

# Microplastic detection in milkfish (*Chanos chanos*) from selected aquaculture farm in the Philippines

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**Abstract** – The reliable detection and analysis of microplastics (MPs) are crucial for the development of solutions to mitigate their potential impacts on the environment and human health. However, the removal of biological materials remains a significant challenge as MPs may be obscured by biological matter, complicating their isolation. This study optimized the digestion and extraction of MPs from milkfish parts using KOH combined with a dual-density separation involving NaCl and NaI solutions. Reference micro-polymers were spiked into the fish parts to assess % recovery. Results revealed excellent digestion efficiencies and % recoveries ( $\approx 90\%$ ) in 10% KOH solution. The method was also applied to milkfish collected from Southeastern Mindanao, Philippines. Cellulose, PE, PP, PA, and PET polymers were detected. The average number of MPs found in the meat, fat, GIT, head, and gills were  $1.2 \pm 0.6$ ,  $2.4 \pm 2.6$ ,  $0.5 \pm 1.0$ ,  $2.6 \pm 2.1$  and  $0.3 \pm 1.0$ , respectively.

## I. INTRODUCTION

Microplastics (MPs), plastic fragments with sizes  $< 5\text{mm}$ , have become a hot topic, as evidenced by the growing number of global microplastic studies that demonstrated the ubiquity of MPs in different compartments of aquatic ecosystems, such as surface water and sediments,<sup>[1]</sup> shorelines,<sup>[2]</sup> and oceans.<sup>[3]</sup> Although the perils that arise from plastic fragments are still controversially discussed,<sup>[4]</sup> commonly consumed seafood, including fish, may be at risk of the potential threats brought by microplastic pollution.

The most common challenge encountered during the extraction of microplastics in fish is the complete removal of biological materials and tissues. Due to the minute nature of these particles, they are often trapped or masked

in the samples, making their isolation and identification difficult. To address this, several digestion protocols are being developed and improved. These methods include visual inspection, density separation, sieving, and chemical digestion.<sup>[5]</sup> The works of Karami et al. (2017) stated that the digestion of fish samples in 10% KOH at 40 °C for 72 hours exhibited superior performance among the methods they optimized.<sup>[5]</sup> Süssmann et al. (2021) also determined that KOH-assisted digestion of sardines and mackerels provided a good digestion efficiency of 91.6% with a mean recovery of plastics of 78%.<sup>[6]</sup> Karami et al. (2017) have also reported that synthetic polymers, except polyethylene terephthalate (PET), were adequately resistant to KOH.<sup>[5]</sup>

Overall, it can be said that KOH digestion has an edge over the other previously mentioned digestion methods. In addition, KOH is a readily available and highly affordable chemical to purchase.<sup>[7]</sup> In the Philippines, only a few studies demonstrated the detection of microplastics and intensely focused on the occurrence in river waters, subtidal, and marine sediments.<sup>[8,9,10,11]</sup> One recent study of Similitan et al. (2023) confirmed positive ingestion of five to ten pieces of MPs among 29 out of 30 milkfish samples collected.<sup>[12]</sup> According to the Bureau of Fisheries and Aquatic Resources (BFAR), as stated in the 2022 Philippine Fisheries Profile publication, the top contributor to the total aquaculture production is predominantly milkfish production, which amounted to PhP 46.41 billion or 37.42% of the total value of aquaculture commodities.<sup>[13]</sup> Owing to the vital contribution of the milkfish industry to the country's economy and only a handful of statistical data available for MP ingestion in fish, the Philippine government recognized the need for further research into MP contamination.

In this study, alkali digestion using KOH solution followed by a dual-density separation using saturated sodium chloride (NaCl) and sodium iodide (NaI) solutions were further developed to provide a more practical and economical method of extracting MPs from different parts of milkfish. The occurrence and distribution of MPs present in the collected milkfish samples were also investigated. As a result, the findings of this study may provide valuable information on understanding MP contamination in milkfish, which can have implications for food safety, consumer health, and milkfish farming sustainability.

