A Project entitled

Microplastic accumulation in frozen mussels bought in Hong Kong

Submitted by

Lai Wing Lam

submitted to The Education University of Hong Kong

for the degree of Bachelor of Education (Honours) (Science)

in April 2021

Declaration

I, Lai Wing Lam declare that this research report represents my own work under the supervision of Dr. CHEANG, Chi Chiu, and that it has not been submitted previously for examination to any tertiary institution.

Lai Wing Lam

9 April 2021

Abstract

Microplastics (< 5 mm) are ubiquitous in daily life and microplastic pollution has been a pressing environmental problem around the globe. Research studies had evidence that microplastics are present in a wide range of commercial seafood, putting human health in danger. The level of microplastic contamination in 50 frozen mussels of five origins bought in Hong Kong was investigated. The mussel samples were treated with potassium hydroxide (KOH) at 40°C for 36 – 48h to digest the organic matters in the soft tissues. The digested solutions were filter to obtain the microplastics on 0.6 μm pore size glass fibre filter papers. After drying, they were observed under a stereo microscope for visual identification of microplastics. 590 microplastic items were identified with a range of 0.06 mm to 17.06 mm sizes and mean of 1.18 ± 1.45 mm. The main form of plastic items were fibres and about 86% were smaller than 2 mm. The average abundance of microplastics in all origins was 11.80 ± 14.44 items individual^{-1} and 1.42 ± 2.00 items g^{-1} w. w. Canadian mussels contained the most abundant microplastics while Australian and Canadian mussels had the least. It was predicted that there were consistent microplastic contamination sources based on the shapes and colours of plastic items. No significant correlation was found between wet weight of mussels and microplastic abundance, which was contrary to previous research findings. Without FT-IR analysis, the detection of chemical composition of microplastics was not performed, which made ensuring the plastic nature and tracing of microplastic sources difficult. The annual intake of microplastics for

local mussel consumers was calculated to be 507.93 microplastic items/year. It is advised that people should be more aware of microplastic consumption in diets. Actions should be taken to further investigate into potential health implications on long-term microplastic through food consumption.

Acknowledgements

I would like to express my greatest gratitude to my supervisor, Dr. CHEANG, Chi Chiu, for his patient guidance and constructive advice on this Honours Project. Besides, I want to thank Dr. FOK, Lincoln for his help of refining the research procedures, and his research assistants Theresa and Jade for their generous technical support of providing experiment materials and helping me in statistical analysis as well as emotional support throughout the whole laboratory work. I also have to thank Mr. Yiu, research assistant of Dr Cheang, for his helpful suggestions and support in sampling before the change of research topic. Lastly, I would like to show my heartfelt gratitude to my mother and sister who always cared about my research progress and took good care of me in many aspects. This study would never have been completed successfully without any of their contribution.

Lai Wing Lam

April 2021

Table of Contents

List of Tables

List of Figures

1. Introduction

1.1 Background of microplastic pollution

1.1.1 Introduction to microplastics

Microplastics pollution in marine environment has received much attention in recent years and research studies from around the globe have emphasized the severity of this environmental issue. Microplastics have been found in waters, sandy beaches and muddy shores (Cheung & Fok, 2016; Fok & Cheung, 2015; Fok et al., 2018; Lo, Xu, Wong, & Cheung, 2018; So, Chan, & Not, 2018), which does not only ruin our recreational areas, but also destroys seas and oceans that are home to thousands of marine organisms.

Microplastics are commonly defined as plastic materials with a size less than 5 mm (Tsang et al., 2017) and can occur in several forms such as fragments, films, fibres and pellets (Cole, Lindeque, Halsband, & Galloway. 2011). They are widely utilized in daily life, for example, scrubbing agents in personal care and cosmetic products, packaging materials and construction materials (Cole et al., 2011). Generally, microplastics can be classified into two groups, primary microplastics and secondary microplastics. Primary microplastics are manufactured plastics in the industrial sector whereas secondary microplastics are small plastic items formed by degradation and fragmentation of macroplastics under the action of ultraviolet radiation and physical actions in the environment (GESAMP, 2016).

1.1.2 Pathways of microplastics entering the food chain

Due to the light weight, high durability, high strength and good conductor of heat and electricity, the demand of plastic products becomes increasingly high. Geyer, Jambeck and Law (2007) estimated the global plastic production in 2015 was as high as 380 million tonnes. Among these plastics, Thompson (2006) predicted that a huge portion of 10% plastic production will end up in the oceans via different pathways, including effluent from wastewater treatment plants and irresponsible discharges. Because of the extraordinarily small size of microplastics, Vesilind (2003) justified that wastewater treatment plants which are designed to remove solid wastes could not filter microplastics and eventually release them into the sea. Moreover, littering, river channels, industrial and urban discharges are considered some of the direct means of microplastics entering the aquatic environment (Culin & Bielic, 2016).

Once microplastics have incorporated into the marine environment, they become bioavailable to marine organisms. Since these small synthetic items are likely to be non-biodegradable and remain in the environment for a long period of time (Shimao, 2001), microplastics can enter the food chain by ingestion. Microplastics have been detected in many marine species worldwide, including planktons (Setälä, Fleming-Lehtinen, & Lehtiniemi, 2014), fishes (Avio, Gorbi, & Regoli, 2015; Brate, Eidsvoll, Steindal, & Thomas, 2016; Cheung, Lui, & Fok, 2018; Lusher, Mchugh, & Thompson, 2013), bivalves (Li, Yang, Li, Jabeen, & Shi, 2015; Li et al., 2016; Regurea, Viñas, & Gago, 2019) and crustaceans (Devrise et al., 2015; Gray & Weinstein, 2017). The ingested microplastics were found bioaccumulated inside the bodies of marine organisms, for instance, in the gastrointestinal tract (Cheung et al., 2018; Brate et al., 2016) and even translocated to the circulatory system (Browne, Dissanayake, Galloway, Low, & Thompson, 2008) as well as hepatic tissues (Avio et al., 2015). When marine organisms at higher trophic levels feed on those at lower trophic levels, they also uptake the ingested microplastics passively. As a result, microplastics can be transferred along the food chain by trophic transfer (Wright et al., 2013).

1.1.3 Potential effects of microplastics on marine organisms

Studies have revealed that ingested microplastics pose a threat to the physical well-being of marine organisms, causing both physical and chemical effects. For physical implications, Li (2018) reviewed that consumed microplastics may lead to blockage and damage of digestive tract, and thus result in satiation, starvation, physical deterioration, and in the worst cases, death. Considering chemical effects, due to the large surface-area-to-volume ratio and hydrophobicity, harmful organic pollutants, for example, persistent, bioaccumulative and toxic pollutants (PBTs) and persistent organic pollutants (POPs) are discovered to be adhered to microplastics (Wright, Thompson, & Galloway, 2013). Worse still, Mato et al. (2001) showed that the concentration of hydrophobic organic pollutants can be built up to million times more than that in the aquatic environment. Heavy metals such as copper and zinc were also reported to be adsorbed to microplastics (Brennecke, Duarte, Paiva, Cacador, & Clode, 2016). Long-term exposure to these contaminants can trigger toxic effects including endocrine disruption, oxidative stress, immunotoxicity and chronic diseases by biomagnification (Brennecke et al., 2016; Li, 2018). Beside serving a medium to transfer organic pollutants and heavy metals, Zettler, Mincer and Amaral-Zettler (2013) disclosed that microplastics are also a habitat of waterborne pathogens such as *Vibrio* spp., implying that diseases may be spread among marine organisms via ingestion of microplastics.

1.1.4 Potential effects of microplastics on human health

In fact, marine organisms are not the only victims of this global environmental risk. Being at the top of the trophic levels, humans consume marine organisms as foods in which microplastics are likely to be accumulated. Thus, human health may be jeopardized when they intake seafood along with the accumulated microplastics in their body (Barboza, Vethaak, Lavorante, Lundebye, & Guilhermino, 2018). Although the current understanding of the adverse effects of microplastic consumption on humans are still insufficient, it is speculated that the health implications are similar to those on marine organisms on account of the physical and chemical properties of microplastics as mentioned above. Because plastic products are already made by a diversity of toxic chemicals, microplastics are believed to cause cancers, endocrine disruption and neurotoxicity in humans (Wright & Kelly, 2017). Biomagnification of the chemicals attached to microplastics even exacerbates the toxic effects on humans (Hermabessiere et al., 2017). Barboza et al. (2018) summarised that microplastics smaller than 20 μm have the ability to penetrate into internal human organs whereas those smaller than 0.1 μm can gain access to all organs by crossing cell membranes, blood-brain barrier and the placenta. Consequently, Wright and Kelly (2017) suggested that microplastics can be translocated to secondary tissues, for example, hepatic tissues, muscles and the brain. Furthermore, when the immune system interacts with microplastics, a wide range of negative effects are expected, such as immunotoxicity, immune activation, immunosuppression and abnormal inflammatory responses (Wright & Kelly, 2017). Since microplastics can act as a vector of infectious diseases as discussed previously, it is highly possible that there will be global spread of diseases carried by microplastics (Zettler, Mincer, & Amaral-Zettler, 2013), which may also infect humans as seafood consumers.

