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Assessment and characterization of microplastic contamination in milkfish (*Chanos chanos*) from marine aquaculture systems in the Philippines

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ABSTRACT
This paper investigated the microplastic contamination in milkfish harvested from nine mariculture sites in the Philippines. The digestion of microplastics in the gastrointestinal tract (GIT) was carried out using potassium hydroxide (KOH). Optical microscopy (OM) and Fourier transform infrared (FTIR) spectroscopy were utilized to identify and classify the suspected microplastics in the milkfish samples. Results revealed that five types of polymers were identified, namely polyethylene (PE), polypropylene (PP), polyamide (PA), polyethylene and cellulose-based polymers. Sampling site 2 obtained the highest average microplastic concentration of 14.77 + 0.0 particles/fish. This study offers a meaningful contribution to understanding microplastic contamination on tropical aquaculture species and highlights the urgency of implementing effective management strategies to address this escalating environmental concern.

Section: RESEARCH PAPER

Keywords: microplastics; milkfish; FTIR; marine environment

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1. INTRODUCTION

Nowadays, the diverse applications of plastics have led to an increase in their production. However, this elevation in plastic manufacturing does not always equate to proper disposal and management [1]. Plastic waste, which contributes to debris in the marine environment, may disintegrate into smaller sizes known as microplastics (1 μm to > 5 mm) [2] through abiotic degradation, UV radiation, heat, chemical, and mechanical stresses [3]. Various investigations have also proven the varying concentration of microplastics in coastal and underwater sediments [4]–[5].

The occurrence of microplastics has emerged as a major concern [6] due to their potential environmental threats. Microplastics in coastal areas reaching the seabed can be taken up by aquatic fauna [7]–[8]. Numerous studies show evidence that microplastics are present in the digestive tracts of aquatic invertebrates, fish, clams, and mussels [9]–[10]. Marine organisms absorb these microplastics as they are mistaken for food due to their close resemblance to the size of zooplankton [7], [11]. Additionally, microplastics can inadvertently penetrate aquatic creatures, specifically fish, by being absorbed through their gills, consequently entering the gastrointestinal tract (GIT) [9] of the fish. These microplastics present in the GIT of the fish may persist for days to weeks after being swallowed or ingested. The retention of microplastics in such parts allows their migration to other parts of the fish, including its meat [12]. Hence, microplastic consumption not only harms the aquatic

environment, but also threatens humans' food consumption safety, since fish is regarded as a significant source of protein containing essential amino acids by humans [13]–[14].

Microplastic investigations in the Philippines are still beginning to expand. The country which significantly contributed to a share of global plastic—producing 163 million plastic sachets and 45 million thin-film bags [10] leading to becoming the third largest contributor of marine plastic debris [15]—has only conducted limited research on microplastic contamination and has a narrow scope of understanding its effects on flora and fauna, which is evident from the limited research programs and knowledge of its implications for environmental and human health. Recent local studies of microplastics in aquatic species include the investigation of microplastics in market fish samples from a highly urbanized area of Tacloban City [16], the identification of microplastics in milkfish collected from Butuan Bay [17], and the quantification of microplastics in rabbitfish and mullet from the river of Eastern Visayas [18]. Most of these studies focus on one or a few sampling sites, which represents a knowledge gap identified in local research.

The Philippines' fisheries are ranked 13th among the world's major fish-producing countries [14], contributing significantly to global fish production [19]. Locally called bangus, milkfish (*Chanos chanos*) is considered a national fish [20], and is cultured mainly in marine waters, freshwater, and brackish water lakes [14], [20]. As aquaculture plays a vital role in the country's overall fishery output, including milkfish production, the need for microplastic research and its local monitoring in commercial seafood is highly significant.

To date, there is no local research documenting the detection of microplastics in milkfish freshly harvested from marine cage aquaculture systems. In this paper, the detection and classification of microplastics in the GIT of marine milkfish were determined. This study provides the first regional, multi-site comparison and monitoring of microplastic contamination in aquaculture environments for *Chanos chanos*, employing Fourier transform infrared (FTIR) spectroscopy for polymer confirmation. Valuable data were generated from this investigation, enhancing a better understanding of microplastic contamination in aquatic organisms and its potential impacts on ecological and human health, sustainability, and food safety.

2. MATERIALS AND METHODS

2.1. Sampling

The milkfish samples used in this study were collected from nine marine aquaculture locations in various regions of the Philippines (Figure 1). All milkfish samples were collected from the marine fish cages of the specific sites (for further details of the sampling sites, please refer to Table S1[1]). Site visualization was extracted using QGIS software 3.44.4. A random sampling method was used to collect the samples, wherein 30 milkfish were collected from each location. Specifically, fish were selected without prior bias, by randomly choosing individuals from different sections of the cages, to ensure that each fish had an equal probability of being included in the study. This approach minimizes sampling bias and helps ensure that the collected specimens are representative of the overall population at each site. Once collected, each sample was covered with aluminium foil to avoid contamination.

