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Original Article

Occurrence, characterization, and hazard assessment of microplastics in edible tissues of commercial fishes from public wet markets in Cebu Province, Philippines

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Abstract: This study examined the occurrence, characterization, and hazard risk of microplastics (MPs) in the edible tissues of commercial fish from public wet markets in Cebu Province, Philippines. Fish samples from eleven species were collected from eight municipalities (Argao, Bogo City, Carcar City, Daanbantayan, Liloan, Naga City, Oslob, and Toledo City) and analyzed for microplastics using microscopy and ATR-FTIR. Three hundred eighty-nine MP particles were identified, with benthic fish (N = 197) showing a slightly higher count than pelagic fish (N = 192). Argao had the highest MP count (N = 60), while Daanbantayan had the lowest. *Euthynnus affinis* had the highest MP concentration (19%, N = 74), followed by *Scarus psittacus* and *Cypselurus opisthopus* (16%, N = 64 each). Of the samples, 30% were confirmed as microplastics, revealing 15 distinct polymer types, including polyvinyl alcohol (PVA) (20%), polyacetylene (PA) (17%), and polyvinyl chloride (PVC) (7%). The polymer hazard index (PHI) indicated that polyethylene (PE) and polyethylene terephthalate (PET) posed a "High" risk, while acrylonitrile-butadiene-styrene (ABS), PVC, and polyurethane (PU) were classified under "Extreme Danger". These findings suggest that local agricultural practices, laundry activities, and waste disposal contribute to MP contamination in fish tissues, warranting further investigation into the health implications of MP consumption.

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Introduction

Since the 1950s, around nine billion tons of plastic have been produced globally; between 5.3 and 14 million tons of plastic waste enter the ocean annually from coastal areas and tributaries, while only 9% are recycled and 12% are incinerated (Jambeck et al., 2015). Plastics are crucial in various industries, including food preservation, construction, electronics, and healthcare. However, the increasing human population and persistent demand for plastic products drive production and waste generation (Alabi et al., 2019).

As the third-largest contributor to plastic waste worldwide, the Philippines generates an estimated 2.7 million tons annually, with 20% ending in the ocean (World Bank Group, 2021). Single-use plastic sachets significantly contribute to this issue due to their affordability and convenience (Sy-Changco et al., 2011). In areas lacking proper waste disposal facilities, packaging waste is often discarded on land or into waterways, where surface runoff transports it to aquatic habitats (Gorme et al., 2010). As a result, the country's fishing, shipping, and tourism industries are at risk due to its extensive coastline and the high volume of floating oceanic trash (PlastiCount Pilipinas, 2022).

Nonbiodegradable plastics take years or even millennia to decay, eventually breaking down into microplastics with diameters of less than 5 mm (National Oceanic and Atmospheric Administration, 2015K'arrman et al., 2016). A wide range of aquatic organisms easily ingests these tiny particles, as they resemble the size of their natural prey (Galloway et al., 2017). Like heavy metals, microplastics have potentially toxic effects and can bioaccumulate in aquatic organisms, including marine fish, and humans

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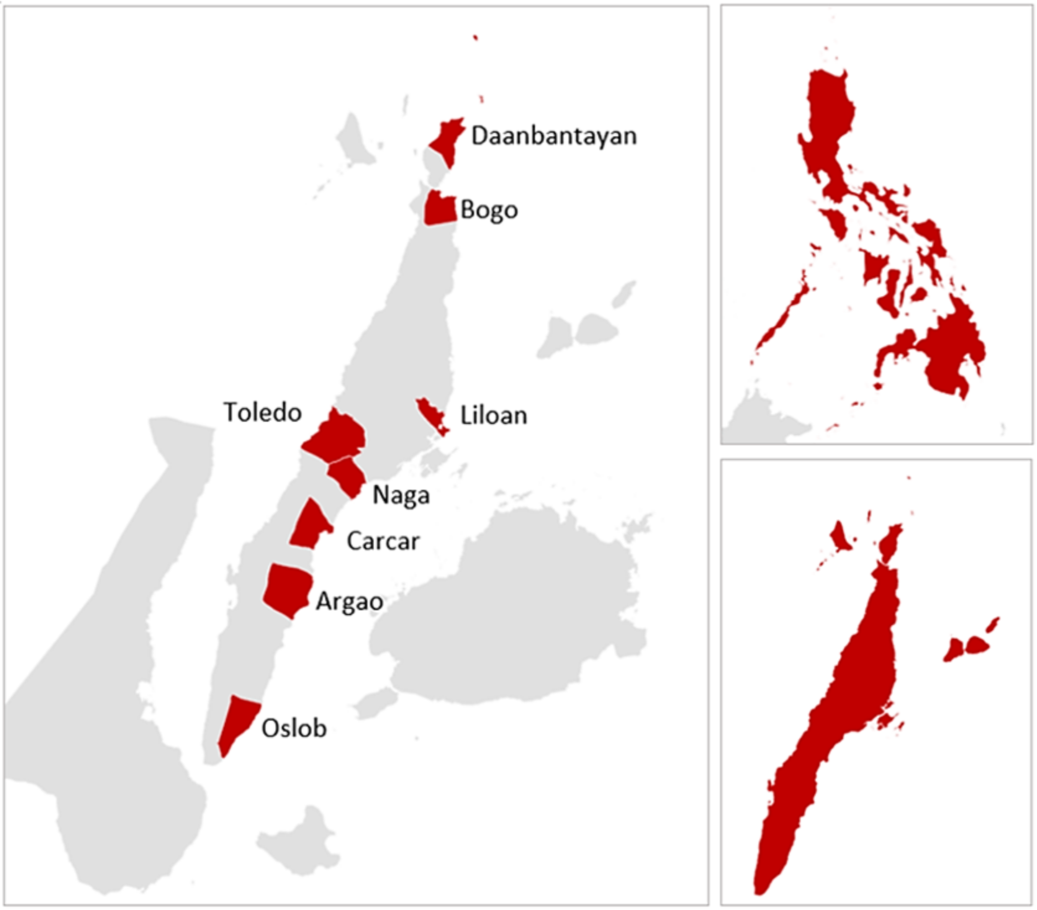

Figure 1. Map of Cebu province showing the eight sampling sites.

