



## Article

# Microplastic Pollution in Sea Turtle Nests on the Beaches of Nautla and Vega de Alatorre, Veracruz

Belem Anahy Estrella-Jordan, Fabiola Lango-Reynoso <sup>\*</sup>, María del Refugio Castañeda-Chávez ,  
Jesús Montoya-Mendoza and David Reynier-Valdes

Tecnológico Nacional de México, Instituto Tecnológico de Boca del Río, Boca del Río 94290, Mexico

\* Correspondence: fabiolalango@bdelrio.tecnm.mx; Tel.: +52-22-9227-9327

**Abstract:** Microplastic contamination has become a topic of interest and concern worldwide due to its persistence and the possible effects it may cause to the environment. When microplastics are present, they can alter their physical properties, negatively affecting the surrounding fauna, such as sea turtles that use the beaches to nest in the sand. In this study, the exposure of sea turtle nests to microplastics on the beaches of Nautla and Vega de Alatorre, Veracruz, one of the main nesting areas for the green turtle *Chelonia mydas*, as well as Kemp's ridley turtle *Lepidochelis kempii* from the Gulf of Mexico, was determined. Sand samples were obtained directly from the nests in situ on four beaches in the area and from two nesting pens, revealing the presence of microplastics in 100% of the nests in situ, with an average abundance of  $2.43 \pm 2.66$  #MP/kg SS and a concentration of  $0.00672 \pm 0.02286$  mgMP/kg SS, predominantly the form of foam, white in color, and from 1 to 2 mm in size.

**Keywords:** nesting area; plastic waste; handling pens; sand



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## 1. Introduction

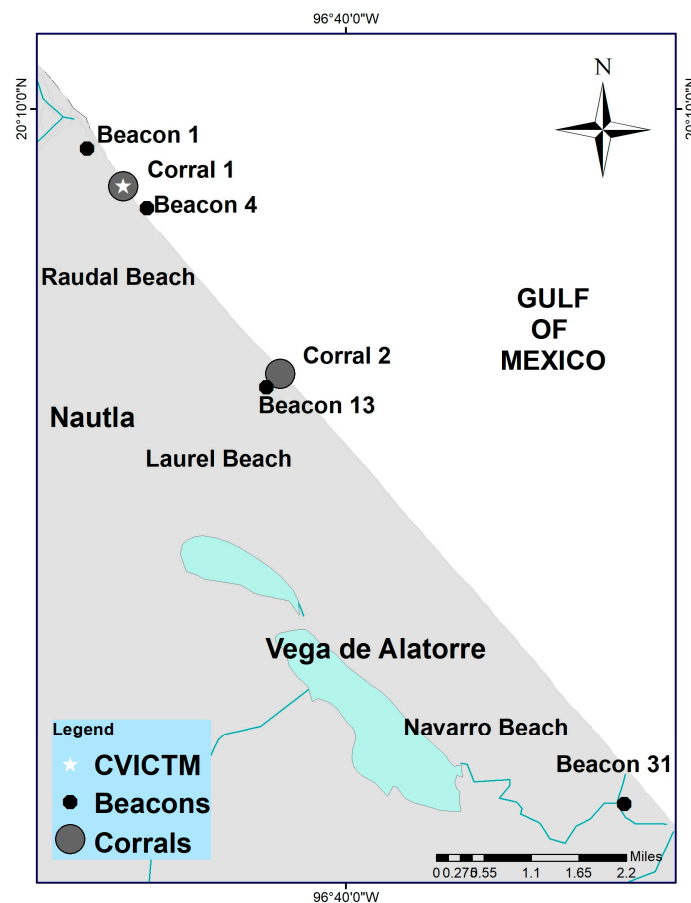
At present, the use of plastic products has become essential for human life [1]. Due to their great versatility, we can find them in cosmetics, clothing, and packaging, among many other uses [2]. Plastics are made up of polymer chains [3] that are obtained from the synthesis of fossil fuels, mainly oil, gas, and coal, which can be molded, applied as a coating, extruded, and have multiple applications [4]. The word “plastic” comes from the Greek “Plastikos” and the Latin “Plasticus”, which means that it can be cast in different ways [3]. Its great variety of uses has caused the continual increase in its consumption, which is why approximately 300 million tons of plastic are produced each year, of which 13 million end up in rivers and oceans [5]. This, in turn, causes them to accumulate in coastal areas, thus damaging ecosystems and marine organisms, such as sea turtles, of which thousands of deaths a year occur due to suffocation, entanglement, or ingestion [6]. The sea covers most of our planet, so there is a belief that it has an infinite dilution capacity and can serve as a gigantic dumping ground for all the waste produced by humans [7]. Unfortunately, it is not the case that the waste that enters bodies of water can remain suspended for hundreds of years, becoming incorporated into aquatic biota or deposited on the bottom and becoming incorporated into marine sediments [8]. Most plastic products accumulate in the environment due to their low degradation capacity, difficulty in recycling, and poor final disposal [9], producing irreparable damage to ecosystems and affecting the surrounding fauna [5]. Plastic materials, for the most part, have great physical stability, but over time, they can be subjected to physical and chemical erosion, which causes them to fragment into smaller particles called microplastics [9], whose size is less than 5 mm [10]. These are considered emerging pollutants since they are distributed in different environmental components [11] and constitute the most abundant and harmful fraction of plastic waste [12], currently being one of the biggest environmental concerns in the world [13].