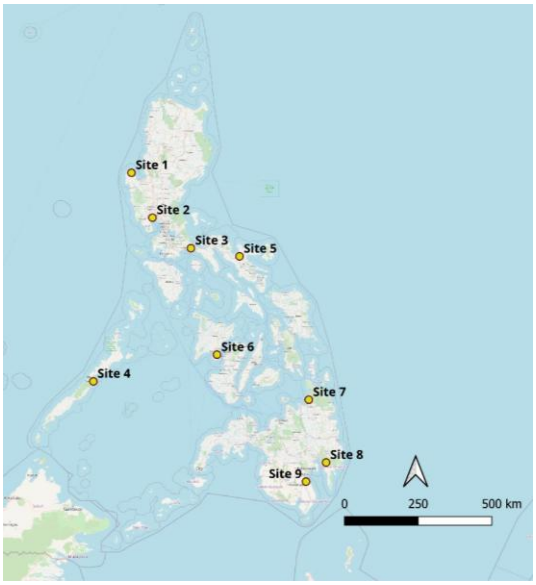

Figure 1. Sampling sites of milkfish from nine marine aquaculture cages.

2.2. Digestion of the GIT of milkfish

The mass and size (length and width) of the milkfish samples were first weighed and measured from snout to tail tip. Each sample was dissected before digestion. The procedure was performed as follows: (i) the fish was descaled and rinsed with distilled water, (ii) a sample was cut from the vent toward the head, slightly off the midline, and (iii) the GIT—including the esophagus, stomach, and intestines—was removed and placed in a non-plastic container. Digestion was carried out following the method described by [21]–[22]. Briefly, five (5) grams of GIT were treated with a strong base (10 wt. % KOH) and were kept inside the oven at 40 °C for 48 hours. The digested samples were then filtered under a vacuum manifold using glass microfiber filters to remove the excess solution and other organic matter. The filtered samples were oven-dried overnight before the extraction and characterization of microplastics. The vacuum filtration set-up was fabricated by Noveaulab Asia Corporation, Philippines, and the glass microfiber filter papers used during filtration had a 1.2 μm pore size and a 47 mm diameter.

2.3. Isolation and characterization of microplastics

The dried, filtered samples were visually examined using optical microscopes (Carl Zeiss Stemi 2000-C Stereo Microscope and Carl Zeiss Axio Scope A1 High-Power Compound Microscope) with varying magnifications of 32 × to 100 ×, depending on the size of the detected microplastics. Suspected microplastics were carefully isolated, noting their sizes and colours. These suspected microplastics were identified using Fourier transform infrared spectroscopy–Attenuated Total Reflectance (FTIR-ATR) and Perkin Elmer Spotlight 200 FTIR-Microscope (μ-FTIR). The analysis was obtained from 4000 - 600 cm^{-1} for 20 scans with a 4 cm^{-1} resolution and a data interval of 4 cm^{-1}. Spectrum Library Search Plus (Version 6.3.5.0176), from Perkin Elmer, Inc., was used as the reference spectral database. Standard polymers, including Polyamide (PA),

[1] Tables labeled with an 'S' are provided in the Supplementary Material at the end of tne article and contain the data underlying the statistical analyses presented.

polyethylene terephthalate (PET), polyvinyl chloride (PVC) resins, polypropylene (PP), polyethylene (PE), and polystyrene (PS), were also tested through FTIR as spectral references for the actual isolated microplastics. The criteria for polymer identification were based on the following: a spectral match of greater than 70 % [23] and a comparison of the acquired spectra with those reported in the published literature.

2.4. Quality control procedure

To prevent microplastic contamination, the sample preparation and analyses were conducted in a laboratory with controlled ambient conditions. To further minimize the contamination, the analysts during the entire analysis were wearing proper protective equipment, including cotton laboratory gowns, electrostatic discharge (ESD) smock gowns and shoes, and nitrile gloves. All materials and equipment used during sample preparation and analysis, including reagent bottles, dissecting tools, and vacuum filtration manifolds, were thoroughly washed with filtered distilled water before use, and care was taken to ensure that none were made of plastic. Procedural blanks per digestion set were incorporated into the analysis as part of the quality control measure to determine the possibility of environmental and background contamination. Additionally, during the filtration, a control blank of wet glass microfiber filter paper was exposed to air for possible airborne particle contamination. Blank corrections were incorporated for the identified microplastics in the actual samples.