## II. MATERIALS AND METHODS

### A. Materials

To prepare standard MPs, six (6) polymers, widely applied in many industrial products and considered principal sources of the most common plastic wastes, were downsized to around 1000-2000  $\mu\text{m}$ . These polymers include PA (Nylon) and PET, which were originally provided by the American Society for Testing and Materials (ASTM) as proficiency testing samples; PS pellets were retrieved from Manly Plastics Inc.; PVC resins were provided by Crown Asia Chemicals Corporation; PE and PP pellets were from JG Summit Holdings, Inc. For grinding plastic polymer pellets (except PVC resins), the Heiko vibrating sample mill T1-100 and Retsch SM 200 cutting mill were used. The downsized polymers were further sieved in a 1-mm mesh. Potassium Hydroxide (KOH, Analytical Reagent Grade) was purchased from Belman Laboratories. For the salt solutions used for density separations, both Sodium Iodide (NaI, Analytical Reagent Grade) and Sodium Chloride (NaCl, Analytical Reagent Grade) were purchased from Chemline Scientific Corporation. For filtration, Cole Palmer EW-02924-20 (Advantec 353100) vacuum filtration manifold was used to filter samples simultaneously. The vacuum manifold was bought from Noveaulab Asia Corporation. Glass microfiber filter papers (with 1.2  $\mu\text{m}$  pore size and 47 mm diameter) were purchased from Analytical and Sample Prep Machines, Inc., while Whatman Grade 1 filters (Standard, 11  $\mu\text{m}$  pore size) were bought from Labotech Trading.

### B. Sampling

Random sampling was performed from a brackish water fishpond. The site, with code SEM-15 for anonymity reasons, is located Southeastern Mindanao, Philippines. The team coordinated with the Bureau of Fisheries and Aquatic Resources (BFAR) Regional Office and the Municipal Agriculture Office (MAO) of the Local Government Unit (LGU) regarding the legalities and protocols of microplastic research. The sizes and weights of the collected samples ( $n=30$ ) were measured before analysis.

### C. Optimization and extraction protocol

In this study, alkali digestion using KOH was optimized for the digestion of different parts of milkfish samples. The optimized digestion parameters were determined based on the optimal digestion temperature, duration, KOH concentration, and extent of structural and morphological changes to spiked reference plastic polymers. With slight modification, the digestion method follows the work of Karami et al. (2017) [5] and proceeded as follows: first, samples were soaked in different concentrations of KOH solutions (10-20% concentrations) and incubated in an oven at varying digestion durations (24, 48, and 72 hours) at 40 °C. The samples were then filtered, washed, and oven-dried for at least 24 hours at 80 °C.

Recognizing the potential interference of residues with the efficient isolation and extraction of MPs, a post-digestion protocol was employed for the head and gills parts. This protocol uses a dual-density separation technique using NaCl and NaI salt solutions, which are both environmentally friendly and relatively affordable.[14] The head and gills samples, which exhibited a considerable amount of recovered undigested materials, saturated NaCl was used, followed by 4.4 M NaI solution[5] to allow the separation of the high-density plastic polymers. During re-suspension, it was essential to agitate the solution to release trapped MPs in the recovered debris while ensuring no left-over materials were broken into smaller, lighter fragments. The resulting supernatant solution was carefully separated by decantation and filtration using a vacuum manifold. All filter papers used were glass microfiber filter papers (pre-dried to constant weight). The samples were then dried in an oven for at least 24 hours at 80 °C.

### D. Digestion Efficiency and % Recovery

The suitability and efficiency of the improved method were validated by calculating the digestion efficiency and % recovery of spiked reference MPs in meat, fats, GIT, head, and gills using Equations 1 and 2:

$$\text{Digestion Efficiency (\%)} = \frac{w_i - (w_a - w_b)}{w_i} \times 100 \quad (1)$$

where  $w_i$  = initial weight of sample,  $w_a$  = weight of filter after filtration, and  $w_b$  = initial weight of filter.

$$\text{Recovery (\%)} = \frac{w_a - w_b}{w_i} \times 100 \quad (2)$$

where  $w_a$  = weight of filter after filtration,  $w_b$  = initial weight of filter, and  $w_i$  = initial weight of spiked MPs.

### E. Characterization of microplastics

#### E. 1 Optical microscopy

Digital optical microscopes (Carl Zeiss Stemi 2000-C Stereo Microscope and Carl Zeiss Axio Scope A1 High-Power Compound Microscope) were used to visually

inspect samples at varying magnifications. The isolated MPs were classified either as a “fiber” (a particle with a length-to-width ratio of >3) or “fragment” (a particle with a length-to-width ratio of <3)<sup>[15]</sup> with varying colors, all noted down.

