Sustinere
Journal of Environment and Sustainability
Volume 5 Number 2 (2021) 90-102

Print ISSN: 2549-1245 Online ISSN: 2549-1253
Website: https://sustinerejes.com E-mail: sustinere.jes@iain-surakarta.ac.id

RESEARCH PAPER

Detection and quantification of microplastics from cultured green mussel *Perna viridis* in Bacoor Bay, Cavite, Philippines

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Article history:
Received 8 February 2021 | Accepted 19 August 2021 | Available online 31 August 2021

Abstract. Microplastic contamination in the aquatic environment is a worldwide problem endangering aquatic organisms and human health. However, few reports were published in the Philippines especially in different edible fishery products. Hence, an investigation to report its prevalence in edible fishery products, especially in a fishery-dependent country, is necessary. This study was conducted to detect, characterize, and quantify microplastics from cultured Green mussel *Perna viridis* in Bacoor Bay, Cavite, Philippines. Samples (*n=63*) were collected from the inner, middle, and outer parts of Bacoor Bay. Isolation and characterization were conducted using wet peroxide oxidation-assisted density separation and stereomicroscopy, respectively. Results show a low concentration of microplastics from *P. viridis* cultured in Bacoor Bay. The highest microplastic count was observed from the inner bay (0.41 particle/$g_{wet\ weight}$) followed by the middle bay (0.40 particle/$g_{wet\ weight}$), then the outer bay (0.27 particle/$g_{wet\ weight}$). The majority of microplastics in all sites were fibers (61%), color red (29%), and were dominated with > 10 to 50 µm length. This study revealed that microplastic is prevalent in Bacoor bay. Further study on confirming the microplastic polymers from *P. viridis* cultured in Bacoor bay is recommended.

Keywords: green mussel; microplastics; aquatic environment; pollution

1. Introduction

Plastic pollution has been a worldwide problem and has become ubiquitous in the aquatic environment. The alarming problem of plastics was aggravated due to its efficiency in many commercial products alongside the lack of proper waste management (Ang & Sy-Changco, 2007; Magalang, 2014; Ryan et al., 2009; Yurtsever & Yurtsever, 2018). Dramatically, 5-13 million tons

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DOI: https://doi.org/10.22515/sustinere.jes.v5i2.166

of plastic waste enters the marine environment every year driven by the large-scale use of plastic products (Jambeck et al., 2015). Larger plastics undergo UV radiation exposure, degradation, and physical abrasion fragmenting them into smaller microplastics (Browne et al., 2007). Microplastics are small plastic debris with a usual size of <0.5 μm in diameter (Fendall & Sewell, 2009; Sanchez et al., 2014; Thompson et al., 2004). Microplastics are further categorized into primary and secondary. Primary microplastics are manufactured as minute plastic material while secondary plastics are results of large plastic abrasion and degradation (Andrady, 2011; Browne et al., 2007; Thompson et al., 2004). Despite the smaller size of microplastics, they are vectors of highly hydrophobic contaminants and endocrine-disrupting chemicals which heightened their adverse impact on the aquatic environment (Chen et al., 2018, 2019). On top of that, microplastics can enter the food chain and subsequently be transferred to higher trophic levels triggering concerns on ecosystem services and human health implications (Hantoro et al., 2019).

In the Philippines, one of the severely affected by several anthropogenic pressures is Manila Bay, including its inlet Bacoor Bay (Cruz & Shimozono, 2021; Rodolfo & Siringan, 2006). Bacoor Bay has been known for its mussel industry. The present area cultivated for mussels is around 382.4 hectares and is considered as one of the Philippines' top mussel producers. The aforementioned bay, however, faces a tremendous amount of deterioration driven by different anthropogenic pressures. Studies have documented that Bacoor Bay is contaminated with heavy metals, antibiotic resistance pathogens, and plastics (Argamino & Janairo, 2016; Dumalagan et al., 2010; Tabo et al., 2015). Apart from anthropogenic pressures coming from nearby cities, Manila Bay pollutants also affect the water quality of Bacoor Bay. These pollutants, severely affecting the bay, are an indicator of poor solid waste management and could lead to further impacts not just on aquatic ecosystems but also on human health should be addressed.

