

## Investigation of Microplastic Contamination in Sediments, Water and Aquatic Biota in Lake Beratan, Tabanan Regency, Bali Province – Indonesia

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### ABSTRACT

Microplastics (MPs) has become a very serious global threat, especially to the health of animals, humans and the environment. In this study we aim to investigate the contamination of MPs in sediment, air, and aquatic biota collected from the waters of Lake Beratan in Bali Province, Indonesia. Lake Beratan is one of the strategic areas that continues to develop as a tourist destination, agriculture and fisheries, as well as a source of water for daily needs. Sediment, water, and aquatic biota samples were collected from 4 stations that represent the Beratan Lake area. Each sample obtained was then followed by the pre-treatment stage using 5 M NaCl solution. Furthermore, the extraction process for each sample used wet oxidation peroxidation (WPO) with the addition of Fe (II) catalyst. The highest average weight of MPs in the sediment samples was obtained at the 1st sampling point of 2.53% which was dominated by soft fragments. The percentage of MPs weight in the water samples was varied at each sampling point with the highest obtained at the 4<sup>th</sup> point of 99.2% and was dominated by hard-shaped fragments at all sampling points. The collected aquatic biota was also contaminated by MPs of 68% in fish and 36.40% in shrimp. The types of MPs found in the digestive tract of aquatic biota are in the form of fragments, filaments, films and foam. These results indicate that the aquatic environment of Lake Beratan, Bali has MPs content that varies in sediment, water and aquatic biota which are often consumed by the local community. This must be of particular concern and further investigation, especially regarding the environmental management of the lake area and the relevant authorities in formulating regulations to reduce the harmful effects of MPs contamination.

**Keywords:** microplastics, sediment, water, freshwater contamination, Beratan Lake.

### INTRODUCTION

Plastic is a basic material that is often used in the manufacturing of household appliances and other objects because of its relatively low manufacturing cost, durability and outstanding structural characteristics. Plastics manufacturing has experienced a very rapid increase since its start in the 1950s and is projected to continue into the next decades (Geyer et al., 2017). Behind its advantages, plastic also acts as a source

of contaminants that have permeated all environmental matrices, especially in aquatic environments (Khan et al., 2018). This happens because of its durable structure, plastic can last in the environment for hundreds of years (Hermabessiere et al., 2017). Environmental pollution caused by plastic has become a major concern around the world and is unlikely to disappear in the near future (MacLeod et al., 2021).

Although plastics are considered to be inert particles, plastics called macroplastics (>2,000 µm)

can fragment over time into smaller fractions to further form microplastics (MPs) (Bermúdez and Swarzenski, 2021). The decomposition of plastic sizes into MPs occurs under the influence of UV radiation, weathering processes, and natural aging in the environment. These MPs particles can be found in all aquatic ecosystems and in drinking water. MPs range in size from 1  $\mu\text{m}$  to 5 mm. Microplastics can come from various sources such as industry, textiles, food packaging, and waste plastic products (Tsering et al., 2022).

In aquatic ecosystems, MPs contamination has been found in seawater (Zobkov et al., 2019), lakes (Koelmans et al., 2019; Uurasjärvi et al., 2020; Whitaker et al., 2019), mangrove waters (Paes et al., 2022), and beach and bottom sediments (Leslie et al., 2017; Matsuguma et al., 2017). Based on the literature review report, MPs contamination in moving water such as rivers is more common than in calm waters such as lakes. On the other hand, the study also limited data on MPs contamination in lake ecosystems in rural areas (Talbot and Chang, 2022). It is also interesting that MPs have also been found in various aquatic biota including shellfish and fish (Yuan et al., 2019), and have been found to be able to contaminate the entire planktonic food chain (Setälä et al., 2014). On the other hand, MPs can be vectors that provide pathways for contaminants and other diseases to spread to the environment and can more easily affect human health (Besseling et al., 2019), but more research is needed, especially on the risks of MPs exposure to human health (Sripada et al., 2022; Thomas et al., 2021).