Microplastics are composed mainly of materials, such as nylon, polyester, polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PU), and polyamide fiber, among others [14], and are classified into primary microplastics and secondary microplastics [10]. Primary microplastics are those that are intentionally manufactured smaller than 5 mm for industrial use, such as microbeads and pellets or pellets, which are used for the manufacture of soaps, cosmetics, toothpastes, and exfoliants, among others [15]. Secondary microplastics are those derived from larger plastic products that are fragmented by physical, chemical, or biological causes, of which they are mainly in the form of fibers, fragments, films, granules, beads, and foams [16]. Among the environmental impacts caused by microplastics, it is found that they can affect the key habitats used by sea turtles by transporting toxic substances and altering the properties of sediments that affect their temperature and permeability [17]. The nesting sites of sea turtles depend on physical factors, such as the substrate, temperature, and humidity [18]. Likewise, the location and distribution of the nests is important for the brood to be successful; there are some variables that facilitate oviposition, such as fine sand beaches, moderate slopes, and good humidity and drainage [19]. Since plastics have a higher specific heat than sand [20,21], when these are combined with sand, they can increase in temperature, especially if the pigment of the plastic is dark [20]. This could potentially affect the nesting environment of sea turtles and, consequently, affect hatchling sex ratios and reproductive success of nests, as well as coastal areas. Thus, their environment may be further affected by toxic substances released by microplastics when heated [16,22]. Monitoring of microplastic contamination has been increasing in recent years, but it still represents a great challenge since validated and standardized methods are still under development, making it difficult to reproduce and compare results [23]. Currently, the studies carried out on microplastics are focused mainly on water, soil, beach, sediments, and various organisms, such as fish and crustaceans. However, the presence of microplastics in sea turtle nests in Mexico has not been documented, so due to the potential impacts of microplastics in the sea turtle hatchery environment, it is of the utmost importance to determine the exposure of nests to microplastics, to address this, and to provide a baseline for future studies the presence of microplastics in sea turtle nests in Nautla and Vega de Alatorre Beaches, Veracruz.

## 2. Materials and Methods

### 2.1. Study Area

The study area is located in the coastal zone of the municipalities of Nautla and Vega de Alatorre, where the Veracruz Center for Research and Conservation of the Sea Turtle (CVICTM) is located. Its facilities are located in the town of El Raudal de las Flores, Nautla, and the work area is sectioned by beacons every 500 m, with a total of 15.5 km of coastline between the two municipalities. The study area was chosen because it is one of the main nesting areas for sea turtles in the state of Veracruz. The sampling sites correspond to 4 beacons (1, 4, 13, and 31) from 3 different beaches (1 and 4 belong to El Raudal Beach, 13 to Laurel Beach, and 31 to Navarro Beach) and two management pens for the protection of the clutches belonging to the CVICTM, the first located in Raudal Beach (Beacon 3) and the second in Laurel Beach (Beacon 13) (Figure 1). The samplings were carried out in November 2021, when the sea turtle nesting season was about to end.



