UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF MICROPLASTICS POLLUTION AND ITS ACCUMULATION IN SELECTED AQUACULTURE PRODUCTS

SITI NORSUHAILA BINTI MOHD NAMRAN

ASSESSMENT OF MICROPLASTICS POLLUTION AND ITS ACCUMULATION IN SELECTED AQUACULTURE PRODUCTS

BY

SITI NORSUHAILA BINTI MOHD NAMRAN

Thesis submitted in fulfilment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

ACKNOWLEDGEMENT

Firstly, I am grateful to Allah s.w.t for giving me strength and patience throughout my thesis and final year project. Without His blessing, I would not be able to start or do anything.

I want to express my deepest gratitude to my supervisor, Associate Professor Dr. Sarva Mangala Praveena, for her immeasurable guidance, time, encouragement, and support, especially in monetary form, from the beginning of the final year project (FYP) until its completion. It is fortunate to be allowed to be under her supervision, and I appreciate and thank her for sharing knowledge in the research and thesis writing process.

Next, I would like to express an appreciation to the Laboratory of Environmental Health in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, for providing help and facilities throughout all laboratory works.

Furthermore, I would like to extend my sincere appreciation to my beloved family, who always support and believe that I can finish my final year project. Other than that, special thanks also go to my FYP mates and close friends, especially Ummi Sabariah and Aqilah Amirah, for helping throughout the sample extraction process at the laboratory.

Last but not least, thank you so much to anyone who had helped me, whether directly or indirectly, throughout the journey of completing this project report and my bachelor's degree. May Allah repay the good deeds for being part of this project.

ABSTRACT

ASSESSMENT OF MICROPLASTICS POLLUTION AND ITS ACCUMULATION IN SELECTED AQUACULTURE PRODUCTS

SITI NORSUHAILA BINTI MOHD NAMRAN

Introduction: Aquaculture species was reported to be contaminated with microplastics (MPs) by a large number of studies all around the world. The widespread occurrence of MPs in those harvested species intended for human consumption increases human health concerns. However, there is insufficient data regarding MPs occurrence in aquaculture products from Malaysia since this issue is rarely investigated. **Objectives:** This study determine the occurrence of MPs and evaluate their characteristics and composition in a group of commonly consumed aquaculture species from local freshwater and marine aquaculture hatcheries in Selangor, Malaysia. **Methodology:** A total of 28 samples consisting of six (6) species: Red Nile tilapia (*Oreochromis niloticus*) (TM: n = 5), African catfish (*Clarias gariepinus*) (K: n = 5), Mozambique tilapia (*Oreochromis mossambicus*) (TH: n = 5), Jade perch (*Scrotum barcoo*) (JP: n = 5), Hybrid grouper (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) (HG: n = 5), and Whiteleg shrimp (*Litopenaeus vannamei*) (P: n = 3) were collected from local freshwater and marine aquaculture hatcheries in Selangor, Malaysia, between March to April 2021. Fish (muscles, gills, liver, gastrointestinal tract) and shrimp (muscles, stomach, intestines, outer layer) were screened for the presence of MPs using NaCl hypersaline solution and hydrogen peroxide (H_2O_2). The extracted particles were examined through microscopic imaging and Fourier-transform infrared spectroscopy (FTIR) for their morphological characteristics' identification (shape, color and polymer composition). **Result and Discussion:** Particles extracted from all the species screened ranged between 11 and 496 particles per individual. Their accumulation was highest in TM (496) > TH (300) > JP (29) > K (23) > HG (22) > P (11). Besides, the highest number of particles was detected in the gastrointestinal tract (GIT) of TM (334), followed by GIT of TH (264). The particles extracted from aquaculture species were mostly in fragment and irregular form, with a mixture of colored and colorless. Polypropylene (PP), nylon (NY), low-density polyethylene (LDPE) and polyvinyl chloride (PVC) were among the plastic polymers detected among the extracted particles from aquaculture species, suggesting that the particles possibly originated from aquaculture structures used at the sites. **Conclusion:** Microplastics detected in freshwater and marine aquaculture products from Selangor, Malaysia. Further investigation is needed to understand better how aquaculture structures utilized / aquaculture activities could influence MPs accumulation in sites and MPs in aquaculture species.

Keywords: microplastics, aquaculture, freshwater, marine, species

ABSTRAK

PENILAIAN PENCEMARAN MIKROPLASTIK DAN PENGUMPULANNYA DALAM PRODUK AKUAKULTUR TERPILIH

SITI NORSUHAILA BINTI MOHD NAMRAN

Pengenalan: Spesies akuakultur telah dilaporkan tercemar dengan mikroplastik (MP) oleh sebilangan besar kajian di seluruh dunia. Percemaran MP yang meluas dalam spesies yang diternak bertujuan untuk konsumsi manusia telah meningkatkan kebimbangan terhadap kesihatan manusia. Walau bagaimanapun, terdapat kekurangan maklumat berkenaan pencemaran MP dalam produk akuakultur dari Malaysia kerana isu ini kurang mendapat perhatian umum. **Objektif:** Kajian ini menilai jumlah dan ciri-ciri MP dalam kumpulan spesies akuakultur terpilih dari tempat penternakan akuakultur air tawar dan air masin tempatan di Selangor, Malaysia. **Metodologi:** Sebanyak 28 sampel yang terdiri daripada enam (6) spesies: Tilapia merah Nil (*Oreochromis niloticus*) (TM: n = 5), Keli Afrika (*Clarias gariepinus*) (K: n = 5), Tilapia hitam Mozambique (*Oreochromis mossambicus*) (TH: n = 5), Jade perch (*Scrotum barcoo*) (JP: n = 5), Kerapu hibrid (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) (HG: n = 5), dan udang Whiteleg (*Litopenaeus vannamei*) (P: n = 3) dikumpul dari tempat penternakan akuakultur air tawar dan air masin tempatan di Selangor, Malaysia, antara bulan Mac hingga April 2021. Ikan (otot, insang, hati, saluran gastrointestinal) dan udang (otot, perut, usus, lapisan luar) disaring untuk mengesan kehadiran MP dengan menggunakan larutan hipersalin NaCl dan hidrogen peroksida (H_2O_2). Partikel yang diekstrak diperiksa melalui pengimejan mikroskopik dan spektroskopi inframerah transformasi Fourier (FTIR) untuk mengenalpasti jumlah dan ciri morfologi mereka (bentuk, warna dan komposisi polimer). **Keputusan dan Perbincangan:** Partikel yang diekstrak dari semua spesies yang disaring adalah antara 11 dan 496 partikel per individu. Pengumpulan partikel paling tinggi dikesan dalam TM (496)> TH (300)> JP (29)> K (23)> HG (22)> P (11). Selain itu, jumlah partikel tertinggi dikesan di saluran gastrousus (GIT) TM (334), diikuti oleh GIT TH (264). Partikel yang diekstrak dari spesies akuakultur sebahagian besar dalam bentuk serpihan dan tidak mempunyai bentuk yang seragam, dengan campuran berwarna dan tidak berwarna. Polipropilena (PP), nilon (NY), polietilena berketumpatan rendah (LDPE) dan polivinil klorida (PVC) merupakan polimer plastik yang dikesan di antara partikel yang diekstrak dari spesies akuakultur, menunjukkan bahawa partikel itu berkemungkinan berasal dari struktur akuakultur yang digunakan di lokasi penternakan. **Kesimpulan:** Mikroplastik dikesan dalam produk akuakultur air tawar dan laut dari Selangor, Malaysia. Penyelidikan lebih lanjut diperlukan untuk memahami dengan lebih baik bagaimana struktur akuakultur yang digunakan / kegiatan akuakultur mempengaruhi pengumpulan MP di lokasi dan dalam spesies akuakultur.

Kata kunci: microplastik, akuakultur, air tawar, air masin, spesies

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ALDFG	Abandoned, lost or otherwise discarded fishing gears
ARC	Aquaculture Research Centre
DOFM	Department of Fisheries Malaysia
FAO	Food and Agriculture Organization of the United Nation
FTIR	Fourier-Transform Infrared Spectroscopy
GESAMP	Group of Experts on the Scientific Aspects of Marine Environmental Protection
GIT	Gastrointestinal tract
H_2O_2	Hydrogen Peroxide
HCL	Hydrochloric Acid
IVF	In-vitro fertilization
MPs	Microplastics
MSFD	European Union's Marine Strategy Framework Directive
MPs	Microplastics
NaCl	Sodium hydroxide
NaI	Sodium iodide
$NaSO_4$	Sodium sulphate
NGS	National Geographic Society
NY	Nylon
NOAA	National Oceanic and Atmospheric Administration
HDPE	High-density polyethylene
LDPE	Low-density polyethylene
PE	Polyethylene

PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
SEAFDEC	Southeast Asian Fisheries Development Center
UPM	Universiti Putra Malaysia
UV	Ultraviolet
$ZnCl_2$	Zinc chloride

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Microplastics (MPs) are small plastic particles with a length of fewer than five millimeters (0.2 inches) that are found in the ecosystem as a result of plastic pollution (Rogers, 2020). Microplastics vary in size, shape, colour, and density based on the polymer used and are typically classified based on their origins: primary and secondary (FAO, 2017). Primary MPs is defined as plastic particles that were purposely generated in a size range below five millimeters for industrial and commercial purposes, including plastic pellets used in industrial manufacturing, microbeads in cosmetics and personal care products, and plastic fibres shed from clothing or other textiles, such as fishing nets (GESAMP, 2015). On the other hand, secondary MPs are microscopic particles formed when larger plastic items, such as plastic bags, water bottles, and fishing equipment, degrade (GESAMP, 2015). Exposure to environmental conditions, such as wind abrasion, UV light exposure, temperature, and ocean waves, is the most common cause of secondary MPs deterioration (National Geographic Society, 2019).

Aquaculture is a method of breeding, raising, and harvesting aquatic organisms and plants in the aquatic environment under controlled conditions (NOAA, 2021). According to the Food and Agriculture Organization of the United Nations (FAO) (2019), Malaysia's aquaculture industry had contributed 26% of total fish production in 2014, with a promising increase in export revenue last year (6,374.99 tonnes compared to 5,986.18 tonnes on the previous year). Since this industry has shown rapid development over the years, demand for aquaculture products is expected to continue growing in line with the increasing pattern of population growth and incomes (FAO, 2008). In addition, there are two major aquaculture categories, which is freshwater and marine aquaculture (NOAA, 2021). Freshwater aquaculture is a farming method in inland water bodies like rivers, lakes, and brackish water (Li & Liu, 2018). On the other hand, marine aquaculture, also known as mariculture, is another farming method in the marine environment, which uses seawater as a breeding medium, for example, the open ocean, an enclosed section of the ocean, or in tanks, ponds or raceways which are filled with seawater (Mariculture, 2021).

Plastic material is most preferred in the development of the aquaculture sector because it offers greater strength, durability and is relatively inexpensive (Lusher et al., 2017). FAO (2017) states that abandoned lost or otherwise discarded fishing gears (ALDFG) are the primary source of plastic waste in the sectors. These structures and facilities are mostly made of plastic materials, tend to degrade over time and pollute aquaculture sites, particularly those in sheltered areas or tanks where microplastics cannot easily be flushed out into the open ocean (NOAA, 2017). As a result, microplastic accumulation is likely to be more severe in aquaculture sites than in the wild, which later contribute to the accumulation of MPs in aquatic species as they can mistake microplastics for food (Mathalon and Hill, 2014; NOAA, 2021). Additionally, the fish meal can also influence the occurrence of MPs in aquaculture sites, and these particles possibly end up in cultivated species through direct ingestion or food chain (Castelvetro et al., 2021).

1.2 Research Problem

So far, several studies have discovered MPs debris in aquaculture species from the worldwide aquaculture industry. A study by Feng et al. (2019) stated that all aquaculture fish in Haizhou Bay, China, consist of many MPs abundance, and the MPs accumulation in the skin or gills was higher than in the digestive tract. Besides, two bivalve species cultured in Germany and France was contaminated with MPs, with an average plastic load of 0.36 ± 0.07 particles per gram tissue (mussel) and 0.47 ± 0.16 particles per gram tissue (oyster) (Van Cauwenberghe and Janssen, 2014). Moreover, a recent study carried out by Priscilla and Patria (2020) also reported the digestive tract of milkfish (*Chanos chanos*) in Jakarta Bay, Indonesia, containing MPs as many as 3,005 particles per individual for a total abundance value of 9.58±3.3 particles per gram. However, there is insufficient information regarding MPs morphological characteristics and polymer composition in aquaculture species from Malaysia.

To date, there are several studies documenting MPs occurrence in aquatic species, such as commercial marine fish (Karbalaei et al., 2019), commercial freshwater fish (Karami et al. 2017, Sarijan et al. 2019), and canned seafood (Karami et al. 2018). However, the studies on microplastics in Malaysia are limited and still in their early stages. Therefore, little is known about MPs occurrence in aquaculture species since no study has focused on assessing MPs pollution and its accumulation in aquaculture products.

1.3 Study Justification

The findings from this study have provided insight into MPs pollution and its accumulation in selected aquaculture products from local aquaculture hatcheries in Selangor, Malaysia. The samples include commonly consumed aquaculture species such as African catfish (*Clarias gariepinus*), Red Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*Oreochromis mossambicus*), Jade perch (*Scortum barcoo*), Hybrid grouper (tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) and Whiteleg shrimp (*Litopenaeus vannamei*). Since the aquaculture sector provides animal protein sources intended for human consumption and contributes an annual growth rate of about 10 per cent in the last five years (FAO, 2019), these findings evaluate the MPs risk in selected aquaculture products that could impact human health.

1.4 Conceptual Framework

Figure 1.1 shows the conceptual framework for this study. The independent variable (IV) is the total number of particles extracted from aquaculture products collected from local aquaculture hatcheries, while the dependent variable (DV) is the MPs characteristics and polymer composition.

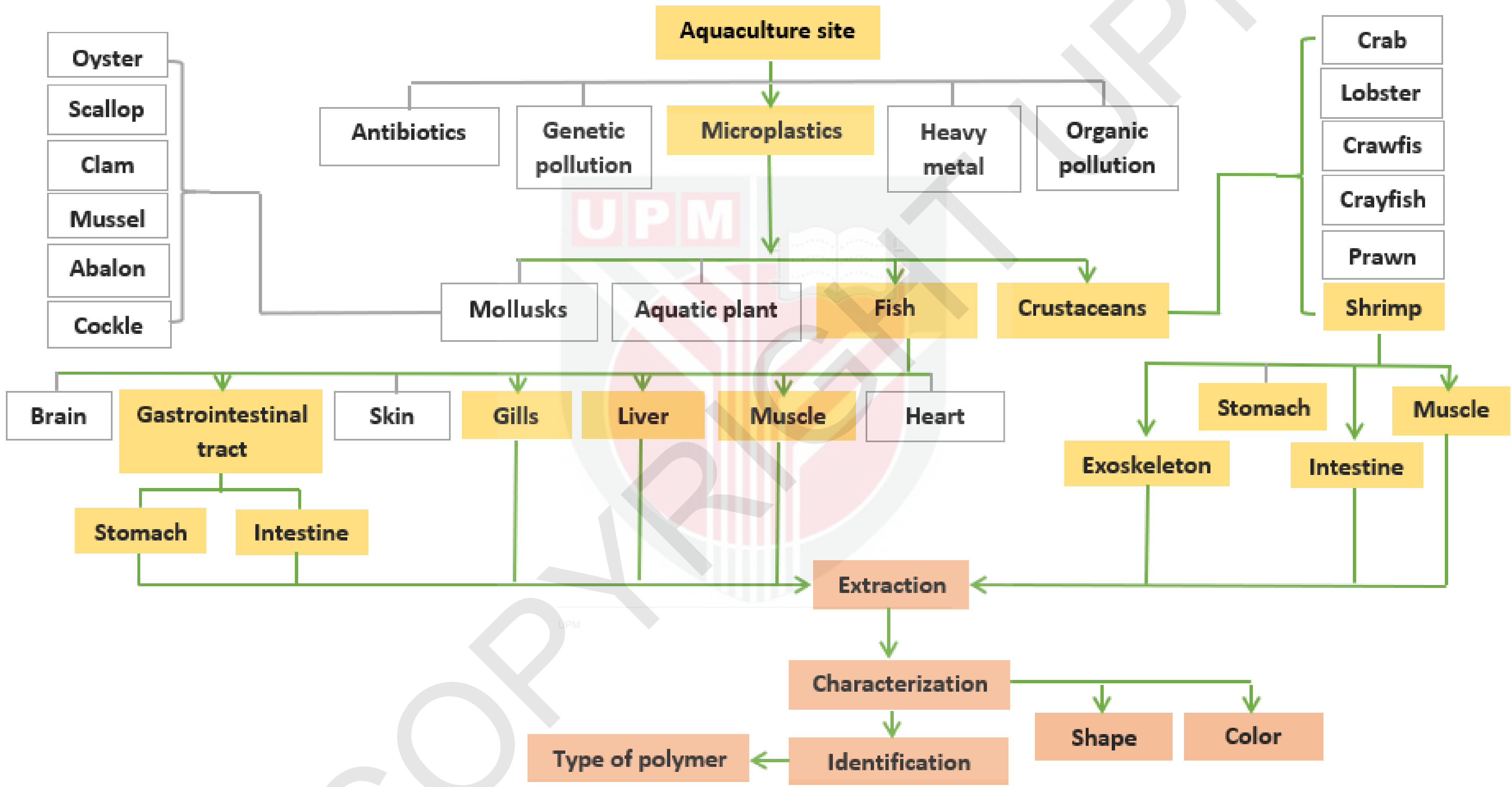


Figure 1.1: Conceptual framework

1.5 Research Objectives

1.5.1 General Objective

To assess MPs pollution and its accumulation in selected aquaculture products (fish and prawn) from local aquaculture hatcheries in Selangor, Malaysia.