1.2 Microplastics in seafood

To pave the way for more detailed exploration of potential health implications on humans, assessing the abundance of microplastics in the seafood is the very first step. Previous research studies reported that microplastics were usually detected in a wide range of bivalves, crustaceans and commercially important fishes. Among the 25 most important marine species in the worldwide sea fishing industry mentioned by the Food and Agriculture Organization of the United Nations (2016), as many as 11 species were revealed to be contaminated by microplastics.

Considering the microplastics accumulation in bivalves, Vandermeersch et al. (2015a) found an average abundance of 0.13 ± 0.14 microplastic items g^{-1} of wet weight in commercial mussels, *Mytilus edulis* and *M. galloprovincialis*, collected from mussel farms and shops in five European countries, namely Denmark, France, Italy, Spain and The Netherlands. The number of fibres observed in these mussels varied from 0.00 item g^{-1} to 0.29 items g^{-1}. De Witte et al. (2014) retrieved a higher abundance of microplastics from Belgian commercial mussels, *Mytilus edulis*, with an average of 0.35 fibres g^{-1}. In Canada, Mathalon and Hill (2014) compared the microplastic abundance in *M. edulis* wild mussels in Nova Scotia to that in farmed mussels. They counted an average number of 75 microplastics individual^{-1} in farmed mussels, which was higher than that in wild mussels with 34 microplastics individual^{-1} on average. Van Cauwenberghe and Janssen (2014) recovered 0.36 ± 0.07 microplastics g^{-1} from the same mussel species but from a German farm. A study in China investigated microplastics in 9 commercial bivalves bought from a fishery market in Shanghai (Li et al., 2015). The total number of microplastics ranged from 2.1 to 10.5 items g^{-1} and 4.3 to 57.2 items individual^{-1}. Significantly high numbers of fibers were observed in all bivalve species, comprising of more than 50% of the extracted microplastics.

For other shellfish species, Van Cauwenberghe and Janssen (2014) recovered 0.47 ± 0.16 particles g^{-1} in *Crassostrea gigas* oysters from France. Five mollusc species, including bivalves and gastropods collected from the Persian Gulf in the Middle East were discovered to contain 0.2 to 21.0 microplastics g^{-1} and 3.7 to 17.7 microplastics individual^{-1} (Naji, Nuri, & Vethaak, 2018). Similarly, the most common type of microplastics was fibres which were more than half of the total items, followed by fragments, about 26%. A pilot study in Taiwan focused on three popular seafood species, hard clam *Meretrix lusoria*, oyster *C. gigas* and loligo squid *Loliginidae* spp., and showed an average microplastic abundance of 0.1167 items g^{-1}, 0.1079 items g^{-1} and 0.0390 items g^{-1} in each respective species (Chen, Lee, & Walther, 2020).

Regarding commercial fishes, Avio et al. (2015) studied 5 commercial Adriatic fish species, including European pilchard *Sardina pilchardus*, spiny dogfish *Squalus acanthias*, European hake *Merlucius merlucius*, red mullet *Mullus barbatus* and tub gurnard *Chelidonichthys lucernus*, which contained 1 to 1.78 items per positive individual. More than 50% recovered microplastics were fragments. In Norway, the Atlantic cod *Gadus morhua* was found to be microplastic polluted in 9 out of 302, approximately 3% of examined fish stomachs (Brâte et al., 2016). A higher percentage of 5.5% among 290 pelagic and dermal fishes from the North Sea and Baltic Sea was detected to contain microplastics (Rummel et al., 2016). Rochman et al. (2015) sampled fishes and shellfish from markets in Indonesian and the USA. About 28% in USA species and 25% in Indonesian species were contaminated with microplastics while the average number of plastic items per individual was 0.5 and 1.4 respectively. Two important edible fish species in the eastern coast of Brazil, king mackerel *Scomberomorus cavalla* and sharpnose shark *Rhizoprionodon lalandii*, were also uncovered to ingest plastic pellets from 2 to 6 items per individual, with 1 to 5 mm sizes (Miranda & Carvalho-Souza, 2016). Microplastics were revealed in about 20% of 26 commercial fish species caught from the Portuguese coast, among which more than 60% were fibres and 34.2% were fragments (Neves, Sobral, Ferreira, & Pereira, 2015). The average microplastic abundance was 0.27 ± 0.63 items individual^{-1}. In Hong Kong, Cheung, Lui and Fok (2018) studied the microplastic accumulation in wild and captive flathead grey mullets (*Mugil cephalus*), a commercially important fish in the local. Wild mullets were found to contain more microplastics than in captive mullets, with 4.3 items individual^{-1} in the former species and 0.2 items individual^{-1} in the latter group.

It should be noted that the samples in the above research studies were lively caught, bought from fishery markets or from supermarkets. For those brought from supermarkets, whether they were packaged or alive was unknown. Even though Li, Green, Reynolds, Shi and Rotchell (2018) sampled mussels from supermarkets from the UK and established a distinctive difference in the microplastic abundance between live (0.9 items g^{-1}) and processed mussels (1.4 items g^{-1}), the processed samples could have been only frozen or even cooked that was not clearly indicated.

In brief, the extent of microplastic contamination in seafood varied depending on the origins. The most commonly found plastic items were fibres and some were fragments, and their sizes were substantially smaller in shellfish species than in fishes. Table 1 summarises the above-mentioned previous research on microplastics in seafood.

Table 1: Summary of previous research on microplastics in seafood

Species	Sources	Major shapes of microplastics	Sizes of microplastics	Abundance of microplastics (Mean/range)	References
Shellfishes					
Mytilus edulis, *M. galloprovincialis*	Denmark, France, Italy, Spain, The Netherlands	fibres	23% < 20 μm 77% < 50 μm	0.00 - 0.29 items g^{-1}	Vandermeersch et al., 2015a
Mytilus edulis	Belgium	fibres	200 μm - 1500 μm	0.35 fibres g^{-1}	De Witte et al., 2014
Mytilus edulis	Canada	fibres	Not mentioned	Farmed: 75 items $individual^{-1}$ Wild: 34 items $individual^{-1}$	Mathalon & Hill, 2014
Mytilus edulis	German	not mentioned	5 - 10 μm (the most abundant group)	0.36 ± 0.07 microplastics g^{-1}	Van Cauwenberghe & Janssen, 2014
9 commercial bivalves	China	fibres	5 μm – 5 mm (the most common: < 250 μm)	2.1 to 10.5 items g^{-1}, 4.3 to 57.2 items $individual^{-1}$	Li et al., 2016
Crassostrea gigas	France	not mentioned	16 – 20 μm	0.47 ± 0.16 particles g^{-1}	Van Cauwenberghe & Janssen, 2014
5 mollusc species	the Persian Gulf in the Middle East	fibres	10–25 μm (the most abundant group)	0.2 to 21.0 microplastics g^{-1}, 3.7 to 17.7 microplastics $individual^{-1}$	Naji, Nuri, & Vethaak, 2018
Mytilus edulis	the UK	fibres	73 μm – 4.7 mm	0.9 items g^{-1} (live) 1.4 items g^{-1} (processed)	Li, Green, Reynolds, Shi, & Rotchell, 2018
Meretrix lusoria, *Crassostrea gigas*	Taiwan	fragments	20 – 800 μm	*Meretrix lusoria*: 0.1167 items g^{-1} *Crassostrea gigas*: 0.1079 items g^{-1}	Chen, Lee, & Walther, 2020

