

DETECTION OF MICROPLASTICS IN COMMERCIALY IMPORTANT BIVALVES IN TINORIAN RIVER, ILOILO, PHILIPPINES

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Abstract

Microplastics (MPs) are tiny plastic particles with a diameter of less than five millimeters that have become pervasive in the environment. These pollutants infiltrate ecosystems, posing significant risks to marine life, particularly filter-feeding organisms. Filter feeding is a technique where animals use water flow to capture small food particles or organisms. This feeding mechanism is vital for bivalves such as *Crassostrea iredalei* (slipper-shaped oyster) and *Perna viridis* (green mussel), which are widely distributed in the environment and highly sensitive to waterborne contaminants. These bivalves serve as reliable indicators of microplastic pollution. Although MPs are abundant in marine environments, there is a lack of comprehensive data regarding their presence and characteristics in bivalves. To extract MPs, potassium hydroxide (KOH) infused with sodium lauryl is used for digestion, and sodium chloride (NaCl) is employed as a flotation reagent. In this study, a hot-needle or hot-point test was used to examine particles' melting or deformation behavior. The results show that MPs were detected in 10 out of 15 samples (66.66%) of *Crassostrea iredalei* and 11 out of 15 samples (73.33%) of *Perna viridis*. The predominant types of microplastics identified were fibers, fragments, and films. These findings highlight the need for further research, particularly using Fourier Transform Infrared (FTIR) spectroscopy, to confirm and accurately detect microplastics.

Keywords: Bivlaves, Detection, Microplastics, Tinorian river

INTRODUCTION

Microplastics (MPs) are little plastic particles that have increased in the environment and have a diameter of less than five millimeters. These minuscule pollutants find their way into ecosystems and negatively impact marine life. These come from microbead products or larger plastic particles breaking down. Microplastics are a major concern for both human and animal health, hence study must be done on them and find ways to mitigate their effects to preserve ecological balance and promote sustainable practices. Plastic is any synthetic or semi-synthetic organic polymer.

Plastics always contain carbon and hydrogen, even though plastics can also be manufactured from almost any organic compound. Most industrial plastic is produced from petrochemicals (Helmenstine, 2020). Plastics with a diameter of less than five millimeters (0.2 inches), which is smaller than the typical pearl used in jewelry (Brown, 2023). Microplastics have a major impact on the ecology, particularly on animals that rely on filter feeding for nutrition. Some animals adopt a method called filter feeding, in which animals search for food by swimming through the water or by moving through it and collecting food particles and other microscopic products out of it. (Perez, 2022). After pushing the water through a sieve, the organism filters through the particles and releases unnecessary materials (Perez, 2022). Bivalve (class Bivalvia), is any of the more than 15,000 species of mollusks that belong to the phylum Mollusca that are distinguished by having a shell that is separated from front to back into left and right valves (Morton, 2024). Since most bivalves

rely on their gills for sustenance, these organs have significantly expanded to accommodate their secondary-derived role. In bivalves, the two processes—clearance and filtration—are managed separately. (Gosling, 2015). The World Bank estimates that the Philippines uses over 163 million sachet pieces daily.

Every year, the nation produces an astounding 2.3 million tons of plastic waste (Pustadan, 2022). In 2010 the Philippines ranked third in the world for the amount of plastic waste it dumped into the oceans; in 2016, the nation mismanaged 1.01 million tonnes of plastic debris but there is hardly any monitoring of the microplastic contamination in the country (Ozorio *et al.*, 2021,). With the species spread over freshwater, terrestrial, and marine habitats, mollusks are one of the most diverse groups of invertebrates and the second-largest phylum after Arthropoda. (Nybakken and McClintock, 1997; Sierwald *et al.*, 2018). Currently, there is a dearth of efficient treatment techniques even though marine microplastic pollution is becoming a worldwide pollution crisis and getting worse (You *et al.*, 2021). Bivalves are employed as indicators in the biomonitoring of environmental quality because bivalves can be exposed to a broad variety of organic and inorganic particles (Moreschi *et al.*, 2020). When it comes to removing microscopic plastic particles from the environment, MPs can be effectively collected by bivalves (Ringwood, 2021c). The majority of field sampling methods have a harder time sampling these particle sizes, bivalves can fill in the gaps and also show bioreactivity (Ringwood, 2021d). For biomonitoring investigations in marine habitats, mussels have been employed extensively because of several benefits, including their large geographic distribution and ease of accessibility. Additionally, bivalves have proven to be a valuable instrument for assessing MPs contamination in both field and laboratory investigations (Da Costa *et al.*, 2022).