1.5.2 Specific Objectives

I. To determine the occurrence of MPs in selected aquaculture products: African catfish (*Clarias gariepinus*), Red nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*Oreochromis mossambicus*), Jade perch (*Scortum barcoo*), Hybrid grouper (tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) and Whiteleg shrimp (*Litopenaeus vannamei*).

II. To evaluate the characteristics and composition of MPs in selected aquaculture products species.

1.6 Research Hypotheses

I. Microplastics are detected in both of marine and freshwater aquaculture products from local aquaculture hatcheries in Selangor, Malaysia.

1.7 Definition of Variables

Independent variable (IV): Total number of particles extracted from aquaculture products.

Dependent variable (DV): Microplastics characteristics (color, shape) and polymer composition (PP, PE, PET, PVC, HDPE, LDPE).

CHAPTER 2

LITERATURE REVIEW

2.1 Microplastics

Microplastics (MPs) are small plastic particles (Figure 2.1) with a diameter of less than five millimeters. In practical terms, this definition has been established and frequently used since it is the size at which many aquatic biota species consume food (GESAMP, 2015). The US National Oceanographic and Atmospheric Administration (NOAA) and the European Union's Marine Strategy Framework Directive (MSFD) have both recognized this wide definition of MPs. In addition, the entry routes for MPs into the environment will be determined by their application as stated in Table 2.1.

Figure 2.1: Microplastics

Table 2.1: Route of entry for MPs into the environment (Source: FAO, 2017)

Primary MPs	
Microbeads from cosmetic and personal care products	Wastewater
Microplastics from abrasive blasting	Atmosphere and wastewater
Pre-production resin pellets or nurdles that used as raw material in plastic manufacturing	Accidental loss from spills during transportation and transshipment process, runoff from processing plants
Secondary MPs	
Plastics from textiles	Wastewater during washing process or through air when drying (Browne et al., 2011; Napper and Thompson, 2016);
Plastics used in agricultural applications	Surface runoff from soil
Abrasion of tyres	Air and surface runoff
Plastic items from landfills	Atmosphere, surface runoff, rivers and the ocean by wind

Once those MPs are released into the aquatic environment via waste, they can transfer into the effluent streams and are small enough to be filtered in certain wastewater treatment plants. The majority of them would then be carried to seas by rivers, while the remaining would stay in potable water and freshwater environments, including such isolated water systems as remote mountain lakes (Browne et al., 2010; Free et al., 2014). This situation showed that microplastics in the water environment had influenced the possible uptake of microplastics by aquatic organisms through bioaccumulation and biomagnification (Cole et al., 2011; de Sá et al., 2018). As a result, MPs in the marine and inland waterways are accidentally consumed by many commercially valuable aquatic animals and accumulated in their bodies (FAO, 2017).

2.1.1 Morphological Characteristics of Microplastics

Microplastics are primarily characterized by their morphological characteristics, which include size, shape, and color (FAO, 2017). Size is a critical factor when analyzing MPs as it determines the spectrum of species that it may impact (FAO, 2017). MPs size limits are generally said to range between one millimeter and five millimeters since it is the size at which many aquatic biota species consume food (GESAMP, 2015). Nonetheless, in certain research, larger MPs are classified as one millimeter to five millimeters, whereas smaller MPs are described as less than one millimeter (Van Cauwenberghe and Janssen, 2014). Since the sizes of the MPs studied and the size classifications differed significantly among studies, a direct comparison of MPs abundance in precise size ranges is said to be impossible.

In addition, most studies reported varied shapes of MPs, and the majority of them did not have consistent shape categories. However, all detected shapes of MPs are taken into account, namely fragments, fibres, beads, foams and pellets (Lusher et al., 2017; EFSA, 2016) (Table 2.2). Figure 2.2 shows the shapes of collected MPs from inland waterways in China (Qinghai Lake and Three Gorges Reservoir) (Wu et al., 2018). So far, fragments have previously been reported as the dominating shape in various environmental media (Eriksen et al., 2013; Cózar et al., 2015), whereas fibres appeared to be more numerous in urbanized areas (Wu et al., 2018). As reported by Su et al. (2016), the most common microplastics detected in Lake Hovsgol were fragments and films, which accounted for 78 percent of all microplastics, while fibres and fragments were highly significant in samples from Taihu Lake. Also, Wang et al. (2018) have observed an abundance of fiber, granule, film, and pellet in Wuhan's inland freshwaters, with fibres accounting for 52.9–95.6 percent of the total plastics found.

Table 2.2: Categories used when classifying MPs by shape (Source: FAO, 2017)

Shape classification	Other term used
Fragments	Irregular shaped particles, crystals, fluff, powder, granules, shavings, flakes, films
Fibres	Filaments, microfibers, strands, threads, lines
Beads	Grains, spherical microbeads, microspheres
Foams	Polystyrene, Expanded Polystyrene
Pellets	Resin Pellets, nurdles, pre-production pellets, nibs

Figure 2.2 Shapes of typical microplastics (Source: Wu et al., 2018)

Aside from that, another way to categorize microplastics is by their color. MPs colors are documented in studies and reported throughout a broad range. Some studies, for example, describe MP colors in detail, such as transparent, blue, and black, while others reveal MPs to be colored or colorless/transparent. Yin et al. (2019) categorized the MPs in the samples according to their color and divided into six color classifications: transparent, black, white, red, blue and other. According to Que et al. (2018), colored MPs are more likely to be swallowed by aquatic organisms compared with transparent MPs, which will cause damage to their health (Qu et al., 2018). Nevertheless, visual identification of MPs cannot be relying entirely on color as their colors can differ from the original plastic product in response to degradation and exposure to environmental factors (Wu et al., 2018).

2.1.2 Microplastics Polymers

There are various plastics polymers, namely Polyvinyl Chloride (PVC), Polyethylene Terephthalate (PET), High-Density Polyethylene (HDPE), Low-Density Polyethylene (LDPE), Polypropylene (PP), Polystyrene (PS), Nylon (NY) and so on. These plastic polymers differ in terms of density and origin sources (Table 2.3). Polymer density of MPs has been identified as the primary factor that influences buoyancy, distribution, and bioavailability of MPs in the water column (Wang et al., 2016), as well as possible interactions with organisms (Wright et al., 2013). Positively buoyant plastics (those with a density less than a solution) will float at the top of the water column, whereas negatively buoyant plastics (those with a density greater than a solution) would sink to the bottom (Browne et al. 2013; Wright et al. 2013) (Figure 2.3). For example, PE (0.91-0.94 g/cm^3) and PP (0.90-0.92 g/cm^3) floats in seawater (approximately 1.027 g/cm^3) due to their lower density and are accessible for pelagic species. In contrast, PET (1.34–1.39 g/cm^3) and PVC (1.16 – 1.30 g/cm^3) sinks rapidly to the seafloor for having higher density and are mainly available for benthic species (Weber et al. 2018).

Table 2.3: Specific density of plastic polymers and different water types, and its common applications (Source: GESAMP, 2015)

Plastic type	Common applications	Density (g/cm³)
Low-density polyethylene (LDPE)	Plastic bags and packaging materials	0.91 - 0.94
High-density polyethylene (HDPE)	Pipes, net, ropes, storage containers, plastic equipment	0.95 – 0.97
Polypropylene (PP)	Rope, bottle caps, fishing gears, strapping	0.90 – 0.92
Polystyrene (expanded) (PS)	Cool boxes, floats, cups	1.01 – 1.05
Polystyrene (PS)	Utensils, containers	1.04 – 1.09
Polyvinyl chloride (PVC)	Film, pipe, containers	1.16 – 1.30
Polyamide or Nylon (NY)	Fishing nets, rope	1.13 – 1.15
Polyethylene terephthalate (PET)	Bottle, strapping, textiles	1.34 – 1.39
Polyester resin + glass fibre	Textiles, boats	>1.35
Cellulose Acetate	Cigarette filters	1.22 – 1.24
Pure water		1.000
Brackish water		1.005 – 1.012
Sea water		1.027

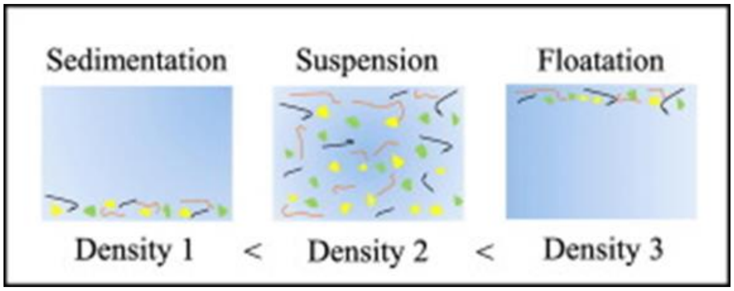


Figure 2.3 Polymer density influences the position of MPs in water column

2.2 Aquaculture industry

Aquaculture is an important sector that provides animal protein sources for human consumption (FAO, 2018). Since its development in the past centuries, aquaculture has been one of the fastest-growing industries in the food production sector. It is also playing a vital role in food supply and security, nutrition, income, employment, and contributing to economic growth and development for millions of people worldwide (FAO, 2018). Table 2.4 summarizes the global production of aquatic food animals by capture fisheries and aquaculture in 2016. Based on the data, capture fisheries contribute for 53 percent (90.9 Mt.), while aquaculture produces 47 percent (80 Mt.) of aquatic products (FAO, 2018). Even though capture fisheries still produce more food for human consumption than aquaculture (Edwards et al., 2019), it is not denied that aquaculture also contributes to global fisheries production to fulfil the demand for food sources (Boyd & Davis, 2020).

Table 2.4: Global production by capture fisheries and aquaculture of fish, crustaceans and mollusks in 2016 (Source: FAO, 2018)

Production category	Production quantity (million tonnes)
Inland capture fisheries	11.6
Marine capture fisheries	79.3
Total capture fisheries	90.9
Inland aquaculture	51.4
Marine aquaculture	28.7
Total aquaculture	80.0
Total fisheries and aquaculture	170.9
Human consumption	151.2
Non-food uses	19.7

According to the Food and Agriculture Organization of the United Nations (2018), the major aquaculture producer were Asian countries (China, Indonesia, India, Vietnam, and Bangladesh), which contributes to 89.4 percent of total global production in 2016, followed by Europe (3.67%), South America (3.38%), Africa (2.48%), North America (0.81%) and Oceania (0.26%). Furthermore, about 543 species groups of animals were documented in the FAO aquaculture database, including 362 finfish, 104 molluscs, 62 crustaceans, six amphibians and reptiles, and nine aquatic species animals' invertebrates (FAO, 2016b). Three major animal groups comprised of fish, crustacean (prawn, shrimp, crab, lobster, crayfish, crawfish) and mollusc (clam, oyster, mussel, cockle, abalone, scallop) in Figure 2.4 accounted for about 82.8 percent of global aquaculture production (FAO, 2016b). Besides, amphibians, reptiles, and aquatic invertebrates contribute another 17.2 percent (FAO, 1996).

Crustacean Fish Mollusc

Figure 2.4 Three major animal groups in aquaculture industry

The aquaculture sector in Malaysia has been recognized as a potential way to meet increasing demand in the domestic and global markets based on climate suitability, the potential production area and availability of technology (DOFM, 2017). The data on the total aquaculture production in Malaysia from 1980 to 2019 was shown in Figure 2.5, whereas the aquaculture production by categories for human consumption was summarized in Figure 2.6. The significant increase in production can be seen since its development in the 1920s, and the highest aquaculture production (373,034 tonnes) was recorded in 2010, comprising 155,399 tonnes of freshwater fish, 24,326 tons of marine finfish, 103,943 tonnes of marine shrimp and 89,366 tonnes of molluscs. In 2012, this sector had come up with 302,886 tonnes of production valued at RM 2,559 million and recorded an annual growth rate of about 10 percent in the last five years (FAO, 2019). Based on the production pattern, freshwater fish are the most popular aquaculture products, followed by marine finfish, prawn/shrimp, and molluscs.

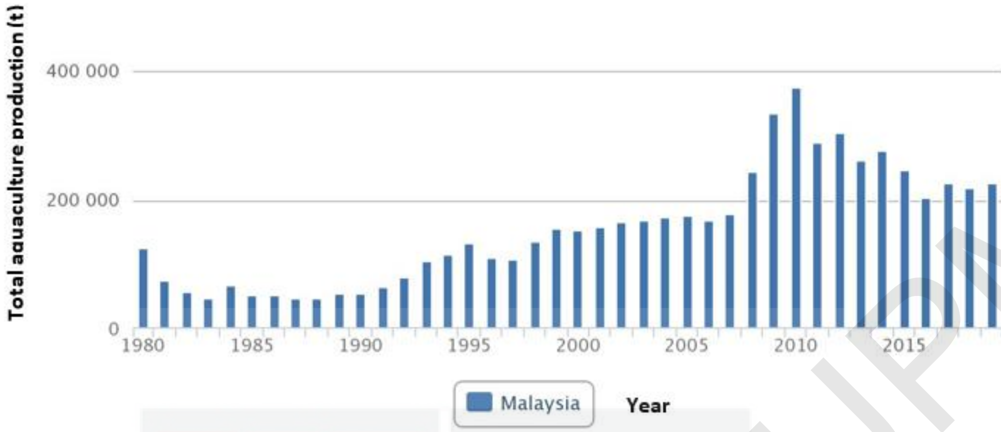
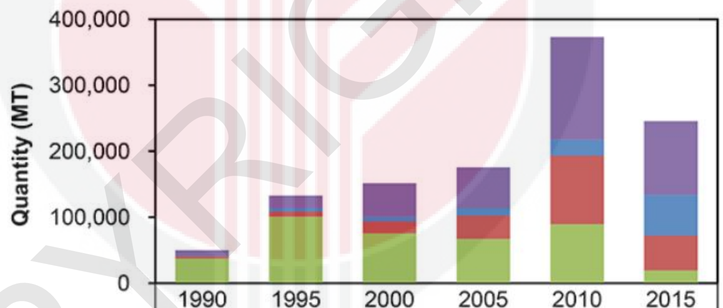


Figure 2.5: Total aquaculture production for Malaysia 1980-2019 (tonnes)

(Source: FAO FishStat, 2020)



	1990	1995	2000	2005	2010	2015
Freshwater fish	9,689	18,499	50,688	62,007	155,399	112,145
Marine finfish		5,761	7,966	11,259	24,326	61,492
Marine shrimp	3,045	7,406	17,418	35,104	103,943	52,571
Mollusks	37,514	101,080	75,695	67,425	89,366	19,372
Total (MT)	50,248	132,746	151,767	175,795	373,034	245,580

Figure 2.6: The aquaculture production for human consumption by categories in Malaysia in 1990-2015 by quantity (MT) (Source: SEAFDEC, 2017)

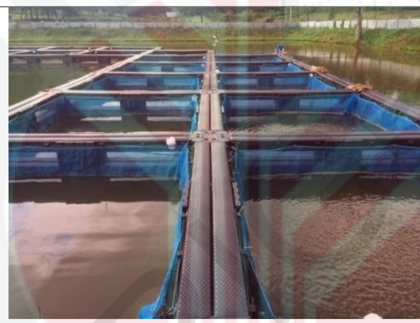
2.2.1 Freshwater aquaculture

Freshwater aquaculture refers to farming aquatic species for economic purposes in inland water bodies, including ponds, rivers, lakes, and brackish water. This type of breeding was dominated by several species of finfish, especially carps and tilapias (FAO, 2018). The freshwater aquaculture industry has contributed for 29 percent of world aquaculture production (44.3 million tonnes) in 2011 (Boyd, 2013) and representing around 63 percent of world aquaculture production (48 million tonnes) in 2015 (FAO, 2019). Yusoff (2015) documented that Malaysia's freshwater aquaculture provided 163,757 tonnes of products in 2012, valued at RM992 million at production, which comprised freshwater catfish (Clarias sp.), black and red tilapia (Oreochromis spp.), riverine catfish (Pangasius sp.), and giant freshwater prawn (*Macrobrachium rosenbergii*) as main cultured species.

So far, according to SEAFDEC (2017), freshwater aquaculture in Malaysia was dominated by the earthen pond system, net cages, and concrete tanks, covering 6,152 ha. The earthen pond culture developed throughout the country, covering the largest area of 4,769 ha and producing more than 80 percent of the freshwater aquaculture production (FAO, 2017). The following standard system is the floating net-cage culture, which involves modern polyethene cages in lakes, reservoirs and ex-mining pools, occupying 2,734 ha. Other than that, concrete breeding tanks, followed by canvas and polyethene materials, is also preferred in breeding freshwater species. Table 2.5 shows different types of culture system in the freshwater hatcheries in Malaysia.

Table 2.5: Types of culture system used in freshwater aquaculture in Malaysia

(Source: SEAFDEC, 2017; Aquaculture Research Centre, n.d.)

Type of culture system		Advantages/Limitation
Earthen pond		- preferred for large-scale fish farming projects - low construction cost - allow rapid growth of species
Net-cage		- simple water quality management - high construction cost
Concrete tank		- easy management - short harvesting process - high construction cost
Canvas/PP tank		

2.2.2 Marine aquaculture

Marine aquaculture, also known as mariculture, is another farming method in the marine environment, which uses saltwater as a breeding medium. This type of culture is mainly utilized in the open ocean, an enclosed section of the ocean, or tanks, ponds, or raceways filled with seawater (Mariculture, 2021). In 2012, this method contributed about 139,129 tonnes valued at RM1,566.78 million within a 7,978-ha cultured area (Yusoff, 2015). The main cultured species were marine prawns (*P. monodon* and *P. vannamei*), cockles (*Anadara granosa*), marine finfish, mussels (*Perna viridis*) and other species. Many systems are employed in mariculture, but the preferred systems are earthen ponds, net cages and rafts (mussel and oyster), and bottom culture (cockle) and long line (seaweed). Marine fish or finfish are largely harvesting in cages or tanks (Yusoff, 2015). Figure 2.7 shows two different systems utilized in local mariculture hatcheries.

