Research Article

Presence of Microplastics and Additives Detected in Digestive Systems of Freshwater and Saltwater Fish Consumed by Humans in Puebla, Mexico

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ABSTRACT

Aim: This study demonstrates the presence of microplastics and associated compounds in fish consumed in Puebla a state in Mexico. **Background:** When plastics are improperly disposed of, they easily reach bodies of water, where they break down into tiny pieces called Micro Plastics (MPs), causing a serious environmental problem. **Materials and Methods:** Samples were taken from freshwater fish: rainbow trout (*Oncorhynchus mykiss*) and eurasian carp (*Cyprinus carpio*) from fish farms, as well as from marine fish: grey mullet (*Mugil cephalus*) and marine tilapia (*Oreochromis* spp) purchased from the local market. The intestinal contents were examined using three detection techniques: density separation, stereoscopic microscopy, and gas chromatography/mass spectrometry. **Results:** It was found that 46.3% of the microplastic particles were black, and 85.8% of the totals were fibers. The predominant plastic was Polyethylene Terephthalate (PETE), with a total average abundance of 5.2±1.1 MPs per individual, and plastic additives, primarily phthalic acid, were detected, attributed to various anthropogenic sources contaminating the ocean. **Conclusion:** Consumption of freshwater fish is recommended due to their lower levels of microplastics and additive compounds.

Keywords: Fish, pollution, microplastic, waste.

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INTRODUCTION

Industrial development increases the production of materials, including plastics manufacturing, aiming to meet the needs of a population seeking a fast-paced life, generating easily accessible products and providing immediate well-being, without adequately considering the high generation of waste.[1]

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The mass production of plastic has led to a pollution issue, with the main reasons being poor waste management, excessive use by society, and limited recycling activity. It is estimated that currently, seven billion tons of plastic have been generated worldwide, with less than 10% being recycled.[2]

Today, single-use plastic materials are produced extensively. Approximately 36% of plastics produced are used for packaging food. When disposed of, a small portion of these plastics are incinerated; however, around 71% of discarded plastics end up in the ecosystem.[3] The main entry pathways include open dumps, discharge into river systems, runoff from sediments, release of industrial waste, wind, and catastrophic events.[4,5]

Once in the sea, plastics tend to fragment and become Microplastics (MPs) due to exposure to sunlight, wind, and the mechanical energy of waves, with a size ≤5 mm,[6,7] this causes an ecological imbalance; the ocean has the ability to absorb carbon dioxide from the environment and can reduce atmospheric carbon by up to 50%. However, the presence of microplastics on the ocean surface reduces this absorption capacity, slowing down the rate at which carbon is extracted from the sea surface to the depths.[8,9]

Microplastic pollution is a serious issue due to its omnipresence in all ecosystems, including the ocean and in demersal and pelagic fish. Studies have revealed the presence of MPs in the digestive system, gills, liver, and soft tissues of clams, among other organisms, allowing them to enter the food chain and eventually reach humans.[8,10] Much of the research focuses on marine fish due to their economic importance and the large-scale impact on the ecosystem. However, there is limited research conducted in freshwater ecosystems where the morphotype, size of microplastics, and their chemical composition are crucial for integration into a sensitive system.[9] Microplastics increase heterotrophic bacterial activity and the breakdown of dissolved organic matter, thereby increasing bacterial respiration and oxygen consumption. They can also form thicker, heavier biofilms that enable microplastics to sink to the seafloor, releasing more complex organic compounds.[11,12]

There are studies confirming the presence of microplastics in fish consumed by humans,[9,13,14] if microplastics are present inside the human body, smaller particles can reach cells,[15] microplastics have been found in the heart,[16] blood,[17] human placenta[18] and lung tissues.[19] Additionally, microplastics can transport microorganisms, heavy metals, and various toxic chemicals,[18] including environmental pollutants and plastic additives such as plasticizers, flame retardants, dyes, heat stabilizers, catalysts, lubricants, antioxidants, and foaming agents.[20] This makes them vectors of contaminants.[19] Such as Endocrine Disruptors (EDs), which are non-natural chemicals or mixtures that interfere with hormonal activity.[20] Microplastics and nanoplastics serve as indicators of environmental contamination due to their status as PBT (Persistent, Bioaccumulative, and Toxic) pollutants, highlighting the importance of demonstrating their persistence in remote locations.[10,21,22]

The objective of this study is to demonstrate the presence of microplastics and associated compounds in freshwater and saltwater fish in the state of Puebla, Mexico that could be affecting human and environmental health in this area. Samples of freshwater fish were obtained from fish farms, while samples of saltwater fish were collected from markets.

BACKGROUND

In Mexico and elsewhere in the world, the issue of microplastics has recently been studied, demonstrating their presence in water, soil, and ocean-derived foods, aiming to assess their extent, raise awareness among society, and seek solutions in collaboration with governments, private and public institutions, and affected communities.

Fish consume plastics or microplastics attracted by their colors, either through primary or secondary ingestion. These particles can enter the intestine and then migrate to various fish body tissues, such as the hemolymph and hemocytes, thus becoming food for other fish and magnifying pollution.[9]

The fish studied in this research have the following characteristics: the eurasian carp (*Cyprinus carpio*) inhabits slow-flowing inland waters with abundant vegetation. Its maturity age is influenced by the latitude and altitude of its habitat, and its reproductive success is linked to water levels that rise and flood terrestrial vegetation, providing food sources.[23] Rainbow trout (*Oncorhynchus mykiss*) reaches maturity as a fully pelagic species. While its natural habitat is freshwater, it can migrate to the sea if necessary and primarily feeds on invertebrates and small fish.[24] The flathead grey mullet (*Mugil cephalus*) is a euryhaline pelagic species that forms schools and feeds by filtering microscopic algae and organic detritus.[25] Finally, the marine tilapia (*Oreochromis* spp) originates from Africa, and its growth is affected when temperatures drop below 15°C.[26]

