MICROPLASTIC CONTAMINATION IN GREEN MUSSEL AQUACULTURE AT STRAITS OF JOHOR

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DEDICATION

This is especially dedicated to my beloved parents, dearest one, siblings, close friends and supervisor.

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ABSTRACT

Microplastics are anthropogenic pollutants present in various morphology of less than 5 mm in diameter. Primary microplastics are manufactured in the form of microbeads and synthetic fibre used in various product. Meanwhile, secondary microplastic resulted from the breaking down of larger plastic material into smaller fragments. The widespread of microplastic contamination in marine environment creates a concern on its impact toward the food chain. In this study, microplastic contamination in seawater and farmed green mussel (*Perna viridis*) at Strait of Johor was investigated. Result from the investigation showed a positive contamination of microplastic in seawater and green mussel samples from the aquaculture sampling location. On average, microplastics present in green mussel samples collected from Kampung Pasir Putih was 0.58 ± 0.25 items/g and 1.95 ± 1.14 items/individual. Meanwhile, the abundance of microplastic in seawater samples was 8 ± 3.85 items/L. Fibres were the most common microplastic followed by fragments, with various size and colour. The result from this study can be used as a baseline level of microplastic contamination in green mussel aquaculture located at Straits of Johor. It is a concern that the presence of microplastic in seafood might bring negative consequences to human health. Therefore, intensive assessment of microplastic contamination in seafood should be conducted for better understanding on the impacts as well as for future reference.

ABSTRAK

Mikroplastik merupakan bahan pencemar yang dihasilkan oleh manusia bersaiz kurang daripada 5 mm diameter. Mikroplastik yang diklasifikasi sebagai mikroplastik primer dihasilkan dalam bentuk manik mikro dan serat sintetik yang digunakan dalam pelbagai produk. Manakala mikroplastic sekunder terhasil daripada bahan plastik yang lebih besar kepada serpihan kecil. Penyebaran pencemaran mikroplastik dalam persekitaran marin menimbulkan kebimbangan terhadap impaknya kepada rantai makanan. Dalam kajian ini, pencemaran mikroplastik dalam air laut dan kupang (*Perna Viridis*) yang diternak di Selat Johor telah dikaji. Hasil daripada kajian menunjukkan pencemaran mikroplastik adalah positif dalam sampel air laut dan kupang dari lokasi persampelan. Purata mikroplastik yang terdapat dalam sampel kupang yang diambil dari Kampung Pasir Putih ialah 0.58 ± 0.25 item/g dan 1.95 ± 1.14 item/individu. Sementara itu, purata mikroplastik dalam sampel air laut ialah 8 ± 3.85 item/L. Serat merupakan mikroplastik yang dominan diikuti oleh serpihan dengan saiz dan warna yang berbeza. Hasil kajian ini boleh digunakan sebagai maklumat tahap asas pencemaran mikroplastik dalam kupang yang diternak di Selat Johor. Adalah menjadi kebimbangan bahawa kewujudan mikroplastik dalam makanan laut mungkin membawa kesan negatif kepada kesihatan manusia. Oleh itu, penilaian intensif terhadap pencemaran mikroplastik dalam makanan laut perlu dijalankan untuk pemahaman yang lebih baik mengenai kesannya serta menjadi rujukan pada masa hadapan.

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LIST OF ABBREVIATIONS

UTM	-	Universiti Teknologi Malaysia
ATR	-	Attenuated Total Reflection
P. viridis	-	*Perna viridis*
M. edulis	-	*Mytilus edulis*
SPI	-	Society of Plastics Industry
n/a	-	Not Available
POPs	-	Persistent Organic Pollutants
PAHs	-	Polycyclic Hydrocarbons
PCBs	-	Polychlorinated Hydrocarbons
µ-FTIR	-	Micro Fourier Transform Infrared
GPS	-	Global Positioning System
QReC	-	Quality Reagent Chemical
w.w.	-	Wet Weight
s.d.	-	Standard Deviation
DOFM	-	Department of Fisheries Malaysia
PET	-	Polyethylene
HDPE	-	High Density Polyethylene
PVC	-	Polyvinyl Chloride
LDPE	-	Low Density Polyethylene
PP	-	Polypropylene
PS	-	Polystyrene
GPS	-	Global Positioning System
rpm	-	Revolution per minute