Research indicates that oysters are more likely to consume and accumulate smaller-sized microplastics and varied colors of microplastic particles (Kristine & Jambre, 2022). The Philippine Cupped Oyster *C. iredalei* is capable of ingesting and accumulating microplastics of diverse types, colors, sizes, and shapes in its soft tissue. Additionally, it can be inferred that oysters are capable of consuming spherical, thread-like, and unevenly shaped microplastics. Bivalves usually spend most of their lives in one location. On that account, bivalves will encounter direct local environmental conditions and therefore will depict actual representations of microplastic contamination in such an area. In the Philippines, oysters and mussels are popular shellfish that are mostly raised for local consumption (Morta, 2024). The Philippines' annual per capita oyster production index in 2021 was approximately 137.7%, which was higher than the base record of 100% in 2018. Since 2016, the Philippines' oyster output has progressively increased (Balita, 2024). The most popular species in the Philippines is the green mussel, or *Perna viridis*. PSA stated that the Philippines produced 19,228.96 MT in total in 2020. With 8,534.16 MT of mussels produced, Western Visayas constituted the leading region; the province of Capiz produced 7,945.70 MT of mussels. Cavite and Samar are additionally high-producing provinces, with 5,654.23 MT and 4,077.14 MT, respectively (Mussel – Industry Strategic Science and Technology Plans (ISPs) Platform, 2024.). The two most widely farmed mollusk species in the Philippines are the green mussel (tahong) and the slipper-shaped oyster (talabang tsinelas), mostly for Filipino consumption of their meat (Tanay *et al.*, 2022). Oysters and mussels are of crucial importance in their ecosystems and human economies, indicating environmental health and providing essential resources to their coastal communities. The issue of microplastics requires collective management and research efforts on pollution and its long-term effects on bivalves and, more inclusively, overall marine ecosystems.

No comprehensive data shows the status of the microplastics in Tinori-an River. The study regarding the intake of microplastics of commercially important bivalves namely *Crassostrea iredalei*

(Talaba) and *Perna viridis* (Tahong). Furthermore, there is no information presented on the specific kind of microplastics that are present in the Tinori-an River; The number of different kinds of microplastics found in bivalves in the Tinori-an River; and studies on the range of microplastics discovered in bivalves in the Tinori-an River.

General Objectives

To determine the presence of microplastics on commercially important shellfishes.

Specific Objectives

1. To determine the presence of microplastics in *Crassostrea iredalei* (Talaba) and *Perna viridis* (Tahong),
2. To test the presence of suspected microplastics in *Crassostrea iredalei* (Talaba) and *Perna viridis* (Tahong) using a Hot-needle test under the stereomicroscope, and
3. To identify the types of microplastics present in *Crassostrea iredalei* (Talaba) and *Perna viridis* (Tahong).

Significance of the study

This study was conducted to benefit the following Fisher folks and Residents - To be aware and educate them about the microplastics present in the area.

Future Researchers - This study will become the baseline information of their research regarding similar studies.

Students - This study can serve as a source of additional knowledge regarding bivalves (*Crassostrea iredalei* and *Perna viridis*) and microplastics.

Local Government Units, Non-Government Organizations, and National Government Agencies - This research will inform LGUs, NGOs, and NGAs of the status of bivalves (*Crassostrea iredalei* and *Perna viridis*) with the possible presence of microplastics in the area.

