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DOI: 10.15343/0104-7809.202448e15762023I

Identification of microplastic polymers found in the digestive tract of fish from Lake Amatitlán, Guatemala

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Abstract

The objective of this research was the identification, through Fourier transform infrared spectroscopy combined with attenuated total reflectance (FTIR-ATR), of microplastics extracted from the gastrointestinal tract of fish collected in Lake Amatitlán, to determine the main polymers and the possible origin of contamination by these materials. 68 microplastics were analyzed, corresponding to 10% of the total microplastics extracted. These originated from 36 specimens, 35 of the species *Oreochromis niloticus* and one of *Parachromis managuensis*, of which 1 to 5 microplastics per specimen were analyzed. The polymers identified were polypropylene (PP), nylon, high-density polyethylene (HDPE), polyethylene terephthalate (PET), latex, polystyrene (PE), low-density polyethylene (LDPE) and polyurethane (PU). Polypropylene (32), high-density polyethylene (13) and nylon (10) were the most frequent polymers. Possible plastic items that may have caused microplastics include toys, laboratory equipment, buckets, food packaging, pipes, insulation for cables, textiles, ropes and furniture that are produced in the plastics industry, located mainly on the south side of Guatemala City. Furthermore, the fish species in the present study support important fisheries, which raises human health concerns, since the ingestion of fish that consume plastics has the potential to increase the body load of dangerous chemicals, as they adhere superficially to the plastics in the environment and are subsequently bioaccumulated.

Keywords: FTIR-ATR. Lake Amatitlán. Microplastics. Fish. Polymers.

INTRODUCTION

Lake Amatitlán is the fourth largest body of fresh water in Guatemala (15.2 km^2), being one of the most important water resources in the country, as it is used for recreation mainly local tourism, for irrigating agricultural crops in the basin, for transportation and for fishing, and for water sports, among other economic and social activities. Located in the Department of Guatemala, 27 km south of Guatemala City, its importance is accentuated by the large surrounding population, covering the municipalities of Villa Nueva, San Miguel Petapa, Villa Canales and Amatitlán[1,2] which total more than two million inhabitants.

The aforementioned lake is in an eutrophic state and highly contaminated by various pollutants, such as heavy metals[3] and cyanotoxins[4]. Furthermore, it stores a large amount of solid waste. It is estimated that annually, around 48,350 m^3 of floating solid waste are

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removed from this lake. In this class of waste, there have been few studies on microplastics, both in quantitative and qualitative aspects. The high concentrations of organic matter, as well as nutrients that come from wastewater discharges, have favored the ecological imbalance of the lake and the trophic chain, increasing the populations of phytoplankton and aquatic plants, which has led to its eutrophication. This overpopulation of microalgae and macrophytes in the lake affects the balance of its ecosystem, causing damage to hydrobiological resources and the people who benefit from them[3].

There are approximately 900 industries in the basin, including textiles, agrochemicals, chemicals, electroplating, metallurgical, food, tanning, soap, cosmetics and ceramics, among others, distributed mainly in the south of Guatemala City and in the municipality of Villa Nueva. However, treatment systems for industrial wastewater and hazardous waste from different production processes (chromium VI, arsenic, lead, cyanide, etc.) are scarce, meaning that all of these reach the lake[2]. Therefore, Lake Amatitlán is highly contaminated with pollutants such as heavy metals, as arsenic and mercury were recently found in fish muscles from the lake[3]. Similarly, quantifiable levels of cyanotoxins such as microcystins have been reported[4].

Microplastics, plastic particles measuring 5 mm or less, were discovered in the 1970s in North America and have been found in bodies of water such as oceans, seas, lakes and rivers[5,6]. There are two types of microplastics: primary ones (manufactured with a size equal to or less than 5 mm) and secondary ones derived from chemical degradation (oxidation), physical degradation (heat, UV light, mechanical action) and/or microbial degradation of large plastic objects[7].

Once microplastics enter ecosystems, the main risks associated with them are their bioavailability in aquatic organisms[8]. In 2019, 295 microplastics were reported in sediment cores from Lake Amatitlán, identified as lines, fragments and films[9]. Oliva-Hernández *et al.*[10] have found microplastics in the digestive tract of 97% of fish collected in Lake Amatitlán, from which microplastic samples were analyzed by FTIR-ATR in the present study. The FTIR-ATR technique allows the identification of the functional groups present in a molecule through the wavelengths at which they absorb infrared radiation, providing a characteristic spectrum for each polymer[11].

The presence of microplastics has been reported in different bodies of water. In a study on microplastics, carried out in Monterrico, Guatemala, 644 microplastics were found in 295 fish belonging to 15 species. The particles found were classified as fibers, fragments and films[12]. In the Amazon River estuary, Brazil, 228 microplastics were found in 26 fish from 14 species, the main polymers being identified by Fourier transform infrared spectroscopy combined with attenuated total reflectance (FTIR-ATR) were polyamide, rayon and polyethylene[13]. In the Gulf of Mexico, 8% of freshwater fish and 10% of estuary fish were reported to have ingested microplastics, with 535 specimens classified as fibers, fragments and films[14]. On an urban beach in Brazil, it was found that 55% of 214 fish ingested microplastics, mainly polyester fibers[15]. In the central Pacific of Costa Rica, 100% of the filter-feeding fish analyzed (30 individuals) contained microplastics (on average, 36.7 pieces), mainly fibers[16]. Microplastics have also been found in the stomachs of commercial crustaceans in Chile, with a frequency of occurrence of 27%[17].

The ingestion of microplastics is harmful to aquatic biodiversity. In organisms, microplastics can damage cells, obstruct intestines, affect gills, cause neurotoxic damage, reduce immunity and affect fish reproduction[7,18]. Furthermore, they can transport invasive species and adsorb contaminants such as pesticides and heavy metals, increasing their toxicity[19,20], and the possibility of being bioaccumulated

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and biomagnified, affecting species populations at different levels of the trophic network. Therefore, in addition to the consequences for the environment of Lake Amatitlán, these effects extend to living beings that consume contaminated organisms, including humans.

Since there is an information gap regarding microplastic contamination in Lake Amatitlán, it is important to identify and understand the possible sources of these contaminants in the lake, as understanding the type and abundance of microplastics in the ecosystem will help in efforts to identify and mitigate the sources of plastic contamination in aquatic life. Studies on contamination given the critical importance of Lake Amatitlán as a vital water resource in Guatemala and the significant challenges related to its contamination by various pollutants, such as the presence of microplastics, emerge as an additional and little explored concern. This study aims to fill this knowledge gap by identifying and quantifying microplastics in Lake Amatitlán, with special attention to the polymers involved. Understanding the extent of microplastic contamination in this ecosystem will provide essential information for the preservation of aquatic life and contribute to effective plastic pollution mitigation strategies in the lake.