LIST OF SYMBOLS

°C	-	Celsius
HNO_3	-	Nitric Acid
Na_2O_2	-	Sodium Peroxide
H_2O_2	-	Hydrogen Peroxide
NaCl	-	Sodium Chloride

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Plastics are among materials that pollute our natural environment due to its properties of slow degradability and tendency to promote the transfer of chemical contaminants released through its product chemical constituents. It is estimated that plastic debris amount found in the marine environment have increased throughout the years where approximately 0.5 million tons a year were reported in 1960 and increased to 30 million tonnes a year in 2013 (Waite *et al*., 2018; Avio *et al*., 2016). The huge difference of plastic debris reported shows a reflection towards human dependency on plastic materials in daily lives. Moreover, the advancement of technology especially in factory has led to the production of various plastic products available in the existing market to support the demand from consumer. Population lifestyle is one of the key factors which contribute to the tremendous usage of plastic material in the present day. Concerns on plastic pollution in the marine environment has long existed as it affects the consumption of marine organisms that mistakenly interpret it as food. Consumption of the plastic debris has resulted in death of the organisms due to failure in their digestive system. Though plastic has various advantages, but its excessive production significantly contributes towards environmental pollution and more recently sparks the debate on microplastic pollution in our natural environment.

Plastic particles of fragments which is less than 5 mm in size is defined as microplastic (Farrell and Nelson, 2013; Li *et al*., 2016; Qu *et al*., 2018). Microplastic can be classified into primary and secondary where primary microplastic are manufactured in plastic industry in the form of microbeads, synthetic fibres and pellets which are being widely used in cosmetics, personal hygiene and household products. Meanwhile, secondary microplastic resulted from breaking down of larger plastics into smaller fragments or particles. Fragmentation of larger plastic product into smaller size

is possible through continuous exposure of sunlight on the plastic surface, collision of plastic material with hard surfaces and wave action. Both classifications of microplastic pose threats to natural environment due to its smaller size that can be uptake by aquatic organisms and consequently affect the food chain. The size of microplastic enable it to be easily ingested by micro size aquatic organisms and accumulate within the tissues of filter feeder organism. Apart from the contamination of aquatic organisms with microplastic, several researchers also showed its existence in bottled water, surface water, sediment and sand. The primary environmental risks associated with microplastics is their suspected bioavailability for marine organisms (Li *et al*., 2016). Among the vulnerable organisms toward microplastic contamination are filter feeders such as clams, oysters and mussels. These organisms have limited movement and tend to stay in the same spot throughout their life unless being moved to somewhere else by natural forces or human being. Mussels are marine organisms which are prone to exposure of microplastics pollution. This is related to its extensive filter feeding activities that exposed them directly towards microplastics present in the surrounding environment (Li *et al*., 2016). They feed on microscopic creatures by filtering substantial amount of seawater where various pollutants are available in the water column. Material that float in the marine waters will be filtered by the filter feeder especially materials in micro size.

Sources of microplastics pollution is related with its surrounding environment activities and will differ in terms of concentration by referring to the availability of plastic debris in that specific locations. Microplastics may as well end up in water column through the release of treated water from wastewater treatment plants. Even though wastewater treatment plants are responsible to remove various pollutants from used water or also known as grey water, unfortunately the smaller size microplastics can easily bypass the existing treatment system which have been widely practiced. In the case of cultured or farmed aquatic organisms for commercial purposes, contamination of microplastics inside its tissue and gut can be affected by the cultivation method such as cultivating mussels on suspended plastic ropes. Apart from the theory of microplastics contamination in mussel tissue through direct ingestion, adherence is another way for mussels to uptake the microplastic (Kolandhasamy *et al*., 2018). Since, mussels are also food for other organisms it plays the role of transferring

microplastics towards the marine trophic web to secondary and tertiary consumer (Renzi *et al*., 2018; Farrell and Nelson, 2013; Khoironi *et al*., 2018). Mussels are also important in providing information on microplastic pollution concentration as it acts as a bioindicators organisms and usually being used for biomonitoring program. The special factor of green mussel causing it to be chosen as a biomonitoring agent is because they accumulate many pollutants slightly higher than the actual concentration in water (Hadibarata *et al.*, 2012; Nicholson and Lam, 2005). Moreover, mussels are also consumed by human where contaminated mussels might put the consumer's health at risk. The chemical additives added to plastics during manufacture which may leach out upon ingestion can potentially cause serious health problems to humans (Jiang, 2018). Hence, there is a need to conduct extensive research in this field to better understand its overall impacts to the environment.

