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Baseline

Surface layer microplastic pollution in four bays of the central Mexican Pacific

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ABSTRACT

Surface microplastics were sampled monthly in four tropical bays (Manzanillo, Santiago, Navidad and Cuastecomates) of the central Mexican Pacific during March 2017 to February 2018. Microplastic concentrations ranged between 0.01 and 1.05 particles/m^2 with a median per bay ranging between 0.26 and 0.40 particles/m^2. Raman spectroscopy registered polypropylene (40%), polyethylene (40%) and polyester (20%) polymers. Fibers dominated all samples, except for Manzanillo where fragments numerically dominated during the rainy season (Jun-Oct). Fiber concentration was not significantly different among bays or seasons, likely associated with continuous wastewater discharge. Fragment concentrations were significantly higher in Bahía Manzanillo and Santiago than the other two bays. Non-metric multidimensional scaling showed distinct distribution of Manzanillo samples (which has important port activities) as compared to Santiago, Navidad, Cuastecomates (where tourism economic activities predominate). This first direct comparison of sea surface microplastic concentration among four bays in Mexico provides a baseline to study impacts on marine zooplankton in this tropical ecosystem.

Pollution stemming from plastic products in marine ecosystems is a global problem (Andrady, 2011; Thompson, 2018). An estimated 4.8–12.7 million MT of plastic entered in the ocean in 2010, and that amount is predicted to increase (Jambeck et al., 2015). Degradation of plastics lasts decades to centuries in marine habitats. Abrasion, solar radiation, and biological processes cause fragmentation over time into micro-particles (Andrady, 2011). Microplastic (MP, <5 mm in diameter) concentrations in the ocean have increased over time due to these secondary micro-fragments and primary micro-waste like microspheres from cosmetics and cleaning products (GESAMP, 2015). Ocean currents and winds transport microplastics around the world (Lusher, 2015; Wang et al., 2016), crossing political boundaries that result in an emerging contamination problem of international concern due it's long-term persistence and accumulation (Andrady, 2011; Avio et al., 2017; Fiedler and Lavín, 2017).

A wide variety of organisms with diverse types of feeding strategies, such as zooplankton (Cole et al., 2013), macro invertebrates (Wright et al., 2013), elasmobranchs (Germanov et al., 2018) and marine mammals (Besseling et al., 2015) are vulnerable to microplastic ingestion in pelagic and benthic habitats. Animals can ingest microplastics directly from the environment or indirectly via microplastic contaminated prey. Feeding appendages of zooplankton can become obstructed, space for energy reserves reduced, and feeding rates can decrease when microplastics are present in their natural habitats (Nelms et al., 2018). Besides the negative effects of particles that cannot be digested, microplastics can release additives and toxic monomers or can absorb a large amount of hydrophobic toxins, that accumulate in tissues and organs of marine organisms, causing detrimental effects to the endocrine and/or immune system (Wang et al., 2016).

The international scientific community has been increasing attention on microplastics in marine ecosystems (Pan et al., 2019). However, in the Mexican Pacific, research is relatively recent and scarce.

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Microplastic concentrations have been quantified on Mexican beaches in Ensenada (Silva-Iñiguez and Fischer, 2003), Huatulco, Oaxaca (Retama et al., 2016), on the Baja California Peninsula (de Jesus Piñon-Colin et al., 2018), and in 22 beaches located along the Mexican Pacific and Gulf of California (Alvarez-Zeferino et al., 2020). Three studies reported microplastics concentrations in sea surface waters in Bahía de La Paz (Fossi et al., 2015), Bahía Banderas (Pelamatti et al., 2019), and Bahía de Todos Santos (Ramírez-Álvarez et al., 2020). The present study is the first effort to directly compare sea surface microplastic concentrations in different bays along central Mexican coasts.

The coastal region of the central Mexican Pacific includes a dry season (Nov-May) with upwelling events that favor primary production, and a rainy season (Jun-Oct, hurricane season) when the water column is warmer, stratified, and phytoplankton abundance is typically lower than during the dry season (Ambriz-Arreola et al., 2012). The river discharges increase during the rainy period along the central Mexican Pacific where sediments suspended in the water column of the rivers can reach distances of ~60–300 km offshore (covering up to 44,000 km^2) (Martínez-Flores et al., 2011).

Microplastic spatial and temporal distribution per types was investigated for one year, comparing four bays on the central Pacific coast of Mexico (Manzanillo, Santiago, Navidad, and Cuastecomates) (Fig. 1). The urban populations in Bahía Navidad and Cuastecomates are smaller (<15,000 inhabitants) than those of Manzanillo and Santiago (>130,000 inhabitants) (Instituto Nacional de Estadistica y Geografía, 2010). The Port of Manzanillo is one of the busiest ports in the Pacific coast of Mexico for containerized cargo and cabotage. Populations in the four bays obtain income mainly from mostly foreign tourism during Dec-Mar and mostly national tourism during Easter and summer holidays. We propose the hypothesis that sea surface microplastic concentrations have a positive association with the human population size located around each bay and therefore have higher concentrations in Bahía Manzanillo and Bahía Santiago than in Bahía Navidad and Bahía Cuastecomates.

