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Microplastic detection and identification in edible bivalves from Dolores and Oras, Eastern Samar

Aljon Victor G. Nibalvos[1*], Cristina T. Nibalvos[2], Maricar T. Obina[3]
[1,2,3]Eastern Samar State University, Borongan City, Eastern Samar, Philippines, 6800; avgn.research@gmail.com (A.V.G.N.)

Abstract: Microplastics are very minute plastic fragments that contaminate the environment and the organisms around it. One such important organism is bivalves. In Oras and Dolores, bivalves, specifically, *Corbicula fluminea* or "bibe" are well known for its economic importance. Henceforth, this study was conducted to detect the presence of microcontaminants or microplastics in the bivalves, being sold in the localities of Oras and Dolores, Eastern Samar. Digestion and Filtration procedures were used to attain consistent results with emphasis on current quality controls. Results revealed the presence of microplastics in the bivalve species collected on both sampling sites. Furthermore, it was found out that most microplastics were microfibers, mostly are filamentous and has the transparent/white to blue coloration. This results suggest the contamination of these non-biodegradable to edible bivalves which may pose health threats once eaten or utilized for various use.
Keywords: *Bivalves, Eastern Samar, Microcontaminants, Microfibers, Microplastics.*

1. Background of the Study

The municipalities of Oras and Dolores in the Northern portion of Eastern Samar is the center of bivalve commerce in the province, there, bivalves are being collected from its rivers. With the increase in commerce and living in both municipalities, Oras and Dolores have become the center of their civilization. The rivers in the two municipalities give food and livelihood through the consumption and selling of bivalves, or locally known as *Bibe* which is scientifically identified as *C. fluminea.*

Obina et al. (2016) identified, assessed and physically described the presence of two (2) economically-important bivalves coming from Dolores and Oras, Eastern Samar. Their paper highlighted the population of the bivalves which are mainly used as food consumption by its inhabitants and are usually being sold at the municipalities' public markets.

With the increase in population of where these bivalves are usually being harvested, there is a trend in the increase in the use of various materials and instruments that are needed by the people living in the areas for its everyday commodity. One of these is the polymeric material known as plastic.

These materials have become a part of every people's lives. It can be everywhere, from hard polystyric-type, to the soft fibrous and filamented polypropylenes. These non-biodegradable instruments can almost be of any shape and sizes. The UNEP (2015) underscored that the high volume and the quality which makes this material so useful can also be harmful to the environment, especially to marine environment.

Siirla, E. (2013) elucidated that plastics have become a valuable commodity and an important part of everyday life, more so that global plastic production has increased from 5 million tons in the 1950's to over 250million tons in 2006. Moreover, Cole et al. (2011), Andrady, A. (2011), and Moore, CJ. (2008) stated that plastics are polymers which are a chain of molecules that are derived from small molecules of monomers that are extracted from oil or gas

The National Oceanography and Atmospheric Administration (NOAA) (n.d.) also highlighted the major negative effects can be outlined by the contamination of marine environments with microplastics.

* Correspondence: avgn.research@gmail.com

Valentine (2014) and Cole (2011) also stated that the consumption of plastics and microplastics by marine animals can lead to false satiation, starvation and death.

Microplastics are typically defined as plastic particles measuring less than 5 mm in size, plastic materials smaller than this measure are considered nanoplastics. There are various types of plastics. They can be either a primary or secondary microplastics. A primary microplastic is an intentionally manufactured small plastic particle with sizes ranging from a few micrometers to 5 millimeters. They are directly produced for specific purposes and applications.

Examples of primary microplastics include microbeads and microfibers. On the other hand, secondary microplastics are formed as a result of the degradation and fragmentation of larger plastic items. Secondary microplastics can originate from various sources, including plastic bottles, bags, packaging materials, fishing gear, and other plastic debris. Over time, exposure to environmental factors like sunlight, wave action, and mechanical forces can break down larger plastics into smaller particles. Secondary microplastics can vary in size, ranging from millimeters to nanometers.

The main route of microplastics to the marine environment is the effluent from sewage and storm water generated in areas contains a significant amount of plastic. This pose some difficulties for treatment because many sewage treatment plants are not able to capture and treat plastic materials that are less than .5mm in diameter (Andrady, A., 2011; Moore, CJ., 2008). These plastics and microplastics become an even greater threat to the marine environment.

According to Li et al. (2022), microplastic contamination is an emerging global threat for various marine organisms. Marine invertebrates such as bivalve mollusks are more susceptible to the widespread presence of microplastics due to their limited abilities to escape from pollution exposure and they can readily ingest environmental pollutants like microplastics through their filter-feeding behaviors.

Since microplastics are small and ubiquitous, thus they can be ingested by a wide range of marine animals from small-size planktons to larger marine mammals. Microplastics and their associated pollutants can transfer into the animal's bodies and pass through the food chain, posing potential risks to marine organisms and human health Hence, microplastic contamination in the oceans is an emerging threat for the global challenge and has received great concerns in the world (Gunaalan et al., 2020).

The ingestion and retention of microplastics of filter-feeder organisms represent a risk for the final consumers and the environment. Biomonitoring is necessary to deal with the effects of plastic material pollution. The selection of the monitored organisms strongly affects the relevance of the results and the understanding of the environmental conditions (Brown et al., 2008).

In the areas of Dolores and Oras, in the province of Eastern Samar, upland barangays along rivers are prevalent, as they locally call it "*iraya*", although remotely situated from the main municipality or *poblacion*, the resident also use volumes of plastics which are which are frequently thrown into the body of water. With its poor waste management, a certain amount of these wastes can be brought into the river system and sometime settle into the river bed and degrade which in turn could be ingested by various living organisms such as bivalves. Hence, this research was conducted to detect and characterize the presence of microplastics in economically important bivalves being sold in the markets of Dolores and Oras, Eastern Samar.