Scope and Limitations

1. The study covers the downstream of Tinori-an River; The assessment of the microplastics in the Tinori-an River and limited only to three (3) areas in downstream of Tinori-an River.
2. The study also covers only cultured bivalves namely *Crassostrea iredalei* (Talaba) and *Perna viridis* (Tahong) in Tinori-an River.
3. The study's limitations include a maximum of 3 grams of meat taken from the *Crassostrea iredalei* (Talaba) and *Perna viridis* (Tahong) in the Tinori-an River.

LITERATURE REVIEW

Microplastics

The development of microplastics (MPs) as persistent pollutants is raising serious concerns about the health of the environment. A microplastic is a plastic particle that has gotten into the environment and caused harm, measuring smaller than 5 mm in diameter.

Threats of Microplastics

Marine life is harmed by microplastics in several ways. Microplastics block an animal's digestive tract when microplastics enter its intestines after being mistaken for food by the animal. This prevents the animal's organs from functioning properly, which can lead to death (Berendzen,

2022). Furthermore, microplastics go up the food chain when larger species eat smaller ones that have already consumed microplastics. Greater animals accumulate plastics and the toxins the species swallow as a consequence.

Types of Microplastics

Primary microplastics include plastic fibers used in synthetic textiles (like nylon), plastic pellets (sometimes called nurdles) used in industrial manufacturing, and microbeads found in personal care items (Rogers, 2024). Plastic particles known as primary microplastics are discharged into the environment directly. Primary microplastics are microscopic synthetic polymers used as exfoliants in various processes: chemical formulations, grinding products, maintenance of various plastic products, and manufacturing of synthetic clothing. Microbeads are another type of primary plastic (size < 2 mm) (Ziani *et al.*, 2023). Corresponds to the fragmentation of larger plastic debris (>5 mm) through a combination of physical, chemical, and biological processes. This distinction enables the potential identification of certain sources of microplastics, making it possible to act upstream to reduce their presence in the environment (Ziani *et al.*, 2023). Secondary microplastics instead form by the degradation and weathering of larger plastic items, entering the environment as mismanaged or improperly disposed of waste (Plastics Europe, 2022).

Widespread Distribution of Microplastics

The widespread occurrence of microplastics (MP) has become a major marine issue as the world's plastic production keeps speeding up. (Coyle, et al., 2020). The Philippines produces 2.7 million tons of plastic garbage annually, of which 20% is thought to wind up in the ocean. The Philippines, a country made up of more than 7,500 islands, has coastal people whose livelihoods depend heavily on fishing, shipping, and tourism. These industries are especially vulnerable to the effects of marine debris. (World Bank, 2021). Microplastics (MPs) have been found in the marine ecosystem, which is home to a diverse spectrum of species at various trophic levels and habitats (Diepens & Koelmans, 2018). Both direct and indirect effects of microplastics (MPs) on bivalves can be seen, influencing the physiology, habitat structure, food supplies, and organic pollutant persistence (Khanjani *et al.*, 2023).

Bivalves Consumption

Bivalves that consume MPs may have direct physiological effects as well as indirect habitat structure changes due to reduced food sources and more effective organic pollution transfer (Zhang *et al.*, 2020). The bivalves contain fragments, film, and fiber-type microplastics. The FTIR investigation, which identifies polyamide fibers as the most prevalent polymer in the bivalves, confirms this. The bivalves were also observed to have a variety of shapes and hues (Yu *et al.*, 2023).

