RESEARCH PAPER



Microplastics Abundance, Characteristics, and Risk in Badagry Lagoon in Lagos State, Nigeria

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Abstract

Microplastics are widely used to manufacture diverse products such as textiles, skin care products, and household products such as detergents and soaps. However, microplastic pollution and its potential health risks are raising concerns worldwide. This study characterized and determined the safety of microplastics in water and sediments obtained from three locations, namely Ibeshe, Amuwo Odofin, and Ojo along Badagry lagoon, Lagos, Nigeria. The samples of the lagoon's surface water and sediments were treated and analyzed for the abundance of microplastics, as well as their shapes, sizes, and types of polymers. The risk index of the polymers in the microplastics was also estimated. Microplastics were found to be more abundant ($p \le 0.05$) in the sediments (283–315 particles/kg) than in the surface water (108–199 particles/L). In both the water and sediments at all the locations, the dominant shapes were fibers (52%-90%), followed by fragments (3%-32%) and films (1%-25%). In order of significance, the microplastic size range of 0-100µm and 100-500µm dominated the surface water, while the size range of 1000-5000µm and 500-1000µm dominated the sediments at all the locations. The dominant polymers in both the water and sediments at all the locations were polyethylene, polypropylene, and polyamide, while the least was polystyrene. In both the water and sediments at all the locations, the dominant risk score among the polymers is III (moderate risk). The results obtained suggest that microplastic pollution poses environmental and health risks to the lagoon, aquatic organisms, and humans. As such, the lagoon required microplastic remediation and control.

Keywords: fibers, lagoon, microplastics, polyethylene, polymers

INTRODUCTION

Microplastics are tiny pieces of plastic with a size ranging from 1 to 5 mm (0.2 inch) in length and are released into the environment through plastic pollution (Frias and Nash, 2018). Microplastics consist of carbon and hydrogen atoms bound together in polymer chains (Rogers, 2020). They also contain some additives such as polybrominated diphenyl ethers, tetrabromobisphenol A (TBBPA), and phthalates, all of which can leach from plastics into the environment (Rogers, 2020). Microplastics can be categorized into types: primary and secondary microplastics (Lehtiniemi *et al.*, 2018). Primary microplastics are microscopic (less than 1 mm) microplastics called microbeads that are manufactured for the production of products such as facial scrubs, exfoliators, cleansers, soaps, detergents, and plastic fibers used in synthetic textiles (e.g.,

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nylon), among others (Miraj *et al.*, 2019). One gram of a facial scrub can contain an average of 20,860 microbeads (Cheung and Fok, 2017). Primary microplastics enter the environment by discarding the mentioned products as waste or accidental loss in industry or transportation. Secondary microplastics, which are produced from the photo-degradation of bigger plastics, are significantly more prevalent than primary microplastics (Hale *et al.*, 2020). This breakdown is mediated by solar ultra violet radiation, wind, currents, and other natural factors (IUCN, 2022). Microplastics can have regular or irregular shapes and come in a variety of shapes, including fragments, spheres, and fibers (Hartmann *et al.*, 2019). Microplastics continue to break down and, over time, become nanoplastics (Hartmann *et al.*, 2019). Thus, microplastics are a transitional state between macrodebris and nanomaterials (Besseling *et al.*, 2018).

In recent times, microplastics have been widely distributed in the environment, so much so that the magnitude of their pollution and possible consequences are raising concerns globally (Bonanno and Orlando, 2018; Koleayo *et al.*, 2021). Microplastics are non-biodegradable and are thus persistent, making them accumulate in the environment (Rogers, 2020). Over 300 million metric tons of plastic are produced annually, of which not less than 14 million metric tons end up in the ocean every year (IUCN, 2022). In Nigeria, over 60 million plastic sachets of water are consumed and disposed of daily (Dumbili and Henderson, 2020). Countless single-use plastic shopping bags and takeaway packs are also disposed of on land and water bodies (Dumbili and Henderson, 2020). These microplastics may accumulate in the seabed sediments by sinking through the water column or through currents and sediments carried down continental slopes (Barrett *et al.*, 2020). From the water or sediments, microplastics can lodge in the digestive tracts and tissues of many species of wildlife, including fish and shellfish (Smith *et al.*, 2018). Humans and other animals can also breathe in microplastics, which can get into their lungs (Amato-Lourenço *et al.*, 2020).