1.1. Objectives of the Study

This study detected the presence of microplastics in bivalve samples which were collected in the markets of Oras and Dolores, Eastern Samar. More specifically, this study;

1. Identified the species of bivalves being sold in Oras and Dolores, Eastern Samar.
2. Classified the present microplastics in bivalve samples as:
    a. Primary microplastics
    b. Secondary microplastics
3. Identified the types of microplastics in bivalve samples as:
    a. Microbeads
    b. Microfibers
    c. Microfilms

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   d. Microfragments
4. Determined the physical structure of the microplastics in bivalve samples as:
   a. Angular
   b. Filament
   c. Round
   d. Other shape
5. Determined the color of the microplastics in bivalve samples as:
   a. Black
   b. Blue
   c. Green
   d. Red
   e. Transparent/white
   f. Other colors
6. Computed the total and average number of microplastics found in the total bivalve samples collected.

2. Methodology

2.1. Research Design

This descriptive study used qualitative analysis in detecting and estimating the present microplastics in bivalve samples from the municipalities of Dolores and Oras, Eastern Samar with the use of flesh digestion, density separation, filtration and Microscopic Technique.

2.2. Locale of the Study

This study was conducted at the Chemistry Laboratory of the Biology Department of the College of Science. Bivalve samples were collected from the markets of Dolores and Oras, Eastern Samar.

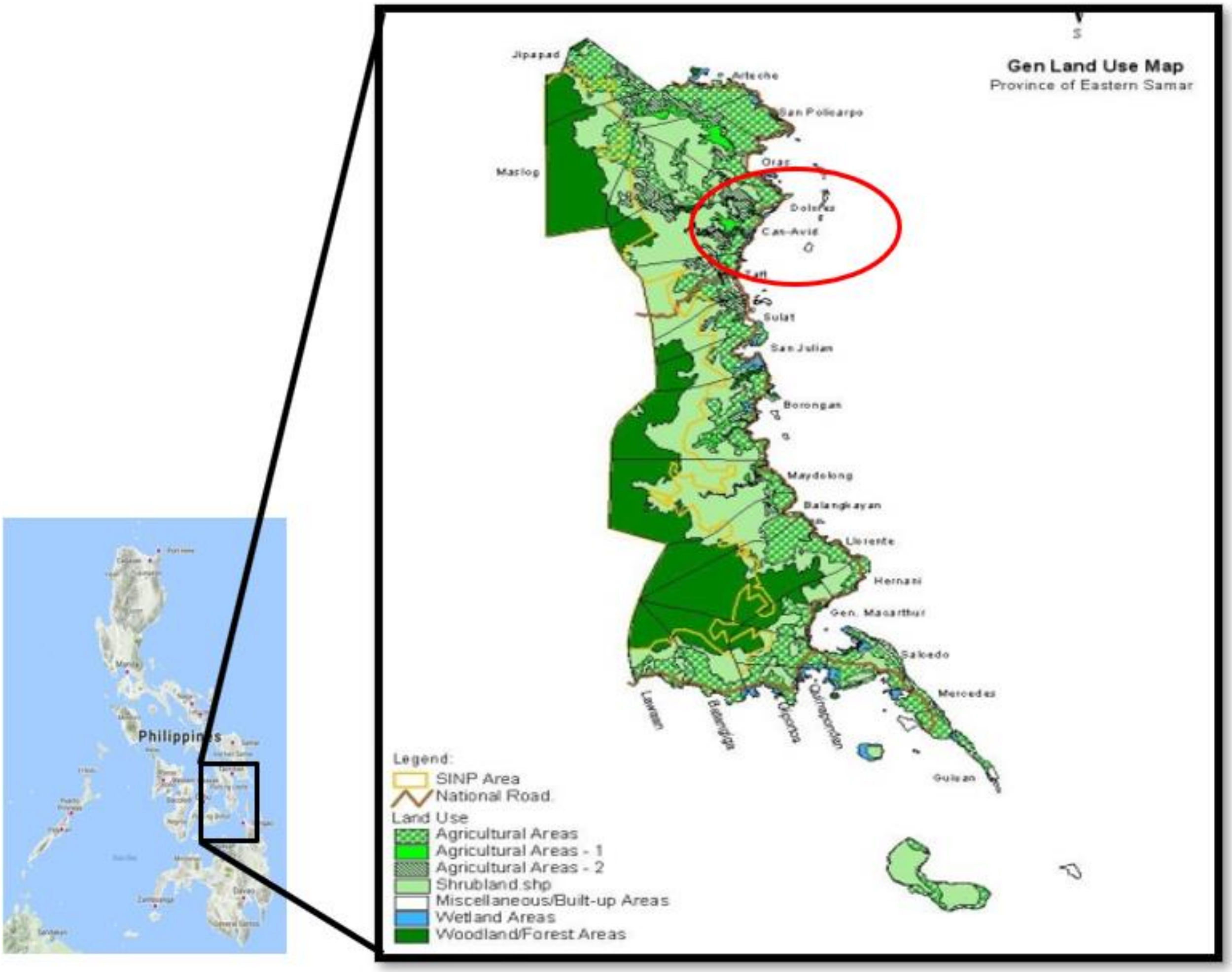

Figure 1.
Location Map of Dolores and Oras, Eastern Samar
Note: *Encircled red (maps.google.com).