In blank correction, a minimum of three procedural blanks per batch were incorporated into the analysis as part of the quality control measure to determine the possibility of environmental and background contamination. The blank correction was employed using equation (1):

$$Total\ MP = \underline{x}_{\text{isolated MP}} - \underline{x}_{\text{MP in blank}}, \quad (1)$$

where the total microplastic count reported is the difference between the detected mean of the isolated microplastic from the filter paper ($\underline{x}_{\text{isolated MP}}$) and the mean of the detected microplastic in procedural blanks. Additionally, during the filtration, a control blank of wet glass microfiber filter paper was exposed to air for possible airborne particle contamination. After drying, the filtered samples were placed in a petri dish covered with aluminum foil and stored in a controlled room before the extraction and analysis of microplastics.

3. RESULTS AND DISCUSSIONS

3.1. Concentration of microplastics in the GIT of milkfish

Table 1 presents the average weight and standard length of milkfish samples collected at each site, along with their corresponding unadjusted microplastic counts and the average concentrations of identified microplastics in the GIT. The average identified microplastics were calculated as the total particles for each individual polymer type, minus the count of particles in controls for each individual polymer type [23]. Based on the data, all sampling sites showed the presence of microplastics in the GIT of the fish, which serves as the primary pathway for microplastic ingestion [24]. Site 2 accumulated the highest average microplastic concentration of 14.77 ± 0.01 particles/fish with an average milkfish size among the other samples (367.8 ± 111.9 g in weight and 26.1 ± 2.5 cm in length). Meanwhile, sites 3 (0.73 ± 0.03 particles/fish) and 4 (0.73 ± 0.02 particles/fish) obtained the lowest average number of identified microplastics. It is quite notable that site 4 also garnered the largest milkfish samples (595.5 ± 204.6 g) collected, among other sites.

Table 1. Weight and height of milkfish and their identified microplastics.

Site	Average weight, g	Average standard length, cm	Unadjusted microplastics, particles	Average microplastics, particle/fish
1	469.02 ± 93.22	28.23 ± 2.38	365.00	8.50 ± 0.12
2	367.79 ± 111.85	26.10 ± 2.49	485.00	14.77 ± 0.01
3	394.29 ± 37.17	27.33 ± 0.3	62.00	0.73 ± 0.03
4	595.54 ± 204.63	29.53 ± 3.04	52.00	0.73 ± 0.02
5	108.80± 30.12	17.70 ± 1.81	505.00	10.50 ± 0.21
6	495.35 ± 137.02	30.27 ± 2.72	186.00	6.20 ± 0.10
7	466.89 ± 126.14	29.17 ± 3.98	243.00	4.24 ± 0.08
8	369.37 ± 47.09	27.17 ± 1.20	107.00	2.67 ± 0.06
9	293.11 ± 46.42	25.07 ± 1.34	61.00	1.72 ± 0.06

**± values are represented as standard error of mean (SEM)*

A Kruskal–Wallis test was conducted to assess differences among sites in average fish size (height and weight) and unadjusted microplastic counts, as presented in Table S2 and Table S3. The results showed no statistically significant differences among the sites regarding fish height, weight, or microplastic counts ($p = 0.433$). This indicates that neither fish size nor microplastic abundance varied significantly across the sampling locations. Accordingly, no evidence of a relationship was observed between milkfish body size and microplastic presence in the gastrointestinal tract. These findings suggest that microplastic ingestion is not dependent on fish size within the sampled population; however, no further inference regarding environmental drivers can be made based on this test alone. The abundance of microplastics in fish appears to be independent of body weight or length, and is instead influenced primarily by the level of plastic pollution in the surrounding water body [25].

The variability in microplastic concentration across sites suggests that environmental factors, such as pollution sources, hydrodynamics, and aquaculture practices, have a greater influence on ingestion than fish size. No clear relationship was observed between fish size and microplastic load, as larger fish showed relatively low counts. Differences in standard deviations further indicate uneven exposure among individuals, reflecting heterogeneous microplastic distribution across sites.

3.2. Comparison of this study with the published literature

The concentrations of microplastics ingested by milkfish (*Chanos chanos*) in this study were compared with those from previous studies (Table 2). It is observed that sites 3 and 4 showed trace concentrations of microplastics in milkfish compared to other locations, while site 2 obtained the highest detected microplastics. A possible reason for the abundance of microplastics in site 2 is its geographical location—it lies in a water passage near Manila Bay, Philippines, a known large body of water, and on which plastic pollution makes up 90 % of litter [28]. However, this study focuses solely on milkfish samples obtained from the aquaculture farm, with no assessment of environmental matrices such as water and sediments from Manila Bay. Consequently, no direct measurements of microplastic concentrations in the bay were conducted, limiting the ability to establish correlations between environmental levels and microplastic accumulation in the sampled fish.