Monthly sea surface zooplankton samples were collected from March 2017 to February 2018 in four bays: Manzanillo, Santiago, Navidad, and Cuastecomates (Fig. 1). The May 2017 sampling was cancelled due to the high phytoplankton concentration that clogged the nets within 30 s of the net tow. A total of 44 zooplankton samples were collected throughout the year (11 samples per bay). The net tows were carried out on a small boat with an offboard motor (locally known as pangas) using two identical neuston nets (named nets A and B) of 57 cm length, 30 cm diameter with a 250 μm mesh size. The nets were placed 1 m away from both sides of the boat towing during 10–15 min along a straight path at 3.7–5.5 km/h speed while ensuring that half of the net was maintained out of the sea surface water to exclusively sample the neuston layer. Geographic coordinates were logged with a GPS at the beginning and end of each zooplankton tow to calculate the total distance sampled expressed in meters.

The outside of the net was rinsed with seawater after each net tow using a submersible water pump to concentrate the sample in the cod-end. Subsequently, the cod-end was carefully removed over a bucket to avoid spillage and poured into a 250 μm mesh to concentrate the sample. A stainless-steel spoon was used to transfer (without scraping) part of the sample to a labeled glass bottle. After depositing most of the sample, the mesh was inverted and rinsed from the outside, nothing was poured directly on the sample, using approximately 200 ml of 90% alcohol for posterior laboratory analysis.

The precipitation time series was analyzed averaging the monthly value between 2017 and 2018 from the three meteorological stations located nearest each bay obtained from CONAGUA, Servicio Meteorólogico Nacional website (https://smn.conagua.gob.mx/es/climatologia/informacion-climatologica/informacion-estadistica-climatologica).

The work area and laboratory equipment were cleaned and sterilized with alcohol before and after each analysis to avoid plankton sample contamination. A cotton lab coat was worn to avoid microplastic contamination of the sample from textile fibers. The microplastic were

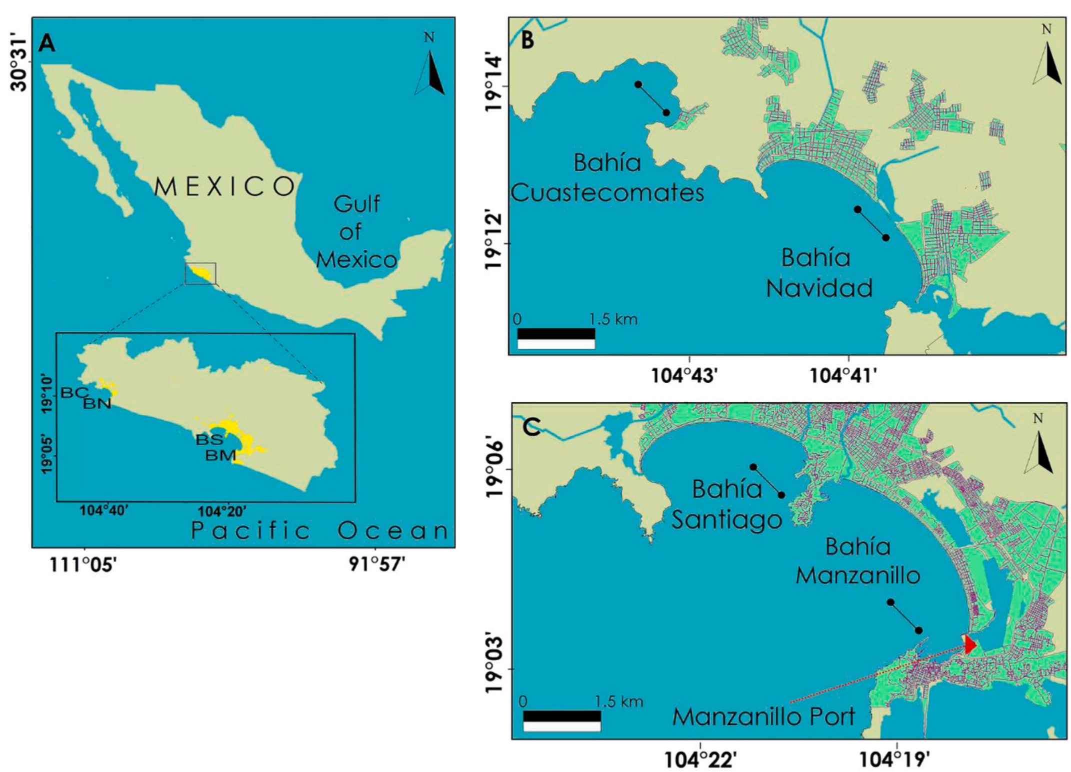

Fig. 1. Area of study in A) Central Mexican Pacific, Mexico, showing a zoom of the sampling sites in the bays of B) Cuastecomates (BC) and Navidad (BN), and C) Santiago (BS) and Manzanillo (BM).