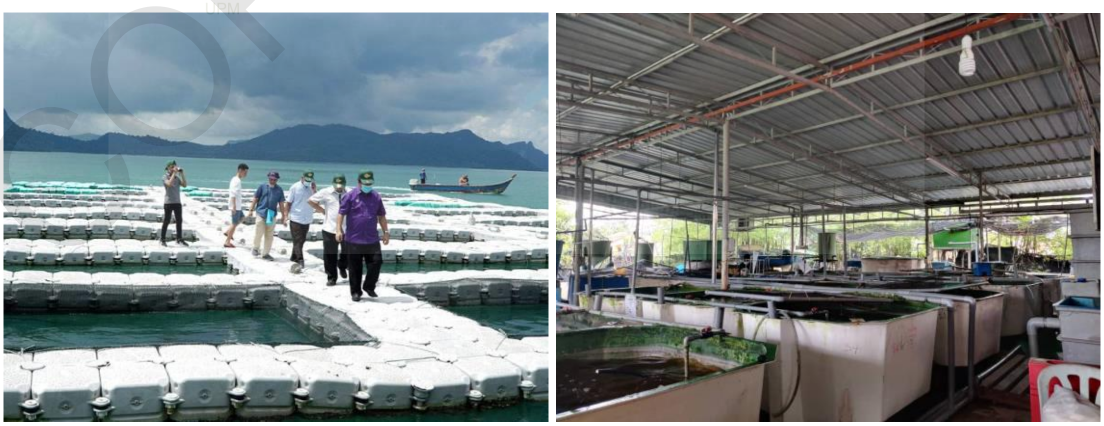


Figure 2.7: Mariculture hatcheries (a) net cage system in open ocean, (b) tank system

2.3 Factors contribute to the occurrence of microplastics in aquaculture

There are several factors contribute to the occurrence of MPs in aquaculture include aquaculture structures and facilities and fish meals.

2.3.1 Aquaculture structures and facilities

An aquaculture facility could be constructed with different types of structures. The type of structure could be determined by several elements, including the species to be cultivated, the scale of operation, site location, financing resources, and climate. Since the plastic industry significantly influences the development of the aquaculture sector, most traditional structures and facilities have already been replaced with synthetic or semi-synthetic materials, which provide better strength and durability (Valdemarsen, 2001). Aside from that, Valdemarsen (2001) also stated that plastics material is preferred from small domestic facilities to highly technical systems because they are cheap and easily sourced and maintained. Lusher et al. (2017) reported that aquaculture structures such as ropes, floats, buoy, nets, cages, pond lining, fish feeders and fish tanks at the sites, as well as seafood packaging and transportation, are primarily made of plastics (Figure 2.8).

Figure 2.8: Fishing structure and facilities used in aquaculture sites

Unfortunately, through continuous exposure to direct UV light, wave action, abrasion and temperature changes, a proportion of these materials utilized in aquaculture sites may undergo degradation and deterioration process over time. Extreme weather conditions can also cause extensive damage to aquaculture facilities, resulting in enormous amounts of microplastics debris at the sites at times. Besides, abandoned, lost, or otherwise discarded fishing gear (ALDFG) has been identified as the primary source of plastic debris in the aquatic environment (FAO, 2016b). Because of its durability, plastic will take a long time to break down, and the debris will further accumulate and pollute the sites (Lusher et al., 2017).

2.3.2 Fish meal

Fish meal (Figure 2.9) is a nutrient-rich feed product mainly used to feed farm animals in aquaculture and intensive animal farming, mainly fish and shrimp. It recognized as an excellent source of protein and other nutrients such as essential fatty acids, phospholipids, cholesterol, minerals, and specific vitamins, with minimal carbohydrate content (Tacon et al., 2009). According to The Fish Site (2006), the application of fishmeal to animal diets encourages fast growth, minimizes feeding costs, and promotes nutrient uptake, digestion, and absorption. Besides, fishmeal can be produced from practically any seafood species, although it is commonly produced from wild-caught, tiny marine fish with a high percentage of bones and oil, which is then processed and manufactured.

Figure 2.9: Fish meal in pellet form

Nevertheless, recent studies have revealed the accumulation of MPs in fish meal intended for aquaculture species consumption. But since MPs particles were known to be nearly ubiquitous in the marine environment, MPs can end up in fish commercialized for direct human consumption and the industrial production of fishmeal through ingestion of direct MPs from the environment or through the food chain (Castelvetro et al., 2021). Furthermore, as Hanachi et al. (2019) indicated, the marine-derived fish meal may be a source of MPs that could be passed to cultured fish, posing a concern for aquaculture. In 2020, Pennino et al. discovered MPs in wild-caught fish, including *Sardina pilchardus* and *Engraulis encrasicolus*, primarily used to manufacture fish meals. Similarly, fishmeal prepared from whole Indian mackerel (*Rastrelliger kanagurta*) specimens contains 200–300 MPs per kg.

Additionally, MPs concentrations in the processed fishmeal appear to be significantly higher than in captured fish, which comprised 123.9 ± 16.5 MPs per kg of fishmeal, predominantly polyethylene and solely seem to consist of secondary MPs (Thiele et al., 2021). On the other hand, MPs occurrence in four varieties of marine-derived commercial fish meal in Iran was investigated by Hanachi et al. (2019), including salmon, sardine, and kilka fishmeal. They detected around 4000–6000 microplastics per kg of fish meal, with fragments being the most common shape of MP detected. Apart from that, a total of 336 MPs were found in three brands of commercial fish meal from Malaysia (Karbalaei et al., 2020). Fragments were the dominant form of MPs (78.2%), followed by filaments (13.4%) and films (8.4%), whereas polyethylene and polypropylene were the dominant polymers detected. This study reveals that the use of MP contaminated fish or shellfish in fish meal manufacturing can expose cultured organisms to high amounts of MPs.

2.4 Methods of microplastics separation

Several MPs separation methods have been developed and used in previous studies, known as density gradient separation, acid and alkaline digestion, and enzymatic digestion. Density gradient separation is a common method used to separate MPs from samples that use density gradient solutions such as sodium chloride (NaCl), sodium iodide (NaI), and zinc chloride ($ZnCl_2$). By applying this method, MPs float or sink status in the density gradient solutions was observed. Indeed, low-density MPs float to the surface when density gradient solution is added to the sample. For example, polypropylene (density 0.90-0.92 g/cm^3) will float in gradient solutions with a greater density, as mentioned in Table 2.6. However, the effectiveness of MPs separation depends on the density of gradient solutions. Claessens et al. (2013) stated that this method is only effective for MPs polymers with a density lower than the density of the saturated saline concentration. Based on polymer density stated in Table 2.6, plastics such as PVC (density 1.16 – 1.30 g/cm^3) or polyethylene terephthalate (density 1.34 – 1.39 g/cm^3) will not float in concentrated NaCl solution (1.2 g/cm^3).

Table 2.6: Density gradient solutions

	NaCl	NaI	NaSO4	ZnCl2
Density (g/cm)	1.2	1.5-3.67	2.66	2.91

Alkaline digestion, also known as bases digestion, is another common method. Several studies (Karami et al., 2016; Kühn et al., 2017; Bessa et al., 2018a; Sarijan et al., 2019) have used this technique for MPs extraction by utilising strong alkaline solutions, such as potassium hydroxide (KOH), sodium hypochlorite (NaClO), and sodium hydroxide (NaOH). Acid digestion, on the other hand, refers to the extraction of MPs using strong acid solutions such as nitric acid (HNO3), sulphuric acid (H2SO4), hydrogen peroxide (H2O2), and hydrochloric acid (HCl) in open or closed systems at high temperature and pressure (Claessens et al., 2013; Nuelle et al., 2014; Avio et al., 2015; Karami et al., 2016). Aside from that, enzymatic digestion involving Proteinase-K is also used in MPs extraction and general protein digestion in biological materials (Lindeque & Smerdon, 1999; Cole et al., 2014; Karami et al., 2016). The protocol, advantages and limitations for each method were summarized in Table 2.7 based on previous studies.

Table 2.7: Microplastics separation and extraction techniques in previous studies

Method	Protocol	Advantages	Limitations
Density gradient separation	• NaCl hypersaline solution (1.2 g/cm^3) (Claessens et al., 2011; Avio et al., 2015; Adeogun et al, 2020) • Saturated NaI (1.8 g/cm^3) and ZnCl2 (1.8 g/cm^3) (Li et al,	• Easy to be prepared in a density range of 0.8–1.8 g/cm^3 and feasible to measure the apparent density of MPs (Li et al, 2018) • Improved the detection and	• Not suitable for the extraction of high-density polymers (Claessens et al., 2013; Coppock et al., 2017) • $ZnCl_2$ had a larger uncertainty in measuring density

	2018) • Concentrated NaCl solution (1.2 kg L^{-1}) (Hidalgo-Ruz et al., 2012) • Concentrated NaCl, NaI and ZnCl2 solution (1.2-1.8 g/cm^3) (Coppock et al., 2017)	analysis step (Sarijan et al., 2019) • ZnCl2 (1.5 g/cm^3), able to extract MPs from sediments with a mean recovery rate of 95.8% in a single step (Coppock et al., 2017)	due to a higher surface tension (Claessens et al., 2013 • Costly (Nuelle et al., 2014) and time-consuming (Thompson et al., 2004)
Alkaline digestion	• 10 M NaOH at 60°C (Cole et al., 22014), • 5 M of NaOH (Karami et al., 2017) • 10% KOH (Karami et al., 2017; Sarijan et al., 2019) • 5% NaClO (Karami et al., 2017)	• KOH is the most suitable solution to digest GI tract of fish (Bessa et al., 2018a; Kühn et al., 2017)	• NaOH caused unsatisfactory digestion efficiency at all temperatures (Karami et al., 2016). • KOH degraded MPs and reduced their recovery rate (Karami et al., 2016)
Acid digestion	• 22.5 M HNO_3 (Claessens et al., 2013) • 5% or 37% HCl, 5% or 69% HNO_3 (Karami et al., 2017) • 30% H_2O_2 at 55 ºC for 7 days (Nuelle et al., 2014) • 35% H_2O_2 (Karami et al., 2017)	• H_2O_2 is a more effective agent for removing biogenic material from sediment samples than HCl or NaOH (Nuelle et al., 2014) • Treatment with HNO_3 produce high extraction yield for polystyrene spheres, and nylon fibers	• Not appropriate to extract all types of MPs polymers (Claessens et al., 2013). • HCl and HNO_3 degraded MPs and obtaining a low percentage of extraction yield (Avio et al., 2015)

	• 15% H_2O_2 at 50 ºC overnight (Avio et al., 2015)	(Claessens et al., 2013). • H_2O_2 digestion did not affect the particles in terms of plastic bleaching (Avio et al., 2015).	• HCl and HNO_3 caused unsatisfactory digestion efficiency at all temperatures (Karami et al., 2016). • Use of 35% H_2O_2 over a relatively long exposure may interfere MPs extraction process (Nuelle et al., 2014), degraded MPs and reduced their recovery rate (Karami et al., 2016)
Enzymatic digestion	• Proteinase-K (Lindeque & Smerdon, 1999; Cole et al., 2014)	• Rapid and efficient method proved capable of digesting >97% of MPs in zooplanktons samples without impacting MPs (Cole et al., 2014). • Allowing practical MPs isolation and low risk of external contamination (Cole et al., 2014).	• Not a cost-effective approach for larger organisms (Cole et al., 2014)

2.5 Previous Microplastics Studies

The occurrence of MPs in aquatic species has been reported worldwide. Researchers discovered MPs particles in various species and locations, including aquaculture sites, open ocean, coastal regions, market and fishing areas. Table 2.8 tabulate the findings of worldwide studies related to MPs accumulation in aquatic species. According to previous studies, many commercial aquatic species are often contaminated with MPs, comprising fragment and fibre as dominant shapes, whereas polyethylene and polypropylene are dominant polymers. For example, Karbalaei et al. (2019) detected MPs ranged between 0.2 and 34.9 mm in nine out of eleven samples from commercial marine fish species in Malaysia, with polyethylene as the primary plastic polymer. Recently, the presence of MPs was also reported in the commercially important species, Indian mackerel (*R. kanagurta*) (Karami et al., 2017a), oxeye scad (*S. boops*) (Karbalaei et al., 2019), Atlantic salmon (*S. solar*) (Liboiron et al., 2019), tongue sole (*Cynoglossus abbreviatus*) (Abbasi et al., 2018), shrimp scad (*Alepes djedaba*), and pickhandle barracuda (*Sphyraena jello*) (Akhbarizadeh et al., 2018). On the other hand, MPs was also identified in the livers of small size fish, such as European anchovies (*Engraulis encrasicolus*) (Collard et al., 2017), spotty-face anchovy (*S. waitei*) (Karami et al. (2017a) and Janapese anchovy (*E. japonicas*) (Tanaka and Tadaka, 2016). These findings have led to increasing concerns about the potential impacts of microplastics on human health.

Table 2.8: Summary of studies reporting the occurrence of microplastics in aquatic species

Species name	Location	Size range (μm)	Dominant shape	Dominant polymer	Reference
African catfish (*C. gariepinus*)	Malaysia	149 – 40,000	Fragment	[a]PE	Karbalaei et al. (2019)
Grass carp (*C. Idella*)	Malaysia	149 – 40,000	Fragment	PE	Karbalaei et al. (2019)
Indian mackerel (*R. kanagurta*)	Malaysia	149 – 40,000	Fragment	PE	Karbalaei et al. (2019)
Orange-spotted grouper (*E. coioides*)	Malaysia	149 – 40,000	Fragment	PE	Karbalaei et al. (2019)
Spotty-face anchovy (*S. waitei*)	Malaysia	NR	Fragment	[b]PP	Karami et al. (2017a)
Greenback mullet (*C. subviridis*)	Malaysia	NR	Fragment	PP	Karami et al. (2017a)
Belanger's croaker (*J. belangerii*)	Malaysia	NR	Fragment	PP	Karami et al. (2017a)
Indian mackerel (*R. kanagurta*)	Malaysia	NR	Fragment	PP	Karami et al. (2017a)
Oxeye scad (*S. boops*)	Malaysia	149 – 40,000	Fragment	PE	Karbalaei et al. (2019)
Mullet	Indonesia	20 – 100,000	Fibre	[d]NR	Hastuti et al. (2019)

Species name	Location	Size range (μm)	Dominant shape	Dominant polymer	Reference
(*M. cephalus*)					
Clupeidae (*A. chacunda*)	Indonesia	20 – 100,000	Fibre	NR	Hastuti et al. (2019)
Siganidae (*S. canaliculatus*)	Indonesia	20 – 100,000	Fibre	NR	Hastuti et al. (2019)
Balistidae (*A. stellari*)	Indonesia	20 – 100,000	Fibre	NR	Hastuti et al. (2019)
Atlantic cod (*G. morhua*)	Canada	>1000	Fibre	PE, [c]PVC	Liboiron et al. (2019)
Atlantic salmon (*S. solar*)	Canada	>1000	Fibre	PE, PVC	Liboiron et al. (2019)
Capelin (*M. villosus*)	Canada	>1000	Fibre	PE, PVC	Liboiron et al. (2019)
Shrimp scad (*A. djedaba*)	Iran	<100, 100-5000, >5000	Fibre	NR	Akhbarizadeh et al. (2018)
Orange-spotted grouper (*E. coioides*)	Iran	<100, 100-5000, >5000	Fibre	NR	Akhbarizadeh et al. (2018)
Pickhandle barracuda (*S. jello*)	Iran	<100, 100-5000, >5000	Fibre	NR	Akhbarizadeh et al. (2018)
Christmas wrasse (*T. tribobatum*)	South Pacific	<300,000, 300,000 – 500,000, >500,000	Fragment	NR	Forrest and Hindell (2018)

Species name	Location	Size range (µm)	Dominant shape	Dominant polymer	Reference
Coronation grouper (*V. louti*)	South Pacific	<300,000, 300,000 – 500,000, >500,000	Fragment	NR	Forrest and Hindell (2018)
Surge wrasse (*T. purpureum*)	South Pacific	<300,000, 300,000 – 500,000, >500,000	Fragment	NR	Forrest and Hindell (2018)
Daisy parrotfish (*C. sordidus*)	South Pacific	<300,000, 300,000 – 500,000, >500,000	Fragment	NR	Forrest and Hindell (2018)
Black surgeonfish (*A. gahhm*)	Saudi Arabia	2700	Fibre	PP, PE	Baalkhuyur et al. (2018)
Klunzinger's wrasse (*T. rueppellii*)	Saudi Arabia	1930	Fibre	PP, PE	Baalkhuyur et al. (2018)
Bluestripe snapper (*L. kasmira*)	Saudi Arabia	2160	Fibre	PP, PE	Baalkhuyur et al. (2018)
Smalltooth emperor (*L. microdon*)	Saudi Arabia	1480	Fibre	PP, PE	Baalkhuyur et al. (2018)
Tang's snapper (*L. carnolabrum*)	Saudi Arabia	1870	Fibre	PP, PE	Baalkhuyur et al. (2018)
Indian mackerel (*R. kanagurta*)	India	<500	Fibre, Fragment	PE	Karthnik et al. (2018)
Java Rabbitfish (*S. javus*)	India	<500	Fibre, Fragment	PE	Karthnik et al. (2018)

Species name	Location	Size range (µm)	Dominant shape	Dominant polymer	Reference
Threadfin Sea Catfish (*A. arius*)	India	<500	Fibre, Fragment	PE	Karthnik et al. (2018)
Common Ponyfish (*L. equulus*)	India	<500	Fibre, Fragment	PE	Karthnik et al. (2018)
Acoupa weakfish (*C. acoupa*)	Brazil	<5000	Filaments	NR	Ferreira et al. (2018)
Catfish (*H. littorale*)	South america	1000 – 12,000	Fibre	NR	Silva-Cavalcanti et al. (2017)
Atlantic cod (*G. morhua*)	North atlantic	>1000	Fragment	NR	Liboiron et al. (2019)
Janapese anchovy (*E. japonicas*)	Japan	100 - 7000	Fragment	PP, PE	Tanaka and Tadaka (2016)
Blue rockfish (*S. mystinus*)	USA	NR	Fibre	NR	Rochman et al. (2015)
Pacific sanddab (*Ci. sordidus*)	USA	NR	Fibre	NR	Rochman et al. (2015)
Common sole (*S. solea*)	Portugal	2170 - 4810	Fibre	PP, PE	Neves et al. (2015)
Common torpedo (*T. torpedo*)	Portugal	2170 - 4810	Fibre	PP, PE	Neves et al. (2015)

[a] PE: Polyethylene, [b] PP: Polypropylene, [c] PVC: Polyvinyl chloride, [d] NR: Not reported.