One of the extensively used bioindicators of coastal aquatic health is Green mussel, *Perna viridis*. A wealth of data has been published on the utilization of bivalves, including *P. viridis*, as a bioindicator of coastal water pollution (Ding et al., 2021; Klasios et al., 2021; Strehse & Maser, 2020; Van Cauwenberghe et al., 2015; Ward et al., 2019). The use of *P. viridis* as a coastal pollution bioindicator can be attributed to its exceptional characteristics (Li et al., 2019). First, the global distribution and accessibility of *P. viridis*, makes the comparison of data to other literatures straightforward. Second, *P. viridis* provides a habitat for other aquatic organisms linking the pelagic and benthic environments. Furthermore, *P. viridis*, as filter feeders, can accumulate and uptake microplastics, making them an excellent route of xenobiotics across trophic levels (Cole et al., 2013; Thompson et al., 2004; Van Cauwenberghe & Janssen, 2014; Ward et al., 2019). Importantly, *P. viridis* has been an important source of protein for the human population. Hence, consumers can be indirectly exposed to microplastics and associated harmful contaminants, highlighting the risk of chemical contamination which could jeopardize human health (Vandermeersch et al., 2015). Although the potential risk of chemical exposure from microplastics to marine organisms and humans is not yet determined to date (Van Cauwenberghe & Janssen, 2014).

Microplastic pollution has been already reported in the Philippines by numerous studies (Argamino & Janairo, 2016; Deocaris et al., 2019; Espiritu et al., 2019; Limbago et al., 2021). While there is a growing literature on the presence of microplastics in the country, little has been published on the ingestion of microplastics by common edible bivalve species like green mussel *P. viridis*.

Although, the study of Argamino & Janairo (2016) already reported the presence of microplastics from Bacoor City, Cavite, the aforementioned study, however, did not quantify and characterize the detected microplastics while this is an important factor in assessing the degree of plastic pollution impact. Moreover, Argamino & Janairo (2016) collect samples from the seafood terminal which could be contaminated with microplastics during transport and/or harvests. Hence, to augment and support the already published study, this study was conducted. The objective of this study is to detect, characterize, and quantify microplastics from cultured green mussels *P. viridis* cultured in Bacoor Bay, Cavite, Philippines. Thus, further investigating the degree of microplastic pollution on different parts of Bacoor Bay.

2. Methodology

2.1. Sample Collection and Sampling Sites

A total of sixty-three (63) mussel *P. viridis* (four months culture period) were collected from Bacoor Bay, Cavite, Philippines. The samples were directly collected from the sites using stainless steel scalpels and forceps.

In order to provide an ample representation of the bay, as shown in Figure 1, sampling sites were haphazardly selected from the inner bay (14°28'31.94"N 120°56'52.30"E), middle bay (14°28'49.40"N 120°55'52.70"E), and outer bay (14°29'11.32"N 120°56'58.83"E). In each sampling point, seven (7) mussels were randomly collected with three (3) replicates. Collected samples were then placed in a zip-lock bag and were transported into the laboratory through a cooler with ice for subsequent analysis.