separated manually from the organic matter and visually categorized using a Zeiss Stemi® DV4 stereomicroscope. The plastic particles were counted, classified by color, and categorized by type of particle (fragments, film, spheres, fiber, and foam), and size per millimeter interval (0.25–1.0 mm, 1.1–2.0 mm, 2.1–3.0 mm, 3.1–4.0 mm, and 4.1–5.0 mm). A second Petri dish was used as a control to determine the degree of laboratory environmental contamination of microplastics to the sample. Particles found in the control were classified according to type, and the same number (ranging from 7 to 36 particles; samples which took longer to separate had more particles in the control) were then discarded from the plankton sample total. Concentration of microplastics (particles/m^2) was calculated with the number of microplastic particles divided by the product of opening width (m) × transect length (m) (Gewert et al., 2017).

Raman spectroscopy was used to identify the chemical structure (type of polymer) of a random subset of fibers, fragments and films separated from the net samples (accounting for 0.1% of the total number of particles collected) to confirm the presence of synthetic polymers as opposed to organic polymers in the plankton samples. The peaks observed on a Raman spectrum correspond to the molecular vibration of both individual and groups of chemical bonds. This was used as fingerprint for a material, allowing for its identification based on comparisons with known spectra. The samples were analyzed using a Raman spectrometer (Horiba HR800) at a wavelength of 785 nm and over a range of 100 to 3500 cm^{-1} at the Center for Nanoscience and Micro and Nanotechnology (Instituto Politécnico Nacional, Mexico City). Spectrums were visualized using the KnowItAll software (academic edition) from Bio-Rad and compared with known polymer spectra. Collected particles with a hit quality index (HQI) > 75% of coincidence with a plastic polymer were considered microplastics.

Frequency distribution of total microplastic concentration, per sizes, and per types were first checked for normality using the Shapiro-Wilks test. Non-parametric statistical analyses were used because all p-values were <0.001. A Kruskal-Wallis test was used to check for significant differences of the concentration of microplastic per type and size among the four bays. When significant differences of median concentration of microplastic per type and size was observed with Kruskal-Wallis test, a Mann-Whitney U test defined the differences. The Kruskal-Wallis and Mann-Whitney U tests were performed with Statistica 10.0 software (Stat Soft). Non-metric multidimensional scaling (NMS) was used to define the spatial and temporal distribution of the microplastic concentrations in the four bays during 2017–2018. This non-parametric statistical analysis requires few assumptions about the type of relationship (e.g. linear, modal) present in the data. The microplastic matrix (MP) had 44 rows (samples) and 20 columns (variables) categorized per sizes (≤1.0 mm, 1.1–2.0 mm, 2.1–3.0 mm, 3.1–4.0 mm, and 4.1–5.0 mm) and types (fragments, film, spheres, fiber, and foam). Microplastic variables with $<n = 3$ frequency in the total number of samples were removed from the matrix (spheres size classes 2, 3, 4 mm; foam size classes 3, 4 mm). The environmental matrix had 44 rows and 6 columns including 3 quantitative variables (average monthly precipitation, total precipitation 3 days before sampling, and tide height at time of sampling) and 3 categorical variables (bays, seasons, and bays seasons). The Sorensen distance measure was applied for the NMS, and the significance of the axes were checked using a Monte Carlo test. The NMS was calculated using the PCORD v6.0 software (MjM Software Design) (McCune et al., 2011).

Median microplastic concentration of each of the four bays ranged between 0.26 and 0.40 particles/m^2 (Fig. 2). The highest microplastic concentration (1.05 particles/m^2) was observed in Bahía Santiago and the lowest (0.01 particles/m^2) in Bahía Cuastecomates (Fig. 3). Although precipitation was significantly higher during the rainy season than during the rest of the year, total microplastic concentration was not significantly different among the four bays between rainy and dry seasons due to the variable concentration of fibers in the surface sea water (Kruskal-Wallis; $p > 0.05$) (Fig. 2). However, the concentration of microplastic fragments was significantly higher in Manzanillo and Santiago than in Navidad and Cuastecomates (Mann-Whitney U; $p < 0.05$).