1.2 Problem Statement

Microplastics contamination have been reported in various organisms, sediment, sand and water such as its occurrence in South Korea, India, Slovenia, South Africa, Taiwan, Spain and etc. (Rezania *et al.*, 2018). At present, there is limited literature available on microplastics contamination in green mussel aquaculture in Malaysia, specifically at Straits of Johor area. Conducting a research in this background will contribute to the availability of reference for microplastics contamination in Straits of Johor. Straits of Johor or also known as Tebrau Straits is located between Peninsular Malaysia and Singapore. Among the major river tributaries which empty into Johor Straits includes Johor River, Pulai River, Tebrau River and Senibong River. These river tributaries can be the cause that spreads microplastic by flowing plastic debris from the source of pollution which travel through the river. Rivers are known to be a land-based source of microplastics into the marine environment and has been estimated that 80% of the plastic found in the ocean comes from land-based sources (Bessa *et al.*, 2018). Along the straits, there are various establishment of fishing village communities which contribute to the supply of fisheries and aquaculture products for the nation. The Government of Malaysia has identified aquaculture to be one of the major sources to increase production in the

fisheries sector to meet domestic demand and expanding its export potential in coming years. In 2014, the aquaculture industry in Malaysia generated an output of about 520,514 tonnes valued at RM 3.47 billion where it contributed to 26 % of total fish production (Department of Fisheries Malaysia, 2018). It is estimated that in 2020 the production from aquaculture sector will yield about 1.443 million metric tonnes.

Besides fish aquaculture, another popular aquaculture activity is the cultivation of green mussel (*Perna viridis*). Green mussels either wild or farmed can be found in shallow waters along the west coast of peninsular Malaysia. They have become a seafood resource especially in Malaysia which at one time, exported them and are harvested commercially in Indo-Pacific region owing to their dense and fast growth (Hadibarata *et al.,* 2012). Cultivation of green mussel in Sungai Melayu Johor located at Johor Straits is also famous for tourist boat attraction in eco-tourism sector. As pollutant such as microplastics can accumulate in the tissues of green mussels, it may cause an increase concern in human health due to its popularity in the seafood dish. The larger the size of green mussels the higher concentration of microplastics contamination should be expected. Besides from the fact that mussels are vulnerable to the microplastics contamination, it is also a vector for the transfer of microplastics into human food chain (Li *et al.,* 2018). The physical ingestion of microplastic by organism leads to blockage of the intestinal tract, inhibition of gastric enzyme secretion, reduction of feeding stimuli, decrease in steroid hormone levels, delay in ovulation and lack of reproduction (Kolandhasamy *et al.,* 2018; Wright *et al.,* 2013; Canesi *et al.,* 2015). Once ingested, microplastic can potentially transferred from the digestive tract to circulatory systems of the organisms (Van Cauwenberghe *et al.,* 2015). Several studies have shown that microplastics can affect the health of marine organisms but the potential risk for human health is still uncertain.

The study on animals and marine organisms can serve as a reference and guidelines in order to increase the awareness on potential risk of microplastics towards human health when consuming organism which have been contaminated by such pollutant. Consuming only one green mussels might considerably pose little risk but since green mussels are widely available in the market and people are continuously consuming it, there is a high probability of experiencing potential health risk when

consuming mussels in huge amount which is contaminated by microplastic. Upon the consideration for environmental and commercial importance of green mussel in Straits of Johor it is important to conduct investigation on the microplastics contamination occurrence in that region. There are still many unanswered questions in this study area as microplastics are considered as a new emerging contaminant in the natural environment where it starts to attract attention of researcher into the field. Relatively, the correlation of microplastics in organisms with their living environment is still unclear and more extensive research should be conducted.