CHAPTER 3

RESEARCH METHODOLOGY

This cross-sectional study has investigated MPs occurrence in muscles, liver, gastrointestinal tracts (GITs) and gills of five commonly consumed fish species which are African catfish (*Clarias gariepinus*), Red Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*Oreochromis mossambicus*), Jade perch (*Scortum barcoo*), and Hybrid grouper (tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*). This study also assessed MPs occurrence in muscles, stomach, intestine and outer layer (exoskeleton) of a prawn species, Whiteleg shrimp (*Litopenaeus vannamei*). The aquaculture species were collected from freshwater and marine aquaculture hatcheries around Selangor, Malaysia.

3.1 Chemicals

There are two chemicals been utilized in this study, including 30% Hydrogen Peroxide solution (H_2O_2) (J.T.Baker) and Sodium Chloride (NaCl) (Bendosen). NaCl hypersaline solution (1.2 g/cm^3) was prepared by dissolving 300 g NaCl in 250 mL of hot distilled water.

3.2 Sample collection

In this study, ten samples comprising two freshwater species were obtained from a local freshwater aquaculture hatchery: African catfish (*Clarias gariepinus*) (K: n = 5) and Red nile tilapia (*Oreochromis niloticus*) (TM: n = 5). Also, another 18 samples comprising four marine species were collected from a marine aquaculture hatchery: Mozambique tilapia (*Oreochromis mossambicus*) (TH: n = 5), Jade perch (*Scortum barcoo*) (JP: n = 5), Hybrid grouper (tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*) (HG: n = 5) and Whiteleg shrimp (*Litopenaeus vannamei*) (P: n = 3). Table 3.1 shows the aquaculture species collected from aquaculture hatcheries.

Table 3.1: The aquaculture species collected from freshwater and marine aquaculture hatcheries.

Type of aquaculture	Species	
Freshwater	African catfish (*Clarias gariepinus*)	

	Red nile tilapia (*Oreochromis niloticus*)	
Marine	Mozambique tilapia (*Oreochromis mossambicus*)	
	Jade perch (*Scortum barcoo*)	

Hybrid grouper (Tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	
Whiteleg shrimp (*Litopenaeus vannamei*)	

Those selected species, excluding hybrid grouper species, are the most preferred and frequently consumed seafood in Malaysia and are usually used in other studies (Ahmad et al., 2016; Anual et al., 2018, Karbalaei et al., 2019). Also, hybrid grouper is a new type of grouper that was cross-bred by fertilising the eggs of the tiger grouper (*Epinephelus fuscoguttatus*) with the sperm of the giant grouper (*Epinephelus lanceolatus*) through the in-vitro fertilisation (IVF) technique. During sample collection, the samples have been randomly selected from a tank. Then, the samples were transported to the laboratory and maintained at −20 °C in the freezer until further analysis.

3.3 Sample preparation

The samples were thawed at room temperature and repeatedly rinsed with distilled water. Samples morphometric data, including total length, was measured with a measuring ruler, while the whole-body weight and body part weight was measured with an electronic analytical balance (GR-200) (A&D Instruments). Table 3.2 shows the average weight and length of samples.

Table 3.2: Average total weight and length of selected aquaculture species

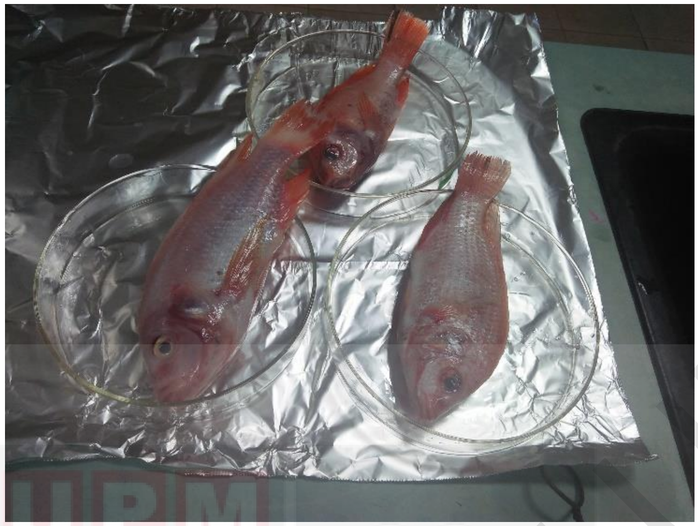
Common name	Species	Aquaculture hatcheries	Number of replicates	Average weight (g)	Average length (cm)
African catfish	*Clarias gariepinus*	Freshwater	5	121.61	31.4
Nile tilapia	*Oreochromis niloticus*	Freshwater	5	132.42	19.5
Mozambique tilapia	*Oreochromis mossambicus*	Marine	5	178.72	19.5
Jade perch	*Scortum barcoo*	Marine	5	210.05	21.4
Hybrid grouper (Tiger grouper X giant grouper)	(*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	Marine	5	500.39	31.5
Whiteleg shrimp	*Litopenaeus vannamei*	Marine	3	35.03	-

Dissection then was carried out by using scissors and blades after the fish scales were removed. The fish samples were filleted, and the edible portions of muscles were collected. Then, the muscles, livers, gills, and GITs of fish were gathered and weighted.

Besides, the outer shells (exoskeleton), muscles, stomach and intestines of shrimp samples were also taken and weighted. Table 3.3 shows the average weight of their body parts. Steps for the sample preparation procedure are shown in a flowchart in Figure 3.1, while the collected body parts of each species were shown in Table 3.4.

Table 3.3: Average total weight of body part of selected aquaculture species

Common name	Species	Code	Body part	Number of replicates	Average weight (g)
African catfish	*Clarias gariepinus*	K M	Muscle	5	45.05
		K GL	Gills	5	6.44
		K GIT	GIT	5	6.34
		K L	Liver	5	0.24
Red nile tilapia	*Oreochromis niloticus*	TM M	Muscle	5	25.36
		TM GL	Gills	5	5.18
		TM GIT	GIT	5	5.72
		TM L	Liver	5	1.08
Mozambique tilapia	*Oreochromis mossambicus*	TH M	Muscle	5	55.90
		TH GL	Gills	5	4.35
		TH GIT	GIT	5	17.99
		TH L	Liver	5	0.39
Jade perch	*Scortum barcoo*	JP M	Muscle	5	75.87
		JP GL	Gills	5	2.52
		JP GIT	GIT	5	2.15
		JP L	Liver	5	2.08
Hybrid grouper (Tiger grouper X giant grouper)	(*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	HG M	Muscle	5	34.60
		HG GL	Gills	5	2.85
		HG GIT	GIT	5	1.43
		HG L	Liver	5	1.29
Whiteleg shrimp	*Litopenaeus vannamei*	P M	Muscle	3	21.09
		P E	Exoskeleton	3	13.45
		P S	Stomach	3	0.20
		P I	Intestine	3	0.29



The samples were thawed at room temperature and rinsed with distilled water.

The whole-body weight and length were measured with an electronic analytical balance.

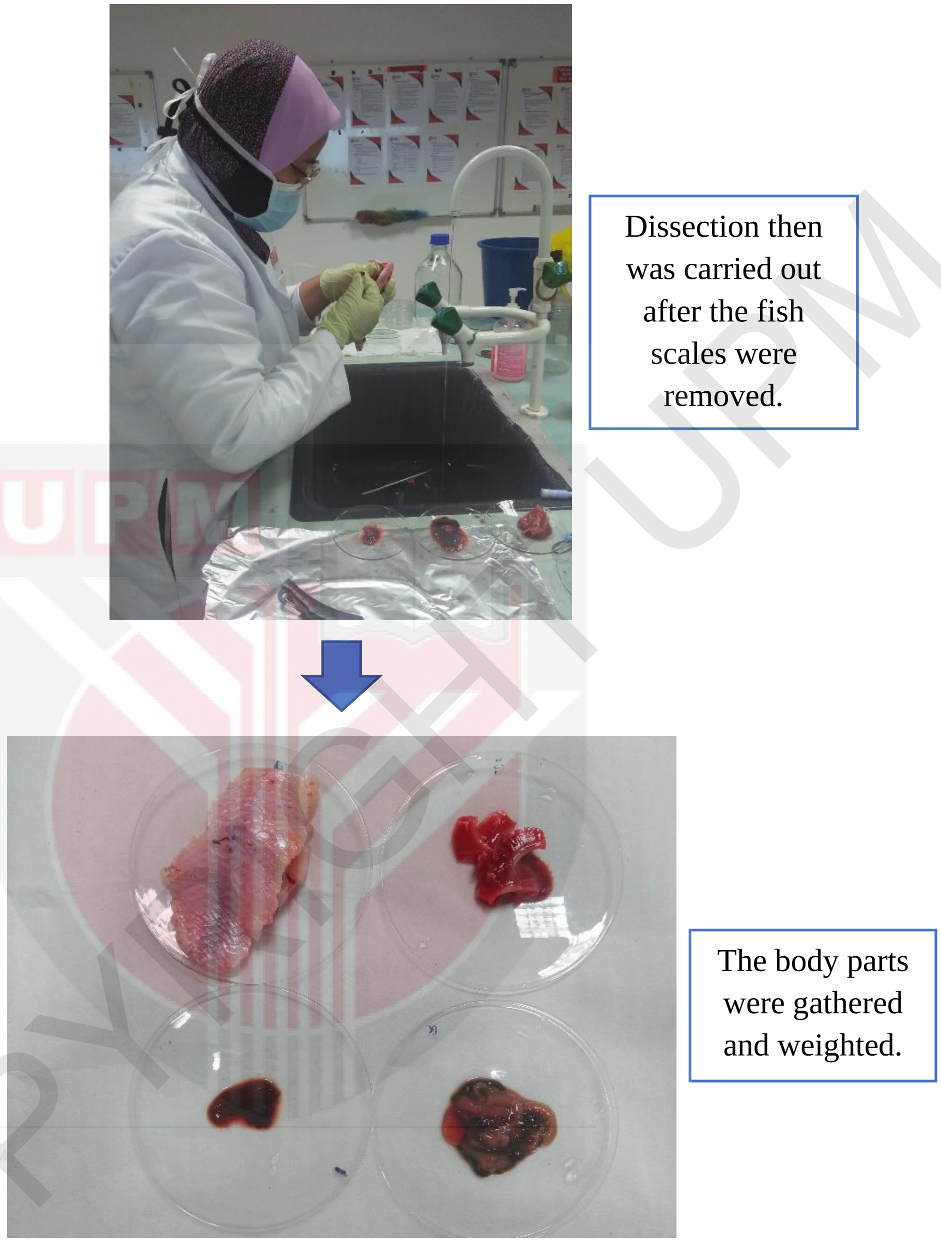
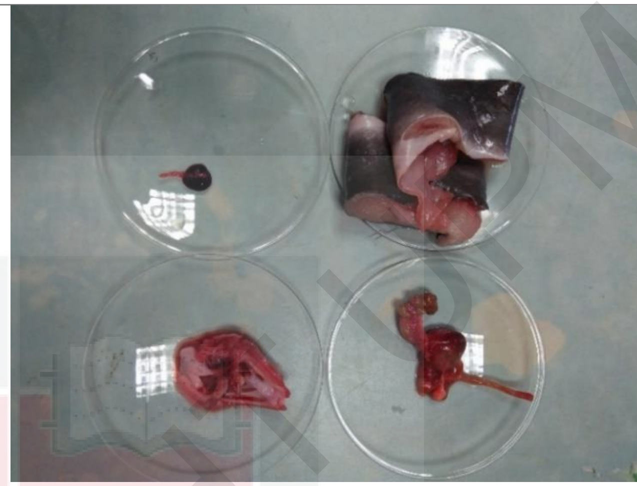
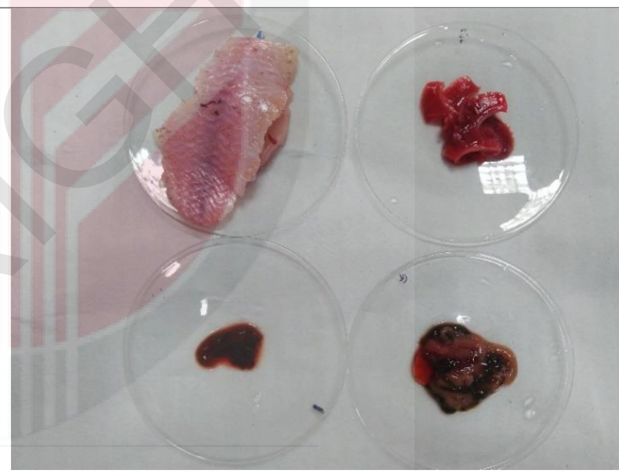
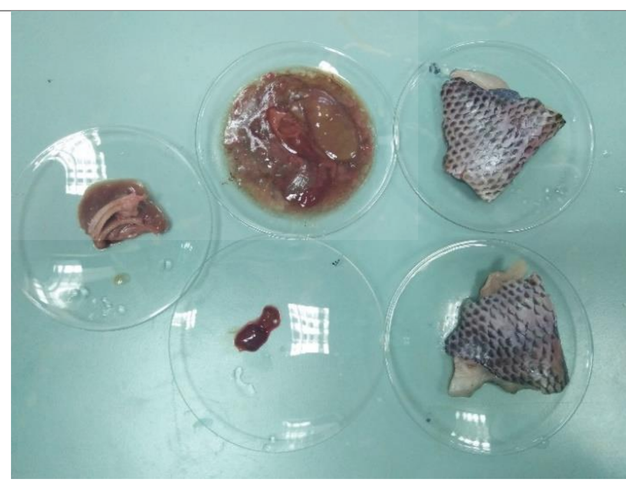
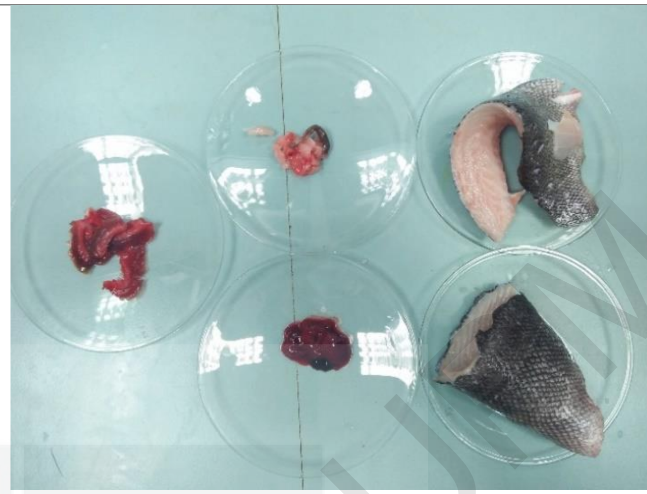
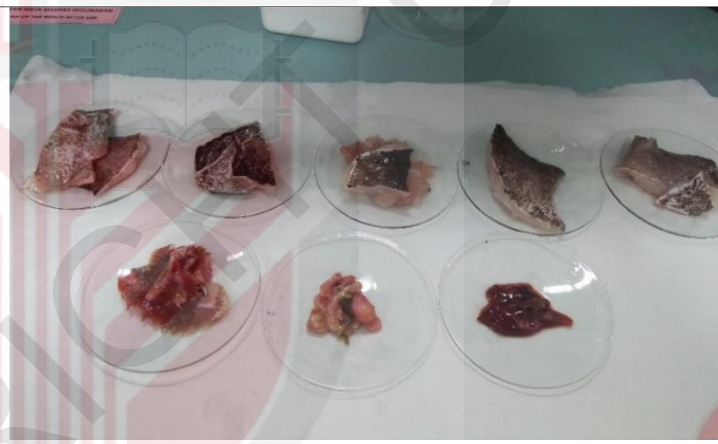
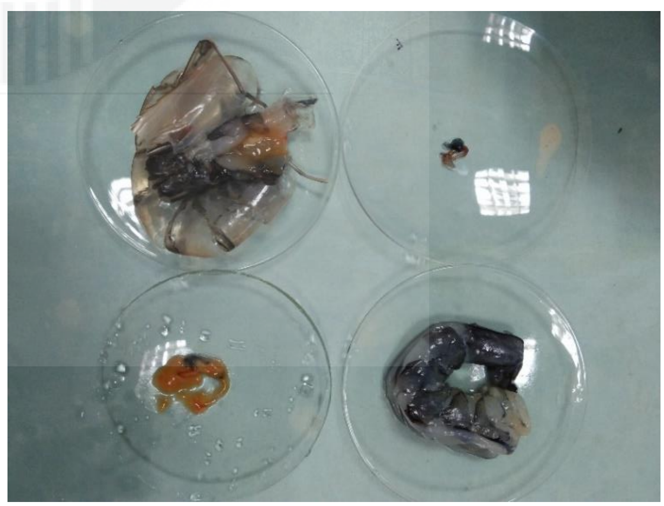