Bali one of the largest tourist destinations in Indonesia both domestically and abroad because of its natural and cultural beauty (Permatasari et al., 2022). However, current conditions show that Bali is struggling with the problem of plastic waste which continues to increase. A 2021 report shows that as much as 33,000 tons of plastic waste pollutes the waters in Bali including rivers, lakes and marine ecosystems (Systemiq, 2021). Plastic waste that has entered sea waters is unlikely to spread far and will head to other beaches in the Indonesian archipelago causing a build-up of waste on the beaches (Joe, 2021). People's habit of throwing plastic waste carelessly, especially near rivers and lakes, is a major factor in MPs contamination in aquatic ecosystems. Environmental factors such as rainfall, wind, and other factors affect the spread of the plastic waste. Finally, through a long process (physics, chemistry,

and biology) breaks down macroplastic into small pieces (Sajjad et al., 2022).

Lake Beratan is one of the most iconic lake ecosystem in Bali which is known for its natural beauty and strategic location adjacent to the route between Tabanan and Buleleng Regencies. Lake Beratan has various supporting facilities for tourism, especially water tourism which includes boat facilities, floating restaurants and lodging adjacent to the lake environment. Based on this, Lake Beratan is prone to be area with high levels of plastic pollution, so it is very important to investigate MPs pollution in sediment, water, and aquatic biota in this lake. Recently, research on MPs contamination has been reported at several tourist beaches in Badung Regency such as Doublesix Beach, Kuta Beach, Melasti Beach, Mengiat Beach, and Tanjung Bena Beach with three types of MPs (film, fiber, and fragments) (Mauludy et al., 2019). A total of 13 types of MPs polymers with the most common fragment forms were reported in the waters of Bena Bay, Bali (Suteja et al., 2021). Likewise, reports of MPs found in commercial marine biota such as lemuru fish, mackerel, flying fish, and layur fish collected from the waters of the Bali Strait indicate MPs from several polymers such as polyvinyl chloride (PVC), polyamide (PA), polycarbonate (PC), and polyethylene (PE) (Sarasita et al., 2020). However, to the best of our knowledge, there has been no study of MPs contamination in the aquatic environment of Lake Beratan in Bali Province.

The purpose of this study is to determine the percentage, average weight, number of fragments, and types of MPs in sediment, water, and aquatic biota collected from Lake Beratan, Tabanan Regency, Bali Province. We discuss this issue based on the results of field observations and laboratory analysis of sediment, water and aquatic biota samples in Lake Beratan which are then compared to other lake environments in the world (Parvin et al., 2022). To our knowledge, this is the first study to assess MPs contamination in Lake Beratan, Tabanan Regency, Bali Province.

## MATERIAL AND METHODS

### Area of study

The samples are taken from Lake Beratan, which is located in the Bedugul area, Candikuning Village, Baturiti District, Tabanan

Regency, Bali Province. This area is dominantly used as an area for agriculture, nature recreation (ecotourism), and fish farms. In addition, Lake Beratan is also included in the area that is consecrated by religious circles in the Province of Bali. Beratan Lake is very vulnerable to anthropogenic pollution caused by the activities of the surrounding community. We chose several sampling points that were adjusted to the sediment, water, and aquatic biota from the lake environment so that we could know the overall MPs situation. Sampling of sediment, water and aquatic biota (fish and shrimp) were collected from 5 stations representing the Lake Beratan area (Figure 1).

### Pre-treatment of microplastics

Samples were collected using Ekman Grab. At each station, 3 samples will be taken with a distance of  $\pm 5$  m. The three samples from each station are composited and air-dried. Samples of sediment, water and aquatic biota that have been collected are labeled and put into sterile airtight plastic clips for sediment and aquatic biota samples while glass bottles are for water samples (Rosiana et al., 2022; Wasilah et al., 2021). The samples are then placed into a cool box to prevent exposure to sunlight or environmental influences. 5 M NaCl solution was prepared by dissolving solid NaCl using distilled water with a stirrer. The tools used are sterilized beforehand to prevent MPs contamination outside the test sample.