The main issue with plastics is their high durability and, secondly, their toxicity, which leads to bioaccumulation in water and marine organisms, causing adverse effects on their development by reducing growth rate, reproduction, shape, size, volume, and density. Plastics most found in aquatic environments include Polystyrene (PS), Polyethylene Terephthalate (PET or PETE), High-Density Polyethylene (HDPE), Polypropylene (PP), Polyvinyl Chloride (PVC), and Low-Density Polyethylene (LDPE). These plastics can leach compounds such as phthalates, Polybrominated Diphenyl Ethers (PBDEs), bisphenol A, perfluoroalkyl, perfluoroalkyl, alkylphenols, and alkylphenol ethoxylates. Chemicals added to plastics and microfibers can have impacts on human health and the environment.[10,21]

Plastics, due to their varying density from their polymeric formation and added chemical compounds, can be

found floating in water or settled on the seafloor, making them more accessible to various aquatic groups.[27] The presence of plastic material causes various ecological imbalances that have been documented; for example, when zooplankton feeds on phytoplankton and excretes it, Microplastics (MPs) within fecal fragments slow down sinking rates, preventing carbon deposition on the seafloor, thus reducing carbon sequestration.[11]

Among the investigated studies, alkaline, acidic, enzymatic, and oxidative digestions are employed to remove organic matter adhering to microplastics.[28] Alkaline digestion was chosen for this study due to its cost-effectiveness and accessibility, with reports indicating losses from effervescence or interference using hydrogen peroxide and sodium thiosulfate.[29]

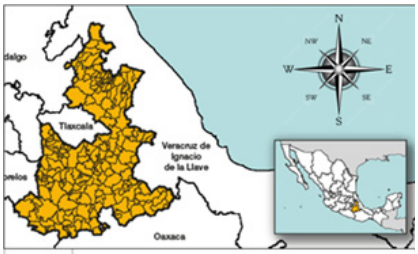
The non-parametric Kruskal-Wallis test,[30] was used in this study, which examines whether there is a statistically significant difference between the medians of three or more groups. This test is considered a very important tool compared to ANOVA; however, it is applied to non-parametric tests because it does not make assumptions about the defined parameters, such as the mean, variance, or the distribution of the data. This test determines whether the independent groups have the same mean in the ranks by assigning a rank to each value and using those ranks to verify if the data from each group come from the same distribution. It´s compares k random samples obtained from k possible populations. The Dunn procedure is a statistical analysis used to identify which pairs of means are significantly different from each other. It works by adjusting the alpha level the level of statistical significance) to consider the number of pairs of means being compared. It´s was applied to assess differences between treatments applied to independent populations.[31]

MATERIALS AND METHODS

Sample collection

Four sampling locations were selected to obtain saltwater fish (2) and freshwater fish (2) as shown in Figure 1, with the aim of comparing the presence of microplastics across different ecosystems. The first and third groups were sampled from fish markets within the city (Latitude 19.05142024988416, Longitude -98.19685142907417 and Latitude 19.0515658773825, Longitude -98.19713062121444). These fish originate from the states of Veracruz, Tabasco, and Campeche, meaning they are from the Gulf of Mexico, information obtained through interviews with suppliers. The second sampling location was a fish farm (Latitude 18.988362807227197, Longitude -98.48043430823724) located at the foothills of the Popocatepetl volcano, and the fourth sampling site was a tourist fishing zone (Latitude 19.317003932284788, Longitude -98.47076553636921) where fish are raised in spring water.

a)



Group	Geographic coordinate system	
	Latitude	Longitude
1	19.05142024988416	-98.19685142907417
2	18.988362807227197	-98.48043430823724
3	19.0515658773825	-98.19713062121444
4	19.317003932284788	-98.47076553636921

b)

Figure 1: Sampling points. a) view of geographical distribution, b) Location and coordinates of sampling sites.

The samples were transported in a cooler at 4°C. The samples were classified by species, weight (g), and total length (cm).[32] The animals were dissected using stainless steel scissors, making an incision from the anal opening to the mouth,[33] the intestines of the animals were obtained intact, recovering the entire contents[34] and were stored in aluminum foil.

Processing of gastrointestinal tracts

The intestinal samples were transported to the Institute of Microbiological Sciences at the Benemérita Universidad Autónoma de Puebla (ICUAP) for processing and detection of microplastics.[29] The digestive tracts were cut into 2-centimeter segments and placed in vials with a 3:1 ratio of 10% potassium hydroxide (KOH analytical reagents) solution.[29,35,36]

Then they were placed in an oven at 60ºC for 2-5 days for complete digestion, the time necessary according to the amount of organic matter present in the sample, the digested sample must dissolve the fat, obtaining a consistency that allows its filtration. The digested samples were subsequently vacuum filtered through a Buchner funnel and a Kitasato flask (Pyrex). The filter paper used in each filtration was stored in aluminum foil, and excess moisture was removed in the oven (QL-10GCE brand Felisa).

Identification of microplastic morphotypes

Sediment content on each filter paper was examined using a ZEISS stereoscopic microscope (VE-S5C) to 20X and 40X,[37] Particle search was conducted in a zigzag pattern. Particles identified as potential plastics had to meet the criteria of not disintegrating under pressure,[34,38] and displaying uniform color throughout. [39] Particle counting was performed, documenting their color and shape (fibers, fragments, spheres). Subsequently, particles found in each sample were transferred to test tubes along with scraped filter paper. For each batch of samples analyzed, solvent blanks were processed in the same manner as the samples to assess potential external contamination.