Fishes					
5 commercial Adriatic fish species	Adriatic Sea	fragments	< 0.1 mm (the most abundant group)	1 – 1.78 items per positive individual	Avio et al., 2015
Gadus morhua	Norway	fibres	3.2 – 41.7 mm Mean: 14.1 mm	9 out of 302 samples (3%)	Brâte et al., 2016
pelagic and dermal fishes	the North Sea and Baltic Sea	fragments	200 μm – 2.2 mm	Demersal fishes: 0.03 ± 0.18 items individual^{-1} Pelagic feeders: 0.19 ± 0.61 items individual^{-1}	Rummel et al., 2016
fishes and shellfishes	the USA, Indonesia	fibres	*For the USA's:* 0.01 – 2.1 mm Mean: 6.3 mm *For Indonesia's:* 0.1 – 4.5 mm Mean: 3.5 mm	the USA: 0.5 items individual^{-1} Indonesia: 1.4 items individual^{-1}	Rochman et al., 2015
Scomberomorus cavalla, Rhizoprionodon lalandii	Brazil	pellets	1 – 5 mm	2 - 6 items individual^{-1}	Miranda & Carvalho-Souza, 2016
26 commercial fish species	Portugal	fibres	0.217 – 4.81 mm Mean: 2.11 mm	0.27 ± 0.63 items individual^{-1}	Neves, Sobral, Ferreira, & Pereira, 2015
Mugil cephalus	Hong Kong	fibres	0.1 mm – 12 mm < 2 mm (the most abundant group)	Wild: 4.3 items individual^{-1} Captive: 0.2 items individual^{-1}	Cheung, Lui, & Fok, 2018

1.3 Objectives and hypothesis

In view of the health drawbacks on microplastic consumption and the limited knowledge about microplastic pollution in seafood that is available to Hong Kong consumers, it is meaningful to study the microplastic abundance in our food in order to estimate the severity of eating contaminated seafood. Among the seafood, mussels are extensively deployed as bioindicators in monitoring the marine environment, such as the Mussel Watch Program in the US (NCCOS, 2017), the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Commission, 2021), the United Nations Environment Programme for the Assessment and Control of Pollution in the Mediterranean Region (IW:LEARN, 2021), and the Environmental Protection Department biological indicator monitoring programme (EPD, 2004) in Hong Kong. It is because mussels are widely distributed worldwide, easy to obtain and have high tolerance to salinity (O'Connor, 1998). Moreover, mussels are filter feeders and have high ventilation rate of water-borne substances, making them a vulnerable marine species to microplastic intake (Browne et al., 2008). On the other hand, mussels were considered contaminated seafood in concern by a European database (Vandermeersch et al., 2015b). Therefore, mussels are marine organisms which are both susceptible to microplastic contamination and a medium of introducing microplastics into human diet. Since Li et al. (2018) proved that processed mussels were more contaminated than live mussels, it is of high significance to examine the microplastics in frozen mussels available in Hong Kong in an effort to fill the current research gap and raise citizens' awareness of food safety, especially seafood.

This study aims to investigate the extent of microplastic contamination in frozen mussels bought in Hong Kong. Specifically, it is to establish any discrepancy in microplastic abundance between mussels from different origins and to investigate the relationship between the physical properties of mussels, for example, length of shells and wet weight, and the abundance of ingested microplastics. It is expected that there will be differences in microplastic abundance between mussel samples originated from various countries as they are exposed to different extent of microplastic pollution, and this has been evident when comparing the findings of previous research. It is also predicted that there will be a positive correlation between the wet weight of mussels and the microplastic abundance as shown in Cheung's et al. (2018) study that larger mullets ingested more microplastics. A similar trend is anticipated between the length of mussel shells and the microplastic abundance as it is commonly acknowledged that larger shells are associated with larger soft tissues. Concerning the types of microplastics, fibres are most likely to appear in mussels like other shellfishes listed above. In this way, the majority of microplastics observed is also foreseen to fall into the group of the smallest size.

2. Methodology

The procedures were mainly adapted from Cheung's et al. (2018) research but modifications were done to improve the results. The figure below shows a flow chart indicating the major steps of this study.

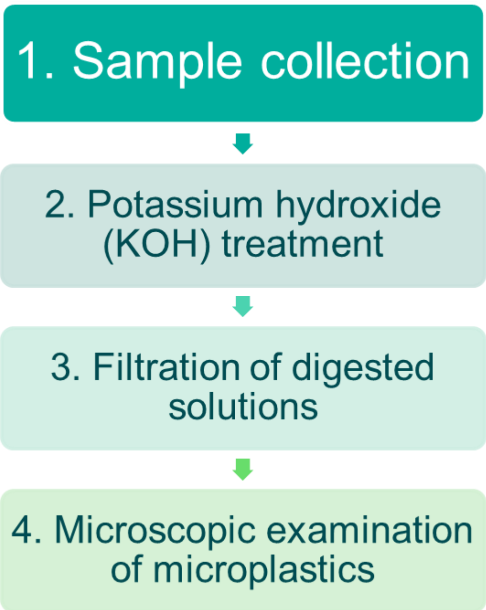


Figure 1: A flow chart of this study

2.1 Sample collection

A total of 50 uncooked frozen mussels were bought from supermarkets or online in Hong Kong. They came from five different countries, including Australia, Holland, Canada, New Zealand and the USA. Ten mussels were randomly selected for each country. Table 2 lists out the origins, species and purchase sources All sampled were sold in plastic packages as shown in Table 3. The package of the

USA samples (sample U) was not shown because it was only a transparent plastic box. The samples were frozen at - 40°C in a refrigerator before further treatment.

Table 2: Origins, species and purchase sources of mussel samples

Origins	**Species**	**Purchase sources**
Australia	Blue mussels (*Mytilus galloprovincialis*)	Supermarket
Holland	Blue mussels (species not specified)	Supermarket
Canada	Blue mussels (species not specified)	Snacks chain store
New Zealand	Green-lipped mussels (*Perna canaliculus*)	Supermarket
USA	Blue mussels (species not specified)	Online

Table 3: The packaging of frozen mussels from different origins

			
Blue mussels from Australia	Blue mussels from Holland	Blue mussels from Canada	Green-lipped mussels from New Zealand

2.2 Extraction of microplastics from mussel samples

Instead of using hydrogen peroxide (H_2O_2) to extract microplastics from soft tissues of mussels as in Cheung's et al. (2018) work, potassium hydroxide (KOH) was used since Thiele, Hudson and

Russell (2019) proposed that 10% potassium digestion method could improve filtration at single-digit pore size. It was also recognised as the most cost-effective and the fastest method for digesting bivalve tissues. Besides, an incubation temperature at 40°C or lower could minimise the structural damage of plastic polymers that would have the least effect on visual identification at the latter stage (Thiele et al., 2019). To prevent contamination, all the liquids, including deionized water and potassium hydroxide, were filtered by 0.6 μm pore size glass fibre filter paper (GB-100R, Advantec, Japan) before use while all containers and appartus were rinsed three times with filtered deionized water prior to use. Samples and containers were covered with aluminium foils immediately when they were not in use. A background blank was performed by soaking a filter paper with filtered deionized water and placing it near the working area to detect any microplastics flowing in the air. No microplastics were observed in the background blank.

2.2.1 Potassium hydroxide (KOH) treatment

The mussel samples were defrosted in a warm water bath. The shells were opened and all the inner contents were removed using dissection scissors and knives. The surface of the soft tissue was rinsed with filtered deionized water before measuring its wet weight, the shell length and shell width. The soft tissues were put into a beaker and was covered with aluminium foil. 10% potassium hydroxide was added into each beaker to digest the organic matters. The required volume of potassium hydroxide was calculated by multiplying the wet weight of each mussel by 10 times. The beakers were covered again and put into an oscillation incubator at 40°C at 80 rpm for 36 to 48 hours, depending on the digestive effect of the soft tissue.

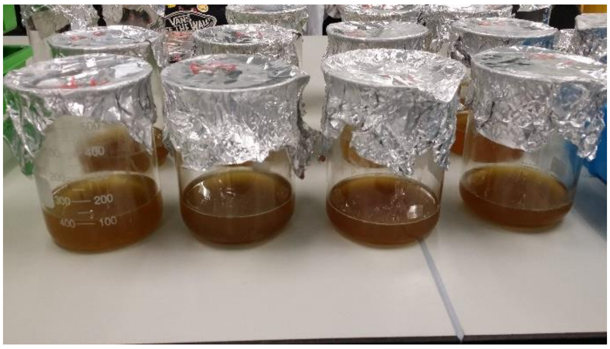
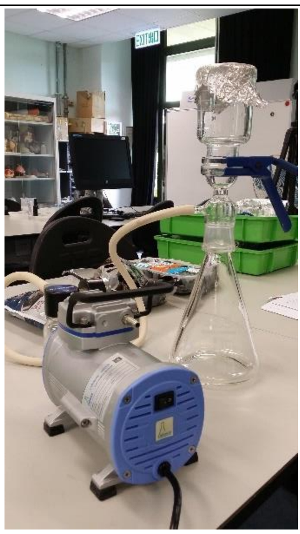
2.2.2 Filtration of digested solutions

After the digestion process, the remaining solutions were filtered over a 0.6 μm pore size glass fibre filter paper using a filter set and vacuum pump. The resulted filter paper was then put into a clean petri dish with a cover and dried in an oven at 40°C for about 24 hours.