Figure 3.1: The steps for sample preparation procedure

Table 3.4: The collected body parts of selected aquaculture products

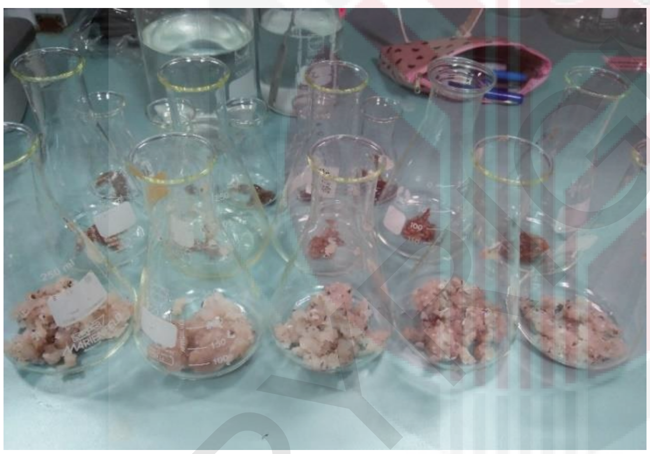
Type of aquaculture	Species	
Freshwater	African catfish (*Clarias gariepinus*)	
	Red nile tilapia (*Oreochromis niloticus*)	
Marine	Mozambique tilapia (*Oreochromis mossambicus*)	

Jade perch (*Scortum barcoo*)	
Hybrid grouper (Tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	
Whiteleg shrimp (*Litopenaeus vannamei*)	

3.4 Microplastics extraction

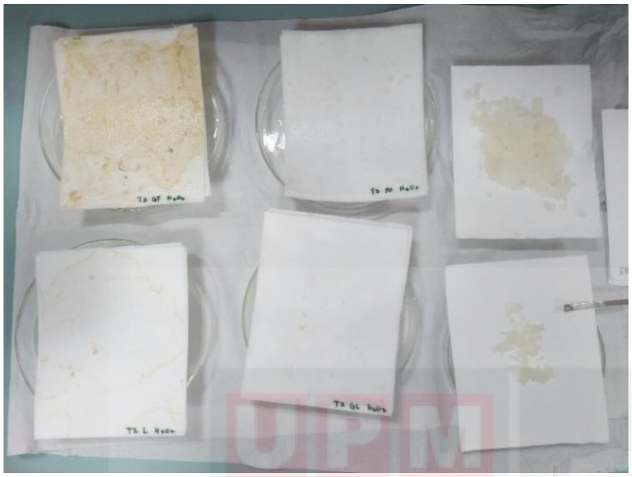
Microplastic isolation from sampled fish and shrimp was carried out according to the method reported by Avio et al. (2015) and Adeogun et al. (2020), with slight modification. The collected samples were sliced and placed individually into 250 mL conical flasks. NaCl hypersaline solution (1.2 g/cm3) was added, and the flasks were sealed with aluminium foil. The sample solution was stirred for a few minutes, incubated at room temperature overnight and filtered manually over glass microfibre filters EPM 2000 450 µm (Whatman, England). Afterwards, the samples were immediately transferred into a petri dish, digested with 30% H2O2 solution and subsequently incubated at room temperature overnight. The samples were after that filtered over 450 µm glass microfibre filters. The filter papers used in NaCl and H2O2 filtration were allowed to dry in the oven at 20 ºC and appropriately stored for further analysis. Steps for the sample preparation procedure are shown in a flowchart in Figure 3.2, while the digestion process for each species was shown in Table 3.5.

The collected samples were sliced.



The samples were placed individually into 250 mL conical flasks.

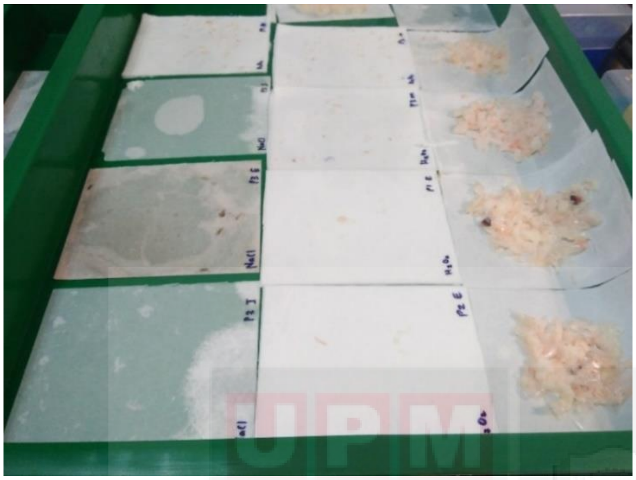
NaCl hypersaline solution (1.2 g/cm^3) was added and the flasks were sealed with aluminium foil.



After incubated at room temperature overnight, the samples were filtered manually over glass microfiber filters EPM 2000 450.

The samples were digested with 30% H_2O_2 solution and incubated at room temperature overnight.

The samples were filtered over 450 µm glass microfibre filters.

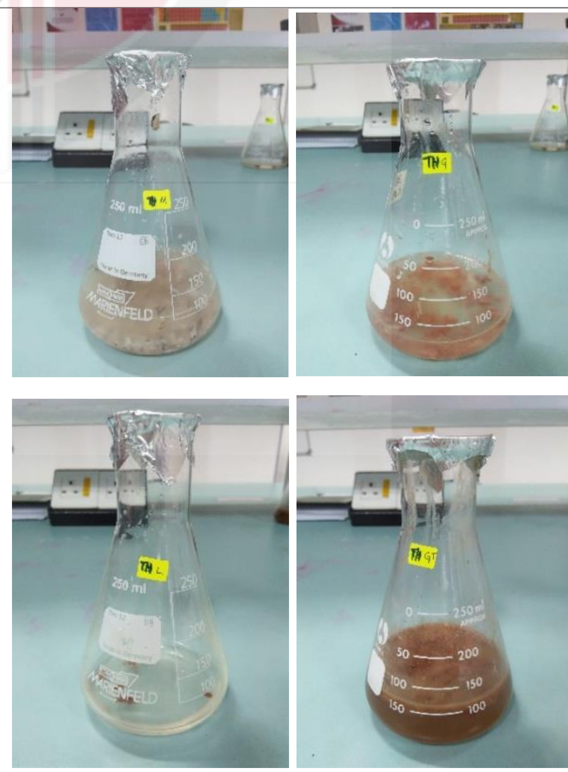


The filters were allowed to dry in the oven at 20 ºC.

The dried filters were stored properly for further analysis.

Figure 3.2: The steps for microplastics extraction procedure

Table 3.5: The digestion process of selected aquaculture products

Type of aquaculture	Species	
Freshwater	African catfish (*Clarias gariepinus*)	
	Red nile tilapia (*Oreochromis niloticus*)	
Marine	Mozambique tilapia (*Oreochromis mossambicus*)	

	Jade perch (*Scortum barcoo*)	   
	Hybrid grouper (Tiger grouper X giant grouper) (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	
	Whiteleg shrimp (*Litopenaeus vannamei*)	

3.5 Visual Identification of the Samples

Visual inspection of filtered samples was conducted using Nikon Eclipse E200 LED MV RS microscope in combination with a BestScope International Limited camera and KJS Show software (magnification of x40). The morphological features of extracted particles that resembled plastic debris, such as shape, size, and colour, were assessed.

3.6 Plastic Polymer Analysis

The polymers of extracted particles were assessed using Thermo Scientific Nicolet 6700 FTIR Spectrometer. Point and shoot analysis with manually operated FTIR micro-scope using a single element MCT-A liquid-nitrogen-cooled detector for speed was applied. In addition, spectra of the unknown particles in each sample were obtained and compared from 500 to 4000 cm−1 to a spectral database of synthetic polymers (Thermo Scientific OMNIC Spectra and Essential FTIR ®Spectroscopy Toolbox software).

3.7 Quality control steps

All analysis and filtration were conducted inside a fume hood (EFH-5A1-ESCO) to prevent potential MPs contamination in samples. Ethanol 70% was used to clean surfaces before and after work was conducted. The same cotton laboratory coats, face masks, and disposable nitrile gloves were worn during all experimental processes. All solutions used in microplastics extraction, especially NaCl hypersaline solution, were prepared fresh on the day of analysis while all glassware and apparatus, including dissecting set, were appropriately washed and rinsed several times with distilled water. Furthermore, any direct contact of samples with plastic materials was avoided during all processes. For example, the plastic parafilm was replaced with aluminium foil, and the samples were placed in the glass petri dish instead of the plastic petri dish. Apart from that, distilled water was boiled using a steel kettle for chemical preparation, and the chopper board was entirely covered with aluminium foil during the dissection process. In addition, three procedurals blank samples (without tissues and filtered water) were processed using identical laboratory methods. No visible particles were observed in the blank shows the absence of contaminants.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Particles Extracted in Selected Aquaculture Products

4.1.1 Freshwater Aquaculture Species

A total of 224 filtered samples was observed under the Nikon Eclipse E200 LED MV RS Microscope with a magnification of x40. Table 4.1 summarized the result from the identification of particles extracted from freshwater aquaculture samples.

Table 4.1: The particles extracted from freshwater aquaculture samples.

Species	Body part	Number of particles	Shape	Colour
Red Nile tilapia (*Oreochromis niloticus*)	Muscle	81	Fragment Fibre Line Film Irregular	Red Blue Black Transparent Other (brown, yellow)
	GIT	334	Fragment Fibre Line Irregular	Red Blue Black Transparent

	Gills	48	Fragment Fibre Line Irregular	Red Blue Black Transparent Other (green)
	Liver	33	Fragment Fibre Line Film	Red Blue Black Transparent
African catfish (*Clarias gariepinus*)	Muscle	5	Fragment Irregular	Black
	GIT	10	Fragment Fibre Irregular	Black
	Gills	6	Fragment Irregular	Black
	Liver	2	Fragment	Black

Based on the data, the amount ranged from 2 to 334 particles. The highest number of particles identified in the gastrointestinal tract (GIT) of Red Nile tilapia (*Oreochromis niloticus*). Another high number of particles found in muscle, gills and liver of the same species. In contrast, a low number of particles extracted from African catfish (*Clarias gariepinus*), with only ten particles identifies in GIT, six particles in gills, five particles in muscle and two particles in the liver. So far, the total number of particles extracted from Red Nile tilapia (496 particles/individual) is relatively higher than African catfish (23 particles/individual). Besides, five types of shape (fragment, fibre, line, film and irregular) observed among the extracted particles. The total number of particles based on detected shapes is shown in Table S1 (refer Appendix 1). Fragments accounted for dominant shape (235 particles), followed by irregular (192 particles), fibres (56 particles), lines (30 particles) and films (3 particles) as shown in Figure 4.1. Also, the color of extracted particles has identified in this study. Black particles were the most dominant in all samples. Figure S2 (refer Appendix 2) shows the images of some extracted particles from freshwater aquaculture samples.

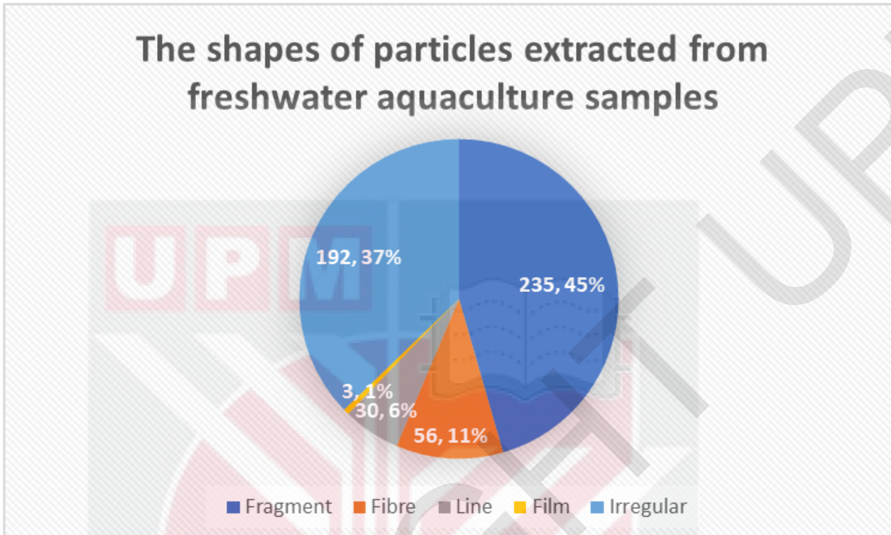


Figure 4.1: The proportion of shapes among particles extracted from freshwater aquaculture samples

Variations in particles shape and color extracted from aquaculture species likely reflect the source of origin and the sites themselves. Fragments of fishing nets and the degradation of plastic bags might be the most significant sources of microplastics in aquaculture site (Foekema et al., 2013). Hard plastic and outer packaging might be the source of fragments, whereas plastic bags might be the primary source of films (Thompson et al., 2003). Pellini et al. (2018) also stated that fishing nets and fishing gear causes the abundance of lines to enter the aquatic environment. Figure 4.2 shows the possible sources of extracted particles from freshwater species based on the aquaculture site.

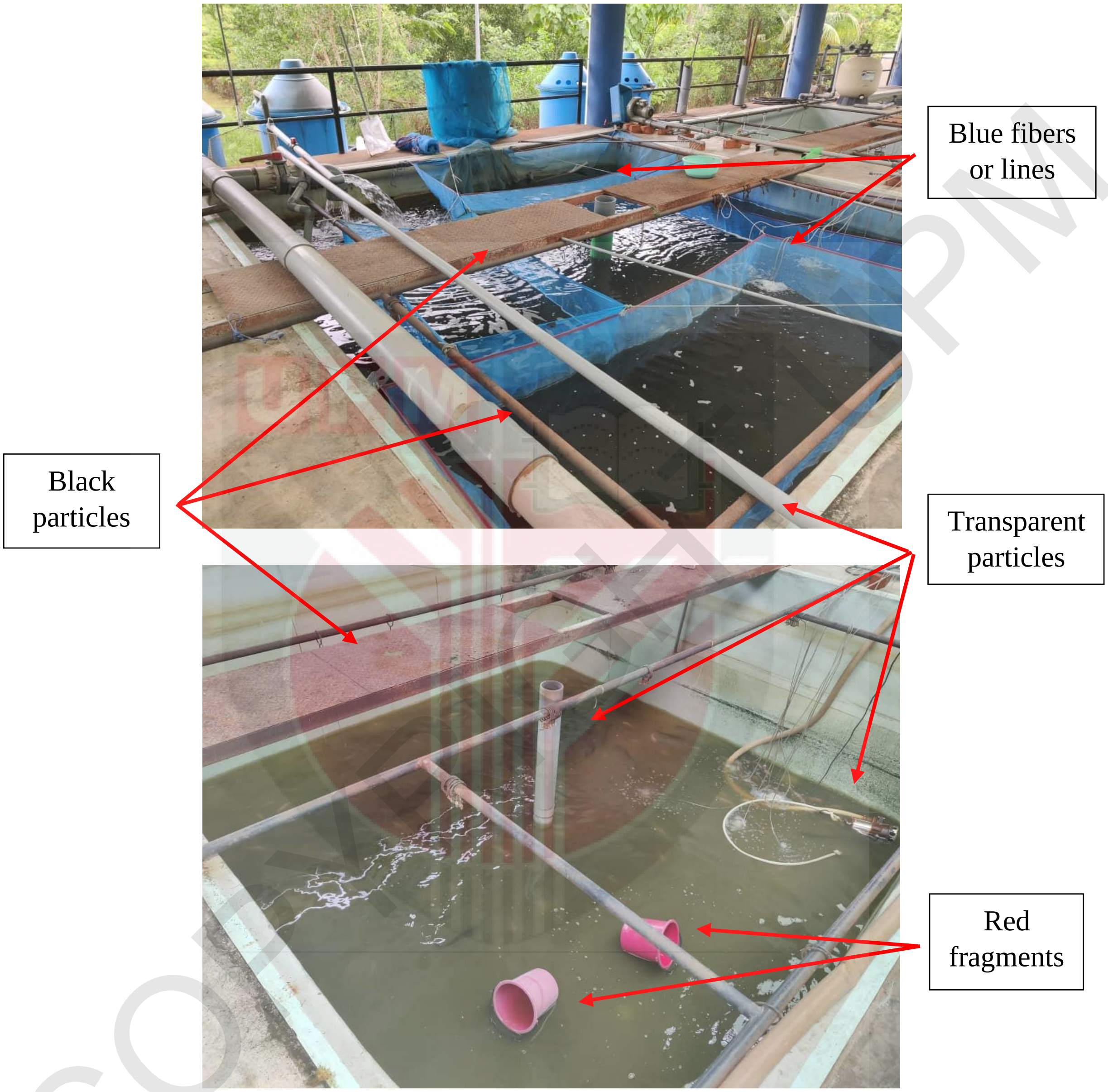


Figure 4.2: The possible sources of particles extracted from freshwater aquaculture samples

Based on the images, the possible source of particles found in freshwater samples could originate from aquaculture structures, especially the pile and fishing nets, since their color is quite similar to the extracted particles. Deterioration of plastic piles and plastic bags could result in fragments and films, while degradation of fishing nets could pollute the site with fibres and lines. The possible source of transparent particles might be plastic bags, typically used in the early stage of breeding, packaging and transportation. Nevertheless, many-colored particles, especially some lines and films, might lose color during or after entering the aquatic environment (Yin et al., 2019). Also, the number of particles found in Red Nile tilapia is relatively high compared to African catfish. The different number of particles found in species may depend on different ingestion rates and fish consumption behavior (Farrell and Nelson, 2013; Liboiron et al., 2016).

4.1.2 Marine Aquaculture Species

The result from the identification of particles extracted from marine aquaculture samples was summarized in Table 4.2.