Fibers and fragments were the dominant forms in nearly all sampling stations in the four bays (>50% of total per sample) during dry and rainy seasons (Fig. 3), except in Manzanillo where significant high fragment concentration was observed during the rainy season (Mann-Whitney U; $p < 0.05$). The highest film concentrations were observed in Manzanillo

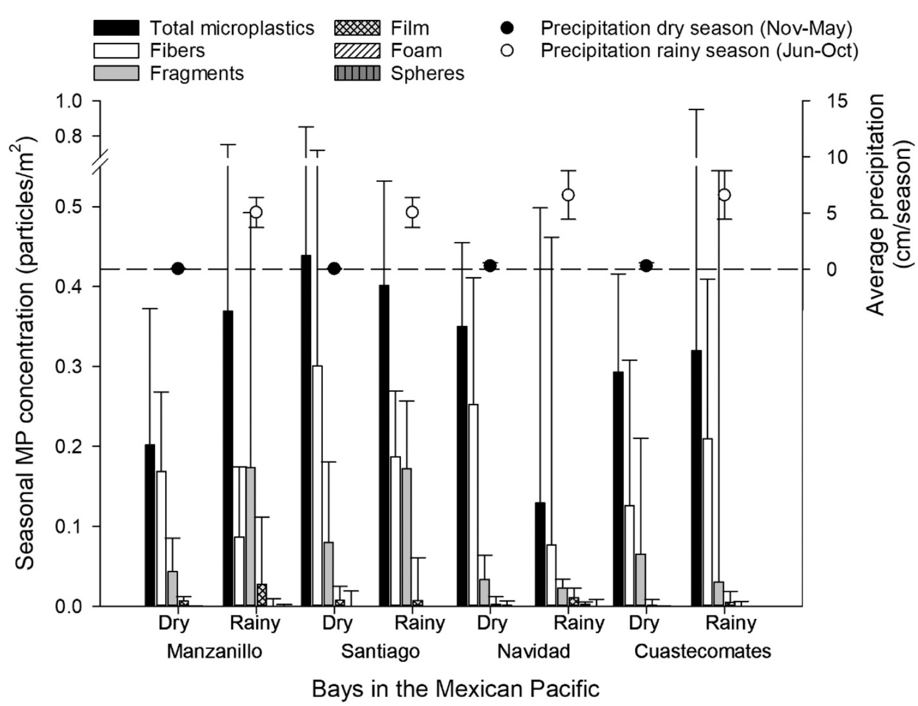

Fig. 2. Median concentration (particles/m^2) of total microplastic, fibers, fragments, film, foam, and spheres per dry and rainy season and per bay (bars). Vertical line is 75% of the interquartile range. Circles indicate mean and range seasonal precipitation values in each bay.