2.3 Microscopic examination of microplastics

The filter papers were observed under a stereo microscope (Olympus, SZ61) with a magnification range of 6.7X to 45X with 10X eyepieces to identify the shapes, sizes and colours of microplastics. Images were taken for records. Several selection rules for microplastics were followed as suggested by Hidalgo-Ruz, Gutow, Thompson and Thiel (2012). Firstly, no cellular or organic structures should be observable. Secondly, only microplastic items with equal thickness along the entire piece should be categorized as fibres. Thirdly, the identified microplastics should have clear and homogenous colours throughout. Lastly, if the microplastic item is suspected to be transparent or white colour, it should be examined under high magnification to ensure its colour. The shapes of microplastics were classified into four groups, namely fragments, films, fibres and pellets. The sizes microplastics were divided into three main categories, small (with sizes less than 2 mm), large (with sizes 2 mm to 5 mm) and mesoplastics (with sizes larger than 5 mm). Table 4 presents photos in some major steps of this study.

Table 4: Photos showing some major steps of this study

 Defrosting and removing the soft tissues of mussel samples.	 Resulted solutions after hydrogen peroxide treatment.
 Setup of vacuum filter to retain microplastics.	 The stereo microscope used for visual identification of microplastic items on filter papers.

2.4 Statistical analysis

All the collected data was analyzed by IBM SPSS 26 software platform. Since the microplastic abundance data did not form a normal distribution, non-parametric tests were conducted to establish any significant difference in microplastic abundance between the five origins. Kruskal-Wallis H Test was used to compare between the five groups, followed by Mann-Whitney U Test to perform pairwise comparison. The significant level was set to 0.05. For the same reason, as the shell length and wet weight data did not approach to a normal distribution, Spearman Correlation Test, a non-parametric test, was run to find out any correlation between length of shells, wet weight, and the abundance of ingested microplastics. The microplastic abundance was presented by the number of microplastic items found per individual mussel sample. The significant level was set to 0.05.

3. Results

3.1 Abundance of microplastics in mussels

Table 5 shows the descriptive data of physical properties, including shell length, shell width and wet weight of 50 mussel samples from 5 different origins while Table 6 shows the descriptive data of abundance of microplastics in mussels in terms of the number of microplastic items per individual sample and the number of items per wet weight of soft tissues. A total number of 590 plastic items were found in 50 mussel samples. Nearly all samples were contaminated with microplastics, except one sample from Australia, revealing that 98% of mussel samples contained plastic items. The average abundance of microplastics was 11.80 ± 14.44 items per individual sample and 1.42 ± 2.00 items per g of wet weight. The average abundance in terms of per individual deviated far away from median (7.44 items), suggesting that most mussels only contained about 7 plastics items and only a few contained exceptionally more items. The mean of the number of items per unit wet weight was 2 times more than the median number 0.65 items g^{-1}. This also pointed to the same trend that some mussels ingested much higher number of plastic items. Taking a closer look at each of the mean microplastic abundance of the five origins uncovers that Canadian mussels were mostly affected by microplastic pollution in both calculations of microplastic abundance (24.2 items $individual^{-1}$ & 4.12 items g^{-1}) while the least affected batches were from Australia and Holland, with approximately 4 items per individual and 0.5 items per unit wet weight. Although mussels from New Zealand was ranked the second polluted batch in terms of per individual, its microplastic abundance per wet weight was twice the abundance of the American samples.

Table 5: Descriptive data of physical properties of mussel samples

Origins	Physical properties	Mean	Median	SD	Max.	Min.	Range
Australia	Shell length (mm)	81.30	82.00	3.13	86.00	77.00	9.00
	Shell width (mm)	40.60	40.00	2.12	44.00	38.00	6.00
	Wet weight (g)	10.70	11.40	2.35	13.60	6.60	7.00
Holland	Shell length (mm)	61.30	61.50	1.83	64.00	59.00	5.00
	Shell width (mm)	26.50	27.00	1.84	29.00	22.00	7.00
	Wet weight (g)	7.74	7.25	1.96	11.50	4.80	6.70

Canada	Shell length (mm)	66.10	66.50	5.11	72.00	56.00	16.00
	Shell width (mm)	29.70	29.00	3.62	37.00	25.00	12.00
	Wet weight (g)	5.70	5.50	1.17	7.30	3.60	3.70
New Zealand	Shell length (mm)	107.90	106.00	7.33	122.00	99.00	23.00
	Shell width (mm)	46.50	46.50	2.92	53.00	43.00	10.00
	Wet weight (g)	26.33	24.50	5.59	36.50	19.00	17.50
USA	Shell length (mm)	67.20	65.50	4.44	78.00	63.00	15.00
	Shell width (mm)	33.10	33.00	2.51	38.00	30.00	8.00
	Wet weight (g)	7.44	7.20	1.55	10.50	4.90	5.60

Table 6: Descriptive data of abundance of microplastics in mussels

Origins	Abundance of microplastics	Mean	Median	SD	Max.	Min.	Range
Australia	No. of items per individual	4.10	5.00	2.13	7	0	7
	No. of items per unit wet weight	0.43	0.44	0.25	0.76	0.00	0.758
Holland	No. of items per individual	3.90	4.00	1.60	7	1	6
	No. of items per unit wet weight	0.55	0.50	0.27	1.04	0.09	0.96
Canada	No. of items per individual	24.20	16.00	19.99	61	7	54
	No. of items per unit wet weight	4.12	2.48	3.04	8.62	1.43	7.19
New Zealand	No. of items per individual	17.80	10.50	18.71	67	1	66
	No. of items per unit wet weight	0.68	0.44	0.70	2.47	0.04	2.43
USA	No. of items per individual	9.00	8.50	4.88	21	4	17
	No. of items per unit wet weight	1.35	1.09	1.10	4.29	0.53	3.76
All	No. of items per individual	11.80	7.00	14.44	67	0	67
	No. of items per unit wet weight	1.42	0.65	2.00	8.62	0.00	8.62

After Shapiro-Wilk Test for normality, the p-values of the physical properties and the abundance of microplastics of mussels from the five origins are listed in Table 7. As the significant value was 0.05, all the parameters when considering all the five groups did not reach a normal distribution ($p < 0.05$). The non-parametric test, Kruskal-Wallis H Test, was chosen to compare the microplastic abundance between the five groups. Test statistics resulted that the p-values (Asymp. Sig. 2-tailed) of the two forms of microplastic abundance were 0.000 (< 0.05). Therefore, it can be concluded that there are significant differences between the five groups of microplastic abundances.

Table 7: Shapiro-Wilk Test results of physical properties of mussels and abundance of microplastics

Origins	**p-values of Shapiro-Wilk Test**				
	Shell length	Shell width	Wet weight	No. of items per individual	No. of items per unit wet weight
Australia	0.335	0.145	0.567	0.275	0.450
Holland	0.219	0.028	0.280	0.609	0.789
Canada	0.512	0.743	0.651	0.031	0.013
New Zealand	0.298	0.235	0.178	0.001	0.001
USA	0.025	0.624	0.647	0.033	0.001
All	0.000	0.023	0.000	0.000	0.000

Mann-Whitney U Test was conducted to look for any significant difference in microplastic abundance between each pair of origins. Table 8 shows the test results of the abundance of microplastics per individual. The p-value of the Australia-Holland pair was 0.537 (> 0.05), so there was no significant difference between the two origins. The p-value of 0.622 also suggested that there was no significant difference between Canada and New Zealand pair. Moreover, USA and Canada pair (p-value of 0.058) and USA and New Zealand pair (p-value of 0.143) also showed no significant difference but with a smaller extent. All other pairs that resulted in p-values less than 0.05 had a significant difference between each other.

For the Mann-Whitney U Test results of the abundance of microplastics per unit wet weight in Table 9, Australia and Holland pair still did not show a significant difference (p-value: 0.496). Australia and Canada, Holland and Canada, USA and Australia, and USA and Holland pairs also had consistent results as the abundance of microplastics per individual, which showed a significant difference. Nevertheless, New Zealand pairs and the USA and Canada pair had opposite results compared to the previous results.