### Microplastics extraction

#### Sediment extraction

Sediment samples were then separated using a 5 mm sieve to separate the MPs component from other materials. The samples were then processed using wet peroxidation (WPO) with the addition of Fe (II) catalyst to separate the organic matter without changing the MPs flakes. Wet oxidation peroxide sample mixture was added with NaCl to separate heavier materials for sinking and floating MPs.

#### Water extraction

Water samples were collected using a 0.3 mm plankton net pulled by a boat with a speed of 1-3 knots. The water sample collected from the bottle section which is placed at the end of the plankton net is collected and filtered using a metal sieve to separate MPs from other materials. Followed by a wet peroxidase oxidase process as in the sediment procedure. MPs resulting from the floating wet peroxidase oxidase process were collected by filtering using a 0.3 mm filter paper, dried and this plastic material was taken and weighed using an analytical balance to determine the volume of MPs in the sample (Mahenda et al., 2021).

#### Aquatic organism extraction

The analysis of MPs in aquatic biota follows the procedure of Kovač Viršek et al., (2016) with modifications. In short, biota samples such as fish and shrimp are cleaned using a soft brush and clean water to reduce contaminants attached

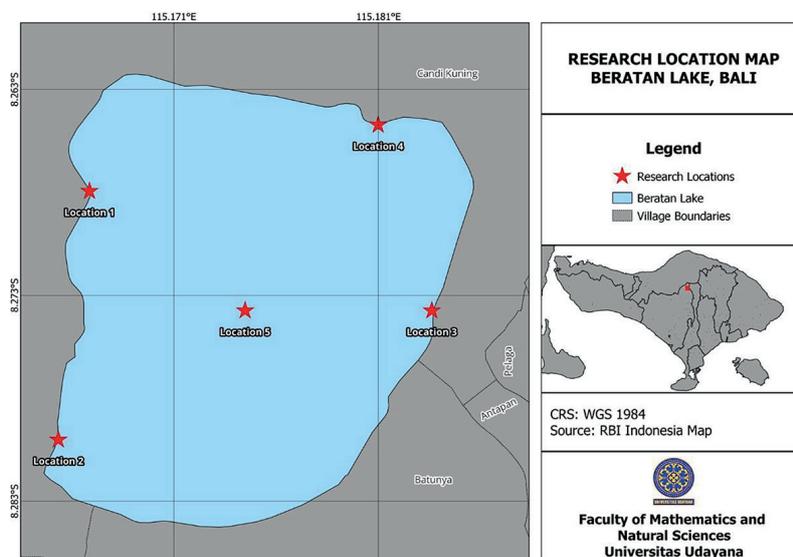


Figure 1. The sampling locations of this study presented with “star” symbol

to the surface of the biota's body. The remaining cleaning water is then filtered to obtain materials that stick to the organisms' bodies for further analysis. To find out the accumulation of MPs in biota's bodies, surgery was performed and parts of the digestive tract were taken for analysis. The digestive tract of each biota is then placed in a polypropylene jar. Anthropogenic waste is separated from its stomach contents referring to the study of Foekema et al., (2013) the digestive tract is placed in a jar and a 10% KOH solution in distilled water is added for 3× the volume of the digestive tract, and followed by incubation for 12 hours at 60°C to destroy organic matter. The solution was then filtered using filter paper with a mesh size of 0.3 mm in a Buchner vacuum filter to separate the solution. The filtered MPs were air-dried, then processed by wet peroxide oxidation with the addition of Fe (II) catalyst to separate the organic matter. NaCl was added to the mixture of wet peroxide oxidation samples so that the heavier material could sink and the MPs would float. MPs resulting from wet peroxidases that floated were collected by filtering using a 0.3 mm filter paper, air-dried and the MPs material was taken and weighed using an analytical balance to determine the volume of MPs in the sample.

### Microplastics analysis and identification

The Whatmann paper containing the sample that had dried in the previous procedure was weighed and the sample was transferred to a sterile petri dish and then weighed again to determine the dry weight of the sample. Samples were identified at 40–100× magnification under a stereo microscope (Olympus SZ) equipped with a digital camera and optilab software. For each sample, MPs members are determined in terms of shape, color, and size categories. The structural features of the MPs were identified visually following the procedure from Prata et al., (2020) and the six size categories used followed the procedure from Simon-Sánchez et al., (2019) namely: <50 µm, 50–100 µm, 100–200 µm, 200–300 µm, 300–500 µm, and >500 µm.

