Determination of Microplastics in Surface Waters of Kāne‘ohe Bay, O‘ahu

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By

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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For my parents, Kristie and Sam Darin, for generously supporting me in every way possible and for never faltering in their encouragement and reassurance.

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ABSTRACT

The popularization of plastics following the end of World War II undoubtedly changed global consumerism and yielded many benefits, but in turn created a significant issue of plastic pollution that has now reached the farthest corners of the Earth, from polar ice caps to hydrothermal vents. Microplastics, plastic materials of 5mm in size or less, are particularly detrimental to marine and terrestrial life alike, and are becoming increasingly more abundant in the ocean. This study characterizes the concentration of microplastics in surface waters within size classifications ≥1mm, 500μm – 1mm and 200 – 500μm in Kāneʻohe Bay, Oʻahu, Hawaiʻi. Samples were collected using a plankton net, and contents from net tows were treated with hydrogen peroxide to remove most of the organic matter. Suspected plastics were individually identified, and compositions were confirmed using Raman Spectroscopy and Fourier Transform Infrared Spectroscopy. Raman and FT-IR ultimately detected no plastics, and composition of materials in samples consisted of organic material only, indicating one of the following potential explanations: plastics are not present in the bay, plastics reside deeper in the water column, plastics are located closer into the bay by the populated shoreline, plastics reside on the surface sporadically and sampling times did not coincide with plastic presence, or that circulation patterns aggregate plastics in a specific area away from the sampling location.

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LIST OF ABBREVIATIONS

MMT	Millions of Metric Tons
FT-IR	Fourier Transform Infrared
PE	Polyethylene
LDPE	Low Density Polyethylene
HDPE	High Density Polyethylene
PP	Polypropylene
PS	Polystyrene
km	Kilometers
yd	Yards
m	Meters
µm	Micrometer/Micron
mm	Millimeter
ft	Foot
ml	Milliliter
DI	Deionized

1.0 INTRODUCTION

1.1 THE PLASTIC PROBLEM

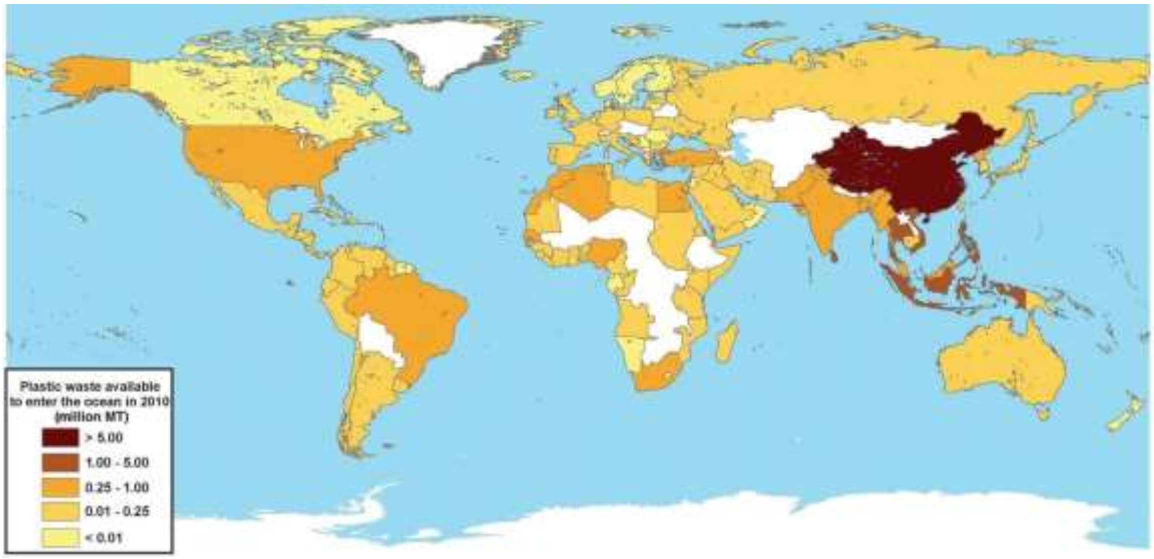


Figure 1: Estimated mass of mismanaged plastic waste [millions of metric tons (MT)] by country, countries without data are colored white (Jambec, 2015).

Utilization of plastics, synthetic polymer-based materials, emerged following the end of World War II, and have undoubtedly transformed quality of life for the global population (Frienkel, 2011). Nowadays plastics dominate the market in the forms of goods and packaging, and due to mismanagement such as low recycling rates and poor disposal systems, have consequently been found throughout terrestrial and marine environments (Shiu et al., 2021). **Figure 1** depicts a quantification of mismanaged plastic waste that is available to enter the ocean by country, with China and Indonesia topping the charts with 8.82 MMT/yr and 3.22 MMT/yr of mismanaged plastic waste, respectively (Jambec, 2015). It is estimated that between 4 and 12 million tons of plastic enter the ocean annually (Picó and Barceló, 2019). Based on this, plastic debris is expected to outweigh fish biomass by 2050 (Picó and Barceló, 2019). Plastics are typically not biodegradable, and are extremely persistent once released into the environment. Instead of breaking down completely, large plastics tend to degrade into smaller and smaller pieces, each new fragment with the same chemical composition as the original. Fragments that are ≤5mm in any direction are typically defined as microplastics (Masura et al., 2015), though there is a lack of consensus on the size parameters that define the term (Picó and Barceló, 2019).

The term microplastics was formally introduced by Thompson et al., (2004), in the first study to characterize microplastics in the ocean. The study examined sediment collected from beaches, as well as estuarine and subtidal sediments around Plymouth in the United Kingdom. The sediments were separated by density, and any particulates that differed in density from the bulk of the sediment were isolated and identified using Fourier Transform infrared (FT-IR) spectroscopy (Thompson et al., 2004). The research concluded that microplastics were present in 23 out of 30 sediment samples taken, and nine different polymer types were identified. Notably, Thompson et al. (2004) only examined microplastics that differed in appearance and density from that of the sediment sampled. Due to this, a full quantification of microplastics using more discerning techniques is expected to be much larger than the results of the Thompson et al. study, 2004.

Presently, microplastics have been detected in even the most remote regions of the world, from deep sea sediments in the Antarctic (Van Cauwenberghe et al., 2013) to the beaches of the most remote oceanic islands (Montiero et al., 2020). Properly characterizing microplastics in marine waters remains a challenge however, due to the small size as well as similarities of plastic polymers to organic and other types of matter (Picó and Barceló, 2019).