Table 8: Mann-Whitney U Test results of the abundance of microplastics per individual

Origins	p-values of Mann-Whitney U Test				
	Australia	Holland	Canada	New Zealand	USA
Australia		0.537	0.000	0.002	0.007
Holland			0.000	0.002	0.003
Canada				0.622	0.058
New Zealand					0.143
USA					

Table 9: Mann-Whitney U Test results of the abundance of microplastics per unit wet weight

Origins	p-values of Mann-Whitney U Test				
	Australia	Holland	Canada	New Zealand	USA
Australia		0.496	0.000	0.880	0.002
Holland			0.000	0.496	0.007
Canada				0.001	0.004
New Zealand					0.015
USA					

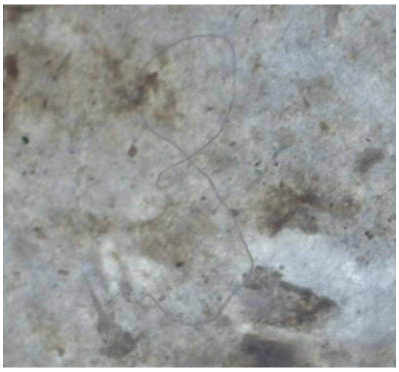
3.2 Shapes, sizes and colours of microplastics in mussels

Among 590 identified plastic items, 573 items were fibres, composing an extremely high percentage of 97.12% (573 items) of all items as presented in Figure 2. Fragments made up of 2.54% (15 items) of the total but were not found in New Zealand samples. Films (2 items, 0.34%) were only observable in Australia samples and no pellets were extracted in all samples. The example appearances of different identified shapes of microplastics are shown in Figure 3.

Figure 2: Shapes of microplastics in mussel samples

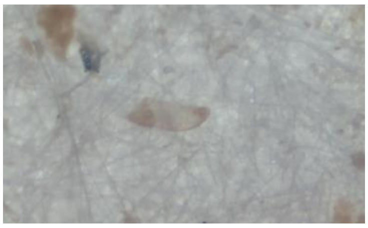
A blue fibre

A white fibre



A transparent fibre

A blue fibre



A brown film

Figure 3: Photos of some identified microplastics

The sizes of identified microplastics ranged from 0.06 mm to 17.06 mm and the mean size was 1.18 ± 1.45 mm (Table 10). The major group of size was small (< 2 mm) microplastics which composed of 85.59%, followed by large items (2 to 5 mm) with 11.69%. The least number was found in mesoplastics (> 5 mm) with as low as 2.71%. Figure 4 shows the distribution of sizes in samples.

Table 10: Descriptive data of sizes of microplastics in mussels (unit in mm)

Origins	Mean	Median	SD	Max.	Min.	Range
Australia	1.35	0.63	1.68	8.65	0.08	8.57
Holland	1.22	0.87	1.28	5.90	0.11	5.79
Canada	0.92	0.74	0.82	6.70	0.08	6.62
New Zealand	1.34	0.70	1.67	12.36	0.06	12.30
USA	1.44	0.79	2.06	17.06	0.10	16.96
All	1.18	0.74	1.45	17.06	0.06	17.00

Figure 4: Sizes of microplastics in mussel samples

The identified microplastics in mussel samples were in 6 different colours, transparent, blue, brown, red/pink, white and black. The pie chart in Figure 5 shows the colour distribution of microplastics. Nearly half of the plastic items were transparent (48.47%, 286 items) and slightly over a quarter of the items were blue (25.93%, 153 items). The third mostly found colour was red or pink with close to one sixth of them (16.10%, 95 items), followed by brown microplastics (8.31%, 49 items), which had about half of the number of the red or pink items. The least numbers were white and black microplastics with only 0.68% (4 items) and 0.51% (3 items) respectively.

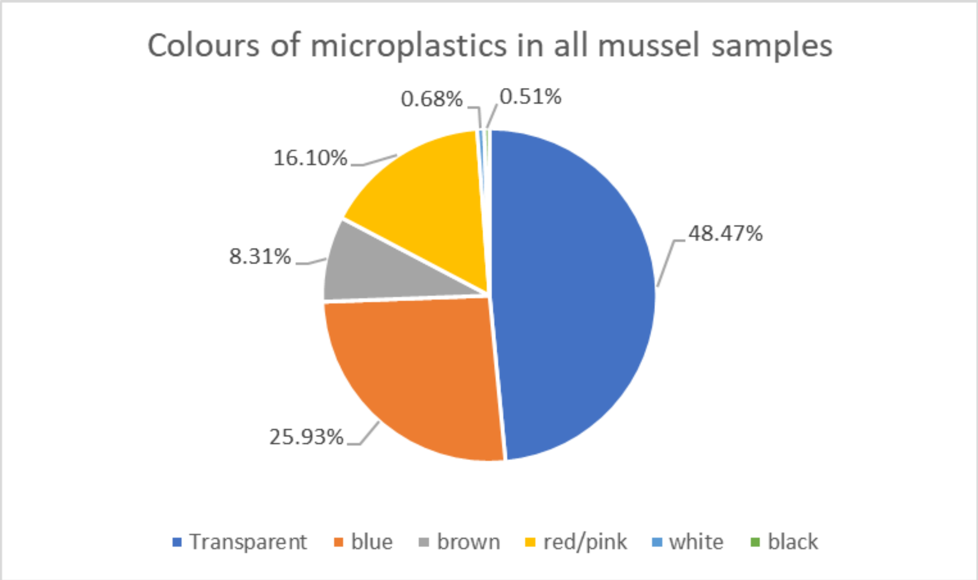


Figure 5: Colours of microplastics in all mussel samples

3.3 Abundance of microplastics and physical properties of mussels

Due to the non-normal distribution of the data, Spearman Correlation Test was performed to establish any significant correlations between shell length and wet weight, shell length and microplastic abundance per individual, and wet weight and microplastic abundance. The results are listed in Table 11. Significant correlation could only be established between shell length and wet weight ($p < 0.05$, $r = 0.759$), implying a strong positive correlation. There was no significant correlation between microplastic abundance per individual and shell length or wet weight ($p > 0.05$). Considering the strength of correlation, both correlations had a very weak or negligible positive correlation ($r = 0.278$ & $r = -0.099$).

Table 11: Spearman Correlation Test results of shell length, wet weight and microplastic abundance per individual

Correlations	p-value	Correlation coefficient (r-value)
Shell length & wet weight	0.000	0.759**
Shell length & microplastic abundance per individual	0.050	0.278
Wet weight & microplastic abundance per individual	0.492	-0.099

** Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

4.1 Comparison of microplastic accumulation between mussels from different countries

In terms of the number of items per individual sample, this study found less microplastics compared to above-mentioned previous research involving shellfish species (Li et al., 2016; Mathalon & Hill, 2014; Naji et al., 2018). However, when considering the number of items per unit wet weight, the microplastic abundance discovered in this study was much higher than most of these research works (Chen et al., 2020; De Witte et al., 2014; Li et al., 2018; Van Cauwenberghe & Janssen, 2014; Vandermeersch et al., 2015a), but was still beyond the lower border of 2.1 items g^{-1} in Chinese bivalves reported in Li's et al. (2016) research. Looking to each origin, Canadian mussels in this study were highly contaminated with microplastics (24.2 items per individual). Nevertheless, in comparison with Mathon's and Hill's (2014) work on mussels from the same country, the microplastic abundance of Canadian mussels in this study was 72% less than their farmed mussels (75 items per individual).

This may be because of different habitats and species of the mussels. Served as a bioindicator, mussels are believed to have the ability to reflect the extent of microplastic pollution in its surrounding aquatic habitat environment and the severity varied from place to place. Despite being filter feeders, the species of Canadian mussels herein was unknown, so their feeding habit such as filtration rate may have a slight difference from other studies'. Comparing the microplastic abundance of American mussels to Rochman's et al. (2015) research on the same country's fish and shellfish species (0.5 items per individual), this study on microplastic abundance on American mussels yielded 18 times the previous one. The difference can be explained by the inclusion of fishes' data in Rochman's et al. (2015) research. From Table 1, it is not difficult to spot that microplastic abundance in shellfishes was generally higher than that in fishes. Combining fishes' data could have been underestimated the microplastic abundance in shellfish species.

