PUP Journal of Science and Technology (PUPJST)
Volume 17 (2024), (pp. 25-44)

Original Article

Assessment of Microplastics in Fish GI Tracts and Shallow Water Sediments in Pugad Baboy Mangrove Area, Kawit, Cavite Using Fourier Transform Infrared-Attenuated Total Reflectance Spectroscopy

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Received: 03 May 2023; Accepted 23 Jul 2023; Available online: 08 October 2024

Abstract: Plastic pollution has been an ongoing environmental problem overwhelming people worldwide. Microplastics (MPs) are defined by their small sizes (<5mm) and are continuously pressing concerns due to the threats they bring to the ecosystem. The scarcity in microplastics study limits the resources for future researchers for methods and applications to explore. This study assessed, characterized, and quantified microplastics contamination in fish gastrointestinal (GI) tracts and sediments in the Pugad Baboy Mangrove areas of Kawit, Cavite. Sediments and fish GI tract samples were treated using a modified methodology. The collected microplastic particles under investigation were separated through density separation using $ZnCl_2$ followed by base digestion using KOH. MP particles were examined using an optical microscope and characterized through Fourier Transform Infrared– Attenuated Total Reflectance (FTIR-ATR) spectroscopy. Results showed that four polymer identities were detected in the particles – polyethylene, polypropylene, polystyrene, and polyethylene terephthalate. A total of 67 fragments were isolated from which approximately 26.9% exhibited irregular shapes, 35.8% had a rounded and pellet-like appearance, and 37.3% were filaments. A high recovery rate of at least 90% upon analysis was recorded and the implementation of the proposed modified methodology is strongly recommended based on recovery and results obtained. These findings provided valuable insights into the physical characteristics and distribution of microplastics in the studied environment.

Keywords: *Microplastics, plastics, Pugad Baboy Mangrove, FTIR-ATR, Raman spectroscopy*

1. INTRODUCTION

Since the development of synthetic plastic polymers in the early 1900s, it has been regarded as an everyday necessity since most of the materials that people use in their daily lives are made of plastics and is attributed to their well diverse and

versatile range of applications (Neal & Andrady, 2009). Despite the growing dependency, the same properties that made plastics a breakthrough of a discovery are also those that pose problems in the long run – their durability and versatility make them non-degradable for a long period of time, with some types requiring at least a decade before they start to show signs of decomposition. Different types of synthetic polymer plastics exist at the present time – *polyethylene terephthalate* (PET), *polyethylene* (PE), *polypropylene* (PP), *polyvinyl chloride* (PVC), and *polystyrene* (PS) to name a few, and are characterized by the differences in their structures and their accompanying applications (Kundu et al., 2021). What comes after using these plastics has then become a top concern since they are now considered threats to the health of the people and the animals, as well as their uncontrolled accumulation in the environment.

Microplastics (MPs) are synthetic plastics that appear smaller and are much harder to control due to their size and difficulty to collect. Studies of the current state of microplastics pollution reveal that MPs can easily release harmful and toxic chemicals from the disintegration of initial additives incorporated, tracing back its origin to its production (Issac & Kandasubramanian, 2021). This propelled the interest of researchers worldwide to embark on studies focusing on the assessment of microplastics in certain environments, covering general to specific samples.

Over the years, researchers have investigated worldwide the estimated quantity of plastics that go from land to different parts of the environment with an emphasis on bodies of water such as the ocean (Jambeck et al., 2015). With China ranking first, the Philippines ranks third among the top most contributors of plastic wastes that are scattered to different parts of the environment, particularly in the ocean. The country produces an average of 0.5 kg of plastic wastes per day per person, and these wastes usually find themselves in water bodies when disposed inappropriately (Jambeck et al., 2015). The continuous increase in plastic production coupled with a very slow rate in degradation and improper waste disposal could result in the overwhelming accumulation of plastics even in marine environments.

In 2022 alone, the Philippines has generated at least 2.7 million metric tons of plastic wastes, which can be found on landfills, aquatic bodies, ingested by animals from both wet and dry locations, and even in the atmosphere. Moreover, plastics can disintegrate and transform into smaller particles such as microplastics and nanoplastics, which have sizes too small to be seen by the naked eye. Microplastics are classified as primary MPs and secondary MPs, depending on the nature of existence. *Primary MPs* are those that are synthetically created to be small, and thus exists in the environment as microstructures, for applications such as cosmetic and personal care products (microbeads), synthetic textiles (plastic microfibers), industrial applications (plastic pellets) and many more. *Secondary MPs* are bits and fragments of macroplastics that have degraded overtime due to chemical, physical, and mechanical reasons (Ivleva, 2021). Degradation of larger plastics through mechanical actions leads to the build-up of plastic-fragment contaminants, referred to as microplastics (Alimba & Faggio, 2019).