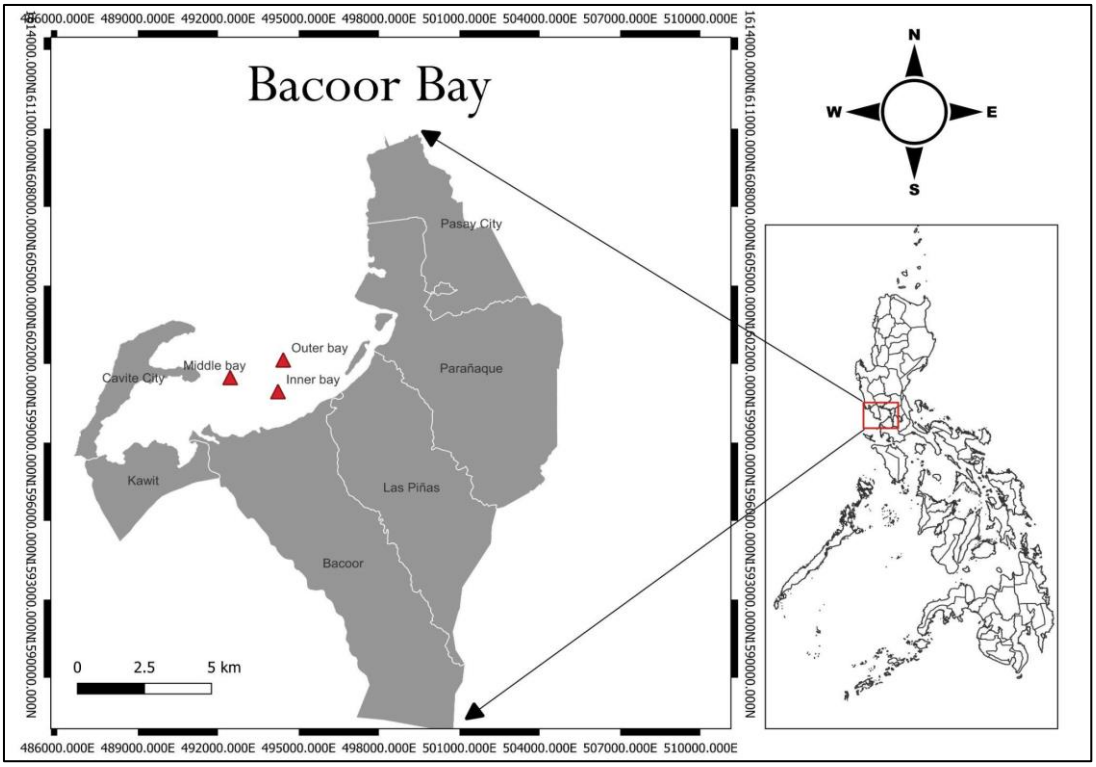

Figure 1. Study site with three sampling stations: Inner Bay, Middle Bay, Outer Bay of Bacoor Bay, Cavite, Philippines.

2.2. Extraction of Microplastics

During the study, a series of measures of quality control was practiced minimizing the background contamination. Researchers wore laboratory coats, distilled water was filtered using a GF/C filter, and glass containers, if not in use, were covered with aluminum foils (Jiang et al., 2018).

Mussels were shucked using stainless steel scalpel and forceps, exposing the soft tissue. After opening the shell, the length of the mussel soft tissue was measured using calipers and was weighed using a digital weighing scale. Then soft tissue was rinsed with filtered distilled water to remove the intervalve water and all soft tissues were placed in Petri dishes. Then, tissue samples were kept in a -20°C deep freezer until further analysis.

Subsequently, the wet peroxide oxidation procedure of mussel meat was conducted through the addition of 100 mL 30% (v/v) hydrogen peroxide hastened by 65°C incubation for 24 h. During density separation, 200 mL of saturated NaCl solution (35 g/100 mL) was co-added to the solution. The samples were then stirred, mixed, and were kept overnight to allow the separation of tissue from denser plastic debris. After an overnight density separation, aqueous components of the samples were vacuum filtered through Ultipor N filter paper (0.45 μm). The filter papers were then stored in clean Petri dishes that were covered with lids after filtration.

2.3. Stereomicroscopy and Microplastic Quantification

Suspected microplastics were categorized into (i) fragments (ii) pellets and (iii) fibers. Microplastics were categorized and characterized in accordance with Murphy (2018). Microplastic fragments are characterized with angular to sub-rounded, uniform, color, non-reflective; pellets are circular, have a uniform color, non-reflective, and fibers are elongated, with approximately uniform color, with constant width, often with tattering or splitting. Moreover, cellular structures should not be present in all identified microplastics.

Each identified microplastics were then counted, photographed, and measured using a calibrated stereomicroscope. The longest dimensions (measured in μm) and the colors of the identified plastic items were also recorded.

2.4. Statistical Analysis

Statistical tests for normality were performed using the software program SPSS version 23.0 and a confidence limit of 95% ($p = 0.05$). Shell length (mm), soft tissue length (mm), and soft tissue weight (g) were presented as mean ± standard deviation. Analysis of Variance (ANOVA) and LSD post hoc tests were used to determine the significant difference in the number of microplastic between sites.

