Microplastic Contamination of Mollusks (Shellfish) in Sagay Marine Environment

Anabelle E. Villaceran [1*] , Jocelyn D. Bantigue[2], Roselyn D. Usero [3], Angelo T. Layson[4]

1. A. Doctor of Philosophy in Technology Management, Head of the Food Technology Research and Development Center of Northern Negros State College of Science and Technology, Sagay City, Negros Occidental, Philippines
2. A Doctor of Philosophy in Environmental Science, Dean of Graduate School, Northern Negros State College of Science and Technology
3. Bachelor of Science in Chemical Engineering, Manager Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory, Bacolod City, Negros Occidental, Philippines
4. Master of Criminal Justice Education, Faculty President, Northern Negros State College of Science and Technology

Abstract

Ten univalves and fifteen bivalves of mollusks (shellfish) were identified from the coastal, islands, and reefs during the low tide of inter-tidal and sub-tidal areas of the Sagay Marine environment and subjected to microplastic contamination assessment. Transecting and collecting of samples were done using Global Positioning System (GPS). A minimum of 500 grams of the wet weight of shellfish meat and the extraction and packing in containers were done from the sampling stations and directly delivered to Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory. Sterilization using wet peroxide oxidation (WPO) chemical digestion and optical microscopy were methods used in shellfish microplastic extraction and identification. Of 25 mollusk (shellfish) meats, 7 or 28% were positive for microplastic fiber contamination. Of the 7 microplastic fiber contaminated mollusks (shellfish), 6 or 24% of them were bivalves, namely: tumid venus *(Gafrarium pectinatum*), ark clam *(Anadara antiquata),* cultured oysters *(Ostreidae),* hooded oysters *(Saccostrea cucullata),* ventricose ark *(Arca Ventricosa)* and green mussels *(Perna viridis*) thriving in the inter-tidal and sub-tidal coastal areas of Bulanon; oysters and hooded oysters from Punta Roma, Old Sagay and Tagnipis, Taba-ao proper of Taba-ao and Vito; the 1 or 4% was univalve which was the trapezium horse conch *(Pleuroploca trapezium)* thriving in the mangrove sediments of the island of Molocaboc Daku. That microplastic fiber came from worn-out clothes. The reefs of the Sagay Marine Environment had zero microplastic contamination. The microplastic contamination index of commercially identified mollusks (shellfish) in the Sagay Marine was 28%. Univalve and majority of bivalves mollusks (shellfish) that thrive in the mangrove sediments and in the brackish water of Sagay Marine Environment were more microplastic contaminated.

Keywords: Sagay marine environment, microplastic contamination, mollusks, food safety, univalve, bivalves, wet peroxide oxidation

1 INTRODUCTION

Microplastic (MPs) contamination in shellfish species affects food security, food safety, and human health. Recent studies have shown that microplastic ingested by shellfish can cause adverse effects and can raise potential health risks to higher tropic levels of organisms including humans. Chronic exposure to polyvinyl chloride MPs induces liver injury and gut microbiota, and this proves that MPs hinder the life span of animals and humans, (Li, Q., Ma, C., Zhang, Q., Shi, H., 2021; World Economic Forum; The Guardian Angel; The Economic Times; Scientists from Plastic Action Centre; and National Geographic Organization).

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. These particulates have been found everywhere, from the top of Mount Everest down to the Mariana Trench, the deepest part of the sea, as well as, there are 600 pieces of MPs in 1 kg of salt (NaCl), 90% in the stomachs of seabirds, 52% in the stomachs of sea turtles, and 50 to 500 per 10 grams in human stool, (Amobonye, A., Bhagwat, P., Raveendran, S., Singh, S., & Pillai, S., 2021; Schwabl, P., Sebastian, Dipl-Ing, S.K., Königshofer, P., 2019; Boucher, J. & Friot, D., 2014; Plastic Action Center, World Economic Forum & National Geographic Society).