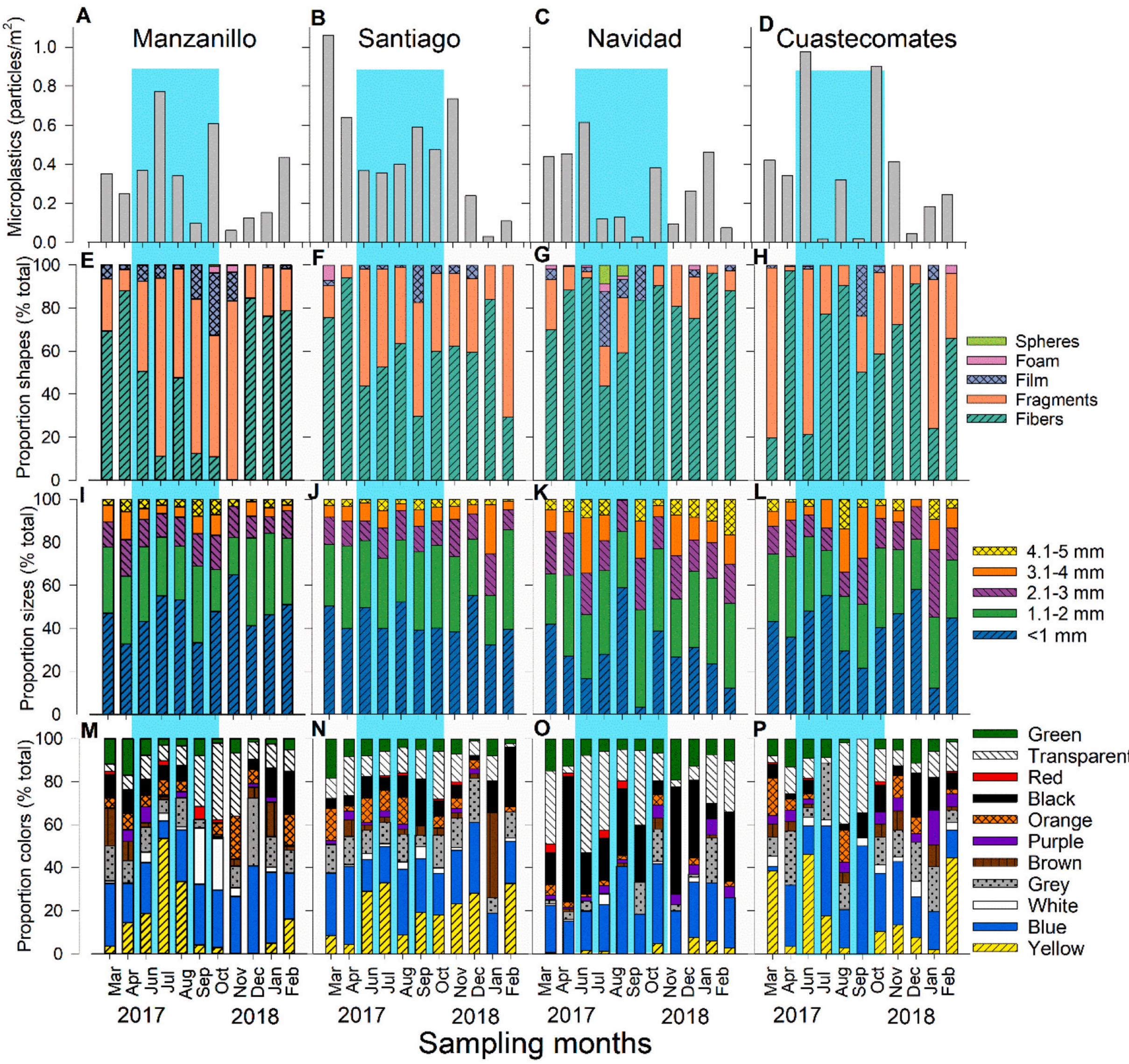

Fig. 3. Total microplastic concentrations (A-D), shape proportion (E-H), size proportions (I-L), and color proportions (M-P) collected at bays of Manzanillo, Santiago, Navidad and Cuastecomates located in the Central Mexican Pacific. Blue background = rainy season. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and Santiago during the rainy season (0.03–0.05 particles/m^2) (Figs. 2, 3). The highest percentage of films in samples of the four bays was registered during the rainy season associated with increased river runoff (Figs. 2, 3). Sphere and foam concentrations were extremely low in the four bays. Particles of microplastic with sizes <2 mm were always >50% in all samples (Fig. 3). Colors were highly variable; yellow, blue and transparent the most common shades, although the lowest concentration of of yellow microplastics in Bahía Navidad was notable (Fig. 3).

The temporal variability of microplastic concentration per type and per size among the bays was analyzed using NMS (Fig. 4). The final stress was 10.6 and both axes were significant ($p < 0.05$). The coeficient of correlation values (r^2) indicated the multidimensional scaling was not correlated with seasonal precipitation. No clear pattern could be observed through the season or bay×season categories, so the samples were categorized per bay. There were two gradients mostly related to forms of particles along the two axes, with particle size not being a relevant factor, except for foam pieces. Axis 1 had a gradient primarily defined by the concentration of the spheres. Along the axis 2, sampling stations with a gradient from numerically dominant fibers to fragments/film particle types were observed. The particle size frequency distribution of Bahía Manzanillo samples was significantly higher than in the other three bays, being the most clearly defined by fragments and film. The other three bays showed slight spatial shifts in the multivariate space. Samples of Bahía Cuastecomates were most closely associated with fibers and foam. Bahía Navidad was mostly dominated with fibers and spheres, and Bahía Santiago had high proportion of film, fragments, and foam microplastic (Fig. 4).

The Raman spectroscopy confirmed there were microplastic within the sea surface water samples (Fig. 5) also including organic materials, particularly red cellulose fibers which were neglected from the analysis due to their low concentrations. Three polymer types were registered from the plastic particles analyzed: polyester (20%), polyethylene (40%) and polypropylene (40%). Fragments were either polypropylene or polyethylene, while fibers were either polyethylene or polyester. All films analyzed were polyethylene. Differences among the Raman spectra of the same type of polymer but with different forms or colors was also noted. For example, the spectra of a polyethylene transparent fragment (Fig. 5A) were distinct from polythylene yellow film (Fig. 5D).

Microplastic contamination in the central Mexican Pacific is within the range of other regions of the world providing evidence of the