Although the health hazards of microplastics are currently sketchy, they have been categorized into physical and chemical effects (Claudia *et al.*, 2020). The physical effects are influenced by microplastics' sizes, shapes, and concentrations, while the chemical effects depend on the toxic compounds in the microplastics (Claudia *et al.*, 2020). Toxic compounds in microplastics include additives and polymeric raw materials as well as chemicals absorbed from the environment (Hahladakis *et al.*, 2018). Birds, fish, and other aquatic organisms often mistake floating microplastics for food and ingest them, resulting in less food intake, therefore less energy, and even toxicity (Lusher *et al.*, 2017). Microplastics can bioaccumulate through the food chain, from zooplankton and small fish to predators (Rogers, 2020). Humans can ingest microplastics through the consumption of aquatic life, and they have been detected in human stools, tissues, and organs (Rogers, 2020). Microplastic pollution can harm food safety and quality, human health, and tourism on the coast. It also contributes to climate change.

Considering the threat posed by microplastic pollution, there is a need to determine the pollution status of every body of water. This will help to develop effective pollution control and remediation strategies as well as policies. There is a dearth of documented studies on microplastic pollution in Badagary Lagoon in Lagos, Nigeria. This study determined the abundance, sizes, and polymer types of microplastics found in surface water and sediments of Badagry Lagoon in Lagos, Nigeria. It also looked at the health risks of the polymers.

MATERIALS AND METHODS

This study was carried out on Badagry Lagoon in Badagry Town, Lagos State, Nigeria (Figure 1). Lagos, the state's capital, is one of the world's fastest growing megacities and the most industrialized in Nigeria, making it an economic haven in Africa. Lagos' landmass is very small, totaling about 3577 km², consisting of several water bodies, including the lagoons, creeks, rivers, streams, and estuaries. In its northern and eastern parts, Lagos borders Ogun State, and



Fig. 1. Map of Badagry lagoon in Lagos indicating the sampling locations

in its southern and western parts, it borders the Atlantic Ocean and the Republic of Benin, respectively. The climate and vegetation of the state are mainly tropical and wet.

Badagry town is the headquarters of Badagry local government, located along the Atlantic Ocean. The Badagry lagoon is about 60 km long and 3 km wide and lies between longitudes 3°0' and 3°45' E and latitudes 6°25' and 6°30' N (Figure 1). The lagoon's water is mildly brackish. The lagoon is among the stretch of several lagoons and creeks that dot Nigeria's coastline from the Republic of Benin to the Niger Delta in Nigeria. The Badagry Lagoon delivers several ecosystem services, including fishing, aquaculture, trading, sand dredging, flood protection, transportation, tourism, and cultural activities, among others. Unfortunately, it receives enormous industrial and domestic waste, among which is plastic waste. This waste could have an impact on the lagoon's water and life, as well as humans that use and consume the lagoon's water and aquatic organisms. Hence, the need for the current study.

Samples of Badagry lagoon's water and sediments were collected at three locations (Ibeshe, Amuwo Odofin, and Ojo) between October 2021 and February 2022. The water samples were collected below the water surface in a pre-cleaned jar. The sediments were collected with a Van veen grab sampler at about 20 cm deep into a pre-washed jar, and all samples were stored at 4 °C in the laboratory pending analyses.

Each of the water samples was filtered with glass fiber filter paper and then digested with 50 ml of hydrogen peroxide and agitated for 5 days. The sediments were weighed, oven-dried, weighed again, and digested as done for the water samples. The digest was transferred into a separating funnel containing an aqueous potassium formate solution. At the end, the lowest water phase in the funnel was filtered with a nanopore inorganic membrane filter (pore size: 0.2μ m) to obtain the microplastics. The filter was covered, air-dried at room temperature, and

Risk category Polymer abundance Polymer type Score Polypropylene (PP) <10 I 1 Polyethylene (PE) 11 10-100 Π 30 100-1000 III Polystyrene (PS) Ethylene vinyl acetate (EVA) 22 >1000 IV Polyamide (PA) 47

Table 1. Microplastic polymer score (Lithner et al., 2011)

Table 2. Mean abundance of microplastics in Badagry Lagoon water and sediments

Location	Water (particles/L)	Sediment (particle/kg)	P-value	
Ibeshe	199.0±65.5	243.3±49.7	0.403	
Amuwo Odofin	157.7±53.0	315.7±27.3	0.010^{*}	
Ojo	108.0±18.7	283.0±40.3	0.002*	

Note: values on the same row with an asterisk are significantly different at $p \le 0.05$ (Student's *t*-test)

stored in an airtight container in order to prevent contamination.