(Geolin et al., 2021). Ingesting MPs harms marine organisms physically and toxicologically, hindering their ability to perform essential eco-physiological functions and causing physical damage and stress (Courtene-Jones et al., 2022). Additionally, MPs can obstruct digestion and transfer pollutants to fish and invertebrates (Galafassi et al., 2021). Therefore, it is crucial to quantify, characterize, and assess the risk posed by microplastic ingestion in commercial fish from wet markets; hence, this study aimed to examine the occurrence, characterization, and hazard risk of microplastics in the edible tissues of commercial fish from public wet markets in Cebu Province, Philippines.

Materials and Methods

Study site: Public wet markets across eight municipalities of Cebu Province, Philippines, were selected for this study to represent the island's geographical distribution: (1) Argao Public Market, (2) Bogo City Public Market, (3) Carcar City Public Market, (4) Daanbantayan Public Market, (5) Liloan Public Market, (6) Naga City People's Market, (7) Oslob Public Market, and (8) Toledo Public Market (Fig. 1). Fish samples from these markets were sourced from local fishermen who caught them in nearby waters of the Cebu Strait. The fishes were treated following the USC Institutional Animal Care and Use Committee guidelines.

Sample collection: Eleven representative commercial fish species were collected from the eight public markets on Cebu Island, Philippines. Three were non-pelagic fish species, while the others belonged to the pelagic fish category. Non-pelagic fish are typically bottom-dwelling species found on or near the ocean floor, usually in rocky or boulder-strewn habitats. The non-pelagic species frequently caught by local fishermen and sold in the public markets include

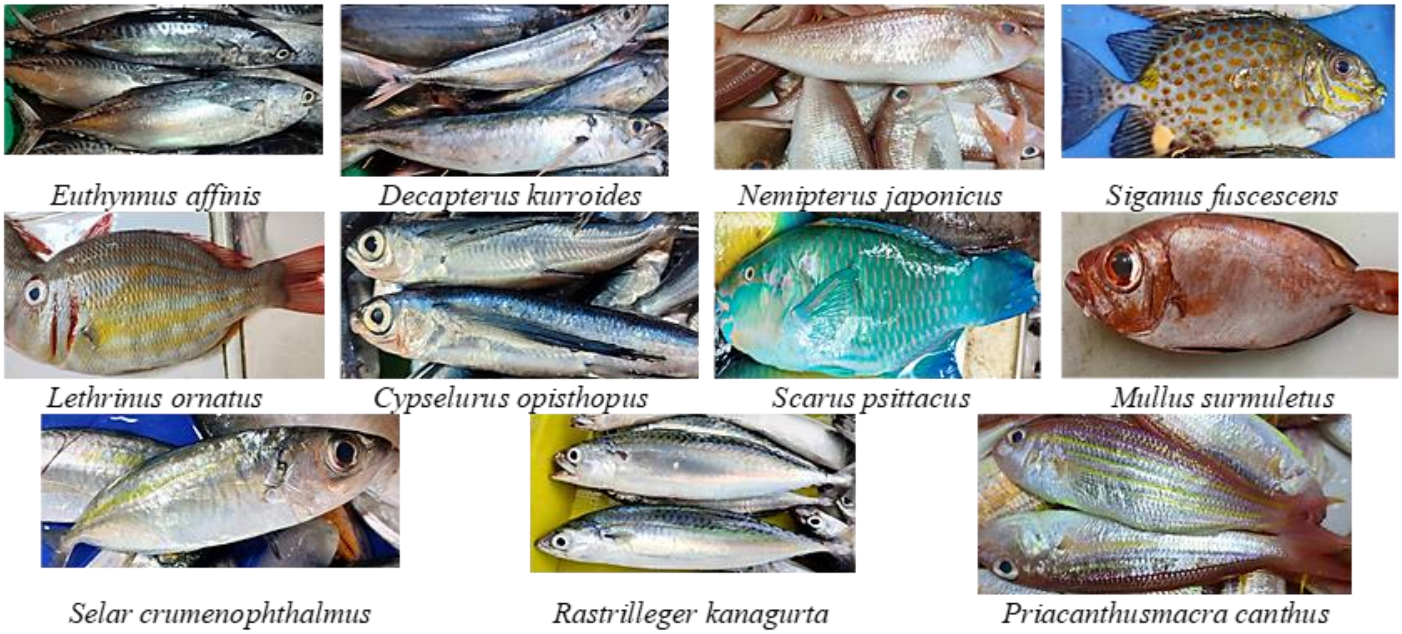

Figure 2. Eleven fish species were collected in the public wet markets.