Microplastics' Philippine Status

Nearly 35,580 tons of trash are produced every day in the Philippines, where solid waste management is a major issue. Approximately 15–60% of these wastes are burned and dumped in the ocean or in pen areas, which is not an appropriate method of disposal (Castillo & Otoma, 2013). This has been estimated that 35 percent of the debris made of plastic in the ocean comes from the Philippines. The archipelago country, with more than 7,000 islands, produces 356,371 metric tons of plastic waste in the sea each year (Bhandari, 2023). Microplastics in bivalves have caused

widespread concern due to their potential health risk to humans (Ding, *et al.*, 2021). For a very long time, bivalves have been used as good bioindicator organisms to characterize the possible local, regional, national, and global effects of anthropogenic stressors such as pollution, hypoxia, and algal toxins (Ringwood, 2021).

Bivalves as Bioindicator

Bivalves like mussels and oysters are suitable bioindicators for microplastic pollution because of their wide distribution, vital ecological niches, susceptibility to microplastic uptake, and close connection with marine predators and human health (Li, *et al.*,). Mussels are widely spread, simple to get, and very resilient to a variety of environmental factors, including salinity, temperature, oxygen saturation, and the availability of food (O'Connor, 2002). Estuarine habitats are destroyed as a result of the pollutants' degradation of the water quality, which lowers biodiversity and depletes fishing resources (Xiao, *et al.*, 2014b). As bioindicators, oysters are frequently employed to track chemical pollutants and trace metals in marine environments. Because of their broad distribution and restricted mobility, oysters—filter-feeding bivalves that collect these substances—are useful markers of local environmental pollution. Filter-feeding bivalves, such as oysters, mussels, and clams, have been identified in the marine ecosystem as good sentinels of local environmental pollution because of their wide geographic distribution, restricted mobility, and high capacity to bioaccumulate chemical pollutants (Zhu *et al.*, 2020).

Extraction of Microplastics

Acid based extraction

ICES has released a draft protocol for the breakdown of organic matter together with the separation of microplastics and sample processing (ICES, 2015). The process aimed to contribute to the standardization of current practices by recommending the use of a 4:1 ratio of HNO₃ to HClO₄ as digesting agents (ICES, 2015).

Extraction and Digestion of *Crassostrea iredalai* (Talaba) and *Perna viridis* (Tahong)

The typical method for obtaining mussel lipid extracts is to extract the meat, either fresh or freeze-dried. Complex lipids are broken down into single fatty acids by chemical (KOH) or enzymatic (lipase or protease) hydrolysis (Almonacid *et al.*, 2014).

Utilizing potassium hydroxide (10%) and hydrogen peroxide (30%) to break down organic matter, as well as seawaters and sediments with hydrogen peroxide (30%), the soft tissues of oysters were examined using optical microscopy, digital camera, and micro-ATR-FTIR (Wang *et al.*, 2021). An anionic surfactant called sodium lauryl sulfate (SLS) is naturally found in coconut and/or palm kernel oil. Typically, it is made up of a combination of sodium alkyl sulfates, mostly lauryl. SLS is utilized as a fat emulsifier and decreases the surface tension of aqueous solutions (Sodium Lauryl Sulfate (SLS) - Chemical Safety Facts, 2022). Emulsification is the process of dissolving fat into smaller blood cells to allow easier meal digestion and enzyme function. By separating fatty acids and glycerol into forms that are easily absorbed, fat emulsification facilitates the digestion of fat (What Is Emulsification of Fats, 2022).

Identification of Microplastics

The hot needle test, also known as the hot point test, is a low-cost, useful technique that is widely used to help researchers identify suspected microplastics under optical microscopy by

examining their physical melt or deformation behavior Beckingham et al., 2023. Using the module from Hudson River Park POP (2022), this will be the guide in identifying the different types of suspected microplastic.

RESEARCH METHOD

Research Design

This study utilized descriptive research design to examine the presence of microplastics in economically important bivalves using the Acid Based Extraction Method. It will determine the different types of microplastics present in the *Crassostrea iredalei* and *Perna viridis*.

Locale of the study

The sampling of *Crassostrea iredalei* and *Perna viridis* will only cover the downstream of the Tinori-an River wherein bivalves are cultured by the locals.