The water and sediment membrane filters were examined under a digital microscope for visual counting (abundance) and determining the shapes and sizes of the microplastics. The abundance of the microplastics in the water was expressed as the number of particles per liter, while the sediment samples were expressed as particles per kg. The shapes of the microplastics were classified as fibers, fragments, and films. The microplatics' sizes and polymers were determined by FTIR spectroscopy and classified as 0-100 μ m, 100-500 μ m, 500-1000 μ m, and 1000-5000 μ m.

Non-plastic materials were used for sampling at the lagoon to prevent microplastic contamination. Plastic-free laboratory wears such as coats and gloves were worn during analyses, and all materials and instruments used were washed thoroughly with ultrapure water. All chemicals used were filtered with a glass microfiber membrane with a 0.45 μ m pore size and stored in glassware covered with aluminum foil to prevent microplastic cross-contamination.

The risks of microplastics in the water and sediments were calculated from the risks of polymers in the samples using equation 1.

 $PRI = \sum PPT \times PS$

(1)

From equation 1, *PRI* stands for polymer risk index, *PPT* represents the percent of polymer types collected at each sampling station, and *PS* is the polymer score (Table 1). Table 1 also shows polymer abundance and risk category.

Excel software version 22 was used to present values as mean \pm standard deviation (SD). The graphs were drawn using Minitab software version 16.0. The Student's *t*-test was used to evaluate the significance difference between microplastic abundance in the sediments and surface water, in which $p \le 0.05$ was considered statistically significant.

RESULTS & DISCUSSION

Table 2 shows the abundance, shapes, and sizes of microplastics in the water and sediments obtained from Badagry Lagoon. In the water samples, Ibeshe had the highest concentration of microplastics (199 particles/L), followed by Amuwo Odofin (157 particles/L), and Ojo (108 particles/L). In the sediment samples, Amuwo Odofin had the highest concentrations of microplastics (315 particles/kg), followed by Ibeshe and Ojo (283 particles/kg each).

The percentage distribution of microplastic shapes in the water and sediments is depicted



Fig. 2. Shapes of microplastics in water samples obtained from Badagry Lagoon



Fig. 3. Shapes of microplastics in sediment samples obtained from Badagry Lagoon

in figures 2 and 3. In the water samples (figure 2), Ojo had the highest percentage of fibers (83.02%), followed by Amuwo Odofin (61.78%) and Ojo (53.27%). Microplastic fragments were detected in Ibeshe at 33.67%, Amuwo Odofin at 26.75%, and Ojo at 15.09%. Microplastic films were present in Ibeshe, Amuwo Odofin, and Ojo at 13.07%, 11.46%, and 3.77%, respectively. In the sediment samples (figure 3), Amuwo Odofin had the highest percentage of fibers (88.89%), followed by Ojo (71.18%), and Ibeshe (53.91%). Ojo had the highest percentage of microplastic fragments (27.78%), followed by Ibeshe (21.81%), and Amuwo Odofin (6.61%). Microplastic films were recorded in Ibeshe at 24.28%, Amuwo Odofin at 4.44%, and Ojo at 1.04%, respectively. Overall, fibers, fragments, and films, in that order, were the dominant shapes in both the water and sediments.

Figure 4 reveals the percentage distribution of microplastic sizes in the water samples in which Ibeshe had the highest percentage (67.38%) of the 0-100 μ m range of microplastic size, followed by Amuwo Odofin (54.73%), and Ojo (48.92%). With 30.47%, Ojo recorded



Fig. 4. Size distribution of microplastic particles in the surface water samples obtained from Badagry Lagoon