Dolores is a municipality located in the province of Eastern Samar, Philippines. As of the 2020 census, it has a population of 44,626 people. The municipality is classified as a 3rd class municipality and is part of the Eastern Visayas region. Dolores is known for its coastal location, bordered on the east by the Pacific Ocean, and it features numerous beaches and small islands. The municipality covers an area of 308.58 square kilometers (119.14 square miles), constituting about 6.68% of Eastern Samar's total area. It is situated at approximately 12° 2' North latitude and 125° 29' East longitude, with an average elevation of 7.1 meters (23.3 feet) above sea level. Dolores is divided into 46 barangays extending upstream, which are the smallest administrative divisions in the Philippines (Dolores | Province of Eastern Samar, n.d.).

The economy of Dolores is primarily agricultural, with a significant portion of the population engaged in farming and fishing. The municipality also experiences a tropical climate, with temperatures ranging from 22°C (72°F) to 30°C (86°F) throughout the year. The area receives an average annual rainfall of around 1,506 millimeters (59.3 inches) (Dolores | Province of Eastern Samar, n.d.).

Oras is a municipality located in the province of Eastern Samar, Philippines. It is part of the Eastern Visayas region and falls under the lone legislative district of Eastern Samar. As of the 2020 census, Oras had a total population of 37,451 individuals, spread across 42 barangays. The municipality covers an area of 188.7 square kilometers, with an elevation ranging from sea level to 138 meters above sea level.

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The economy of Oras is classified as the 3rd municipal income class, indicating moderate economic activity. The poverty incidence in the area was reported to be 39.66% in 2021 (Oras | Province of Eastern Samar, n.d.).

Both localities have upstream barangays where majority of their freshwater bivalves are harvested. Their rivers are somehow extends significantly to other municipalities, but in terms of bivalve collection, the rivers from both municipalities are thriving.

2.3. Data Gathering Procedures

The following step-by-step processes were used in the study to detect the presence of microplastics in bivalve samples from Dolores and Oras, Eastern Samar.

2.4. Gathering of Bivalve Samples and Identification

Bivalve samples were gathered from the local market of Dolores and Oras, Eastern Samar; samples were commercially identified and brought. As per identification, *C. fluminea* samples were collected between the months of May and June 2024. These samples were kept in a sterilized and disinfected hard plastic to minimize contamination. Sample preservation was done to maximize the integrity of the sample using ice and freezers. The samples were labeled and were brought into the laboratory for digestion and microscopic analysis. Representative sample from each kind of identified bivalve samples was done having a total of 10 bivalve samples per locality. Data result from Obina et al. (2016) on bivalve biodiversity from the identified locale was used to determine as accurately as possible, the scientific names of bivalve samples.

2.5. Sample Preparation

Bivalve samples were taken to the laboratory for dissection and identified sample preparation followed the procedure by Bahri, et al., (2020) with minor modifications. All equipment was sterilized with distilled water. The bivalve samples were dissected and the soft tissues inside their shell were taken to extract the microplastics and weighted. The soft tissue samples was then inserted into 250 mL beakers and given a 30% NaOH solution up to 3 times the volume of the tissue. The sample is left to stand for 14 days or until full disintegration. Then the sample slurry was stirred at 1500 rpm for 15 minutes to homogenize and filtered first in a 0.15 mm filter mesh and seconded by a 30 micron nylon mesh as final filter with the help of a vacuum pump. The residue was then collected and observed under a stereo microscope at 40x magnification.

2.6. Microscopic Identification of Microplastics

Wet Sampling and Microscopy was done to all bivalve samples collected and prepared.

Materials:

Stereomicroscope at 40x magnification
Tweezers, forceps, alcohol lamp and needles
Microplastic identification reference materials
Disposable gloves and lab coat (as required for safety)

Prepare a clean and dedicated workspace in the laboratory or controlled environment to avoid contamination during the analysis. Wear disposable gloves and a lab coat to maintain a sterile working environment and prevent contamination. Dissolved bivalve soft tissue samples was filtered using a 30 micron filter mesh. The filter mesh was allowed to stand and placed unto a Petri Dish. The Petri dish was then examined under a stereomicroscope at 40x magnification. The dish was scanned from side to side, moving across the sample area to search for particles that match the characteristics of microplastics, such as shape, color, and texture. Use the microscope's focus and illumination controls to obtain a clear view of the particles. Any particles that appear consistent with microplastics based on their size, shape (e.g., fragments, fibers), and visual appearance was recorded. Optionally, observed particles with microplastic identification reference materials or images to assist with accurate identification was also done for comparison and confirmation using the Microplastics Sampling and

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Processing Guidebook of the Mississippi State University Extension Service. Images was captured on potential microplastics using a digital camera attachment for further analysis or documentation.

Process was repeated with multiple other filters and Petri dishes to different representative digested bivalve tissue samples for thorough examination. The characteristics and quantities of identified microplastics, including their size, shape, color, and any additional relevant observations was recorded and properly documented. 30 micron mesh filter was cleaned with distilled water for further use and debris left from the previously observed filter mesh was properly disposed with adherence to laboratory's waste management protocols. Collected data to assess the presence, abundance, and characteristics of microplastics in the samples were analyzed and interpreted.

2.7. Quality Control

Quality control of this study was done following the methods by Egessa et al, (2020) with some modifications as stipulated by Arcadio et al. (2022). During microscopy, all the wet samples analyzed was kept covered in glass Petri dishes. Background contamination from laboratory sources via the air and laboratory tools and equipment was tested using procedure blanks made from nylon filter contained in the Petri dishes and distilled water. At each stage of sample collection and analysis, the Petri dishes were left open to the air. The contents of control Petri dishes was processed and screened for microplastic contamination. The procedural blanks were also used as reference containing no microplastics.