MATERIALS AND METHODS

Fish collection

The fish were collected with the help of artisanal fishermen, who caught them in various locations on Lake Amatitlán, using a fishing net. Collections were carried out between 5 am and 10 am in the morning, selecting specimens with more than 15 cm in total length. Sampling was carried out in the months of October and December 2020 and January 2021, obtaining a total of 70 individuals, 65 of which were of the species *Oreochromis niloticus* (tilapia) and five individuals of the species *Parachromis managuensis* (guapote). The fish were transported to the laboratory, where they were stored frozen for later processing. The biometric measurements of the analyzed fish are shown in Table 1A of Supplementary Material.

Extraction and purification of microplastics from the digestive tract of fish

The digestive tract was weighed, dissected and transferred to a 250 ml glass beaker. To decompose the organic matter, 25 ml of a 1 mol l^{-1} NaOH solution was added and heated at 50°C for 8 hours. If organic matter remained after this procedure, another 10 ml of NaOH solution was added in different successive portions until the sample was completely dissolved. Samples were mixed with a glass stirring rod to facilitate alkaline digestion. Each sample was diluted with 125 ml of ultrapure water and vacuum filtered through a *Whatman* brand glass fiber filter, 47 mm in diameter and 45 µm in porosity[21].

Selection of microplastics

The selection of analyzed microplastics was made based on their size, using as criteria the potential of the instrumental technique used (FTIR-ATR), which for reading, it is necessary to cover the total attenuation reflection crystal (ATR crystal) of 1 mm. Therefore, only 68 microplastics larger than this limit were analyzed, corresponding to 10% of the total microplastics found in fish collected in Lake Amatitlán. These microplastics originated from 36 specimens, 35 of which were from the species *Oreochromis niloticus* and one from the species *Parachromis managuensis*.

Microplastic identification

Microplastics were analyzed using FTIR-ATR equipment, brand *PerkinElmer Precisely* and model *Spectrum One FTIR Spectrometer*

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with the universal accessory ATR (*PerkinElmer*, Inc., United States of America). The microplastics were manipulated with stainless steel precision tweezers and placed on the ATR crystal for reading. The spectra were obtained in percentage transmittance, with a spectral range of 4,000 to 600 cm^{-1} through 4 scans. Each analytical cycle lasted 2 minutes. All results were properly stored on a flashdrive. *PerkinElmer Spectrum IR* Version 10.6.1 software and the *PerkinElmer* polymer library were used. For spectral analysis, the composition of each microplastic was identified based on work carried out by Jung *et al.*[22] and Li *et al.*[23]. The analyzed microplastics were photographed with a *Steren* 1000x USB digital microscope (*Eletrónica Steren*, S.A. de C.V., México).

RESULTS

Microplastics were found in 97% of the 70 fish collected. 68 microplastics were analyzed, corresponding to 10% of the total microplastics extracted. These microplastics originated from 36 specimens, 35 of which were from the species *Oreochromis niloticus* and one from the species *Parachromis managuensis*, of which 1 to 5 microplastics per fish were analyzed.

In Table 1, the composition of each microplastic analyzed by FTIR-ATR is presented; the identification was carried out based on the main absorption bands, which together are specific to each type of polymer, and which also best corresponded with the existing files in the polymer library of the *PerkinElmer* spectrometer. Oliva-Hernández *et al.*[10] have classified morphologically the microplastics found in the fish under study, by evaluating the shape of isolated particles larger than 1 mm seen under the microscope[10] and their distribution among the five main categories used by researchers[24]; this information is presented in Table 1.

Table 1 - Identification of microplastic polymers found in the digestive tract of fish from Lake Amatitlán using infrared spectroscopy (FTIR-ATR). Guatemala City – Guatemala, 2023.

Fish number	Microplastic Morphology[1]	Absorption bands (cm^{-1})	Identification of polymer type[2]
1	Line/Fiber	3290, 2940, 2860, 1640, 1540, 680	Nylon
1	Fragment	2955, 2910, 2830, 1460, 1380, 1170	PP
1	Fragment	2955, 2910, 2840, 1460, 1380, 1160	PP
3	Line/Fiber	3298, 2935, 2870, 1640, 1540, 690	Nylon
3	Line/Fiber	3298, 2925, 2855, 1630, 1540, 1460	Nylon
3	Film	2915, 2845, 1470, 1460, 730, 720	HDPE
3	Fragment	2950, 2910, 2830, 1460, 1380, 1170	PP
3	Fragment	2915, 2845, 1475, 1465, 730, 720	HDPE
4	Line/Fiber	2950, 2915, 2840, 1460, 1380, 1170	PP
5	Foam	2915, 2845, 1470, 1460, 730, 720	HDPE

to be continued...

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... continuation Table 1

Fish number	Microplastic Morphology[1]	Absorption bands (cm^{-1})	Identification of polymer type[2]
5	Line/Fiber	2950, 2915, 2835, 1460, 1380, 970	PP
5	Fragment	2950, 2915, 2835, 1460, 1380, 970	PP
6	Film	2910, 2845, 1470, 1460, 730, 720	HDPE
6	Fragment	2950, 2910, 2835, 1455, 1380, 1170	PP
8	Fragment	2950, 2915, 2835, 1455, 1380, 1170	PP
9	Fragment	2910, 2845, 1475, 1475, 1465, 730	HDPE
9	Fragment	2960, 2915, 2855, 1670, 1450, 1380	Latex
10	Film	2915, 2845, 1470, 1460, 730, 720	HDPE
10	Film	2915, 2850, 1470, 1460, 730, 715	HDPE
10	Film	2915, 2845, 1470, 1460, 730, 720	HDPE
10	Film	2915, 2845, 1475, 1465, 735, 720	HDPE
11	Fragment	2950, 2910, 2840, 1460, 1380, 1170	PP
13	Fragment	2915, 2850, 1465, 1460, 1375, 715	LDPE
14	Line/Fiber	1710, 1240, 1100, 720	PET
14	Line/Fiber	2950, 2915, 2835, 1455, 1380, 1165	PP
17	Fragment	2950, 2915, 2835, 1455, 1380, 805	PP
19	Fragment	2950, 2910, 2835, 1455, 1375, 1170	PP
19	Fragment	2950, 2915, 2835, 1460, 1380, 1170	PP
22	Line/Fiber	2950, 2915, 2840, 1460, 1380, 1170	PP
22	Fragment	2950, 2915, 2835, 1460, 1370, 1170	PP
22	Fragment	2950, 2915, 2835, 1460, 1370, 1170	PP
22	Fragment	2915, 2845, 1470, 1460, 730, 720	HDPE
23	Line/Fiber	3298, 2930, 2860, 1630, 1540, 1470	Nylon
26	Line/Fiber	1715, 1240, 1100, 730	PET
30	Film	2915, 2845, 1470, 1460, 730, 715	HDPE
31	Line/Fiber	3298, 2930, 2860, 1635, 1540, 1465	Nylon
31	Fragment	2950, 2915, 2840, 1460, 1380, 1165	PP
32	Fragment	2950, 2915, 2835, 1460, 1380, 1170	PP
32	Fragment	2950, 2910, 2835, 1455, 1375, 1170	PP
34	Foam	2870, 1730, 1535, 1460, 1220	PU
34	Line/Fiber	1715, 1250, 1105, 730	PET