Fig. 5. Size distribution of microplastic particles in the sediment samples obtained from Badagry Lagoon

the highest percentage of microplastics in the size range of $100-500\mu m$, followed by Ibeshe (25.08%), and Amuwo Odofin (19.14%). In the microplastic size range of $500-1000\mu m$ and $1000-5000\mu m$, Amuwo Odofin recorded the highest percentages (12.59% and 13.54%), followed by Ojo (10.59% and 10.01%), and Ibeshe (3.43% and 10.10%), respectively. Figure 5 shows the percentage of microplastic sizes in the sediment samples in which the highest percentage of the $1000-5000\mu m$ microplastic size was observed in Ibeshe (54.18%), followed by Ojo (48.76%), and Amuwo Odofin (45.17%). Amuwo Odofin had the highest percentage of microplastics in the size range of $500-1000\mu m$ (30.09%), followed by Ibeshe (25.45%), and Ojo (25.32%). In the particle size range of $100-500\mu m$, Ojo accounted for 18.99%, Amuwo Odofin (14.05%), and Ibeshe (10.61%). The microplastic size of 0-100 μm was 10.68% in Amuwo odofin, followed by Ibeshe (9.73%), and Ojo (6.93%). Overall, the microscopic size range of $0-100\mu m$ was the most dominant size in the surface water, while the size range of $100-5000\mu m$ was the most dominant in the sediments.



Fig. 6. Percentage abundance of polymers in surface water obtained from Badagry lagoon



Fig. 7. Percentage abundance of polymers in sediment obtained from Badagry lagoon

Figures 6 and 7 show the percentage distributions of polymer types in Badagry lagoon's surface water and sediments. In the surface water, polyethylene was the dominant polymer (32.51% in Ibeshe, 27.17% in Amuwo Odofin, and 24.68% in Ojo), followed by polypropylene (27.07% in Ojo, 25.05% in Amuwo Odofin, and 24.84% in Ibeshe), polyamide (29.14% in Ojo, 21.66% in Amuwo Odofin, and 14.70% in Ibeshe), ethylene vinyl acetate (18.22% in Ibeshe, 16.10% in Amuwo Odofin, and 12.74% in Ojo), and polystyrene (9.73% in Ibeshe, 10.02% in Amuwo Odofin, and 6.37% in Ojo). The most abundant polymer in the sediments was polyethylene (30.51% in Ojo, 29.57% in Amuwo Odofin, and 25.75% in Ibeshe), polyamide (19.19% in Ojo, 19.57% in Amuwo Odofin, and 17.87% in Ibeshe), ethylene vinyl acetate (14.34% in Ojo, 16.73% in Amuwo Odofin, and 18.56% in Ibeshe), and polystyrene (6.26% in Ojo, 7.39% in Amuwo Odofin, and 9.28% in Ibeshe).

Tables 3 and 4 reveal the hazard risk index of the polymers in the surface water and sediments.

	Polypropylene	Polyethylene	Polystyrene	Ethylene vinyl acetate	Polyamide
Ibeshe	24.84	357.61	291.90	400.84	690.90
Amuwo Odofin	25.05	188.87	300.60	354.20	1,488.02
Ojo	27.07	271.48	191.10	280.28	1,369.58
Risk level	II	III	III	III	IV

Table 3. Hazard risk index of polymers in the surface water obtained from Badagry lagoon

Note: I = very low risk, II = low risk, III = moderate, IV = high, and V = very high (Yaun et al., 2022)

Table 4. Hazard risk index of polymers in the sediments obtained from Badagry lagoon

	Polypropylene	Polyethylene	Polystyrene	Ethylene vinyl acetate	Polyamide
Ibeshe	25.75	313.94	278.40	408.32	839.89
Amuwo Odofin	26.74	325.27	221.70	368.06	919.79
Ojo	29.70	335.61	187.80	315.48	901.93
Risk level	II	III	III	III	III

Note: I = very low risk, II = low risk, III = moderate, IV = high, and V = very high (Yaun et al., 2022)

In the surface water, polyethylene, polystyrene, and ethylene vinyl acetate from all the locations recorded risk level III, while polypropylene showed risk level II, and polyamide showed risk level IV. In the sediments, polyethylene, polystyrene, ethylene vinyl acetate, and polyamide recorded risk level III in all the locations, and polypropylene showed risk level II.