Moreover, marine fish samples accumulate more microplastics due to the scale and volume of marine pollution carried by maritime trading, coastal population, and fishing activities [29]. Hence, the variation in the concentration of

Table 2. Comparison of microplastics in milkfish from different studies.

Site	Average microplastics, particles/fish	References
Cebu	11*	[26]
Babatngon, Leyte	9.7*	[16]
Nasipit, Agusan del Norte	5.4 ± 3.7	[17]
Butuan, Agusan del Norte	10.3 ± 3.2	[17]
Citarum River, Indonesia	2.7 ± 2.2	[27]
1	8.50 ± 0.12	*This study*
2	14.77 ± 0.01	*This study*
3	0.73 ± 0.03	*This study*
4	0.73 ± 0.02	*This study*
5	10.50 ± 0.21	*This study*
6	6.20 ± 0.10	*This study*
7	4.24 ± 0.08	*This study*
8	2.67 ± 0.08	*This study*
9	1.72 ± 0.08	*This study*

**Declared as particles*

ingested microplastics across different regions can be impacted by several factors that include human activities and the type of environment [30]. With that, sites that are nearer to the mentioned sources may exhibit higher microplastic concentrations due to the greater runoff and direct contamination [31]. High concentrations of microplastics ingested through food and water pose potential risks to human health. These particles can carry toxic chemicals and pollutants, which may contribute to adverse effects on the respiratory and digestive systems. Although research is still ongoing, the increasing accumulation of microplastics raises concerns about long-term health impacts and the development of chronic diseases. However, this study is limited to microplastics isolated from the GIT of milkfish, which restricts comprehensive risk assessment for human exposure, as the edible tissues of the milkfish were not analysed. Furthermore, a limitation of this study is that the correlation between microplastic concentration and population density was not assessed. Future research should focus on elucidating the relationships between environmental and anthropogenic factors and the abundance of microplastics ingested by fish. Such investigations could provide a more comprehensive understanding of the drivers of microplastic accumulation in aquaculture systems.

3.3. Colour of isolated microplastics

Figure 2 presents the distribution of the physical characteristics of the microplastics in terms of colour and shape. Black emerged as the dominant colour with 44.41 %, followed by blue (24.56 %) as the second and red (10.36 %) as the third most prevalent microplastic colour. In contrast, violet (0.58 %), orange (0.72 %), and pink (1.20 %) are the three least dominant colours. The dominance of black, blue, and brown colours has correlated with the result of the colours of the microplastics in the study conducted in Butuan, Mindanao [32]–[33]. Otherwise, compared to the study conducted in Visayas [16], orange, blue, and white were the most represented colours of ingested microplastics. However, microplastics demonstrate a wide spectrum of colours in environmental settings and aquatic organisms due to their function and purpose [34]. For instance, black and gray are from synthetic textiles [35], blue and orange are accounted for laundry, fishing nets, and plastic packaging [36], white and transparent are from degraded plastic bags, food containers, and bottles, while green and yellow are attributed to bottle caps and marine debris [36]. Moreover, the faded colour

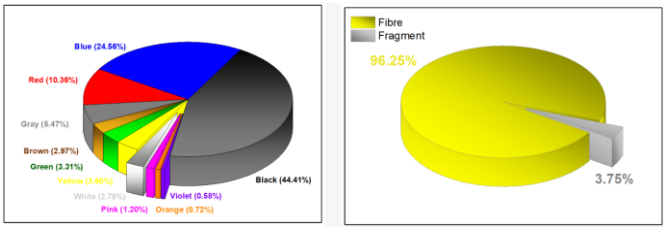

Figure 2. (L-R) Colour and shape of isolated microplastics in the GIT of milkfish.

of microplastics is likely due to the process of weathering and degradation, further indicating prolonged exposure to sunlight [32], [34], [37]. Furthermore, blue and green-coloured microplastics could be highly ingested by marine animals as they are mistaken for food [38]. All the mentioned colours were present across the studied microplastics regardless of the sampling site, with a statistically significant variation in colour distribution among the milkfish samples (f value = 3.74; $p < 0.00178$) (see Table S4 and Figure 1).