to be continued...

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... continuation Table 1

Fish number	Microplastic Morphology[1]	Absorption bands (cm^{-1})	Identification of polymer type[2]
34	Fragment	2965, 2915, 2855, 1670, 1445, 1380	Latex
39	Line/Fiber	3300, 2930, 2865, 1635, 1540, 1465	Nylon
40	Fragment	2950, 2915, 2835, 1460, 1380, 1170	PP
40	Fragment	2955, 2910, 2830, 1455, 1380, 1170	PP
41	Line/Fiber	1715, 1250, 1100, 720	PET
43	Line/Fiber	2950, 2915, 2835, 1460, 1380, 1170	PP
45*	Fragment	2950, 2915, 2835, 1460, 1375, 1175	PP
46	Line/Fiber	3299, 2830, 2860, 1635, 1535, 1465	Nylon
47	Fragment	2970, 2910, 2850, 1660, 1450, 1380	Latex
49	Fragment	2950, 2915, 2835, 1460, 1380, 1160	PP
51	Fragment	3025, 2845, 1600, 1495, 1455, 1030	PS
52	Film	2950, 2915, 2840, 1460, 1380, 1170	PP
53	Film	2915, 2845, 1475, 1465, 730, 715	HDPE
53	Film	2915, 2850, 1475, 1465, 730, 715	HDPE
53	Fragment	3020, 2850, 1600, 1495, 1450, 695	PS
54	Film	2950, 2915, 2835, 1455, 1375, 1165	PP
54	Film	2950, 2915, 2835, 1460, 1380, 1170	PP
59	Line/Fiber	3300, 2920, 2860, 1640, 1540, 1470	Nylon
59	Fragment	2950, 2915, 2840, 1455, 1385, 1170	PP
59	Fragment	2950, 2910, 2835, 1455, 1375, 1170	PP
60	Line/Fiber	3300, 2930, 2860, 1630, 1540, 1470	Nylon
60	Line/Fiber	2950, 2915, 2840, 1455, 1370, 1170	PP
60	Film	2955, 2910, 2840, 1460, 1380, 1170	PP
65	Line/Fiber	2960, 2910, 2840, 1455, 1380, 1170	PP
65	Line/Fiber	3295, 2930, 2860, 1630, 1540, 1460	Nylon
66	Fragment	2910, 2845, 1470, 1460, 1370, 730	LDPE
66	Fragment	3030, 1600, 1495, 1450, 1030, 695	PS

[1]From "Microplastics in Nile tilapia (*Oreochromis niloticus*) from Lake Amatitlán", by B.E. Oliva, F.M. Santos-Ruiz, M.A. Muñoz-Wug y J.F. Pérez-Sabino, 2021, Revista Ambiente & Água, 16 (5), (https://doi.org/10.4136/ambi-agua.2754).
[2]HDPE= High density polyethylene; LDPE= Low density polyethylene or linear LDPE; PET= Polyethylene terephthalate; PP= Polypropylene; PU= Polyurethane.

*Fish No. 45 is the only one of species *Parachromis managuensis*.

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Of the 68 microplastics analyzed, 21 were morphologically classified as lines, 32 as fragments, 13 as films and 2 as foams. In fish number 3, the maximum amount of microplastics was analyzed, identifying 2 microplastics as nylon, 2 as high-density polyethylene (HDPE) and 1 as polypropylene (PP). This individual, in its entirety, had 9 fibers, 1 fragment and 1 film. In Table 2, it is possible to visualize the distribution of microplastics by type of polymer and morphology. Therefore, there was a greater identification of nylon for lines, polypropylene (PP) for Fragments, high-density polyethylene (HDPE) for Films and the only presence of polyurethane (PU) for foams.

Table 2 - Distribution by type of polymer and morphology of microplastics smaller than 1 mm found in the digestive tract of fish from Lake Amatitlán Guatemala, 2020-2021.

	HDPE	Latex	LDPE	Nylon	PET	PP	PS	PU	Total
Lines/Fibers	-	-	-	10	4	7	-	-	21
Fragments	3	3	2	-	-	21	3	-	32
Film	9	-	-	-	-	4	-	-	13
Foam	1	-	-	-	-	-	-	1	2
Total[1]	13	3	2	10	4	32	3	1	68

[1]Polymer types: HDPE= High density polyethylene; LDPE= Low density polyethylene or linear LDPE; PET= Polyethylene terephthalate; PP= Polypropylene; PU= Polyurethane.

Using the FTIR-ATR spectroscopy technique, the polymers that make up the microplastics found in the digestive tract of the fish under study were identified. Of the total microplastics found in fish collected in Lake Amatitlán, only 10% could be analyzed due to the fact that the FTIR-ATR equipment can only analyze microplastics larger than 1 mm. This limitation did not allow the majority of microplastics found in the fish analyzed to be identified, meaning it was not possible to determine the complete composition of the polymers in the smaller particles, which may have greater toxicity due to their greater surface area.

Characteristic spectra of microplastics from each polymer found

Figures 1 to 8 present photographs and infrared spectra of microplastics representative of the study sample, with a description of the characteristic bands of the functional groups of the identified polymers.