The above situations denote the possibility that MPs might also be present in mollusks (shellfish) in Sagay Marine waters such as the univalves and bivalves. In a recent study of MPs contamination in bivalves of the Philippines, green mussels, and oysters, have proven of positive contents, (Malto, M. & Mendoza, A. 2022; Bilugan, Q., Limbago, J. and Gutierrez, R., 2021; Jambre, K., 2021). On the other hand, Global studies have proven that MPs are one of the primary contaminants in bivalves' shellfish, (Lozano-Hernandez, E., et al, 2021; Covernton, G. A., Collicutt, B., Gurney-Smith, H. J., Pearce, C. M., Dower, J. F., Ross, P. S., & Dudas, S. E., 2019). Lozano-Hernandez, E., 2021; Zhang, F. et al, 2018). In recent observations of Sagay City Health Center, it has been noted that there are cases of food allergens and poisoning in eating shellfish meat and this situation connotes pollution in the land, air, and water around the environment.

In Sagay Marine environment, Sagay City, Negros Occidental, Philippines; shellfish are abundant and shellfish food safety is not yet at the highest level of awareness. Aside from marine water levels of acidity and some heavy metals toxicity, MPs contamination in shellfish is not yet assessed for public food safety.

Sagay Marine Environment is a 32,000 hectares aquatic ecosystem declared as a marine protected seascape in 1995 by virtue of Proclamation Number 592 under the National Integrated Protected Areas System (NIPAS). It comprises the Islands of Molocaboc Daku, Molocaboc Diut, Matabas, and Suyac; Reefs of Carbin, Macahulom, Panal, and other surrounding reefs. It also includes the coastal Barangays of Himugaan Baybay, Old Sagay, Taba-ao, Bulanon, and Vito, (SMR). Living and exposure to aquatic areas and food resources in this place since birth are strong factors in conducting this study.

There are lots of studies on microplastic contamination in shellfish in the Philippines, like the study of Malto, M. & Mendoza, A. 2022 entitles" Suspended Microplastic in Sorsogon Bay Attributing Perna viridis and Atrina pectinata Contamination"; Bilugan, Q., Limbago, J. and Gutierrez, R., 2021 on the "Detection and quantification of microplastics from cultured green mussel Perna viridis in Bacoor Bay, Cavite, Philippines" and Jambre, K., 2021 on the " Density of Microplastics in Philippine Cupped Oyster (Crassostrea iredalei).
The said studies were focused on microplastic contamination in specific bivalve mollusks (shellfish), while this study, "Microplastic Contamination of Mollusks (Shellfish) in Sagay Marine Environment" deals with the identification of the location of shellfish using the GPS (global positioning system), classification of shellfish with the help of local community using science communication tool, transecting the identified and classified shellfish, extraction of shellfish meat and assessment of the commercially consumed univalve and bivalve mollusks in Sagay Marine Environment using wet peroxide oxidation(WPO)chemical digestion and optical microscopy.

This study was conducted in the area covering the Sagay Marine environment at Sagay City, Negros Occidental, Philippines, and samples were analyzed at the Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory. It covered almost 24 months to complete the study.

II. MATERIALS AND METHODS

Materials

The researchers sought permission from the Sagay Marine Reserve Office to assess the different kinds of mollusks and shellfish commercially consumed in the Sagay Marine Environment and the suggested maps and sampling stations were given.

A. Materials and Technologies Used for Gathering Mollusks

Figure 1 shows the materials and technologies utilized during the establishment of the transect and collection of shellfish samples.

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B. Sagay Marine Reserve Map

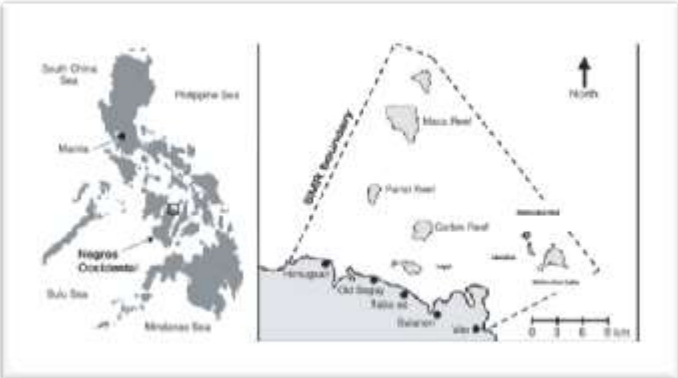

Figure 2 shows the Sagay Marine Reserve Map taken from the Sagay Marine Reserve Office, Sagay City, Negros Occidental, Philippines

