Microplastic Contamination in Cultured Oysters *(Ostreidae)*

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Abstract

Six samples of intertidal long-line cultured oysters in the coastal areas of Sagay Marine waters such as Punta Roma, Old Sagay, Purok Mahogany and Sitio Tagnipis of Taba-ao, Purok Kalubihan and Sitio Tu-ong, Plaridel, Brgy. Bulanon and Vito were identified and mapped using the desire and flow lines technique from three to ten kilometers distance in a zigzag manner and transected during the low-tide hot season; extracted; weighed into 500 grams wet weight; coded; assessed and evaluated the microplastic contamination at Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory Center. Five samples were from the estuarine and densely populated areas and a sample was from the sea which had a ten-kilometer distance from densely population and beyond the mangroves' reserve. Each meat sample was divided into four quadrants on filter paper and underwent a sterilization process using wet peroxide oxidation (WPO) chemical digestion and optical microscopy. Five out of 6 samples or 83% were microplastic contaminated and as evaluated, microplastic fiber was from synthetic worn-out clothes. Intertidal long-line cultured oysters in estuarine and in close distance with densely populated residences were more microplastic contaminated. As observed, the intertidal long-line oyster culture along the area with zero MPs content was beyond the mangrove reserve where macro and microplastics were filtered by the roots of the mangroves.

Keywords: Densely populated area, estuarine, intertidal long-line cultured oyster, microplastic contamination, microplastic fiber, synthetic worn-out clothes, mangroves.

1. Introduction

Microplastics that flow in salty or brackish water are combined with phytoplankton and are mistaken as food and consumed by marine organisms, (Egbeocha et al.,2018). Phytoplankton are microscopic plankton that fluctuate in shape and size. These are crucial to the aquatic ecosystem biology and climate, they can photosynthesis, where they were the primary producer of the aquatic food web, and any alternative in their productiveness ought to have a sizable effect on biodiversity, fisheries, and human food supply, and the pace of global warming, (Henson, S., et al, 2021). In this situation, microplastics floating in water were combined with phytoplankton, so marine organisms usually hibernate in phytoplankton and also their food source.

Microplastic contamination is everywhere, it refers to the presence of tiny pieces including chemical compositions in various environments, land, air, and water (Steele, C., 2020). It comes from different sources such as littering, storms, water runoffs, streams, winds, synthetic clothing fibers, tire dust, and industrial wastes. When heavy rains drop, water flows from upper heights down streams, passing through highly urban and rural residential areas including industries. Rains clean and wipe off all undesirable wastes including chemicals that are scattered in air and land environments and bring these down to rivers, sea, and to the ocean. These can pose threats to wildlife, and marine organisms that are consumed as food by humans and are threatening to human health as they are ingested in the gut of the human body, (national geographic.org). Microplastics are small plastic particulates including heterogeneous groups of particles, varying in size, shape, and chemical composition that measure 100 nanometers to 5 millimeters (< 5 mm), if this measures less than 100nm these are called nano plastics.

The above situation also threatens aquaculture products like oyster culture (Botta, R. et al, 2020); Shruti et al., 2020; Ghosh, A., et al., 2021). The intertidal long-line culture of oysters is common in the coastal areas of Sagay Marine Environment, such as oyster culture in Punta Roma, Old Sagay, and Sitio Tagnipis, in the mangrove areas of Brgy. Taba-ao, Brgy. Bulanon waters, and Brgy. Vito. In this method, oysters are grown on ropes or lines that are suspended in the water column (Bordignon, F., et al, 2020).

Oysters *(Ostreidae)* are bivalve shellfish (mollusk), they are filter feeders that consume algae, phytoplankton, and zooplankton, they don't move and are steady on their place, and food dependent on the different matters and organisms that are present in the water around them (Li, Y., et al, 2012). Oyster microplastic contamination already exists globally and can be existed in any local waters due to environmental pollutants. Wild-caught oysters were found to have over twice the quantity of microplastics compared to oysters raised through aquaculture, possibly indicating the influence of the pristine and productive waters typically associated with oyster aquaculture systems (Wootton, N., et al, 2022).

