and cements (Otero & Meléndez, 2011). Considering the location of the SJBE at the center of a highly urbanized metropolitan area, the aim of this study was to examine the occurrence and abundance of microplastic pollution in the surface waters of the San Juan Bay Estuary system.

Methods

Surface water sampling

The San Juan Bay, Los Corozos, San José and La Torrecilla lagoons were selected to explore the presence and distribution of microplastic pollution in the SJBE since they are the largest water bodies of the system (Figure 1). Because Los Corozos and San José lagoons are directly connected to each other, they were sampled together and, thus, referred to and reported in this study as the same aquatic body. Samples were collected by surface trawl using a 1 m long neuston net with a 363 µm mesh and removable cod end. The net, mounted in a metal frame with a rectangular opening of 50 cm wide by 30 cm high, was towed at a speed of approximately 4.0 knots from the starboard side of a vessel using a metal pole to position the towline outside the bow's wake. Each selected water body was trawled once between April and July, 2016. The sample collecting was time measured, with a stopwatch, for 60–minutes long each sampling. Trawl contents were rinsed with fresh water and preserved in glass jars with 70% isopropyl alcohol to prevent any organic material from decay until they could be separated and inspected at the laboratory.

Visual sorting and separation

Preserved samples were carefully rinsed with distilled water through a stack arrangement set of customized mini sieves with mesh sizes of 0.3 mm, 1 mm and 4 mm, respectively. This allowed sorting the material into three size classes: 0.3–0.9 mm, 1–3.9 mm, and 4–5 mm. Each classification size sample was immersed in 20 mL of 30% hydrogen peroxide (H_2O_2), briefly shaken, and stored in glass vials for seven days since this treatment can successfully digest most of the biogenic material (Nuelle et al., 2014). The wet peroxide oxidation mixture was subject to a density separation in a NaCl (0.3 g/mL) hyper-saline solution to isolate the plastic debris. Individual pieces within each size classification were handled with forceps and visually inspected using a dissecting microscope to be counted and categorized as fragment, foam, line/fiber, pellet, or film according to their appearance.

Microplastics characterization and quantification

Fourier transform infrared (FT-IR) spectroscopy was employed by using an ABB FTLA2000 (ABB Group; Zürich, Switzerland) instrument for the identification of plastics from the collected samples. The FT-IR analyzer was equipped with a high attenuated transverse reflection (ATR) unit containing a ZnSe crystal in which the samples were placed using tweezers. Only particles confirmed as plastics were considered for numerical abundance.

Data analysis

A G-test for categorical data was used to examine if there was any relationship between microplastics concentration and sample location. Due to the small sample sizes a Williams' correction (Gadjusted) was applied to prevent any misjudgment errors with the hypothesis testing. Pearson Product Moment Correlation analysis was employed to investigate the association between microplastics concentration and population density in the study area using data of the U.S. Census Bureau (USCB, 2016). All analyses were performed using R (R Foundation for Statistical

Computing) version 3.3.2. $P < 0.05$ was considered as the level of significance.

Results and discussion

Microplastic particles abundance, spatial distribution and potential sources

Plastic debris was present, in different shapes and sizes, in all three water bodies sampled. However, regarding microplastics concentrations it varied between sites (Table 1). The results revealed concentrations that are in a range similar to that of reports published from other water bodies (Hidalgo-Ruz et al., 2012). But the intensity was relatively high considering the size of each aquatic body sampled and the number of residents inhabiting the watershed area. La Torrecilla lagoon was the most polluted with microplastic debris while San Juan Bay turned out to be the least affected of them all. Plastic density from all water bodies sampled averaged 9,269 particles/km2, composed mostly of 1–3.9 mm plastic fragments (54%), followed by 0.3–0.9 mm (31%) and 4–5 mm fragments (15%). G-test highlighted a significant interaction between sample location and microplastics concentration ($P = 0.0108$). Abundance showed a relationship with anthropogenic activity and improper waste management, although several other variables can be of influence including population density, industrial occurrence and environmental factors (e.g. wind, rain, marine currents and wave action).

A strong negative relationship ($r = -0.9281$) is noticeable with the Pearson Product Moment Correlation analysis of data from all three sites, where microplastics concentration increases as population density decreases and vice versa (Figure 2). The elevated microplastics concentration in La Torrecilla lagoon, despite the low population density (961 inhabitants/km^2) in the area, compared to the other sampling sites and prominent wetland vegetation from the Piñones State Forest nearby, is surprising since other studies have shown a negative relationship between the number of plastic pieces and forested areas, where the number of microplastics decreases as the wooded region expands (Yonkos et al., 2014). But this happening may be partially explained by the high rate of human actions performed in the zone. Recreational fishing and boat sailing are favorite pastimes at the lagoon and can result in elevated amounts of trash inappropriately discarded by visitors. This could validate the sample composition of mostly secondary microplastics, same findings observed by Free et al. (2014) in an aquatic body with comparable settings.