Efficient methods for analysis – identification, characterization, and quantification – are crucial to determine extensively the plastic particles. The *Fourier-Transform Infrared (FTIR) spectroscopy* and *Raman spectroscopy* are regarded as the two most widely used techniques for analysis due to their efficiency and capability for chemical imaging and for presenting simultaneous information of its measured physical and chemical properties. FTIR is the most popular techniques used in the characterization and quantification of plastic particles (Chen et al., 2020) and the most

applicable one in the study of microplastics (Renner et al., 2018) due to the vibrational spectra that enable accurate identification of polymer type and determination of nonplastic particles (Primpke et al., 2020). Meanwhile, the Raman spectroscopy, which is a method based on Raman light scattering on a molecule, is more frequently used nowadays to analyze nanoplastics (Sobhani et al., 2020).

Given that microplastics are currently defined as having micro-sizes of less than 5 mm, of different reported types and visual identification, and remain undissolved and undecomposed in such conditions (RIVM, 2015), researchers are incessantly trying to examine its prevalence in the environment in hopes of attempting to control the long-time damage brought by MPs. The researchers of this study were motivated by the scarcity of microplastics study in the Philippines as well as the diverse but unsystematic methods of quantification and analysis of microplastics used in local areas. Mangroves were chosen as the major location for sampling due to the rich biodiversity of its flora, which provided the researchers ample space in choosing the sampling points. In particular, the *Pugad Baboy Mangrove Area* in Kawit, Cavite was selected because it is densely populated by residents along the perimeter, which is one of the main causes of plastics and/or microplastics accumulation. The province of Cavite is surrounded by a water area of 93,679.3750 ha. Water sources in the province are abundant due to numerous natural springs, rivers, and waterfalls. The Department of Environment and Natural Resources (DENR) states that five (5) municipalities have rich mangrove areas in the province — stretching from Cavite City, Kawit, Noveleta, Rosario, and Bacoor with a total cover of 150.39 ha. Some of the mangrove areas are sources of fishes, prawns, crabs, and shellfish that provide livelihood to the coastal residents. Due to rapid industrialization and having one of the highest annual population growth rates, Cavite province was intensively affected by pollution resulting from improper plastic consumption and disposal.

This study assessed and analyzed the presence of microplastics, which include microbeads and those resulting from breakdown of larger plastics due to weathering, in-shallow water sediments, and GI tracts of selected fish species in Pugad Baboy Mangrove Forest in Kawit, Cavite in order to address the growing concern and extent of microplastics prevalence. Following a modified experimental methodology, the identity and quantity of microplastics in sediment and fish samples were evaluated by doing a recovery analysis using blank and spiked solutions with and without microplastics.

2. METHODOLOGY

This study used a modified methodology that was adapted from related literatures and studies and it consists of three (3) distinct aspects, namely - (a) area scouting, sample collection, and sample preparation, (b) identification and characterization of microplastic particles, and (c) recovery analysis for method validation. The detection and characterization of the microplastics in the mangrove area were done following the experimental and modified methodology from different studies (Hoffman & Turner, 2015; Rodrigues et al., 2020; Pfeiffer & Fischer, 2020; Garcia, 2020). The methodology included the static sampling of soil sediments and fish from the mangrove area, the pretreatment of the samples, extraction, and isolation of the microplastics, and ultimately, the qualitative and quantitative analysis of the microplastic samples using a light microscope and FTIR spectroscopy.

2.1 Sampling sites and ecological profiling

Sediment samples were collected in three selected sites along the coastal waters of the Pugad Baboy Mangrove Forest located in Kawit, Cavite. The selection of three sampling sites served as bases to compare the degree of general contamination in the relative areas. *Site 1* was located *near the residential area* (NR), *Site 2* was 153.75 m far from Site 1 and designated as *far from residential area* (FR), and *Site 3* was in the undisturbed area or mangrove area (MA), 216.95 m away from the second site. These three sampling sites within the mangrove forest were marked in Figure 1.

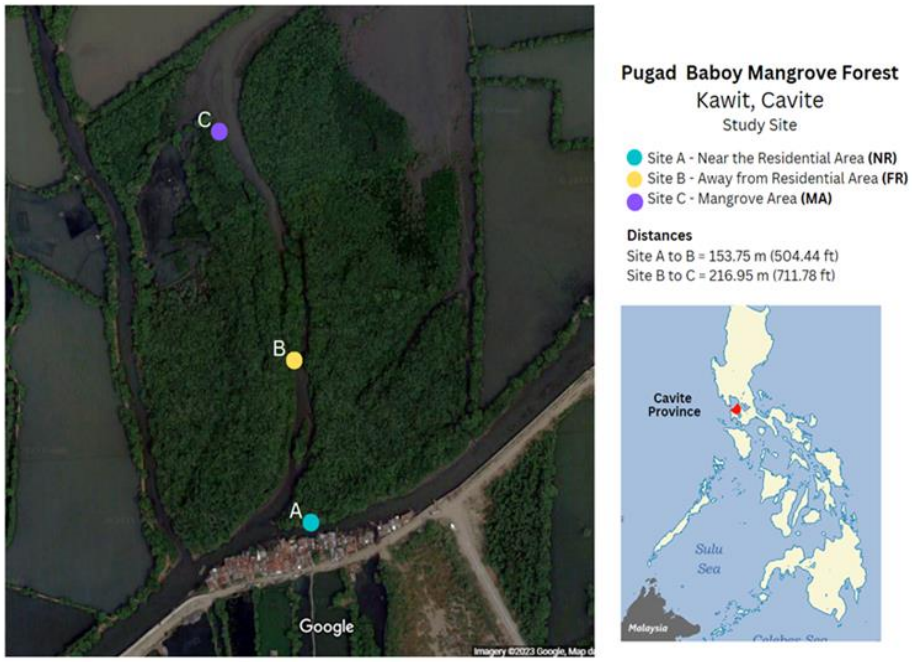

Figure 1. Location of the three sampling stations for microplastic assessment of mangrove sediments and fish along Pugad Baboy mangrove forest.