Sagay Marine Environment coastal upper heights areas are heavily residential and the residential wastewater pipe system is not yet fully established. Environmental observations of this study found that aside from set policies on proper waste management, leaders could not fully control the flowing of liquid household wastes from residences to water streams due to a lack of water pipe systems and disposal of solid and liquid wastes management.

Oysters are the most valuable and marketable seafood product in Sagay City, as observed during Sunday and Tuesday markets, oysters are the most salable seafood products due to their organoleptic properties and appetizing tastes that impact the tastebuds. There were several cases where food allergens stroke in the people in the area particularly the adults because this age group is the ones usually consuming oysters. Food allergy is an immune system reaction that occurs soon after eating a certain food, this can trigger signs and symptoms such as digestive problems, hives, or swollen airways, in some people, a food allergy can cause severe symptoms or even a life-threatening reaction known as anaphylaxis. A situation like this leads the research group to investigate and evaluate properly the reason behind such a situation.

This study focused on the assessment of microplastic contamination of the intertidal long-line oyster culture in the coastal area of Sagay Marine Environment. Furthermore, this study

mapped, transected, and identified the locations of the different samples for further assessment and evaluation of microplastic contamination in intertidal long-line oyster culture. This study was conducted covering the coastal area of Sagay Marine Environment. It took twenty-four months to finish the study and samples were assessed and evaluated at Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory.

2. Materials and Methods

Research Design

This study was conducted to assess the microplastic contamination in intertidal long-line oyster culture in the coastal areas of Sagay Marine Environment. This is descriptive research in quantitative design. A Descriptive was employed because it describes the characteristics of microplastic contamination in shellfish. The descriptive method is to gather information about the present existing condition of a thing (Creswell, 1994). A quantitative design was used because it generates numbers or frequency and percentage of samples contaminated with microplastics. This is a process of collecting and analyzing numerical data (Bhandari, 2022).

Materials

In order to determine the number of samples, mapping, and distribution of coordinates covering the coastal areas of Sagay Marine Reserve from the SMR office and GPS were utilized. A transect rope was used to locate the intertidal long-line cultured oysters, motorboat, knife, scouring knife, samples safety containers, weighing scale, PPE (personal protective equipment), cooler containers, packing tapes, and record notes.

Methods

Preparation

A. Local of the Study

Map showing the location of the six samples of intertidal long-line oyster culture

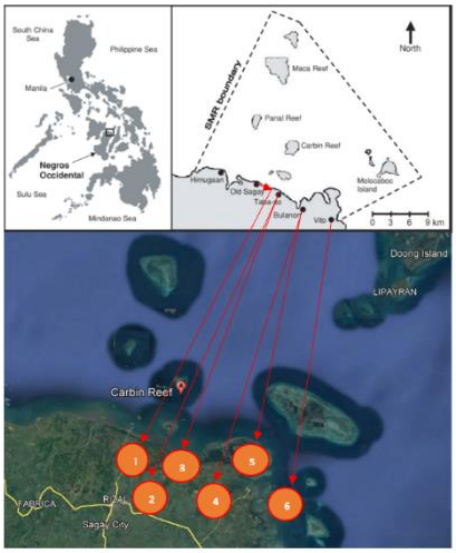

Figure1: Six samples of intertidal long-line oyster culture for microplastic assessment

Figure 1 shows the location of the six samples of the intertidal long-line oyster culture. The mapping of the samples was determined through a one-to-three-kilometer distance and the different downstream of water from the heights and rivers and thickness of residences was considered. Sample 1 was from the intertidal long-line oyster culture from Punta Roma, Old Sagay; Sample 2 was from the intertidal of Purok Mahogany, Brgy. Taba-ao; Sample 3 was

from the intertidal brackish water of Sitio Tagnipis which was with dense population and down streams rivers were observed; Sample 4 was from the intertidal area of Purok Kalubihan, Plaridel; Sample 5 was from the brackish water of Sitio Tu-ong and with dense population and downstream rivers were observed; Sample 6 was from the brackish water of Brgy. Vito. Those locations were set using the GPS (Global Positioning System and Sagay Marine Reserve (SMR) Map. This GPS (Global Positioning System) was the supporting technology utilized to make a vivid picture and interpretation of the sampling locations. Samples shown in Fig.1 were indicated with line segments to further locate, identify, and collect the kinds of samples represented by each location.