Method for separating microplastics by density differences

Distilled water (ASTM Type I) was added to the test tubes obtained from each sample to suspend or settle particles based on the density of each type of plastic, as shown in Table 1. To separate the floated product, a Pasteur pipette was used to transfer it to a different test tube. On the other hand, to recover the sedimented particles, the remaining liquid was evaporated. Subsequently, solutions of 70% methanol (CH_3OH, analytical reagents) and 23% sodium chloride (NaCl analytical reagents) were added to these test tubes respectively, as depicted in Figure 2. Finally, after adding these solutions, the same procedure of product recovery was performed using a Pasteur pipette and evaporation.[35,40]

Table 1: Densities of plastics.[41]

Plastics	Density (g.cm^{-3})
Polypropylene (PP)	0.85-0.92
High-density polyethylene (HDPE)	0.94-0.98
Polyvinyl chloride (PVC)	1.38-1.41
Polyethylene Terephthalate (PETE)	1.38-1.41
Polystyrene (PS)	1.01-1.06

Finally, to identify the type of plastic, gas chromatography technique was employed.[42-45] Previously separated samples were dissolved in 1 mL of HPLC-grade dichloromethane. Plastic identification was performed using reference plastics dissolved in dichloromethane, comparing the chromatographic profile obtained from these references with the problem samples. The samples were analyzed using an Agilent Technologies 7890A GC System equipped with an Agilent 7693 autosampler (G4513A, USA) and an Agilent Technologies 5975C mass detector (MSD, USA). Analytes were separated on a ZB-50 column (L=30 m ID=0.25 mm FT=0.25 µm), composed of 50% phenyl and 50% dimethylpolysiloxane. The oven temperature ramp started at 60ºC with a 12ºC/min increase until reaching 194ºC, held for 2 min,

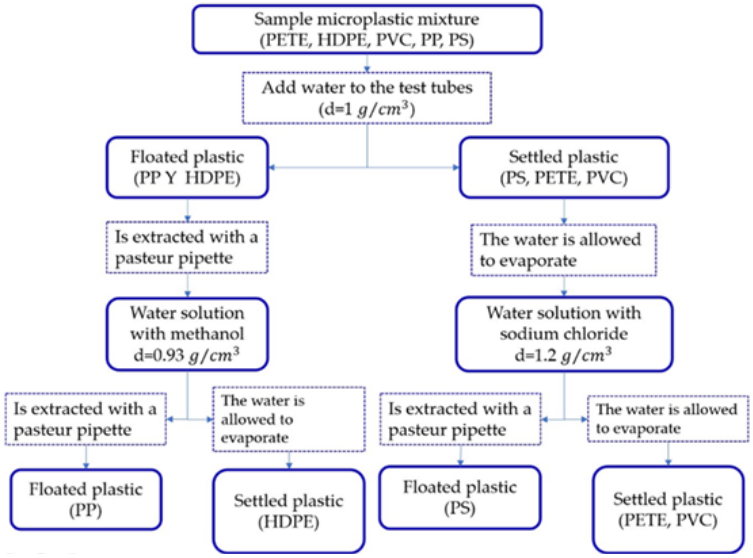


Figure 2: Separation diagram of MPs, by difference in densities (Own).

followed by a 10ºC/min increase to 295ºC and held for 4 min. The injection volume was 1 µL, helium was used as the carrier gas, and the instrument's response was verified using an auto tune according to internal procedures. Solvent blanks were run prior to analysis until an appropriate baseline was achieved. All materials used were glass.

Data analysis

The data from the microplastic pieces were discrete variables with a non-normal distribution. The Kruskal-Wallis non-parametric test was applied for analysis.[30] Subsequently, multiple comparison tests were conducted using the Dunn procedure. Both tests were performed at a significance level of a=0.05 using RStudio.

Measures to avoid cross contamination

Mainly glass and stainless steel materials were used, under a laminar flow hood,[46] and they were rinsed with distilled water before each use. Throughout the laboratory procedure, cotton lab coats were worn to prevent shedding of synthetic fibers. Working areas were cleaned with methanol, and air currents were minimized. Whole fish were used to ensure there was no external contamination; they were washed and dried before dissection. At all times, digestive systems and obtained filter papers were covered with aluminum foil. The same procedure was applied to quality control samples (solvent blanks) to demonstrate absence of external contamination.[37,47]

RESULTS

Species of fish obtained and number of microplastics detected

A total of 42 specimens were obtained, comprising 4 fish species: The flathead grey mullet (*Mugil cephalus*), eurasian carp (*Cyprinus carpio*), tilapia (*Oreochromis* spp.), and rainbow trout (*Oncorhynchus mykiss*), distributed across groups as shown in Table 2. The weight of the fish ranged from 239 to 800 g, and their length varied

Table 2: Number of species per sample group and microplastic pieces content.

Species	Figure	Quantity (no. of fish)	Weight (g)	Length (cm)
Group 1				
Flathead grey mullet (*Mugil cephalus*).		5	281-418	29.2-34
Group 2				
Rainbow trout (*Oncorhynchus mykiss*).		6	260-380	26.5-35
Group 3				
Flathead grey mullet (*Mugil cephalus*).		1	239	28.5
Eurasian carp (*Cyprinus carpio*).		11	301-518	26.5-34
Tilapia (*Oreochromis* spp).		10	264-384	24-26.5
Group 4				
Rainbow trout (*Oncorhynchus mykiss*).		9	430-800	36-46

between 24 cm and 46 cm. These measurements represent average values for the study samples, allowing estimation of fish size. Out of 42 individuals, microplastic pieces were detected in the digestive tract of 36 upon initial examination of their intestines, representing an 85.7% presence of plastic material detected without any specific treatment in the sampled fish.

Types of microplastic morphotypes

Once identification of plastic type and shape was completed using the stereoscopic microscope, a total of 218 pieces of Microplastics (MPs) were found. Of these, 16.1% were red, 46.3% black, 19.3% white, 3.2% green, 12.8% blue, and 2.3% pink.

Fish obtained from the sea may acquire these microplastics from the waters they inhabit, particularly in the Gulf of Mexico, known for its extensive commercial port activity, which leads to a significant presence of plastic materials in its ecosystem. Figure 3, Presents three types of microplastic shapes found.