1.3 Objective of Study

Research in microplastics is currently trending specifically in year 2018 where various literatures in this background being published covering ranges of objectives. Among the objectives are to investigate microplastic concentration in surface water, sediment, sand and marine or freshwater organisms, identifying microplastic features in terms of shape, size and colour as well as studying the trophic transfer of microplastic in the food chain. Overall, the studies showed evidence of microplastic contamination in their sample which raise a concern to conduct further research in this background in order to better understand its variations, properties, distribution and impacts towards natural environment. Hence, this study aims to investigate the occurrence of microplastic contamination in green mussels aquaculture located at Straits of Johor. It should be noted that state of Johor is one of the biggest producers of fisheries and aquaculture product in Malaysia hence there is importance to conduct study in this field. In order to achieve these aims the following objectives are formulated:

(a) To investigate the presence and abundance of microplastic in farmed green mussel and surface seawater.

(b) To determine the physical characteristics (shape, colour and type) of microplastic in farmed green mussel and surface seawater.

1.4 Scopes and Limitations of Study

In this study, research on microplastic contamination was conducted on farmed green mussels and surface seawater from selected aquaculture located at Straits of Johor. The selected location was Kampung Pasir Putih, Pasir Gudang, Johor. Since, aquaculture is among the main contributors to the Johor fisheries product for local demand as well as for export purpose, there is a need to evaluate the occurrence of microplastics contamination in the seafood supply. Only one particular living condition type of organism is chosen in this study hence there is no comparison of farmed green mussel data with wild green mussels which evolve independently in the natural environment. Different modes of cultivation might affect the rate of microplastics pollution where in farmed green mussels the suspended rope used in the whole operation can be considered as source of microplastics pollution. Hence, both the farmed and wild mussels may have different sources of microplastics pollution which reduces its quality by accumulating such pollutant.

This study focuses to investigate the abundance and physical characteristics of microplastics pollution within the farmed green mussel and its living environment as stated in section 1.3. Laboratory works consists of digestion of organic tissue, separation of microplastic from the dissolve liquid of digested organic tissue, and observation as well as validation of microplastic using several instruments available in the laboratory of School of Civil Engineering and Faculty of Science, Universiti Teknologi Malaysia (UTM). Stereomicroscope was used to identify the abundance and physical characteristics of microplastics fragment or particle and attenuated total reflection (ATR) spectroscopy were used to validate whether the selected microplastic indeed originates from plastic or from organic materials. Another limitation of this study is that, the sampling location will only cover one area at Straits of Johor which specifically located at Kampung Pasir Putih, Pasir Gudang, Johor. The selection of sampling location is based on the land use of that region where Pasir Gudang is an area of various industrial activities.

1.5 Significance of Study

It is a concern that anything exist in the natural environment balance each other where for example if a species of marine organisms is affected by microplastics pollution which lead to its extinction might alter the living environment of other species. Certain predator organism that prey on the vulnerable organism which already decreased in number thus need to change its diet for survival. In worse condition if the lower trophic level organism become extinct, the higher trophic level may face the same condition. With nonstop production of plastic, there is no guarantee that microplastics pollution is going to stop in the future. In fact, more microplastics will be available in the natural environment throughout the coming years adding to the amount of microplastics which already existed. Among the plastics material which cannot be avoided in human lives is the usage of product packaging, tyres, synthetic fabric and many more. Currently there is still no environmentally friendly material which can replace the usage of plastic material in our everyday lives and when the amount of microplastics becoming more abundant in the future it will increases the rate of toxicity in contaminated organisms. Since, Johor Straits is famous with fisheries and aquaculture industry making it vulnerable to the environmental and commercial impact caused by microplastics pollution.

Nowadays, information can be spread out easily with the help of technological devices and the awareness on microplastics pollution by the public might cause a doubt in the quality of green mussel supply in the market whether it contain high level of contaminant or not. By conducting research in this field, it may help better understanding on the physical characteristics of microplastics, abundance, sources and most importantly aid knowledge in order for the government sector or interested body to combat further pollution caused by microplastics. In general, this study will add in knowledge on the occurrence of microplastics contamination in green mussel aquaculture at Straits of Johor and information provided from this study can serve as a baseline data for understanding on the extent of microplastics contamination along Straits of Johor. By comparing data and result of microplastics abundance in farmed green mussels taken from Straits of Johor with existing literature at different country, it will help to further broaden knowledge on the extent of contamination. The result of

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