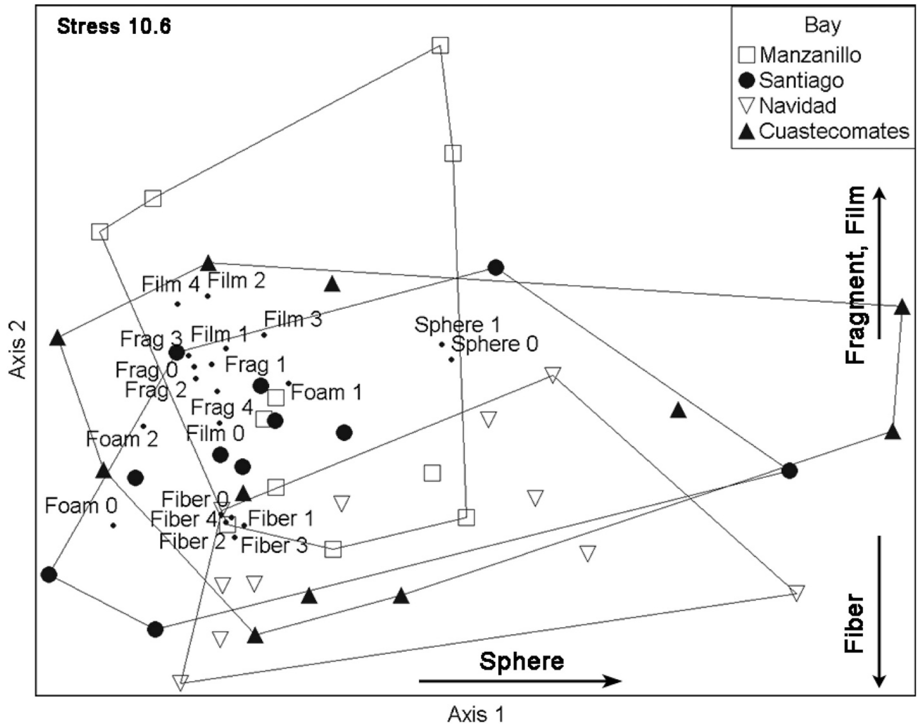

Fig. 4. Non-metric multidimensional scaling plot showing the variability of microplastic per size and type at Manzanillo, Santiago, Navidad and Cuastecomates bays located in the Central Mexican Pacific. Symbols show samples Size intervals of microplastic particles (0.25–1.0, 1.1–2.0, 2.1–3.0, 3.1–4.0, 4.1–5.0 mm) per type (Fiber, Film, Foam, Fragment, Sphere).

widespread problem of these particles in the world ocean. The present study provides a baseline for microplastic monitoring and ongoing research of the effect of microplastics on tropical zooplankton. Synthetic polymers in the form of microplastic were found in the four bays during the rainy and dry seasons showing a high site-to-site variability. Average microplastic concentrations (0.45 particles/m^2) observed in the present study were within the range of concentration observed in other regions reporting high concentrations of microplastic using similar methods. Gewert et al. (2017) reported mean microplastic concentrations of 0.42 particles/m^2 near Stockholm and 0.047 particles/m^2 in offshore areas, while Moore et al. (2001) reported an average of 0.33 particles/m^2 in the North Pacific Central Gyre. These two studies collected microplastic with a slightly larger mesh size of 333–335 μm than the present study (250 μm), but concentrations of microplastic overlap among regions. The microplastic concentration of the present study cannot be directly compared with the concentrations reported on the Mexican Pacific coast in Yelapa, Jalisco located at the southern region of Bahía Banderas (Pelamatti et al., 2019) and Bahía de Todos Santos, Baja California (Ramírez-Álvarez et al., 2020) because those studies calculated the concentration in cubic meters (m^3). Pelamatti et al. (2019) found microplastics in only about half the samples collected, while in Todos Santos, Baja California and this study microplastic were present in all the samples collected, as is commonly found in other microplastic research. This could be because the Yelapa time series is in the south of Bahía Banderas, near a tiny town only accessible through a rural road and by sea.

Proportions of particle shapes in the four bays were similar to those reported in Latin America (Kutralam-Muniasamy et al., 2020), with fibers the dominant particles in most of the sea surface samples. An important source of microplastic in coastal regions stems from wastewater contaminated by fibers from washing clothes (Browne et al., 2011), and high concentrations of microplastic have been reported near wastewater treatment plants in Bahía Todos Santos where the large Port city of Ensenada, Baja California with a population around 500,000 is located (Ramírez-Álvarez et al., 2020). The towns adjacent to the four bays of the present study have year-round residential populations and are also tourism destinations. The population at Bahía Navidad and Cuastecomates currently does not have a functioning sewage treatment plant, and wastewater is generally released directly into the bays. Cuastecomates, the most enclosed bay but with the smallest population, and no direct terrestrial runoff, also had the largest variability of microplastic concentration in sea surface samples, with both the lowest as well as nearly the highest (just below Santiago) observed. This variability is related to moment of sampling and sewage discharge of the urban settlement (including a large hotel which takes up a quarter of the beach). Therefore, effluent discharge is probably much higher and more direct in Bahía Navidad and Cuastecomates, even though the population in these towns are considerably smaller than those of Bahía Manzanillo and Santiago. The lack of urban development planning in Bahía Manzanillo also might result in some clandestine discharges of wastewater into the bay (Ahumada-Martínez et al., 2018); and there is probably a similar situation in Bahía Santiago. Sewage and sewage effluents released directly (in different concentrations based on residual treatment capacity) into all four bays are probably the reason why fibers were present throughout the year with no significant seasonal or spatial differences within and among the bays; therefore, this hypothesis is rejected.