High-density polyethylene (HDPE) was identified in 13 microplastics, mainly of the film type, due to the presence of absorption bands at 2915 and 2845 cm^{-1}, which correspond to the stretching of the C-H bond, at 1472 and 1462 cm^{-1}, which correspond to the bending of CH_2, and at 730 and 717 cm^{-1}, which correspond to asymmetric bending in the plane of CH_2. It is worth mentioning that the predominant morphological classification for HDPE was film. In Figure 1, the IR spectrum of a microplastic identified as HDPE is presented.

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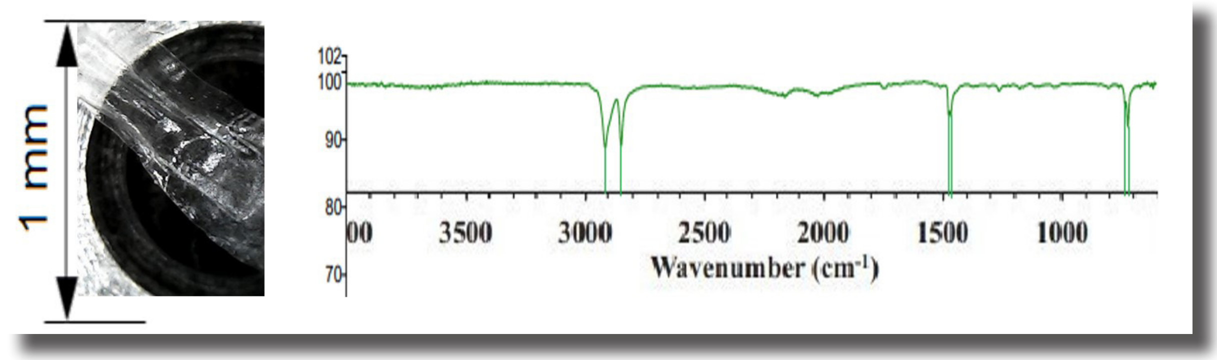

Figure 1 - Photograph (left) and IR spectrum (right) of a film-like microplastic found in fish No. 3 (*Oreochromis niloticus*, *Linnaeus*, 1758 - female tilapia) identified as high-density polyethylene (HDPE). Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

Latex was identified in 3 microplastics classified as fragment, due to the presence of absorption bands at 2960, 2920 and 2855 cm^{-1}, which correspond to the stretching of the C-H bond, at 1667 cm^{-1}, which correspond to the stretching of the C-C double bond, at 1447 cm^{-1}, which corresponds to the bending of CH_2, and at 1376 cm^{-1}, which represents the bending of CH_3. In Figure 2, the IR spectrum of a microplastic identified as latex is shown.

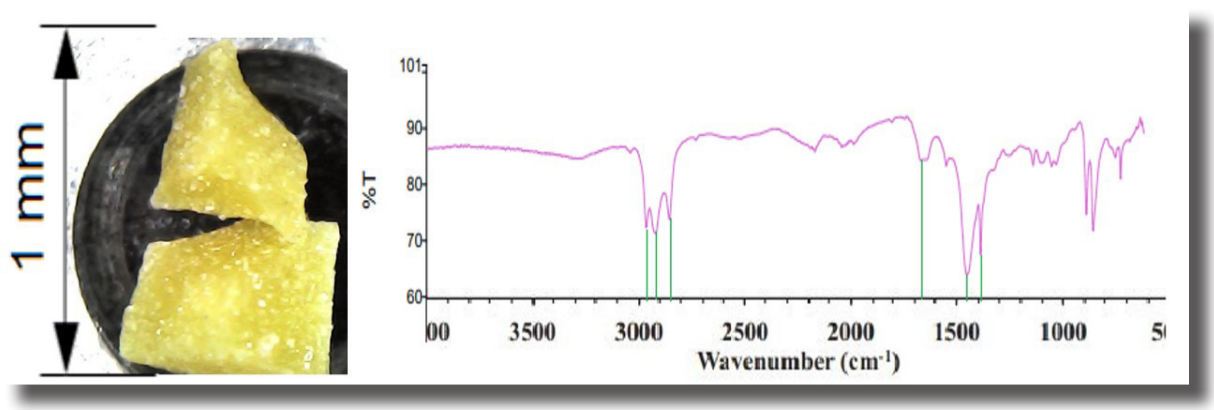

Figure 2 - Photograph (left) and IR spectrum (right) of a fragment-like microplastic found in fish No. 9 (*Oreochromis niloticus*, *Linnaeus*, 1758 – female tilapia) classified as latex. Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

Low-density polyethylene (LDPE) was identified in 2 microplastics classified as fragment, due to the presence of absorption bands at 2915 and 2845cm^{-1}, which correspond to the stretching of the C-H bond, at 1467 and 1462 cm^{-1}, which correspond to the bending of CH_2, at 1377 cm^{-1}, which represents the bending of CH_3, and at 730 and 717 cm^{-1}, which correspond to asymmetric bending in the plane of CH_2. In Figure 3, the IR spectrum of a microplastic identified as LDPE is shown.

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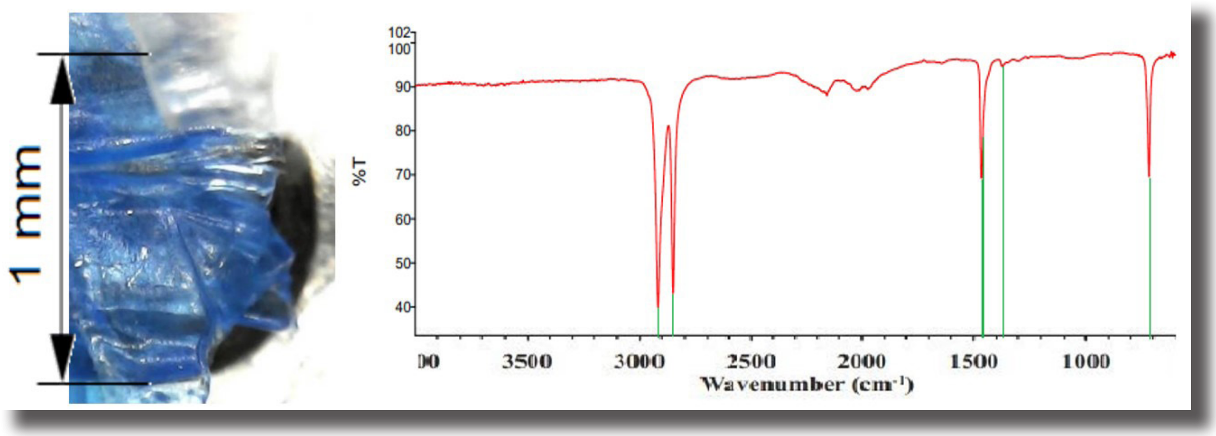