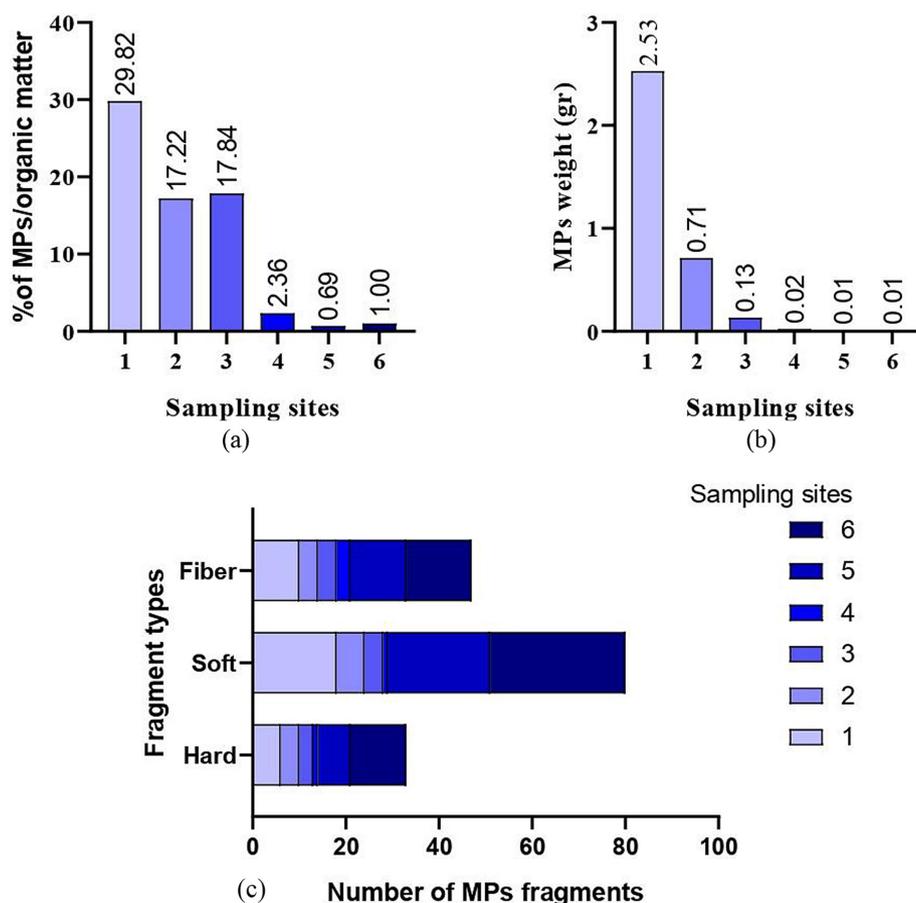
## RESULT AND DISCUSSION

### Microplastics on sediment

The highest average percentage of MPs content was found in organic matter in sediment samples was at sampling point 1, which was 29.82%.

The highest percentage of MPs content was found at sampling points 3 and 2, respectively 17.84% and 17.22%. The lowest percentage of MPs content was found at sampling points 4 to 6, namely 2.36%; 0.69%; and 1.00% (Figure 2a). Furthermore, the average weight of MPs in the highest sediments was also found at sampling points 1–3 respectively of 2.53 grams; 0.71 grams; and 0.13 gram (Figure 2b). The lowest average MPs weight was also found at three sampling points, namely 4–6 each of 0.02 gram; 0.01 grams; and 0.01 grams. Based on these findings, it can be estimated that sampling locations 1–3 have high exposure to contaminants including MPs which subsequently accumulate in sediments and organic matter in the Lake Beratan environment. MPs pollution sources originating from agricultural areas and tourism activities significantly affect the amount of accumulation in sediments and lake biogeochemical cycles (Shi et al., 2022).