### E.2 FTIR Spectroscopy

Vibrational spectra of isolated MPs were obtained using Fourier Transform Infrared (FTIR) Spectroscopy. Isolatable microplastics (>100 μm) were analyzed using a Perkin Elmer Frontier Spectrometer via the Attenuated Total Reflectance (ATR) technique. Non-isolatable MPs, due to their small sizes (<100 μm), were analyzed using Perkin Elmer Spotlight 200 FTIR-Microscope (μ-FTIR) in reflectance mode. To ensure comprehensive analysis, all suspected MPs in this study were first analyzed in μ-FTIR before ATR analysis, and relevant data were retrieved accordingly during identification. The analysis was obtained from 4000-600 cm<sup>-1</sup> for 20 scans and 4 cm<sup>-1</sup> resolution with a data interval of 4 cm<sup>-1</sup>. For the ATR technique, force gauge pressure was at a minimum of 80 Newton (N) for best contact with the crystal. Moreover, all spectra obtained were baseline-corrected to improve the quality without distorting the band intensities. Spectrum Library Search Plus (Version 6.3.5.0176), from Perkin Elmer, Inc., was used as the reference spectral database.

### F. Blank correction

This study implemented procedural blanks to account for contamination from sample handling, reagents, dust, and other airborne particles. To improve the accuracy of data reporting and quality assurance, a procedural blank correction was done in three replicates, which followed the work of Lindeque et al. (2020).<sup>[16]</sup> The total average number of MPs (*Ave MP/sample*) was calculated by the mean of the detected microplastic in procedural blanks ( $\bar{X} MP_{blank}$ ) subtracted from mean of the detected microplastic of the samples ( $\bar{X} MP_{sample}$ ), as shown in Equation 3:

$$\begin{aligned} Ave MP_{sample} = & (\bar{X}_{p1} MP_{sample} - \bar{X}_{p1} MP_{blank}) \\ & + \dots + (\bar{X}_{pn} MP_{sample} - \bar{X}_{pn} MP_{blank}) \end{aligned} \quad (3)$$

where p= type of polymer.

### G. Statistical analysis

All statistical analyses were performed using SPSS statistical software (IBM, Version 27). Data were tested for normality and homogeneity using Levene's and Shapiro-Wilk's tests. A non-parametric ANOVA (Kruskal-Wallis test) was used to compare univariate groups in the datasets. It was used to compare the recovery rates of MPs across the different fish parts and recoveries in the different fish parts across each type of polymer. The same tests were performed to compare the digestion efficiency in different fish parts. Significant differences

between groups were determined when the probability level,  $p < 0.05$ .

## III. RESULTS AND DISCUSSION

### A. Optimization of alkali digestion

Meat samples were digested in 10-20% KOH solutions and individually spiked with 0.1 g of randomly cut, pink-colored PE MPs (n=3) with an average particle size of 334 μm ± 85 μm. It was found that digestion at 40 °C for 48 hours using 10% KOH solution (Table 1) provided practical and reasonable results. No substantial changes were observed in the surface morphology of the recovered MPs after digestion, as observed using an optical microscope with 200x and 500x magnifications. Additionally, structural changes of the polymers were monitored via FTIR-ATR spectroscopy. From the spectra, prominent peaks attributed to PE were identified with a library match of 98.4% (Perkin Elmer Spectrum Search Plus Library, 2009). Based on the morphology and FTIR results, 10% KOH digestion solution proved to be non-destructive towards PE polymers. Thus, digestion in 10% KOH at 40 °C for 48 hours was selected as the final parameter for the digestion of milkfish samples.

The digestion efficiency and % recovery was calculated from the digestion of meat, fat, GIT, head, and gills (in 6 replicates) using the optimized digestion parameter (48-hour and 40 °C digestion). The meat, fat, and GIT samples showed >95% digestion efficiencies and % recoveries, while the head and gills samples exhibited comparable digestion efficiencies of 90.0% ± 0.9% and 93.3% ± 0.7%, respectively (Tables 2 and 3). Seemingly, the % recoveries calculated from different parts of the samples exceed the estimated 80%-100% recovery. The observed >100% recovery values in all samples can be attributed to the heterogeneity of the milkfish samples. GIT, head, and gills exhibited visible undigested remains, which were not separated during density separation. Even meat and fat samples revealed traces of undigested materials.