Table 4.2: The particles extracted from marine aquaculture samples

Species	Body part	Number of particles	Shape	Color
Mozambique tilapia (*Oreochromis mossambicus*)	Muscle	21	Fragment Lines Irregular	Red Blue Black
	GIT	264	Fragment Lines Irregular	Red Blue Black Transparent
	Gills	1	Irregular	Other
	Liver	14	Irregular	Black
Jade perch (*Scortum barcoo*)	Muscle	1	Fragment	Red
	GIT	16	Fragment Irregular	Red Blue
	Gills	9	Fragment Lines Irregular	Red Black Transparent
	Liver	3	Fragment Irregular	Blue Black Other (Brown)
Hybrid grouper (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	Muscle	10	Fragment Fiber Irregular	Red Black Other (Yellow)
	GIT	8	Fragment Fiber Irregular	Red Black
	Gills	4	Fragment Irregular	Black Transparent
	Liver	0	-	-
Whiteleg shrimp (*Litopenaeus vannamei*)	Muscle	6	Fragment Fiber Irregular	Red Black
	Stomach	2	Irregular	Black
	Intestine	0	-	-
	Exoskeleton	3	Fragment Irregular Fiber	Red Black Transparent

Based on the data, the amount ranged from 1 particle to 264 particles. The highest number of particles was found in the GIT of Mozambique tilapia (*Oreochromis mossambicus*), and the second-highest was identified in the muscle of the same species. Another high number of particles was found in the GIT of Jade perch (*Scrotum barcoo*). However, no particles detected in the liver of Hybrid grouper (*Epinephelus fuscoguttatus X Epinephelus lanceolatus*) and intestine of Whiteleg shrimp (*Litopenaeus vannamei*). To date, Mozambique tilapia accounted for the highest number of extracted particles (300 particles/individual), followed by Jade perch (29 particles/individual), Hybrid grouper (22 particles/individual) and Whiteleg shrimp (11 particles/individual).

Apart from that, four types of shape (fragment, fiber, line and irregular) were identified in these extracted particles. The total number of particles based on detected shapes is shown in Table S3 (refer Appendix 3). Irregular shape accounted for the dominant shape of the particle extracted (338 particles), followed by fragment (40 particles), lines (14 particles) and fibers (4 particles), as shown in Figure 4.3. Many colored particles have been found in marine aquaculture samples, especially in some fragments and fibers while black particles were mostly detected in irregular shape. Red and black particles were widely distributed in most of the samples. Abundance of particles extracted from Mozambique tilapia (*Oreochromis mossambicus*) was identified in black with irregular shape. In contrast, fewer particles presented in other marine species reflect precise shape and color, as shown in Figure S4 (refer Appendix 4).

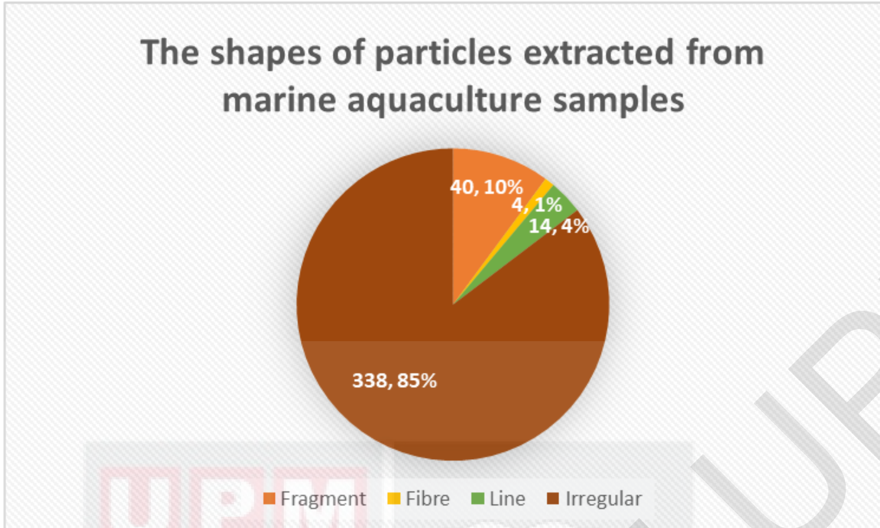


Figure 4.3: The proportion of shapes among particles extracted from marine aquaculture samples

There are several possible sources of particles in the mariculture site, as shown in Figure 4.4. The fragment in marine samples could originate from existing aquaculture structures, including the plastic pipes and air pump used to create bubbles that remove undesirable gases from the water in the tank. Besides, the fishing nets could be the primary source of fibres and lines in the sites, which later accumulate in marine species as they could appear similar to the food. Another possible source of particles in marine species is the structure of the tank. Deterioration of plastic tank over time could lead to the accumulation of particles in the species. As shown in Figure 4.4, some part and color of the tank were already degraded, especially on the interior side. Aside from that, the source of water supplies could also contribute to particles in species. Mariculture is known for using seawater as the breeding medium. However, numerous amounts of MPs have been detected in the ocean and coastal water as MPs can enter the marine ecosystem mainly through the atmosphere, surface water and wastewater (Desforges et al., 2014; Andrady, 2011; Isobe et al., 2017; Avio et al., 2017; Allen et al., 2020). These findings have shown that existing MPs from seawater in mariculture sites can end up in the species.

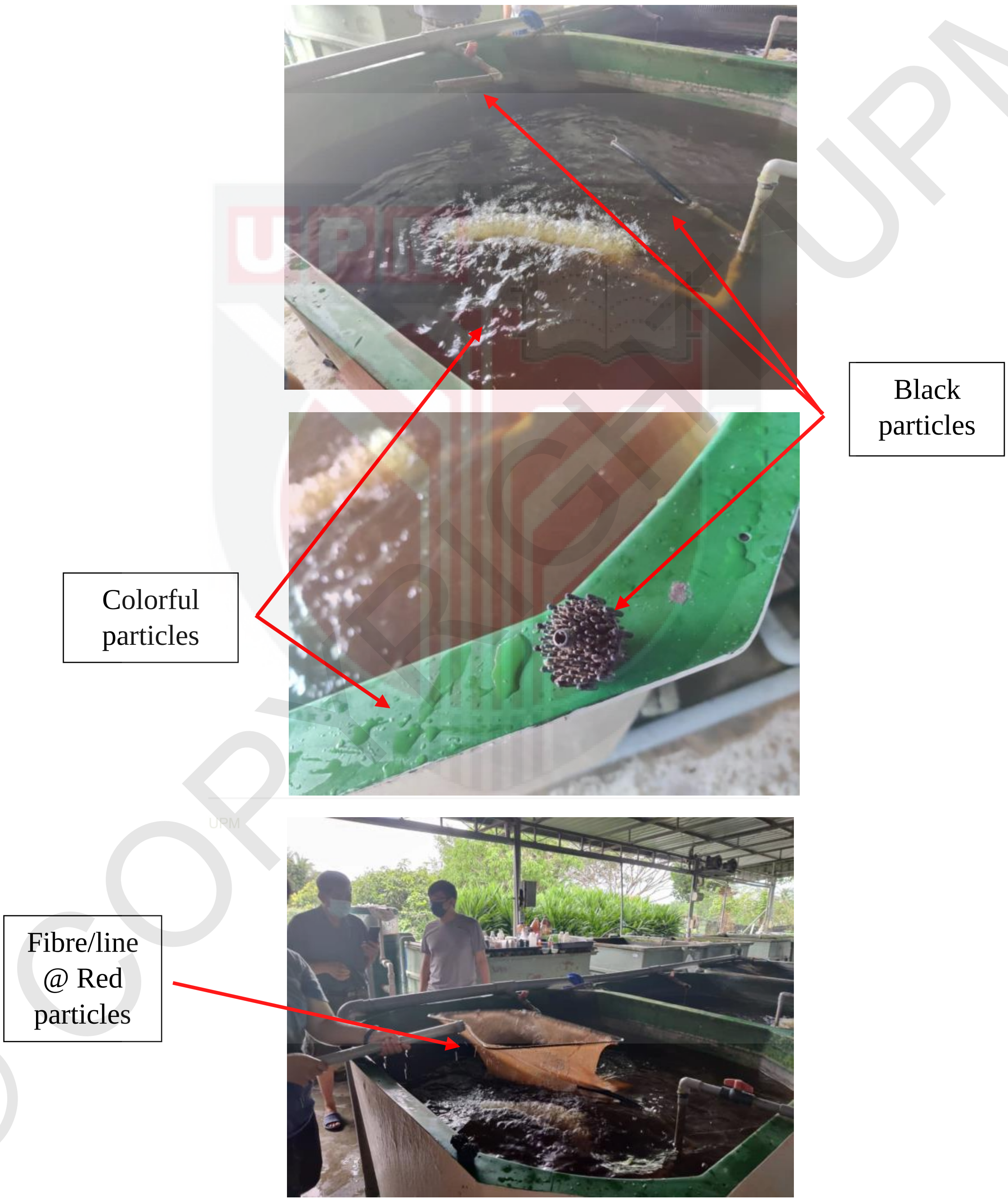


Figure 4.4: The possible sources of particles extracted from marine aquaculture samples

4.2 Polymer Composition of Extracted Particles in Selected Aquaculture Products

4.2.1 Freshwater Aquaculture Species

Ten filters comprised of freshwater samples were selected through visual identification and the polymers of extracted particles were determined with FTIR spectrometry. Table 4.3 shows the results of the FTIR spectrometry of particles extracted from freshwater samples.

Table 4.3: The polymer composition of extracted particles in selected aquaculture products from freshwater aquaculture

Sample	Species	Body part	Extraction	Polymer composition of extracted particles
1	Red Nile tilapia (*Oreochromis niloticus*)	Muscle	H_2O_2	Nylon
2		GIT	H_2O_2	PP
3		Liver	H_2O_2	PP
4		Gills	H_2O_2	LDPE
5		GIT	NaCl	PP
6	African catfish (*Clarias gariepinus*)	GIT	NaCl	PVC
7		Muscle	H_2O_2	PP
8		GIT	H_2O_2	Nylon
9		Liver	H_2O_2	Nylon
10		Gills	H_2O_2	LDPE

Four types of polymers with different chemical composition were confirmed. Figure 4.5 to Figure 4.14 shows the FTIR and microscopic images of extracted particles in Sample 1-10 with their polymer types. The primary plastic polymers were polypropylene (PP) followed by nylon (NY), low-density polyethylene (LDPE) and polyvinyl chloride (PVC).

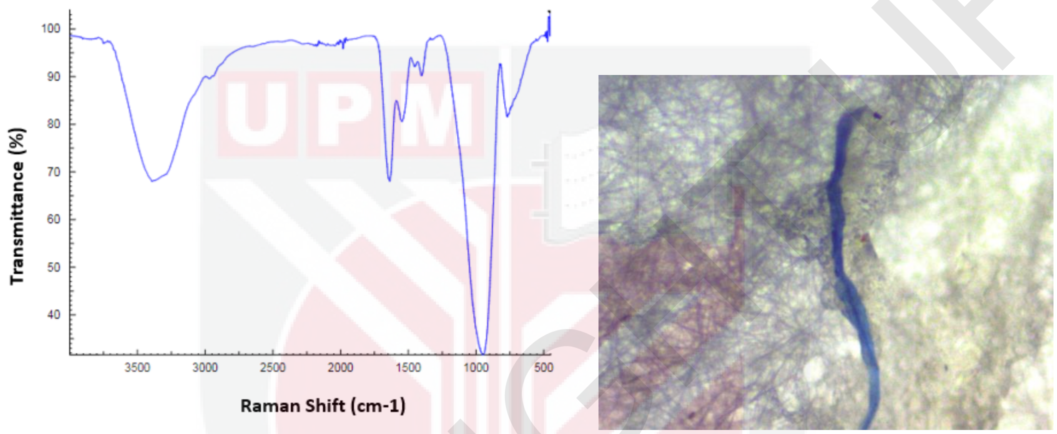


Figure 4.5: FTIR and microscopic images of extracted particles in Sample 1 (Muscle of Red Nile tilapia) – Nylon

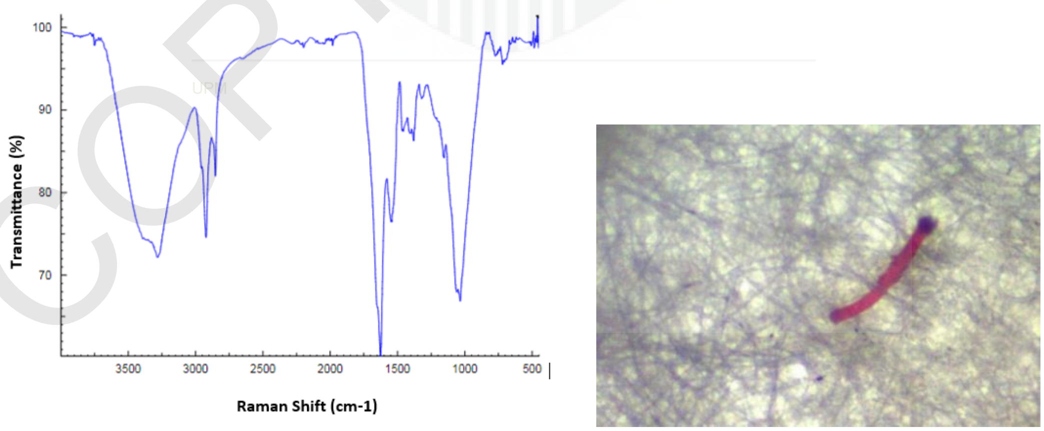


Figure 4.6: FTIR and microscopic images of extracted particles in Sample 2 (GIT of Red Nile tilapia) – Polypropylene

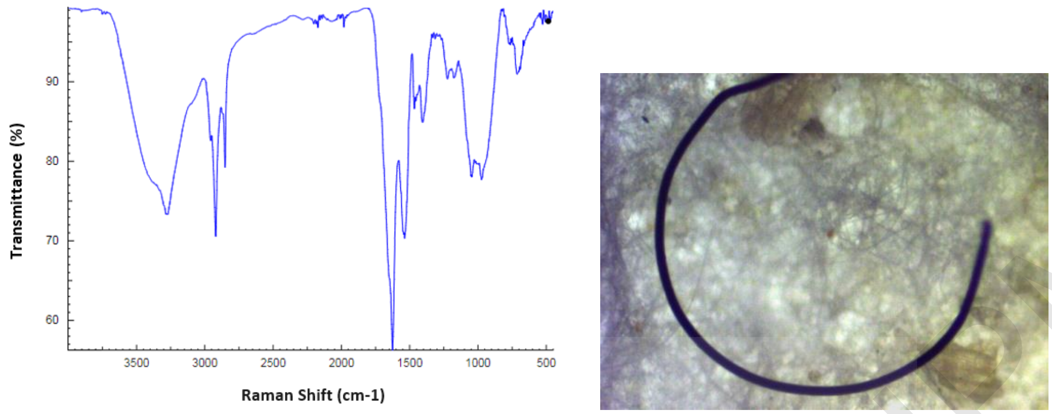


Figure 4.7: FTIR and microscopic images of extracted particles in Sample 3 (Liver of Red Nile tilapia) – Polypropylene

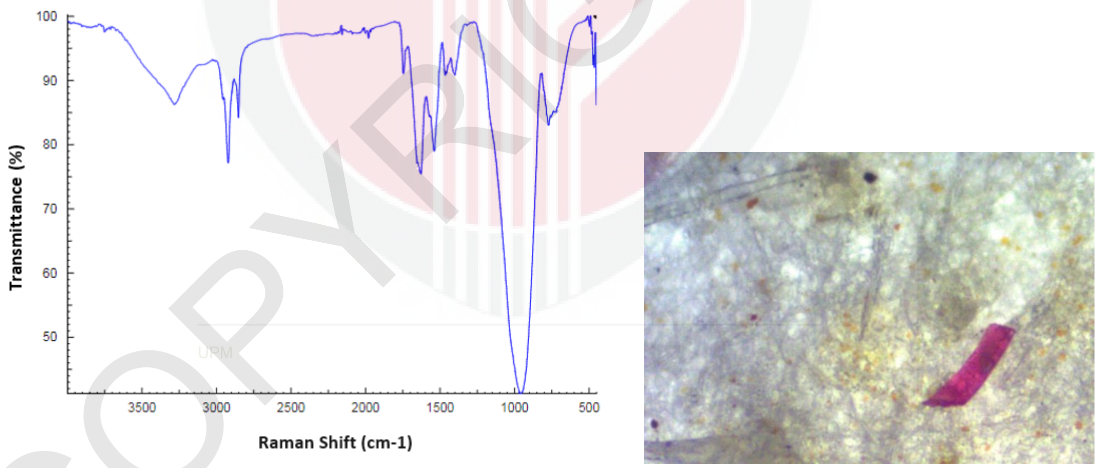


Figure 4.8: FTIR and microscopic images of extracted particles in Sample 4 (Gills of Red Nile tilapia) – LDPE

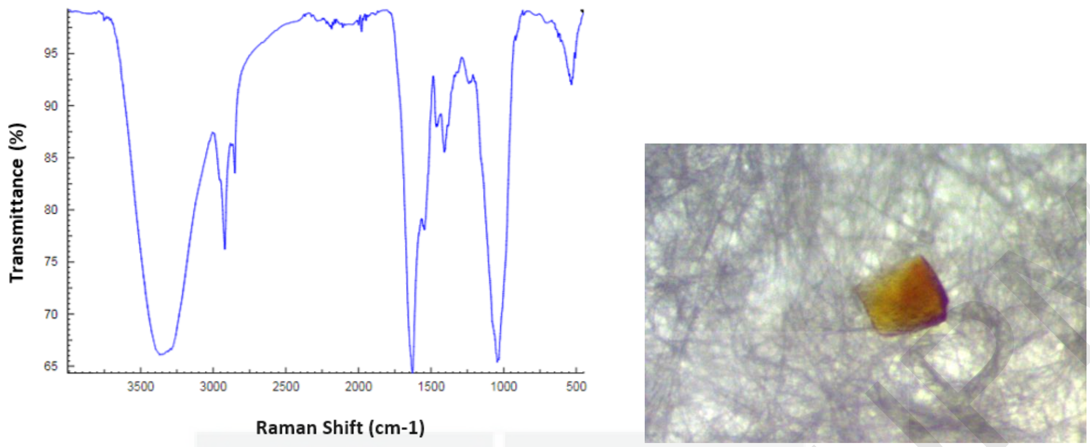


Figure 4.9: FTIR and microscopic images of extracted particles in Sample 5 (GIT of Red Nile tilapia) – Polypropylene

