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Microplastics in Nile tilapia *(Oreochromis niloticus)* from Lake Amatitlán

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Bessie Evelyn Oliva-Hernández*; Francis Mariel Santos-Ruiz; Manuel Alejandro Muñoz-Wug; Juan Francisco Pérez-Sabino

Facultad de Ciencias Químicas y Farmacia. Escuela de Química. Universidad de San Carlos de Guatemala,
Ciudad Universitaria, Edificio T12, zona 12, 01012, Ciudad de Guatemala, Guatemala.
E-mail: francissantos94@gmail.com, mwmanuel@yahoo.com, fpsabino@usac.edu.gt
*Corresponding author. E-mail: bessieoliva@yahoo.com

ABSTRACT

Microplastics are plastic particles smaller than 5 mm found in the environment, which can cause health problems for aquatic organisms and humans, being classified as emerging pollutants. In Guatemala, Lake Amatitlán is one of the most polluted lakes as it receives discharges of wastewater, treated and untreated, as well as other waste from Guatemala City and other major urban populations. In a recent study, microplastics were found in sediments in Lake Amatitlán, so it was necessary to determine whether the lake's fish are affected by these pollutants, which can be harmful to their health. This research aimed to determine the microplastics in fish from Lake Amatitlán, for which 65 specimens of Nile tilapia (*Oreochromis niloticus*) were collected in October and December 2020, and January 2021. The collected fish were dissected in the laboratory, where the number and type of microplastics were analyzed in the digestive tract, after their separation by digestion and filtration. Lines or fibers were the most common type of microplastics, found in 63 specimens (96.9% of the analyzed specimens), ranging from 0 to 27 lines/fibers per individual. The frequency of other types of microplastics found were 0-3 fragments/individual, 0-2 films/individual, and 0-4 foams/individual, while microspheres were not found in any specimen. The presence of microplastics in tilapia from Lake Amatitlán is an indicator of contamination in the lake by pollutants that could affect the trophic network and represents a risk for the fish consumers, requiring the attention of environmental and health authorities.

Keywords: emerging pollutants, Guatemala, pollution.

Microplásticos em tilápia do Nilo (*Oreochromis niloticus*) do Lago Amatitlán

RESUMO

Microplásticos são partículas plásticas menores que 5 mm encontradas no meio ambiente, que podem causar problemas à saúde dos organismos aquáticos e humanos, sendo classificados como poluentes emergentes. Na Guatemala, o Lago Amatitlán é um dos lagos mais poluídos, pois recebe descargas de águas residuais, tratadas e não tratadas, bem como outros resíduos da Cidade da Guatemala e de outras populações urbanas. Em um estudo recente, foram encontrados microplásticos em sedimentos do Lago Amatitlán. Então era necessário saber se os peixes do lago estão ingerindo microplásticos, que podem ser prejudiciais. Assím, o objetivo desta pesquisa foi determinar os microplásticos em peixes do Lago Amatitlán, para o qual foram coletados 65 exemplares de tilápia do Nilo (*Oreochromis niloticus*) nos meses de outubro e dezembro de 2020 e janeiro de 2021. Os peixes foram dissecados no laboratório, onde o número e tipo de microplásticos foram analisados no trato digestivo, após sua separação por digestão e filtração. Linhas ou fibras foram o tipo de microplástico mais comum, sendo encontradas em 63 espécimes (96,9%), variando de 0 a 27 linhas / fibras por indivíduo. A frequência de outros tipos de microplásticos encontrados foi de 0-3 fragmentos / indivíduo, 0-2 filmes / indivíduo e 0-4 espumas / indivíduo, enquanto microesferas não foram encontradas em nenhum espécime. A presença de microplásticos na tilápia do Lago Amatitlán é um indicador de contaminação do lago por poluentes que podem afetar a rede trófica e representa um risco para os consumidores de peixe, exigindo atenção das autoridades ambientais e sanitárias.

Palavras-chave: contaminação, contaminantes emergentes, Guatemala.