Figure 3 - Photograph (left) and IR spectrum (right) of a fragment-type microplastic found in fish No. 13 (*Oreochromis niloticus*, *Linnaeus*, 1758 – female tilapia) classified as low density polyethylene (LDPE). Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

Nylon was identified in 10 microplastics classified as lines, due to the presence of absorption bands at 3298 cm^{-1}, which corresponds to the stretching of the N-H bond; at 2932 and 2858 cm^{-1}, which correspond to the carbon-hydrogen stretching; at 1634 cm^{-1}, which corresponds to the stretching of the double C-O link; at 1538 and 1274 cm^{-1}, which correspond to the bending of NH and the stretching of the C-N bond; at 1464, 1372 and 1199 cm^{-1}, which correspond to the bending of CH_2, and at 687 cm^{-1}, which corresponds to the bending of the NH and the bending of the C-O double bond. In Figure 4, the IR spectrum of a microplastic identified as nylon is shown.

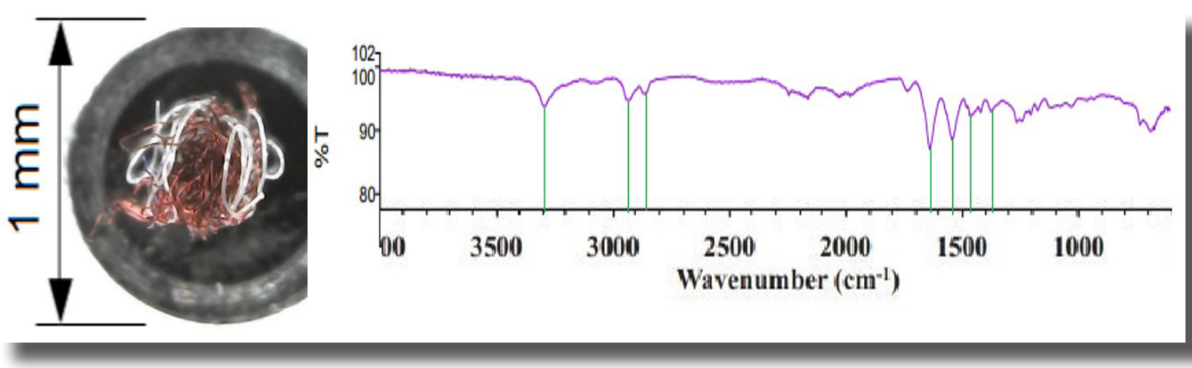

Figure 4 - Photograph (left) and IR spectrum (right) of a fiber-like microplastic found in fish No. 23 (*Oreochromis niloticus*, *Linnaeus*, 1758 – female tilapia) classified as nylon. Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

Polyethylene terephthalate (PET) was identified in 4 microplastics classified as lines, due to the presence of absorption bands in 1713 cm^{-1}, which corresponds to the stretching vibration of the carbonyl group; at 1241 and 1094 cm^{-1}, correspond to the vibration of the C-O bond; and at 720 cm^{-1}, which corresponds to out-of-plane bending of the aromatic C-H bond. In Figure 5, the IR spectrum of a microplastic identified as PET is shown.

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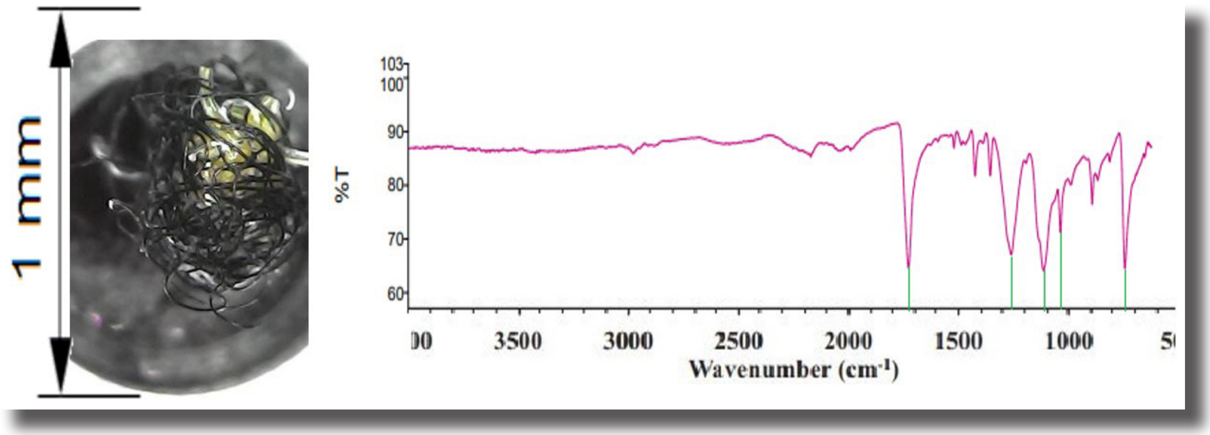

Figure 5 - Photograph (left) and IR spectrum (right) of a fiber-like microplastic found in fish No. 34 (*Oreochromis niloticus*, *Linnaeus*, 1758 - female tilapia) classified as polyethylene terephthalate (PET). Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

Polypropylene (PP) was identified in 32 microplastics, mainly of the fragment type, due to the presence of absorption bands at 2950, 2915 and 2838 cm^{-1}, which correspond to the stretching of the C-H bond; at 1455 cm^{-1}, corresponding to the bending of CH_2; em 1377 cm^{-1}, corresponding to the bending of CH_3; at 1166 cm^{-1}, corresponding to the bending of CH, as well as asymmetric in-plane bending and stretching of the C-C bond; at 997 cm^{-1}, which corresponds to asymmetric bending in the plane of CH_3, the bending of CH_3 and the bending of CH; at 972 cm^{-1}, corresponding to the asymmetric in-plane bending of CH_3 and the stretching of the C-C bond; at 840 cm^{-1}, corresponding to the asymmetric bending in the plane of CH_2 and the stretching C-CH_3; and at 808 cm^{-1} which corresponds to the asymmetric bending in the plane of CH_2, the C-C stretching and the C-CH stretching. In Figure 6, the IR spectrum of a microplastic identified as PP is shown.