Another reason that may explain our findings in La Torrecilla is the lack of easy outlet of Los Corozos/San José lagoons towards the ocean. Since extreme sedimentation and accumulation of rubble in the Martín Peña Channel prevents water exchange with San Juan Bay (PEBSJ, 2000), it appears that water is headed, inevitably, to La Torrecilla bringing plastic debris with it. In addition, La Torrecilla has a very small ocean outlet aside the highly irregular shape and small surface area (2.46 km^2) of the lagoon relative to San Juan Bay (13.27 km^2) and Los Corozos/San José lagoons (4.57 km^2), which may concentrate the microplastic amount. Low density consumer plastics are buoyant and contained to the surface (Cole et al., 2011), thus they may be concentrated by La Torrecilla lagoon's small surface area rather than be diluted by its volume. However, not all microplastics are buoyant (Kukulka et al., 2012), which suggests that differences in the sources and composition of microplastic pollution or in the intensity of organisms accumulated on them, known as biofouling, may also be important drivers of microplastic density on the water surface.

On the other hand, San Juan Bay turned out to be the least affected by microplastics of all the three water bodies sampled. Unlike La Torrecilla and Los Corozos/San José lagoons, low human activity is performed at the bay no matter the great population density in the area (2500 inhabitants/km2) due to its location in the middle of San Juan, Guaynabo, Cataño and Toa Baja municipalities. The San Juan Bay area is characterized by a high degree of industrialization that includes a sewage treatment plant, an electric power plant, an old landfill, a regional airport, and substantial shipping traffic, aside its low water quality (PEBSJ, 2015). All this do not make it prone for sport and/or recreational activities like the other water bodies sampled. Eriksen and colleagues (2013b) were among the first researchers to find a direct relationship between microplastics concentration, industrial activity and population density. But this relationship has not always been clear since Klein et al. (2015) have also found low numbers in highly industrialized and populated areas.

Sewage treatment plants are known point sources of small plastic particles and fibers (Browne et al., 2011). Nonetheless, the Puerto Nuevo Waste Water Treatment Plant located near the shore of San Juan Bay does not seem to have a direct effect considering the low amount of 0.3–0.9 mm plastic particles found. Additionally, the small concentration of 1–3.9 mm and 4–5 mm microplastics cannot be explained by a direct emission from industrial areas since they are also comprised of secondary microplastics and do not appear to be originally produced of miniature size. For these reasons, neither industrial activities nor sewage treatment plant locations look to be good indicators of microplastic pollution in the monitored area. Furthermore, the Municipality of San Juan Landfill closed down in 2000 and has been in compliance with state and federal environmental regulators (JCA, 2000); thus, a direct implication of polluting the San Juan Bay with microplastics seems unlikely.

Microplastics concentration can be influenced by wind and rain. Strong winds can increase the mixing and partitioning of plastic particles in the highest levels of the water column (Browne et al., 2010; Collignon et al., 2012). Likewise, bad weather and flash floods can transport litter to water bodies substantially increasing the concentration of plastics on the water surface (Moore et al., 2002; Yonkos et al., 2014). Coincidently, in this study samples at Los Corozos/San José and La Torrecilla lagoons were taken shortly after rain events which could have had some effect regarding the higher microplastics concentration found compared to San Juan Bay where good weather prevailed during sampling.

Type of microplastic particles

Plastic fragments, films and pellets dominated the microplastic composition in all three aquatic bodies sampled except for foam and line/fiber, which only occurred at Los Corozos/San José lagoons, and La Torrecilla lagoon, respectively (Figure 3). Spheres and pellets were just found in the lowest size fraction, while films, lines and fibers were obtained only in larger sizes. The size class representing particles 1–3.9 mm was more abundant than any other size accounting for 54% of the total particle count. These particles are most likely the result of the degradation and fragmentation of household debris, such as bottles, bags, wrappers, or other plastic products. Plastics can be easily broken by overexposure to UV light, which is why they degrade faster when they are on dry land than in water, a heavy reason for their prevalence in aquatic environments (Gregory & Andrady, 2003). This, however, is a dynamic process influenced variously by characteristics of the material like polymer composition, size, shape, and density as well as the aqueous environment like salinity and temperature (Andrady et al., 2011). Climatic and/or meteorological conditions like rain and prevailing or episodic wind can also favor plastic fragmentation through mechanical degradation by turbulence of the marine environment and sand abrasion (Kukulka et al., 2012). The small abundance of lines and fibers found in this study was unexpected, especially the total absence of them in San Juan Bay with the Puerto Nuevo Sewage Treatment Plant in close proximity. It is known that synthetic fibers can be emitted through washing processes and are not completely removed by sewage treatment (Browne et al., 2011). The Puerto Nuevo sewage plant discharge its effluents 1.6 km offshore from the mainland out to the open sea (Quiñones & Guerrero, 2004), which could explain the lack of this type of particles. The only line/fibers found were at La Torrecilla lagoon and because of their physical appearance they may be fishing gear remainders. This might be expected considering the high volume of sport fishing that takes place at the lagoon.