4.2 Compositions of microplastics in mussels

Since Fourier transform infrared spectroscopy (FT-IR) instrument at this university cannot be used to detect the polymer type of microplastics with a very small scale, it posed obstacles to the prediction of microplastic sources based on the chemical compositions. However, some microplastic items with the same colours and shapes appeared in the mussel samples repetitively. For example, transparent cylindric fibres and transparent flat fibres (Figure 6) as well as blue fibres (Figure 7) existed consistently in Australian mussels. Other samples also contained similar transparent fibres within their origins (Figure 8, 9, 12 & 14). Apart from transparent colour, distinctive blue fibres with similar appearance were found in all origins (Figure 7, 10 & 13). A considerable number of red and pink fibres were observed particularly in Canadian mussels (Figure 11). Thus, it is highly possible that these fibres came from the same sources of microplastics within their origins such as in the mussel farms or the packaging process. Unfortunately, the FT-IR analysis could not be conducted to detect whether these similar plastic items were composed of the same chemicals, so the exact sources of microplastics remained unclear.

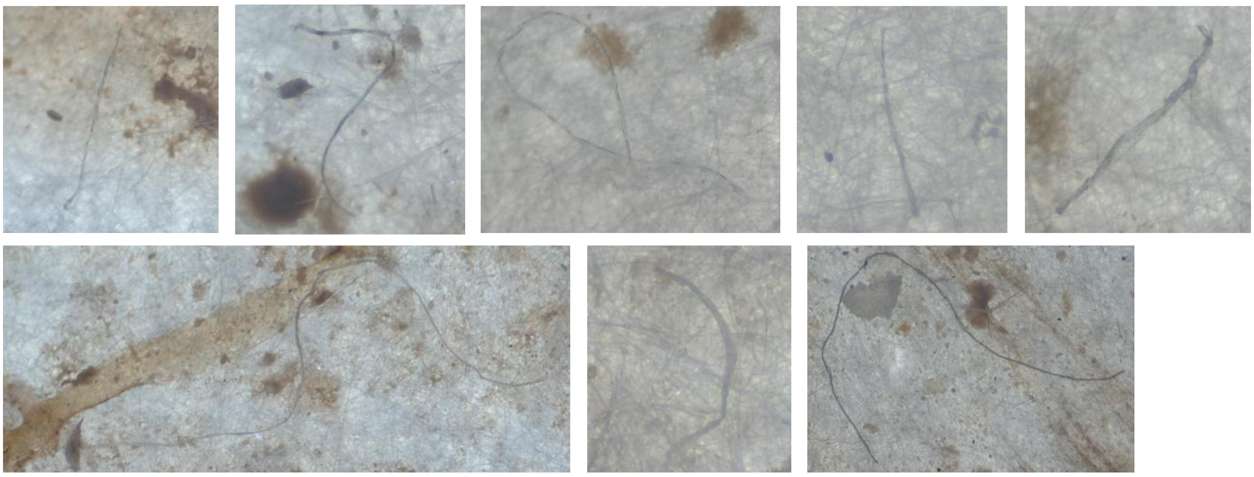


Figure 6: Transparent fibres found in Australian mussels

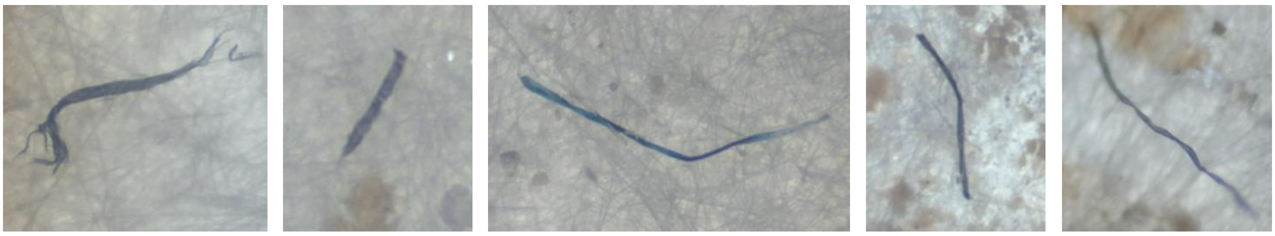


Figure 7: Blue fibres found in Australian mussels

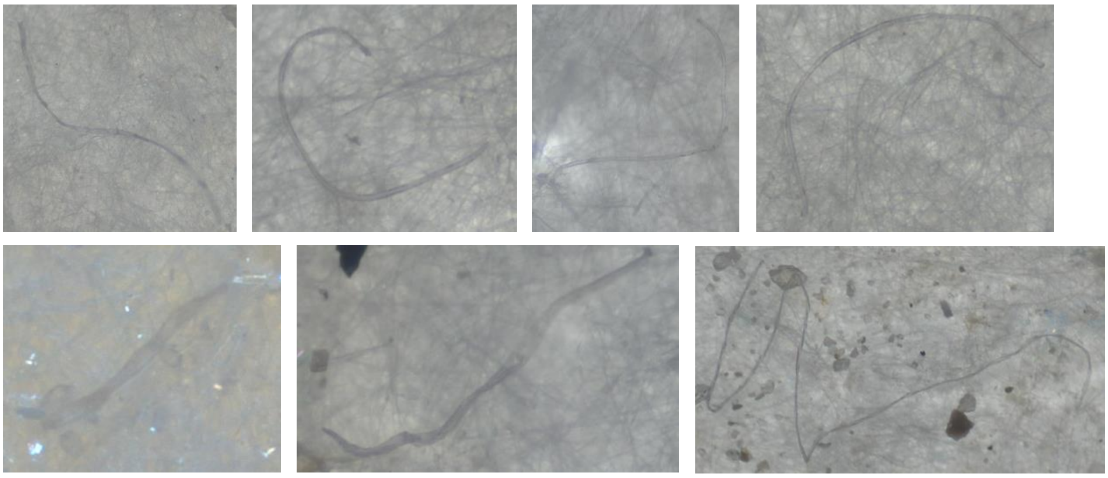


Figure 8: Transparent fibres found in Holland mussels

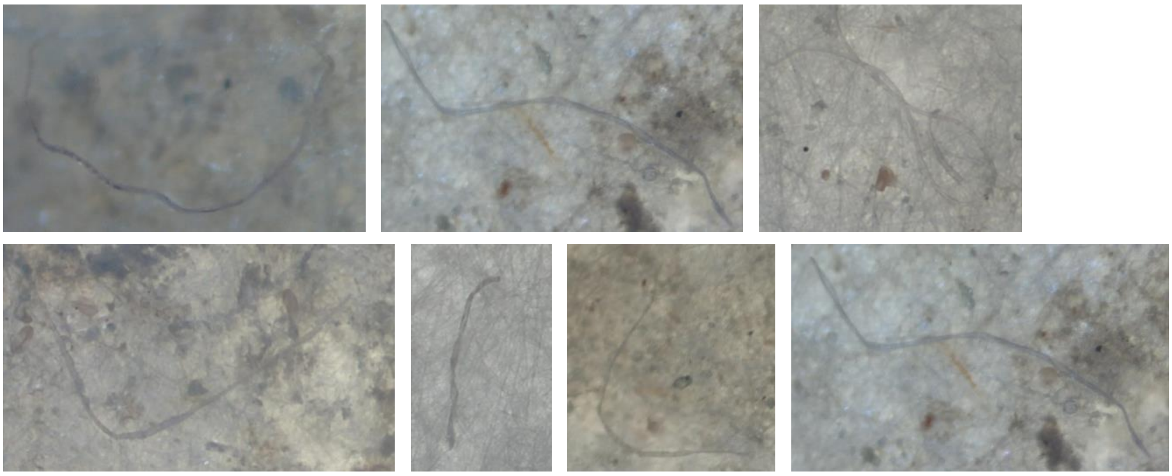


Figure 9: Transparent fibres found in Canadian mussels

Figure 10: Blue fibres found in Canadian mussels

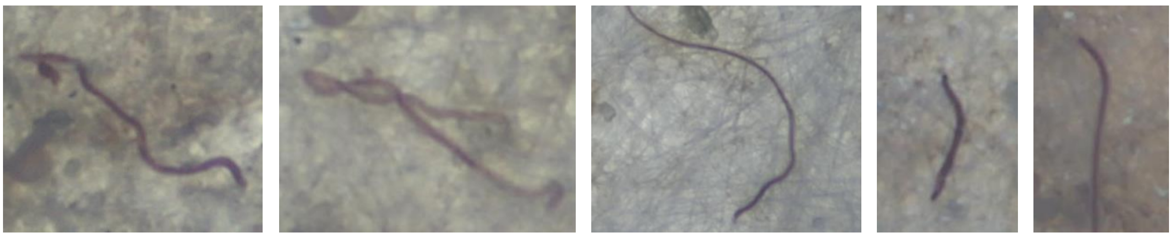


Figure 11: Pink/red fibres found in Canadian mussels

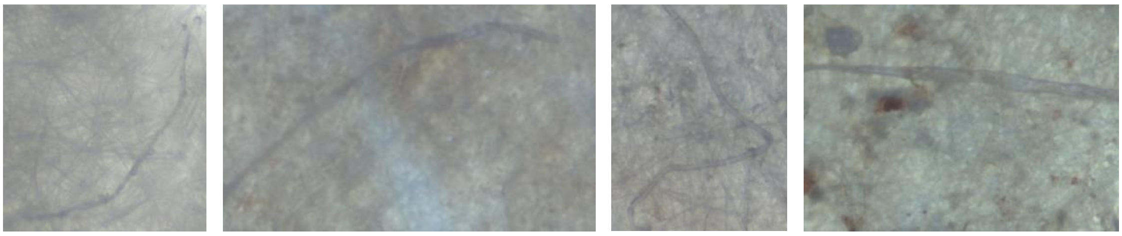


Figure 12: Transparent fibres found in New Zealand mussels

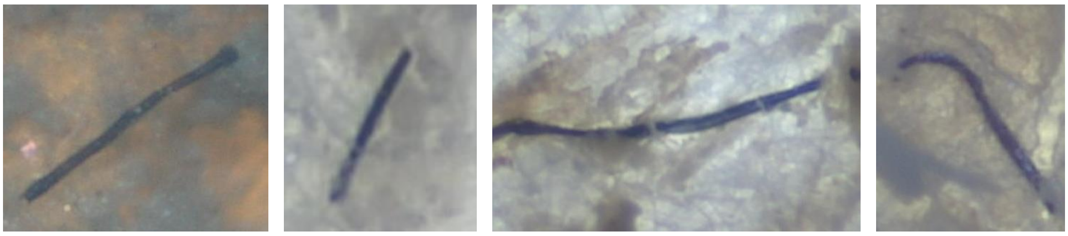


Figure 13: Blue fibres found in American mussels

Figure 14: Transparent fibres found in American mussels

For the size of microplastics, the range identified in this study (0.06 mm – 17.06 mm) only overlapped with those in several research pieces on shellfish species, for example, in Belgium (De witte et al., 2014) (200 µm - 1500 µm), China (Li et al., 2016) (5 µm – 5 mm), the UK (Li et al., 2018) (73 µm – 4.7 mm) and in Taiwan (Chen et al., 2020) (20 – 800 µm). The median size and the smallest size of microplastics in this study were 0.74 mm and 0.06 mm, referring that 50 % of the microplastics fell in this size range. The size range in Chen's et al. (2020) research in Taiwan was the closest to the most popular range in this study. While the lowest boundary (0.073 mm) in Li's et al. (2018) was similar to that in this study (0.06 mm), the mean herein (1.18 mm) was the nearest to the largest size of microplastic (1.5 mm) found in De Witte's et al. (2014). Other studies such as in Vandermeersch et al. (2015a), Van Cauwenberghe and Janssen (2014), and Naji, Nuri and Vethaak (2018) observed microplastic sizes in the scale of micrometres (μm) as small as 5 μm (= 0.005 mm). This could be because of the magnification of the sterero microscope used for visual examination of microplastics. The highest magnification possible for records in this study was 45X with 10X eyepieces, so microplastics as small as in single micrometres were not observable. In contrast, larger microplastics were found in this study. The longest fibre with 17.06 mm was in American mussel, which exceeded the upper limit of microplastic sized in all of the above-mentioned studies on shellfish species.

4.3 Correlations between abundance of microplastics and physical properties of mussels

Although it was ensured that the shell length of mussels was strongly and positively correlated to their wet weight, the wet weight did not have a significant correlation with the microplastic abundance per individual overall. This result did not match with the prediction in the hypothesis as reported by Cheung et al. (2018) that a positive correlation between physical properties of grey flathead mullets, for instance, body weight, and the microplastic abundance was found. Similarly, insignificant correlation also occurred in shell length and microplastic abundance. This finding suggested that the distribution of microplastics in these mussel samples did not hinge on the shell length or the wet weight of mussels but occurred randomly. It rejected the common belief that larger mussels must ingest more microplastics.

4.4 Implications of mussel consumption on human health