3.4. Shape and size of isolated microplastics

In terms of shape, the microplastics are categorised into two main forms—fiber and fragment. The shapes of isolated microplastics (Figure 2) revealed that fiber-shaped microplastics were predominant, constituting 96.25 % of the total count. On the other hand, fragment-shaped microplastics accounted for 3.75 % of the total microplastics. Results also show that the fiber-shaped microplastics measured from 697 µm to 1600 µm, while the fragment-shaped microplastics measured from 112 µm to 537 µm. These findings are consistent with studies conducted in southern Taiwan [39], Hong Kong [40], and the South China Sea [41], where fiber-type microplastics were found to dominate in marine species. Moreover, the fiber-shaped microplastics may originate from laundry wastewater and marine-based synthetic ropes and fishing nets [36]. In contrast, the fragment-shaped microplastics are likely derived from thick plastic, such as bottles and plastic bags [42]. Furthermore, a statistically significant difference was observed among the shapes of the isolated microplastics (f value = 15.43; $p = 0.0011$) (see Table S5). This suggests that the variability of isolated microplastic sizes and shapes across each site comes from the differences in the degradation process. The large fragments and fibers are indicating a recent degradation from fishing and domestic activities, while smaller microplastics indicate an advanced and longer degradation process.

3.5. Polymer-type distribution of microplastics

As observed in Figure 3, a total of five polymer types were classified: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polyamide (PA), and cellulose. Cellulose constitutes the highest frequency among all types, with 81.22 % of the total isolated microplastic particles. It is followed by PET (14.29 %), PA (1.82 %), PP (1.72 %), and lastly PE (0.96 %), as the least abundant classified polymer. The cellulose-based microplastics were classified as natural cellulose (81.29 %) and regenerated synthetic cellulose (18.71 %). The classification of cellulose was based on the Spectrum Library Search Plus (Version 6.3.5.0176), from Perkin Elmer, Inc., considering > 70 % match. Microplastics exhibiting a spectral match of > 70 % with “alpha cellulose” and “cellulose” were classified as natural cellulose, whereas those showing a > 70 % match with rayon were categorized as regenerated synthetic cellulose. These

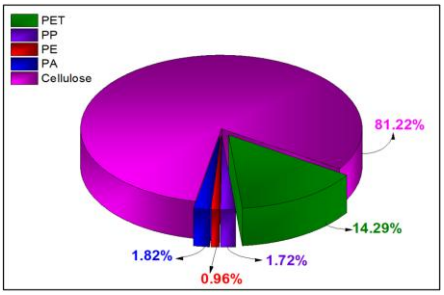

Figure 3. Polymer classification of identified microplastics in the GIT samples.

polymers may originate from the breakdown of plastic pellets, food and beverage packaging, such as bottles, sewage sludge, fishing nets, and ropes [29]. The result of this study is comparable to the studies of [43]–[44], wherein cellulose and rayon have been considered the dominant composition of microplastics. Moreover, statistical treatment (Table S6) suggests that the microplastics found in milkfish samples varied significantly based on polymer type (f value = 68.59; $p \leq 0.001$).

3.6. FTIR analysis of microplastics

Figure 4 depicts the spectra of the standard and isolated microplastics. The spectrum of the isolated microplastic in Figure 4a shows similar peaks to those of the standard PET with a library match of 93 %. Both standard PET and the isolated microplastic exhibited peaks at 2969 cm^{-1} and 2920 cm^{-1}, which are attributed to the C–H stretching vibrations [45]. Moreover, PET was further confirmed when both spectra displayed peaks representing C=O stretch (1710 cm^{-1}), C=C bending vibrations (1341 cm^{-1}), C–O bending vibrations (1247 cm^{-1} and 1095 cm^{-1}), and C–H bending vibrations (1017 cm^{-1}, 871 cm^{-1}, and 724 cm^{-1}) [45]. In Figure 4b, the isolated microplastic generated a 91 % library match and exhibited a spectrum comparable to the standard PP. CH_3 stretching vibrations (2953 cm^{-1} and 2877 cm^{-1}) were visible from both spectra of the isolated microplastic and standard PP. Sharp CH_2 peaks present from both spectra at 2921 cm^{-1} and 2849 cm^{-1} validated that the isolated microplastic is present as PP [45]. From the spectra in Figure 4c, prominent peaks attributed to PE were identified with a library match of 94.5 %. Both isolated microplastic and standard PE exhibited peaks at 2914-2848 cm^{-1}, which is attributed to the C–H stretching vibrations of the PE polymer [46]. Additionally, the peaks at 1471 cm^{-1} and 716 cm^{-1}, due to the C–H stretching vibrations, and CH_2 bending vibrations, respectively, further confirm that the isolated microplastic is considered as a PE polymer [46]. The detected PA microplastic presented in Figure 4d exhibited peaks similar to those of the standard PA polymer. The peak around 3302 cm^{-1} is attributed to the N–H stretching vibration of the amide functional group [47], while the peaks at 3084 cm^{-1}, 2924 cm^{-1}, and 2864 cm^{-1} are attributed to the C–H stretching vibrations. The peak at 1629 cm^{-1} is due to the C=O stretching of amide I, while the peaks at 1533 cm^{-1} and 1460 cm^{-1} are due to the N–H bending vibrations of amide II. The C–H bending vibrations at 1377 cm^{-1} and C–N bending vibrations at 1264 cm^{-1} prove that the isolated microplastic is considered a PA polymer [47]. In Figure 4e, the microplastic exhibited characteristic peaks of cellulose, as indicated by broad O–H stretching vibrations at 3315 cm^{-1}. Additionally, C–H stretching vibrations are also evident at 2896 cm^{-1}. The cellulose-based polymer is further validated by the C–O–C stretching vibration at 1163 cm^{-1} and C–O–C bending vibrations at 1107 cm^{-1}, 1028 cm^{-1}, and 897 cm^{-1} [48].