Polymer composition of the separated microplastics

Although several methods have been employed to identify microplastic polymers, like Raman spectroscopy, scanning electron microscopy, fluorescence microscopy, density-based tests and even by simple visual inspection of the particles, the use of infrared spectroscopy is strongly recommended for small plastic fragments because it can determine the chemical composition of unknown particles with high reliability (Hidalgo-Ruz et al., 2012). Fourier transform infrared (FT-IR) spectroscopy analysis allowed determination of which particles initially identified as microplastics were actually polymeric and discard those which not. Fragments captured produced spectra with distinct peaks of polyethylene (PE), polypropylene (PP) or polystyrene (PS), the only synthetic polymers found (Figure 4), and confirmed with others from known composition plastics as well as from additional spectra of other studies. The largest abundance, in terms of particle number, was represented by PE and PP (Figure 5). But the same could not be said of PS which was only found in Los Corozos/San José lagoons.

Even though the fingerprint region (500–1500 cm^{-1}) of the spectrum can contain a complex set of absorptions unique to each polymer, sometimes they can be hard to interpret visually. But their comparison with references of known materials can allow the identification of a specific compound. PE was identified by two mid strong signals of approximately 680 cm^{-1} and 1450 cm-1 in the spectrum followed by a distinctive strong double signal of 2800–2900 cm-1 corresponding to the long hydrocarbon (C–H) chain of the polymer. For PP particles, a series of weak signals from 600–1200 cm^{-1} followed by a mid strong twin signal around 1300–1450 cm^{-1}, from the bending C–H in the molecule, was detected. Confirmation of its identity was possible with the presence of a continuous four-peak "hand" like signal in the 2700–3000 cm^{-1} region of the spectrum. In contrast, PS produced a spectrum slightly similar to PE, but a very strong double signal of 680–750 cm^{-1}, which represents the C–H and CH_2 bonds, respectively, was fundamental to correctly identify the polymer.

The high level of abundance of PE (75%) relative to PP (24%) and PS (1%) is no surprise since PE, considering all its varieties that are manufactured, is the most widely used plastic polymer in the world (PlasticsEurope, 2019). Also, its low specific density and floating ability allow the widespread distribution in aqueous systems, same traits that share with PP and PS. Consequently, these three polymers are also the most frequently identified in studies of aquatic environments polluted with synthetic particles. The absence of other type of polymers is possibly caused by their less frequent usage but could also be explained by different transport mechanisms in water systems due to the lack of buoyancy of some of them.

Limitations

Ease of access was a key factor when selecting the sampling sites. The SJBE watershed is located almost entirely in urban and suburban areas, or in forest protected areas. Therefore, not every aquatic body is suitable for sailing and take samples. Weather also played an important role during the sampling process. Since the seasonal change is very limited in the tropics, the time of the year is marked by a wet (or rainy) season and a dry season. Sampling took place at the end of the dry season and beginning of the rainy season. Hence, in order to evaluate the extent of microplastic pollution in the estuary and the potential influence of rainfall and floods, comprehensive studies are needed during both seasons.

Conclusions and future remarks

This study provided and initial insight on the occurrence of surface water microplastic pollution in Puerto Rico given that, at the time of the sampling, no previous data was available. Also, knowing the type of microplastics that overflows in our water bodies can help in efforts to identify and mitigate the sources of plastic contamination in aquatic environments. The survey evidenced the existence of neustonic (surface waters) microplastics in all three water bodies sampled. Although, due to the preliminary status of this project and the importance of our findings a more detailed investigation comprising all the water bodies of the SJBE is recommended. La Torrecilla lagoon was the most polluted with microplastic particles, with a possible overflow of plastic debris from Los Corozos/San José lagoons due to their lack of a direct outlet into the Atlantic Ocean. These results were later reaffirmed by recent findings of microplastic contamination in nearby beaches (InterNewsService, 2019). Synthetic polymers in the estuary, composed of PP, PE and PS, seems to come from improper waste management and related anthropogenic activities like sports and recreational pastimes which can compromise the environmental health of the entire aquatic system.