**Figure 1.** Sampling sites, Nautla, Veracruz.

## 2.2. Sampling

Sampling consisted of taking samples from 6 in situ empty nests from each of the sites and from 9 nests from each management pen. Three samples were collected from each nest made up of the wall and bottom of the nest; for this, we first searched for the nest in the dune area, which is where the turtles mainly nest. In the selected beacons, “beds” were searched for, and the name was given to the trail left by the turtles in the space where they made the nest.

When a bed was found, a search was conducted around it with the help of a broomstick, introducing the stick into the sand. Once the place was located where the stick entered without much difficulty and where the sand was not compacted, we proceeded to dig approximately 30 cm with the help of a metal shovel and then by hand to look for the shells. Once the shells were found, they were removed from the nest, and 3 samples were taken with the help of a metal cylinder with a capacity of 1.8 kg and garden shovels. The depth at which the sediment sample was taken and the location of the nest were documented; then, the shells were returned to the nest, and the previously removed sand was returned. To take samples from the nests in management pens, 9 nests were randomly selected from each pen; then, with the help of a metal shovel, the sand was removed to a depth of 60 cm, where a cavity was made to deposit the nests of turtle eggs that were endangered in situ. Once at a depth of 60 cm, 3 composite samples of the wall and bottom of each nest were taken with the help of a sampler shovel. Once all the samples were collected, they were transferred to the Aquatic Resources Research Laboratory (LIRA) of the Technological Institute of Boca del Rio Veracruz (ITBOCA), for analysis and to obtain results.

### 2.3. Sample Drying

The drying process of the samples was carried out based on the methodology proposed by Mohamed Nor and Obbard (2014). Each sample was placed on aluminum trays with a capacity of 3 kilos, which were dried at 60 °C for 24 h [24]. Once completely dry, a kilogram of each sample was taken.

### 2.4. Extraction of Microplastics

Once the one-kilogram samples were dry, they were subjected to the sieving process [25] with the help of 3 sieves with mesh openings of 5, 2.3, and 0.9 mm. The sample was sifted to separate the excess sand from the particles if the particles were larger than 1 mm. The materials contained in the sieves were labeled to undergo visual evaluation by stereoscopic microscopy, then a saturated NaCl solution was prepared at a density of 1.2 g/cm<sup>3</sup> [26]. The sample was added by shaking it for one minute, and then it was allowed to settle. The microplastics that were found floating were removed with metal tweezers and left to dry at room temperature. For the identification of microplastics by stereoscopic microscopy, it was taken into account that they did not have visible organic structures, that their color was uniform and opaque, that they did not crumble to the touch, and in the case of fibers, that were not segmented [27,28]. Once dry, the microplastics obtained were counted and weighed. The result was expressed in #MP/Kg SS (pieces of microplastics per kilogram of dry sediment), and their concentration expressed per mgMP/Kg SS (milligrams of microplastics per kilogram of dry sediment) according to the table of units used to report concentrations of microplastics as described by Álvarez et al. (2020) [25]. Because the samples presented a considerable amount of organic matter, mainly roots and leaves, 30% hydrogen peroxide was used to rule out false positives [29] since organic matter oxidizes and turns yellowish and brown when it comes in contact with hydrogen peroxide.

### 2.5. Classification of Microplastics

The classification of microplastics was carried out visually with the help of a lamp and a stereoscopic microscope. For each sample, the microplastics were quantified and weighed, and for each microplastic, its size was classified as 1–2, 2.1–3, 3.1–4, or 4.1–5 mm. Corresponding to the interval from 1 to 5 mm [25], their color (white, blue, gray, yellow, orange, green, pink, red, purple, black, or transparent) [30], and their type (fragment, fiber, pellet, foam, or film) [14] were classified.