3. Results and Discussion

3.1. Microplastic Concentration

Microplastics were detected on cultured *P. viridis* from Bacoor Bay, Cavite, Philippines. As shown in Table 1, there is a low concentration of microplastics in Bacoor Bay with only 0.41 particles/$g_{\text{wet weight}}$ in inner bay, followed by the middle bay (0.40 particle/$g_{\text{wet weight}}$), then the outer bay (0.27 particle/$g_{\text{wet weight}}$).

The concentration of microplastics detected in Bacoor bay is lower as compared to published literature. In this study, the concentration of microplastics ranges from 0.27 – 0.41 particles/$g_{wet\ weight}$ and is lower as compared to 3 particles/$g_{wet\ weight}$ (Li et al., 2015), 0.9 to 4.6 particles/$g_{wet\ weight}$ (Li et al., 2016), 4.44 particles/$g_{wet\ weight}$ (Courtene-Jones et al., 2017), and 6.2–7.2 particles/$g_{wet\ weight}$ (Renzi et al., 2018). Differences in plastic contamination in different regions could be a plausible explanation for the variation of microplastic contents in mussels. However, the sampling and extraction methods, and temporal variability may also affect the data.

Table 1. Number of microplastic debris per tissue weight ± mean standard deviation on three sampling points of Bacoor Bay, Cavite, Philippines

Site	Wet tissue length (mm)	Shell Length (mm)	Wet tissue weight per individual (g)	Microplastic Particles/tissue weight (g)
Inner Bay	44.00 ± 7.56	51.57 ± 9.17	3.99 ± 1.70	0.41
Middle Bay	46.90 ± 8.67	53.90 ± 8.95	5.10 ± 2.41	0.40
Outer Bay	45.62 ± 13.11	54.29 ± 14.55	5.92 ± 3.25	0.27

It is also noteworthy to raise that there are differences in microplastic concentration across sampling points, with higher concentration in inner bay and middle bay compared with outer bay. Nonetheless, the concentration difference is not statistically significant. A higher concentration of microplastics in the inner bay could be attributed to its proximity to urbanized areas where anthropogenic activities are prevalent. In soothe, however, there are other factors affecting the differences of microplastics across sampling sites, including water currents and microplastic densities (Frère et al., 2017; Wang et al., 2020).

3.2. Microplastic Types

All microplastic categories such as pellets, fibers, and fragments were detected from all sampling stations at Bacoor Bay. The microplastic distribution in all sampling stations are 47% fragments, 38% fiber, 15% pellet in the inner bay; 61% fiber, 23% fragments, 16 % pellet in the middle bay; and 73% fiber, 18% pellet, 9% fragments in the outer bay (Figure 2). Consequently, fibers dominate the microplastic category in *P. viridis* tissue cultured in Bacoor Bay, Cavite, Philippines. Microplastic types detected in this study are shown in Figure 3.

The abundance of fragments and fibers across all sites reported in this study was consistent with previous results in blue mussel *Mytilus edulis* (Van Cauwenberghe et al., 2015; Van Cauwenberghe & Janssen, 2014). Fibers and fragments have a variety of possible sources, either be larger plastics from degraded or abraded larger plastics, which could result in their abundance in aquatic environments (Gewert et al., 2015; Wagner et al., 2014). This could be linked with the massive use of oxo-biodegradable polyethylene plastic bags in the Philippines that are easily degraded and turned into microfragments. The abundance of fibers, on the other hand, could be linked to commercial laundry washing considering synthetic textiles as one of the primary sources of microfiber pollution in the ocean (De Falco et al., 2019). However, it is also important to mention that the prolific use of polypropylene ropes of many mussel farmers in growing green mussels in Bacoor Bay may contribute greatly to fiber abundance.