Methods

Preparations

A. Locale of the Study

Maps showing the Different Sampling Locations of the Mollusk (Shellfish) Samples in Sagay Marine Environment

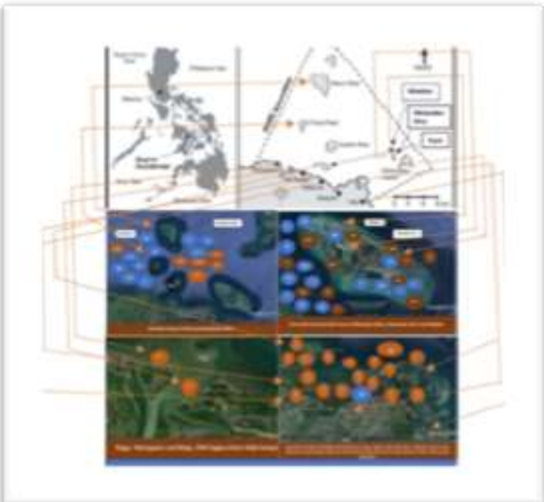

Fig 3. Sagay Marine Environment Map Showing the Different Sampling Locations

Figure 3 above shows the local of the study indicated with line segments were the sampling locations in Sagay Marine environment. Starting from the left of the bottom of the figure shown above are the inter-tidal areas of Toble, Himugaan Baybay, and Sitio Lu-ok, Old Sagay; next, the right side of the bottom is the inter-tidal area of Punta Roma of Old Sagay, inter-tidal area of Sitio Tabunan of Taba-ao, inter-tidal area of Sitio Tagnipis of Taba-ao, the inter-tidal and sub-tidal areas of Suyac Island of Taba-ao, inter-tidal and Sub-tidal areas of Bulanon and its annex Island, inter-tidal and sub-tidal areas of Vito; the upright part of the figure is inter-tidal and sub-tidal areas the Islands of Molocaboc Daku, Molocaboc Diut, Matabas; And up left part of the figure is Reefs of Panal and Macahulom. Indicated in blue are univalve samples and indicated in orange are bivalve samples. Each type of mollusk (shellfish)in every sampling station was collected, identified, and analyzed.

Those locations were set using the GPS (Global Positioning System and Sagay Marine Reserve (SMR) Map. This GIS-generated map of the sampling stations served as a guide in locating the samples of mollusks (shellfish) that were randomly gleaned or picked by the divers. This GPS (Global Positioning System) and GIS (Geographic Information System) were the support technologies utilized to make a vivid picture and interpretation of the sampling locations and mollusks (shellfish). Samples in different Islands and Coastal Barangays which were shown above were indicated with line segments to further locate, identify, classify, and collect the kinds of samples represented by each location. In Panal and Macahulom Reefs, 10 out of 16 or 62.50% of samples were univalve. Macahulom and Panal Reefs are the deepest sampling locations where the majority of the samples were taken from 20 meters to 35 meters deep. In Molocaboc Daku, Molocaboc Diut, and Matabas Islands, 15 out of 27 or 55.56% of samples were univalve. Molocaboc Daku, Molocaboc Diut, and Matabas Islands were sampling locations that measure from 1 meter to 20 meters deep. In the inter-tidal and sub-tidal areas of Punta Roma, Old Sagay, Sitio Tabunan, Sitio Tagnipis, and Suyac Island, of Brgy. Taba-ao; including the Brgys of Bulanon and its annex Island and Vito, 17 out of 18 or 94.44% of samples were bivalves. The said sampling stations are estuarine where waters are brackish and siltation is usually trapped under or near the mangroves. In Sitio Toble, Himugaan, and Sitio Lu-ok, Old Sagay inter-tidal (coastal)areas, the only sample taken was the Asiatic Hard Clam which was a bivalve. This area is estuarine where waters from the Himugaan River and sub-tidal waters are connected. The commercially identified mollusks (shellfish) of Sagay marine environment were tested, analyzed, and simplified into 25 samples.