B. Data Gathering Procedure

1. Mapping, Identifying and Transecting of Samples in Different Locations

Figure 2: Mapping, Identifying, and Transecting of Samples

Figure 2 shows the mapping, identifying and transecting of samples. In Punta Roma, Old Sagay, sample was taken during the early morning low tide. On the next morning, sample 2 and 3 were taken from Sitio Tagnipis, and Purok Mahogany of Brgy. Taba-o; sample 4 was taken from Brgy. Plaridel, sample 5 from Brgy. Bulanon and sample 6 from Brgy. Vito.

2. Extracting, Weighing, Packing and Coding of Intertidal Long-line Oyster Culture Samples

Figure 3: Extraction, Weighing, Packing and Coding of Intertidal Long-line Oyster Culture Samples

Figure 3 shows the samples' extraction, weighing, packaging and coding of intertidal long-line oyster culture. Acquisition was done during the low tide and directly delivered to a safe house near the shoreline. Extraction was done safely and weighed into 500 grams wet weight

and packed using the slider top ziplock bag and coding for quick identification of the type of sample.

C. Data Analysis Procedure

1. Assessment of microplastic content in the intertidal long-line oyster culture at Sagay Coastal Areas

a. Wet Peroxide Oxidation (WPO)Chemical Digestion Technique

Laboratory safety practices and policies for handling samples were observed before conducting the assessment and evaluation of microplastic contents. The sample was placed on filter paper ; placed in the beaker which contained a fraction of the collected sample, this was added with 20mL of aqueous 0.05M Fe (II); added with 20mL of 30% hydrogen peroxide, cautioned was observed, the solution can be boiled violently when heated up to more than 75 ^{o}C); the mixture was then placed on laboratory bench at room temperature for 5 minutes prior to the proceeding on the next step; stirring bar was added to the beaker and covered with a watch glass; heated into 75 ^{o} C on a hot plate; when gas bubbles were observed at the surface, then beaker was removed from the hot plate and placed in the fume hood until boiling subsided, there was an overflowing reaction so distilled water was added to stop the reaction; it was then heated up to 75 ^{o} C for an additional 30 minutes; when the natural organic material was visible, it was added with another 20mL of 30% hydrogen peroxide and repeated until there was none natural organic material at all; the 6g of salt (NaCl) per 20mL of sample was added to increase the density of the aqueous solution (-5M NaCl); heated the mixture up to 75 0C until the salt was dissolved.

b. Density Separation and Optical Microscopy

The WPO solution was transferred from the WPO process to the density separator; the WPO beaker was rinsed with distilled water to transfer all remaining solids to the density separator; covered loosely with aluminum foil and allow the solids to settle overnight; inspected visually the settled solids for any MPs if there were present, then drained the settled solids from the separator and removed MPs using forceps, and archived these MPs contaminants properly for further evaluation; placed the collected floating solids in a clean 0.3 -mm custom sieve. Lastly, Optical Raman Microscopy was used by a trained licensed laboratory technician for further identification of the types of microplastics.

c. Screenshots of the Microplastic Fiber in Oyster Samples

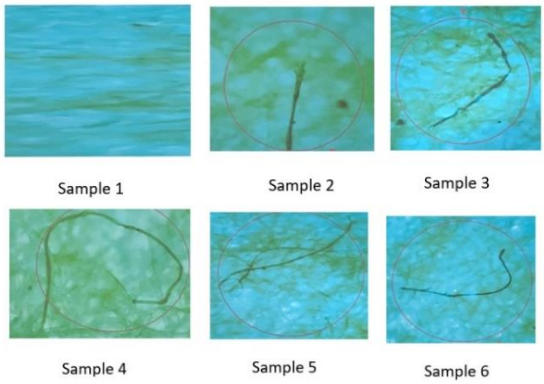

Figure 4: Screenshots of the Microplastic Contamination Assessment of Intertidal Long-line Oyster Culture in the Coastal Areas of Sagay Marine Waters

Figure 4 shows the screenshots of the microplastic assessment of the intertidal long-line oyster culture where sample 1 which came from intertidal long-line oyster culture of Punta Roma, Old Sagay had no microplastic content. Sample 2 from the Prk. Mahogany of Taba-ao with quadrant 1 had microplastic content; Sample 3, Sitio Tagnipis of Taba-ao with quadrant 3 had microplastic content; Sample 4 of Prk. Kalubihan and Tuong near the river of Plaridel with quadrant 2 with microplastic content; Sample 5 of Brgy. Bulanon with quadrant 4 had the microplastic content; and sample 6 of Brgy. Vito filter paper quadrant 1 had the MP's content.