Different kinds of garbage float and pollute most of the areas of the mangrove. As shown in Figure 2, the profusion of accumulated garbage from one sampling point to another is evident. Because the mangrove area is the farthest, it is expected to have less accumulation of garbage compared to the two. However, the water current has brought the garbage from end to end, therefore making the mangrove area polluted as well.

The second sampling point was far from the residential area (Figure 2b). Although the accumulation of garbage there is not as obvious compared to the near-residential area, a variety of not only plastic wastes but undegraded materials such as big appliances, clothing that have been washed away by the current, and other wood wastes can be found surrounding the place. Plastic wastes dominate the rest due to its striking colors, as well as having distinct designs such as shampoo packaging, bottles from beverages sold in the market, baby diapers, and many more.

The third sampling site is near the residential area. As expected, the amount of garbage accumulation in this sampling site is greater compared to the previous two sites. Some of the residents can be seen treating the mangrove lake as their personal disposal site for their trash which then accumulates on the edge of the mangrove lake, while some are carried away by the slight current and the tide which also explains how the two sites above are infested with microplastics. For the purpose of the study, the three area sites have shown a great possibility of acquiring microplastics based on the observed quality of the estuary although, no water testing was done before the sampling process.

Figure 2. Photos captured in Pugad Baboy Mangrove Forest in Kawit, Cavite. (a) Mangrove Area (MA), (b) Far from residential area (FR), and (c) Near Residential Area (NR).

2.2 General schematic diagram

The overall procedure was adapted from the proposed methods of different studies (Argamino & Janairo, 2016; Dehaut et al., 2016, Espiritu et al., 2019, Navarro et al., 2022). Some reagent modifications were applied based on the studies (Karami et al., 2016; Hidalgo-Ruz et al., 2012) related to optimizing analytical methods for microplastic extraction and analysis. Sediment and fish samples underwent a series of steps of preparation and pretreatment prior to analysis. The main reagent for chemical digestion for both sediment and fish was supplemented by KOH, based on the digestion solution assessment of some studies (Pfeiffer & Fischer, 2020; Karami et al., 2016). Slight modifications from related studies were adapted in creating the experimental design to provide optimum results in assessing MPs from the samples.

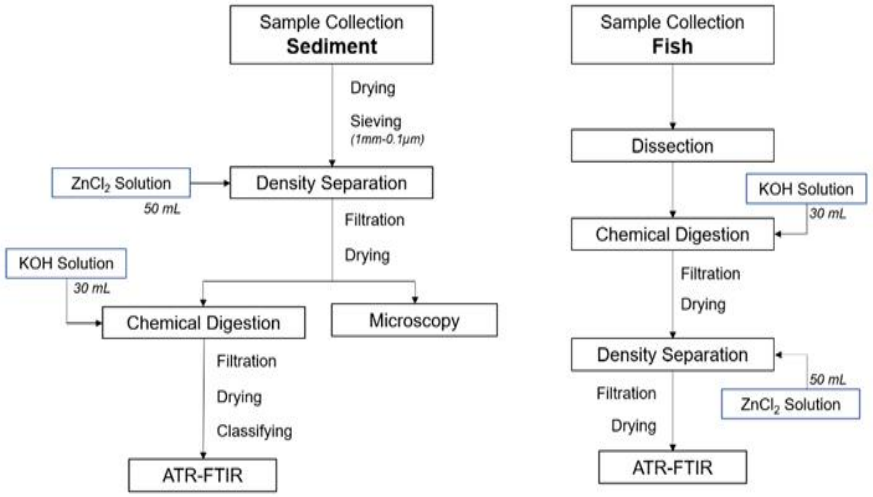

Figure 3. General schematic diagram for microplastic analysis in (a) sediments and (b) fish species obtained from Pugad Baboy Mangrove Forest.

2.3 Sample collection

Mangrove sediment samples were collected through random sampling in each sampling site (near residential, far from residential, and in mangrove area) using a 5-quart stainless-steel colander. The samples were stored separately in a labeled 1L glass jar and the jars were sealed with aluminum foil before closing to prevent the sample from contamination and exposure to light. The jars were kept in a cooler (0-4°C) and were transported for laboratory processing and analysis.