The Sankey diagram in Figure 4 illustrates the relationship between the quantity, shape, and color of microplastic pieces found per sampling group.

The Sankey diagram illustrates the relationship between microplastic morphotypes and the number of microplastics found per fish group, with fibers being the predominant form and spheres and fragments in lesser proportions. It should be noted that plastic films were not detected.

To obtain the average number of microplastics per examined fish, we used the Mean Abundance (MA) and

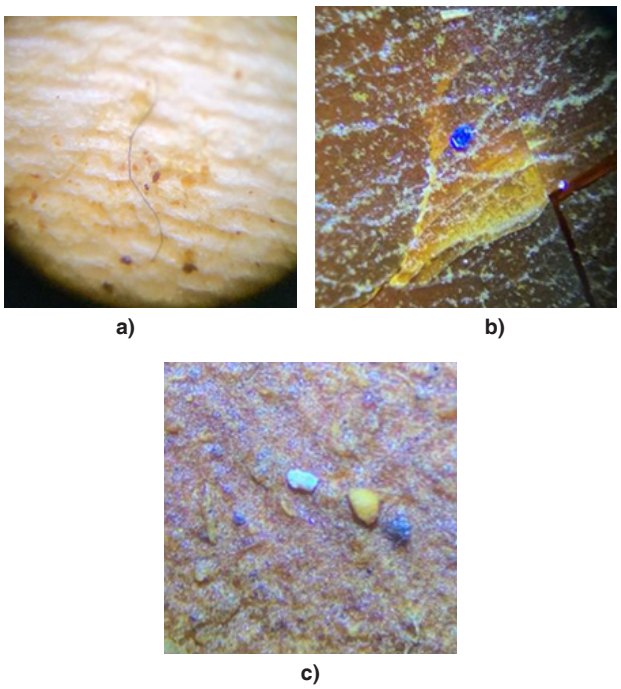


Figure 3: Shapes of microplastics found in the samples: a) Fiber, b) Fragment, c) Sphere at 40x magnification. (Own work)

Standard Error (SE). Results were expressed as MPs/individual, as shown in Table 3. The following equations were employed

$$MA = \frac{\text{Total of MPs}}{n} \quad (1)$$

$$SE = \frac{\sigma}{\sqrt{n}} \quad (2)$$

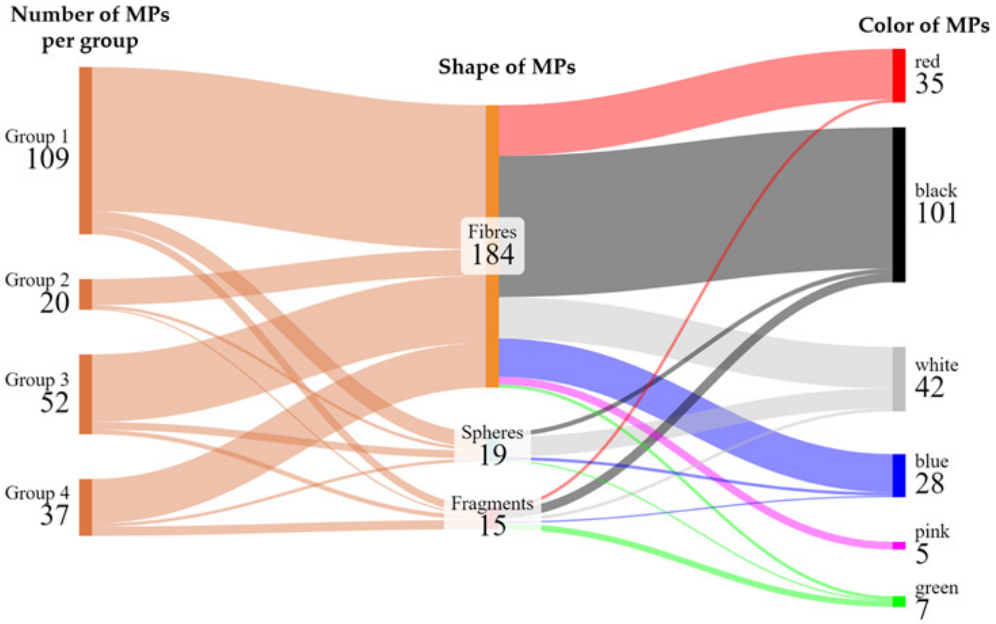


Figure 4: Sankey diagram of visual characterization data. (Own work)

Table 3: Mean abundance of microplastics per sampled fish group.

No. Group	Total of MPs/group	Mean abundance (± SE)[1]
1	109	21.8 (±3.5)
2	20	3.3 (±0.4)
3	52	2.4 (±0.7)
4	37	4.1 (±1.3)
TOTAL	218	5.2 (±1.1)

[1]Standard error.

Statistical treatment of MPs found Standard error

Kruskal-Wallis non-parametric test was used, with a significance level of α=0.05, using the following hypotheses:

Null hypothesis

The number of microplastics in fish is the same across all 4 fish groups.

Alternative hypothesis

The number of microplastics in fish differs at least for one of the groups.

It obtained a chi-square value of 16.115, with 3 degrees of freedom and a *p*pvalue of 0.001074. Since the *p*-value <α, the null hypothesis was rejected, indicating significant differences were found in at least one of the fish groups. Subsequently, using the Dunn's multiple comparisons test (1964),[31] it was found that groups 1 and 3 are different. Fish from these groups were obtained from fish and seafood markets within the state of Puebla, highlighting significant differences in microplastic presence between these groups. This can be observed in the box plot graph showing the microplastic content in Group 1 and Group 3 (Figure 5).

In the case of groups 2 and 4, their behavior shows homogeneous content with fewer than 10 microplastic pieces, suggesting lower exposure to microplastic contamination.