3. Results and Discussion

After thorough microscopic identification and analysis, the following data were obtained:

Only one species of bivalve was obtained to be commercially available in the areas of Oras and Dolores, Eastern Samar. This bivalve is *Corbicula fluminea* which is locally known to as "bibe". A total of 10 bivalve samples from each locality were commercially brought and their soft tissues were separated from their hard shell via simple dissection. The soft tissues were weighed (wet weight) and the data can be seen below:

Table 1.
Weight of Soft tissues of bivalve samples.

No.	Bivalve samples from	
	Oras, Eastern Samar (g)	**Dolores, Eastern Samar (g)**
1	13.13	10.90
2	12.40	9.61
3	15.61	8.89
4	11.75	9.90
5	16.75	11.47
6	15.41	7.63
7	13.94	7.77
8	12.14	5.61
9	14.37	4.97
10	18.98	6.67
Total	144.48	83.42

It can be seen from the table above that the total weight of the soft tissues from bivalves coming from Oras were considerably more than those that were collected from Dolores, Eastern Samar. It is worth noting that the C. fluminea species being sold in the markets of Dolores Eastern Samar are much smaller than those coming from the rivers of Oras, hence this result.

Several factors could lead to this data, Aquino et al. (2021) identified that Environmental Factors such as water quality and seasonality plays a vital role to how freshwater bivalves grow.

Aquino et al. (2021) elaborate that the quality of the water in which the bivalves live plays a crucial role in their health and growth. Parameters such as temperature, total hardness, and organic content of the substrate can significantly impact the density and relative abundance of bivalves. For instance, higher temperatures and organic content in the substrate can favor the growth and reproduction of certain species. Moreover, seasonal changes in environmental conditions, including water temperature and availability of food sources, can affect the growth patterns and thus the weight of bivalves. Studies have shown fluctuations in bivalve populations based on seasonal variations

Mayor et al. (2016) also highlighted the biological characteristics which are factors in the growth of freshwater bivalves. According to them, species differences bivalves may respond differently to environmental conditions. For example, the shell length and depth have been found to be ideal estimators for the increase in weight for some species, while others might rely more heavily on shell length alone. Also, growth patterns of bivalves, including their condition index and reproductive status, are critical determinants of their weight. Negative allometric growth patterns indicate that as bivalves grow larger; their shells do not scale proportionally with their soft tissues, which can affect their overall weight.

But most importantly, Mayor et al. (2016) emphasized that harvesting and Exploitation of humans can significantly impact the weight and sustainability of bivalve populations. Overharvesting can lead to reduced meat yields and negatively affect the growth and condition of the remaining individuals. Finally, the potential for aquaculture or artificial propagation of these species is another factor that could influence their weight through managed breeding programs aimed at improving growth rates and meat yield.

Figure 2.
Bivalve samples.

3.1. Microplastics Classification

The microplastics observed from the sample were classified either as primary or secondary microplastics, the results can be seen below:

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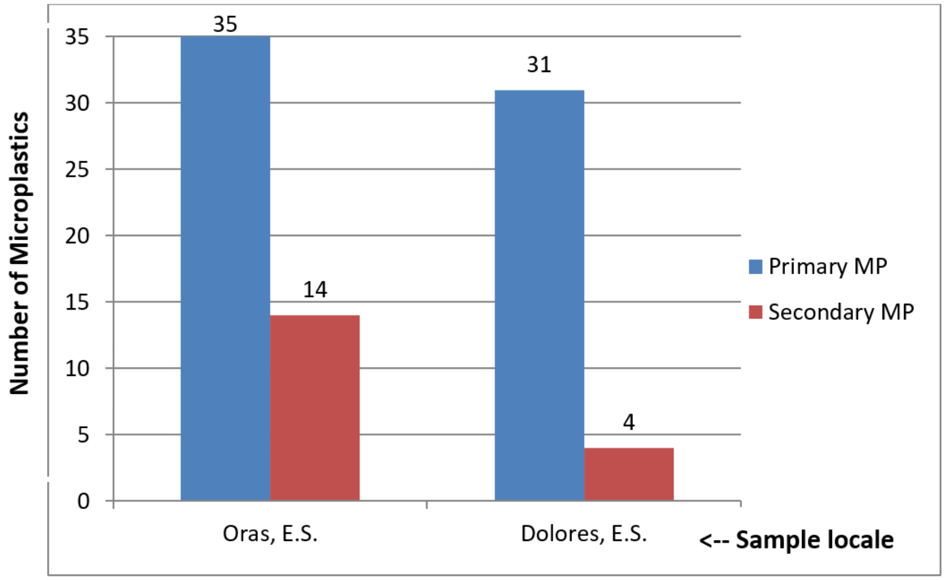

Figure 3.
Classification of Microplastics from the two (2) localities.

It can be seen from the chart above that most of the microplastics observed were primary microplastics. This data is supported by the statement from the National Geographic (n.d.) that most primary microplastics are tiny particulates that are designed for commercial use, as well as microfibers that are shed from clothing and other textiles such as fishing nets. It is also observed that Oras samples contain the highest number of primary microplastics than in Dolores samples.

Only a small number of microplastics were observed to be secondary MPs; these are particles that result from the breakdown of larger plastic items such as water bottles and plastic bags.