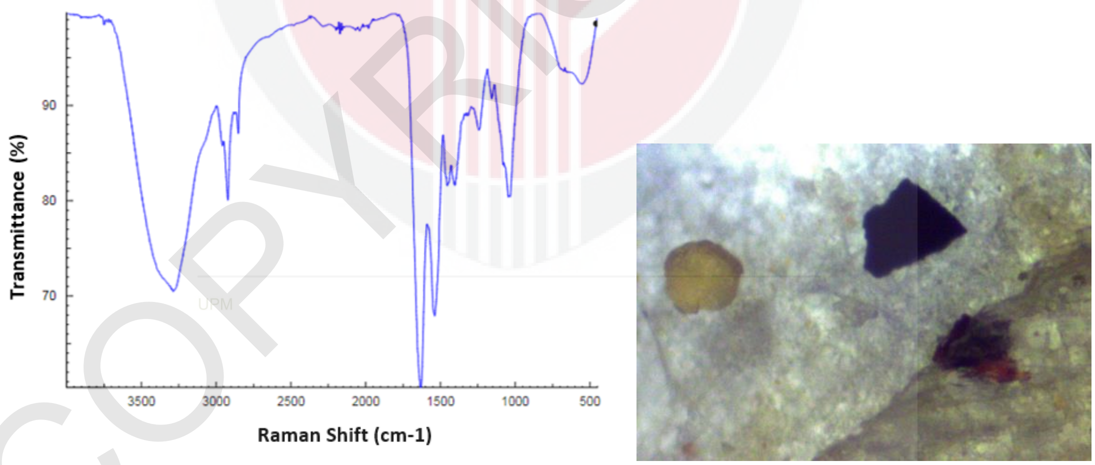


Figure 4.10: FTIR and microscopic images of extracted particles in Sample 6 (GIT of African catfish) – Polyvinyl chloride

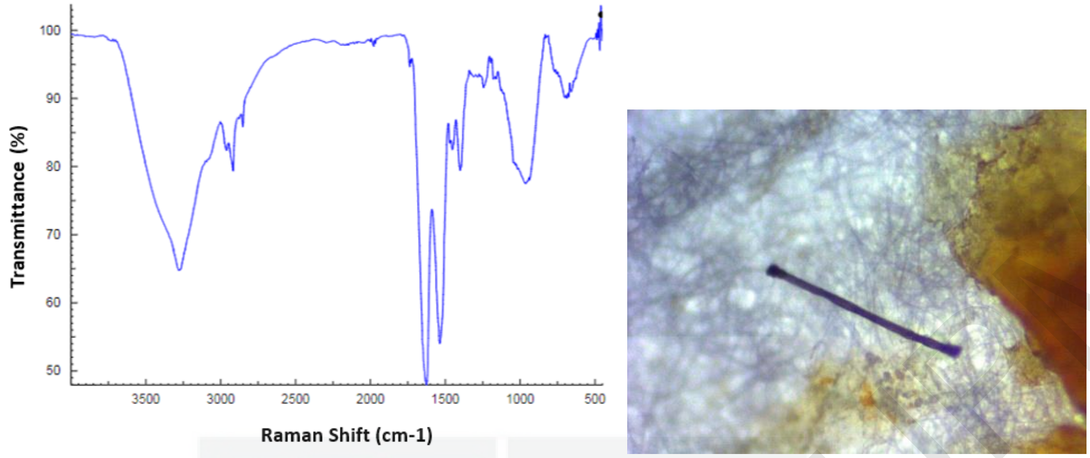


Figure 4.11: FTIR and microscopic images of extracted particles in Sample 7 (Muscle of African catfish) – Polypropylene

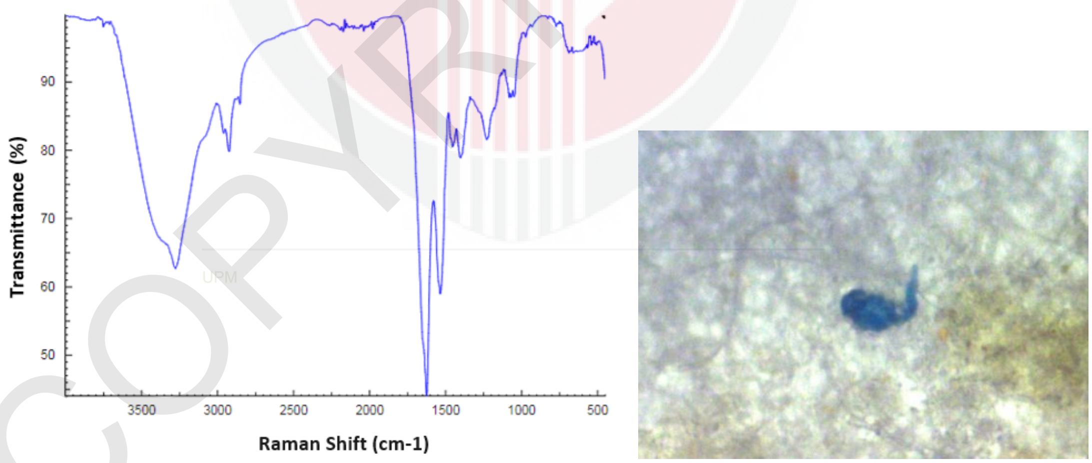


Figure 4.12: FTIR and microscopic images of extracted particles in Sample 8 (GIT of African catfish) – Nylon

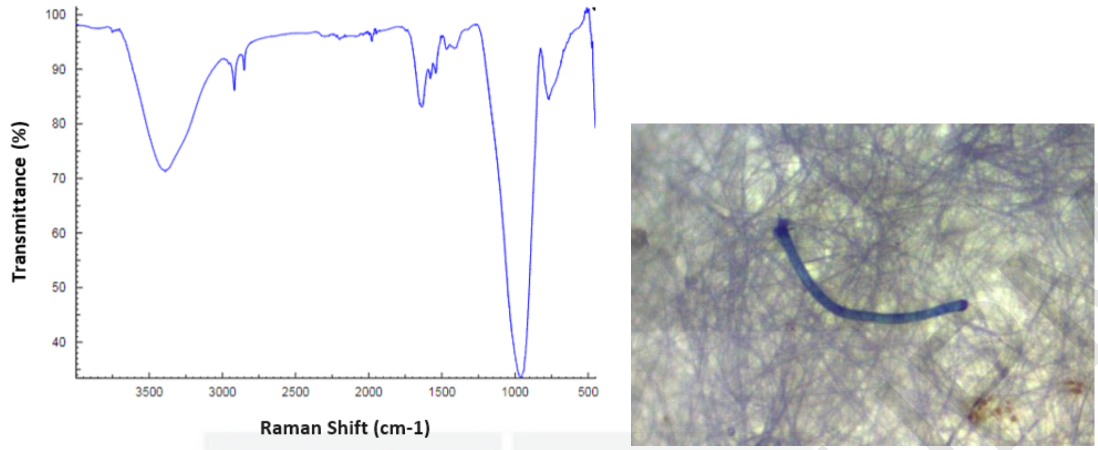


Figure 4.13: FTIR and microscopic images of extracted particles in Sample 9 (Liver of African catfish) – Nylon

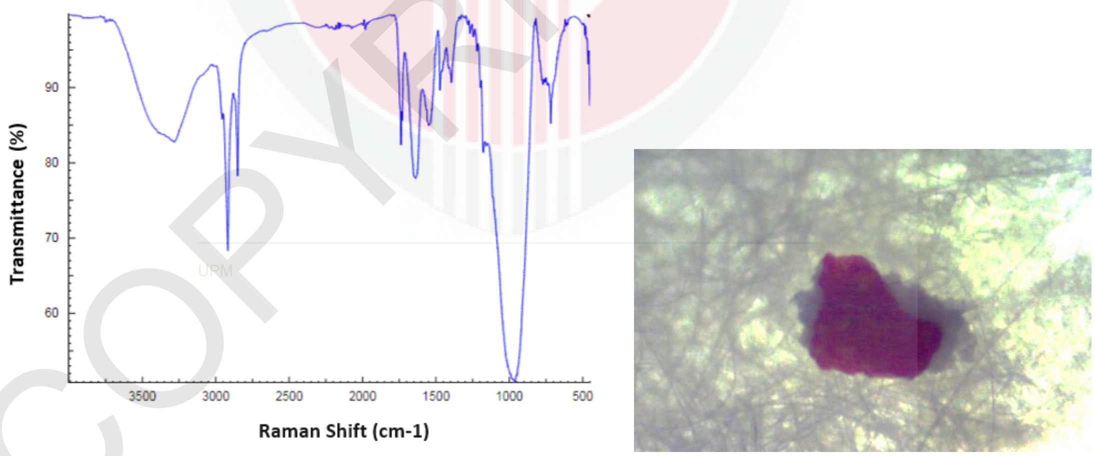


Figure 4.14: FTIR and microscopic images of extracted particles in Sample 10 (Gills of African catfish) – LDPE

Polypropylene (PP) was found in GIT and liver of Red Nile tilapia and muscle of African catfish. According to GESAMP (2015), PP is a primary material used in rope, bottle caps, and fishing gears. Since PP has a lower density (0.90 – 0.92 g/cm^3) than freshwater utilized in the sites (approximately 1.00 g/cm^3), it tends to float on the upper column of the freshwater and is accessible for aquatic species by ingestion. Thus, PP polymer identified in freshwater species could be originated from the aquaculture structures used in the breeding sites. Other than that, nylon (NY) was identified in the muscle of Red Nile tilapia, GIT, and liver of African catfish. Nylon is the typical material used in aquaculture cages as it has a slightly stronger initial breaking load compared to polyester and has a better working force due to high polymer density (1.13 – 1.15 g/cm^3) (AquaFeed, 2018). However, deterioration of NY is possible over time due to the direct exposure to ultraviolet (UV) and abrasion, which later accumulated in the sites and end up in aquaculture species. Therefore, the presence of NY in freshwater species is possibly come from aquaculture structure like cages.

Apart from that, low-density polyethylene (LDPE) was recognized in the gills of Red Nile tilapia and African catfish. This type of polymer was commonly applied in plastics bags, pipes, nets, ropes, storage containers, and plastic equipment due to low polymer density (0.91-0.97 g/cm^3) (GESAMP, 2015). Because LDPE is denser than freshwater, degradation of LDPE products at the sites results in their deposition on the freshwater surface. Hence, the LDPE could potentially end up in the gills of pelagic fish through inhalation. Besides, a particle extracted from the GIT of African catfish has been recognized as polyvinyl chloride (PVC). PVC is a typical polymer utilized in plastic products such as plastic pipes and containers as it has a higher polymer density (1.16 – 1.30 g/cm³) (GESAMP, 2015). Nevertheless, the particle possibly sinks into the bottom of sites after degraded from main structures and taken up by freshwater species through ingestion.

4.2.2 Marine Aquaculture Species

The polymer composition of extracted particles from marine species was supposed to identify with FT-IR spectrometry during this study period. However, due to the implementation of 'Movement Control Orders' (MCOs) to reduce the deadly spread of COVID-19, the identification is impossible to be done in time.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, abundance number of particles has been detected in selected aquaculture products from local aquaculture hatcheries in Selangor, Malaysia comprised of African catfish (*Clarias gariepinus*) (23 particles/individual), Red Nile tilapia (*Oreochromis niloticus*) (496 particles/individual), Mozambique tilapia (*Oreochromis mossambicus*) (300 particles/individual), Jade perch (*Scortum barcoo*) (29 particles/individual), Hybrid grouper (tiger grouper X giant grouper) (*Epinephelus fuscoguttatus X Epinephelus lanceolatus*) (22 particles/individual) and Whiteleg shrimp (*Litopenaeus vannamei*) (11 particles/individual). The highest number of particles identified in the gastrointestinal tract (GIT) of Red Nile tilapia (334 particles). Another high number of particles found in GIT of Mozambique tilapia (264 particles), followed by muscles and gills of Red Nile tilapia (81 and 48 particles). In contrast, no particles were detected in the liver of Hybrid grouper and intestines of Whiteleg shrimp.

The particles extracted from freshwater aquaculture species were predominantly in fragment form followed by irregular, fibres, lines and films. The black particles were mostly observed in all samples. Polypropylene (PP), nylon (NY), low-density polyethylene (LDPE) and polyvinyl chloride (PVC) were among the plastic polymers detected among the extracted particles, suggesting that the particles originated from aquaculture structures and facilities used in the sites such as fishing nets, ropes, plastic pipes and cages or tanks. On the other hand, irregular shape accounted for the dominant shape among the particles extracted from marine aquaculture samples, followed by fragment, lines and fibers. The particles with irregular shape usually resemble in black color while the colored particles have been found in fragments and fibers. Red and black particles were widely distributed in most of the samples. Nevertheless, the polymer identification for particles extracted from marine aquaculture samples was pending due to implementation of MCO.

5.2 Limitations

This study is subject to several limitations. The major limitation in this study is the limited time for polymer analysis. As Movement Control Orders (MCOs) was continuously implemented to reduce the deadly spread of COVID-19, the complete polymer identification for extracted particles from aquaculture species is impossible to be done in time. Moreover, the extracted particles were selected through microscopic imaging for polymer identification with FTIR spectrometry. However, the selection process could pose a risk for selection bias, and the results might deviate from the actual situation as there were numerous particles present in the samples, yet, only ten significant particles were chosen since the high cost of FTIR spectrometry identification limits the complete polymer identification of all extracted particles. Besides, a low number of samples were collected due to restricted access to the sampling sites during MCO. This situation could lead to sampling bias, which interferes with the samples' representativeness and accuracy.

5.3 Recommendations

Based on the limitations, it is necessary to find more economical and rapid polymer identification methods in the future in order to obtain more detailed and accurate experimental data, especially when conducting experimental studies during MCO. Moreover, we recommended future studies to investigate the occurrence of MPs in various aquaculture species from different aquaculture locations. The investigation involving a high number of species could reduce the bias and increase the accuracy of the results. Aside from that, this study provides baseline data of microplastic pollution in aquaculture species. Hence, further investigation is needed to understand better how aquaculture structures utilized / aquaculture activities could influence MPs accumulation in sites and MPs in aquaculture species.

REFERENCES

Adeogun, A. O., Ibor, O. R., Khan, E. A., Chukwuka, A. V., Omogbemi, E. D., & Arukwe, A. (2020). Detection and occurrence of microplastics in the stomach of commercial fish species from a municipal water supply lake in southwestern Nigeria. Environmental Science and Pollution Research, 1-11.

Avio, C. G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., ... & Regoli, F. (2015). Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environmental pollution*, *198*, 211-222.

Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Marine Environmental Research, 111, 18-26.

Barboza, L. G. A., Vethaak, A. D., Lavorante, B. R., Lundebye, A. K., & Guilhermino, L. (2018). Marine microplastic debris: An emerging issue for food security, food safety and human health. *Marine pollution bulletin*, *133*, 336-348.

Bour, A., Avio, C. G., Gorbi, S., Regoli, F., & Hylland, K. (2018). Presence of microplastics in benthic and epibenthic organisms: influence of habitat, feeding mode and trophic level. Environmental Pollution, 243, 1217-1225.

Castelvetro, V., Corti, A., Bianchi, S., Giacomelli, G., Manariti, A., & Vinciguerra, V. (2021). Microplastics in fish meal: contamination level analyzed by polymer type, including polyester (PET), polyolefins, and polystyrene. Environmental Pollution, 273, 115792.

Cole, M., Webb, H., Lindeque, P. K., Fileman, E. S., Halsband, C., & Galloway, T. S. (2014). Isolation of microplastics in biota-rich seawater samples and marine organisms. Scientific reports, 4(1), 1-8.

Department of Fisheries Malaysia. (2017). Malaysian Fishing Industry Scenario-Fishery Sector Performance and Achievement. Retrieved from: https://www.dof.gov.my/index.php/pages/view/42

Ding, J., Zhang, S., Razanajatovo, R. M., Zou, H., & Zhu, W. (2018). Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (Oreochromis niloticus). Environmental pollution, 238, 1-9.

FAO 2008-2021. National Aquaculture Sector Overview. Malaysia. National Aquaculture Sector Overview Fact Sheets. Text by Mazuki Hashim. In: FAO Fisheries Division [online]. Rome. Updated. [Cited 5 June 2021].

Feng, Z., Zhang, T., Li, Y., He, X., Wang, R., Xu, J., & Gao, G. (2019). The accumulation of microplastics in fish from an important fish farm and mariculture area, Haizhou Bay, China. Science of The Total Environment, 696, 133948.

Hanachi, P., Karbalaei, S., Walker, T. R., Cole, M., & Hosseini, S. V. (2019). Abundance and properties of microplastics found in commercial fish meal and cultured common carp (*Cyprinus carpio*). Environmental Science and Pollution Research, 26(23), 23777-23787.

Karami, A., Golieskardi, A., Choo, C. K., Larat, V., Galloway, T. S., & Salamatinia, B. (2017). The presence of microplastics in commercial salts from different countries. Scientific Reports, 7(1), 1-11.

Karami, A., Golieskardi, A., Choo, C. K., Romano, N., Ho, Y. B., & Salamatinia, B. (2017). A high-performance protocol for extraction of microplastics in fish. Science of the Total Environment, 578, 485-494.

Karbalaei, S., Golieskardi, A., Watt, D. U., Boiret, M., Hanachi, P., Walker, T. R., & Karami, A. (2020). Analysis and inorganic composition of microplastics in commercial Malaysian fish meals. Marine Pollution Bulletin, 150, 110687.

Karbalaei, S., Golieskardi, A., Hamzah, H. B., Abdulwahid, S., Hanachi, P., Walker, T. R., & Karami, A. (2019). Abundance and characteristics of microplastics in commercial marine fish from Malaysia. Marine Pollution Bulletin, 148, 5-15.

Li, L., Li, M., Deng, H., Cai, L., Cai, H., Yan, B., ... & Shi, H. (2018). A straightforward method for measuring the range of apparent density of microplastics. Science of The Total Environment, 639, 367-373.