The current study was carried out to determine the levels, spread, and risk of microplastics in the surface water and sediments obtained from Badagary Lagoon in Lagos, Nigeria. Table 2 shows that sediments from the lagoon had more microplastics than the surface water at all the sampling locations. This result is consistent with that of Olarinmoye et al. (2020), who detected more microplastics in sediments than in water obtained from Lagos lagoon. Peng et al. (2018) also found more microplastics in the deep bottom water of the Mariana Trench in the Pacific Ocean than those in the subsurface water. Moreover, Li et al. (2020) detected more microplastics in sediments than in surface water in the Yangtze Estuary, Chongming Island, China. The detection of microplastics in both the surface water and sediments in this study portends danger for aquatic life and organisms that consume them. Microplastic pollution can change the water chemistry, ecology, and life span of an aquatic ecosystem. Furthermore, microplastics can accumulate in aquatic organisms and cause tissue damage, oxidative stress, and antioxidant depletion (Bhuyan, 2022). Aquatic organisms may also show reproductive and neurotoxic damage as well as growth retardation through blockage of the digestive system and changing feeding patterns (Bhuyan, 2022). Microplastics in water may eventually be deposited on land, altering its geochemistry and causing environmental stress (Allouzi et al., 2021). Once ingested by humans or other terrestrial animals, microplastics can induce several health hazards (Rahman et al., 2021). Microplastics can spread harmful microorganisms and toxic chemicals, like heavy metals, to humans (Rahman *et al.*, 2021).

Figures 2 and 3 show that, in both the sediments and water samples obtained from all the locations, fibers were the most dominant shape of the microplastics, followed by fragments and films in that order. This result is in line with that of Olarinmoye *et al.* (2020), who reported the dominance of microplastic fibers in water and sediments obtained from Lagos Lagoon. Lenaker *et al.* (2018) and Jorquera *et al.* (2022) also reported the dominance of fibers in the water and sediment samples obtained from Milwaukee River Basin and Chilean fjords, respectively. Additionally, Xu *et al.* (2018) reported the dominance of fibers, followed by fragments and films,

in the samples of water and sediments obtained from Changjiang Estuary in China. According to Olarinmoye *et al.* (2020), the probable sources of fibers in lagoons include domestic wastewater, sewage disposals, laundering, erosional discharges, urban runoffs, and water currents from an adjoining ocean. In contrast to the results of the current study, Ilechukwu *et al.* (2019) found more fragments in sediments obtained from four beaches in Lagos, Nigeria. Fred-Ahmadu *et al.* (2020) also detected more fragments in sediments obtained at various sampling locations in the Atlantic Ocean, Lagos. Similarly, in the study conducted by Plastic Atlas (2020), the most dominant microplastic shape found in water samples at a Lagos beach was fragment. According to Plastic Atlas, the main source of microplastics at Nigerian beaches is plastics dumped by tourists and washed off onto the beaches as secondary microplastics. In addition, plastics can be transported by rivers and storm water from inland to the ocean and end up on shores as microplastics.

Figures 4 and 5 show the sizes of the microplastics in the surface water and sediments, respectively. In the surface water (figure 4), light microplastics, mainly 0-100µm and 100-500µm, were the most dominant microplastics, while in the sediments, dense microplastics, mainly 1000–5000µm and 500–1000µm, were the most dominant. This result is in line with a systematic review and meta-analysis comprising 39 articles by Erni-Cassola et al. (2019) in which polymers segregate in the water column down to the sediments based on density, with lower density dominating the surface water and denser microplastics found majorly in the sediments. Vermaire et al. (2017) also reported higher concentrations of dense microplastics in sediments than in the surface water in Ottawa River, Canada, and its tributaries. Moreover, Li et al. (2021) reported higher levels of dense microplastics in sediments than in surface water in Guangdong Coastal Areas, South China. Lenaker et al. (2019) also reported decreased deposition of low density microplastics in the water column to the sediments in Milwaukee River Basin in Wisconsin, United States, while high density microplastics did the opposite. However, the results contradict those of Li et al. (2020), who observed a higher concentration of dense microplastics in sediments than in the surface water in Yangtze Estuary, China. Furthermore, the result contradicts that of Blankson et al. (2020), who found that both the light and dense microplastics were distributed evenly in the surface water and sediments of the high velocity Densu River in Ghana. In the same study, Blankson and colleagues found that dense microplastics floated in the stagnant water of a dam in Ghana. This demonstrates that several factors, including microplastic weight and water velocity, influence the distribution of microplastics in the water column and sediments (Shamskhany et al., 2021). Rivers with a high velocity flow will deposit fewer microplastics in the sediments, while low velocity water will do the opposite (Olarinmoye *et al.*, 2020). Regarding density, dense microplastics, especially those that are denser than seawater (1.02 g/cm³), will likely sink to the bottom and build up in the sediments (Xu *et al.*, 2018).

