LEVELS OF MICROPLASTICS IN MUSSELS (*Perna viridis*) AND MANILA CLAM (*Venerupis philippinarum*) IN BACOOR CITY, CAVITE AND CALATAGAN, BATANGAS, PHILIPPINES

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Abstract

The concern over the ocean's microplastic research and general awareness has grown over the past few years. Microplastics come from a variety of sources, including larger plastic debris that has broken into resin pellets used in the production of plastics or microbeads used in cosmetic and health products. This study determined the number of microplastics in mussels (Perna viridis) and Manila clams (Venerupis philippinarum) collected from Bacoor City, Cavite, and Calatagan, Batangas; and determined the consumption pattern of mussels and Manila clams. The samples were collected from the study sites, and the meat was extracted from the shell. The meat samples were alkaline, and were digested, filtered, and oven-dried. A light microscope was used to separate the microplastics from other debris. The purified microplastics were weighed using an analytical balance. A survey was conducted to determine the consumption pattern of the consumers. The results showed that the mussels from Bacoor City, Cavite have the highest amount of extracted microplastics. Most of the respondents from Bacoor City consumed 0.99g per year because of eating mussels daily. Majority of the respondents from Calatagan consumed 0.0170g per year because of eating Manila clams six times a month. Most of the respondents consume mussels and Manila clams frequently, while some of them consider this a staple food. Microplastics were present in Manila clams and mussels collected from the Bacoor, Cavite, and Calatagan, Batangas. The researchers recommend the conduct of in-depth research on microplastics in Philippine seas and aquatic life to reduce the health danger to people and the ecosystem.

Keywords*: manila clams, microplastics, consumption pattern, environmental health*

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1.0 Introduction

In the past few years, the concern has increased for the research on microplastics in the ocean and the community's awareness about it. The increase in the number of plastics amassed in the environment is mainly caused by their inert, slow degradation rate and improper disposal of plastic waste. Even with their benefits, the impact of plastic on ecological degradation leads to the ingestion of plastic by marine organisms. One of the species that can be used to detect microplastics is the mussel. Jambeck *et al.* (2015) and Van Cauwenberghe *et al. (*2014) recorded seven million tons of plastic ended up in the ocean in 2010. The plastic particles found in the marine environment mainly come from land-based sources through mismanaged disposal of waste directly into the body of water, while marine aquaculture and fisheries contribute 20 percent of these pollutants (McKinsey & Company and Ocean Conservancy 2015). The majority of microplastics in the oceans are secondary products resulting from the degradation and fragmentation of mesoplastics or larger fragments. Secondary microplastics result from the degradation and subsequent fragmentation of larger plastics (Mathalon *et al.,* 2014) Furthermore, microplastics are susceptible to contamination by water-borne organic pollutants and the leaching of potentially toxic plastic additives known as "plasticizers." Aquaculture of mussels can be traced back to 1955, initiated by the Philippine Bureau of Fisheries and Aquatic Resources in Binakayan, Cavite.

Before the expansion of the study, the researchers came up with this type of research to find out the positive and negative impacts of Microplastics on humans and the environmentitself and, in turn, provide a basis for imminent research dealing with aquatic studies in the Philippines. Hence, this study's general objective is to determine the levels of microplastics in mussels (*Perna viridis*) and Manila Clam (*Venerupis philippinarum*) collected in Bacoor City, Cavite, and Calatagan, Batangas, Philippines, and assess the health risk to humans from the two sites. Specifically, this study sought to (1) determine the number of microplastics in mussels (*Perna viridis*) and Manila clam (*Venerupis philippinarum*) collected in Bacoor City, Cavite and Calatagan, Batangas; and (2) determine the local community's consumption pattern of mussels and Manila clams.

2.0 Methodology

Sample collection. Three kilograms of mussels were collected from mussel farmers from Barangay Alima, Sineguelasan and Talaba II, Bacoor City, Cavite. These three barangays have the highest production of mussels in the City of Bacoor, Cavite. Three kilograms of Manila clams were collected from Manila clam farmers from Barangay Balitoc, Quilitisan, and Poblacion Uno, Calatagan. These barangays were known to culture Manila clams exclusively. All samples were stored in polyethylene bags, kept in an ice box, and transported to Cavite State University. The mussels and Manila clam samples were stored until analyzed.

*Weighing of collected samples***.** The samples of mussels and Manila clams were weighed with shells and without shells.