Based on the type of MPs fragments found, soft fragments were the most dominant, especially at sampling location 1; 5 and 6 in a row – 18 in total; 22; and 29 fragments. The same thing also happened to the dominant fiber type fragments found at sampling location 1; 5; and 6 each by 10; 12; and 14 fragments (Fig. 2c). Meanwhile, hard type fragments are rarely found at each sampling location, but their existence still needs to be highlighted. MPs fragments with a diameter of <5 mm are anthropogenic pollutants that are commonly found in sediments. The chemical composition of MPs particles that are dominant in sediments includes polyethylene (PVC), polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC) (Dodson et al., 2020). A similar view was expressed by a previous study which found MPs contamination of 65 MPs/kg of sediment on the three west coasts of Lake Superior, which was dominated by polyester fiber (Davidson et al., 2022). MPs particles have a relatively long degradation period and the strong chemical stability of their particles causes them to easily move through food webs in aquatic ecosystems and eventually get buried in sediments (Yang et al., 2022). It should also be considered that the number of MPs found in the lake environment depends on the residence time of the water and the size of the water body, and the activity around the lake is played by the density of the human population (Imhof et al., 2013). Due to biological processes, the density of MPs found in aquatic sediments tends to be several times higher than that in water (Haegerbaeumer et al., 2019).



**Figure 2.** MPs contamination found in the sediments of Lake Beratan, Bali Province: (a) average percentage of MPs content in organic matter in Lake Beratan sediments; (b) the average weight of MPs in the sediment at each sampling point; (c) the number of MPs fragments in the form of hard, soft and fiber in the sediment of each sampling point