1. INTRODUCTION

Lake Amatitlán is located 25 km south of Guatemala City. It has an approximate area of 15 km^2, and is surrounded by the municipalities of Villa Nueva, Villa Canales, San Miguel Petapa and Amatitlán. It is one of the most polluted water bodies in Guatemala, as it receives the waste water from Guatemala City through the Villalobos River, its major tributary. High levels of nutrients, pesticides and heavy metals have been found in the lake, which have caused the lake´s eutrophication and high risk of diseases for human beings (Basterrechea, 1997; van Tuylen, 2020; Knedel *et al.*, 1999). Since the 1990s, different decisions have been made to reduce lake pollution, which have decreased the input of traditional pollutants (López, 2013); However, emerging pollutants, including microplastics, had not been considered, of which several have polluted the water for decades, and only recently have begun to get any attention (Geissen *et al.*, 2015).

Microplastics are all plastic particles smaller than 5 mm found in the environment (Bellasi *et al.*, 2020), either by direct discharge or by the degradation of larger plastic materials (Barboza *et al.*, 2020). The intensive and ubiquitous use of plastic in the last century has given rise to extensive contamination of water bodies worldwide (Espinosa Ruiz *et al.*, 2016), generating microplastics from different sources, either as primary microplastics that are small plastics produced as raw material or end- product or as secondary microplastics that are produced from the disintegration of plastics of a larger size (Thiele *et al.*, 2021). Microplastics are an emerging pollutant of growing concern, not only because of their direct negative effects, but because they are assumed to increase the exposure of aquatic organisms to associated chemicals. These pollutants are ingested by aquatic organisms, in which they can cause health problems and even death, as well as bioaccumulation and biomagnification of microplastics in the food chain (Cole *et al.*, 2011; Xu *et al.*, 2020). The ingestion of microplastics affects the physiology and behavior of aquatic animals and introduces this pollutant to the trophic chains (Espinosa Ruiz *et al.*, 2016). Microplastics can also adsorb pollutants such as heavy metals and organic pollutants, facilitating their entry into the food chain and increasing the risk for aquatic organisms and humans (Bollaín Pastor and Agulló, 2019; Wilkinson *et al.*, 2017). Due to their size and color similar to those of some natural prey, microplastics are ingested by aquatic organisms such as fish, invertebrates or even aquatic birds, and can cause negative effects to them (Yuan *et al.*, 2019).

The Nile Tilapia (*O. niloticus*) was introduced into the lakes of Guatemala, including Lake Amatitlán, between 1950-1960, in order to promote the economic growth of the basin inhabitants. Different studies have shown that Lake Amatitlán is highly polluted with heavy metals; recently arsenic, mercury and cadmium were found in the muscle of fish in the lake (González, 2010; Cano Alfaro, 2018) as well as quantifiable levels of cyanotoxins, such as microcystins (Romero-Oliva *et al.*, 2014); however, no studies had been carried out to identify and quantify microplastics. Most of the studies on contamination by microplastics have been carried out in the oceans, meanwhile the studies in continental waters are still insufficient (Bollaín Pastor and Agulló, 2019; Yuan *et al.*, 2019). Lakes are the main sinks for microplastics in freshwater ecosystems, as these pollutants can accumulate and remain in sediments for long periods of time. Likewise, lakes can also become sources of microplastics for downstream watersheds (Yuan *et al.*, 2019).

In other countries, microplastics and phthalates have been found in freshwater and saltwater fish (Baini *et al.*, 2017), while recently microplastics were found in sediments from Lake Amatitlán (Romero Oliva, 2019), in water from Lake Petén Itzá (Mejía Saenz, 2019) and water and fish from Lake Atitlán (López, 2013; Sagastume, 2020; Santos Ruíz, 2020), which shows that there is contamination by microplastics in water bodies in Guatemala. In this way, the authors considered it important to determine if the tilapias of Lake Amatitlán are affected by microplastics present in the environment, which could threaten its health and represent a risk for consumers.

The importance of studying these emerging pollutants is based upon the fact that it is unknown, on the one hand, in which environmental components they are found, and on the other, the effects that they may be causing in the aquatic ecosystem and the risk to the health of the human population in contact with water or consuming fish from contaminated water bodies (Geissen *et al.*, 2015). Due to this lack of knowledge, the allowed levels for these pollutants have not yet been included in the water quality standards or in the regulation of wastewater or food. Thus, it is necessary to investigate their presence and identity in order to make proposals for the better disposal of plastic waste.