The dominating fish species (spotted scat fish “kitang”) caught in Pugad Baboy Mangrove is the primary fish sample. The sample was part of the catch by local fishermen on the sampling day. For uniformity, the size of the fish sample ranged from 6.5 – 7.5 in. Fish samples from the catch were wrapped with cheesecloth (katsa) and placed in zipper storage bags. Since the samples were transported for further processing and analysis, the samples were kept in a cooler (0-4°C). Lastly, gastrointestinal tracts were manually removed through dissection and scoured over for suspected microplastic particles.

2.4 Preparation of sediment samples

One-hundred (100) grams of each sediment sample were pre-weighed using a weighing scale in a liter of glass jar and the pre-weighed samples were dried at room temperature overnight. The samples were homogenized by grounding the sediments into a fine powder utilizing a mortar and pestle with a small addition of deionized water until the texture of the mixture was fine. The particles were sieved first to separate large microplastics from smaller ones. The mesh sizes used were 1 mm and 100 µm. The sieved particles were dried again at room temperature until a constant weight was achieved (Espiritu et al., 2019).

Fifty (50) mL of $ZnCl_2$ solution was added to each of the sediment samples for the density separation of MPs. The containers containing the samples were rinsed to transfer all remaining sediment samples with the $ZnCl_2$ (density: 1.4 g/cm^3) solution in their corresponding glass jars. The solution was transferred to each of the jars and left for 4 h at room temperature to let the particles float. The jars were covered with aluminum foil to prevent exposure to light. Floating MPs were collected using a glass dropper into another glass container. To transfer all the recovered MPs, the glass jars were rinsed with distilled water into the container. A repetition of density separation, n = 3 (Rodrigues et al., 2020), were applied to further maximize the recovery of the sample (Browne et al., 2011; Claessens et al., 2011; Martin et al., 2020).

To remove the organic material in the sample and further isolate microplastics, the sediment samples were soaked with 10 mL of 10% KOH solution for 10 h and were dried at room temperature overnight. The solution from chemical digestion was filtered through usual filtration with Whatman filter paper. The containers were washed with a small amount of distilled water while filtrating to retain the presence of microplastics in the container walls (Hidalgo-ruz et al., 2012). The collected microplastics from filter papers were dried at 43.6°C for 30 min.

2.5 Preparation of fish samples

Fish samples first underwent the manual removal of bones for a more efficient chemical digestion procedure, following the standard protocols for animal necropsies including wearing proper protections from infection or injury (Noguera, 2015). In this process, digestive tracts (including the stomach and intestine) of the three fish samples were removed and secured to avoid loss of contents that were stored in separate glass containers. Samples were processed after thawing from being frozen. Methods in extracting MP debris from the gut of fish were adapted (Lusher & Millian, 2018). The intestine section was opened using sterilized scissors and forceps.

The obtained GI tracts from the fish samples were rinsed in a nested sieve, where the potential plastic particles undergo filtration. The samples were placed in a glass container filling up 10% KOH solution in filtered water and the containers were covered with aluminum foil to prevent contamination. Accessing digestion solutions to extract MPs in biological matrices showed that 10% KOH had an optimum recovery rate and least destructive treatment. The digestion process was concluded once the matrix appears clear or yellowish as the organic tissues are completely digested. The potential microplastic particles were filtered through a manual filtration. Following the same method, the filtered materials were subjected to density floatation using $ZnCl_2$ solution (density 1.4 g/cm3) to allow the separation of undissolved organic material (Rodrigues et al., 2020). The MP samples in the filter paper were placed oven dried at 40°C for 1 hr. The samples were manually separated and classified using clean needles according to their sizes prior to further characterization.

2.6 Identification and characterization of microplastics

The procedures used were adapted from the different published literatures, which formed as the basis of this study. In identifying the microplastics from the samples, sorting out the particles were done by visual observation – extracted microplastics particles of less than 5mm were placed in glass containers with labels from each sample source. The suspected microplastic particles that have been extracted upon doing various pretreatment methods were analyzed using two

characterization methods, microscopy and Fourier-Transform Infrared (FTIR) spectroscopy. Some studies have less variation in the instrument settings due to reasons such as (1) the instrument does not belong to the institution of the researchers hence minimal modifications can be done, and (2) the required modification is not available at the moment of use. These reasons leave the researchers with no choice but to work with the available FTIR instrument and its accompanying parameters. FTIR spectroscopy gives identification and characterization to the MP particles by the spectral information of their corresponding polymer structures.

The fragments were analyzed using Agilent Cary 630 FTIR-ATR and the sampling interface crystal attached in the instrument is a type II diamond crystal. To maximize the scanning-to-spectrum capability and improve the signal-to-noise ratio of the resulting spectrum, at least 45 scans per run per microplastic fragment was implemented and scanned over the comprehensive spectral range of 4000 cm to 650 cm. The lines in blue are from the spectral database comparing the similarity in peaks from the samples (in red). Special attention was given to the fingerprint region, where the distinct identification of various microplastics, such as polypropylene (PP) and polyethylene (PE), can be achieved. The resolution is set to 4 cm for easier determination of the level of detail or fine structure that can be observed in the spectrum. The spectrum was analyzed using Wiley's *KnowItAll Analytical.* In determining the particle recovery rates of microplastics, five types of microplastics – polypropylene (PP), poly(ethylene terephthalate) (PET), polyethylene (PE), polystyrene (PS) and poly(vinyl chloride) (PVC) were evaluated. The required microplastics were manually made using a drill or the tool used for driving fasteners or round holes. Drilling holes in the plastic surface create small plastic fragments around the edge of the hole created by the drill. This would serve as the known MPs that underwent recovery analysis. The MPs were then added with filtered water to be analyzed. This process significantly evaluated the effect of isolating microplastics in terms of particle recovery and assessed the potential procedural contamination. The said materials were acquired from the sources shown in Table 1.