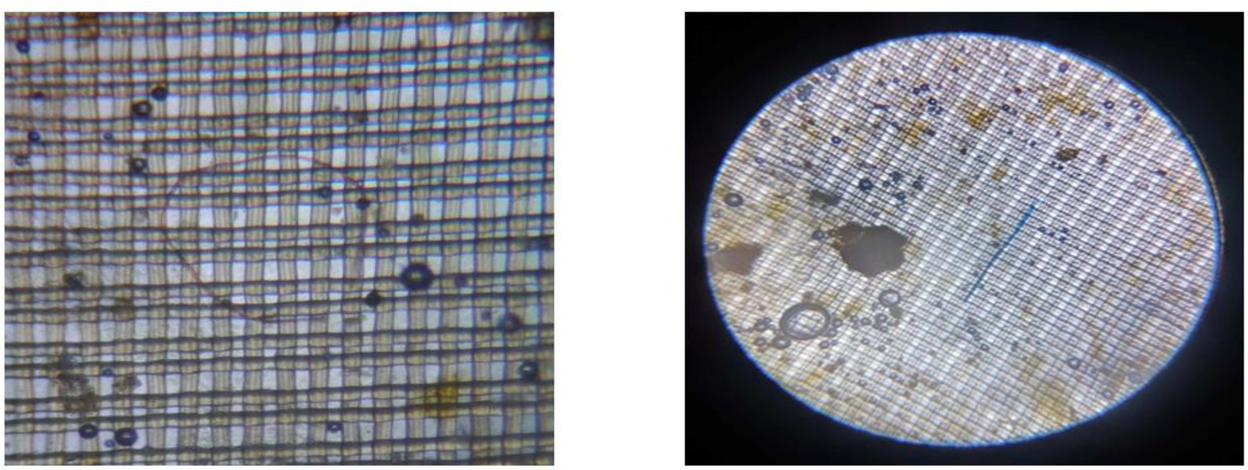

Figure 4.
Microplastics observed (left – red microfiber; right – blue microfiber).

The presence of these microcontaminants may be due to poor waste management where these organisms were collected. Although these materials are less dense than water and have a tendency to float, with poor segregation of these plastics, heavier particualtes in the waste may tend to make these materials sink into the bottom of the river floor and there, these plastics degrade with time due to varying water temperature, pH and other chemical and physical attributes that will break plastics into smaller and smaller pieces. Since *C. flumineas* are filter feeders, upon the feeding process, tiny bits of microplastics are accumulated into their system and are sometimes hard to be processed by the bivalves and therefore bioaccumulate in their tissues.

3.2. Microplastic Identification

Microplastics can also be identified, either as a microbead, a microfilm, a microfiber and or microfragments. Data on the identified MPs can be seen from the figures below:

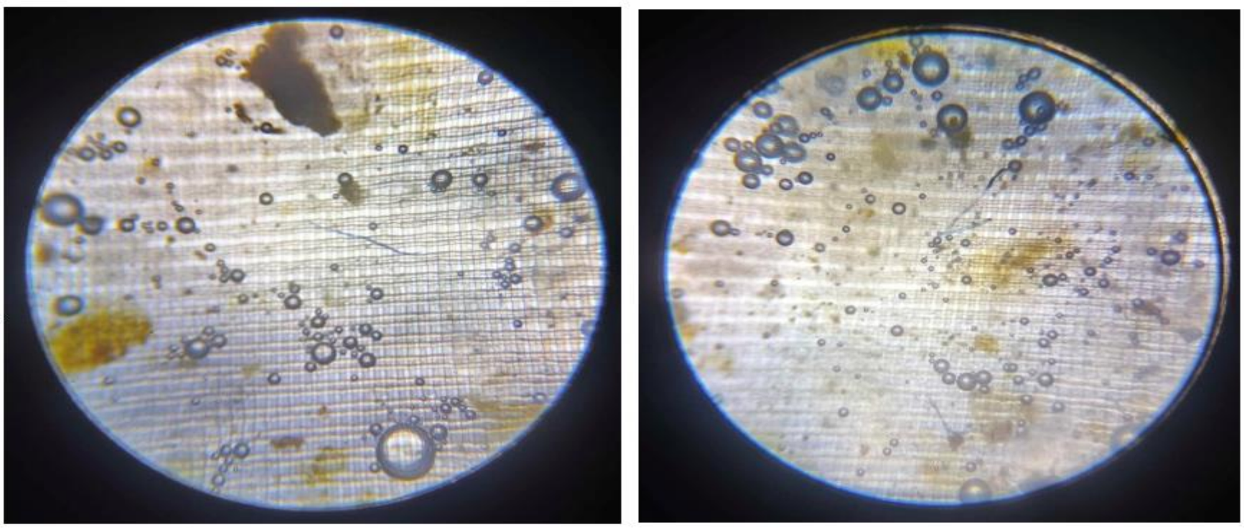

Figure 5.
Microplastics Observed (left – blue microfiber; right – blue biofouled microfiber)