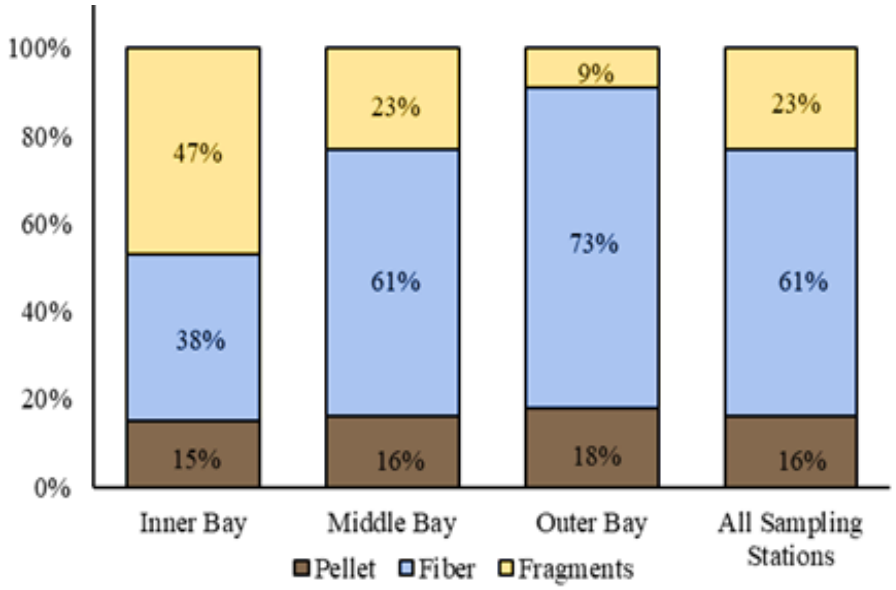

Figure 2. Percentage of microplastic types detected from cultured *P. viridis* in Bacoor Bay, Cavite, Philippines.

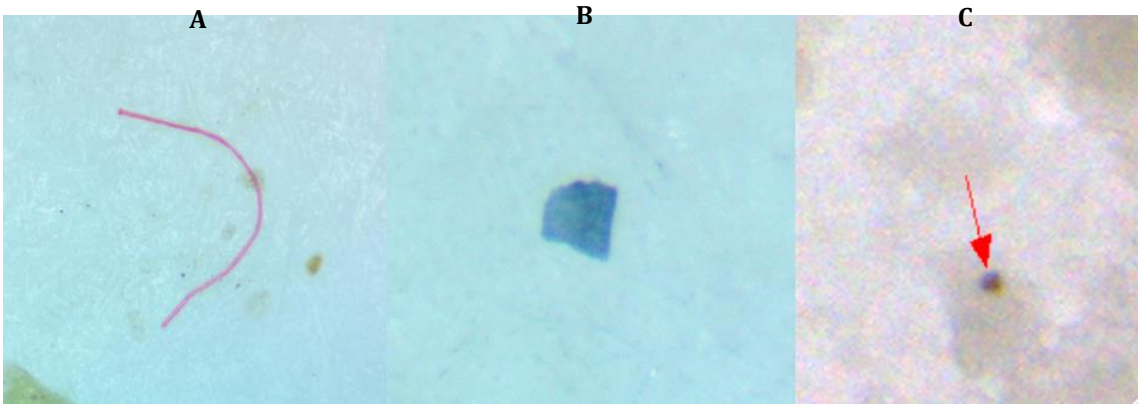

Figure 3. Microplastic types detected from cultured *P. viridis* in Bacoor Bay, Cavite, Philippines. (A) Fiber; (B) Fragments and (C) Pellet.

3.3. Colors of Microplastics

Color is another crucial physical characteristic of microplastics; it is also a good indicator of sea surface residence time, photodegradation, and the degree of degradation (Hidalgo-Ruz et al., 2012; Serranti et al., 2018). Moreover, the colors of microplastics play a role in the organisms' uptake of microplastics (Lusher et al., 2020). In this study, frequent colors of microplastics observed were black, brown, blue, and red with 18%, 19%, 24%, and 29% of the total microplastic count present in *P. viridis* from all sample stations (Figure 4). Specifically, 15% red, 38% brown, 23% black, 6% yellow, 18% blue in inner bay; 33% blue, 25% red 14% yellow, 14% black, 12% brown, 2% transparent in middle bay, and 61% red, 21% blue, 11% brown, 7% yellow in outer bay (Figure 4).