1.2 CLASSIFICATION OF MICROPLASTICS

Microplastics can be categorized as either primary or secondary based on their original manufacture size (Pico et al., 2019). Plastics that are manufactured as microplastics to begin with are known as primary microplastics, and are common as microbeads in personal care products, fibers from synthetic textiles, and as plastic pellets used in industrial manufacturing (Silva et al., 2018). Secondary microplastics are those that form due to the breakdown of larger plastics. The breakdown of plastic can occur due to chemical or physical weathering, and usually a combination of both (Silva et al., 2018).

Additionally, microplastics can be classified based on morphology (Silva et al., 2018). Some groups in this classification include pellets, fragments, foams, fibers, spheres and sheets. The most abundant plastics identified are pellets (70%), followed by fragments (25%) (Silva et al., 2018).

Microplastics can also be classified by chemical composition, or polymer type (Erni-Cassola et al., 2019). The most abundant polymers in manufacturing plastics are polyethylene (PE), polypropylene (PP), and various forms of polystyrene (PS). Among marine surface waters, which is the target of this study, PE and PP are the most numerous due to their low-density. They become increasingly less abundant with depth (Erni-Cassola et al., 2019). **Figure 2** illustrates the abundance of plastic polymer types in different sampling zones.

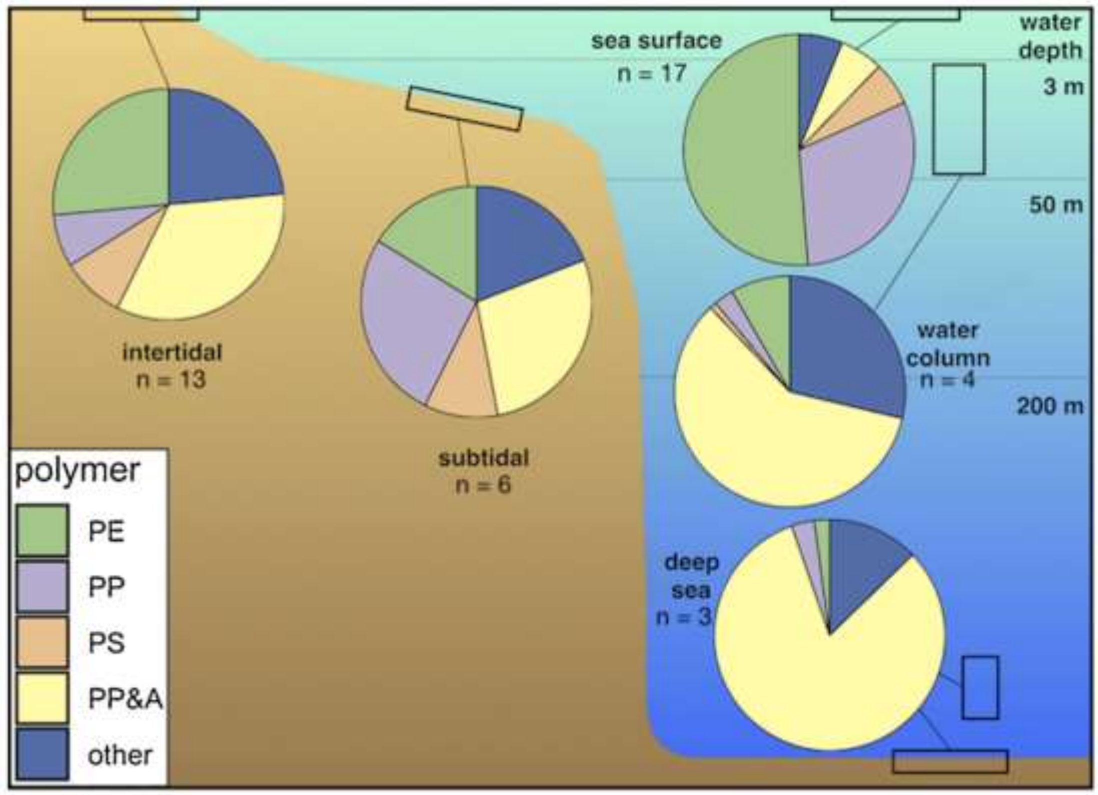


Figure 2: Abundance of polymer types in different marine sampling zones. PE = polyethylene, PP = polypropylene, PS = polystyrene, PP&A = polyester, polyamide and acrylic (Erni-Cassola et al., 2019).

1.3 CONSEQUENCES OF MICROPLASTICS

There are several significant consequences of the presence of microplastics in the marine environment. Countless species of marine and coastal biota ingest microplastics through direct consumption in the case of zooplankton, and through bioaccumulation in the case of larger organisms (Setälä et al., 2014). Ingestion of plastics yields a variety of implications, including blockages in digestive tracts, loss of appetite, and altered feeding behavior (Setälä et al., 2014). Microplastics have the ability to absorb a variety of harmful substances (Chen et al., 2019). Notably, endocrine-disrupting chemicals such as estrogens are commonly detected in marine microplastics, and have serious implications for biota (Chen et al., 2019).

Microplastics pose a significant threat to humans as well. Wild-caught and farmed seafood serves as a primary source of protein for approximately three billion people globally (WWF, 2021). Human consumption of seafood is a major, and perhaps the most significant, pathway for human microplastic exposure (Smith et al., 2018). Though minimal research has been done concerning the implications of human microplastic ingestion, preliminary studies have demonstrated numerous potentially concerning impacts, including disruption of the gut microbiome, toxicity of different sized plastics, transfer of absorbed chemical pollutants, and enhanced inflammatory response (Wright and Kelly, 2017).

1.4 MICROPLASTICS IN HAWAIʻI

The Hawaiian archipelago is located just west of the Great Pacific Garbage Patch, the largest accumulation of plastic waste in the ocean (Blickley et al., 2016). The North Pacific Subtropical Gyre and the northerly trade winds both push floating plastic debris to the Hawaiian Islands, causing an accumulation of plastics throughout the archipelago (Blickley et al., 2016). A comprehensive study of plastic debris in the Main Hawaiian Islands conducted by Brignac et al., (2019) found that surface waters, the focus of this study, were dominated by polyethylene (PE) and polypropylene (PP). Given this, it is reasonable to expect that microplastics, particularly PP and PE, are present in surface waters surrounding the Main Hawaiian Islands.