The overestimation in % recovery values may also be attributed to possible contamination of samples from the processes it underwent.<sup>[17]</sup> Additionally, this could also indicate possible weighing inaccuracies, particle aggregation, or filter loss during analysis, which is considered a limitation of this study. Tables 4 and 5 summarize the results of the Independent Kruskal-Wallis Test and Pairwise Multiple Comparison used to determine the distribution of recovered plastics across different fish parts and vice versa. From the results, the distribution of each type of microplastic polymer across the different fish parts was statistically different. A consistent difference was observed in the recovery values of plastics in the head and gills samples. Moreover, the recovery of PET MPs across all fish parts was determined to be statistically different from the other plastic polymers. This is considerably due to its lost during alkali digestion due to

base-catalyzed hydrolysis, since PET is mostly made up for ester bonds. [5, 18] Despite the exceptional thermal stability of PETs, [19] they can also be degraded by thermal oxidation, photo-oxidation initiated by ultraviolet (UV) light, and hydrolytic cleavage. [20]

In general, the developed method of digestion and extraction of MPs in milkfish was found to be most effective for the meat, fat, and GIT samples. For the head and gills samples, a relatively good digestion efficiency was obtained, while the recovery of spiked MPs was remarkably improved with the addition of a dual-density

**Table 1. Digestion efficiencies (at 24, 48, and 72-hour) and recovery (at 72-hour) in meat at varied KOH concentrations and 40 °C digestion temperature.**

Digestion Solution	% Digestion Efficiency (72 hrs)	% Recovery at 72 hrs	% Digestion Efficiency (48 hrs)	% Digestion Efficiency (24 hrs)
10% KOH	99.3 ± 0.1	100%	98.3 ± 0.2	93.3 ± 1.6
12% KOH	99.7 ± 0.1	100%	95.8 ± 0.6	97.1 ± 0.8
15% KOH	99.8 ± 0.1	30%	-	-
18% KOH	99.9 ± 0.0	Dissolved	-	-
20% KOH	99.9 ± 0.0	Dissolved	-	-

separation method.

**Table 2. Digestion efficiency and % recovery of spiked PE, PP, PS in meat, fat, GIT, head, and gills samples at 10% KOH and 48-hour digestion at 40 °C.**

Sample	Digestion efficiency %	% Recovery of Microplastic Reference Polymers		
		PE	PP	PS
Meat	98.3±0.2	101.9±2.2	98.4±4.7	101.3±0.8
Fat	95.9±1.0	114.2±5.3	108.8±1.7	110.5±9.3
GIT	96.0±2.4	113.2±7.0	120.9±8.8	97.3±3.3 <sup>b</sup>
Head <sup>a</sup>	90.0±0.9	123.7±2.8	114.9±6.6	112.8±3.9
Gills <sup>a</sup>	93.3±0.7	119.0±14.3	117.1±11.8	106.3±9.0

<sup>a</sup> Performed dual-density separation using saturated NaCl and 4.4 M NaI  
<sup>b</sup> n=4

**Table 3. Digestion efficiency and % recovery of spiked PA, PET, PVC in meat, fat, GIT, head, and gills samples at 10% KOH and 48-hour digestion at 40 °C.**

Sample	Digestion efficiency %	% Recovery of Microplastic Reference Polymers		
		PA (Nylon)	PET	PVC
Meat	98.3±0.2	101.1±4.7	90.3±2.7	103.6±1.1
Fat	95.9±1.0	103.9±2.1	97.5±1.5	102.0±1.3
GIT	96.0±2.4	103.1±1.9	101.3±1.4	107.0±4.0
Head <sup>a</sup>	90.0±0.9	113.2±4.4	97.6±6.5	115.0±11.4
Gills <sup>a</sup>	93.3±0.7	120.0±7.1	105.5±12.7 <sup>b</sup>	109.0±11.0

<sup>a</sup> Performed dual-density separation using saturated NaCl and 4.4 M NaI  
<sup>b</sup> n=4

**Table 4 Distribution of the % recoveries in different Fish Parts across Plastics using Kruskal-Wallis Test.**

Samples	Distribution of % recovery in different Fish Parts across Plastics
Meat	Kruskal-Wallis Test ( $p$ value = 0.001 < 0.05)
Fats	Kruskal-Wallis Test ( $p$ value = 0.000 < 0.05)
GIT	Kruskal-Wallis Test ( $p$ value = 0.000 < 0.05)
Head	Kruskal-Wallis Test ( $p$ value = 0.001 < 0.05)
Gills	Kruskal-Wallis Test ( $p$ value = 0.363 > 0.05)