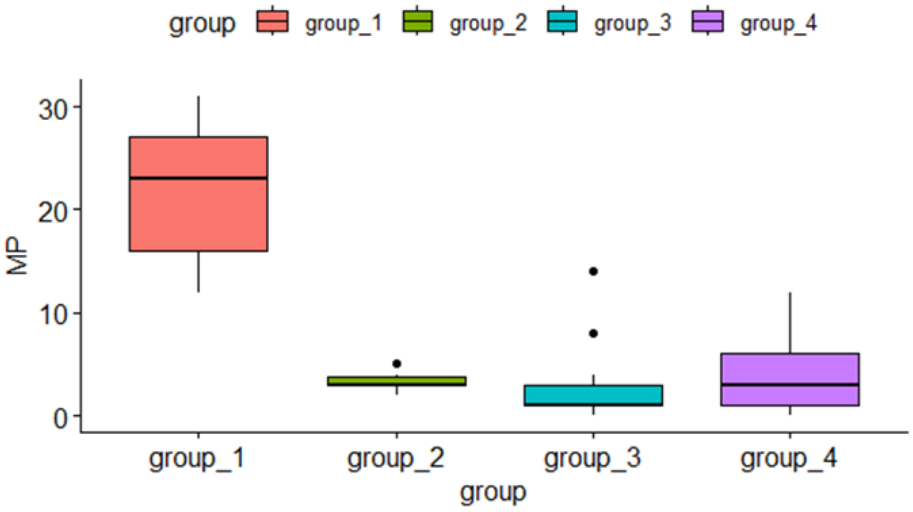


Figure 5: Box plot of the 4 fish groups. (Own work)

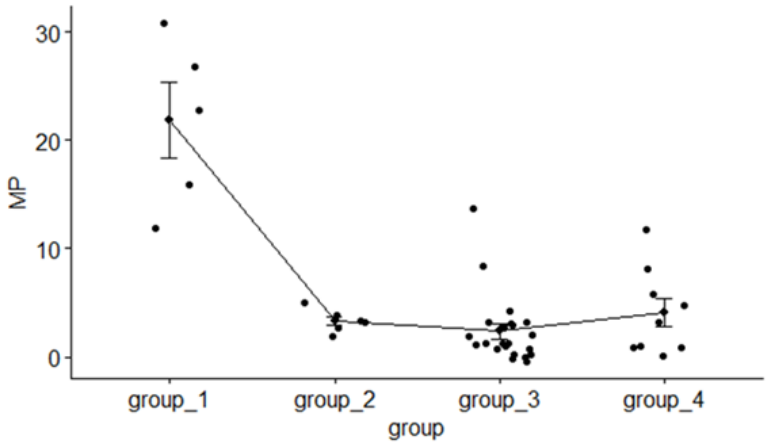


Figure 6: Trend line of the 4 fish groups. (Own work)

In Figure 6, fish from groups 1 and 3 showed greater dispersion in microplastic content. This is because in marine environments, plastic availability is higher, and fish may mistake colored plastic particles for food. This dependence varies depending on the depth from which the fish are caught and the level of pollution in their environment, indicating varied exposure to microplastics. In contrast, fish raised in farms have controlled diets with commercial pellets or are caught with hooks that are also carefully made.

Results of microplastic separation using the density difference method

Microplastics made of PETE, HDPE, PVC, PP, and PS showed varying behaviors in water, methanol solution, and sodium chloride solution, as detailed in Table 4. It is noteworthy that, according to[35] using 70% alcohol does not degrade the microplastics (MPs) and allows for obtaining solutions with densities similar to those reported for plastic materials.

Table 4: Behavior of generated microplastics.

Plastic type	1º Behavior with water (d=1)	2º Behavior with liquid of different density
PETE	The particles settled	The particles settled in a sodium chloride solution at 23%.
HDPE	The particles floated	The particles settled in a methanol solution at 70%.
PVC	The particles settled	The particles settled in a sodium chloride solution at 23%.
PP	The particles floated	The particles floated in a methanol solution at 70%.
PS	The particles settled	The particles floated in a sodium chloride solution at 23%.