2.0 SITE SELECTION

Kāneʻohe Bay, which lies along the Northeast coast of Oʻahu, Hawaiʻi, is the largest sheltered body of water in the Hawaiian islands (Jokiel, 1991). The bay spans 7.9km from the Northeast point of the bay to the Southwest, and stretches 4.3km in width. Kāneʻohe Bay can be divided into two depth zones: a shallow (1.5-6.1m) zone, characterized by stream delta formation and input of dredge soils, and a deeper (12-14m) zone, associated with a flat lagoon bottom (Jokiel, 1991). The inner portion of Kāneʻohe Bay is largely protected from ocean storms and large swells.

Figure 3: Map of Kāneʻohe Bay and surrounding landmarks

Net tows were conducted in the Sampan channel, depicted in **Figure 3**. The Sampan channel is a natural channel that has not been dredged, and stretches across the reef flat out to sea (Jokiel, 1991). It accommodates small boats, and there is typically some boat traffic on a regular basis. The channel has a depth of approximately 2m and bottom cover is dominated by large coral rubble (Jokiel, 1991). On incoming tides, water from outside the bay flows into the Sampan channel and then over the fringing reef (Jokiel, 1991). On outgoing tides, the opposite occurs, and water flows from barrier reefs to the Sampan channel, and then out to the open ocean (Jokiel, 1991). This channel was chosen as the site to conduct plankton tows due to the regular inflow and outflow of surface waters. **Figure 4** below depicts the routes taken for the two plankton tows (inner and outer) conducted during each field outing for this study. Yellow lines indicate tow routes.

Figure 4: Plankton tow routes along the inner and outer sections of the Sampan Channel. Coordinates mark approximate start and end locations of each tow.

3.0 METHODS

Data processing methodology is based on the 2015 method developed by the National Oceanic and Atmospheric Administration (Masura et al., 2015). The authors state that this method is applicable for the determination of common surface plastics such as PP and PE.

3.1 DATA COLLECTION

Data were collected using a $706.86cm^2$ area plankton net with a mesh size of 53µm, secured athwartship of the 21ft Boston Whaler used for field work. A boathook was secured to the center of the boat fore and aft, extending off of the starboard (right) side. As depicted in **Figure 5**, a yellow polypropylene line was tied from a cleat in the front of the boat, looped around the outboard end of the boathook, and then tied to the plankton net, allowing it to be pulled 3-5m behind the boat, but out of the propwash directly behind the boat. **Figure 6** shows the net trailing behind the boat, just below the surface of the water. Careful watch was taken to ensure that the net stayed behind the boat and did not dip too far below the surface of the water.

Figure 5: setup for plankton tow net. Rope was secured from cleat on bow to end of boathook to net 3-5m off of stern.

Figure 6: depiction of plankton net drifting 3-5m off of stern during a plankton tow.

As discussed in Section 2.0, two tow routes were taken along the Sampan channel. The objective of this was to cover one route at the bay end of the channel, and one additional route toward the outside of the channel closer to the open ocean. This would ideally account for any difference in tides on a given day, as most of the water coming in and out would be between the two tow routes. Tows were taken for 10 minutes, as determined in the first field outing as the ideal time for sufficient data collection. Sufficient tow time was determined based on total amount of material collected in the net at the end of each tow. Tows longer than 10 minutes had too much organic material to effectively dissolve so any plastics could be identified.

During each plankton tow, data collected included a start and end time, total time (always 10 minutes), GPS start and end coordinates, and average boat speed, given in knots. The volume of water sampled (in m^3) was calculated using these data based on the following equation:

$V = d*A$, where d is the distance determined from the beginning and ending coordinates in meters, assuming current is negligible, and A is the area of the net in m^2.

Figure 7: Retrieval and draining of the plankton net. Net was rinsed with seawater to ensure that all material collected was deposited into the collection cup, pictured at the base of the plankton net.

Once tows were completed, the net was brought in to the boat and rinsed with clean seawater using a squirt bottle to gather any material stuck to the inside of the net into the collection cup located at the base of the net. **Figure 7** depicts the retrieval and draining of the plankton net. The collection cup was then removed from the net and contents were emptied into glass sample jars. The collection cup was rinsed with seawater until all contents from the cup were deposited into the sample jar. Samples were labeled with a tow number and date, and brought back to the lab in the Marine Science Building. Samples were stored in the laboratory refrigerator until they could be processed, shown in **Figure 8**.

Figure 8: Collection and storage of material from plankton tows. Samples were stored in refrigerator in glass sample jars until processing.

3.2 WET PEROXIDE OXIDATION

The purpose of wet peroxide oxidation, the first step in processing the samples, is to oxidize any organic matter and leave behind anything that is synthetic for analysis. The process utilizes a series of reactions using an iron sulfate solution to catalyze the reaction, and then addition of 30% hydrogen peroxide to degrade and dissolve any organic matter (Masura et al., 2015). All processing during wet peroxide oxidation was performed under a working fume hood for safety.

To begin the process, a sample was emptied from the glass jar into a beaker large enough to prevent any overflow or spilling once other reactants were added. The jar was rinsed with deionized (DI) water to ensure all remaining material was deposited into the beaker. Once all material was in the beaker, 20ml of 0.05M Fe(II) solution, the iron catalyst, was added to the sample. After that, 20ml of 30% hydrogen peroxide, H_2O_2, a strong oxidizer, was carefully added to the solution. At this point, the mixture was left alone for five minutes at room temperature in a fume hood before proceeding to the next step. The sample was then covered with a watch glass, placed on a hotplate, and heated to 75ºC, careful not to overheat to avoid violent boiling. Once bubbles started to appear, the sample was removed from the hotplate and allowed to cool. If the reaction appeared to have the potential to boil over, DI water was added to subdue the reaction. Once cool, the solution was added to the hotplate once again, and allowed to gently boil at 75ºC for an additional 30 minutes (**Figure 9)**.

After the 30 minutes, the sample was examined, and if organic matter was still visible, an additional 20ml of hydrogen peroxide was added, and the sample underwent the same boiling process until all obvious organic matter was removed. The sample was then allowed to cool for 20 minutes in the fume hood for sieving.