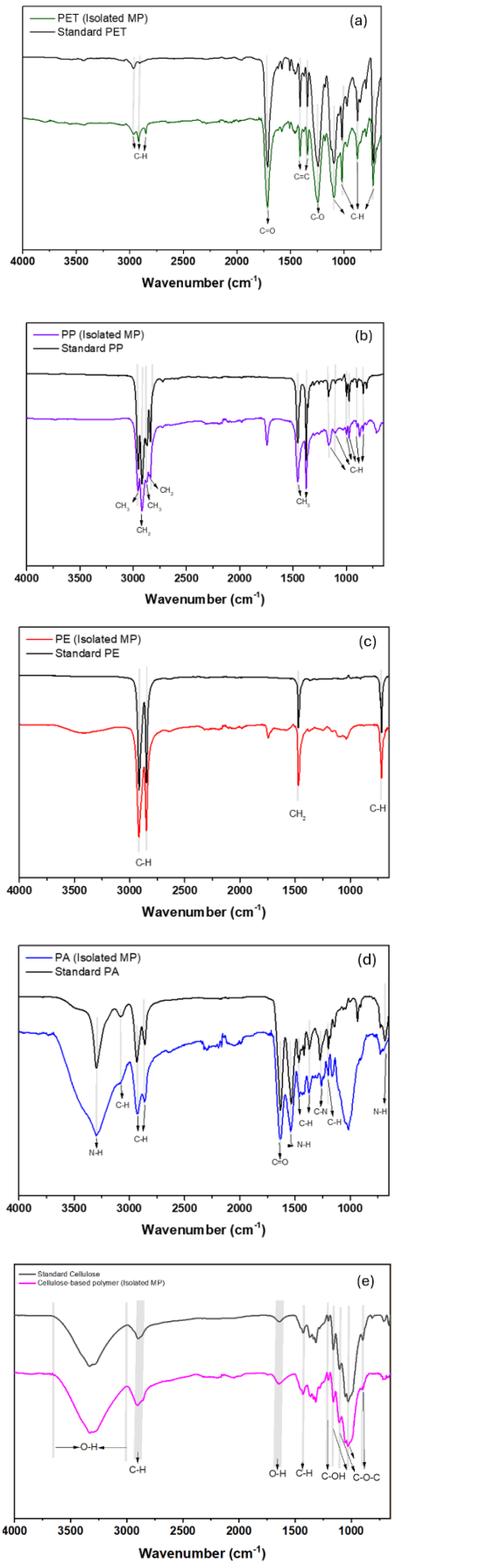

Figure 4. (1) FTIR spectra of standard and isolated (a) PET, (b) PP, (c) PE, (d) PA, and (e) cellulose.

4. CONCLUSION

This study provides critical insights into microplastic contamination in milkfish harvested from marine aquaculture systems in the Philippines. Fish is a vital food commodity, yet it may be increasingly threatened by microplastic pollution. The potential human health implications associated with microplastic exposure through fish consumption represent an undeniably emerging concern. The findings reveal the prevalence of microplastics in local mariculture systems and underscore the urgent need to inform fisherfolk and local communities about the implementation of effective mitigation strategies, as well as to explore alternative fishing practices that reduce microplastic risks while maintaining best aquaculture practices.

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Supplementary Material

Nine (9) marine aquaculture sites were collected with milkfish samples. **Table S1** shows the site description and its corresponding geographical coordinates.

Table S1. Marine sampling sites of milkfish samples.