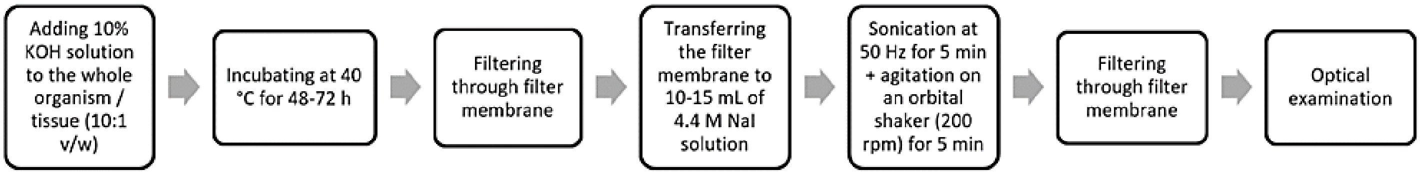

Figure 3. Flow diagram of microplastic extraction protocol (Karami et al., 2017)

Lethrinus ornatus, *Scarus psittacus*, *Siganus fuscescens*, *Nemipterus japonicus*, *Priacanthusmacra canthus*, and *Mullus surmuletus*. The pelagic zone of the ocean encompasses everything from the surface to just short of the bottom. The most common catches in the pelagic category in the studied markets included *Euthynnus affinis*, *Selar crumenophthalmus*, *Cypselurus opisthopus*, *Decapterus kurroides*, and *Rastrilleger kanagurta* (Fig. 2). All fish samples were securely wrapped in aluminum foil and placed in labeled polyethylene bags to avoid possible microplastic contamination. These were then stored in coolers with ice (0-4°C) to prevent degradation and were transported to the laboratory for dissection and processing.

Fish processing and digestion: The fish samples were gutted, and the meat and skin were collected. The fish meat was minced into small pieces and stored at -20°C. The protocol for the tissue digestion of fish samples was adopted from Karami et al. (2017) (Fig. 3). Briefly, the pooled samples were added to 60 mL (1:10 w/v) KOH in a sealed petri dish. The petri dishes were incubated at 40°C for 48 to 72 hours. The digests were filtered through Whatman No. 540 filter paper using a vacuum pump. The filter membranes obtained from the previous step were transferred into another set of Petri dishes filled with 10-15 mL of 4.4 M NaI solution. The bottles were sonicated at 50 Hz for 5 minutes, followed by agitation on an orbital shaker (200 rpm) for 5 minutes. The supernatants were collected and filtered through Whatman No. 541 filter membranes, and the samples were then ready for optical examination.

Optical identification and characterization of microplastics: The filter papers were examined under a microscope, and microplastics were counted, measured, and photographed using software (Motic, China). MPs were classified based on their size, shape, and color, according to Jabeen et al. (2017). Hard angular pieces were classified as fragments, elongated

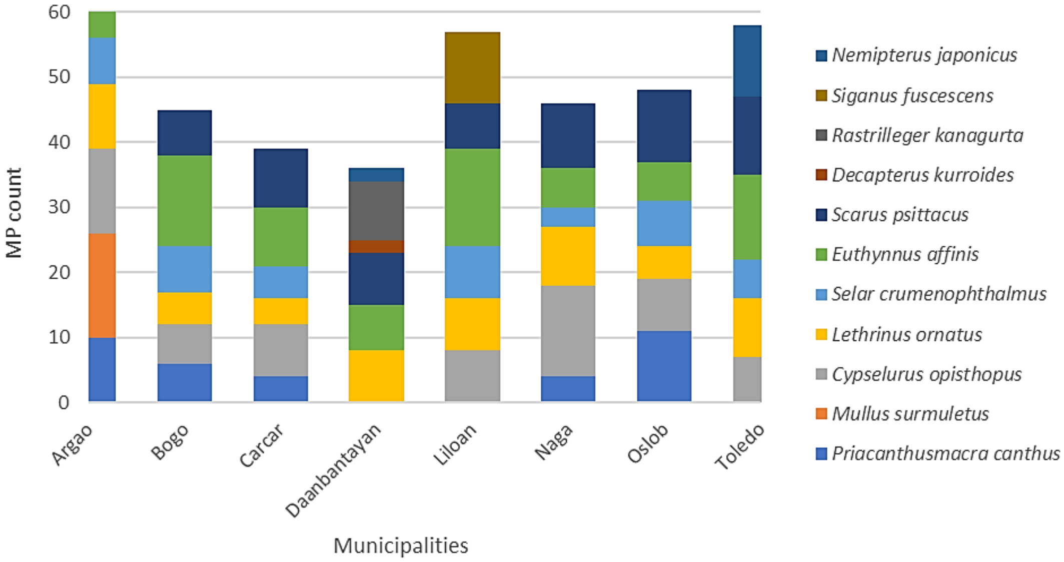

Figure 4. Occurrence of MPs in different fish species per sampling site.

threadlike particles as fibers, and flat flexible pieces as sheets (Jabeen et al., 2017). The size of each particle was measured along its longest length using Accu-Scope 3000 LED Series with magnification 10X/0.2500/0.17.

The MPs were classified into six different size groups: 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1.0, and 1.0-5.0 mm. Particles smaller than 0.100 mm were difficult to analyze using ATR-FTIR; therefore, only particles greater than 0.100 mm were randomly selected for analysis using an Attenuated Total Reflectance Fourier Transform Infrared spectrometer (Shimadzu).

Before analyzing each batch of samples, a background scan was conducted. The samples were scanned in absorption mode over a 400-4000 cm^{-1} wavenumber range. Three scans were performed for each sample, and the spectra were matched with spectral libraries in OMNIC software. Spectral matches with greater than 90% similarity were automatically accepted, while matches below 80 were individually examined and compared with the available literature on polymer spectra (Cowger et al., 2020).

Contamination control: Before the experiment, filter papers were inspected under a microscope to ensure they were free from MPs contamination. All reagents were filtered using Whatman No. 1 filter paper, which was also examined for contamination. Equipment was washed with pre-filtered water, and alcohol was used to clean the work surface and prevent cross-contamination. During sample collection, Petri dishes with unused, wet control filters were left open to capture airborne MPs, and controls with distilled water and filter papers were included throughout each batch of sample processing (n = 15). These controls were examined microscopically, and any detected particles were recorded. Control blanks containing 150 mL of 10% KOH were processed alongside each batch, and particles found in these blanks or matching particles were considered contamination and excluded from analyses. The study was conducted in a facility with limited air movement to minimize airborne contamination, and samples were only exposed to air briefly during transfers. Containers remained covered at all other times.