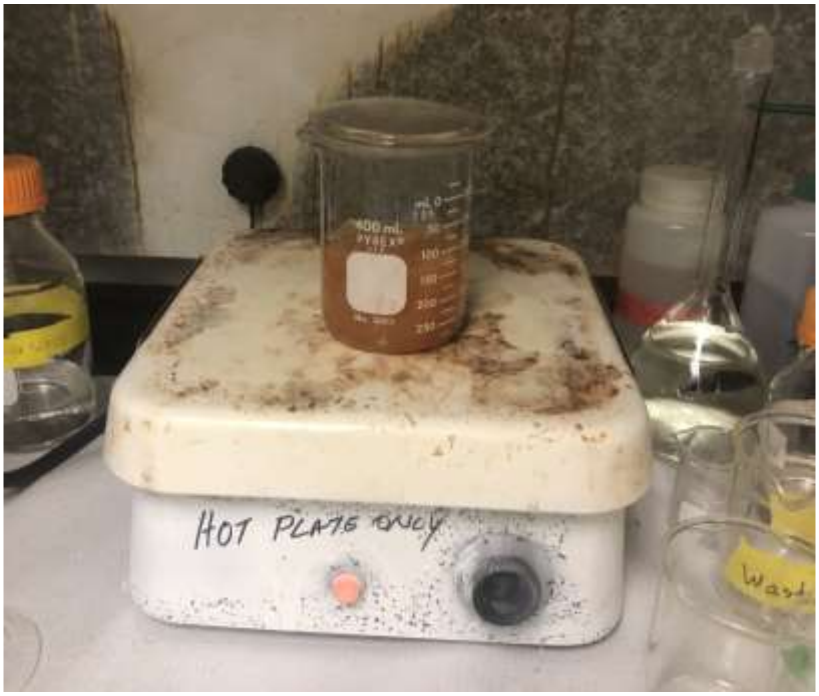


Figure 9: Wet peroxide oxidation reaction in sample. Sample is exposed to an iron catalyst and 30% hydrogen peroxide, and set over heat at 75ºC for 30 minutes to oxidize organic matter.

3.3 SIZE SEPARATION

Following the wet peroxide oxidation, cooled samples were separated by size using sieves to divide materials into three size classifications. Sieve sizes selected were 1mm, 500µm, and 200µm. Each solution was run through the 1mm sieve first, and all material that fell through was collected into another beaker. Materials caught in the sieve were rinsed out with DI water into a separate beaker labeled “1mm”. The remaining solution was poured into the 500µm sieve , and the process was repeated for the 200µm sieve. This left three beakers with materials in 1mm, 500µm and 200µm size classifications ready for preparation to be mounted on slides for visual analysis.

3.4 VISUAL ANALYSIS

After processing, samples from each tow were mounted onto three separate slides for the three different size classifications. A syringe was used to inject the sample components onto a 25mm filter, and then sample was allowed to air dry, leaving only solid materials behind on the filter. Filters were mounted on slides and labeled with the tow number and appropriate size classification.

Once slides were prepared and labeled, they were examined under a microscope at 200x magnification. Each sample was carefully inspected over its entire area, and any materials suspected of being plastic were marked with a location and a description of the material. Factors for identification of microplastics were taken from Hidalgo-Ruz et al. (2012). **Table 1** below contains common characteristics in microplastics that were used to identify suspected plastics (Hidalgo-Ruz et al., 2012). Plastics identified can be either in the form of pellets or fragments. Shapes of pellets typically take the form of cylindrical figures, disks, ovoid, or spheruloid shapes, and are usually flat (Hidalgo-Ruz et al., 2012). Fragments are typically rounded, subrounded, angular or subangular in shape (Hidalgo-Ruz et al., 2012). In general, characteristics to look for in microplastic shape include irregular, elongated, degraded, rough, and pieces with broken edges (Hidalgo-Ruz et al., 2012). There are various pigmentations that are common amongst plastics, as summarized in **Table 1** (Hidalgo-Ruz et al., 2012). Examples of materials that were suspected plastics from samples are depicted in **Figure 10**. A total count of suspected plastics was determined for each size classification of each sample.

Table 1: Common characteristics of microplastics, including color and shape for pellets, fragments, and plastics in general. Taken from Hidalgo-Ruz et al. [2012].

Common Characteristics of Microplastics for Microscope Identification	
Shape	Color/Pigmentation
<u>Pellets</u>: cylindrical, disks, flat, ovoid, spheruloids	Transparent, crystalline White, Opaque
<u>Fragments</u>: rounded, subrounded, subangular, angular	Clear-white-cream, Tan Red, Orange, Blue
<u>In General</u>: irregular, elongated, degraded, rough, and broken edges	Black, Gray, Brown Green, Pink, Yellow