Concerning food safety, the Centre for Food Safety (2010) published the Final Report of Hong Kong Population-Based Food Consumption Survey that was conducted between 2005 and 2007. The report revealed that the mean daily consumption of mussels for local citizens was 0.43 g/day for all respondents and 0.98 g/day for consumers. If the mean abundance of microplastics (1.42 items/g) was taken into account, the estimated annual intake of microplastics for each Hong Kong citizen would be approximately 222.87 items/year for all respondents and 507.93 items/year for consumers. Although these numbers may seem low when they are compared to the annual microplastic intake of Chinese shellfish consumers (100000s items/year), that of European counterparts (11000 items/year) (Van Cauwenberge & Janssen, 2014) and that of Taiwanese (909.8 items/year) (Chen et al., 2020), the health implications should not be overlooked. The EFSA Panel on Contaminants in the Food Chain (2016) stated that microplastics with a size less than 150 μm may pass through the epithelial layer of human digestive tract. Referring to the data in this study, 5.76% of microplastics fell into this range and are likely to be translocated in human bodies, causing disruption to immune system and toxic effects (Wright & Kelly, 2017).

4.5 Errors, limitations and suggestions for further studies

In this study, several errors and limitations were identified that may have impacted the results. Firstly, the sample size was small. Only 50 mussels were bought from supermarket or online and five origins were analyzed, which could not sufficiently represent the eating habit of local people. Secondly, since a pack or box of mussels usually already exceeded the required number of mussel samples for each origin, sampling from the same pack lead to a packaging effect that the microplastic source may vary in each pack and finally the results became biased. More importantly, the human errors during the visual identification of microplastics under stereo microscope using human naked eyes were unavoidable despite convenience and low cost of this method. As this was the stage in which microplastic data was recorded, any underestimation of number of microplastics may have affected the results. Moreover, the magnification may not be high enough to count all the microplastics present in the samples, in particular, those in micrometre scale. There was also difficulty in tracing the sources of microplastics because the FT-IR spectroscopy could not be utilized to detect the chemical composition of the plastic items. Whether the identified items were really plastics was unsure due to the lack of FT-IR analysis, which could not eliminate the human error during the identification process. Minor error included the possibility of losing microplastics during defrosting mussels in the same warm water bath.

For future studies, it is suggested that the sample size should be larger and more origins could be investigated. More packs of mussels can be bought, if the budget allows, to minimize the packaging effect. Stereo microscope with higher magnification could be used to observe smaller microplastics. Besides, FT-IR analysis should be performed to detect the chemical compositions of identified microplastics and to help to trace the sources of microplastic pollution. Future research could focus on other prevailing seafood species or local seafood. Since the health implications of microplastic consumption in humans are still being explored, the chronic effects of long-term microplastic ingestion could be examined.

5. Conclusion

Microplastics pollution is an alarming global issue not only in environmental impacts but also in food safety. Humans as the top of the trophic level are exposed to microplastics in consumption of contaminated seafood. This study investigated the extent of microplastic contamination in frozen mussels from five origins bought in Hong Kong. Totally, 590 microplastics were observed in 50 mussel samples. The average abundance of microplastics was 11.80 ± 14.44 items individual^{-1} and 1.42 ± 2.00 items g^{-1}. The mostly polluted batch was found in Canadian mussels whereas the Australian and Holland mussels were the least polluted. The majority (97.12%) of plastic items were in form of fibres which was in line with many other studies. The range of plastic item sizes was 0.06

mm to 17.06 mm and the mean was 1.18 ± 1.45 mm. Most of them (85.59%) were less than 2 mm. Consistent microplastic sources were possible according to the similar shapes and colours of microplastics. Unlike previous research, no significant correlation was established between wet weight of mussels and microplastic abundance, implying that the distribution of microplastics in mussels is a random event and consuming less mussels may not effectively lower the chance of ingesting the synthetic materials. Based on the data obtained from this study, it was also estimated that as many as 507.93 microplastic items could be ingested annually for Hong Kong seafood lovers, and some can even cross the epithelium of digestive tract if they are small enough. It is hoped that this study could raise the awareness of food safety in local people and call for further research on the potential health risks on chronic exposure of microplastics in food. It is also high time for the authorities to halt illegal disposal of plastics and promote the reduction of plastic use in production of daily products.