Polymer hazard index: The polymer hazard index was calculated to determine the potential risk of these MPs to humans. PHI was estimated using the formula of $PHI = \Sigma Pn \times Sn$, where Pn is the percentage of specific polymers, and Sn is the hazard score of polymers (Lithner et al., 2011). PHI was calculated following the method of Ranjani et al. (2021).

Results

Occurrence of microplastics in fish: Microplastics

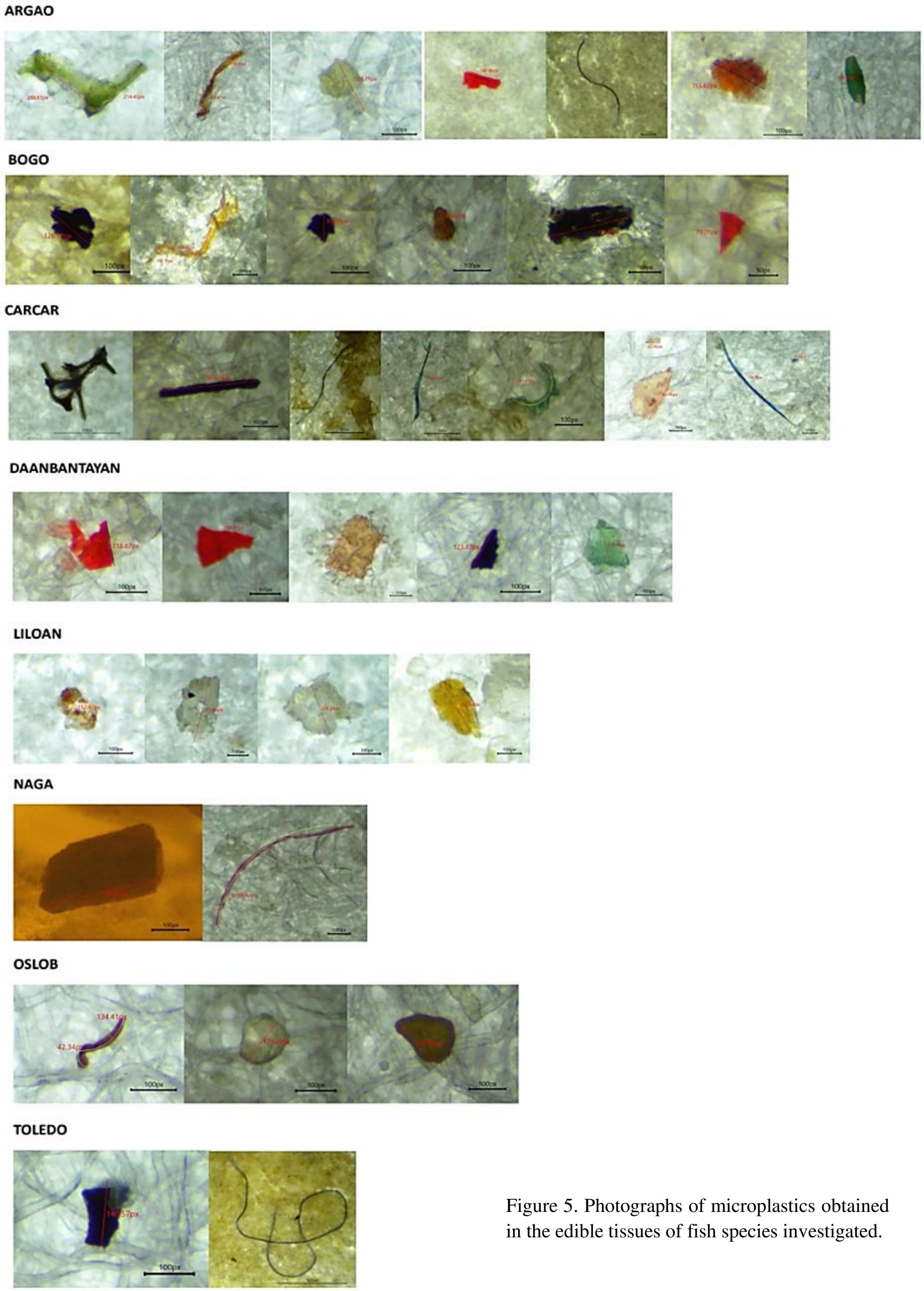

Figure 5. Photographs of microplastics obtained in the edible tissues of fish species investigated.

were detected in all fish samples. After excluding non-verified items and those found in blanks, a total of 389 microplastic particles were identified from the 11 fish species collected in the public wet markets of Cebu Province. Figure 4 shows the occurrence of MPs in different fish species per sampling site, and Figure 5 images of some of the MPs obtained in the study.

Microplastic concentration in edible fishes: Figure 6 illustrates the concentration of MPs in various fish species, expressed as the number of microplastics per