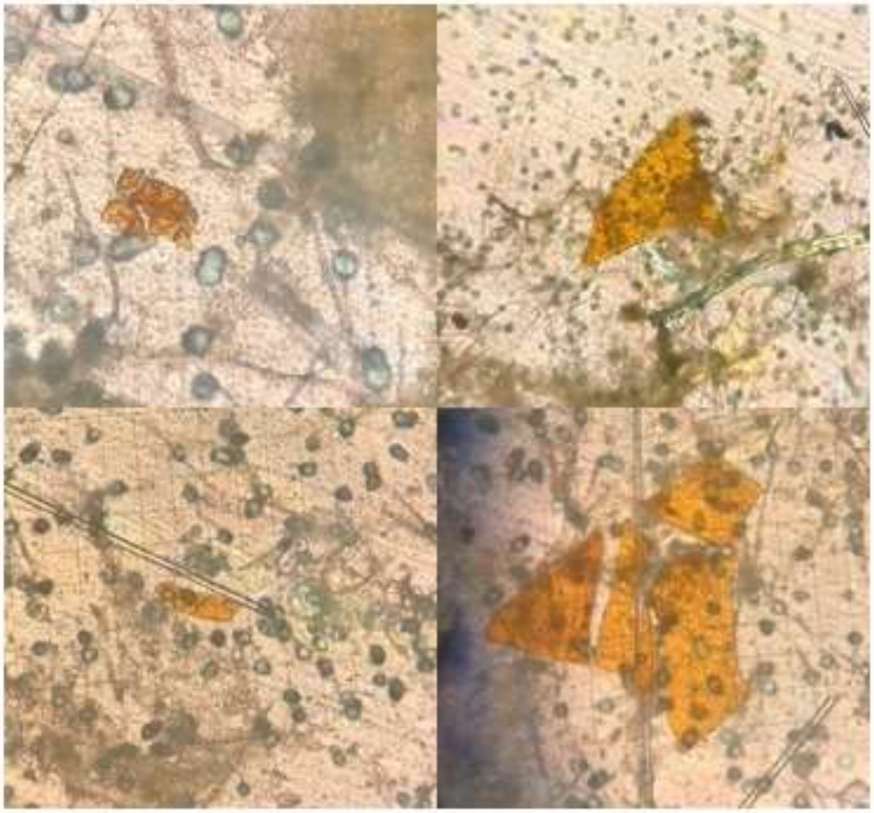


Figure 10: Suspected microplastic fragments in samples

3.5 COMPOSITION DETERMINATION

After visual determination of suspected plastics, the composition of the selected pieces was determined using Raman Spectroscopy and Fourier-Transform Infrared Spectroscopy (FT-IR). Samples were originally tested with Raman Spectroscopy, a technology that utilizes lasers with photon energies ranging from the ultraviolet (UV) to the near infrared (IR) to measure inelastic scattering off of an object, in this case suspected plastics (Do Nascimento, 2018). Raman spectroscopy provides a shift in wavelength of the inelastic scattering, which in turn provides chemical and structural information about the composition of the material (Do Nascimento, 2018). Raman spectra were analyzed using GRAMS/AI spectroscopy software, and comparisons and identifications were made using the Open Specy raman public database.

A secondary test was done using FT-IR at the Hawaiʻi Pacific University Center for Marine Debris Research. FT-IR is slightly different from Raman Spectroscopy in that an infrared spectrum of absorption or emission of a material is obtained (Song, 2016). This is measured by shining a monochromatic beam of light at the desired material and quantifying how much of it is absorbed. FT-IR is often advantageous because it yields a high signal-to-noise ratio for scanning time (Song, 2016).

3.6 VALIDATION OF METHODOLOGY

In order to validate methodology, two tow samples were processed with the addition of three 1mm x 1mm squares of store-bought polyethylene and polypropylene, respectively. The polyethylene was cut from a Ziploc brand plasticware, and the polypropylene was cut from an all-purpose cleaner bottle. Squares were cut from flat surfaces on each material and both the plasticware and the bottle were thoroughly cleaned with soap and water before they were placed in the samples. Store-bought items were used because it is fragments of these materials in forms such as these that are typically found in surface waters as broken-down plastics. The addition of a recognized amount of known polymer types determines whether the methodology to process the samples in any way degrades or damages plastics and confirms the ability of the approach to quantify the plastic content.

4.0 RESULTS

4.1 VISUAL DETERMINATION

Upon visual analysis of slides under the microscope, many pieces were identified as suspected plastic in each sample. **Table 2** depicts the counts of suspected plastic in slides from plankton tows 3-8. **Figure 12** shows a graphical depiction of **Table 2**. The average counts of the 1mm, 200µm and 500µm size classifications were 260.5, 420.2, and 807, respectively. Almost all of the suspected plastics identified appeared to be of the same composition, fragments of amber pigmentation shown on the right in **Figure 11**. The other fragments found were darker in pigmentation, though still amber colored, as shown in the photo on the left in **Figure 11**. Images were taken under a microscope at 200x magnification.

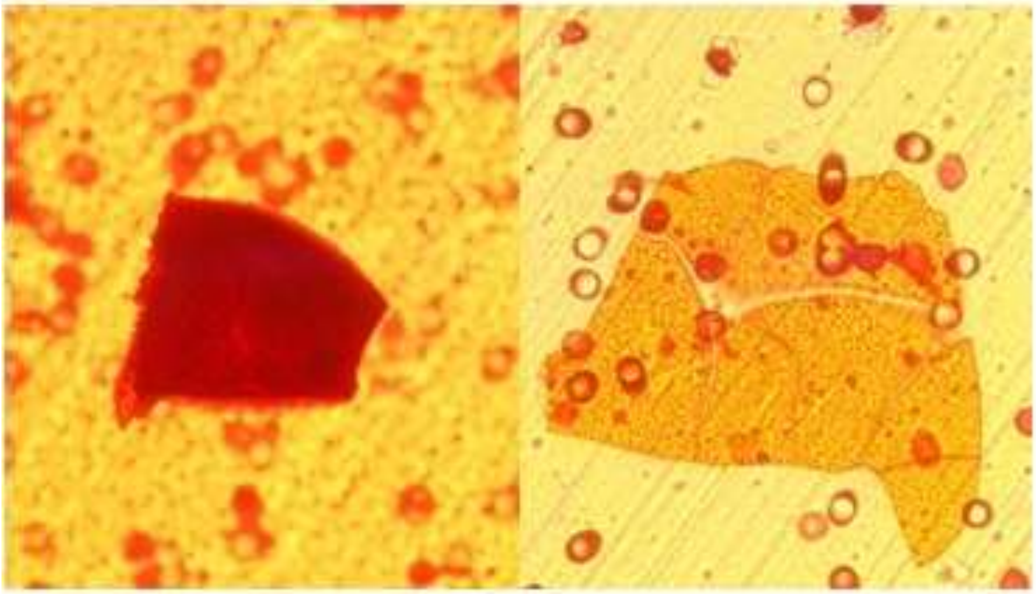


Figure 11: Appearance of suspected plastics identified in tow samples.

Table 2: Summary of counts of plastics for each plankton tow in each size classification. Determined under a microscope at 200x magnification.

Tow Number	Size Classification	Date of Sample Collection	Suspected Plastics Counted
3	1.0mm	5/21/20	75
3	500µm	5/21/20	136
3	200µm	5/21/20	1207
4	1.0mm	5/21/20	261
4	500µm	5/21/20	306
4	200µm	5/21/20	1121
5	1.0mm	5/21/20	240
5	500µm	5/21/20	796
5	200µm	5/21/20	963
6	1.0mm	5/21/20	675
6	500µm	5/21/20	854
6	200µm	5/21/20	1043
7	1.0mm	3/24/21	259
7	500µm	3/24/21	418
7	200µm	3/24/21	390
8	1.0mm	3/24/21	53
8	500µm	3/24/21	170
8	200µm	3/24/21	118

