SORPTION OF HEAVY METALS ONTO POLYETHYLENE MICROBEADS AND ITS EFFECT ON SEABASS

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DEDICATION

Dedicated to my beloved little family, my parents, and my supportive supervisor – DR. SHAMILA BINTI AZMAN. Thank you very much for being positive, helpful, and understanding

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ABSTRACT

Microbeads are one of the causes of microplastic pollution that is currently polluting ocean environment. It enters food chain via ingestion of marine vertebrates and invertebrates. This study aims to elucidate the interactions between polyethylene microbeads and heavy metals as well as to determine the possibility of polyethylene microbeads as heavy metal vectors for juvenile seabass. Two parts of experiments performed in this study, i.e. adsorption and ingestion. For the adsorption study, 10 g of virgin polyethylene microbeads (300 µm) were submerged into 0.2, 0.4, 0.6, and 1.0 µg/mL solutions of cadmium and chromium, and 0.4, 0.8, 1.2, 1.6, and 2.0 µg/mL concentrations of lead in a batch of sorption experiments for 96 hours. In the ingestion experiment, seabass was exposed in control, single, co-exposure, and preloaded experiments. All exposure conditions were similar to the adsorption experiment with 10 g and 5 g of microbeads. Acid digestion and Atomic Absorption Spectroscopy were used to quantify the amount of heavy metal adsorbed on microbeads and accumulated in fish tissues. Maximum adsorption capacity of microbeads were 11 µg/g for Cd, 1.7 µg/g for Cr, and 9.0 µg/g for Pb. The kinetic study concluded that the adsorption of polyethylene microbeads occurred at a pseudo-first-order reaction, which involves physical attraction. Adsorption isotherm fitted the Freundlich model signifying adsorption occurs rapidly and has the tendency to desorb due to weak binding. The rates of heavy metal adsorption onto microbeads were 11, 4.5, and 1.7 mL/g for Cd, Pb, and Cr, respectively, suggesting that Cd had a higher affinity towards microbeads polyethylene than Pb and Cr. In the ingestion study, most of the heavy metal were detected at the skin layer. Control experiment validated that, seabass uptake exposed microbeads via ingestion. The single experiment concluded that uptake of heavy metals in seabass tissues increased with concentration and time. The higher the amount of exposed microbeads, the higher the uptake of heavy metals in the gastrointestinal tract after 48 hours of co-exposure. This indicates that heavy metals were first adsorbed on the microbeads followed by their ingestion by the seabass. In the preloaded experiment, the concentration of heavy metal ions detected in the gastrointestinal tract was higher than the direct exposure in single and co-exposure. The uptake values in the preloaded experiment increased steadily with concentration, time, and quantity of microbeads. Preloaded exposure in this study verified that microbeads-loaded heavy metals were incidentally ingested by seabass during foraging. The incorporation of the three heavy metals in the ingestion study was performed using Minitab 16.0 multi analysis of variance (MANOVA). This study proved that polyethylene microbeads possess the potential to accumulate, transport, and transfer heavy metals from water to intestinal organ, thus increasing risk, threatening the marine food web, and possibly harming other consumers.

ABSTRAK

Manik mikro adalah salah satu sumber pencemaran mikroplastik, mencemarkan persekitaran lautan. Ia memasuki rantai makanan melalui pengambilan makanan vertebrata dan invertebrata. Kajian ini bertujuan untuk memperjelaskan interaksi antara manik mikro polietilena dan logam berat untuk menentukan kemungkinan manik mikro polietilena sebagai vektor logam berat kepada ikan siakap. Dua bahagian eksperimen dalam kajian ini, iaitu penjerapan dan pengambilan makanan. Untuk kajian penjerapan, 10 g manik mikro polietilena tulin (300 µm) direndam dalam larutan 0.2, 0.4, 0.6 dan 1.0 µg/mL untuk kadmium dan kromium, manakala kepekatan plumbum adalah 0.4, 0.8, 1.2, 1.6 dan 2.0 µg/mL dalam kumpulan eksperimen jerapan selama 96 jam. Dalam eksperimen pengambilan makanan, ikan siakap didedahkan dalam eksperimen kawalan, tunggal, pendedahan bersama dan pramuat. Semua keadaan adalah sama dengan eksperimen penjerapan dengan jumlah manik mikro yang digunakan adalah 10 g dan 5 g. Pencernaan asid dan Spektrofotometer Serapan Atom digunakan untuk mengukur jumlah pengambilan logam berat dalam manik mikro dan lapisan tisu ikan. Kapasiti penjerapan maksimum manik mikro adalah 11 µg/g untuk Cd, 1,7 µg/g Cr dan 9,0 µg/g Pb. Kajian kinetik menyimpulkan bahawa penjerapan manik mikro polietilena berlaku mengikut pseudo-tertib-pertama, yang melibatkan tarikan fizikal. Model isoterm Freundlich yang menunjukkan bahawa penjerapan berlaku dengan cepat serta mempunyai ikatan yang lemah. Kadar penjerapan logam berat pada manik mikro adalah 11, 4.5 dan 1.7 mL/g untuk Cd, Pb dan Cr, masing-masing menunjukkan bahawa Cd mempunyai tarikan yang lebih tinggi terhadap manik mikro polietilena berbanding Pb dan Cr. Dalam kajian pengambilan makanan, kebanyakan ion logam berat dikesan pada lapisan kulit ikan. Eksperimen kawalan menyimpulkan bahawa ikan siakap memakan manik mikro. Eksperimen tunggal menyimpulkan pengambilan logam berat dalam tisu ikan siakap meningkat dengan kepekatan dan masa. Semakin tinggi manik mikro yang terdedah, semakin tinggi pengambilan logam berat di saluran usus setelah 48 jam dalam eksperimen pendedahan bersama logam berat dan manik mikro. Ini menunjukkan bahawa manik mikro menyerap logam berat dari persekitaran, kemudian dicerna oleh ikan siakap. Dalam eksperimen pramuat, kepekatan ion logam berat yang dikesan di saluran usus lebih tinggi daripada pendedahan langsung dalam kawalan dan pendedahan bersama. Nilai pengambilan dalam eksperimen pramuat meningkat dengan stabil dengan kepekatan, masa dan jumlah manik mikro dengan jelas. Pendedahan yang dimuatkan dalam kajian ini mengesahkan bahawa, logam berat yang diserap oleh manik mikro secara tidak sengaja ditelan oleh ikan siakap semasa mencari makanan. Ketiga-tiga logam berat dalam kajian penjerapan dan pengambilan makanan dilakukan menggunakan Minitab 16.0 dalam analisis pelbagai varians (MANOVA). Kajian ini telah membuktikan bahawa manik mikro polietilena berpotensi untuk mengumpulkan, mengangkut, menjadi vektor logam berat di persekitaran laut ke organ pengambilan makanan, sehingga meningkatkan risiko dan mengancam jaringan makanan laut, dan mungkin berbahaya bagi pengguna lain.