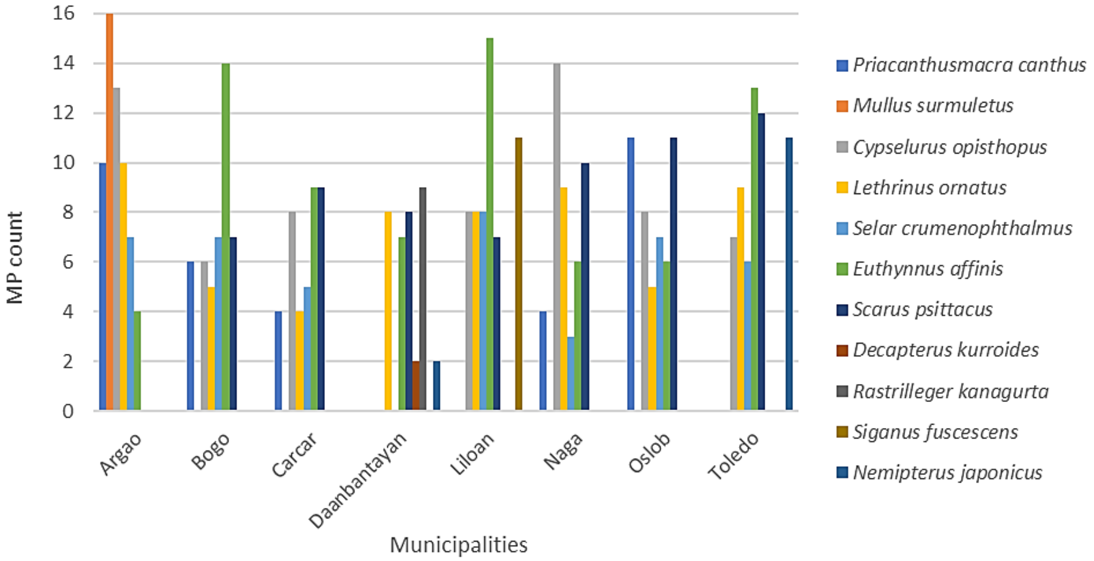

Figure 6. MP concentrations in items per g tissue in fish species in all sampling sites.

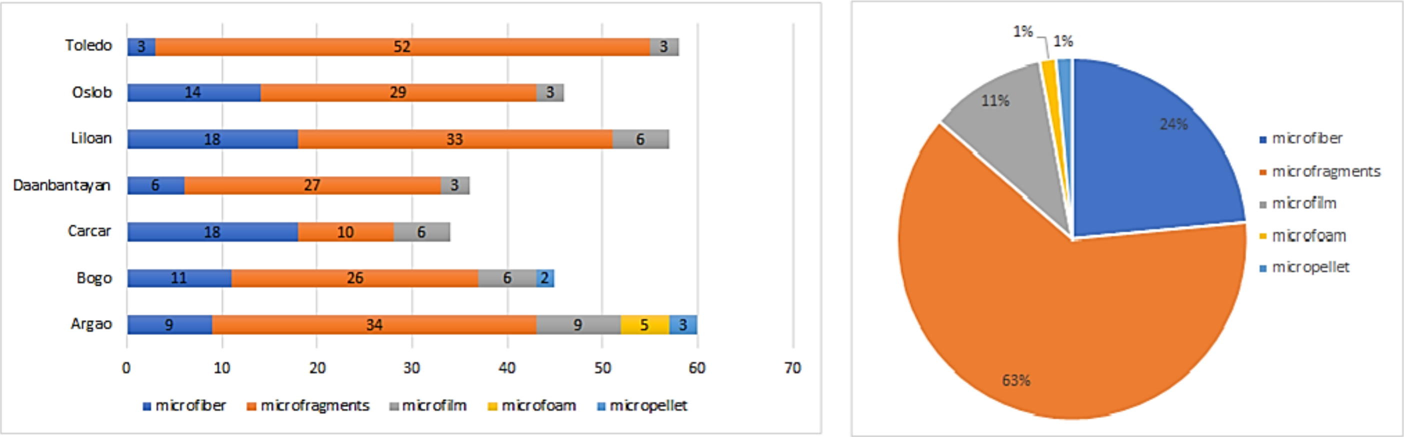

Figure 7. Microplastic shape distribution in Cebu Province. A. Percentage per sampling site; and B. Total Percentage of Microplastic Type in fish muscles and skin.

gram of tissue (items MP/g tissue). The highest MP concentration was recorded in the Municipality of Argao, where *Mullus surmuletus* exhibited 3.2 MPs/g; however, this species was only available in Argao during the sampling period. The second highest concentration was *S. crumenophthalmus* collected from Liloan, with a value of 3.0 MPs/g. Additionally, *E. affinis* and *C. opisthopus* had concentrations of 2.8 MPs/g in Bogo City and Naga City, respectively.

Characterization of the isolated microplastics

Types: A total of 389 MP particles were identified in the muscle and skin of the studied fish species, classified into five types, with the following distribution: 62.80% were micro fragments, 23.51% microfibers, 10.71% microfilmed, 1.49% microforms, and 1.49% micro pellets (Fig. 7).

Size: The sizes of microplastic particles in the water samples were determined using microscopy (iN10MX, ThermoFisher Scientific, USA). Based on size, the detected microplastics were classified into four classes: class 1 (0.1-0.3 mm), class 2 (0.3-0.6 mm), class 3 (0.6-1.0 mm), and class 4 (1-5 mm). The detected MPs in fish exhibited sizes ranging from 0.024 to 3.000 mm, with class 1 as the most abundant size. The second most abundant size category included particles smaller than 0.100 mm. Several MPs were also noted for class 2, while only a few samples were observed in the remaining size classes (Fig. 8).

Color and type**:** The colors of microplastic particles extracted in the edible portions of the fish samples