Different MPs have different compositions that should be considered in selecting treatment methods. There are polymers that are known to be more sensitive to acidic solutions or depending on certain temperatures (Lusher et al., 2017). Most of the preparation and examination has similarities among the MP samples. In this study, to assess the recovery rate of each treatment, the following methods were done. On a petri dish, a small amount of water containing 5 grams of microplastic polymer – PET, PS, PP, LDPE, and HDPE were spiked in 500 mL of distilled water and were checked under a microscope with 20x magnification to ensure the presence of the MPs. Further treatments were done in the spiked sample: sieving, multiple drying, density separation, and chemical digestion. Calculation for the average recovery rates and related standard errors in every MP type showed the number recovered microplastics.

3. RESULTS AND DISCUSSION

The presence of microplastics was detected and identified in all sediment samples collected from three sampling sites - near (NR) and far (FR) from residential areas, and in the mangrove areas (MA) of the mangrove forest of Kawit, Cavite. The

first part of assessing microplastics from sediment samples was through visual observation using a compound microscope. The collected materials were categorized according to color, shape, type, and quantity.

Table 1. List of the representative materials used in spiked solutions for microplastics recovery analysis and their corresponding polymer type.

Polymer	Source	Type
Polyethylene Terephthalate (PET)	Yi Lu Jia Plastics	Flakes
Polyethylene (PE) - LDPE and HDPE	Metroseal Plastic Industries	Pellets
Polypropylene (PP)	Tupperware	Drill : Powder-like
Polystyrene (PS)	Tupperware	Drill : Powder-like
Polyvinyl chloride (PVC)	Metroseal Plastic Industries	Powder

Table 2. Total number of MPs from sediments (MA, FR, and NR) and fish samples collected.

	Irregular shapes	Pellet	Filament	TOTAL
MA	6	7	9	22
FR	3	8	7	18
NR	8	8	9	25
Fish	1	1	-	2
TOTAL	18	24	25	67

3.2 Assessment of microplastics in sediments according to color, shape, type, and quantity

Part of assessing the acquired microplastics is by quantifying the total isolated particles and characterizing them according to their physical forms. The results of the analyses of sediments and fish samples for the presence of MPs are shown in Table 2.

A total of 67 suspected MPs were isolated and characterized into three classifications – irregular shapes, pellet, and filament particles. From this total, 65 of which were collected from sediment samples, while two particles were from fish samples. Among the three area sites, the highest number of MPs was found in NR samples, followed by MA and FR having the least number of MPs isolated.

The observed microplastics appeared in different colors – most of which are blue, green, white, and red. The type of MPs found in sediment also varied in shape – from fragments and filaments that have irregular, elongated, degraded, and crumpled edges, to circular MPs that have rigged ends when seen under the microscope.

3.3 Microscopic level of analysis

Continuation of the visual investigations of the isolated fragments is through microscopic observation. Figure 4 shows the images as having significant characteristics that could explain the possible degradation processes such as photolytic, mechanical, and biological degradation in the marine environment (Andrady, 2011).

It is evident that in terms of the MPs sizes, bigger particles may be an indicator of shorter residence time compared with smaller particles that are presumed to have undergone longer degradation time. Since mechanical integrity highly depends on the average molecular weight of the polymer, any significant extent of degradation inevitably integrated the material into becoming brittle enough to fall apart into powdery fragments. (Espiritu et al., 2019; Andrady, 2011).

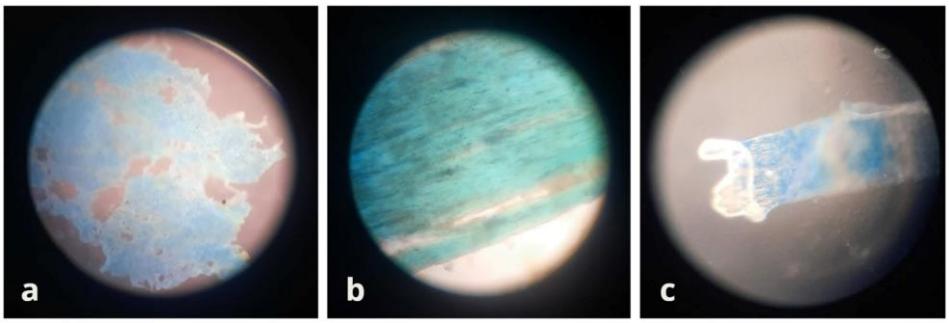

Figure 4: Microscopic images of the particles: (a) jagged fragment, (b) splitting and tearing of edges, and (c) discoloration of surface particles.