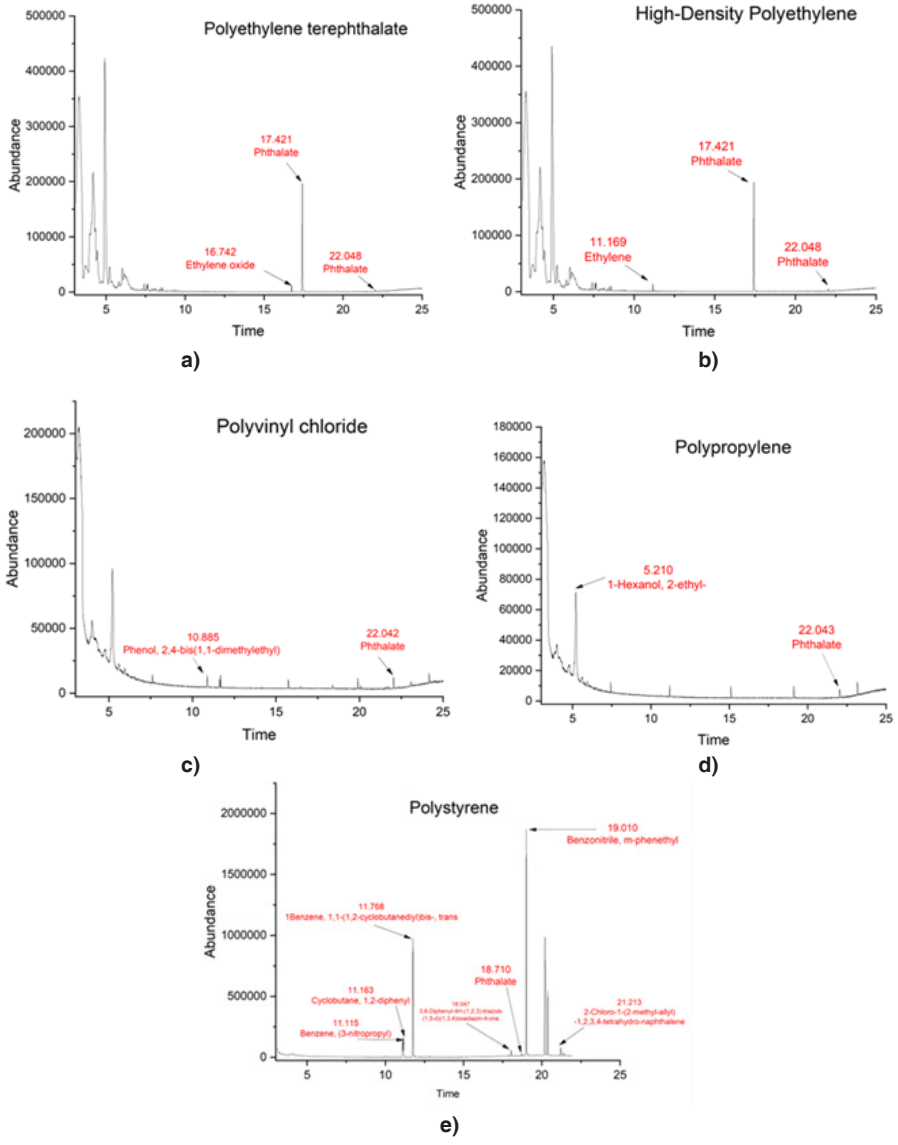


Figure 7: Chromatographic profile of: a) Polyethylene terephthalate; b) High density polyethylene; c) Polyvinyl chloride; d) Polypropylene; e) Polystyrene. (Own work)

The density separation aimed to identify microplastic particles from each type of plastic; however, even with the aid of an optical microscope, their recognition was not possible. This led us to identify the components found in each fraction using gas chromatography, which had the advantage of ensuring samples clean enough not to be masked by compounds inherent to the fish.

Results of microplastic identification by GC

In the gas chromatograph, profiles were conducted for Polyethylene Terephthalate (PETE), Polyvinyl Chloride (PVC), High-Density Polyethylene (HDPE), Polystyrene (PS), and Polypropylene (PP). Figure 7 displays the chromatograms of the reference polymers used for identifying the type of plastic in each sample. However, plasticizers were also found, which are additives used in many products to impart characteristics such as lubrication, color, flexibility, ductility, etc.,[5] these are also shown in Table 5.

Following this, the fractions of the samples were analyzed. The results were compared with the chemical compounds of reference plastics from Table 5, where some plastics such as PETE, PP, and plasticizing chemical compounds were classified. Additionally, new chemical compounds identified as additives were found in the samples, as shown in Figure 8 a) with 2,3,3-trimethylpentane and 1-pentanol, and in Figure 8 b) with acetophenone.

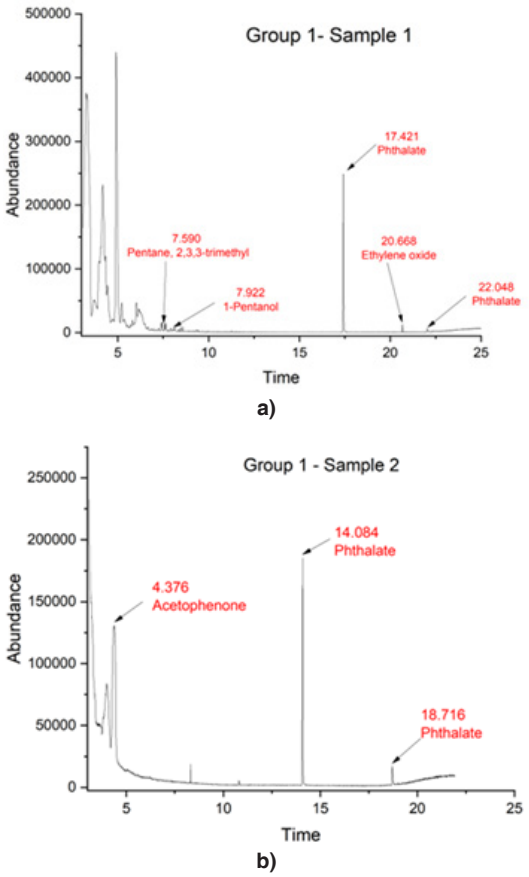


Figure 8: Example of chromatogram obtained from: a) sample 1; b) sample 2. (Own work)

Table 5: Chemical compounds of reference plastics.

Types of plastics	Chemical compounds indicating the type of plastic	Plasticizing chemical compounds	References
Polyethylene terephthalate (PETE)	- Ethylene oxide	- Butyl hexyl phthalate. -1,2-benzenedicarboxylic acid mono(2-ethylhexyl) ester.	[48]
High density polyethylene (HDPE)	- Polyethylene	- Butyl hexyl phthalate. -1,2-benzenedicarboxylic acid mono(2-ethylhexyl) ester.	[49]
Polyvinylchloride (PVC)	-2,4-bis(1,1-dimethylethyl) -Phenol	1,2-benzenedicarboxylic acid mono(2-ethylhexyl) ester.	[49]
Polypropylene (PP)	2-ethylhexan-1-ol	Phthalic acid, 2-hexyl ester.	[49]
Polystyrene (PS)	-3-nitropropyl benzene -1,2-diphenyl cyclobutane -1,2-difenil ciclobutano -3,6-diphenyl-4H-(1,2,3) triazolo(1,5-d)(1,3,4)oxadiazin-4-one - m-phenethyl Benzonitrile -2-chloro-1-(2-methyl-allyl)-1,2,3,4-tetrahydronaphthalene	Mono(2-ethylhexyl) ester of 1,2-benzenedicarboxylic acid.	[49]

Figure 9 shows the 4 groups and the percentages of the plastics identified in each of them, as well as the plasticizing chemical compounds (phthalic acid) and compounds identified as additives. In all groups there were PETE plastic pieces and only in group 3 there were PP.