To address part of these knowledge gaps, this study identified microplastics in *O. niloticus* from Lake Amatitlán and captured information regarding the size, sex and condition index of the fish consuming microplastics, through a descriptive study, with a non-probabilistic sampling design. The type and quantity of microplastics extracted from the gastrointestinal tract of fish collected by local fishermen during three samplings (October 2020, December 2020 and January 2021) were analyzed in order to prove whether the fish of Lake Amatitlán are ingesting microplastics from the aquatic environment at levels that could represent a risk for biota and human health.

2. MATERIALS AND METHODS

2.1. Fish collection

Fish were collected with the aid of local fishermen, who catch them in various locations of Lake Amatitlán, using a fishing net. The fish were collected at the lake between 05h00 and 10h00, choosing specimens not smaller than 15 cm in length for analysis in the laboratory. During the three samplings, 65 individuals of the species *Oreochromis niloticus* (Nile tilapia) were collected. The fish were transferred to the laboratory where they were stored at freezing temperature for further processing. In the present study, three fish samplings were carried out in the months of October and December 2020 and in January 2021, in Lake Amatitlán,

2.2. Fish measurement

Fish length was measured from mouth to tail, with a precision of 1 mm, using a graduated ruler and the specimens were weighed in an electronic semi-analytical balance with a precision of 0.1 grams, according to the methodology of Cifuentes *et al.* (2012). For all the collected fish, the condition index was determined.

2.3. Extraction and purification of microplastics from the fish gastrointestinal tract

The entire gastrointestinal tract of the 65 collected specimens was dissected and transferred to a 250 ml glass beaker. To digest the organic matter, 25 mL of a 1.0 M NaOH solution prepared in the laboratory (Merck, 99-100% purity) were added to the beaker and heated at 50°C in an electric oven for 15 min. The samples were mixed by means of a glass stirring rod to aid alkaline digestion. Each sample was diluted with 125 ml with ultrapure water and vacuum filtered through a 47 mm diameter Whatman brand fiberglass filter 45 μm pore size (Roch *et al.*, 2020).

2.4. Microplastics classification

The filters with the recovered microplastics were examined with a dissection microscope (Kyowa Optical, Model SD-2PL) counting the number of each type of microplastics (lines/fibers, fragments, films, microspheres and foams) present in the gastrointestinal tract of the analyzed specimens (Roch *et al.*, 2020).

2.5. Analysis of results

The results of the measurements, weights, number and type of microplastic were classified and tabulated, describing what was found in each specimen. The condition index was calculated for each individual according to the Equation 1.

$$K = 100\,(W\,/\,L^3) \qquad (1)$$

Where W is the wet body weight, in grams and L the length in cm (Cifuentes *et al*., 2012). The microplastics determined were classified by type of microplastic in body length intervals of the specimens calculated using the Sturges equation, resulting in seven intervals of 1.3 cm in length. Because the two groups of larger fish had four (22.1-23.4 cm) and two individuals (23.4-24.7 cm), they joined a group of fish with length equal to or greater than 22.1 cm.

The median and interquartile range (IQR) were calculated for each group, since the resulting dispersion was too large to use average and standard deviation, a non-parametric one-way analysis of variance (Kruskal Wallis) was performed. ($P < 0.05$), using STATA16 software. Four analyzes were run according to the type of microplastic: lines, fragments, films and foams. In the case of the films, there were no results. The most relevant analysis is that of the fibers: it showed that there is a difference according to the length.

3. RESULTS AND DISCUSSION

Table 1 shows the results of the determination of sex, weight, length, condition index and different types of microplastics for the 65 fish collected. Of the 65 specimens collected, 51 were female and 14 male. The lengths of the specimens were between 15.6 and 24.2 cm, with an average of 19.31 cm and the body weights were between 67.12 and 287.09 g, with an average of 147.0 g per specimen. The average for the condition index (K) was 1.97 g cm^{-3} ranging from 1.57 to 2.56 g cm^{-3}. Microplastics were found in 63 of the 65 specimens analyzed with an average of 9.71 microplastics per individual. The lines/fibers (Figures 1 and 2) were the most common type of microplastics, as there were from 2 to 27 lines or fibers per specimen. One to five fragments were found in 28 specimens, one or two films were found in 12 specimens, and one to four foams were found in 18 specimens. No microspheres were found in any specimen. The results demonstrate that tilapia is ingesting microplastics from the aquatic environment of Lake Amatitlán independently of the weight and length of the specimen. Only two females (44 and 57) did not present any microplastic. The condition index also shows a wide dispersion, unrelated to sex or presence of microplastics.