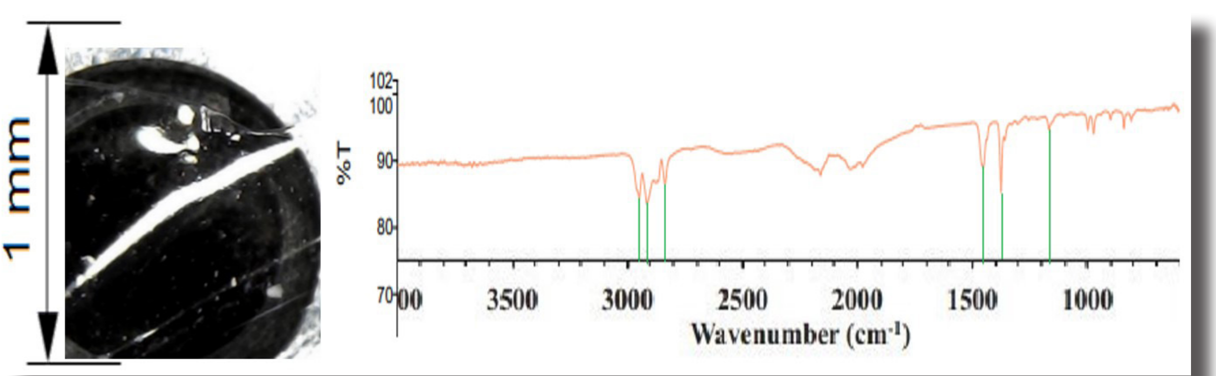

Figure 6 - Photograph (left) and IR spectrum (right) of a fragment-type microplastic found in fish No. 45 (*Parachromis managuensis*, *Günther*, 1867 - male guapote) classified as polypropylene (PP). Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

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Polystyrene (PS) was identified in 3 microplastics of the fragment type, by the presence of absorption bands at 3024 cm^{-1}, which corresponds to the stretching of the aromatic C-H bond; at 2847 cm^{-1}, representing the stretching of the C-H bond; at 1601 and 1492 cm^{-1}, corresponding to the stretching of the aromatic ring; at 1451 cm^{-1}, corresponding to the bending of CH_2; at 1027 cm^{-1}, which corresponds to the bending of aromatic CH; at 694 cm^{-1}, corresponding to out-of-plane bending of aromatic CH; and at 537 cm^{-1}, which corresponds to out-of-plane bending of the aromatic ring. In Figure 7, the IR spectrum of a microplastic identified as PS is shown.

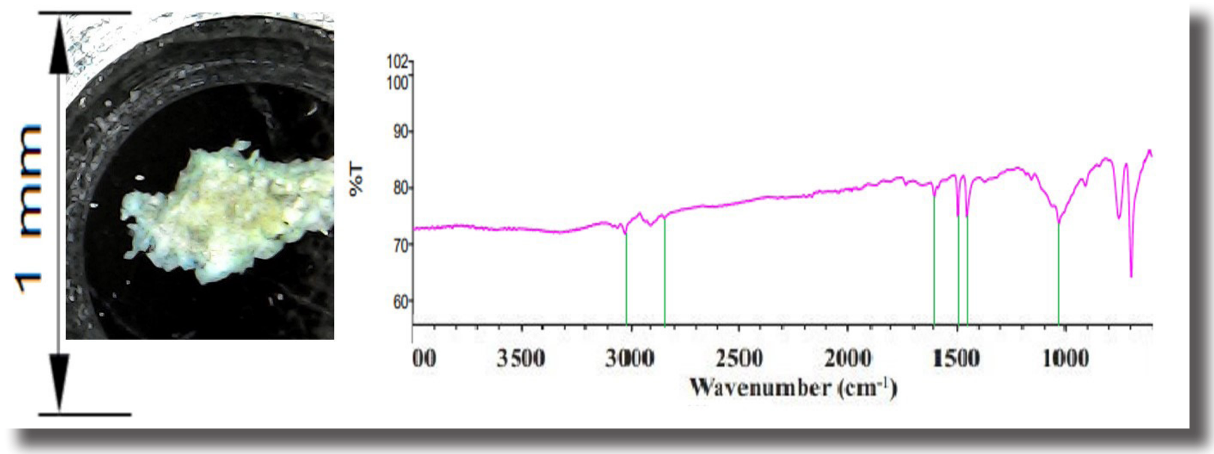

Figure 7 - Photograph (left) and IR spectrum (right) of a fragment-type microplastic found in fish No. 51 (*Oreochromis niloticus*, *Linnaeus*, 1758 - female tilapia) classified as polystyrene (PS). Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

Polyurethane (PU) was identified in a microplastic classified as foam, by the presence of absorption bands at 2865 cm^{-1}, corresponding to the stretching of the C-H bond; at 1731 cm^{-1}, corresponding to the stretching of the C-O double link; at 1531 cm^{-1}, which corresponds to the stretching of the C-N bond; at 1451 cm^{-1}, corresponding to the bending of CH_2; and at 1223 cm^{-1}, corresponding to the stretching of the carboxylic acid ester. In Figure 8, the IR spectrum of the microplastic identified as PU is shown.

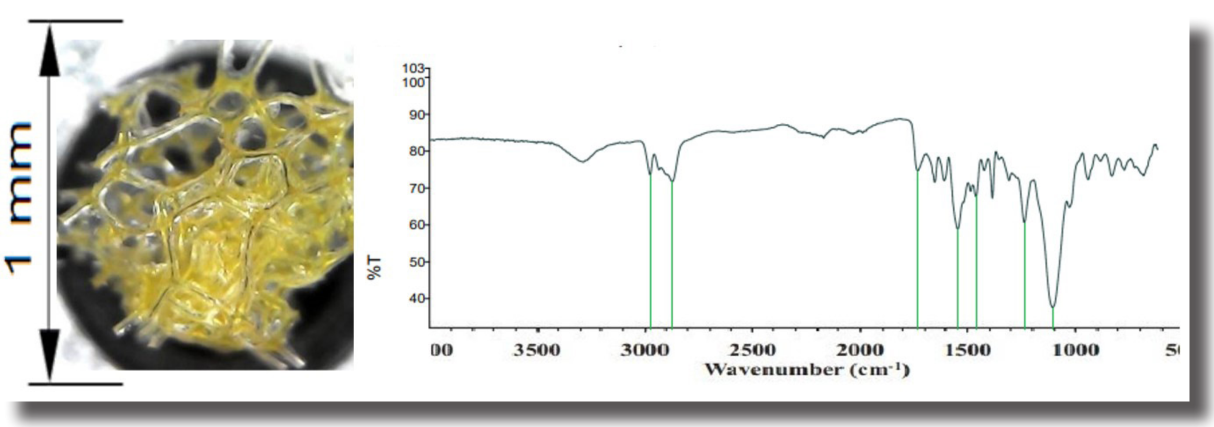

Figure 8 - Photograph (left) and IR spectrum (right) of a foam-like microplastic found in fish No. 34 (*Oreochromis niloticus*, *Linnaeus*, 1758 - female tilapia) classified as polyurethane (PU). Source: Spectrum obtained in FTIR-ATR at the Laboratorio Químico Fiscal, SAT. Guatemala City – Guatemala, 2023.