The distinction between oxidative weathering and mechanical processes could identify surface texture of the fragments (Zbyszewski et al., 2014). Oxidative weathering features made the particles revealed to have discoloration and roughness in edges, which can be observed in Figure 4c, that possibly have undergone photo-oxidation. Severe polymer degradation observed often occurs from photo-oxidation that is exposed to UV-B Radiation. The thermo oxidative in this process often needs further exposure but progresses as long as oxygen is present in the system. However, particles with discoloration and tearing around edges reported (Espiritu et al., 2019) also suggest a faster degradation rate. Meanwhile mechanical weathering processes can lead to surface features such as grooves and fractures, thereby splitting plastic fragments apart, as can be seen in Figure 4a and 4b. Mechanical degradation process is when plastic particles are dragged or scratched during the transport or as it flows during the current. A stress corrosion cracking occurs during this process resulting in the formation of fractures (Lampman, 2003). Degradation of MPs could take an effect in the FTIR Analysis specifically for PP and PE when a presence of hydroxyl group may interfere on FTIR spectra, an indication of oxidation. Peaks that appear around 1700 cm^{-} are attributed to carbonyl groups, such as carboxylic acids,

aldehydes, esters, and ketones can be resulted from photo-oxidation. Additionally, a hydroxyl group, which is responsible for the broad peak in the 3300 cm^- region, is also an indication of oxidation. This is mostly observed for PE and PP types of polymer that are less resistant to chemical weathering (Chen et al., 2021 & Zbyszewski, 2011).

3.4 Efficiency of methodology design: Using $ZnCl_2$ and KOH as reagents for density separation and chemical digestion

One of the aims of this study is to enhance existing methodologies from different studies in microplastics analysis and corroborate suitable steps as well as appropriate reagents for better results. The modified approach demonstrated promising outcomes, with positive results observed throughout the experimental process. Additionally, the recovery analysis conducted further substantiated the effectiveness and reliability of the implemented modifications. The incorporation of the chosen reagents for density separation and chemical digestion provided a better approach of sample preparation and a detailed overview of the obtained results, highlighting the successful application of the modified methodology and its implications for microplastics analysis is explained in this section as well.

The study compiled and evaluated various published literatures to create a simpler way of separating microplastic particles through density separation and chemical digestion. While both methods have been proven effective, the key to obtaining accurate and precise results with minimal interference lies in selecting the most appropriate solutions. To achieve this, we have chosen $ZnCl_2$ as a high-density saline solution for separating microplastics from interferences, particularly in sediment samples. For instance, $ZnCl_2$ was extracted 15 times more sediment particles than other solutions due to their higher density (Mattsson et al., 2022). This is in contrast to low-density solutions like NaCl which are less effective for separating heavier polymers like PVC and PET. We observe a correlation between the density of the solution and plastic polymers. A solution with a density below the density range of plastic polymer will not be efficiently recovered. It has been found that $ZnCl_2$ can be reused at least 3 to 5 times while maintaining its efficiency above 95% to make it a more cost-efficient solution than Sodium iodide (Rodrigues et al., 2020). The findings were also confirmed in one of the studies (Imhof et al., 2012), as higher recovery rates were achieved in the study. In addition to achieving a high recovery rate through density separation in this study, various plastic polymers were successfully retrieved at minimal cost. This was made possible by the solution's density, which covers that of most common plastic polymers, enabling their recovery.

A study conducted by Pfeiffer & Fischer (2020) compared the effectiveness of digesting solutions from marine organisms. Among their findings, KOH showed promising results in digesting biotic tissue. In the same study, they computed the weight difference of the digesting solution shown in Table 3. Nitric acid has a higher difference in weight, indicating a portion of MP particles were chemically degraded aside from interferences. In a separate study (Gulizia et al., 2022). It was discovered that HNO_3 was the most destructive to PS polymer, potentially deforming microplastics. Thus, alkaline digestion, including KOH, did not alter MPs' physical or chemical properties, proving it is a non-destructive solution. This makes KOH a suitable choice for plastic ingestion by marine organisms, as polymers remain unaffected (Kühn et al., 2017). Chemical digestion has the second highest recovery

rate in this study and swelling or degradation of MP particles was not observed as the temperature while digesting did not exceed 60°C.

3.4 ATR-FTIR analysis and results

Microplastic particles were present in the three sampling sites, specifically from the sediment samples. However, only two fish samples contained microplastics particles. Among the tested samples, polypropylene (PP), polystyrene (PS), polyethylene (PE), and polyethylene terephthalate (PET) were identified through instrumentation. Among the polymer types found in the study, PE appeared to have been the most abundant polymer type, identified in more than two samples. One of the suspected samples turned out to be a non-polymeric material, returning a hit quality index (HQI) value of approximately 78% for menthol glucuronide. Further discussion on the identity of this fragment is outside the scope of this study.