Table 1. Determination of weight, length, condition index and microplastics in specimens of *Oreochromis niloticus* collected in Lake Amatitlán.

Fish Code	Sex	Weight (g)	Length (cm)	Condition Index K (g cm^{-3})	Lines/ Fibers	Fragments	Films	Foams	Total
1	Female	121.51	19.5	1.64	11	1	0	0	12
2	Female	139.98	20.0	1.75	18	1	1	0	20
3	Female	89.98	16.3	2.08	9	1	1	0	11
4	Female	173.69	20.6	1.99	23	0	0	0	23
5	Male	125.27	18.7	1.92	11	1	0	0	12
6	Female	91.16	17.3	1.76	18	0	0	0	18
7	Female	73.33	15.6	1.93	3	0	0	0	3
8	Male	119.18	18.5	1.88	6	1	0	0	7
9	Female	143.20	19.3	1.99	8	1	0	0	9
10	Female	104.94	17.0	2.14	12	0	0	0	12
11	Male	111.09	17.8	1.97	7	0	0	0	7
12	Female	150.14	19.2	2.12	22	2	1	0	25
13	Female	167.82	21.0	1.81	22	1	2	0	25
14	Female	172.74	20.4	2.03	16	1	0	0	17
15	Female	217.07	23.3	1.72	5	0	0	0	5
17	Female	215.25	21.7	2.11	9	2	0	0	11
18	Female	186.71	21.0	2.02	5	3	0	1	9
19	Female	210.58	21.1	2.24	27	1	0	0	28
20	Female	178.06	21.6	1.77	8	0	0	0	8
22	Female	212.55	22.0	2.00	4	0	0	0	4
23	Female	180.56	21.5	1.82	8	0	0	0	8
24	Female	228.94	21.8	2.21	7	0	0	0	7
25	Female	273.87	23.0	2.25	14	0	0	0	14
26	Female	181.33	22.0	1.70	10	0	0	0	10
27	Female	198.36	21.0	2.14	10	0	0	0	10
28	Female	205.08	22.5	1.80	20	0	0	0	20
29	Female	197.38	21.5	1.99	9	0	1	0	10
30	Male	168.06	20.5	1.95	7	0	0	0	7
31	Female	170.46	20.7	1.92	18	0	0	0	18
32	Female	284.80	24.0	2.06	9	0	0	0	9
33	Female	195.18	21.5	1.96	15	0	0	0	15
34	Female	67.12	17.8	1.19	4	1	0	1	6
35	Female	234.50	22.9	1.95	10	1	0	2	13
36	Male	160.04	20.5	1.86	8	2	0	0	10
38	Female	173.01	20.2	2.10	4	0	0	1	5
39	Female	181.84	20.5	2.11	2	0	0	0	2
40	Female	186.36	20.5	2.16	4	0	0	0	4
41	Male	123.83	16.5	2.76	4	0	1	3	8
42	Male	287.09	24.2	2.03	12	1	0	0	13
43	Female	132.26	18.5	2.09	4	0	0	1	5
44	Female	154.58	19.9	1.96	0	0	0	0	0
46	Male	211.56	22.0	1.99	3	2	0	0	5
47	Female	203.99	21.6	2.02	7	0	0	0	7
48	Female	97.00	17.5	1.81	4	1	1	1	7
49	Female	112.61	17.8	2.00	4	0	0	0	4
50	Female	89.59	16.5	1.99	3	1	0	1	5
51	Female	116.69	18.0	2.00	6	0	0	1	7
52	Female	118.45	18.3	1.93	11	1	0	0	12
53	Male	99.21	17.0	2.02	7	0	0	3	10
54	Female	110.50	17.7	1.99	16	1	0	0	17
55	Female	102.25	17.9	1.78	14	3	0	1	18
56	Male	121.99	17.5	2.28	3	0	0	0	3