Site Number	Location	Coordinates
1	Northwestern Luzon	16°17'55"N 119°59'12"E
2	Central Luzon	14°55'35"N 120°35'19"E
3	Central Luzon	13°55'02"N 121°48'58"E
4	Luzon Island Group	9°47'27"N 118°37'44"E
5	Southeastern Luzon	13°31'39"N 123°20'44"E
6	Western Visayas	10°35'01"N 122°36'24"E
7	Northeastern Mindanao	8°57'01"N 125°32'26"E
8	Southeastern Mindanao	7°12'16"N 126°02'30"E
9	Southeastern Mindanao	6°31'53"N 125°22'51"E

A Kruskal-Wallis test was conducted to examine the relationship between the average size (height and weight) of microplastics and the unadjusted number of microplastics recorded at each site, as presented in **Table S2** and **Table S3**.

Table S2. Correlation of the average height and weight of milkfish to the number of identified microplastics (unadjusted)

	Sampling Site	**Mean Rank**
Unadjusted microplastics in particles	1	7.00
	2	8.00
	3	3.00
	5	1.00
	5	9.00
	6	5.00
	7	6.00
	8	4.00
	9	2.00
Average weight in grams	1	7.00
	2	3.00
	3	5.00
	4	9.00
	5	1.00
	6	8.00
	7	6.00
	8	4.00
	9	2.00
Average height in centimetres	1	6.00
	2	3.00
	3	5.00
	4	8.00
	5	1.00
	6	9.00
	7	7.00
	8	4.00
	9	2.00

Table S3. Kruskal-Wallis Test Statistics

Description	**Unadjusted microplastics in particles**	**Average weight in grams**	**Average height in centimetres**
Kruskall-Wallis H	8.00	8.00	8.00
df	8	8	8
Asymp. Sig,	0.433	0.433	0.433

Table S4. Welch's ANOVA Results and Levene's Test for Homogeneity: Colours of Microplastics

Welch's ANOVA				
DF Num	*DF Den*	*F-value*		*p-value*
10	11.69964	4.31528		0.03873
At the 0.05 level, the population means are significantly different.				
Homogeneity of Variance Test: Levene's test (Absolute Deviations)				
	DF	*Sum of Squares*	*Mean Square*	*F value*	*Prob > F*
Model	10	51473.96733	5147.39673	13.42071	5.4845E-14
Error	88	33751.62414	383.54118		
At the 0.05 level, the population variances are significantly different.					

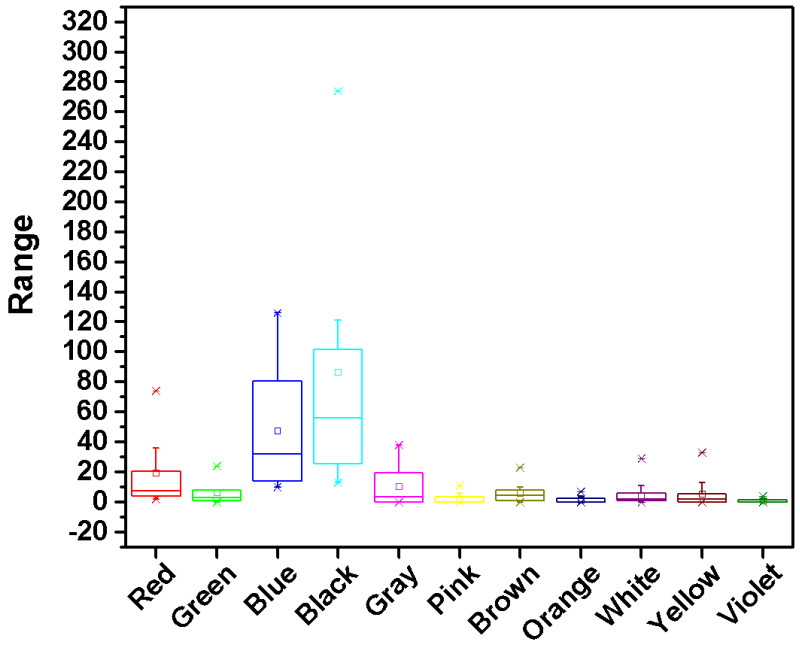

Figure S1. Box Chart: Colours of Microplastics

Table S5. Welch's ANOVA Results and Levene's Test for Homogeneity: Shape of microplastics

Welch's ANOVA			
DF Num	*DF Den*	*F-value*	*p-value*
1	17.09	21.44	0.00024
At the 0.05 level, the population means are significantly different.			

Homogeneity of Variance Test: Levene's test (Absolute Deviations)					
	DF	*Sum of Squares*	*Mean Square*	*F value*	*Prob > F*
Model	1	86250.88889	86250.88889	43.34458	6.29071E-6
Error	16	31838.22222	1989.88889		
At the 0.05 level, the population variances are significantly different.					