### 2.6. Statistic Analysis

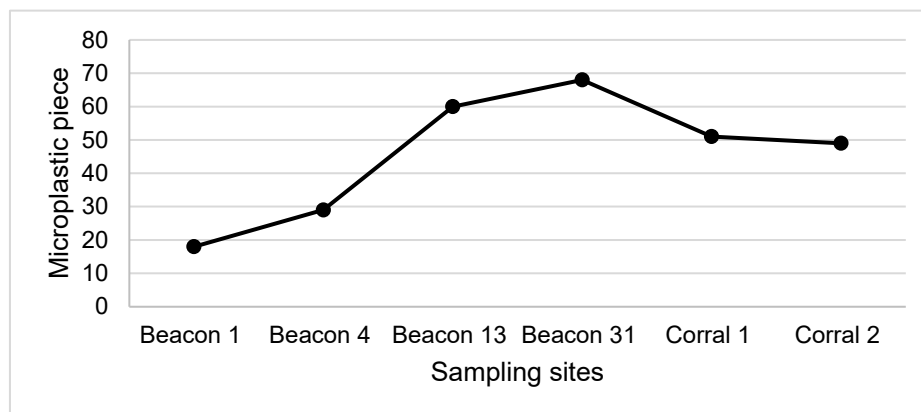
Shapiro–Wilk normality tests were performed to make a decision, taking the beaches as the variables with respect to the amount of microplastics in each of the sampled nests. The analysis was carried out using the PAST and Minitab software with 95% confidence. Subsequently, for the results of the nests in situ, analysis of variance (ANOVA) was carried out, as the data yielded a normal distribution. For the pens, the results showed that the data did not follow a normal distribution, so we proceeded to perform a non-parametric Kruskal–Wallis analysis. With these analyses, it was possible to evaluate whether there were statistically significant differences among the concentrations of microplastics in the nests. Likewise, Tukey comparisons were calculated to evaluate whether there were statistically significant differences among the amount of microplastics in the nests on each beach and to highlight that the nests that were different from each other.

## 3. Results

### 3.1. Abundance of Microplastics in Nests

Microplastics were found at each of the sampled sites, with a total of 275 pieces of microplastics found at all sampled sites (Figure 2). Pieces of microplastics were observed in 100% of the sampled nests, except for Corral 1, where 88.8% of microplastics were observed in the nests. The amount of microplastics among the nests of the sites did not

have statistically significant differences ( $p = 0.173$ ), nor were they observed among the pens ( $p = 0.194$ ), oscillating between a maximum average of  $3.78 \pm 3.50$  #MP/Kg SS presented at Navarro Beach (Beacon 31) and a minimum of  $1 \pm 0.84$  #MP/Kg SS at Raudal 1 Beach (Beacon 1) (Table 1).



**Figure 2.** Abundance of microplastics in each of the sampled sites.

**Table 1.** Average abundance of microplastics (#MP/Kg SS) for the sites sampled in November 2021.

Sampling Site	Average Abundance of Microplastics (#MP/Kg SS)
Beacon 1 Raudal Beach	$1 \pm 0.84$
Beacon 4 Raudal Beach	$1.61 \pm 1.24$
Beacon 13 Laurel Beach	$3.33 \pm 3.10$
Beacon 31 Navarro Beach	$3.78 \pm 3.50$
Pen 1 Raudal Beach	$1.88 \pm 2.37$
Pen 2 Laurel Beach	$1.81 \pm 1.21$

### 3.2. Concentration of Microplastics in Nests

The average concentration of the microplastic pieces was  $0.0067 \pm 0.0042$  mgMP/Kg SS, where the highest concentration of microplastics was found was in Navarro Beach (Beacon 31) with  $0.0139 \pm 0.0445$  mgMP/Kg SS, and the lowest concentration was found in Raudal 1 Beach (Beacon 1) with  $0.0030 \pm 0.0069$  mgMP/Kg SS (Table 2). In total, 0.842 g of microplastics were obtained in all the sampled sites.