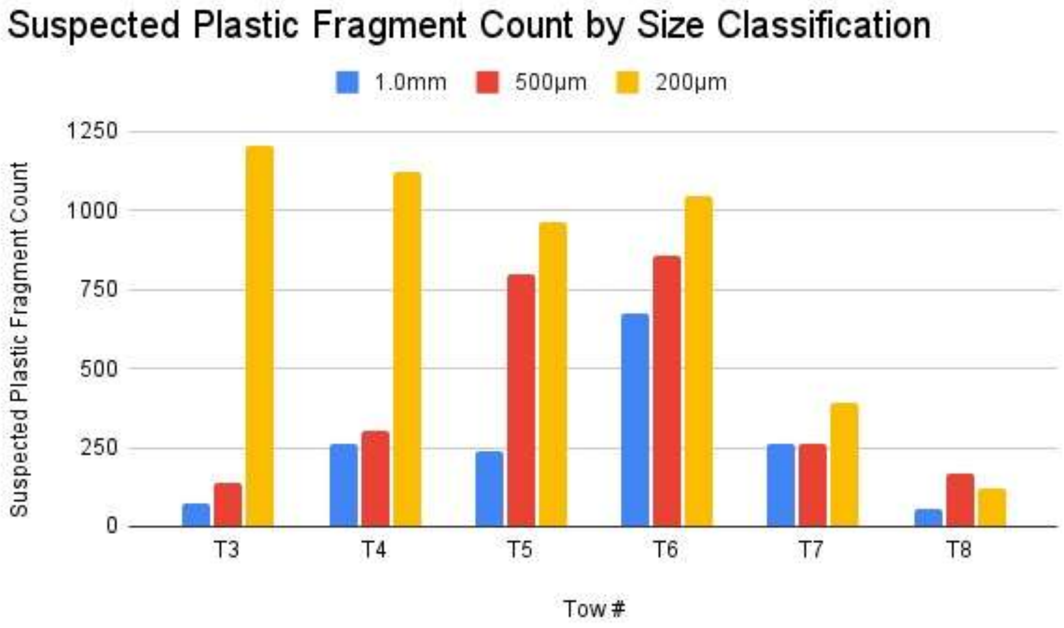


Figure 12: Graphical depiction of table 2. Blue bars represent fragments in 1mm size class, red in 500µm, and yellow in 200µm. Graph includes tows 3-8.

4.2 RAMAN SPECTROSCOPY AND FT-IR

Following visual identification of suspected plastic materials, samples were analyzed by Raman spectroscopy with the goal of determining the composition of the identified fragments. Common plastic polymers that are suspected to be prevalent in surface waters of Kāneʻohe Bay were tested first in order to obtain a baseline. Polymers tested included low density polyethylene (LDPE), high density polyethylene (HDPE), and polypropylene (PP). Virgin plastic pellets of these polymer compositions were obtained from the Hawaiʻi Pacific University Center for Marine Debris Research Polymer Kit 1.0. **Figure 13** depicts the spectra of LDPE.

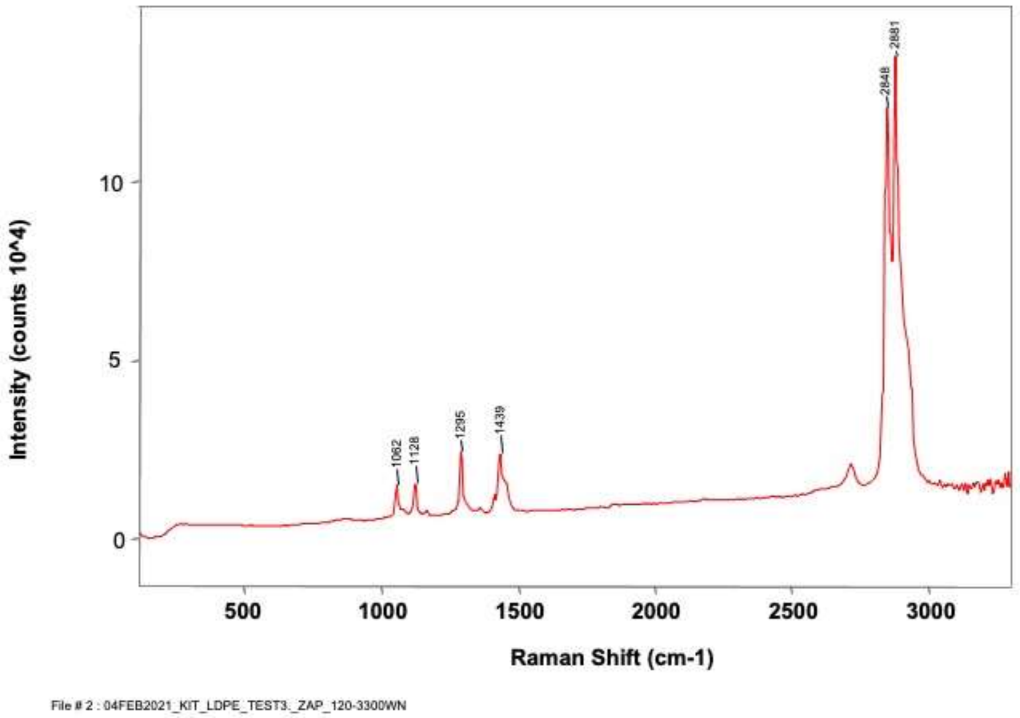


Figure 13: Raman Spectra for LDPE. Virgin plastic sample obtained from HPU Center for Marine Debris Research Polymer Kit 1. Significant peaks on spectra are identified.