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57	Female	95.92	17.0	1.95	0	0	0	0	0
58	Male	91.26	16.7	1.96	5	1	0	1	7
59	Female	99.15	16.5	2.21	3	0	0	0	3
60	Male	76.90	16.5	1.71	5	1	0	4	10
61	Female	83.81	16.1	2.01	3	5	0	1	9
62	Female	93.20	17.4	1.77	2	0	0	1	3
63	Female	98.30	17.0	2.00	6	1	0	1	8
64	Male	105.86	17.8	1.88	6	0	0	0	6
65	Female	94.28	17.1	1.89	5	1	1	0	7
66	Female	106.18	17.4	2.02	5	2	1	2	10
67	Male	101.43	16.0	2.48	3	0	0	0	3
68	Female	88.33	17.8	1.57	1	3	0	0	4
69	Female	92.90	17.0	1.89	4	0	1	1	6
70	Average	147.70	19.32	1.97	8.45	0.68	0.17	0.42	9.71

Source: Proyecto B12-2020.

Figure 1. Dissection of the gastrointestinal tract of two tilapia specimens (19 and 22) for extraction of microplastics by alkaline digestion.

Table 2 shows the results of the different types of microplastics found in the gastrointestinal tracts of specimens of *O. niloticus* from Lake Amatitlán, grouped by ranges of body length. Lines/fibers, fragments, films and foams were found, but microspheres were not found in any specimen. Figure 1 shows two of the specimens caught in Lake Amatitlán with the gastrointestinal tract extracted. Groups 3 and 6 presented the highest medians (9.5 and 11, respectively). In the Kruskal Wallis test (not shown), Groups 3 and 6 were significantly different from the rest of the groups. In the case of Group 6, the longest fish, the high median could indicate bioaccumulation of microplastics related to age. According to the results, lines and fibers are the type of microplastics that are most abundantly ingested by tilapia in Lake Amatitlán. The fragments of Groups 1 and 3 presented medians of 1, while for the other four groups the median was 0. The median of the films for all groups was 0, while for the foams, only Group 1 presented a median of 1 and the other groups had a median of 0. Group 1 of smaller fish presented the highest average condition index median (2.01), as well as the lowest median of lines/fibers (3). However, no really relevant differences between condition indexes are found between groups, indicating equivalent nutrition between them. Regarding fish sex, 79.7% of the captured fish were female and 20.3% were male, both sexes showing wide dispersion in terms of the number of microplastics present and the condition index, so they cannot be related in this study sample.

Table 2. Median and interquartile range (in brackets) of condition index and each type of microplastics found in specimens of *Oreochromis niloticus* from Lake Amatitlán classified by fish body length.

Group	Length Range (cm)	n	Condition index ($g\ cm^{-3}$)	Lines/fibers	Fragments	Films	Foams
1	15.6 -16.9	9	2.01 (0.25)	3 (2)	1 (1)	0 (0)	1 (1)
2	16.9 -18.2	19	1.95 (0.22)	5 (3)	0 (1)	0 (0)	0 (1)
3	18.2-19.5	6	1.96 (0.17)	9.5 (5)	1 (0)	0 (0)	0 (0)
4	19.5 -20.8	11	1.96 (0.24)	8 (7)	0 (1)	0 (0)	0 (0)
5	20.8 -22.1	14	2.00 (0.29)	8.5 (3)	0 (1)	0 (0)	0 (0)
6	≥22.1	6	1.99 (0.26)	11 (5)	0 (1)	0 (0)	0 (0)

Source: Proyecto B12-2020.