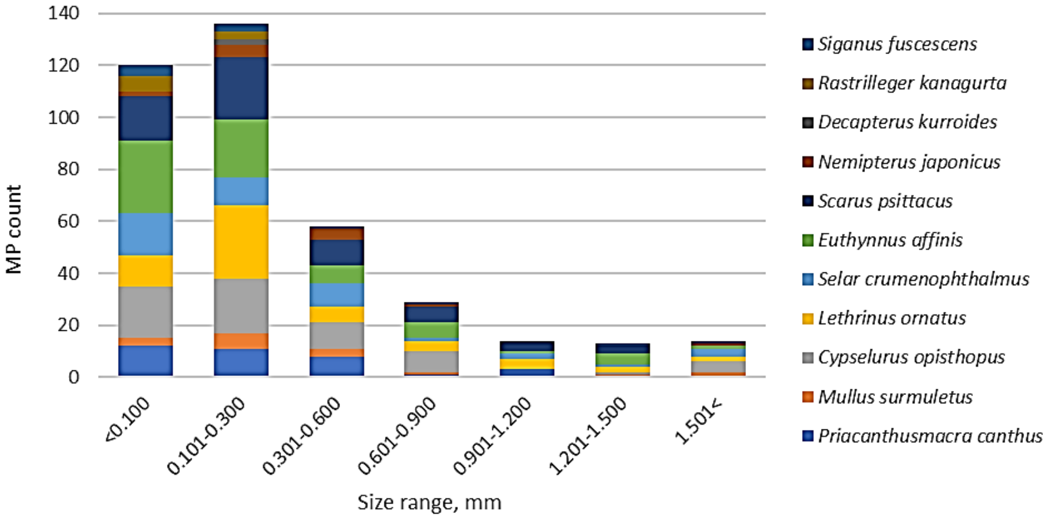

Figure 8. Size distribution of the MPs in the fishes collected.

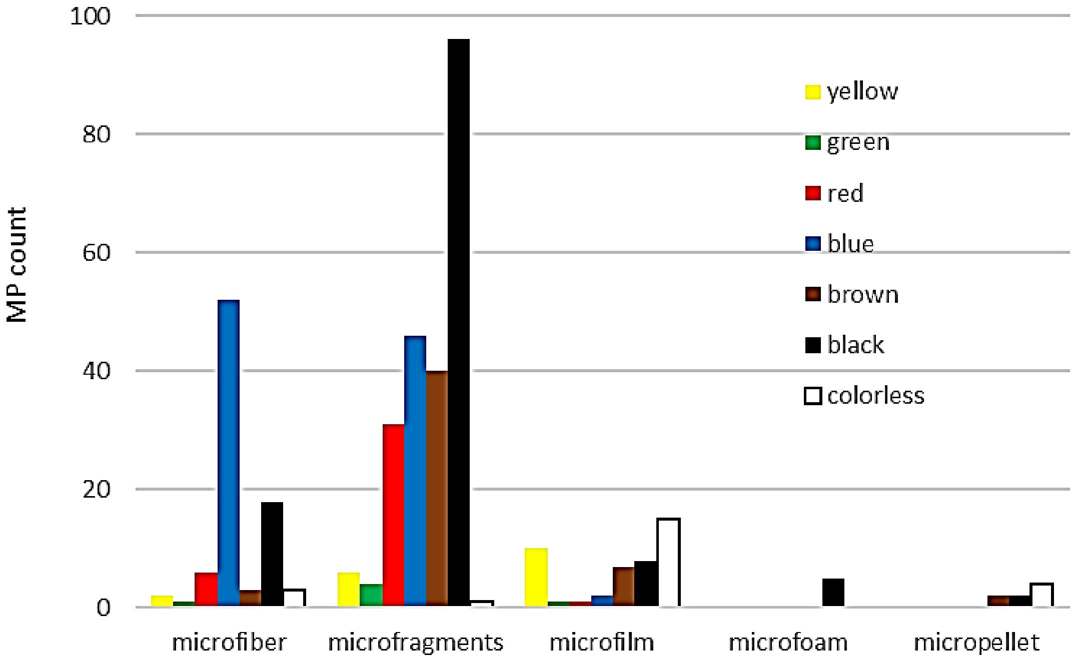

Figure 9. MPs types and color distribution in all fish samples.

included yellow, green, red, blue, brown, black, and colorless (Fig. 9). The most abundant color of microfiber particles recovered in the fish samples was blue, accounting for 61.18%, black at 21.18%, red at 7.06%, and brown and colorless at 3.53%. Yellow and green microfibers represented 2.35% and 1.18%, respectively. Meanwhile, the micro fragment particles ingested by the fish samples consisted of 42.86% black, 20.54% blue, 17.86% brown, 13.84% red, 2.68% yellow, 1.79% green, and 0.45% colorless. Additionally, microfilm particles were predominantly colorless, making up 34.09%, followed by yellow at 22.73%, black at 18.18%, brown at 15.91%, and blue at 4.55%. Green and red microfilms were both at 2.27%.

***Polymer characteristics of microplastics*:** FT-IR spectroscopy was used to determine different polymer types of random representative samples. Representative MPs were visually identified. A total of 15 plastic types were identified, including polyvinyl chloride (PVC), polyacetylene (PA), and polyvinyl alcohol (PVA). The most common were PVA and PA

Table 1. Polymers identified in the present study are classified based on hazard level and Polymer Hazard Index (PHI).

Polymer	**Monomer**	**Hazard**	**Hazard level**	**Score**	**PHI**	**Hazard Category**	**Risk category**
PVC	Vinyl chloride	carcinogenic	1	10,001	66,707	V	Extreme danger
PU	Propylene oxide	carcinogenic, mutagenic	1	13,844	46,101	V	Extreme danger
ABS	Styrene	Acute toxicity	10	6552	21,818	V	Extreme danger
PE	Ethylene	carcinogenic	36	11	37	III	High
PET	Ethylene glycol	acute toxicity	36	4	13	III	High