The NMS plot showed the most notable spatial differences between Manzanillo and the other three bays. Manzanillo is one of the largest shipping ports in Mexico (Secretaría de Comunicaciones y Transportes, 2017), and the sampling site was in front of the port entrance. Santiago, Navidad and Cuastecomates bays are communities with primarily national and international touristic socio-economic activities, with numerous restaurants along the beach that provide service throughout the year. Only Bahía Manzanillo showed significant seasonal differences, with a higher number of fragments during the rainy season than during the dry season. The larger inputs of plastic fragments likely are due to the transport activities carried out at the port; but intense currents result in non-significant differences among the four bays. The difference among touristic bays and a port bay raises the question of the impact of

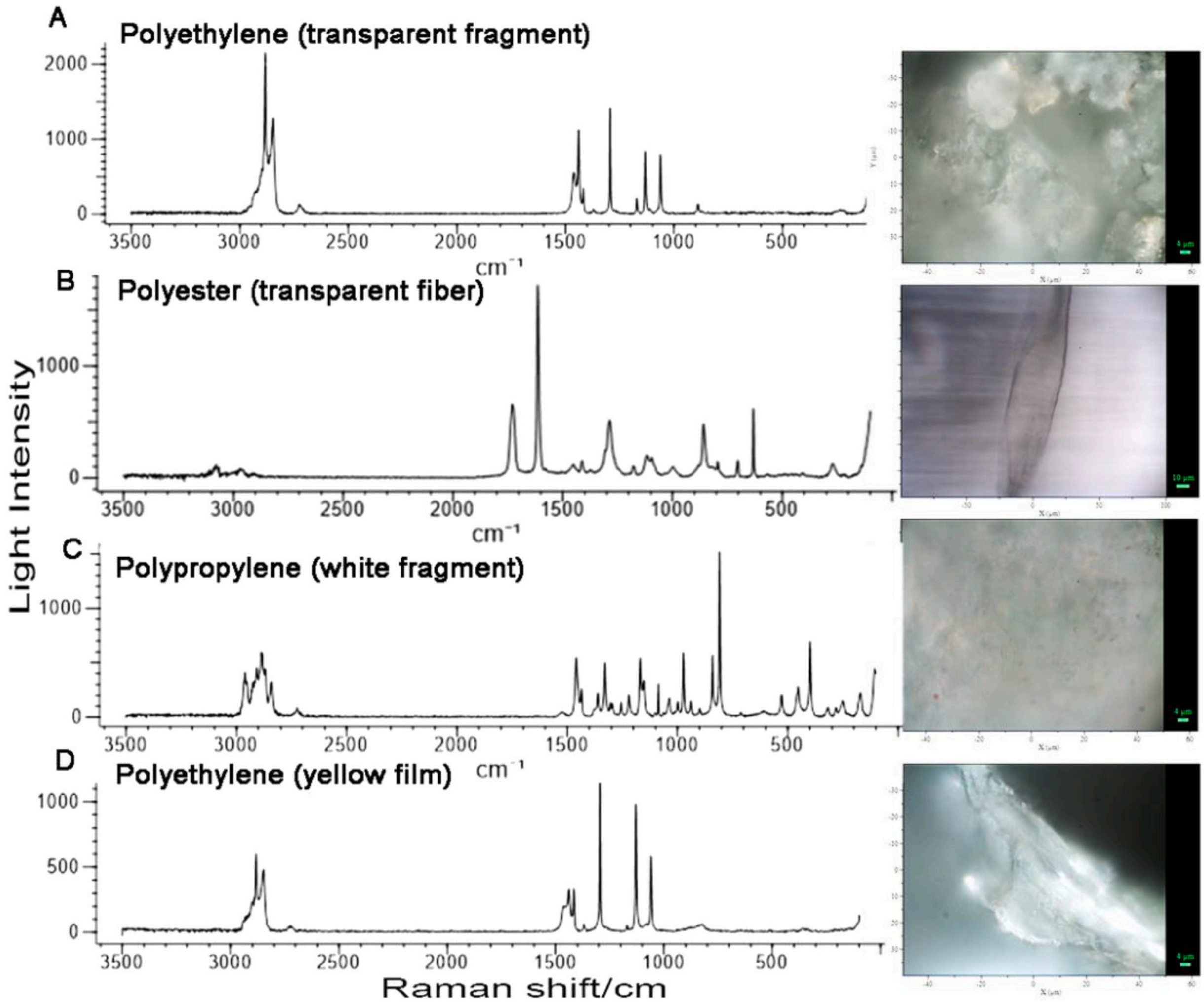

Fig. 5. Polymer composition through Raman spectroscopy of select particles collected in the Central Mexican Pacific A) polyethylene transparent fragment, B) polyester, C) polypropylene, D) polyethylene.

different coastal area use patterns among regions on marine plastic waste contamination, and how they arise. Jang et al. (2020) found microplastic polymers had different concentrations among three sites (urban, aquafarm, and rural) in Korea, generating microplastics from land and marine sources. In the present study, the differences between port *versus* tourism activities were also notable. Tourism activities demand clean beaches, likely reducing the number of plastic items/fragments on beaches that can be washed by large waves, wind and rain that occur in this region. There is not clear evidence which activities at the Manzanillo port are most responsible for generating high concentration of plastic fragments, and why they are more likely to enter the water column during the rainy season. There is also the possibility that the plastics which are washed into the ocean through the river runoff around the touristic beaches are not yet fragmented (macroplastics) which can be more feasibly removed from the bays. These are key questions to implement plans to reduce plastic contamination and suggest that a certain amount of standardization might be possible for management strategies based on use patterns in each bay.