Sampling Collection

Systematic sampling was used in this study to determine the area wherein the bivalves are being cultured. Using a GPS tracking device, the determined locations where *Crassostrea iredalei* are being cultured (10°56'52.80"N and 122°45'45.70"E, and 10°56'53.10"N and 122°45'39.10"E), and the *Perna viridis* are being cultured in (10°56'53.80"N and 122°45'33.70"E) (Figure 1).



Figure 1. Three (3) sampling sites in the downstream of Tinori-an river (Google Earth).

A letter was sent and approved by the local government of Tinori-an requesting the conduct of a study along Tinori-an River. The collection of *Crassostrea iredalei* (Talaba) and *Perna viridis* (Tahong) was done by riding a motor banca and manual gathering of samples. Documentation was conducted during the sampling, and the following data were gathered during sampling: time, date, length, and width of the samples and water tide. Twenty (20) random samples of *Crassostrea Iredalei*

and *Perna viridis* were collected, a total of 40 samples (Figure 2). To preserve their quality and keep them frozen throughout transportation, the collected samples were kept in an ice box.

The samples are immediately taken to Iloilo State University of Fisheries Science and Technology Biotechnology, Science Laboratory, Tiwi, Barotac Nuevo, Iloilo for analysis.

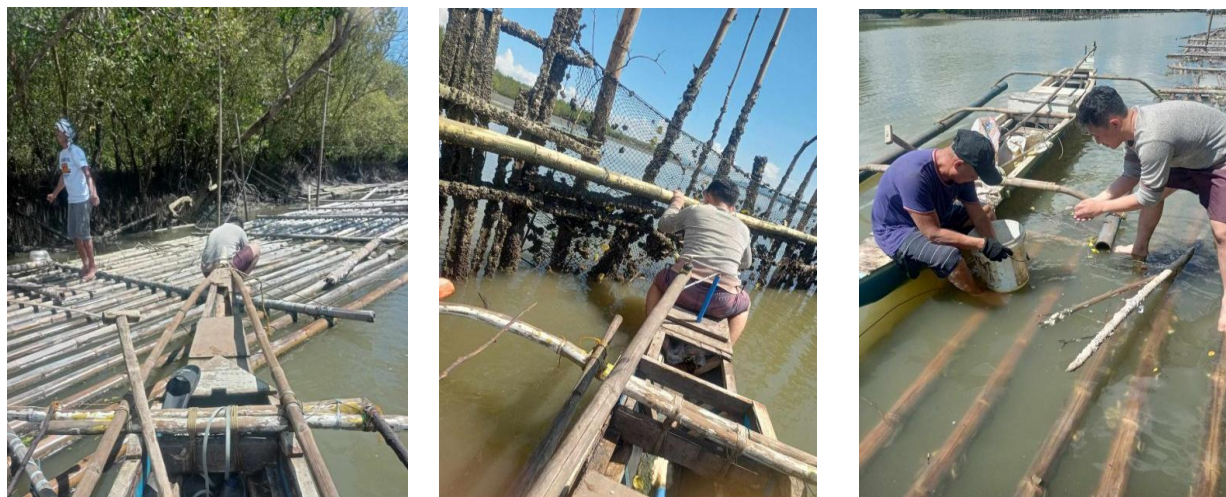


Figure 2. Pictures during sampling.

Analysis

All equipment used in sample preparation and dissection that would come into touch with the samples was thoroughly cleansed using distilled water and rinsed with it. Strict adherence to laboratory analytical protocols was maintained, including the use of personal protective equipment (PPE). To avoid plastic contamination, this process was implemented.

Acid-based Extraction Method

The separation of microplastics was done through acid-based extraction by Enders et al. (2016) with the use of a Potassium Hydroxide (KOH) solution that is also used in the study of Susanne et al. (2017) with some modifications for fat separation using Sodium lauryl sulfate ($C_{12}H_{25}SO_4Na$).