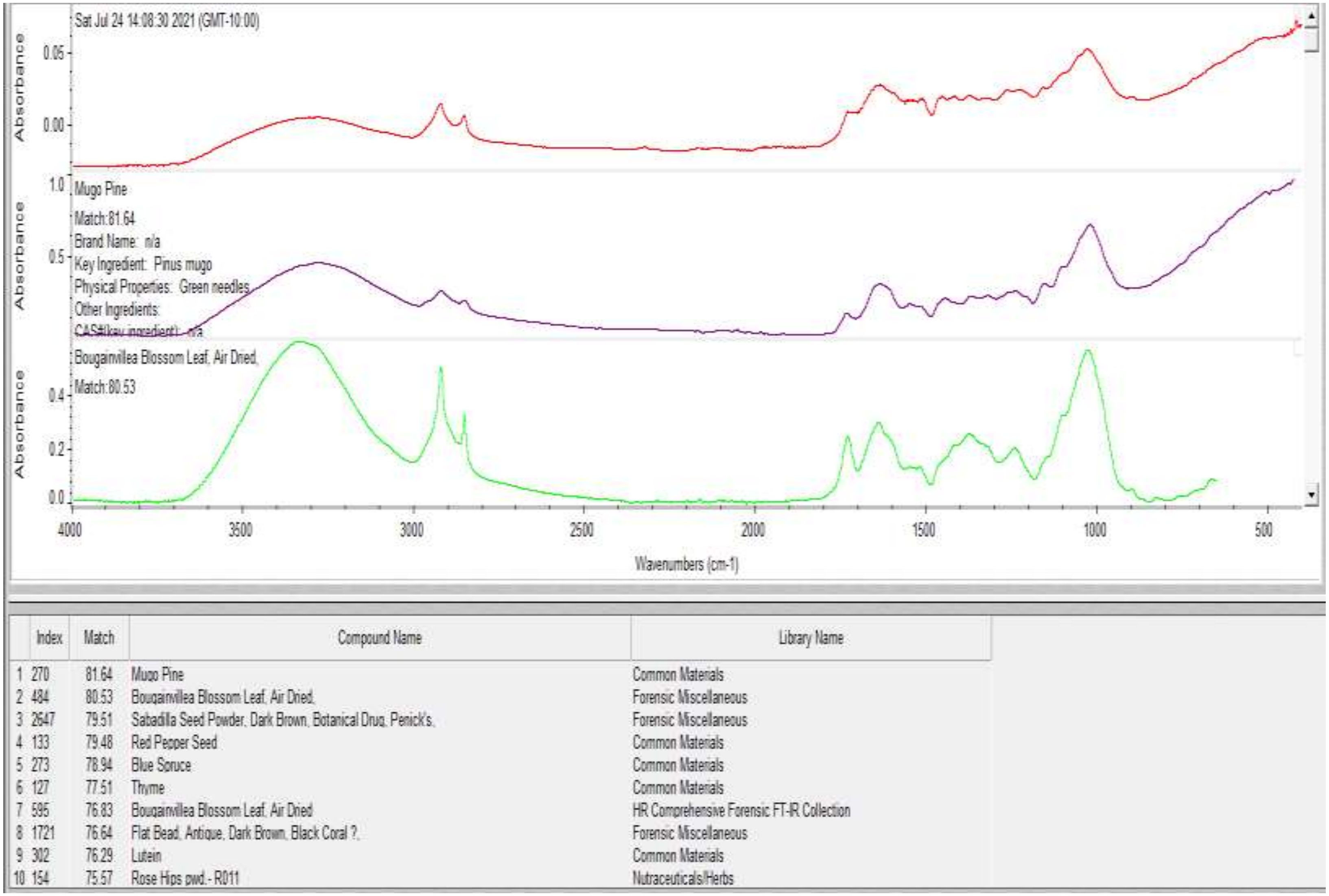
Peak values from spectra obtained from Raman were compared in the Open Specy public Raman database, and did not yield consistent results. **Table 3** provides results pulled from the database for amber-colored fragments from tow 3 in the 200µm and the 500µm size classifications. For each fragment tested, the top five matches are listed. Many of the polymer/material matches were identified as metals (silver, copper, iron and tin), as well as arsenic, manganese, and sulfur. The Pearson’s r correlation value is provided for each match, where a value greater than 0.7 is considered a strong correlation and a likely match. Notably, none of the matches listed were of plastic composition but it was also clear that many of the matches were not indicative of the actual material being tested. This was consistent across all samples.

Table 3: Raman spectroscopy data from tow 3, including 200µm and 500µm size classifications. Includes top five material/polymer matches and Pearson's r correlation for each.

Raman Spectroscopy Data from T3 200µm and 500µm Size Classifications		
Sample Spectra	Material/Polymer Matches	Pearson's r Correlation
T3_200µm_1	Ag As Mn S3	0.74
	Cu Fe0.5 S4 Sn1.5	0.71
	Ivoryblk	0.7
	Lampblak	0.63
	Lampblak	0.63
T3_200µm_2	Ag As Mn S3	0.72
	Ivoryblk	0.69
	Cu Fe0.5 S4 Sn1.5	0.69
	Lampblak (1)	0.62
	Lampblak	0.62
T3_200µm_3	Ivoryblk	0.69
	Cu Fe0.5 S4 Sn1.5	0.66
	Ag As Mn S3	0.64
	Lampblak (1)	0.62
	Lampblak	0.62
T3_500µm_1	Ag As Mn S3	0.81
	Cu Fe0.5 S4 Sn1.5	0.78
	Ivoryblk	0.7
	Mn S	0.69
	As8 S9	0.66

T3_500µm_2	Ag As Mn S3	0.83
	Cu Fe0.5 S4 Sn1.5	0.78
	Mn S	0.7
	Ivoryblk	0.7
	As8 S9	0.66
T3_500µm_3	Ag As Mn S3	0.82
	Cu Fe0.5 S4 Sn1.5	0.78
	Ivoryblk	0.71
	Mn S	0.71
	As8 S9	0.67

In order to confirm that the identified suspected plastics were not plastics, samples were taken to the Hawai‘i Pacific University Center for Marine Debris Research laboratory for analysis using FT-IR. Results of data derived from an amber-colored fragment on FT-IR is shown in **Figure 14**. Matches most closely correspond with mugo pine bark, air-dried bougainvillea blossoms or leaves, sabadilla seed powder, red pepper seed, and blue spruce, all organic matter. Matches with mugo pine bark and air-dried bougainvillea blossoms/leaves have particularly significant Pearson’s r correlations of 81.64 and 80.53, respectively. Bougainvillea grows throughout Hawai‘i, and mugo pine is a common household and yard plant that many homeowners maintain.



	Index	Match	Compound Name	Library Name
1	270	81.64	Mugo Pine	Common Materials
2	484	80.53	Bougainvillea Blossom Leaf, Air Dried,	Forensic Miscellaneous
3	2647	79.51	Sabadilla Seed Powder, Dark Brown, Botanical Drug, Penick's,	Forensic Miscellaneous
4	133	79.48	Red Pepper Seed	Common Materials
5	273	78.94	Blue Spruce	Common Materials
6	127	77.51	Thyme	Common Materials
7	595	76.83	Bougainvillea Blossom Leaf, Air Dried	HR Comprehensive Forensic FT-IR Collection
8	1721	76.64	Flat Bead, Antique, Dark Brown, Black Coral ?,	Forensic Miscellaneous
9	302	76.29	Lutein	Common Materials
10	154	75.57	Rose Hips pwd.- R011	Nutraceuticals/Herbs

Figure 14: FT-IR spectra of suspected plastic fragment. Notably all matches consist of organic materials.