Sea currents and seawater density play a large role in the three-dimensional distribution of the plastics on the ocean. It is assumed here that most of the microplastic contamination is from nearby populations, but it is evident that other sources from farther distances also must contribute, albeit in a so far unknown proportion, to the total microplastic concentration observed in the present study. Low-density particles float near the surface for up to 6–8 months (fibers), or 10–15 years (spherical particles) (Chubarenko et al., 2016), sinking once they lose buoyancy through biological processes like biofouling (Zhang, 2017). Ramírez-Álvarez et al. (2020) sampled at several locations in Bahía Todos Santos, Baja California, Mexico reporting that sea surface currents and wind are the main factor that dominate the dispersion and distribution of microplastic. Local coastal currents patterns and meso-scale processes in the area of study have not been investigated near the coast (Fiedler and Lavín, 2017); which could explain the high variability of concentrations and type of microplastic observed in samples of the same bay and among bays. Polymers reported in the present study (polyesterene, polypropylene and polyethylene) are positively bouyant, explaining their high concentration in the surface of the water column and also indicating their potential transport forced by sea surface currents.

Evidence exists that the intense river plumes occurring during the hurricane season (August–October) in the region of study increase turbidity and Chlorophyll-*a* concentration (Martínez-Flores et al., 2011). Sporadic transport of plankton and sediment in the water column occurs from Cape Corrientes coast towards offshore waters due to river plumes. A plume of river discharge originated from Acaponeta, San Pedro, Santiago, and Ameca in August 2004, covered an area of 44,000 km^2, reaching offshore regions as far ~300 km offshore from the Nayarit coastline (Martínez-Flores et al., 2011). These, relevant process during anomalous high precipitation can transport microplastic far offshore. However, the ecosystem impact on biota from this tropical region is still unknown. Seasonal upwelling and downwelling seasonal dynamics that prevail in the region could also modify near surface dispersion during intense upwelling and concentration nearshore during weak downwelling events (Ambriz-Arreola et al., 2012; Franco-Gordo et al., 2015; Fiedler and Lavín, 2017).

Although the scope of the present study does not encompass zooplanktoon feeding on microplastic particles, there are several regional time series of zooplankton taxonomic groups that feed on phytoplankton and ciliates that are within the size range of microplastic particles (Ambriz-Arreola et al., 2012; Gasca et al., 2012; Franco-Gordo et al., 2015; Kozak et al., 2017). At least nine of the 19 zooplankton taxonomic groups reported in the area of study are primarily filter feeders, making them vulnerable to microplastic ingestion. Non-filter feeding taxonomic groups can also directly ingest microplastic due to the biofilm which can build on them (Vroom et al., 2017), or indirectly through their zooplanktonic prey. Pontellidae copepods, which are known to be neustonic (Heinrich, 1971) are also found in the inshore region of the Mexican Pacific (Kozak et al., 2017). These tend to be relatively larger tropical copepods (3–5 mm), within the size range to be potentially ingesting the smaller microplastic found in this study (0.3–1 mm). Ongoing research is investigating which zooplankton species are actually ingesting microplastic in the central Mexican Pacific (Kozak, unpubl. observations), as it could have important implications for zooplanktophagous predators and overall ecosystem health. *In situ* and experimental feeding studies of zooplankton are needed to quantify the potential impact of these contaminants on the regional zooplankton community.

We tested and rejected the hypothesis that microplastics have a positive, linear association with human populations, where coastal areas would have significantly higher concentrations in bays with larger human populations. The results of the present study show a more complicated relationship, probably involving land use activities (Port *versus* touristic economic activities), residual treatment capacity, sea surface current patterns, connectivity among bays, and upwelling/downwelling seasonal dynamics.

The Port of Manzanillo impacted the type and concentration of microplastic in comparison to bays without ports. Results also showed that wastewater effluents are a significant problem in the four bays and should be addressed in a near future. This is the first study in Mexico to directly compare surface water microplastic concentration and composition among multiple bays, providing a baseline insight into the primary type of anthropogenic use patterns among bays. Identification of such differences will provide information for designing efficient management strategies to reduce contamination in the future.

CRediT authorship contribution statement

Eva R. Kozak: Conceptualization, Investigation, Formal analysis, Writing – original draft, Funding acquisition. **Carmen Franco-Gordo:** Conceptualization, Investigation, Writing – review & editing. **Jorge Mendoza-Pérez:** Investigation, Resources, Writing – review & editing. **Nancy Sánchez-Nuño:** Investigation. **Xenia A. Martínez-Sánchez:** Investigation, Writing – review & editing. **Paola Melo-Agustín:** Investigation, Writing – review & editing. **Gloria Pelayo-Martínez:** Investigation. **Jaime Gómez-Gutiérrez:** Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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