The meat is extracted from the shell collected from 15 randomly picked *Crassostrea iredalai* and *Perna viridis*. A total of 30 pieces of meat are placed in separate petri dishes. A maximum of 3 grams of each sample were used for microplastic isolation.

The ratios of the sample to the Potassium Hydroxide used were 1:6 (w/v) and for Sodium Lauryl Sulfate were 1:3 (w/v). The ratio of Potassium Hydroxide (KOH in grams) to the distilled water (H_2O in milliliter) was 6:60 and for Sodium Lauryl Sulfate was 3:30 (Table 3.1). The solution is mixed and corresponding samples are added to the mixed solution. The mixture samples were then covered, labeled, and incubated at 40°C for 48-hour digestion.

Flotation or Density separation was adopted from the study of Han et al. (2019) with minor modifications. After 48 hours of digestion, a ratio of 6g:20ml solution (Table 3.2) of NaCl is added to the incubated samples and covered and placed a room temperature. After 24 hours of flotation, the mixture samples were vacuum-filtered with a Whatman cellulose filter paper (No. 2).

Microscopic Identification

A stereomicroscope was used to examine the filtered mixture. Conducting a hot needle test based on the study of Beckingham et al., 2023 for the Marine and Environmental Research Institute's Guide to Microplastic Identification.

RESULTS AND DISCUSSION

Length, Width, and Meat Weight

After extracting the meat from the shell, the researchers measured the length, width, and meat weight of *Crassostrea iredalei* and *Perna viridis*. The measurement is based on The Hatchery Culture of Bivalves: A Practical Manual. A caliper was used to measure the length and width of *Crassostrea iredalei* and *Perna viridis*, the results concluded that the average length of *Crassostrea iredalei* is 34.45mm while the average width is 26.41mm and *Perna viridis*, the average length is 36.45 mm and 19.69 mm in width. The purpose of this study is to detect the presence of microplastic in *Crassostrea iredalai* and *Perna viridis*. This goal is addressed by the results that follow, which demonstrate a greater comprehension of the state of microplastics in the Tinori-an River. The analysis of microplastic in *Crassostrea iredalai* exhibit that 66.67%, 10 out of 15 samples exhibit microplastics (Table 1) while in *Perna viridis* 73.33%, 11 out of 15 samples exhibit microplastics (Table 2).

Table 1. Presence of Microplastic in *Crassostrea iredalai*

SAMPLE No.	Positive (1)/ Negative (0)
1	1
2	1
3	1
4	1
5	0
6	0
7	1
8	0
9	0
10	1
11	1
12	1
13	0
14	1
15	1

Table 2. Presence of Microplastic in *Perna viridis*

SAMPLE No.	Positive (1)/ Negative (0)
1	0
2	1
3	1
4	0
5	1
6	1
7	1
8	1
9	1

10	1
11	1
12	0
13	1
14	1
15	0

The samples are tested under the hot needle test during identification, this method exhibits deformation, thermal degradation, and melting during the process. The suspected microplastics are tested, identified, and classified. After testing, microplastics are identified based on the module from HRPK - POP - Microplastics - ID - Guide, 2022. The microplastics found are fragments (Figure 3a), film (Figure 3b), and line (Figure 3c).