DOI: 10.15343/0104-7809.202448e15762023I

In Figure 9, it can be seen that the polymers most frequently found in the microplastics analyzed were polypropylene (PP), mostly of the fragment type (Table 2), high-density polyethylene (HDPE), mostly of the film, and nylon, being of the line type. They presented percentages of 47%, 19% and 15%, respectively, in the total microplastics analyzed. On the other hand, polyurethane (PU) represented 2%, and low-density polyethylene (LDPE) 3%. This is in line with information compiled by Wang *et al.*[25], who mention that polyethylene and polypropylene are frequently found in the digestive tracts of fish, which is also in line with the polymers produced in greater quantities around the world[26].

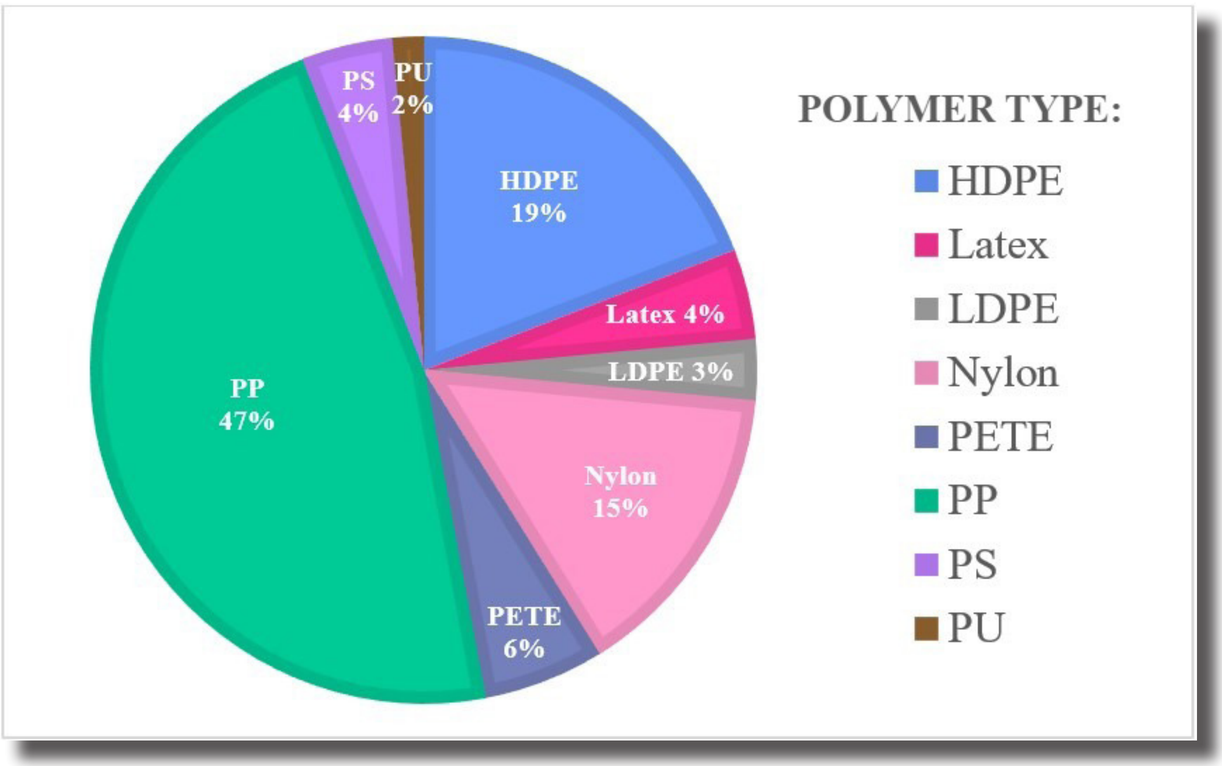

Figure 9 - Percentages of the types of polymers found in microplastics analyzed by infrared spectroscopy (FTIR-ATR). HDPE= high density polyethylene; LDPE= low density polyethylene or linear LDPE; PET= polyethylene terephthalate; PP= polypropylene; PS= polystyrene; PU= polyurethane. Guatemala City – Guatemala, 2020-2021.

DISCUSSION

The results show that Lake Amatitlán contains microplastics that are ingested by fish; In previous studies, microplastics were found in the lake's water and sediments. The two species investigated (*O. niloticus* and *P. managuensis*) are omnivorous, therefore, in addition to the microplastics that they ingest directly, they can incorporate them through food, as it has already been demonstrated that microplastics can be adsorbed on phytoplankton or adsorbed or ingested by macroinvertebrates and thus enter the food chain[27]. Microplastics have been found in the digestive tract of carnivorous and herbivorous freshwater fish in different parts of the world[25], entering organisms through direct

DOI: 10.15343/0104-7809.202448e15762023I

ingestion when mistaken for food, through accidental ingestion during feeding or through transfer through the food chain[21].

The risk to aquatic biodiversity is that, in addition to the toxicity of microplastic additives themselves, such as antibiotics, flame retardants, plasticizers, phthalates, or bisphenol A and nonylphenols, considered endocrine disruptors[28], they can adsorb other toxic contaminants, such as pesticides, metals or persistent organic contaminants[20,28], increasing toxicity to organisms at different trophic levels[27]. Furthermore, it has been suggested that smaller microplastics can be translocated to other organs from the digestive tract, with microplastics having been found in the muscular tissues of fish[27], so that microplastics could reach humans through the consumption of the two study species in the Lake Amatitlán.