Sample preparation. All equipment, storage containers, and laboratory wares were cleaned and rinsed with distilled water. The collected samples were unshelled in preparation for alkaline digestion. All the mussels' and Manila clams' meat were washed with distilled water to ensure that there were no foreign particles in them.

Alkaline digestion. Potassium hydroxide was used to remove microplastics from the body of the mussels and Manila clams. Samples in 250 mL of water and 10% KOH were digested for six hours in the water bath at 60°C (Paul-Pont *et al.*, 2016).

Filtration of digested tissue. Vacuum filtration was done on the digested tissue samples using the Buchner funnel and Whatman Filter Paper Grade 1 (11-micron pore size). The filters were dried for two hours using an oven at 40°C. Dried filters were examined for the presence of microplastics using a microscope (Van Cauwenberghe & Janssen 2014).

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Weighing of microplastics. The dried filters were gently scraped with a wire loop and a small brush to obtain the amount of potential microplastics in the samples. The initial weight of petridish was measured and the potential microplastics were gently transferred for storage before weighing and analyzing through a compound light microscope (Marine & Environmental Research Institute n.d.).

Consumption pattern survey. A structured questionnaire was prepared to determine the consumption pattern of the mussels and Manila clams and estimate the levels of microplastics to its consumers based on their consumption patterns. The questionnaire was subjected to validation and Ethics Review Board evaluation. The purposive quota sampling was employed to select the 150 mussels and the 150 Manila clams consumers, resulting in a total of 300 respondents. All of the respondents who participated in the survey were bivalve eaters, and most of them frequently consume bivalves as part of their protein diet. The consumers were asked about their consumption patterns of mussels and Manila clams to determine the average consumption in Bacoor City and Calatagan, Batangas.

Statistical analysis. Mean, percentage, standard deviation, and range were used to analyze the levels of microplastics in mussels and Manila clams. The survey results were analyzed and interpreted using the data from the answers given by the respondents on the questionnaire sheets.

3.0 Results and Discussion

Table 1 shows the weight of microplastics from mussels (*Perna viridis*) and Manila clams (*Venerupis philippinarum*). Findings reveal that Bacoor City had the highest estimated weight of microplastics because of the nearby urbanized areas like Metro Manila, Las Piñas City, and other cities. This indicates the extent of microplastic pollution ingested by mussels. Some factors that influenced microplastic concentration in mussels were the localized microplastic contaminant levels at the aquaculture farm location, which includes sources from urban centers, effluent discharge, commercial activity, and industrial activity around Manila Bay.

Table 1. Weight of microplastics in mussels (*Perna viridis*) and Manila clams (*Venerupis philippinarum)*

Location	Average Extracted Microplastics Per Kilo of Mussels (with shell) (g)	Estimated Weight of Microplastics Per Piece (g)
Bacoor City Cavite	1.43×10^{-2}	1.375×10^{-4}
Calatagan, Batangas	7.2×10^{-4}	1.18×10^{-5}

The mismanagement of plastic waste is the most significant source of plastic pollution globally (Boucher & Friot, 2017). Plastic waste can enter the marine environment through industrial wastewater (Nor & Obbard, 2014) and the extensive degradation times of plastics enable them to persist for decades, resulting in the abundant distribution of small pieces of plastics called microplastics (Figure 1). This has potential health risks for humans due to the toxic chemicals in the microplastics. The potential effects on humans by alternate ingestion of microplastics can cause chromosome alterations which can lead to infertility, obesity, and cancer (Sharma & Chatterjee, 2017).

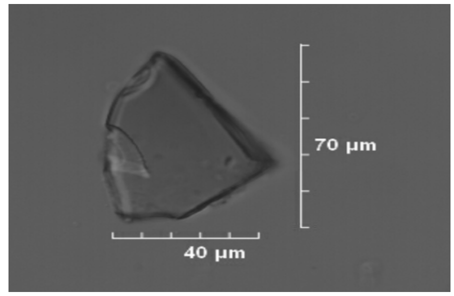

Figure 1. Image of a Microplastic under a Confocal Microscope

Figure 2 shows the amount of mussels and Manila clams consumed in a day by the respondents. Majority of the respondents from Bacoor City, Cavite (66%) and Calatagan, Batangas (81.3%) consume more than 16 pieces of mussels and Manila clams daily. Figure 3 shows the frequency of consuming mussels and Manila clams among the respondents from Bacoor City, Cavite, and Calatagan, Batangas. The majority of the respondents (23.3%) from Bacoor City, Cavite consume mussels three times a week, while the majority of the respondents (31.3%) from Calatagan, Batangas consume Manila clams six times a month. The respondents from Bacoor City, Cavite consume more mussels because most of the male respondents are fishermen and the female respondents are vendors. Fishing is their source of livelihood as they depend on it for food and basic needs. The respondents from Calatagan, Batangas do not consume Manila clams often because they have other sources of food, and they do not depend on seafood for their daily source of food.