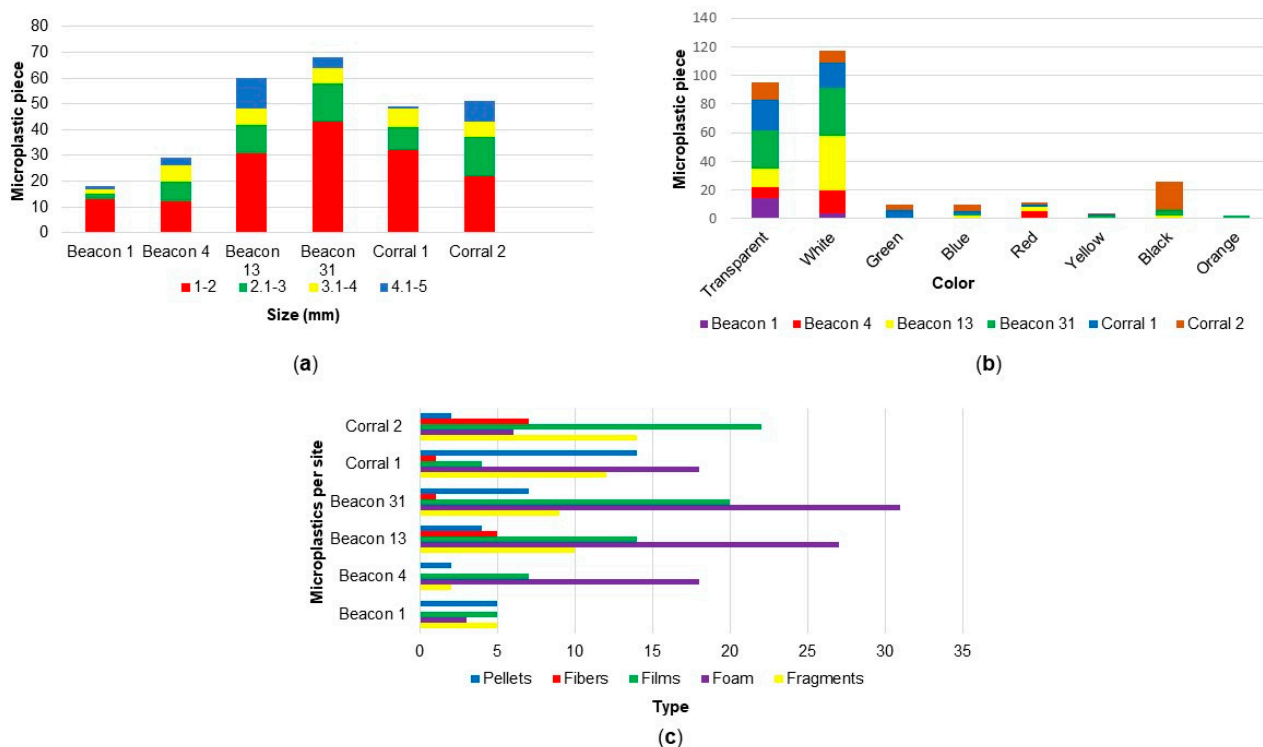
**Table 2.** Average concentration of microplastics (mgMP/Kg SS) for the sites sampled in November 2021.

Sampling Site	Average Concentration of Microplastics (mgMP/Kg SS)
Beacon 1 Raudal Beach	$0.0030 \pm 0.0069$
Beacon 4 Raudal Beach	$0.0038 \pm 0.0037$
Beacon 13 Laurel Beach	$0.0062 \pm 0.0078$
Beacon 31 Navarro Beach	$0.0139 \pm 0.0445$
Pen 1 Raudal Beach	$0.0095 \pm 0.0244$
Pen 2 Laurel Beach	$0.0040 \pm 0.0083$

### 3.3. Classification of Microplastics Found in Nests

Most of the microplastics found were within the range of 1–2 mm, followed by those of 2.1–3 mm (Figure 3a). In Figure 3b, it can be seen that the most abundant color was white, followed by transparent in the nests in situ; in the nesting pens, the most abundant color was transparent, followed by white. Regarding the type of plastic, it was observed

that the most common type was foam, with the exception of Corral 1, where the films predominated, and Beacon 1 in Raudal Beach, where the same percentage of pellets, films, and fragments were observed, with foam having a lower percentage (Figure 3c).



**Figure 3.** Classification of microplastics found by site on the beaches of Nautla, Veracruz: (a) by size, (b) by color, and (c) by type.

Among the results obtained, the presence of microplastics was found in all the nests studied at depths where sea turtle eggs are incubated. This indicates, first of all, that they are plastic particles that have been in the sediment for a long period of time and, secondly, that these could be affecting the success of the broods.