Microplastics can affect the biodiversity of Lake Amatitlán in different ways. Due to their different densities, depending on the polymer that composes them, microplastics that are less dense than water can float, while those that are denser can sink into deeper waters[29]. Identification of microplastic polymers found in the digestive tract of fish collected from Lake Amatitlán helps establish the possible origin of microplastic contamination in the lake. The microplastics analyzed, in which fibers and fragments were the most abundant, presented HDPE, PP and nylon as the main polymers. Due to the type of fragments, the majority correspond to microplastics of secondary origin, that is, produced from the degradation of waste plastic products that were discarded in the environment.

Thus, the microplastics found in Lake Amatitlán probably come from bottle caps, food storage containers, tubes and textile items, with polypropylene (PP) being mainly identified. Additionally, they can originate from pipes, containers, household items, cable insulation and toys, as these are commonly made from high-density polyethylene (HDPE) and textile products and ropes made from nylon[30,31]. In Guatemala, polyethylene is used in the plastics industry for the production of lids for aerosol containers, bottles, tubes for creams, honey bottles, as well as for the manufacture of toys. Polypropylene is used in the manufacture of buckets, bottles, toys, food containers, among others[32], while nylon and polyester are mainly used in the textile industry[33]. All of these products are widely used by the population of the municipalities located in the Amatitlán lake basin. Waste from these products reaches the lake mainly through the Villalobos River, which carries the largest load of wastewater from the southern zone of Guatemala City and other populous municipalities, such as Mixco and Villa Nueva.

It is important to take into account that the FTIR-ATR instrument used does not have the capacity to satisfactorily analyze microplastics smaller than 1 mm. Oliva *et al.*[10] have reported that 96.3% of the fish from Lake Amatitlán analyzed had microplastics, and only 10% of the microplastics were larger than 1 mm, this being the fraction analyzed in this work. Thus, in 90% of the microplastics found in the digestive tract of fish, the constituent polymer could not be determined by FTIR-ATR, meaning their possible originating products are not known. As smaller microplastics have a greater surface area, they could have a greater impact on aquatic organisms, as they could have different interactions with adsorbed contaminants in addition to a different behavior in possible translocation between organs or in their absorption by higher organisms.

The disposal of plastic waste is not adequately regulated in Guatemala, nor have maximum allowable levels for microplastics been established in wastewater and drinking water regulations. Thus, microplastics derived from the disposal of plastic waste in Guatemala City and other municipalities in the basin put fishing production and the biodiversity of Lake Amatitlán at risk. Fishing is an activity that in

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countries like Guatemala contributes to the production of food that partially supports the population's food security[34]. The volume of fishing in Lake Amatitlán, years ago, was 239 tons per year, of which the species *Oreochromis niloticus* (tilapia) represented almost half; There are other species of fish in the lake, tilapia is the species that practically sustains fishing in Lake Amatitlán[35].

It has been reported that microplastics affect the growth and reproduction of fish[27], therefore measures must be taken to mitigate contamination by plastics and other contaminants in Lake Amatitlán, to reduce the risk to fish production and the lake's biodiversity, such as as well as for the health of the population, which is why it is necessary to adopt regulatory measures to improve environmental education in the basin's municipalities, as well as to control plastic discharges. To reinforce these measures, new research should aim to understand the levels and interactions of microplastics in different environmental components, including the presence of microplastics in the different organs of fish and other aquatic organisms.

CONCLUSION

To reduce the entry of microplastics into Lake Amatitlán, regulation of the use of plastics and single-use products must be promoted and implemented, as these will become secondary microplastics. The results of this research can be used in formulating strategies to manage these contaminants and can serve as a basis for proposing guide levels for natural surface water quality standards, as the country currently lacks regulations in this regard.

FUNDING: General Directorate of Research -DIGI-, Project B-12-2020, Universidad de San Carlos de Guatemala.

ACKNOWLEDGEMENTS: To “Dirección General de Investigación -DIGI-, Proyecto B12-2020, Universidad de San Carlos de Guatemala”. To “Laboratorio Químico Fiscal, de la Superintendencia de Atención Tributaria de Guatemala”.

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Conceptualization: Hernández, BEO; Sabino, JFP. Methodology: López, GRQ; Hernández, BEO. Validation: López, GRQ; Sabino, JFP. Statistical analysis: López, GRQ; Hernández, BEO; Sabino, JFP. Formal analysis: López, GRQ; Hernández, BEO; Sabino, JFP. Investigation: López, GRQ; Hernández, BEO; Sabino, JFP. Resources: Hernández, BEO. Writing-original draft preparation: López, GRQ. Writing-review and editing: López, GRQ; Hernández, BEO; Sabino, JFP. Visualization: López, GRQ; Hernández, BEO; Sabino, JFP. Supervision: Sabino, JFP. Project administration: Hernández, BEO.

All authors read and agreed to the published version of the manuscript.

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DOI: 10.15343/0104-7809.202448e15762023I

Received: 01 december 2023.
Accepted: 27 february 2024.
Published: 08 march 2024.

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DOI: 10.15343/0104-7809.202448e15762023I

Supplementary Material

Table 1A - Biometric parameters of fish from Lake Amatitlán in which microplastics from the digestive tract were analyzed. Guatemala City – Guatemala, 2020-2021.

Fish No.	Length (g)	Organism weight (g)	Digestive tract weight (g)
1	19.5	121.51	2.6
3	16.3	89.98	2.62
4	20.6	173.9	1.45
5	18.7	125.27	2.39
6	17.3	91.16	2.2
8	18.5	119.18	2.26
9	19.3	143.24	2.48
10	17.0	104.94	2.55
11	17.8	111.69	3.39
13	21.0	167.82	4.8
14	20.4	172.74	8.99
17	21.7	215.25	7.03
19	21.6	210.58	7.58
22	22.0	212.55	22.0
23	21.5	180.56	3.7
26	22.0	181.33	5.08
30	20.5	168.06	5.95
31	20.7	170.46	7.18
32	24.0	284.5	8.41
34	17.8	67.12	2.83
39	20.5	181.84	3.80
40	20.5	186.36	3.90
41	16.5	123.83	9.14
43	18.5	132.26	3.82
45*	23.5	203.4	6.14
46	22.0	211.56	7.43
47	21.6	203.99	6.28
49	17.8	112.61	1.94
51	18.0	116.69	4.50
52	18.3	118.45	2.98
53	17.0	99.21	3.86
54	17.7	110.50	1.91
59	16.5	99.15	7.23
60	16.5	76.90	3.17
65	17.1	94.28	3.10
66	17.4	106.18	5.38

*Fish No. 45 is the only one of species *Parachromis managuensis*.