The high concentration of red and blue microplastic may be attributed to the rampant use of mesh bags for the initial stocking of mussel spats and polypropylene ropes as the main line in

the longline culture method of culturing green mussels in the sampling areas. Moreover, abundance of red microplastics is the evidence of larger plastics that are degraded into smaller fragments, synthetic fibers from clothes, and other terrestrial sources. On the other hand, the presence of black and brown suggests that the microplastics were long exposed in seawater causing its discoloration (Hidalgo-Ruz et al., 2012). Longer exposure to seawater of the microplastics results in oxidation thus higher diversity of PAHs and PCBs are possible in degraded microplastics, heightening its adverse impact in the aquatic environment (Frias et al., 2010).

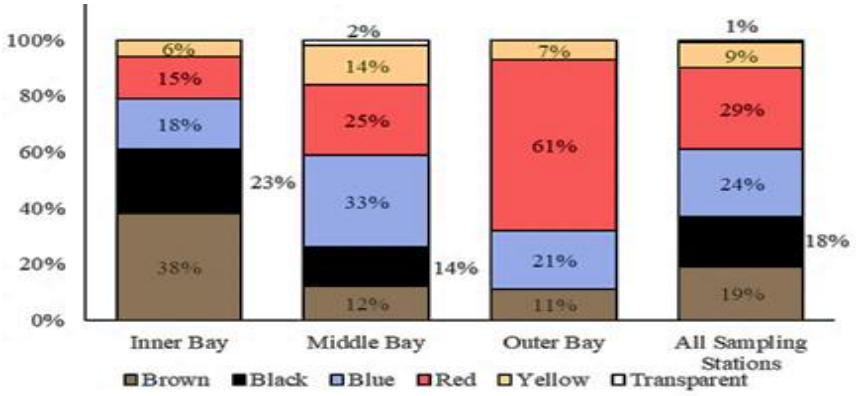

Figure 4. Colors of microplastics detected from cultured *P. viridis* of Bacoor Bay, Cavite, Philippines.

3.4. Size of Microplastics

In terms of size range, 10 to 50 µm dominates the microplastic size detected from cultured *P. viridis* from Bacoor Bay, Cavite, Philippines. Interestingly, the > 10 to 50 µm was uniformly distributed from the outer bay, middle bay and inner bay of Bacoor (Figure 5A-D).