4.3 VALIDATION OF METHODS

In order to validate the sample processing methodology, 1mm squares of PP and PE were added to samples from tows 9 and 10, collected from Kāneʻohe Bay on August 4, 2021. After processing, PP and PE remained completely intact, with no visible sign of any degradation. Examination of a square of PE from tow number 9 under a microscope at 200x magnification is shown in **Figure 15** below, illustrating clear-cut edges and no signs of deterioration. No other plastics were found in these tow samples.

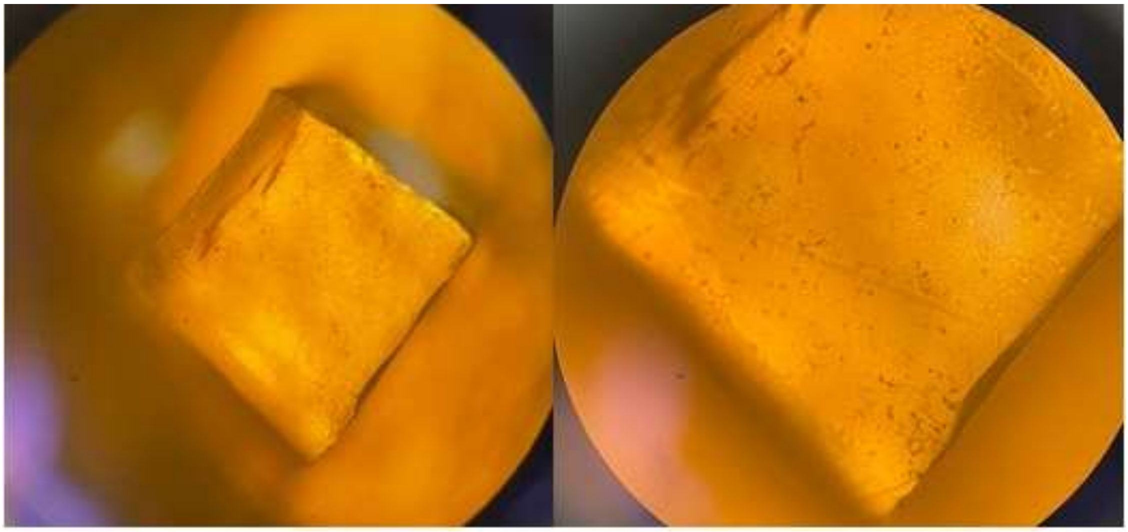


Figure 15: Image of 1mm by 1mm PE square after sample processing at 200x magnification. No visible degradation present on the surface of the plastic.

5.0 DISCUSSION

Although a significant number of fragments were identified as suspected plastics, none of the visually suspected plastics were synthetic materials. Analysis using FT-IR confirmed that the amber fragments that dominated the samples were likely tree bark and leaves and petals from bougainvillea plants. The lack of plastics is surprising, given that Kāneʻohe is very populated along the shoreline, and the bay itself experiences a great deal of boat traffic on a daily basis. Given this level of human interaction, one would expect some degree of plastic pollution to be present.

5.1 CONDITIONS DURING SAMPLING

Although sample site and location were determined to avoid much influence from external conditions, one possible explanation for the lack of plastic in the tow samples could be the weather and conditions on the given sample days. The first tow samples that were processed were collected around noon on May 21, 2020. There was no precipitation 24 hours prior to sampling, and tows were conducted around a high tide of 0.46m at 12:24pm. Winds were notable, with a maximum speed of 33.8km per hour from the Northeast in the afternoon. The second round of tow samples that were processed were conducted on March 24, 2021 in the morning, around 10am. Besides a very light negligible rain of 0.025cm around 8am, there was no rain 24 hours prior to sampling. Winds were similar to that of the first tow, with a maximum speed of 25.4km per hour coming from the Northeast around 2pm. This round of tows was conducted on a rising tide, with a high of 0.68m at 12:22pm.

Wind speeds were similar and precipitation was negligible during net tows. Data collection on the first field outing was performed on a high and then a falling tide, while the second outing was collected on a rising tide. Given this, it is noted that both sample collections took place close to a high tide, suggesting that samples were taken with a large fraction of offshore waters. This may explain a lack of plastics in samples if plastics are more concentrated in bay waters closer to the populated shore.

5.2 SAMPLE COLLECTION PARAMETERS

It is important to note that samples were only taken from the surface of the water. Growing evidence indicates that plastic is much more typically found in deeper waters due to higher densities (Choy et al., 2019), though it is important to note that PE and PP do float and would be found on the surface if present. All tows conducted contained a large amount of organic matter, suggesting that ample material was collected, though none of it contained plastic. Ten-minute tows were assumed to be representative of average surface waters of Kāneʻohe Bay, though the fact that tows were collected near high tides may suggest that the sample was taken from primarily offshore waters that came into the bay during the rising tide.

5.3 PROCESSING TECHNIQUE

Another possible explanation for the lack of plastics present is the way in which the samples were processed. The concern that the processing methodology was dissolving the plastics or breaking them down in a way that made them undistinguishable was addressed by testing the validity of the technique, as outlined in section *3.6: Validation of*

Methodology. A drawback to this approach could be that the only size plastic that was tested was 1mm, but the surface of the 1mm cubes tested did not appear to be altered in any way, so it is reasonable to extrapolate this to smaller fragments and assume that they also would not be altered by the organic matter digestion process. It is likely that the organic matter remaining after processing is due the the thick-walled nature of plant cells (Crawford and Zambrynski, 2000), potentially making it more difficult to break down than other organic material such as plankton.

6.0 CONCLUSION

Interestingly, no plastic was detected within surface net tows conducted within the highly populated Kāneʻohe Bay, Oʻahu. These are surprising results, and warrant additional future research in this location. As previously mentioned, there is growing evidence showing that plastics are typically found deeper in the water column (Choy et al., 2019). It would be interesting to conduct net tows at depth to see if plastics are present deeper in the water column. It might also be useful to sample more inside the bay, closer to the populated shoreline where the source of plastic is thought to be located. Another way to capture this inshore water could be to sample closer to low tides, when the nearshore water is pulled further out in the bay. Another potential explanation for a lack of plastics found could be that the plastics accumulate in slicks located outside of the bay, closer to open ocean. Evidence suggests that smaller plastics are increasingly heavily populating ocean slicks (Gove et al., 2019). If slicks are present on the outside of Kāneʻohe Bay, it is possible that the timing of the net tows conducted did not line up with events when contents of slicks are carried into the bay. It may be helpful to examine the bay opening for a presence of slicks. Lastly, it would be interesting to examine whether there is a presence of plastic debris and microplastics following significant precipitation or a storm.

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