Li, D., & Liu, S. (2018). Water quality monitoring and management: basis, technology and case studies. Academic Press.

Lusher, A., Hollman, P., & Mendoza-Hill, J. (2017). Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO.

National Aquaculture Sector Overview. Malaysia. National Aquaculture Sector Overview Fact Sheets. Text by Mazuki Hashim. In: FAO Fisheries Division [online]. Rome. Updated 2008.

NOAA. What is aquaculture? National Ocean Service website, https://oceanservice.noaa.gov/facts/aquaculture.html, 26/02/21.

NOAA. What is microplastics? National Ocean Service website, https://oceanservice.noaa.gov/facts/microplastics.html, 26/02/21.

Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. Environmental Pollution, 184, 161-169.

PlasticsEurope. (2018). Plastics – the Facts 2018: An Analysis of European Plastics Production, Demand and Waste Data. Retrieved from: https://www.www.plasticseurope.org/en

Prata, J. C., da Costa, J. P., Lopes, I., Duarte, A. C., & Rocha-Santos, T. (2020). Environmental exposure to microplastics: An overview on possible human health effects. *Science of the Total Environment*, *702*, 134455.

Praveena, S. M., Shaifuddin, S. N. M., & Akizuki, S. (2018). Exploration of microplastics from personal care and cosmetic products and its estimated emissions to marine environment: An evidence from Malaysia. Marine Pollution Bulletin, 136, 135-140.

Priscilla, V., & Patria, M. P. (2020). Comparison of microplastic abundance in aquaculture ponds of milkfish Chanos chanos (Forsskål, 1775) at Muara Kamal and Marunda, Jakarta Bay. In IOP Conference Series: Earth and Environmental Science (Vol. 404, No. 1, p. 012027). IOP Publishing.

Rainieri, S., & Barranco, A. (2019). Microplastics, a food safety issue?. *Trends in food science & technology*, *84*, 55-57.

Roch, S., & Brinker, A. (2017). Rapid and efficient method for the detection of microplastic in the gastrointestinal tract of fishes. Environmental Science & Technology, 51(8), 4522-4530.

Rogers, K. (2020, September 8). Microplastics. Encyclopedia Britannica. https://www.britannica.com/technology/microplastic

Sarijan, S., Azman, S., Mohd Said, M. I., & Lee, M. H. (2019). Ingestion of Microplastics by Commercial Fish in Skudai River, Malaysia. EnvironmentAsia, 12(3).

Senathirajah, K., Attwood, S., Bhagwat, G., Carbery, M., Wilson, S., & Palanisami, T. (2021). Estimation of the mass of microplastics ingested–A pivotal first step towards human health risk assessment. *Journal of Hazardous Materials*, *404*, 124004.

Smith, M., Love, D. C., Rochman, C. M., & Neff, R. A. (2018). Microplastics in seafood and the implications for human health. *Current environmental health reports*, *5*(3), 375-386.

Su L, Xue Y, Li L, Yang D, Kolandhasamy P, Li D, Shi H (2016) Microplastics in Taihu Lake, China. Environment Pollution. doi:10.1016/j.envpol.2016.06.036

Thiele, C. J., Hudson, M. D., Russell, A. E., Saluveer, M., & Sidaoui-Haddad, G. (2021). Microplastics in fish and fishmeal: an emerging environmental challenge?. Scientific reports, 11(1), 1-12.

Vethaak, A. D., & Legler, J. (2021). Microplastics and human health. *Science*, *371*(6530), 672-674.

Wang W, Ndungu AW, Li Z, Wang J (2016) Microplastics pollution in inland freshwaters of China: a case study in urban surface waters of Wuhan, China. Sci Total Environ. doi:10.1016/j.

Wikipedia Foundation. (2021, April 16). Mariculture. Wikipedia. https://en.wikipedia.org/wiki/Mariculture.

Wu, C., Zhang, K., & Xiong, X. (2018). Microplastic pollution in inland waters focusing on Asia. In *Freshwater microplastics* (pp. 85-99). Springer, Cham.

Wu, F., Wang, Y., Leung, J. Y., Huang, W., Zeng, J., Tang, Y., ... & Cao, L. (2020). Accumulation of microplastics in typical commercial aquatic species: A case study at a productive aquaculture site in China. Science of The Total Environment, 708, 135432.

Zhang K, Su J, Xiong X, Wu X, Wu C, Liu J (2016) Microplastic pollution of lakeshore sediments from remote lakes in Tibet plateau, China. Environ Pollut 219:450–455.

Zhou, A., Zhang, Y., Xie, S., Chen, Y., Li, X., Wang, J., & Zou, J. (2020). Microplastics and their potential effects on the aquaculture systems: a critical review. Reviews in Aquaculture, 13(1), 719-733.

APPENDICES

APPENDIX 1: The total number of particles extracted from freshwater aquaculture samples by shapes.

Species	Body part	Number of particles	Shape	Number of particles
Red Nile tilapia (*Oreochromis niloticus*)	Muscle	81	Fragment	27
			Fibre	17
			Line	6
			Film	2
			Irregular	29
	GIT	334	Fragment	146
			Fibre	24
			Line	18
			Irregular	143
	Gills	48	Fragment	28
			Fibre	7
			Line	3
			Irregular	10
	Liver	33	Fragment	22
			Fibre	7
			Line	3
			Film	1
African catfish (*Clarias gariepinus*)	Muscle	5	Fragment	2
			Irregular	3
	GIT	10	Fragment	5
			Fibre	1
			Irregular	4
	Gills	6	Fragment	3
			Irregular	3
	Liver	2	Fragment	2

Shapes	Fragment	Fibre	Line	Film	Irregular
Number of particles	235	56	30	3	192

APPENDIX 2: Microscopic images of particles extracted from freshwater aquaculture products

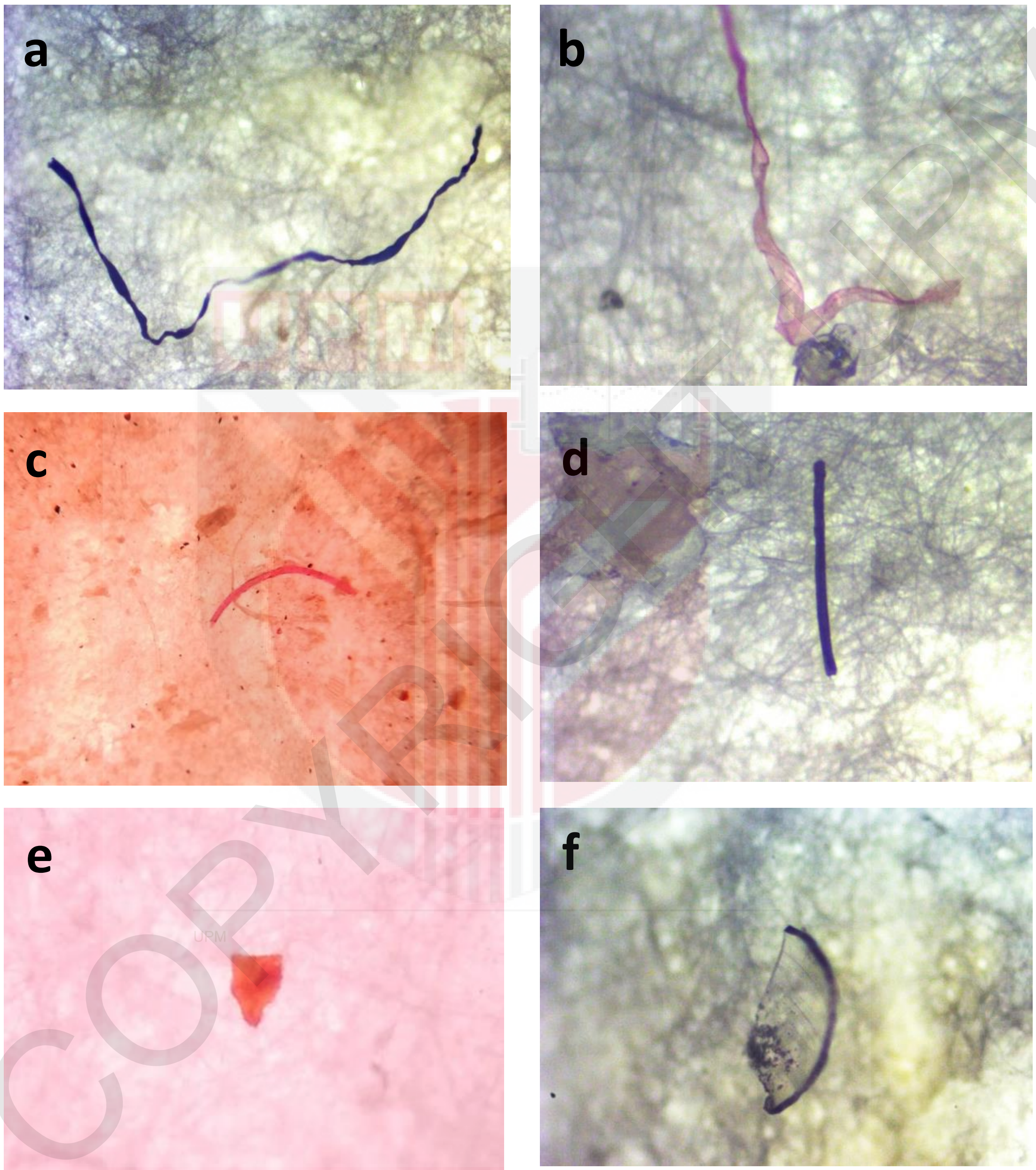


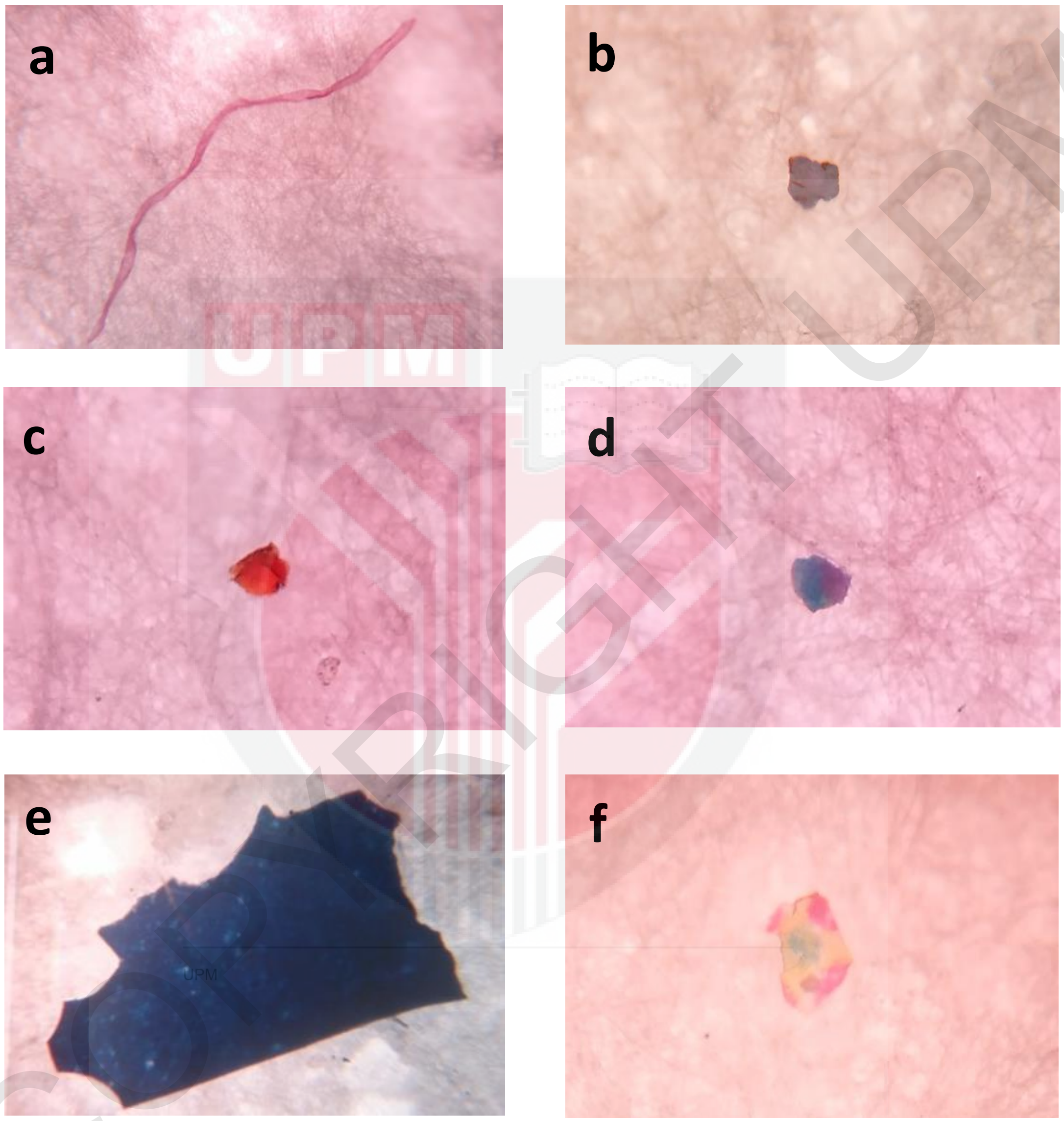
Figure S2: Microscopic images of particles extracted from freshwater aquaculture products: (a) line, (b) line, (c) fibre, (d) fibre, (e) fragment, (f) film.

APPENDIX 3: The total number of particles extracted from marine aquaculture samples by shapes.

Species	Body part	Number of particles	Shape	Number of particles
Mozambique tilapia (*Oreochromis mossambicus*)	Muscle	21	Fragment	5
			Lines	3
			Irregular	13
	GIT	264	Fragment	15
			Lines	9
			Irregular	240
	Gills	1	Irregular	1
	Liver	14	Irregular	14
Jade perch (*Scortum barcoo*)	Muscle	1	Fragment	1
	GIT	16	Fragment	4
			Irregular	12
	Gills	9	Fragment	3
			Lines	2
			Irregular	4
	Liver	3	Fragment	1
			Irregular	2
Hybrid grouper (*Epinephelus fuscoguttatus* X *Epinephelus lanceolatus*)	Muscle	10	Fragment	5
			Fiber	1
			Irregular	4
	GIT	8	Fragment	3
			Fiber	1
			Irregular	4
	Gills	4	Fragment	1
			Irregular	3
	Liver	0	-	-
Whiteleg shrimp (*Litopenaeus vannamei*)	Muscle	6	Fragment	1
			Fiber	1
			Irregular	4
	Stomach	2	Irregular	2
	Intestine	0	-	-
	Exoskeleton	3	Fragment	1
			Irregular	1
			Fiber	1

Shapes	Fragment	Fibre	Line	Film	Irregular
Number of particles	40	4	14	0	338

APPENDIX 2: Microscopic images of particles extracted from marine aquaculture products



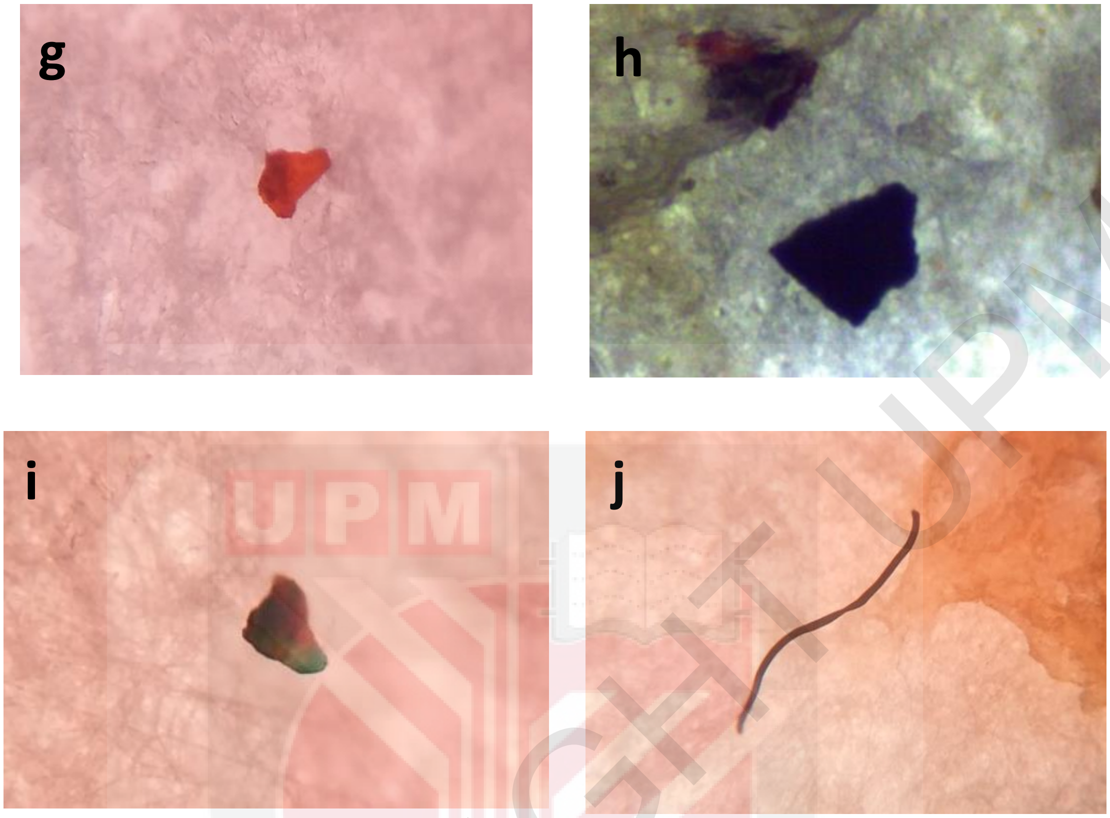


Figure S4: Microscopic images of particles extracted from marine aquaculture products: (a) line, (b) - (i) fragment, (j) fiber.