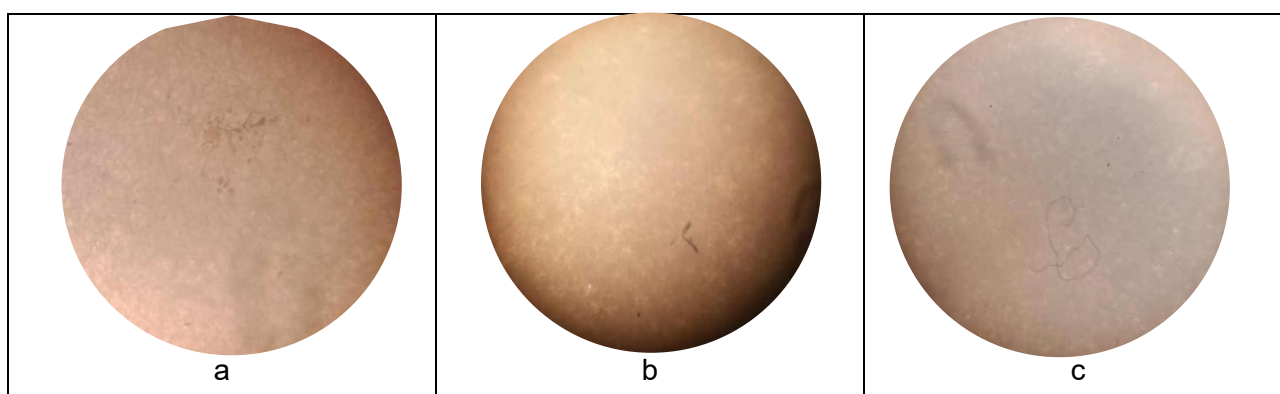


Figure 3. Fragments (a); Film (b); Line (c)

Fragments (Figure 3a) are unidentified, hard, and sometimes may have waxy texture, a large fragment is in the top left, small fragments (right), and pellets are at the bottom. The Film (Figure 3b) is sheet-like and thin in the bottom right. The Line (Figure 3c) appears translucent and in various hues. Line usually takes the form of larger fragments than the microplastic fibers. The most common microplastics present are the Fragments (both in *Crassostrea iredalei* and *Perna viridis*). *Crassostrea iredalei*, exhibits 100%, 10 out of 10 had a presence of Fragment (MP's), while *Perna viridis* exhibits 54.55%. The Film present in *Crassostrea iredalei* is 40% while in *Perna viridis*, 45.45% is present. The line present in *Crassostrea iredalai* is 80% while in *Perna viridis* is 63.64% (Tables 3 and 4).

Table 3. Microplastics Identification in *Crassostrea iredalai*

Sample no.	Microplastics Present
1	Fragments, Line
2	Fragments, Film, and Line
3	Fragments, Line and Film
4	Fragments, Film
5	0
6	0
7	Fragments, Line
8	0
9	0
10	Fragments, Line
11	Fragments and Film
12	Fragment, Line

13	0
14	Fragment, Line
15	Fragment, Line

Table 4: Microplastics Identification in *Perna viridis*

Sample no.	Microplastics Present
1	0
2	Line, Fragment and Film
3	Line, Fragments
4	0
5	Film
6	Line
7	Film, Fragments
8	Fragments, Line
9	Film
10	Line
11	Fragments, Line
12	0
13	Film, Fragments
14	Line
15	0

CONCLUSION

The results of this investigation highlight the widespread existence of microplastics in bivalves and point to a serious environmental issue. The research results indicate heterogeneity in the kinds and forms of microplastics present in bivalves, demonstrating numerous contamination origins. Microplastics that were frequently found included fragments, lines, and film; fragments were the most prevalent form. This emphasizes how important it is to distinguish between different kinds of microplastics. In addition, to compromising a bivalve's health and ability to act as a filter feeder, with the presence of microplastics.

Bivalves are especially susceptible to contamination by microplastics because, as filter feeders, they typically inhabit coastal and estuarine habitats with high pollution levels. Given the commercial importance of bivalves, this contamination has consequences for both food safety and bivalves' consumption in addition to posing dangers to their survival and health.

The discovery of microplastics in bivalves is an essential indication of the threats that plastic pollution could pose to greater systems. The long-term impacts of microplastic buildup on the physiology, reproductive, and ecological roles of bivalves, as well as the potential health risks associated with consuming affected seafood, require further investigation. Better strategies for preserving marine ecosystems and guaranteeing food security will be established with the aid of the gaps highlighted.

This study emphasizes how critical it is to take action on microplastic pollution at its source while collaborating with entrepreneurs, researchers, and governments to find sustainable solutions.

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