**Table 5 Distribution of the % recoveries of Plastics across different Fish Parts using Kruskal-Wallis Test.**

Samples	Distribution of % recovery of Plastics across different Fish Parts
PE	Kruskal-Wallis Test ( $p$ value = 0.003 < 0.05)
PP	Kruskal-Wallis Test ( $p$ value = 0.002 < 0.05)
PS	Kruskal-Wallis Test ( $p$ value = 0.003 < 0.05)
PA	Kruskal-Wallis Test ( $p$ value = 0.000 < 0.05)
PET	Kruskal-Wallis Test ( $p$ value = 0.000 < 0.05)
PVC	Kruskal-Wallis Test ( $p$ value = 0.003 < 0.05)

### B. Occurrence of microplastics in collected milkfish samples

A total of 30 (meat, fats, GIT) and 15 (head and gills) fish samples were analyzed for the presence of MPs. The average fish sample size was 42.72 g ± 5.56 g, while the average maximum standard length was 13.57 cm ± 0.72 cm. The total average number of MPs ingested per fish in meat, fat, GIT, head, and gills samples was calculated to be 1.2 ± 0.6, 2.4 ± 2.6, 0.5 ± 1.0, 2.6 ± 2.1 and 0.3 ± 1.0, respectively. For the procedural blanks, the average MPs

obtained were  $2.3 \pm 2.1$  (meat),  $1.7 \pm 1.2$  (GIT),  $1.3 \pm 0.5$  (fats),  $0.7 \pm 0.9$  (head) and  $1.0 \pm 0.8$  (gills). Cellulose fibers and four (4) synthetic polymers were identified: PP, PE, PET, and PA (nylon). Possible sources of these polymers may include degradation products from plastic pellets, food packaging, beverage containers, to name a few.<sup>[21,22]</sup> Factors such as feeds, the environmental conditions where the fish species are cultured, and alike, may also contribute to the number of MPs that can be found.<sup>[23, 24]</sup> Fish meal/feeds which served as the food source for the collected milkfish samples might also have been contaminated with MPs beforehand, as suggested by several studies.<sup>[25, 26]</sup>

In this study, cellulose is reported as an additional polymer and could not be disregarded entirely since artificial cellulose microfibers (e.g., rayon) are also emerging pollutants,<sup>[27]</sup> which are produced primarily from domestic washings and the textile industry.<sup>[28]</sup> The distribution of the colors of the identified polymers was black (55%), blue (30%), red (11%), and brown (4%). For the polymer type, cellulose comprised 80% of the total isolated particles, followed by PET, which was 16%, while PP, PE and PA made up the minority of the synthetic polymers. Furthermore, the types of MPs found were mostly microfibers (67%) and fragments (33%) as shown in Fig. 2. One implication of microplastic penetration is the damage in the developmental stages of aquatic species as observed in the study of Zhao et al. (2021).<sup>[23]</sup> They further demonstrated that microplastic fibers prevented the food intake of zebrafish adults and caused intestinal damages.<sup>[23]</sup> In addition, plastic additives including phthalates and polyaromatic hydrocarbons (PAH) may induce oxidative stress in fish species that can cause damage to cells and tissues.<sup>[29]</sup>

Table 6 shows the pairwise comparison of the identified microplastics in the different milkfish parts. A consistent significant difference has been observed mostly in the head samples. This is probably due to the greatest concentration of average MPs detected in head part. Evidence of MPs was also observed in the GIT part. This is reasonable since GIT is the most direct pathway of microplastic ingestion and retention in fish.<sup>[28]</sup> It was also observed that most of the identified MPs in the meat and fats samples were similar with the detected MPs in the blank corrections. The number of MPs in these parts were probably due to contaminations from environmental contributions (e.g., airborne particles) during sample preparation and characterization analyses, despite implemented efforts to control cross-contamination.<sup>[30,31]</sup>

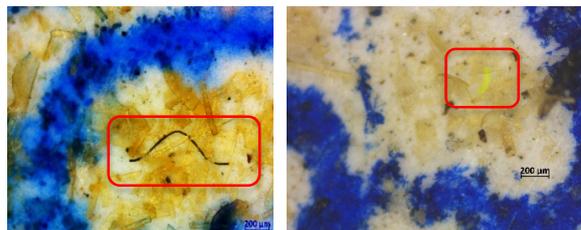


Fig. 2. (L-R) Fiber and fragment types of MPs observed in 50x magnification.