B. Research Design

This study was conducted to assess the microplastic (MPs) contents in both univalve and bivalve which are commercially important shellfish. This is descriptive research in quantitative design. A Descriptive was employed because it describes the characteristics of MPs 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 MPs. This is a process of collecting and analyzing numerical data (Bhandari, 2022).

C. Materials and Data Gathering Procedure

The Bureau of Fisheries and Aquatic Resources assisted in the identification and classification of the different kinds of mollusks and shellfish commercially consumed in the Sagay Marine Environment and the suggested maps and sampling stations were given.

1. Materials and Technologies Used for Gathering Mollusks

Figure 4 shows the materials and technologies utilized during the establishment of the transect and collection of shellfish samples.

One hundred meters, 5mm nylon rope for measuring the depth of the mollusks (shellfish) location;1 kilo plumb bob used to weight the rope; pull-push rule for measuring the rope indicated in the depths of mollusks shellfish; compass for guides of location; GPS (Geographical Positioning System) tracker as support to locate the Islands; Pro camera or undersea camera for taking the profile or 3-D picture of mollusks (shellfish); diver's suits with tempered glasses for divers' body protection; feet paddles to support and protect the feet when diving; GIS mapping iPhone with a camera where GPS tracker is inserted; boat paddles to help the boat move when the motor is not working; pump or motor boat hats for divers' protection from the heat of the sun; 2 motor or pump boats as the carrier of the researchers' group (one as guide and the other one performing the transect)and weighing scale for measuring the samples after excavating the meat from it shell, plastics' containers for samples' packing; styrofoam containers for samples containers packing delivery; record book for record- keeping; ball pens for recordings; paper tapes for coding; packing tapes for packing ; scotch tapes to protect the codes on the samples' containers; scouring knife for shellfish meat extraction; white towels as rags to clean the containers and Sagay Marine Ecosystem as support document of location.

2. Mapping and Distribution of Transect Rope in the Different Sampling Areas.

Fig 5. Mapping and Locating the Identified Mollusks for Collection

Figure 5 above shows the mapping, transect, and collection of mollusk (shellfish) samples in the different sampling locations in Sagay marine environment.

Planning, analyzing, and pointing coordinates on the GIS map were the attempts in the transect and collecting the mollusk (shellfish) samples. Using the GIS map and GPS as a guide, the location of shellfish samples was directed and located with researchers over boarding the 2 pump boats, one for direction and the other one for the transect and collecting samples. The assessment of the location of the samples was successfully done with accomplished recordings in the record book.

3. Collection and Identification of Mollusks (shellfish

Fig 6. Collection of Identified Mollusks in Sagay Marine Environment

Figure 6 above shows the twenty-five mollusks' shellfish that were identified according to their physiological formation, characteristics, and habitation. There were 10 univalves and 15 bivalves shellfish. Each was taken with a profile or 3-D picture and identified with their Local, English, and Scientific names.

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4. Extraction of mollusk (shellfish) meat and packing in a sterilized container

In the acquisition of shellfish in its location, the meat of shellfish was directly extracted using the scouring knife, weighed, placed in sterilized containers, packed, coded, and covered the coding with scotch tapes to protect the coding from moisture. Samples were delivered to Negros Prawn Producers Cooperative Analytical and Diagnostic Laboratory for MPs' assessment