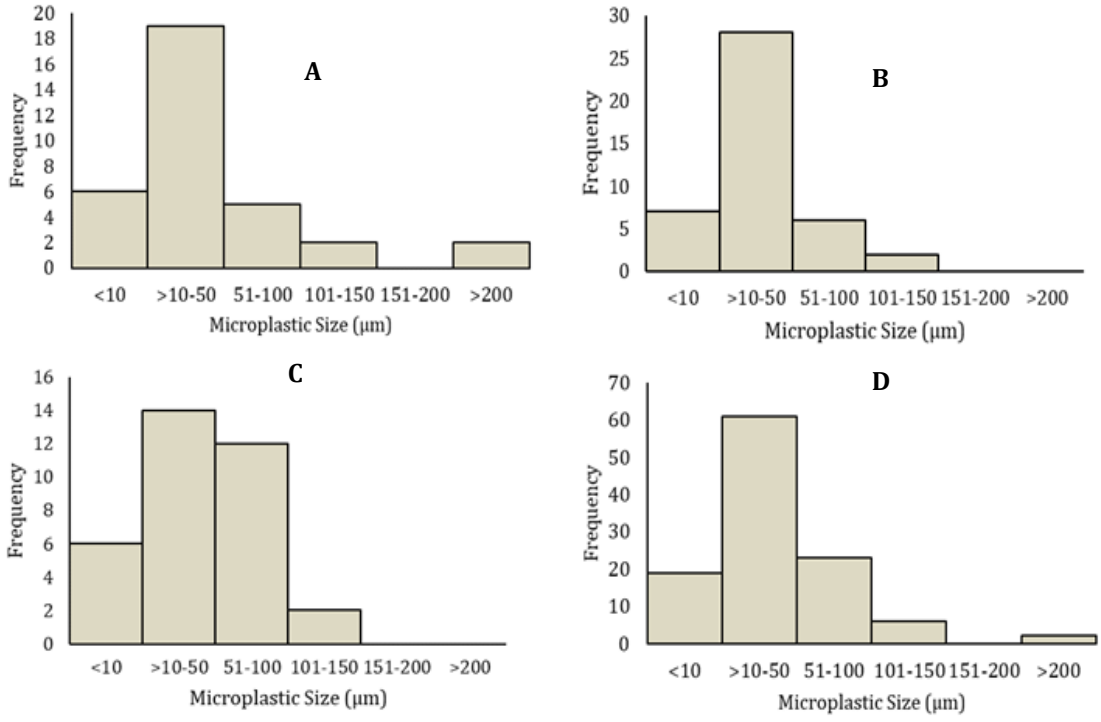

Figure 5. Microplastic size distribution detected from *P. viridis* cultured from Bacoor Bay, Cavite, Philippines. (A) Inner Bay; (B) Middle Bay; (C) Outer Bay and (D) All sites.

Different studies have reported the size distribution of microplastics ingested by bivalve mollusks. Size distribution already reported were <100 μm (Dowarah et al., 2020), < 250 μm (Li et al., 2015), 300 μm (Cho et al., 2021), and 660 μm (Ding et al., 2021). However, this study recovered the dominant smaller microplastic size with only > 10 to 50 μm. This report is alarming seeing that microplastic size is a crucial nominator in determining their environmental impact. Smaller microplastics and nanoplastics has the highest possibility to enter the planktonic food chain, entrained by detritus, and further be distributed to higher trophic levels (Akhbarizadeh et al., 2019; Ballent et al., 2013; Botterell et al., 2019; Cole et al., 2013; Saley et al., 2019). Additionally, the smaller size microplastics have large surface-area-to-volume increasing its bioavailability in aquatic habitat (Botterell et al., 2019). Seeing that, microplastics are vectors of endocrine-disrupting compounds such as the members of "dirty dozen", concern therefore of smaller size microplastics has increased their possible adverse impact and human health effects.

4. Conclusion

This study revealed microplastic ingestion of cultured green mussels *P. viridis* from Bacoor Bay, Cavite, Philippines. Primary results from the study were, (i) low concentration of microplastics, as compared to other published literatures, were detected from all sampling points; (ii) fibers and fragments dominate the microplastics types; (iii) random microplastics colors were detected such as black, brown, blue, and red, and (iv) > 10 to 50 μm dominates the microplastic size. These data indicate plastic pollution in Bacoor bay, and possibly Manila Bay, which could further affect the aquatic environment and human health.

5. Recommendations

While this study supports the results of previously published studies, several limitations were still observed. First, this study might underestimate the actual number of microplastics in *P. viridis* cultured in Bacoor Bay because of excessive foaming observed during wet peroxide oxidation consistent with the observation of Thiele et al. (2019). The foaming hinders the proper quantification of microplastics in the samples. Moreover, difficulties in the identification of microplastic types were also encountered in the study. This underscores the significance of polymer and chemical-based identification techniques such as FTIR and Raman spectroscopy (Jung et al., 2018; Lenz et al., 2015; Simon et al., 2018; Song et al., 2015). The microscopy-based characterization utilized in this study might lead to misidentification and further quantification. Hence, it is further recommended that a standard protocol on the visual identification of microplastics from different samples be developed. In addition, in order to minimize the adverse impact of microplastics on consumers of cultured *P. viridis* from Bacoor Bay, Philippines, further studies verifying the effect of depuration technologies on microplastic counts should be conducted. The result of this study, moreover, highlights the importance to strengthen the implementation of Philippine RA 9003 or the Ecological Solid Waste Management Act and effective Zero Waste programs at the local government levels. The national government must mandate businesses to stop the production of single-use plastic packaging to curb the plastic pollution crisis and further prevent the increase and/or to deter the microplastic count in Bacoor Bay.

Acknowledgement

We would like to acknowledge the Biotechnology Laboratory and Chemistry Research Laboratory of Central Luzon State University for providing the laboratory equipment during the conduct of the study, the local government unit of Bacoor, Cavite for assistance during the sampling, and the Department of Science and Technology (DOST), Republic of the Philippines for providing scholarship grants to the junior authors of this study.

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