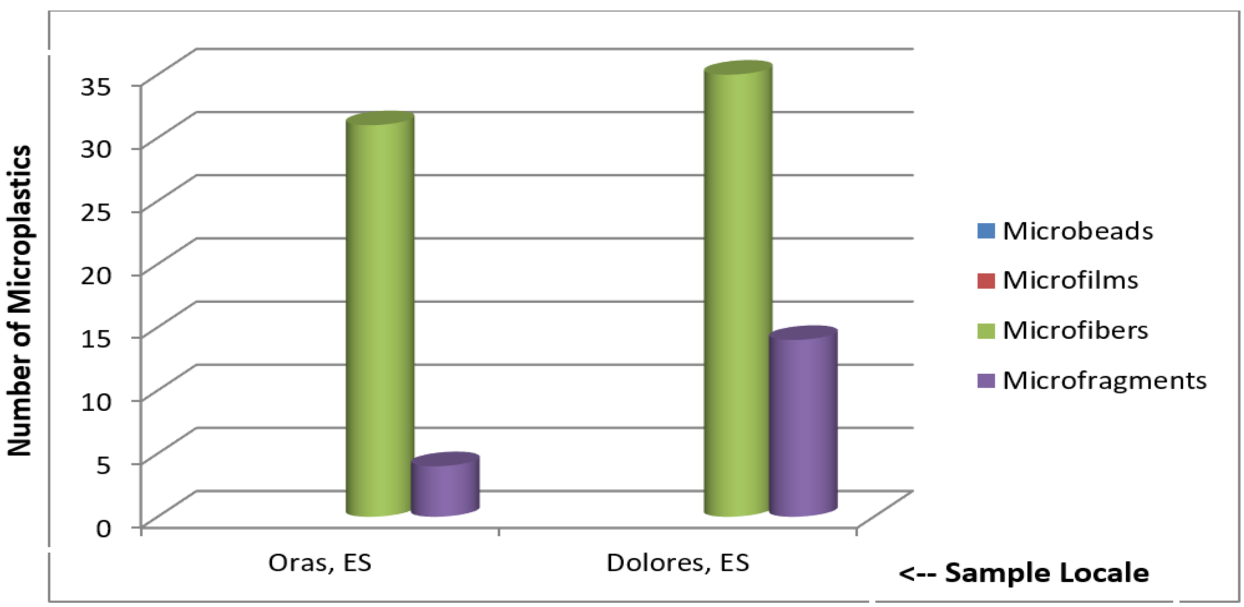

Figure 6.
Identification of Microplastics from the two (2) localities

As seen from the figure above, there were no observed microbeads or microfilms on all samples of bivalves in both localities. The figure above also signifies the prominent presence of microfibers which are usually classified as primary MPs. As observed, only small amounts were identified as microfragments which indicate that most MPs in both areas are microfibers. This result is supported by the statement of Weis, J. (2024) wherein the most common microplastics in the environment are microfibers – plastic fragments shaped like tiny threads or a filament which has many sources, including cigarette butts, fishing nets and ropes.

3.3. Microplastics Physical Structure

Physical structure means the overall physical shape and size of the microplastics, it is round or spherical, angular, filamentous or other shaped MPs which means it goes out the usual shape of most microplastics . Results on the physical structure of the MPs observed can be seen on the figure below:

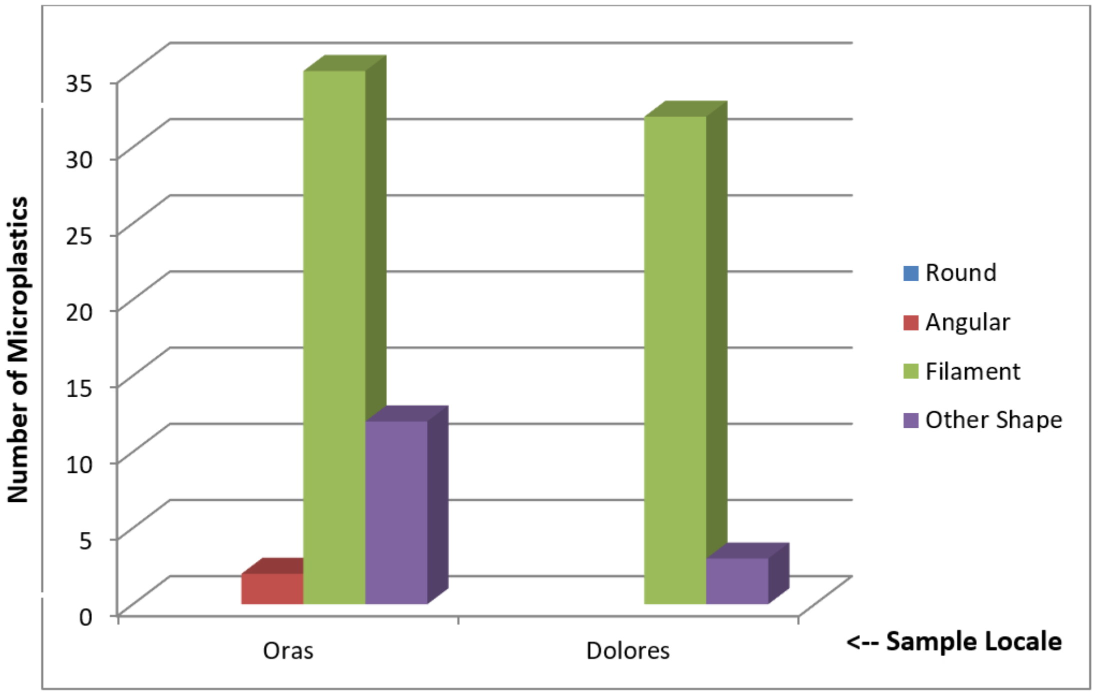

Figure 7.
Identification of Microplastics from the two (2) localities.

It can be observed from the figure above that no rounded MPs were observed, however, 2 angular MPs were observed from the samples coming from Oras, whereas no angular MPs were observed from the Dolores samples. But, the most prominent result here is that high number of filamentous MPs coming from both sampling areas which is in relation to the high number of microfibers observed, also from both localities. This indicates that the most common form of microplastic contaminants in the bivalve samples, comes in the form of filamentous microfibers.