Fig.7 Extraction of Mollusks' (Shellfish) Meat

Figure7 above shows the extraction of mollusk meat (shellfish). Each sample has a minimum of 500 grams of meat wet weight depending on the content of samples upon collecting and extracting from its shells, and those were the 1,670 grams of Baler shell (*Melo melo*); 1,789 grams of Horned helmet shell (*Cassis cornuta*); 500 grams Ramose murex (*Chicoreus ramosus*); 500 grams of Bat volute (*Voluta vespertilio*); 750 grams of Spider Conch (*Lambis cerea/ Lambis L*); 500 grams of Fine-ribbed auger (*Terebra protexta*); 500 grams of Plicate conch (*Strombus labiatus*); 500 grams of Trapezium horse conch (*Pleuroploca trapezium*); 500 grams of Top shell (*Trochidae*); 500 grams of Telescope snail (*Telescopium telescopium*); 800 grams of Small giant clam (*Tridacna maxima*); 500 grams of Pacific Tiger Lucine (*Codakia tigerina*); 500 grams of Tumid venus (*Gafrarium pectinatum*); 500 grams of Scallops (*Pectinidae*); 500 grams of Comb pen shell (*Atrina pectinata*); 500 grams of Mother of pearl (*Placuna placenta*).; 500 grams of ark clam (*Anadara antiquata*); 500 grams of Ducal thorny oyster (*Spondylus squamosus*); 500 grams of Oyster (*Ostreidae*); 500 grams of Hooded oysters (*Saccostrea cucullata*); 500 grams of Asiatic hard clam (*Meretrix meretrix*); 500 grams of Abalone (*Haliotis*); 500grams of Ventricose ark (*Arca Ventricosa*); 500 grams of Mangrove clam *(Dinocardium robustum*); and 500 grams meat wet weight of Green mussels (*Perna viridis*).

5. Coded samples of univalve and bivalve shellfish for MPs assessment

Fig 8. Coded Samples of Mollusk (Shellfish) from the Different Locations of Sagay Marine Environment

Figure 8 above shows the univalve and bivalve shellfish samples which were coded based on the given control numbers by Negros Prawn Analytical Laboratory Inc., recorded in the official record book of the laboratory and also taken as a copy for control purposes when doing the microplastic content assessment. Control Number 530-Baler shell, CN 544 Horned helmet shell, CN 531 – Ramose murex, CN 542- Bat volute, CN 532 – Spider conch, CN 536- Finely ribbed auger, CN 534- Plicate conch, CN 545- Trapezium horse conch, CN 535-Top shell, CN 529-Telescope snail, CN 536 Small giant clam, CN 543- Mother of pearl shell, CN 547- Scallops, CN 540- Pacific tiger Lucine, CN 541- Comb pen shell, 2498X-Asiatic hard clam, 2498B-Tumid venus, 2498 C- Ventricose ark, 2498C-Mangrove clam, 2498D-Green mussel, CNs 546 and 547, Ark clam, CN 543-Ducal thorny oyster, B1, B2, and B4-Cultured oysters, B5-Hooded oysters, and B3- Abalone.

6. Packaged mollusk (shellfish) samples were delivered to Negros Prawn Analytical Laboratory, Inc.

Fig 9. Packaged Mollusks Samples from Sagay Marine Environment

Figure 9 shows the packaged samples of mollusks collected from the different sampling locations of Sagay Marine Environment. The delivery time budget from sampling locations to Negros Prawn Producers Cooperative analytical and Diagnostic laboratory was 1 hour to protect the samples from any heat stress and pollution.

7. Assessment of MPs content in mollusks (Shellfish) from Different Sampling Locations in Sagay Marine Waters

Upon the arrival of the delivery of the shellfish samples, a representative of the laboratory accepted the delivered package and recorded for controlling the coded samples. These were then placed in a cooler to prepare the samples for the wet peroxide oxidation (WPO) chemical digestion and optical microscopy methods. These laboratory assessments were done by trained and licensed laboratory technicians.

a. Wet Peroxide Oxidation (WPO)Chemical Digestion Technique

Since this mixture was highly reactive, proper reviewing and following the laboratory safety practices and policies for handling this mixture were observed before completing the analyses. The samples were placed on filter paper and divided into 4 quadrants; 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 because 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; 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; 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.