DISCUSSION

Morphotypes and nature of microplastics found

Of the microplastic shapes observed, 85.8% were fibers, 6.4% were fragments, and 7.8% were spheres. The shape of secondary microplastics, determined by their

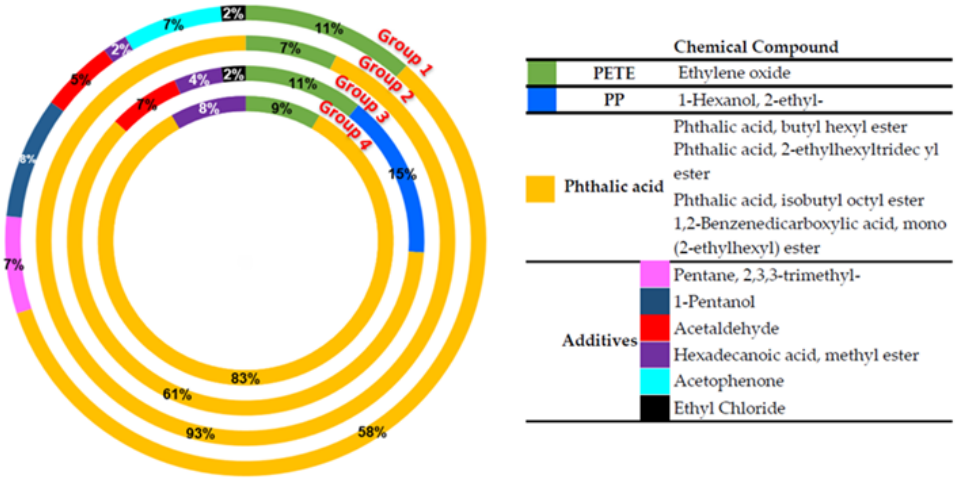


Figure 9: Graph, which represents the major chemical compounds in each group. (Own)

degradation or residence time in the environment, can be used to infer their origin and trajectory. For example, fibers may be linked to the increasing production of synthetic fibers used in clothing, carpets, and debris from larger pieces, as well as from fishing gear such as nets or fishing lines connected to hooks.[50]

There are reports suggesting classification based on color found;[51] however, in this study, we do not attribute color to a specific type of plastic because there is a wide variety of materials that could contribute color. This property of plastic could be important if fish were confused, mistaking it for food. Although white particles are typically associated with polyethylene, this information may be biased, so color should not be used to deduce the type of plastic.[50]

Besides microplastics, remnants of food, excess sand, fin residues, scales, small algae, and exoskeleton fragments were also observed.

This result is encouraging because the small amount obtained, undetectable by simple microscopy, implies that microplastics are present in lower concentrations than reported in other studies.[3,8,52]

The detected additive compounds may be present due to their association with plastics from their manufacturing, from wear and tear of plastic pieces, from the release of enzymes during fish digestion in the digestive tract, or potentially released during the digestion of organic matter found alongside other food remnants in the fish's digestive system.[53] These compounds are listed in Table 6.

Microplastics, invisible to the naked eye, are ubiquitous across all environments. They enter marine and freshwater animals through water.[54-56] Despite Puebla not having a coastline, it consumes both marine and freshwater fish.[57] This region hosts highly demanded fish farms, and to a lesser extent, the population consumes marine products originating from the Gulf of Mexico, specifically from the states of Veracruz, Tabasco, and Campeche, information provided by suppliers who directly transport fish from cooperatives to the distribution market.

Table 6: Chemical compounds identified as additives. (Own work)

Name	Use	Effects on human health	Reference
2,3,3-trimethylpentane	It is a hydrocarbon and isomer of octane that can be found in gasoline.	It causes lung damage and central nervous system depression.	[49]
1-Pentanol	Solvent (resins, petroleum additives, synthetic flavors), lubricant, plasticizer, corrosion inhibitor and antioxidant.	Nausea, dizziness, sore throat and headache.	[49]
Acetaldehyde	It is used in the production of vinyl acetate resins, perfumes, polyester resins, basic dyes and rubber solvent.	It is expected to be carcinogenic, based on studies in experimental animals.	[49]
Methyl palmitate	To manufacture detergents, emulsifiers, stabilizers, resins, plasticizers, and lubricants.	No harmful health effects have been reported.	[49]
Acetophenone	For fragrance of soaps, flavoring in foods and solvent for plastics and resins.	Effects on the central nervous system and impairment of reproductive function.	[49]
Ethyl chloride	Byproduct of vinyl chloride production.	Liver and kidney damage, decreased defensive responses against diseases	[49]

When evaluating freshwater and saltwater fishes, it was found that the total weight variation of the obtained fish species ranged from 239 g to 800 g. Normally, the flathead grey mullets (*Mugil cephalus*) can reach a maximum of 2 kg.[58] For rainbow trouts (*Oncorhynchus mykiss*), their weight ranges between 500 g and 6 kg,[59] euroasian carps (*Cyprinus carpio*) weigh approximately 500 g[57] and have a harvest weight ranging from 300 g to 500 g.[60] In summary, these values are in accordance with the reported standard averages, as for the size variation of the fishes, it ranged from 24 cm to 46 cm. Commonly, mullets can reach up to 45 centimeters in length,[58] rainbow trouts vary between 20 and 40 cm,[59] common carps have a minimum size of 18 cm,[57] and mojarras have a total length between 20 and 25 cm.[61] Similarly, all sizes of the studied fishes are considered within the standard parameters referenced in the literature.