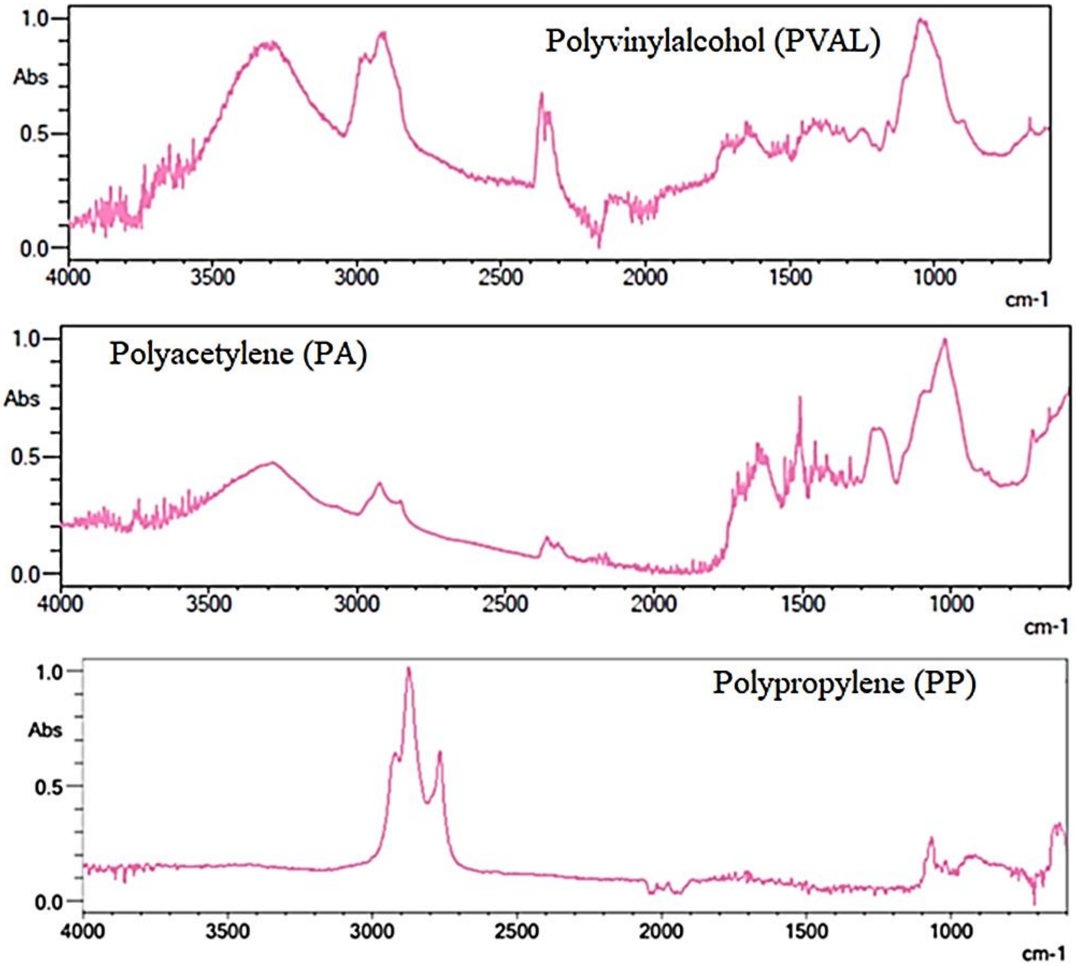

Figure 910. FT-IR spectra of microplastic samples identified as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polyvinyl chloride (PVC).

at 20 and 17%, respectively, followed by polybutene (PB) and polypropylene (PP) at 10% and PVC and polyethylene chlorinated (PEC) at 7%. The remaining polymers—polyether sulfone (PES), nylon, polyurethane (PU), polyethylene terephthalate (PET), polyethylene-polypropylene (PE-PP) copolymer, polyester, and Acrylonitrile Butadiene Styrene (ABS)—were present at 3% (Fig. 10).

***Polymer hazard assessment*:** The polymer hazard index (PHI) values in this study indicated that polyethylene (PE) (PHI = 37) and polyethylene terephthalate (PET) (PHI = 13) fell into the "High" risk category (hazard level III). On the other hand, acrylonitrile-butadiene-styrene (ABS) (PHI = 21.818), polyvinyl chloride (PVC) (PHI = 66.707), and polyurethane (PU) (PHI = 46.101) were classified under the "Extreme Danger" (hazard level V) risk category (Table 1).

Discussions

Occurrence of microplastics in fish: Of the 11 fish species sampled, only six were common across all sampling stations, with three being benthic and three non-pelagic species. The benthic species were *L. ornatus*, *S. psittacus*, and *S. fuscescens*, while the non-pelagic species comprised *N. japonicus*, *P. canthus*, and *M. surmuletus*. The pelagic species included *E. affinis*, *S. crumenophthalmus*, *C. poecilopterus*, *D. kurroides*, and *R. kanagurta*. Benthic fish (N = 197) exhibited a higher microplastic count than pelagic fish (N = 192). The results indicated no significant difference in MP levels between the two groups. This finding agrees with the findings of Keerthika et al. (2024), who reported similar MP levels in pelagic and benthic fish.

Microplastic Concentration in Edible Fishes: *Euthynnus affinis* exhibited the highest concentration of MPs in its muscles and skin, accounting for 19% (N = 74) of the total collected. This was followed closely by *S. psittacus* and *C. opisthopus*, representing 16% (N = 64) throughout the Province of Cebu. These concentrations are lower than the 4 MPs/g reported by Abiñon et al. (2020) for *E. affinis* collected from Metro Cebu. Their study attributed the abundance of MPs to environmental factors (e.g., wave action and water currents) and human activities (e.g., waste disposal and effluents from industrial and municipal facilities).

When compared to other literature, the MP concentrations reported in this study were higher than those for *Capoeta trutta* (1.6 MPs/g), *Alburnus chalcoeides* (1.1±0.7 MPs/g), and *Cyprinion macros*omia (1.1 MPs/g), as noted by Makhdoumi et al. (2021). In contrast, the concentrations were lower than those found in leaping mullets, *Chelon saliens*, on the Aegean coast of Turkey, where the highest concentration reached 4.3 MPs/g (Gundogdu et al., 2020). The findings were comparable to those of Palermo et al. (2020), who reported 3.74 MPs/g in *Sardinella lemuru* in Northern Mindanao, and to Zitouni et al. (2020), who recorded an average of 6.03±0.47 items/g of tissue in *Serranus scriba* collected from the Tunisian coast.