The large number of studies on microplastics carried out in recent years demonstrate the level of awareness on the negative impact that plastic pollution is having on the environment. Some countries, such as the United States with the Microbead-Free Waters Act of 2015 (USC, 2015), have already taken the first steps to halt microplastic contamination by banning their use. However, is clear that these principles must be constantly reinforced if a lessening in the production of this type of waste wants to be achieved.

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Table 1

Microplastic particles abundance, in three size classes, found in three water bodies of the San Juan Bay Estuary.

Location	**Plastic Size**			**Count Total**	**Mean Abundance (particles/km²)**
	0.3–0.9 mm	**1.0–3.9 mm**	**4.0–5.0 mm**		
San Juan Bay	3	13	3	19	5,130
Los Corozos/ San José	16	8	3	27	7,289
La Torrecilla	13	35	9	57	15,389
Count Total	32	56	15	103	NA

Note: NA=not applicable

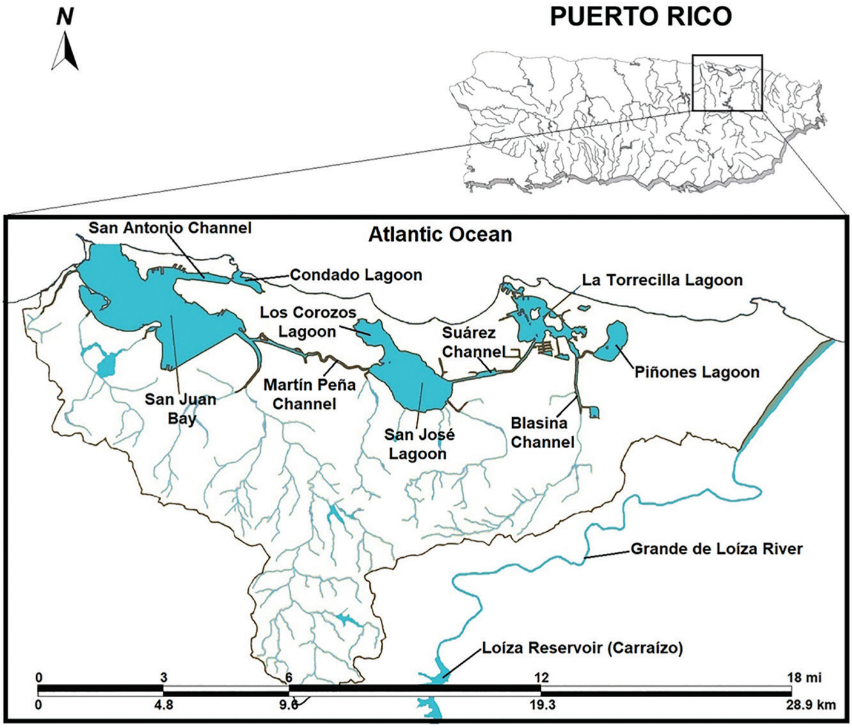

Figure 1. San Juan Bay Estuary watershed located in northeastern Puerto Rico.

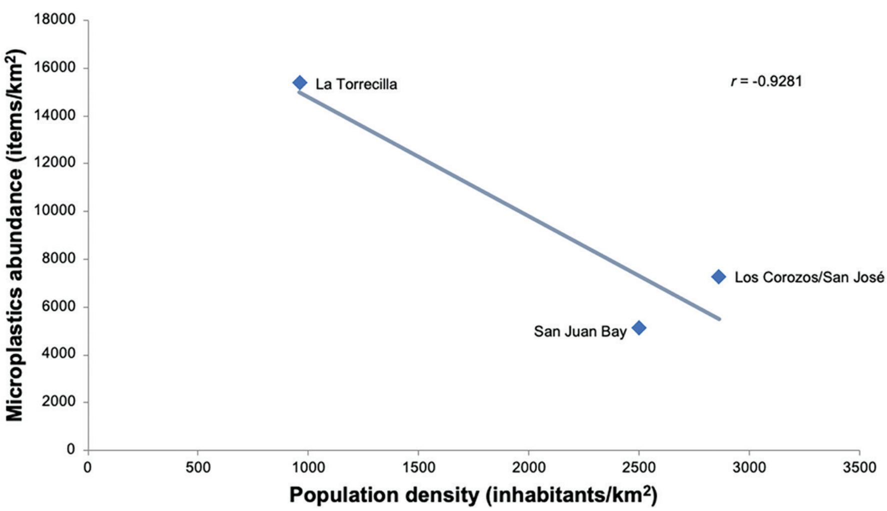

Figure 2. Association between population density in sampling areas and microplastics concentration.