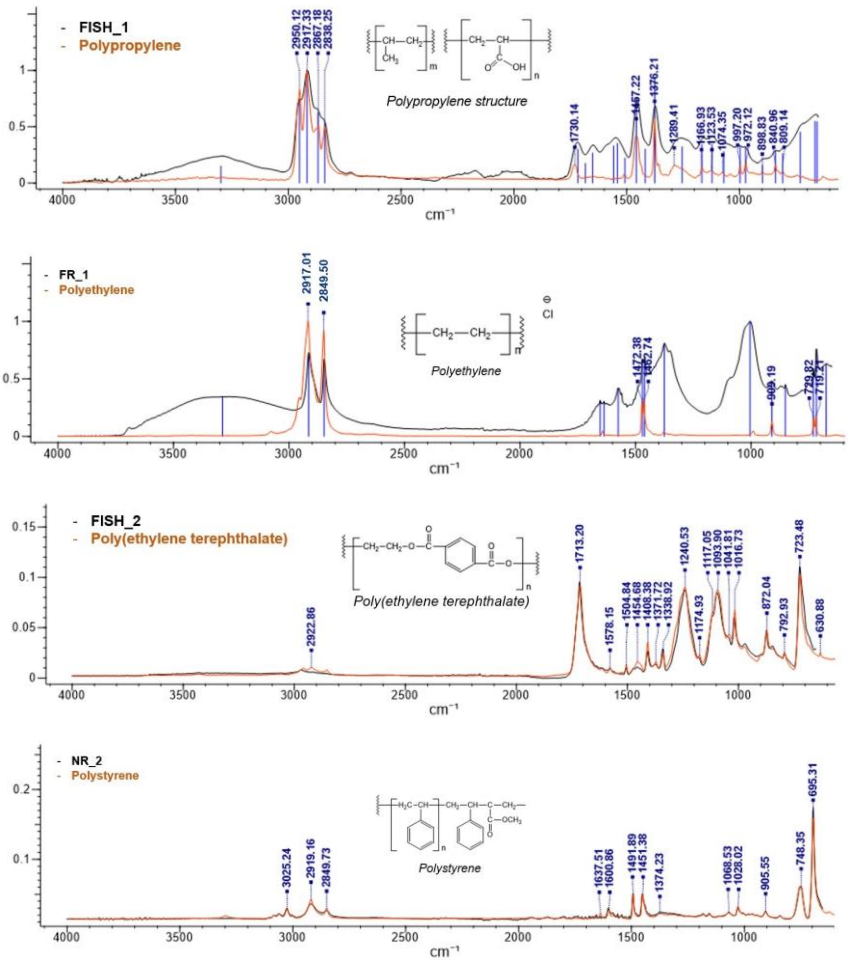

Figure 5. Spectra from the different samples resulting in the four (4) types of polymer identities found.

The ATR-FTIR analysis of the microplastics collected from the gastrointestinal tracts of two fishes showed that PP and PET were found. The spectra for the first fish sample show that with hit quality index (HQI) values of approximately 79% from database mining and 73% from peaks analysis using the ID expert function; it is confirmed for the identity polypropylene, PP. The peak of 2950 cm^{-} corresponded to —CH_3 asymmetric stretching vibrations and significant absorption bands of 2917, 2838, and 1457 cm^{-} assigned to —CH_2 asymmetrical stretching, stretching, and symmetrical bending of the polymer, respectively. A strong absorption peak was observed at 1376 cm^{-} assigned to —CH_3 bending and absorbance peaks at 972 and 997 cm^{-} indicate —CH_3 rocking. The HQI value for this spectrum reveals it matches the standard library spectrum for PP approximately 73%. The fragment found in the other fish is identified as polyethylene terephthalate, PET. The absorption peak present in the region of 2922 cm^{-} is attributed to the response of C—H symmetrical stretching and a characteristic band pointing to the C=O stretching of aliphatic ester carbonyl appeared at around 1713 cm^{-} and the strong C—O ester stretch at the region of 1240.05 cm^{-}. The peaks between 872.04 and 792.93 cm^{-} are attributed to the repeating ethylene units while the absorption at 723.48 cm^{-} highly suggests the vibrations brought by the aromatic benzene ring (Chen, 2021).

Polyethylene, PE, appeared to be identified in three samples of sediments analyzed. PE significantly has a strong peak around 2917-2849.5 cm^{-} indicating an asymmetric and symmetric C—H stretch respectively. Medium to weak intensity bands occurring at around 1472.38-1462.7 cm^{-} in the FTIR spectra of PEs are attributed to CH_2 wagging and scissoring that also appears for MA_1 and FR_2. PE also has almost no methyl groups present but C-C bending can be observed around 729.8 – 719.2 cm^{-} making it different from PP.

The spectrum obtained from one of the sediment samples (NR_2) identified as polystyrene, PS. The structure of PS contains a mono-substituted benzene ring which appears in the region of 3060.1-3024.7 cm^{-} that specifically corresponds to the aromatic C—H vibrations. Saturated C-H stretching usually falls below 3000 (Smith, 2021). The biggest peak in the spectrum at 695.31 cm^{-} can be attributed to out-of-plane ring bending.