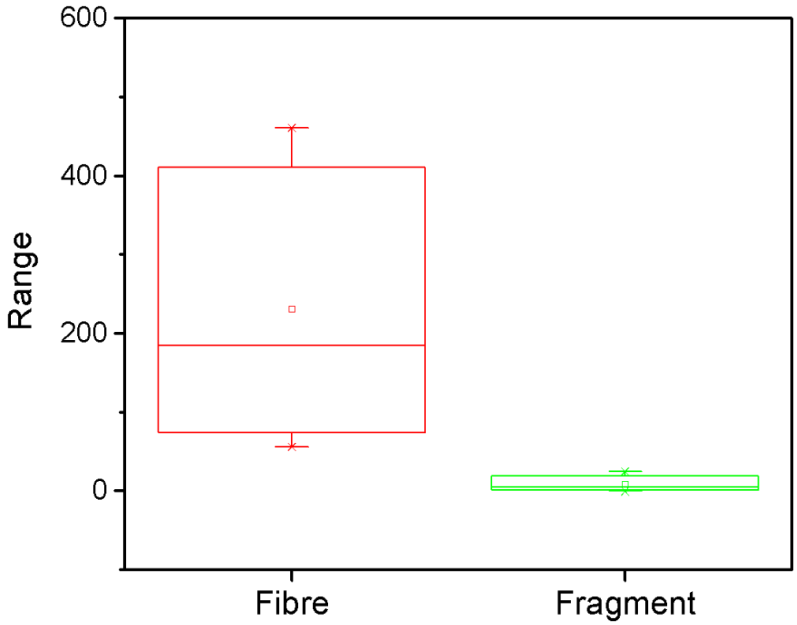

Figure S2. Box Chart: Shape of Microplastics

Table S6. Welch's ANOVA Results and Levene's Test for Homogeneity: Classification of microplastics

Welch's ANOVA			
DF Num	*DF Den*	*F-value*	*p-value*
4	617.10	68.59	< 0.0001
At the 0.05 level, the population means are significantly different.			

Homogeneity of Variance Test: Levene's test (Absolute Deviations)					
	DF	*Sum of Squares*	*Mean Square*	*F value*	*Prob > F*
Model	4	4859.63972	1214.90993	183.66266	0
Error	1345	8897.03928	6.6149		
At the 0.05 level, the population variances are significantly different.					

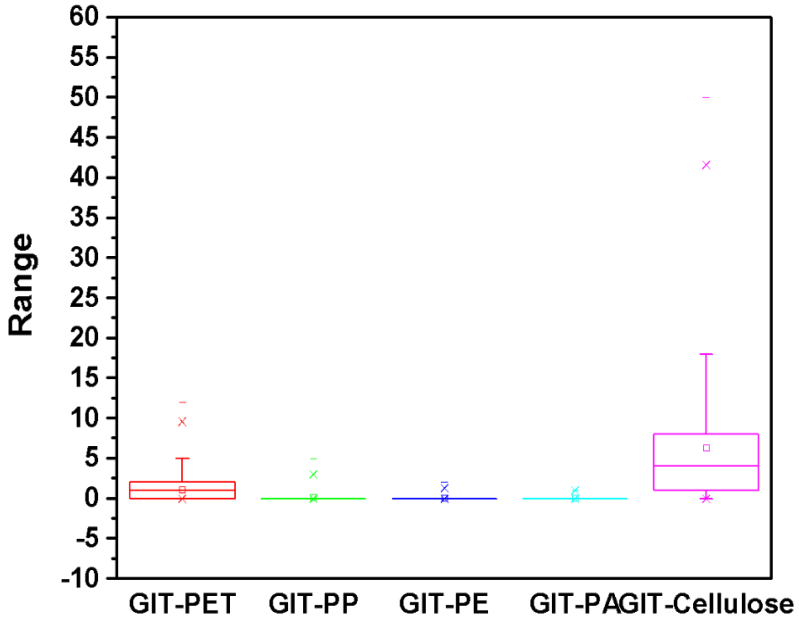

Figure S3. Box Chart: Classified microplastics in GIT of milkfish

Table S7. Average size range of MPs from milkfish samples from the marine water system

Sampling Site	**Shape of MP**	
	Fiber, μm	**Fragment, μm**
1	1461.8 ± 1390.6	320.7 ± 147.1
2	1177.0 ± 107.7	537.3 ± 219.2
3	787.3 ± 529.5	307.1 ± 100.9
4	1147.2 ± 844.8	150.3 ± 0.0
5	823.0 ± 216.1	248.5 ± 0.0
6	697.2 ± 151.9	233.2 ± 62.9
7	819.3 ± 298.9	112.0 ± 39.7
8	1543.9 ± 1009.3	272.4 ± 0.0
9	1595.4 ± 134.5	-