Table 6. Pairwise comparison of identified microplastics in the different milkfish parts

Parts	Values of p				
	Meat <sup>a</sup>	GIT <sup>a</sup>	Fats <sup>a</sup>	Head <sup>a</sup>	Gills <sup>a</sup>
Meat	-	0.034	0.106	0.942	0.004
GIT	0.034	-	0.612	0.071	0.256
Fats	0.106	0.612	-	0.121	0.164
Head	0.942	0.071	0.164	-	0.011
Gills	0.004	0.256	0.106	0.011	-

<sup>a</sup> = 0.050; Significant differences between groups were determined when the probability level,  $p < 0.05$

### C. Characterization of Isolated Microplastics

Fig. 3 presents the FTIR spectra of isolated and identified microplastic polymers (PET, PE, PP, PA, and cellulose) in the milkfish samples. The suspected PET exhibited peaks at  $2912 \text{ cm}^{-1}$  and  $2848 \text{ cm}^{-1}$ , which were attributed to the C-H stretching vibrations. The MP was classified as PET due to a strong C=O stretch and C-O bending vibration at  $1709 \text{ cm}^{-1}$  and  $1235 \text{ cm}^{-1}$ , respectively.<sup>[32]</sup> PE was also confirmed by the presence of C-H ( $2919 \text{ cm}^{-1}$  and  $2847 \text{ cm}^{-1}$ ) stretching vibrations,  $\text{CH}_2$  ( $1470 \text{ cm}^{-1}$ ) and CH ( $715 \text{ cm}^{-1}$ ) bending vibrations.<sup>[33]</sup> Some isolated microplastics were classified as PP, evident in the peaks at  $2914 \text{ cm}^{-1}$  and  $2847 \text{ cm}^{-1}$  attributed to the  $\text{CH}_3$  and  $\text{CH}_2$  stretching vibrations, respectively.<sup>[32]</sup> Additionally, some of the microplastics were identified as PA. The PA spectra in Fig. 3 showed the amide functional group's N-H stretching vibrations at  $3299 \text{ cm}^{-1}$ . Additionally, PA is confirmed due to the presence of C=O stretching of amide I and N-H peaks at  $1537 \text{ cm}^{-1}$  and  $1455 \text{ cm}^{-1}$  attributed to the bending vibrations of amide II.<sup>[34]</sup> Lastly, most of the isolated microparticles are classified as cellulose. As shown in Fig. 2, the cellulose spectra showed a broad O-H peak at  $3310 \text{ cm}^{-1}$  and an O-H bending vibration at  $1160 \text{ cm}^{-1}$ . The cellulose-based polymer was further confirmed through the presence of C-O-C bending vibrations, particularly at  $1025 \text{ cm}^{-1}$ .<sup>[35]</sup>

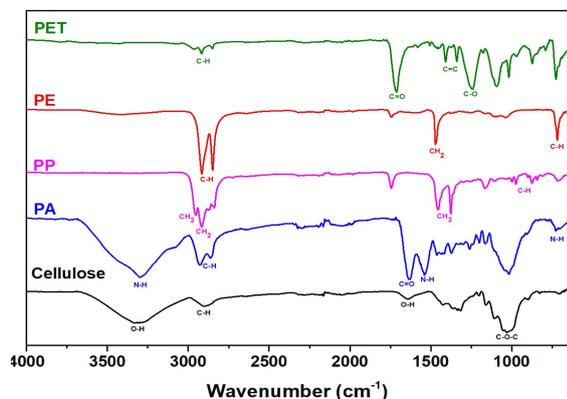


Fig. 3. Representative FTIR spectra of identified microplastics from milkfish samples

#### IV. CONCLUSION

Optimized digestion of fish meat, fat, GIT, head, and gills was successfully carried out in 10% KOH solution at 40 °C for 48 hours. The recoveries of plastic polymers in the head and gills were also significantly improved by a dual-density separation using saturated NaCl and 4.4 M NaI solutions. Optical microscopy and FTIR analyses were instrumental in identifying microparticles ingested by fish samples, including cellulose and four synthetic polymers, PE, PP, PA, and PET. Overall, the digestion and post-digestion protocols employed in this study provided an effective, practical, and economical approach to the digestion and extraction of MPs in different parts of milkfish. Further studies concerning the rapid increase of MPs found in the biota of fish and fishery resources must be conducted. As of to date, no regulatory concentrations of MPs are released in the Philippines. Although this paper briefly touched on the environmental implications of MPs, it is also recommended that future studies place greater emphasis on the potential food safety and bioaccumulation risks posed by MP contamination in the milkfish industry.

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