The site that obtained the highest concentrations of microplastics was Navarro Beach (Beacon 31), with an average abundance of  $3.78 \pm 3.50$  #MP/Kg SS; the one with the lowest concentrations was El Raudal 1 Beach (Beacon 1), with  $1 \pm 0.84$  #MP/kg SS. The ANOVA analysis of variance produced a value of  $p = 0.173$  ( $>0.05$ ), which indicated that there were no statistically significant differences among the sampling sites. Likewise, an ANOVA analysis was performed for each one of the sampling sites, which showed us whether there were statistically significant differences between the nests of Laurel Beach (Beacon 13) and the nests of Navarro Beach (Beacon 31). These were the two sites where the highest concentrations of microplastics were found, and this is related to anthropogenic activities that are carried out in the area—these two beaches have public access, which gives rise to a greater generation and accumulation of waste in the area.

In the pen nests, it was found that Pen 1 obtained a higher concentration of microplastics, with an average abundance of  $1.88 \pm 2.37$  #MP/Kg SS; the Kruskal–Wallis statistical analysis of the pens produced a  $p = 0.194$  ( $>0.05$ ), which indicated that there were no significant differences between the pens.

Regarding the characteristics of the microplastics found in the nests, the majority were 1 to 2 mm in size (56%), were foams (38%), and were white (43%). This indicates that they are plastic particles that have been exposed to the environment for a long time, so their size has been reduced considerably. On the other hand, the type and color of the microplastic indicates that they likely came from Styrofoam products, such as cups or disposable plates



or trays, which accumulate over time in the layers of sand on the beaches. This is why they could be found at depths from 19 to 82 cm.

### 3.4. Implications of This Work

This research work allowed us to address the current problem on the study of microplastics in Mexico by evaluating the presence of microplastics in sea turtle nests on the beaches of Nautla and Vega de Alatorre, Veracruz, one of the main areas Gulf of Mexico nesting. This allows us to establish a baseline for future studies on the implications and consequences of direct exposure of sea turtle eggs and hatchlings to microplastics. There were some limitations for the present study; it was not possible to perform spectroscopic analyses to determine the polymer present in the microplastics found. Likewise, it is worth mentioning that there are still no standardized methods or techniques for obtaining and processing the samples, which limits the comparison of the results.

## 4. Discussion

In total, an average abundance of  $2.430 \pm 2.668$  #MP/Kg SS and an average concentration of  $0.007 \pm 0.023$  mgMP/Kg SS of microplastics were obtained in the nests sampled in situ, and an average abundance of  $1.852 \pm 1.867$  #MP/Kg SS and an average concentration of  $0.0068 \pm 0.018$  mgMP/Kg SS of microplastics were obtained in nests of nesting pens. Although no other studies specifically on the concentration and abundance of microplastics in turtle nesting sites at depths greater than 30 cm have yet been reported in Mexico, the values obtained were comparable to similar studies on Mexican beaches. The results were within the range reported by Cruz-Salas et al., 2020 [28], who obtained an abundance of 1.06 #MP/Kg SS. Likewise, there are the studies carried out by Rosado et al., 2018 (93.27 #MP/Kg SS) [31], Piñon et al., 2018 ( $135 \pm 92$  #MP/Kg SS) [32], and Retama et al., 2016 [33] in which, although they present greater abundance per kilogram of dry sample, their samples were taken from superficial sand. Other studies carried out in turtle nesting areas, such as Beckwith and Fuentes, 2018 in the Northern Gulf of Mexico (FL, USA) [17], Duncan et al., 2018 in Cyprus [34], and Zhang, 2022 in Qilanyu (China) [35], recorded a higher abundance of microplastics at depths of 60 cm than those recorded in the present study. It is worth mentioning that these studies were carried out in sea turtle nesting areas at depths of 60 cm but not directly in the nests, as was carried out in the present study. The results of the classification by size coincide with what was reported by Cruz et al., 2020 in Zipolite Oaxaca Beach [13] and Álvarez et al., 2020 [36], who reported a higher percentage of microplastics in size of 1–2 mm for 35 Mexican beaches. As mentioned in the results, the most abundant color was white (mainly of the foam type, which can be related to disposable plates and cups), followed by transparent (mainly of the film type, which is related to bags and wrappers) for the four beaches, results that coincide with what was reported by Beckwith and Fuentes, 2018 [17], Beltrán et al., 2019 [37], Álvarez et al., 2019 [38], Cruz et al., 2020 [28], Álvarez et al., 2020 [36], Ríos et al., 2021 [39], and Zhang et al., 2022 [35]. The colors with the least presence were green, yellow, and blue (these were mainly in the form of fragments). The most common types of microplastics found were foams, followed by films, which agrees with the study by Zhang et al., 2022 [35], who also found foams in a higher percentage, indicating that the deeper the sand sampling, the higher the percentage of accumulated foams. This may be due to the fact that on the surface, due to the action of the wind, they are easier to transport, but once they accumulate on the sand, they remain for long periods of time. The type of plastic with the least presence in the nests were the fibers; it has been observed that the fibers have a greater abundance in studies carried out in water. The result obtained from the classification by type of microplastic do not agree with what was established by Beltrán et al., 2019 [37], Álvarez et al., 2019 [38], Álvarez et al., 2020 [36], or Ríos et al. 2021 [39], who obtained a greater presence of fragments; in addition, Cruz et al., 2020 [28] obtained a greater presence of fibers in their study.