Characterization of the isolated microplastics

***Types*:** Based on the results, microfragments were confirmed to be the most abundant in the fish tissues, followed by microfibers and then microfilms. This result aligns with the study of Muhdhar et al. (2021), which identified fragments as the most prevalent type of microplastic collected in Indonesia. Similarly, Di Giacinto et al. (2023) observed that fragments were the dominant form in fish species (*Xiphias gladius* and *Thunnus thynnus*) collected from the Mediterranean Sea. Park et al. (2020) also reported that over 94% of all microplastics found in fish from the Han River in South Korea were fragments, with the remainder being fibers. Daniel et al. (2020) similarly found that microfragments were the most common morphotype, comprising 57.8% in edible tissues and 55.6% in inedible tissues. Fragments were also identified as the primary component of floating microplastics in the coastal waters of southwest India (James et al., 2020).

Cole et al. (2011) explained that microplastic fragments provide strong evidence of secondary microplastic provenance, as they are typically produced via the breakdown or fragmentation of larger plastic materials into smaller pieces. Another possible source of micro fragments is from degraded plastic products, which include plastic containers, packaging materials, and cleaning media (Zhang et al., 2015). The abundance of these fragments may be attributed to indiscriminate waste dumping and mismanaged plastic waste (Osorio et al., 2021). Polyethylene (PE) and polypropylene (PP) microplastics, likely sources of these fragments, may have entered the marine environment from packaging, consumer goods, automotive parts, and pharmaceuticals (An et al., 2020). In contrast, Abiñon et al. (2020) found that microfibers constituted 91% of Metro Cebu's most abundant microplastic type. Park et al. (2020) noted that fibers provide further evidence of secondary microplastics, often derived from clothes and other textiles.

Size: The detected MPs in the studied fish exhibited sizes ranging from 0.024 to 3.000 mm, with class 1 as the most abundant size. The second most abundant size category included particles smaller than 0.100 mm. Zhang et al. (2015) attributed this to the fact that plastic naturally degrades into smaller and smaller pieces over time. Several MPs were also noted for class 2, while only a few samples were observed in the remaining size classes. Compared to Abiñon et al. (2020), this study reported fewer microplastics in the fish of Cebu Province, but the sizes of the detected microplastics were smaller. This implies that the MPs in these fishes are more bioavailable, have more significant toxicity, and are easily transported.

***Color and type*:** The black MPs might have come into the environment due to abrasion of tires on the road surfaces as regular wear and tear (Wik and Dave, 2009). The prevalence of blue microfibers in *Crassostrea* sp. has been noted by Dantas et al. (2020), who attributed this to fishing nets or other local products. Other identified color particles such as red, green, blue, yellow, orange, violet, and brown were possibly fragmented from commonly used products such as packaging, toys, household products, sachets, plastic straw ropes, and food wrappers. Colored microplastics detected in the environment could be related to the high consumption of colored plastic products in everyday living (Zhang et al., 2015; Osorio et al., 2021).

***Polymer Characteristics of Microplastics*:** The most abundant polymer detected was the polyvinyl alcohol. It showed the characteristic absorption peaks of at 3380 cm^{-1} attributed to the stretching ν(O–H), 2910 and 2880 cm^{-1} are related to the stretching of ν(C–H2) and ν(C–H), respectively, 1736 cm^{-1} is attributed to the stretching of ν(C=O), 1420 cm^{-1} is due to the bending of δ(CH–O–H), 1320 cm^{-1} is attributed to the wagging π(C–H), 1080 cm^{-1} is related to the stretching of ν(C–O), and 830 cm^{-1} is due to the stretching of ν(C–C). These bands were also reported to be present by Saha et al. (2021) for the PVA and other microplastic in *Mugil cephalus*, *Gerres filamentosus*, *Arius jella*, and *Etroplus suratensis* collected in Sal estuary, Goa, situated on the central west coast of India.

Xiong et al. (2018) reported that PVA usually comes from textiles, laundry detergents, adhesives, and packaging materials. PVC and PA are linked to local agricultural practices, while laundry activities may contribute to PVA, PES, and PET levels. Other community activities, such as trash dumping and recreational bathing in the river, likely increase microplastic presence. Bansal and Singh (2022) also mentioned that PVA has applications in textiles. Jin (2022) pointed out that it was used in biotechnology, such as tissue regeneration, wound dressing, and drug delivery systems.

Polymer hazard assessment: The PHI is a crucial criterion for assessing the risk of microplastics (MPs). Nithin et al. (2022) evaluated the PHI for various fish species from the southeastern coast of India, reporting scores of 1-10, which placed them in risk category II, indicating a medium level of risk. In this study, the PHI values for PE and PVC were notably high due to their elevated hazard scores (hazard score for PE = 11 and PVC = 10,001) (Lithner et al., 2011). Polypropylene (PP) and PET, although identified in the gastrointestinal tract of tilapia with low hazard scores, also fall into the "High" risk category based on their estimated PHI values.

Conclusion

The study highlights the widespread occurrence of microplastics (MPs) in the edible tissues of commercial fish sold in public wet markets across Cebu Province, Philippines, from eight municipalities exhibiting varying levels of MP contamination. The occurrence of microplastics in commercial fish from Cebu Province presents significant environmental and public health concerns. The identified polymers, including those categorized as high-risk and extreme danger based on their polymer hazard index, highlight the urgency for targeted mitigation strategies. When consumed, these polymers may enter the human body, potentially leading to health implications requiring further investigation. Immediate actions such as stricter regulations on waste disposal, improved agricultural practices, and public awareness campaigns are necessary to reduce microplastic contamination in aquatic ecosystems. Continuous monitoring and research into the health effects of microplastic ingestion are crucial to addressing the potential risks to both human health and biodiversity.

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