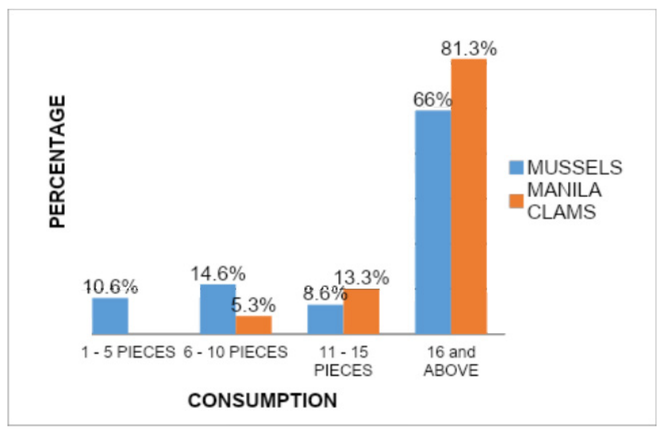

Figure 2. Amount of mussels and Manila clams consumed in Bacoor City, Cavite and Calatagan, Batangas

Table 2 shows that 23.3% of the respondents in Bacoor City, Cavite have an annual estimated consumption of microplastics amounting to 0.99g because of eating mussels three times a week, and 19.3% of the respondents have an annual estimated consumption of microplastics amounting to 0.396g because of eating mussels every day. On the other hand, 31.3% of the respondents in Calatagan, Batangas have an annual estimated consumption of microplastics amounting to 0.0170g because of eating Manila clams six times a month, and 25.3% of the respondents have an annual estimated consumption of microplastics amounting to 0.0850g because of eating Manila clams once a month.

The daily consumption of mussels and Manila clams has a

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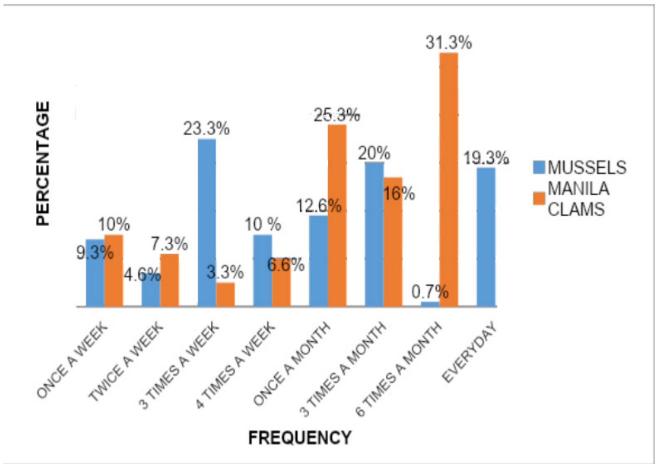

Figure 3. Frequency of consumption of mussels and Manila clams in Bacoor City, Cavite and Calatagan, Batangas

Table 2. Annual estimated consumption of microplastics through eating mussels and Manila clams

Consumption Frequency	Estimated amount of Microplastics Ingested Per Piece of Mussel/Manila Clam (g)			
	5	10	15	20
Bacoor City, Cavite				
Once a week	0.033	0.066	0.099	0.132
Twice a week	0.066	0.132	0.198	0.264
3 times a week	0.099	0.198	0.297	0.396
4 times a week	0.132	0.264	0.396	0.528
Once a month	0.0083	0.0165	0.0248	0.033
3 times a month	0.0248	0.0495	0.0743	0.099
6 times a month	0.0495	0.099	0.1485	0.198
Everyday	0.2475	0.495	0.7425	0.99
Calatagan, Batangas				
Once a week	0.0028	0.0057	0.0085	0.0113
Twice a week	0.0057	0.0113	0.0170	0.0227
3 times a week	0.0085	0.0170	0.0255	0.0340
4 times a week	0.0011	0.0227	0.0340	0.0453
Once a month	0.0007	0.0014	0.0021	0.0028
3 times a month	0.0021	0.0042	0.0064	0.0085
6 times a month	0.0042	0.0085	0.0127	0.0170
Everyday	0.0212	0.0425	0.0637	0.0850