(7287 words)

6. References

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. O., Lundebye , A., & Guilhermino , L. (2018). Marine microplastic debris: An emerging issue for food security, food safety and human health. *Marine Pollution Bulletin, 133,* 336-348.

Brâte, I. L. N., Eidsvoll, D. P., Steindal, C. C., & Thomas, K.V. (2016). Plastic ingestion by Atlantic cod (*Gadus morhua*) from the Norwegian coast. *Marine Pollution Bulletin, 112*(1–2), 105–110.

Brennecke, D., Duarte, B., Paiva, F., Cacador, I., & Clode, J. C. (2016). Microplastics as vector for heavy metal contamination from the marine environment. *Estuarine, Coastal and Shelf Science, 178*, 189–195.

Browne, M. A., Dissanayake, A., Galloway, T. S., Low, D. M., & Thompson, R. C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science & Technology, 42*, 5026-5031.

Chen, J. Y. S., Lee, Y. C., & Walther, B. A. (2020). Microplastic contamination of three commonly consumed seafood species from Taiwan: A pilot study. *Sustainability, 12*, 9543.

Cheung, L. T. O., Lui, C. Y., & Fok, L. (2018). Microplastic contamination of wild and captive flathead grey mullet (*Mugil cephalus*). *International Journal of Environmental Research and Public Health, 15*, 597.

Cheung, P. K., & Fok, L. (2016). Evidence of microbeads from personal care product contaminating the sea. *Marine Pollution Bulletin, 109* (1), 582-585.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin, 62*, 2588-2597.

Culin, J., & Bielic, T. (2016). Plastic Pollution from Ships. *Pomorski Zbornik, 51*(1), 57-66.

Devriese, L. I., Van de Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., ... Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the southern north sea and channel area. *Marine Pollution Bulletin, 98*, 179–187.

de Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., & Robbens, J. (2014). Quality assessment of the blue mussel (*Mytilus edulis*): comparison between commercial and wild types. Marine Pollution Bulletin, 85(1), 146–155.

EFSA Panel on Contaminants in the Food Chain. (2016). Statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal 14*(6), 4501-4531.

EPD. (2004). Biological Indicator Monitoring in Hong Kong. Retrieved from https://www.epd.gov.hk/epd/english/environmentinhk/water/hkwqrc/waterquality/marine-5.html

Food and Agriculture Organization of the United Nations. (2016). *The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All*. Rome.

Fok, L., & Cheung, P.K. (2015). Hong Kong at the Pearl River Estuary: A hotspot of microplastic pollution. *Marine Pollution Bulletin, 99* , 112-118.

Fok, L., Jia, Z. N., Lam, W. L., Li, H. K., Ng, K. L., & Yeung, L. C. (2018). *Microplastics and large plastic debris in Hong Kong waters.* Hong Kong: Greenpeace.

GESAMP. (2016). *Sources, fate and effects of microplastics in the marine environment: Part two of a global assessment*. Kershaw, P. J., & Rochman, C. M. (Ed.). Reports and Studies No. 93.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances, 3* (7), e1700782.

Gray, A.D., Weinstein, J.E., 2017. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (*Palaemonetes pugio*). *Environmental Toxicology and Chemistry, 36*(11), 3074–3080.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., & Duflos, G. (2017). Occurrence and effects of plastic additives on marine environments and organisms: A review. *Chemosphere, 182*, 781–793.

Hidalgo-Ruz, Gutow, L., Thomspon, R. C., & Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environmental Science & Technology, 46*, 3060-3075.

IW:LEARN. (2021). *UN Environment Programme for the Assessment and Control of Pollution in the Mediterranean Region (UNEP/MAP-MEDPOL)*. Retrieved from https://iwlearn.net/iw-projects/organizations/415

Li, J., Green, C., Reynolds, A., Shi, H., & Rotchell, J. M. (2018). Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. *Environmental Pollution, 241*, 35-44.

Li, J., Yang, D. Q., Li, L., Jabeen, K., & Shi, H. H. (2015). Microplastics in commercial bivalves from China. *Environmental Pollution, 207*, 190-195.

Li, J., Qu, X. Y., Su, L., Zhang, W. W., Yang, D. Q., Kolandhasamy, ... Shi, H. H. (2016). Microplastics in mussels along the coastal waters of China. *Environmental Pollution, 214*, 177-184.

Li, W. C. (2018). The occurrence, fate, effects of microplastics in the marine environment. In E. Y. Zeng (Ed.), *Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency* (pp. 133-173). Netherlands: Elsevier.

Lo, H. S., Xu, X. Y., Wong, C. Y., & Cheung, S. G. (2018). Comparisons of microplastic pollution between mudflats and sandy beaches in Hong Kong. *Environmental Pollution, 236*, 208-217.

Lusher, A. L., Mchugh, M., & Thompson, R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin, 67*(1), 94-99.

Mathalon, A., & Hill, P. (2014). Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin, 81*, 69-79.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., & Kaminuma, T. (2001). Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science & Technology, 35*(2), 318–324.

Miranda, D. A., & Carvalho-Souza, G. F. (2016). Are we eating plastic-ingesting fish?. *Marine Pollution Bulletin, 103*, 109–114.

Naji, A., Nuri, M., & Vethaak, A. D. (2018). Microplastics contamination in molluscs from the northern part of the Persian Gulf. *Environmental Pollution, 235*, 113–120.

National Centers for Coastal Ocean Science. (2017). *NOAA Mussel Watch Program: An Assessment of Contaminants of Emerging Concern in Chesapeake Bay, MD, and Charleston Harbor, SC*. Retrieved from https://coastalscience.noaa.gov/project/mussel-watch-program-assessment-chesapeake-bay-charleston-harbor/

Neves, D., Sobral, P., Ferreira, J. L., & Pereira, T. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine Pollution Bulletin, 101*(1), 119–126.

O'Connor, T.P. (1998). Mussel Watch results from 1986 to 1996. *Marine Pollution Bulletin, 37*(1-2), 14-19.

OSPAR Commission. (2021). *OSPAR Convention*.Retrieved from https://www.ospar.org/convention

Reguera, P., Viñas, L., & Gago, J. (2019). Microplastics in wild mussels (*Mytilus* spp.) from the north coast of Spain. *Sciential Marina, 83*(4), 000-000.

Rochman, C. M., Tahir, A., Williams, S. L., Baxa, D., V., Lam, R., Miller, J., … Teh, S. J. (2015). Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific Reports, 5*, 14340.

Rummel, C. D., Loder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.-M., Janke, M., & Gerdts, G. (2016). Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic sea. *Marine Pollution Bulletin, 102*, 134–141.

Setälä, O., Fleming-Lehtinen, V., & Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution, 185*, 77–83.

Shimao, M. (2001). Biodegradation of plastics. *Current Opinion in Biotechnology, 12*(3), 242-247.

So, W. K., Chan, K., & Not, C. (2018). Abundance of plastic microbeads in Hong Kong coastal water. *Marine Pollution Bulletin, 133*, 500-505.

Thiele, C. J., Hudson, M. D., & Russell, A. E. (2019). Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single digit pore size. *Marine Pollution Bulletin, 142*, 384-393.

Tsang, Y. Y., Mak, C. W., Liebich, C., Lam, S. W., Sze, E. T., & Chan, K., M. (2017). Microplastic pollution in the marine waters and sediments of Hong Kong. *Marine Pollution Bulletin, 115*, 20-28.

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. *Environmental Pollution, 193*, 65–70.

Vandermeersch, G., Lourenço, H. M., Alvarez Mu~noz , D., Cunha, S., Diogene , J., Cano Sancho, G., Sloth, J. J., Kwadijk, C., Barcelo, D., Allegaert , W., Bekaert, K., Fernandes, J. O., Marques, A., & Robbens, J. (2015b). Environmental contaminants of emerging concern in seafood e European database on contaminant levels. *Environmental Research, 143*(B), 29-45.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kotterman, M. J. J., Diogene, J., Bekaert, K., Robbens, J., & Devriese, L. (2015a). A critical view on microplastic quantification in aquatic organisms. *Environmental Research, 143*,46–53.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution, 178*, 483-492.

Wright, S. L., & Kelly, F. J. (2017). Plastic and human health: A micro issue?. *Environmental Science & Technology, 51*, 6634-6647.

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the “plastisphere”: Microbial communities on plastic marine debris. *Environmental Science & Technology, 47*, 7137-7146.