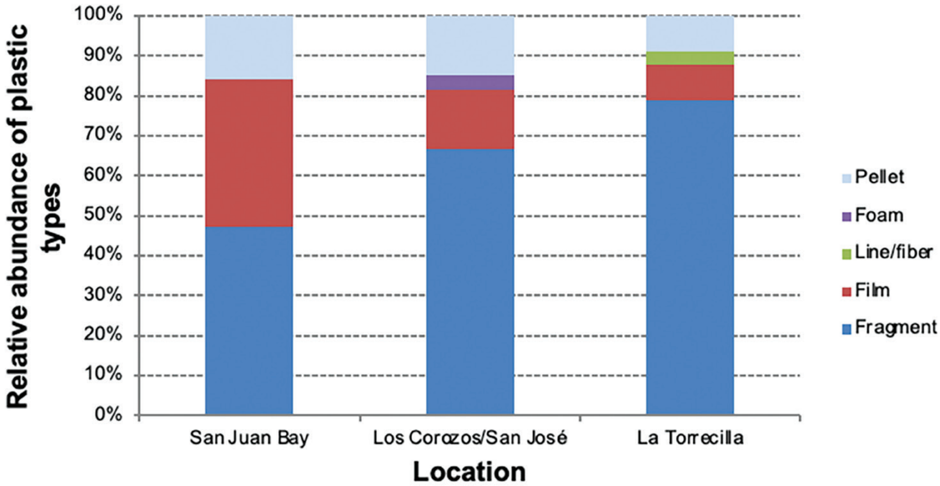

Figure 3. Relative abundance of different types of microplastic particles found in all sampling sites.

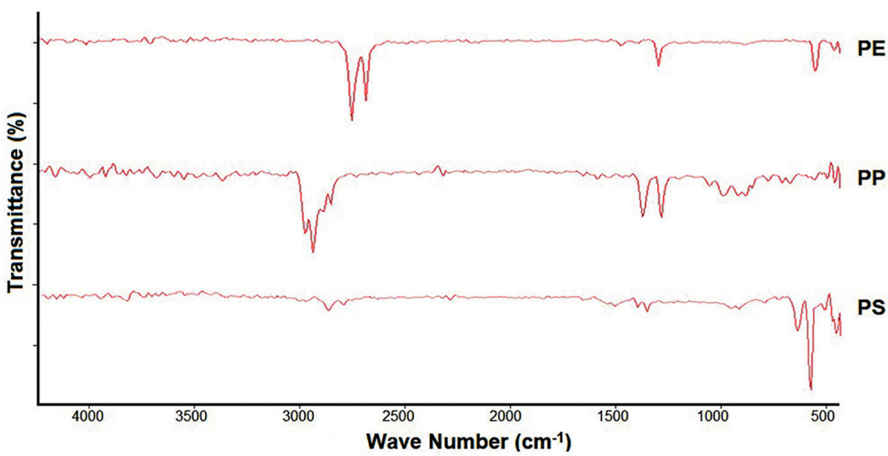

Figure 4. IR spectra of the synthetic polymers found in all sampling sites (PE=polyethylene, PP=polypropylene, PS=polystyrene).

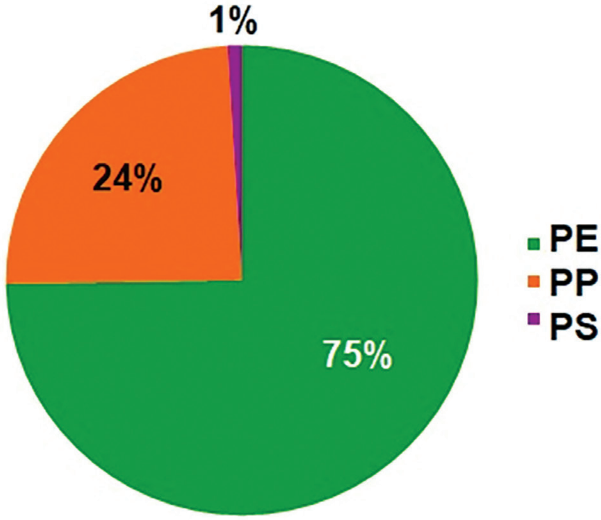

Figure 5. Relative abundance of polymer types identified in all sampling sites (PE=polyethylene, PP=polypropylene, PS=polystyrene).