potential high risk to human health because microplastics may contain toxic chemicals from their production, manufacturing, and surrounding environment (Smith *et al*., 2018). Vethaak & Leslie (2016) argued that the physical effects of microplastic particles observed in human cells and tissues and animal models give an understanding into the potential risks of particle exposure in humans. Previous studies confirm that microplastic particles can cause lung and gut injury, and minute particles can cross cell membranes, blood-brain barrier, and human placenta. Some of the detected effects comprise oxidative stress, cell damage, inflammation, and impairment of energy allocation functions. Thus, it is highly possible that microplastic particles could be dangerous to human health. There are proofs that microplastics can absorb toxic chemicals and then release them into an animal's digestive system, which indicates a negative impact on human health. Also, there is evidence that potentially-toxic plastic nanoparticles may be able to migrate through the intestinal wall during digestion.

The World Health Organization has recently announced a review into the potential human health impacts of small plastic particles. In the marine environment, these plastics break down into smaller fragments, which eventually become microplastics. Mussels are reported to be particularly susceptible to microplastic contamination (Hugh, 2018). Impacts of microplastics on human health are inflammation, diarrhea, irritation of the eye, vision failure, breathing difficulties, respiratory problems, liver dysfunction, cancers, skin diseases, lung problems, headache, unconsciousness, thyroid, coughing, swelling of the throat, vomiting, indigestion, typhoid, stomach ache, food poisoning, birth effect, infertility, hormonal changes, declining sperm count, rashes, allergy, skin cancer, asthma, dizziness, birth effect, genotoxicity, reproductive, cardiovascular, and gastrointestinal problems. Particles cannot penetrate the tissues, but the microplastic goes down the bloodstream and causes diseases (Proshad *et al*., 2018).

The unmitigated release of plastics into the environment affects all terrestrial and aquatic life they come into contact with. The manufacturing of plastic products in plastic industries releases a huge quantity of dangerous gaseous chemicals into the air, including carbon monoxide, dioxin, and hydrogen cyanide. The high amounts of these gases released into the air not only pollutes the air but also causes harmful effects to both human and animal health. Some cause respiratory diseases, nervous system disorders, and reduction of immunity to diseases. Chlorinated plastics can release dangerous chemicals into the surrounding soil, which will seep into groundwater or the surrounding water sources and the ecosystem. When biodegradable plastics are broken down, methane is released, which is a very powerful greenhouse gas that contributes significantly to global warming (Biello, 2011). Polymers in the oceans contribute to global warming by creating a shaded canopy that makes it harder for plankton to grow. The presence of plastic waste in water bodies disturbs the natural flow; it limits the ability of fish to reproduce and destroy helpful organisms (Proshad *et al.,* 2018).

4.0 Conclusions and Recommendations

The findings of this study confirmed that microplastics are present in Bacoor Bay-cultured mussels (*Perna viridis*) and cultured Manila Clams in Calatagan, Batangas. The results confirmed that mussels from Bacoor City, Cavite ingest the highest levels of microplastics, while Manila clams from Calatagan, Batangas ingest the lowest levels of microplastics. Hence, consumers of mussels in Bacoor City, Cavite and Manila clams in Calatagan, Batangas are both exposed to health risks caused by microplastics. The data also reveal that mussels and Manila clams are consumed frequently, while others consider them as staple food. An essential part of the aquaculture business in the Philippines is the mussel industry. The continued demand for quality and safe mussels will contribute to the economic growth of the Philippines through an enhanced and active participation in numerous programs headed by the different government sectors to lessen the percentage of microplastics in the Philippine seas.

With the combined effort involving our national agency headed by the Department of Environment and Natural Resources (DENR) and other stakeholders, the Ecological Solid Waste program should be strictly implemented. On the other hand, intensifying and improving the waste management, advertisements and programs that will promote a clean and safe environment is a good channel to address this pressing issue at hand, and disseminate information about microplastics. For the high-risk areas, putting aquacultures of oysters, mussels, Manila Clams and other bivalves to create natural filters in Manila Bay and Isla Verda passage will be a huge help. Lastly, a more detailed and extensive research about microplastics in Philippine waters and aquatic organisms may be included to prevent greater health risk to humans and the environment.

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