3.6 Recovery analysis

Each polymer type of microplastics - PET, PS, PP, LDPE, and HDPE was weighed one gram each to have a total of five (5) grams as initial mass of the samples for recovery analysis.

$$Percent\ Recovery, \% = \frac{Recovered\ Microplastics\ (in\ g)}{Starting\ Amount\ of\ Microplastics\ (in\ g)} \times 100\% \quad (1)$$

Samples were spiked in 500 mL of distilled water. The samples underwent the same methodology as sediment samples wherein the following sample pretreatment was applied: pre-weighing, sieving, drying, density separation, and chemical digestion. All sample treatments were done after each step to determine what process would decrease the percent recovery. The formula for calculating the percentage recovery is as follows.

Table 3 and Table 4 show the percentage of recovery after applying each method as well as the average percent recovery of sieving, density separation, and chemical digestion as they play a key role in high yield of recovery of microplastic.

Table 3. Percent recovery of sample treatments when applied after each step

Sample Treatment	Mass (g)	Percent Recovery (%)
Initial Weight	5.000	-
Sieving	4.673	93.46
Density Separation	4.618	92.36
Chemical Digestion/Final Weight	4.538	90.76
Average of three treatments	4.610	92.2

Table 4. Percent recovery of sample treatments alone

Sample Treatment	Mass (g)	Percent Recovery (%)
Initial Weight	5.000	-
Sieving	4.673	93.46
Density Separation	4.945	98.9
Chemical Digestion/Final Weight	4.92	98.4

The percent recovery yielded from the sample pretreatment is 90.76% for the whole process (from initial weight to end of sample treatment). Filtration was shown to be the best method for having a high yield of recovery rate of 91.4% and 92.9% for wastewater and sludge samples, respectively (Lares et al., 2019). Sieving and drying are used most frequently for microplastic separation to remove large debris and water content. A percent recovery of over 93% for both was seen (Lares et al., 2019). In contrast to the recovery analysis in this study, the processes were performed after each treatment; thus, transferring and filtering the spiked microplastics decreases the amount of the sample. Some samples were left in the container and filter paper affecting the recovery rate for density separation and chemical digestion. Drying, sieving, and filtration, significantly affect the final particle counts based on the findings (Lusher et al., 2020). Moreover, sieving affects the lowering of percent recovery among the sample treatments due to the presence of larger microplastics prior to replicating microplastics, and some were difficult to grind. Sieving has the lowest percent recovery, while density separation has the highest recovery, followed by chemical digestion. The recovery rates resulting from spiked microplastics validated the efficiency of density separation using $ZnCl_2$ and chemical digestion via KOH as sample pretreatments. Density separation and chemical digestion were emphasized (Duong et al., 2022; Reineccius et al., 2021) to have high recovery rates of microplastics with 87% and 98%, respectively.

4. CONCLUSIONS

Overall, upon examining the Pugad Baboy Mangrove Forest in Kawit, Cavite, the study confirmed the presence of microplastics in the sediments and fish species.

The near residential area sampling site had the greatest number of microplastic particles, followed by the mangrove area, and then the far from residential area sampling sites. This was justified by the distance of the sites from where commercial and residential infrastructures can be seen, as well as the number of activities done in the sampling sites. Upon extensive literature reading, this study provided the first microplastics analysis and evidence in the sediments and fish samples of Pugad Baboy Mangrove in Kawit, Cavite.

The corresponding recovery analysis also showed the percentage recovery for the sample preparation method used in the study. $ZnCl_2$, as the density separation solution, and KOH, as the chemical digestion solution, averaged to at least 90% recovery, showing that the combination of reagents, as well as the modified methodology from sampling up to the spectroscopic analysis gave positive results. It was highly advisable to test all the microplastic particles for a more extensive quantification, identification, and further analysis. Furthermore, to get a more generalized and thorough assessment of microplastic pollution in the research area, it was also critical to increase the sample size and frequency of sampling sessions.

The study was very beneficial not only as an addition to the small list of microplastics studies in the country, but as an environmental and biological marker and warning for the local area of Kawit, Cavite, specifically to those that were directly connected to the mangrove itself, to help spread awareness and influence the people of the potential health risk of plastic garbage, both macro and micro. Various studies have known that microplastics pose threats to water quality as well, which also affects the residents around the mangrove area since most of these residents interact with the mangrove's water on a day-to-day basis.

5. ACKNOWLEDGMENT

The researchers acknowledge the help of the College of Science Laboratory and DKL Laboratory Supplies for providing most of the necessary apparatus and chemicals to make this project possible; as well as Ms. Anna Karen Laserna of the De La Salle University, Laguna for patiently assisting the researchers in the ATR-FTIR analysis of the samples.

The researchers would also like to extend their gratitude towards Mr. Jose Carlos Basilio Taroy, our thesis adviser, for guiding us throughout the process of writing and sharing his expertise and suggestions upon reviewing the study. We may have started the project with limited time, but he was patient enough to be with us until this very moment.

To Pasig Scholars, A Better Chance Foundation, Unbound Foundation, and Iskolar ng Bataan, your help in funding this study is also well appreciated.

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