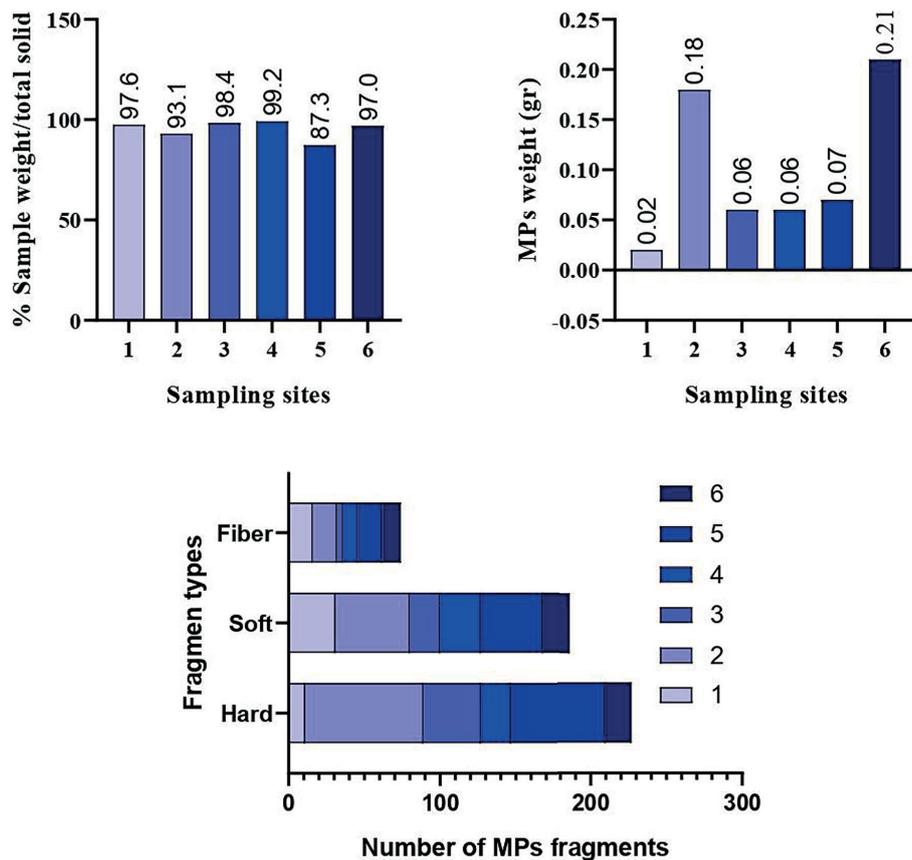
### Microplastics on water

MPs contamination was also found in water samples collected from Lake Beratan. The results of the study revealed that the highest percentage of MPs sample weight obtained from dissolved solids was found at sampling point 4 (99.2%), followed by sampling point 3 (98.4%), and sampling point 1 (97.6%). Meanwhile, the lowest MPs percentage was found at sampling point 5 (87.3%) (Figure 3a). However, overall the MPs sample data on total dissolved solids in the Lake Beratan water column is classified as high or more than 85%. It also reveals that of the total dissolved solids in the waters of Lake Beratan, most of them have been contaminated by MPs. Data regarding the average weight of MPs obtained from the waters of Lake Beratan show an uneven distribution at each sampling location. The highest MPs weight was found at sampling points 6 and 2 with an average value of 0.21 gr and 0.18 gr,

respectively. The lowest MPs weight was found at sampling point 1 (0.02 gr), while at sampling points 3, 4 and 5 the values were close, ranging from 0.06–0.07 gr (Figure 3b).

Based on the category of MPs fragment form, it was dominated by hard, soft and fiber forms and only one fragment was found in the form of foam (at sampling point 3). Interestingly, in contrast to the form of MPs found in sediment samples, the form of MPs found in the waters of Lake Beratan is mostly in hard form, while the sediments are dominated in soft form. The average of all samples, MPs in the hard form found  $37.83 \pm 27.3$  fragments, soft form  $31.0 \pm 12.1$  fragments, and the lowest was the fiber form which was an average of  $13.33 \pm 4.33$  fragments.

Based on the sampling location, the highest concentration of MPs in the form of hard fragments were found at sampling point 2 with 78 fragments and sampling point 5 with 63 fragments. Furthermore, the number of hard fragments found at other points ranged from 11–20 fragments, with



**Figure 3.** MPs contamination found in the water of Lake Beratan, Bali Province: (a) average percentage of MPs content in total dissolved solids in Lake Beratan water; (b) the average weight of MPs in water at each sampling point; (c) the number of MPs fragments in the form of hard, soft and fiber in the water at each sampling point

the lowest number found at sampling point 1. The highest number of soft fragments found was found successively at sampling points 2, 5, and 1 each of 49, 41, and 3 fragments. At the other three sampling points, the number of soft fragments was relatively high at sampling point 6 with 18 fragments and sampling point 4 with 27 fragments. Finally, there were 16 MPs in the form of fiber found at sampling points 1, 2 and 5, while at sampling points 6, 4 and 3 only 12, 10 and 4 fragments were found respectively (Figure 3c).

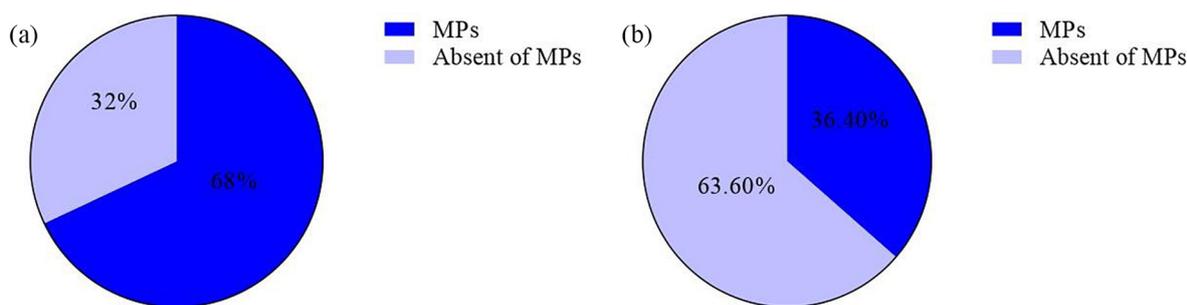
It is known that MPs consist of heterogeneous polymer mixtures of various sizes and shapes that can be distributed unevenly in the aquatic environment. MPs with a higher density compared to water such as polyester (PES) or polyvinylchloride (PVC) will be more prone to sink to the bottom of the waters (Koelmans et al., 2019). Similar reports have also been reported in Lake Kallavesi, Finland, which has a high concentration of MPs in its water samples, which are thought to be close to ports, snow dumps, and WWTP drainpipes (Uurasjärvi et al., 2020). Based on the fragments, our results are certainly different from previous