Within the study area, there are sources of contamination by solid waste, which leads directly to the contamination of plastic waste and, therefore, to contamination by microplastics.

Beacon 1, located on El Raudal Beach, is located at a distance of 771 m from the mouth of the Misantla River, which can be one of the main sources of contamination of the site since it transports plastic waste and other contaminants towards the sea [40]. These come from the surrounding towns through which the river passes, and these can contain bottles, bags, clothes, and containers. At Beacon 4, also located on El Raudal Beach, there is the presence of agricultural activities, in which an endless number of plastic products are used, such as containers for agrochemicals, fertilizers, pipes, and other containers. In addition, agricultural runoff can also incorporate degraded plastic residues from greenhouse films, plastic mulch, irrigation systems, and planter boxes [41]. In Beacon 13, located in Playa Laurel, there are livestock, agriculture, and extractive fishing activities, which contribute to plastic pollution through the generation of fibers from the degradation of ropes and nets [20], and the use of plastic products, such as containers and balls, which degrade due to the action of the sun and salt and generate microplastics throughout their useful life. Likewise, once the life time of the nets is over, they are abandoned adrift, causing what is called ghost fishing. Finally, Beacon 31—located in Playa Navarro, 169 m from the mouth of the Laguna Grande—is a tourist beach, so it has public access, bathrooms, and local shops. There is also livestock, agriculture, and fishing in the zone, with three fishing cooperatives. In addition to the anthropogenic activities in the area, there are sources of plastic pollution derived from meteorological phenomena (rain, wind, and hurricanes) when a meteorological phenomenon occurs, and this contributes to the transport of plastic waste from rural and urban areas, either through the wind or through stormwater runoff, among which is found car tires and road paint [41]. Ocean currents are another source of plastic pollution since plastics, having a low density, are easily carried away by currents and end up accumulating on garbage islands or directly on the beaches.

## 5. Conclusions

The present investigation allowed us to evaluate, by means of their quantification and characterization, the presence of microplastics in turtle nests within the nesting zone of sea turtles under the charge of the Veracruz Center for Research and Conservation of the Sea Turtle (CVICTM), located in the coastal zone of Nautla and Vega de Alatorre, Veracruz.

Compared with other studies carried out at depths of approximately 60 cm, we found that the concentrations of microplastics obtained in the present investigation are lower, but this does not mean that it is not a matter of concern. This warrants intensifying sustainable management alternatives that avoid, as much as possible, the use of plastics, thus avoiding the generation of microplastics.

The study of microplastics in Mexico is an area of opportunity since currently it focuses only on the presence and characterization of microplastics. However, it is necessary to know the impacts that they can have on different ecosystems, their relationship with other contaminants, their presence in the species of greatest human consumption, as well as their role in degradation studies.

Finally, it is recommended to continue with the line of studies of microplastics directly in the turtle nests, since these could be affecting the success of the clutches through toxicology and the sex ratio, in addition to the problems that they can cause the hatchlings that are emerging from the nests.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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