III. RESULTS AND DISCUSSIONS

A. Spatial Distribution and Microplastic Contamination Index in Mollusks (Shellfish)of Sagay Marine Environment

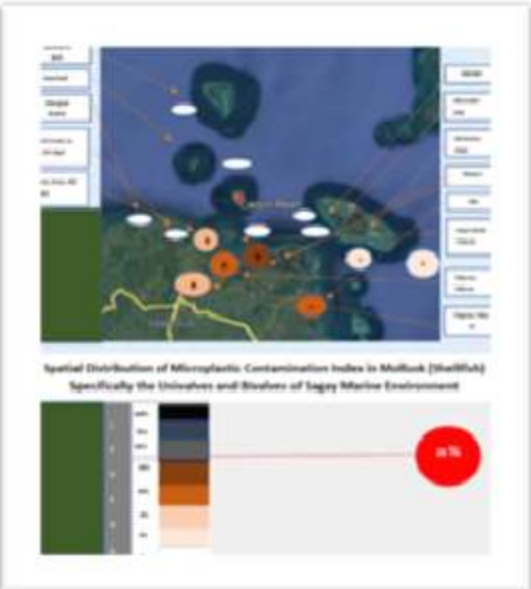

Figure 10: Microplastic Distribution Index of Mollusk (Shellfish) in Sagay Marine Environment

Mollusk (Shellfish)	Coastal	Islands	Reefs	Total	Microplastic Contamination Index
Univalve	0	1	0	1	
Bivalve	6	0	0	6	
				7/25	28%

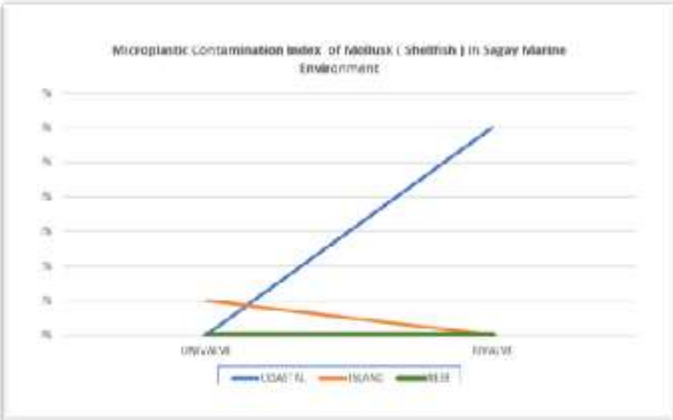

Table 1: Tabular and Graphical Representation of Microplastic Contamination Index Of Mollusk (Shellfish) in Sagay Marine Environment

Table 1 shows the results of the microplastic contamination of mollusks (shellfish) in the Sagay Marine Environment which is supported by Fig. 10 located on the left side which is the spatial distribution of microplastic contamination index of the location of microplastic contaminated mollusks (shellfish). Simplified into 25 mollusks of which 10 or 40% were univalves and 15 or 60% were bivalves. Of 25 mollusk (shellfish) meat, 7 or 28% were positive for microplastic fiber contamination. Of the 7 microplastic fiber contaminated mollusks (shellfish), 6 or 24% of them were bivalves, namely:

tumid venus *(Gafrarium pectinatum*), ark clam *(Anadara antiquata)*, cultured oysters *(Ostreidae)*, hooded oysters *(Saccostrea cucullata)*, ventricose ark *(Arca Ventricosa)* and green mussels *(Perna viridis*) thriving in the inter-tidal and sub-tidal coastal areas from Bulanon; oysters and hooded oysters from Punta Roma Old Sagay and Tagnipis, Taba-ao proper of Taba-ao and Vito; the 1 or 4% was univalve which was the trapezium horse conch *(Pleuroploca trapezium)* thriving in the mangrove sediments of the island of Molocaboc Daku. The reefs of the Sagay Marine Environment had zero microplastic contamination. The microplastic contamination index of commercially identified mollusks (shellfish) in the Sagay Marine environment was 28%.

IV. CONCLUSIONS

Univalve and majority of bivalves mollusks (shellfish) that thrive in the mangrove sediments and in the brackish water of Sagay Marine Environment were more microplastic contaminated.

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CONFLICT OF INTERESTS

The author declares that this article has no actual, potential, or perceived conflict of interest.

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