findings that occurred in the waters of Lake Kallavesi, Finland and the Great Lakes, USA, which were dominated by fiber fragments by 64% and 99%, respectively (Uurasjärvi et al., 2020; Whitaker et al., 2019). Differences in the shape, weight, and size of MPs detected can be due to differences in the types of MPs sources around the study site, such as the existence of an WWTP, high activity of littering, agricultural, industrial and fishing activities (Tsering et al., 2022; Xiong et al., 2022).

### Microplastic on aquatic organisms

MPs contamination found in aquatic biota such as fish and shrimp in the waters of Lake Beratan shows a different percentage. The percentage of MPs contamination obtained from the digestive tract of fish (n = 50 individuals) that was successfully collected was 68% (Figure 4a). This amount is higher when compared to the percentage of MPs obtained from the digestive tract of shrimp, which is equal to 36.4% (Figure 4b).

Interestingly, this study is the first to report MPs contamination on aquatic biota in Lake Beratan,



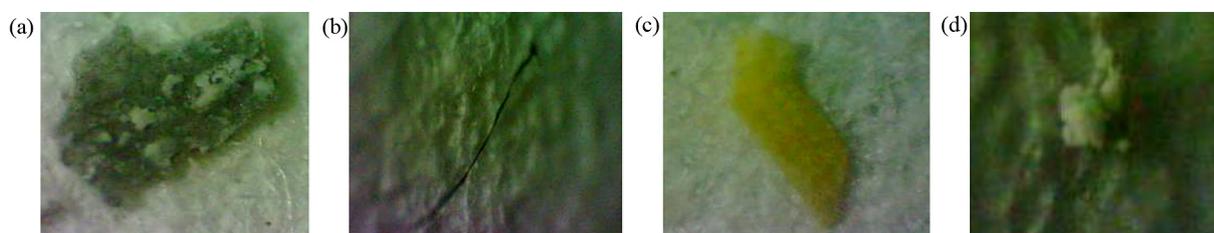
**Figure 4.** Percentage of MPs contamination in aquatic biota collected from Lake Beratan, Bali: (a) the percentage of MPs in the digestive tract of fish (n = 58 individuals), (b) the percentage of MPs in the digestive tract of shrimp (n = 28 individuals)

Bali. Various types of MPs were also found in the digestive tract of fish and shrimp in this study (Fig. 5). A total of four types of MPs were found in fish samples including fragments, fiber/filament, film, and foam. Meanwhile, in the shrimp samples, only fragments, fibers, and films were found. Likewise, the difference in the number of MPs forms found in these two biota is also different.

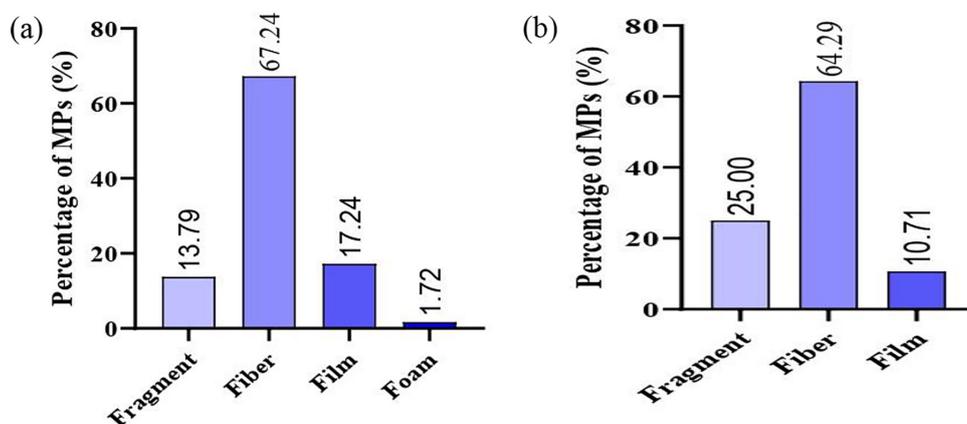
The percentage of fiber forms found in fish samples (n = 58 fish) was 67.24%, the highest when compared to the other three types of MPs, which were fragments (13.79%), film (17.24%), and foam