Figures 6 and 7 reveal the abundance of each polymer type in the water and sediment samples, respectively. In the water samples (figure 6), polyamide, polyethylene, and polypropylene were fairly uniformly abundant in all the stations, followed by ethylene vinyl acetate, while polystyrene was comparatively very low. In the sediment samples, polyethylene was the most abundant polymer in all the locations, followed by polypropylene, while polystyrene was the least. On average, polyethylene and polypropylene were the most abundant polymers in the water and sediment samples, while polystyrene was the least. This result is consistent with that of Olarinmoye *et al.* (2020), who reported the dominancy of polypropylene and polyethylene in water and sediments obtained from Lagos lagoon. In the study conducted by Fred-Ahmadu *et al.*, polyethylene and polypropylene were the most abundant polymers recorded. However, unlike in the current study, polystyrene was abundant too. In the study carried out by Osorio *et al.* (2021) in Manila Bay, West Philippines, polypropylene and polyethylene were also the most dominant. In the systematic review and meta-analysis by Erni-Cassola *et al.* (2019), polyethylene and polypropylene were the most dominant polymers were the most dominant polymer were the most dominant polymer were the most detection in

several studies, polyethylene and polypropylene could be the most dominant polymers in water bodies, including the current study. Polyethylene is made from hydrocarbon fuels like petroleum oil and has a wide range of applications, including plastic bags, plastic films, geomembranes, and containers, such as bottles, among others. Polypropylene is also made from substances that are derived from hydrocarbon fuels and, among its many uses, it is important in the manufacturing of usable textiles (Sewport, 2020). This suggests that most of the microplastics in the Badagry lagoon came from items like polythene bags, plastic bottles, and laundry done in the lagoon's water.

The risk levels of the various polymers detected in the water and sediments are shown in Tables 3 and 4, respectively. In the water samples (Table 3), polyethylene, polystyrene, and ethylene vinyl acetate recorded risk level III, polyethylene showed risk level II, and polyamide showed risk level IV. In the sediments (Table 4), polyethylene, polystyrene, ethylene vinyl acetate, and polyamide had risk level III, while polyethylene showed risk level II in all the locations. This shows that the dominant risk level among the polymers is III. This is consistent with Xu *et al.* (2018), who reported risk level III as the dominant polymer risk in Changjiang Estuary in China. However, Fred-Ahmadu *et al.* (2020) reported the dominance of risk level I in water samples obtained from different sampling locations in the Atlantic Ocean, Lagos. Rakib *et al.* (2021) and Ranjani (2022) reported high-risk polymers in sediments obtained from Karnaphuli River Estuary, Bangladesh and southeast coast of India, respectively. The results of the current study showed that polyamide posed the most significant risk to aquatic organisms and organisms that consume them. Polyamide occurs naturally or artificially and is used in the fabric industry (Sewport, 2022). So, its occurrence in the Badagry lagoon water and sediments could be through clothes laundering.

CONCLUSION

From the results, it can be concluded that Badagry lagoon's surface water and sediments contained abundant microplastics, with sediments having more. The three dominant shapes among these microplastics are fibers, fragments, and films, with fibers being the most dominant. On average, the microplastic sizes were uniformly distributed at all the locations, with sizes ranging from less than 100 to $5000\mu m$. The two most dominant polymer types in both the water and sediments at all the stations were polyethylene and polypropylene, while polystyrene was the least common. The dominant risk score for these polymers is III, which suggests that the microplastics in the lagoon may induce some health and environmental risks. Though polyamide was not common in the lagoon, it has a risk score of IV, indicating that it poses a high risk.

Based on the findings of this study, the following are suggested:

- There is a need for microplastics remediation and control in the lagoon.
- Dumping of plastic materials in and around the lagoon should be discouraged.

• Plastic recovery and recycling should be enhanced to keep plastic materials away from the environment.

• Laundry of fabrics in the lagoon should be discouraged.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct,

data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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