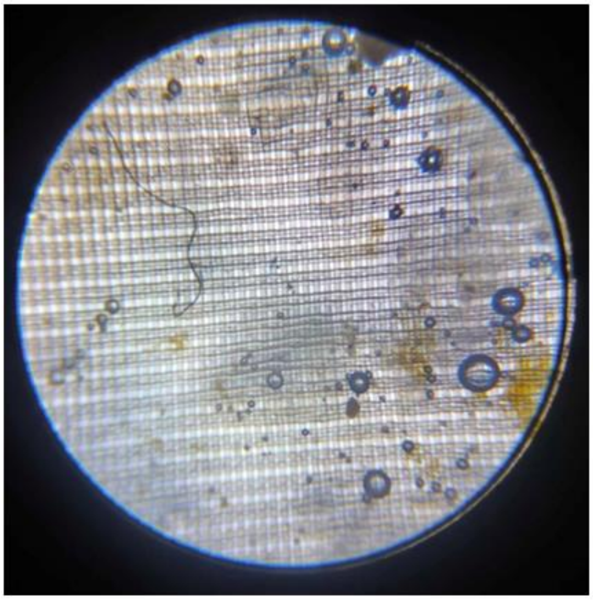
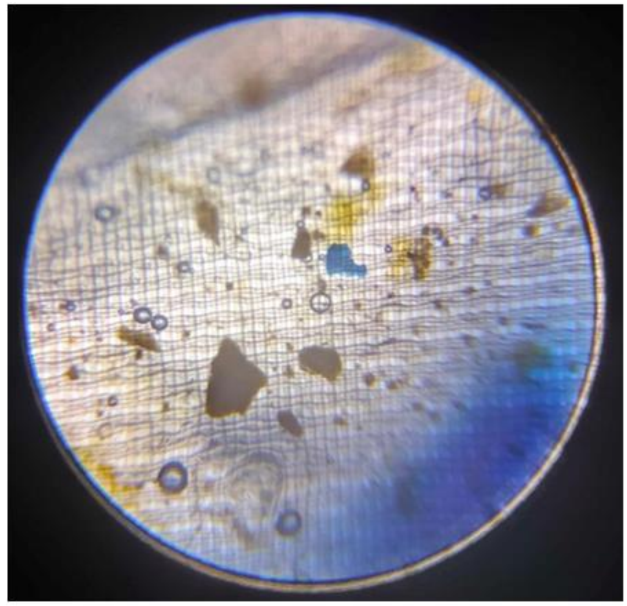

Figure 8.
Microplastics Observed (left – black microfiber; right – blue microfragment).

3.4. Microplastics Color

Microplastic colors vary, they exhibit a wide spectrum of colors, and they can be transparent to black, blue, gray, green, red or purple, and even yellow. Widely depends on where they came from. The results on the color of MPs observed can be gleaned from the figure below:

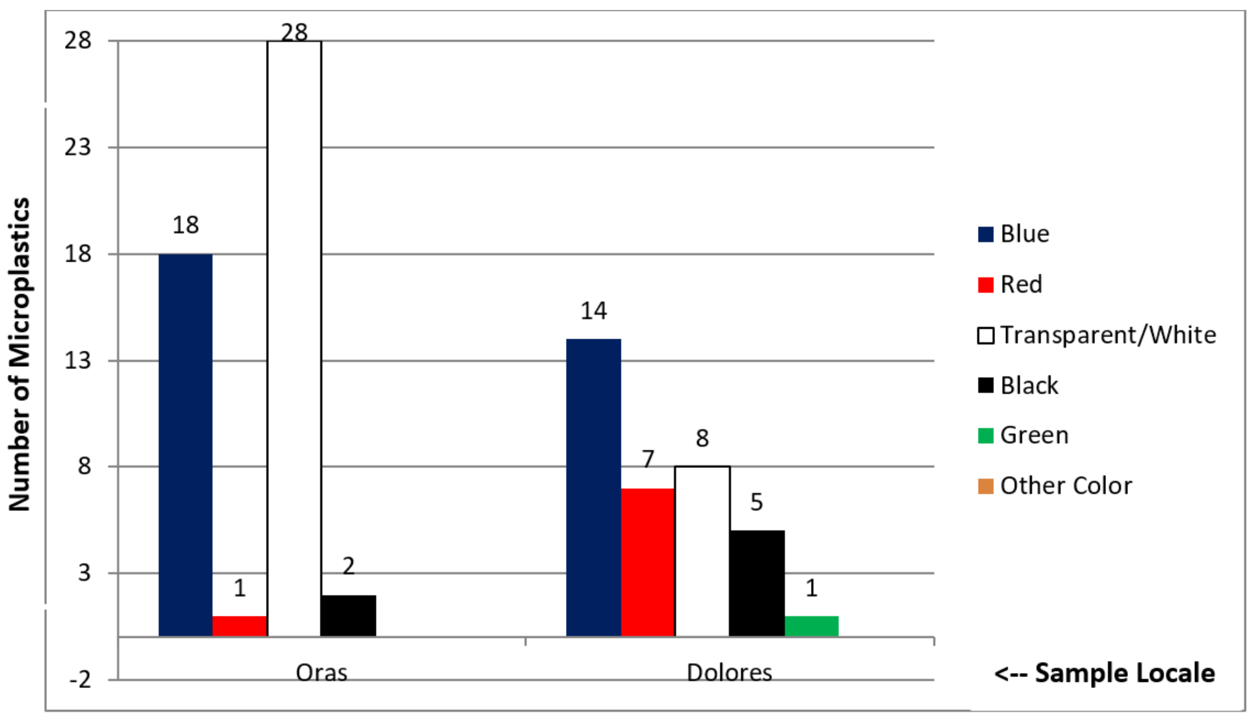

Figure 9.
Identification of Microplastics from the two (2) localities.

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Based on the figure above, it can be seen that transparent/white has the highest number of microplastics observed in Oras samples. However, in Dolores, the samples have the highest number of blue colored MPs, although Oras samples also have a high number of blue colored microplastics. This result is supported by the statement from Wang, Q. (2020) which states that the most abundant color is white or transparent. Moreover, Key, S. (2024) argues that plastics with bright colors such as red, blue and green degrade and form microplastics quicker than those with plainer colors.