(1.72%) (Fig. 6a). The type of fiber was also found to have the highest percentage in the shrimp sample (n = 28), namely 64.29%. In addition, there are fragments of 25% and films of 10.71% (Fig. 6b).

Consumption of MPs by aquatic biota including fish, mammals and invertebrates has been documented in various cases in the field. Our results reveal that MPs contamination has been found in aquatic biota such as fish and shrimp collected in Lake Beratan. This is an early warning regarding the potential impact of MPs consumption by aquatic animals that can affect physiological



**Figure 5.** Types of MPs found in the digestive tract of aquatic biota in Beratan Lake, Bali: (a) fragment, (b) filament, (c) film, (d) foam



**Figure 6.** Percentage (%) of MPs in aquatic biota collected from Lake Beratan, Bali: (a) percentage of MPs species in the digestive tract of fish (n = 58 individuals), (b) percentage of MPs species in the digestive tract of shrimp (n = 28 individuals)

systems and digestive disorders (Rosiana et al., 2022; Schrank et al., 2019). At the food chain level, the transfer of tropic MPs through food webs poses serious ecological and human health risks (Carbery et al., 2018; Nel et al., 2018).

Our findings highlight the MPs found in the digestive tract of fish and shrimp which are dominated by fiber. On the other hand, this species has commercial value and is a food source that can be utilized by the people around the lake for tourism purposes. Therefore, contamination of MPs poses a risk to human health due to the possibility of translocation of plastic particles into the edible tissues of fish or shrimp. Previous studies reported that as many as 35% of fish samples had ingested MPs which were more common in the rainy season than in the dry season and the average particle weight in fish was 0.07 mg/kg.ww in Lake Ziway, Africa (Merga et al., 2020). Analysis of MPs consumption in perch (*Perca fluviatilis*) and vendace (*Coregonus albula*) collected from Lake Kallavesi, Finland showed that 17% of perch and 25% of vendace ingested MPs. Several types of MPs were found mostly in the form of polyethylene, polypropylene, and polyethylene terephthalate which were measured using FTIR (Uurasjärvi et al., 2021). Several cases of MPs contamination have been reported in several aquatic environments in Indonesia, such as cormorants on the coast of Pulau Rambut Wildlife Reserve in Jakarta Bay (Suantani et al., 2022), fish *Hemiramphus far*, *Siganus virgatus*, and *Lethrinus lentjan* collected from island waters Barranglompo, Makassar (Sawalman et al., 2021), digestive tract of fish from the genera *Epinephelus* collected from Pramuka Island, Seribu Island, Indonesia, and MPs contamination in *Gambusia affinis* fish collected from the Brantas River (Buwono et al., 2022).

## CONCLUSIONS

To our knowledge, this is the first study to report MPs contamination in both sediment, water and aquatic biota in the waters of Lake Beratan, Bali Province. This study shows that some of the MPs obtained in the lake environment can be retained in sediments until they are consumed by fish and shrimp in this study. In the studied lakes, more MPs were found in soft sediments. The influence of human activities around the lake which littered and other related activities that caused MPs was also found in samples of water and aquatic

biota (fish and shrimp). Overall, because MPs were found in all samples, it can be concluded that some of the plastic pollution is trapped in lake sediments which may be naturally influenced by the seasons, although further research is needed to prove this.

## Acknowledgments

This research was supported by Udayana University through DIPA PNBP funding for the 2020 and 2021 Academic Years in accordance with the Research Implementation Assignment Agreement Numbers: B/1588-101/UN14.4.A/PT.01.03/2020 and B/96-236/UN14.4.A/PT.01.05/2021.

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