Ethical Considerations

This study had prepared ethical considerations prior to the conduct of the study such as notice to proceed, permission from the local government units specifically from the Sagay Marine Reserve coordinator, Sagay City Department of Agriculture coordinator, Bureau of Fisheries and Aquatic Resources coordinator, Brgy. Captains of Himuga-an Baybay, Old Sagay, Taba-ao, Bulanon and Vito. In the same situations gave remunerations and food packs to pump boat drivers, divers, and communities involved in transecting, identifying, classifying, and extracting the shellfish meat including the seaports in charge. Additionally, this study respects all the communities involved.

3. Results and Discussions

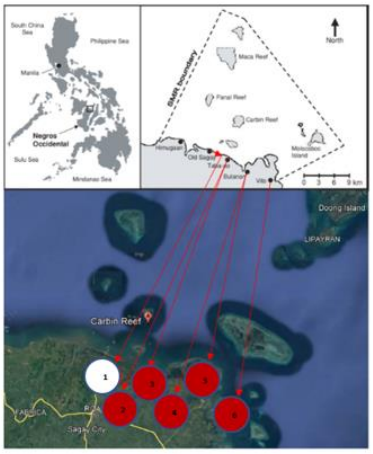

Figure 5: Location of Intertidal Long-line Oyster Culture in the Coastal Areas of Sagay Marine Reserve

Intertidal Long-Line Oyster Culture	Geographical Location	MPs Content
Sample 1	Intertidal far from residences and estuarine	0
Sample 2	Estuarine, densely populated area	1
Sample 3	Estuarine, densely populated area	1
Sample 4	Estuarine, densely populated area	1
Sample 5	Estuarine, densely populated area	1
Sample 6	Densely populated area	1
		5/6 (83%)

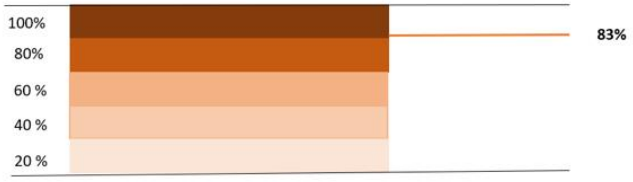

Table 1: Microplastic Contamination Index of Intertidal Long-line Oyster Culture in Coastal Areas of Sagay Marine Environment

Figure 5 shows the location of six samples of the intertidal long-line oyster culture of the coastal areas of the Sagay Marine Environment where samples indicated in red were MPs contaminated and sample indicated in white was zero content MPs. As surveyed and observed, samples in red were estuarine (connections of river and sea water) and densely populated areas. Sample in white was located long distance from densely populated area and estuarine. The MPs contamination index of the coastal areas of Sagay Marine waters was 83%. These MPs fibers as evaluated were from the synthetic fibers of worn-out clothes.

4. Conclusion

Intertidal long-line cultured oysters in estuarine and in near densely populated residences were more microplastic contaminated. As observed, the intertidal long-line oyster culture along the area with zero MPs content was beyond the mangrove reserve where macro and microplastics were filtered by the roots of the mangroves.

5. Recommendations

Oyster culture shall be planted far from or after the mangrove reserve. Since oyster culture in estuarine or near the river was more savory compared with an oyster in salty water, local leaders shall set additional and new policies on proper waste management and proper standard piping and disposal of household solid (residue) and liquid wastes to protect Agri and eco-tourism sites where oyster culture are considered livelihood and one of the valuable economic incomes in the aquaculture industry in the locality.

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Conflict of Interest

The authors declare no conflict of interest.

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