A total of 42 individuals were studied, and at least one microplastic was detected in 36 fish, representing 85.7% of the total. However, there are significant differences compared to research in the central North Pacific, where approximately 35% of the studied fish ingested plastic.[39] In the Red Sea of Saudi Arabia, there was reported low presence of microplastics, with only 14.6% of fish containing microplastics.[62] In contrast, in Lima, Peru, 100% of the studied fish were contaminated with microplastics.[32] These findings suggest that aquatic ecosystems are contaminated with plastic materials that can be ingested by fish. The more fragmented these plastics are, the greater their availability to fauna. In more natural ecosystems such as oceans, there is an increased risk of ingestion of plastic and toxic material in the medium to long term.[63]

In this study, a total of 218 microplastics were found, with black being the predominant color at 46.3%. Similarly, in a study conducted on green turtles in Quintana Roo, 50% of solid plastic residues found in their esophagi were dark in color.[27] In comparison, in the Tyrrhenian Sea (Italy), the predominant color was blue,[63] the color prevalence may be related to the feeding strategies of each fish species and the confusion with their food that shares similar characteristics.[51,64,65]

On the other hand, the shapes of microplastics found in this study were of three types, with fibers being the most abundant at 85.8%. This could be related to the excessive production of synthetic textile fibers,[66] as well as the use, wear, and loss of physical devices used for the capture and collection of marine and freshwater organisms, commonly known as fishing gear.[7,67]

The total mean abundance was 5.2 (±1.1) microplastics per individual, which is similar to the average microplastic count found in marine organisms in Mexico, at 4.5 microplastics per individual.[68] However, there are discrepancies with the study conducted on 26 species from the Red Sea, where the value was 14.4 (±0.3) microplastics per individual,[62] as well as with research on fish in the central North Pacific Gyre, where 2.1 pieces of microplastics per fish were found.[39] The results vary widely between studies due to the diverse range of species and the impacts of various anthropogenic sources contributing different types of microplastics to their ecosystems.

Statistical data shows a significant difference between groups 1 and 3. Group 1 consists of fish obtained from the ocean, whereas group 3 comprises a mix of fish from rivers and others from the sea. This distinction underscores that all fish extraction points have varying concentrations of Micro Plastics (MPs). Reference,[69] explains that multiple factors contribute to the presence of MPs, such as sediment deposits in the ocean facilitated by thermohaline currents. These deposits can lead to higher concentrations of MPs, up to 190 pieces per gram, transported through vertical sedimentation.

The density difference method,[41] utilized in various studies,[40] is an economical and easily detectable technique for assessing high concentrations of Micro Plastics (MPs), making it a presumptive method to gauge MP contamination levels effectively. However, in cases where MPs are less conspicuous, this step might be omitted in favor of flotation techniques followed by CG/MS analysis to detect both MPs and associated additives. This research has shown that MPs are not the only concern; additives present in fish can adsorb into consumable parts and enter human bodies, posing serious health risks to consumers.

Gas chromatography detected in samples from Group 1: PETE plastic, phthalic acid, and 5 types of additives commonly used in polymers to modify color, improve mechanical properties, impart heat resistance, enhance performance, provide flexibility, among other characteristics.[43] Group 2 samples showed PETE and phthalic acid only, suggesting plastic contamination through fishing equipment, dietary habits[70] or atmospheric transport of PETE microplastics[71] Group 3 presented PETE and PP plastics, phthalic acid, and 3 additives: acetaldehyde, methyl palmitate, and ethyl chloride. Lastly, Group 4 exhibited PETE, phthalic acid, and a single additive, methyl palmitate.

Once again, groups 1 and 3 stand out due to their higher quantity of additives, indicating that the microplastics originated from various anthropogenic sources. These compounds are capable of exerting harmful effects on health, such as disrupting the endocrine system[20,72] The quantities of additives added to plastics can vary

significantly, comprising up to 70% of the product.[20] The presence of 5 types of additives in the samples suggests that the fish meat was exposed to these compounds, known for their endocrine-disrupting properties, as these chemicals can leach into the environment.

Freshwater fish and aquaculture farm fish are generally less exposed to plastic particles, making them safer to consume compared to ocean fish. However, even a single piece of microplastic (MP) can contaminate fish meat with additives, plasticizers, or harmful bacteria, which could potentially affect the health of consumers.

CONCLUSION

This study confirms a pathway of Micro Plastic (MP) exposure through fish consumption, even for people living far from the ocean. Despite aquaculture fish being subject to more controlled production conditions, pathways still exist for MPs to enter their ecosystems.[73] According to the data obtained, all ecosystems from which the fish were extracted contain microplastics. However, fish from controlled habitats, such as freshwater and aquaculture farms have a lower probability of exposure, suggesting they may be safer than ocean-caught fish.

Although the digestive system is not typically cooked or consumed along with fish meat, there is MP magnification in the trophic chain among fish of different sizes. When consumed by the final consumer, MPs can be present due to inadequate cleaning of the fish or through absorption of additives during digestion, eventually reaching the consumable parts for humans. Therefore, future research should consider studying digestive tracts alongside fish meat and include a broader range of fish species in investigations.

In conclusion, we agree with authors like[10] who propose that to minimize this impact, it is essential not only to improve waste management and increase recycling but also to reduce the sources of plastic production. This would help attenuate the effects of plastics and their leachates on the environment and health through the ingestion of contaminated fish, which are equally harmful. These findings can support the generation of public policies aimed at controlling the effects of what has been termed the "Plasticene Age".

AUTHOR CONTRIBUTIONS

Conceptualization, M.D.C.A. and G.P.P.; methodology, MDCA, G.P.P.; software, R.P.R; Validation, J.M.R. and A.B.R. formal analysis, M.D.C.A., J.D.S.J. and G.P.P.; investigation, M.D.C.A, and G.P.P.; resources, MDCA and IRPR; data curation, M.E.R.G.; writing-original draft preparation, A.R. and G.P.P.; writing-review and editing, M.D.C.A.; visualization, G.P.P.; supervision, ABR; project administration, M.D.C.A.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Measures were taken to ensure participants' safety and privacy, with the full consent was obtained from participants, who had the freedom to decline participation with full respect.

CONFLICT OF INTEREST

The authors declare no Conflict of interest.

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