3.5. Total Microplastics

A total of 84 microplastics were observed overall in all the samples taken from Oras and Dolores, Eastern Samar. From the total, 49 were detected from the bivalve samples coming from Oras, Eastern Samar, while the remaining 35 MPs were observed from the samples taken from Dolores, Eastern Samar. This numbers indicate that the bivalves collected from the rivers of Oras, Eastern Samar is more contaminated in terms of the number of MPs than those that are harvested from Dolores.

This numerical data also gives us an average of 0.34 MPs/g from Oras and 0.42 MPs/g coming from Dolores. Overall, the average MPs/g of bivalve soft tissues goes at around 0.37 Microplastics/grams of bivalve sample.

4. Discussion

According to Cesarini et al. (2023), the route of microplastic absorption in freshwater bivalves primarily occurs through ingestion and adherence to soft tissues. This process involves several steps wherein, in terms of ingestion, the freshwater bivalves, such as mussels and clams, filter feed by drawing water through their gills, capturing small particles, including microplastics, within their bodies. This method allows them to ingest microplastics directly from the environment. In terms of adherence to soft tissues, this one is beyond ingestion, microplastics can also adhere to the external surfaces of bivalves, particularly to their soft tissues. This mode of uptake is significant because it does not require the microplastics to be ingested but simply comes into contact with the organism's body surface.

Bioaccumulation and Biomagnification is also one particular factor, Cesarini et al. (2023) further elaborated that once absorbed, microplastics can accumulate within the bivalve's tissues. This accumulation can lead to biomagnification, where the concentration of microplastics increases as they move up the food chain. This phenomenon poses risks to higher trophic levels, including humans who consume these organisms. The presence of microplastics in bivalves can have various toxic effects, including genotoxicity and intestinal damage. These effects highlight the potential health risks associated with consuming microplastic-contaminated seafood.

Furthermore, this paper strictly particularizes that the primary routes of microplastic absorption in the C. fluminea samples on Oras and Dolores, Eastern Samar may involve both ingestion and adherence to soft tissues process. Both processes contribute to bioaccumulation and biomagnification of microplastics within the bivalve population. The presence of microplastics in bivalves can lead to adverse health effects, emphasizing the importance of monitoring and managing microplastic pollution in aquatic environments.

Consumption of bivalves contaminated with microplastics poses potential health risks due to the ingestion of both the microplastics themselves and the toxic chemicals they can carry.

According to Smith et al. (2018), humans can be exposed to microplastics through the consumption of seafood, particularly bivalves like mussels, clams, and oysters. This exposure can occur either directly through the ingestion of microplastics or indirectly through the accumulation of toxins adsorbed onto the microplastics. Moreover, they emphasized that the primary concern with microplastic ingestion is the potential for co-occurrence of harmful chemicals that adhere to the plastic surfaces. These chemicals can include persistent organic pollutants (POPs), heavy metals, and other substances that are toxic to humans. The health effects of consuming microplastics are still largely unknown but could potentially include gastrointestinal issues, inflammation, and immune system dysfunction.

Lastly, Smith et al. (2018) stressed the toxicity associated with consuming microplastics is likely dependent on several factors, including the size of the microplastics, the associated chemicals, and the dose. Smaller microplastics (< 10 μm) are of particular concern due to their ability to pass through biological barriers and reach internal organs.

Currently, there has been no regulatory exposure limits for microplastics henceforth, this data can be used to bear support that microplastics and nanoplastics are common contaminants throughout our environment, and that, it may be deemed impossible for humans and animals, especially aquatic organisms to avoid contact with this particulates. This paper articulates that the MPs found in the bivalve samples from both Oras and Dolores, Eastern Samar are below the global average MPs found in bivalves regardless whether freshwater or saltwater species.

5. Conclusions

Based on the results gathered and data analyzed, the following conclusions are herein drawn by the researchers:

1. The bivalve species found in Oras and Dolores, Eastern Samar is the species *Corbicula fluminea*, locally known to as "bibe".
2. Most of the microplastics found in the bivalve species samples in both Oras and Dolores are primary microplastics.
3. The microplastics observed in all samples were mostly microfibers.
4. In terms of physical structure, most microplastics observed were filamentous which are in relation to the high number of the presence of microfibers.
5. The most prominent color of the microplastics observed was transparent or white, in Oras, the most common color was transparent, while in Dolores, the most common color was blue.
6. A total of 84 microplastics were observed overall in all the samples taken from Oras and Dolores, Eastern Samar. From the total, 49 were detected from the bivalve samples coming from Oras, Eastern Samar, while the remaining 35 MPs were observed from the samples taken from Dolores, Eastern Samar. On average, around 4.9 MPs/sample from Oras and 3.5 MPs/sample coming from Dolores. Overall, the average MPs/bivalve goes at around 4.2 microplastics/ bivalve sample.

6. Recommendations

Based on the results and conclusions of this study, the following are hereby recommended:

1. Conduct quantitative analysis on the present microplastics such estimated length of the microfibers,
2. Conduct FTIR spectroscopic analysis of MPs found to further confirm its presence and determine what type of plastics are contaminating these organisms.
3. Conduct a similar study on the surrounding environment in which these bivalves thrive, such as waters and sediments.
4. Conduct a similar study to further affirm or oppose the results of the current study.
5. Conduct a similar study utilizing more number of samples to further intensify the presence microplastics in these organisms.
6. It is also recommended that a more comprehensive waste management be implemented in the areas concerned as improper waste disposal is one of the major factors for microplastics contamination to organisms in marine environments.
7. Also, it is recommended to have a year round monitoring of microplastics in these organisms as a way of determining the increase or decrease of bioaccumulation in their systems.

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