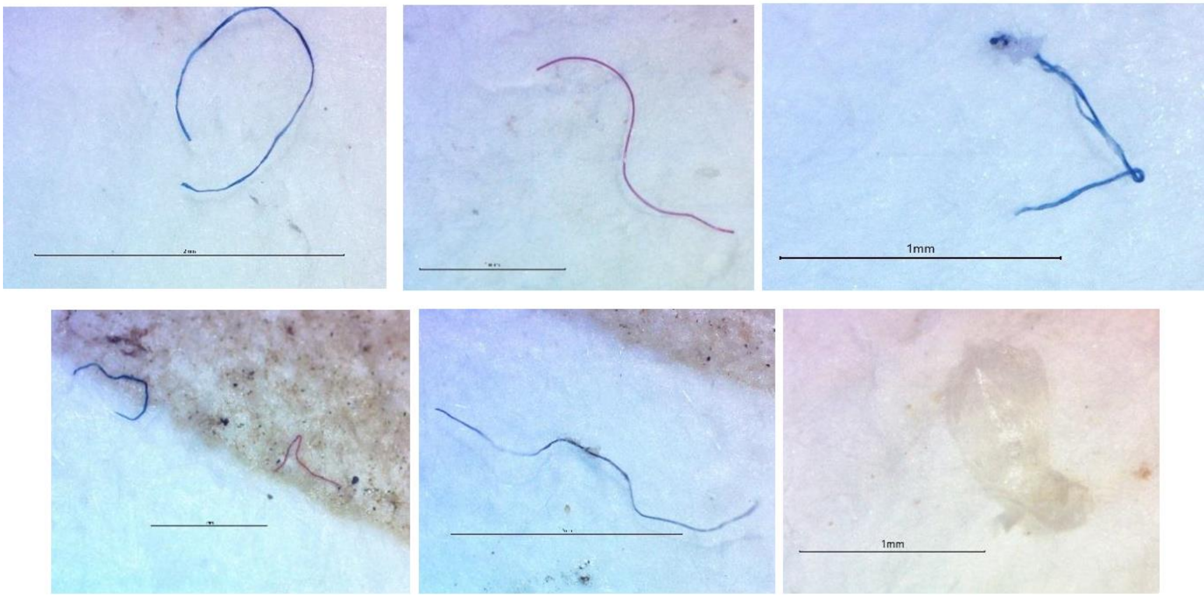

Figure 2. Microscope image of microplastics present in the digestive tract of the fish in Figure 1. Images a, b and c correspond to lines/fibers of individual 19; d and e to lines/fibers and f to a fragment of individual 22 (the scale line corresponds to 1 mm in each image).

The risk of the presence of microplastics in tilapia from Lake Amatitlán is that these pollutants can cause neurotoxicity and oxidative damage to fish (Barboza *et al.*, 2020). Thus, microplastics can reach humans through their consumption. Since tilapia is one of the main products of fishing at the lake and is sold for residents and tourists in the basin. In a recent study, microplastics were found in a sediment core of Lake Amatitlán, with lines being the most abundant microplastics (n = 221), followed by fragments and films (n = 37) (Romero-Oliva, 2019), which agrees with the frequency with which microplastics were found in fish in the present study. No reports of microplastics in water from Lake Amatitlán were found, but the major source of microplastics in the lake and the ingestion by fish could be caused by pollution coming from the Villalobos River that carries treated and untreated wastewater from southern Guatemala City. Since most of the fish analyzed have ingested microplastics, this pollutant represents a risk to the health and growth of the fish, which can cause a decrease in the productivity of the fishing in the lake. Likewise, microplastics can also adsorb other pollutants from the environment, such as pesticides or polynuclear aromatic hydrocarbons, or contain heavy metals, facilitating their transport and entry into organisms and increasing their toxicity (Bollaín Pastor and Agulló, 2019; Vedolin *et al.*, 2018) by being distributed in different organs of fish.

The prevalence of microplastics in tilapia found in this study (96.9%) is higher than that reported for fish in other studies, including 88% in Amazon River fish, 70% in commercial marine fish, or between 39 and 49% for fish of Mediterranean coastal lagoon at the north of Spain (Rodríguez-Sierra *et al.*, 2020), which may be an indicator of high contamination by microplastics in Lake Amatitlán. Thus, the results show that the fish in Lake Amatitlán may be threatened by contamination by microplastics, if actions are not taken for its control by the environmental authorities of the basin.

4. CONCLUSIONS

It was found that tilapias of Lake Amatitlán ingest microplastics from the aquatic ecosystem, with lines / fibers being the most common type of microplastics found in the gastrointestinal tract of the fish analyzed. The presence of microplastics in most of the fish is a good indicator that Lake Amatitlán presents a problem of contamination by these pollutants that can represent a risk for fish health, altering the trophic network and reaching humans. Future studies should address the composition of the microplastics present in fish and in the water of Lake Amatitlán to evaluate possible toxicological effects on the aquatic biota. The results should also be considered by the basin environmental authorities in order to take action to control de contamination by plastics and microplastics.

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