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LIST OF ABBREVIATIONS

AAS		Atomic Absorption Spectrophotometer
Ag	-	Silver
Al	-	Aluminiun
As	-	Arsenic
ASW	-	Artificial seawater
Ba	-	Barium
Br	-	Bromine
Cd	-	Cadmium
Cl	-	Chlorine
Co	-	Cobalt
Cr	-	Chromium
Cu	-	Copper
DDT	-	Dichlorodiphenyltrichloroethane
DEHP	-	Di(2-ethylhexyl) Phthalate
DNA	-	Deoxyribonucleic Acid
DO	-	Dissolved Oxygen
EPA	-	Environmental Protection Agency
FAO	-	Food and Agriculture Organization of the United Nations
Fe	-	Iron
GST	-	Glutathione S-Transferase
HDPE	-	High Density Polyethylene
Hg	-	Mercury
HNO_3	-	Nitric Acid
H_2O_2	-	Hydrogen Peroxide
HCl	-	Hydrochloric Acid
HOC	-	Halogenated Organic Carbons
HPLC	-	High Performance Liquid Chromatography
IDH	-	Isocitrate Dehydrogenase
LDH	-	Lactate Dehydrogenase
LDPE	-	Low Density Polyethylene

MANOVA - Multiple Analysis of Variance
Mn - Manganese
Mo - Molybdenum
MP - Microplastic
Ni - Nickel
PAHs - Polycyclic Aromatic Hydrocarbons
Pb - Plumbum/Lead
PBDEs - Polybrominated Diphenyl Ethers
PBTs - Persistent Bioaccumulative Toxic Substances
PCB - Polychlorinated biphenyl
PE - Polyethylene
PET - Polyethylene Terephthalate
PFASs - Perfluorooctanoic Acid
PFOS - Perfluorooctane Sulfonate
PMMA - Polymethylmethacrylate
POPs - Persistent Organic Pollutants
PP - Polypropylene
PS - Polystyrene
PVC - Polyvinyl chloride
r^2 - Coefficient of Determination
Sb - Antimony
SD - Standard Deviation
Se - Selenium
SM - Synthetic Musks
Sn - Stannum/Tin
Sr - Strontium
SSE - Sum of Squared Estimate of Errors
TC - Tetracycline
Ti - Titanium
U - Uranium
UK - United Kingdom
UPM - Universiti Putra Malaysia
US - United States

USA	-	United States of America
UV	-	Ultraviolet
UV-B	-	Ultraviolet B-Rays
V	-	Vanadium
YSI	-	Yellow Springs Instrument
Zn	-	Zinc
α-HCHs	-	Hexachlorocyclohexanes

LIST OF SYMBOLS

% - Percent

µm - Micrometer

cm^2 - Centimeter Square

cm^3 - Centimeter Cube

m^2 - Meter Square

ng - Nanogram

µg - Microgram

ppm - Part Per Million

ppt - Part Per Trillion

°C - Degree Celsius

J - Joule

Ea - Activation Energy

Kd - Distribution Coefficient

qe - Heavy Metal Adsorbed per Unit Mass at Equilibrium

kt - Pseudo-First-Rate Constant

t - Time

qm - Energy Constant Related to The Heat of Adsorption

Ce - Concentration of Heavy Metals at Equilibrium

kf - Adsorption Capacity

n - Adsorption Intensity

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Plastics are one of the industrial products that are widely used and have successfully replaced several conventional materials such as glass, metal, and wood due to their cost of production, strong, durable, and lightweight characteristics, and easy to produce (DeArmitt, 2017; Thompson et al., 2009). Plastics are constructed through the linking of hydrocarbon monomers that created synthetic polymers. As shown in Figure 1.1, plastic production is growing steadily each year due to its demand and its production had been reported to be up to 348 million tonnes in 2017 (Plastics Europe, 2018), and estimated to climb up to around 33 billion tonnes by 2050 (Rochman et al., 2013).

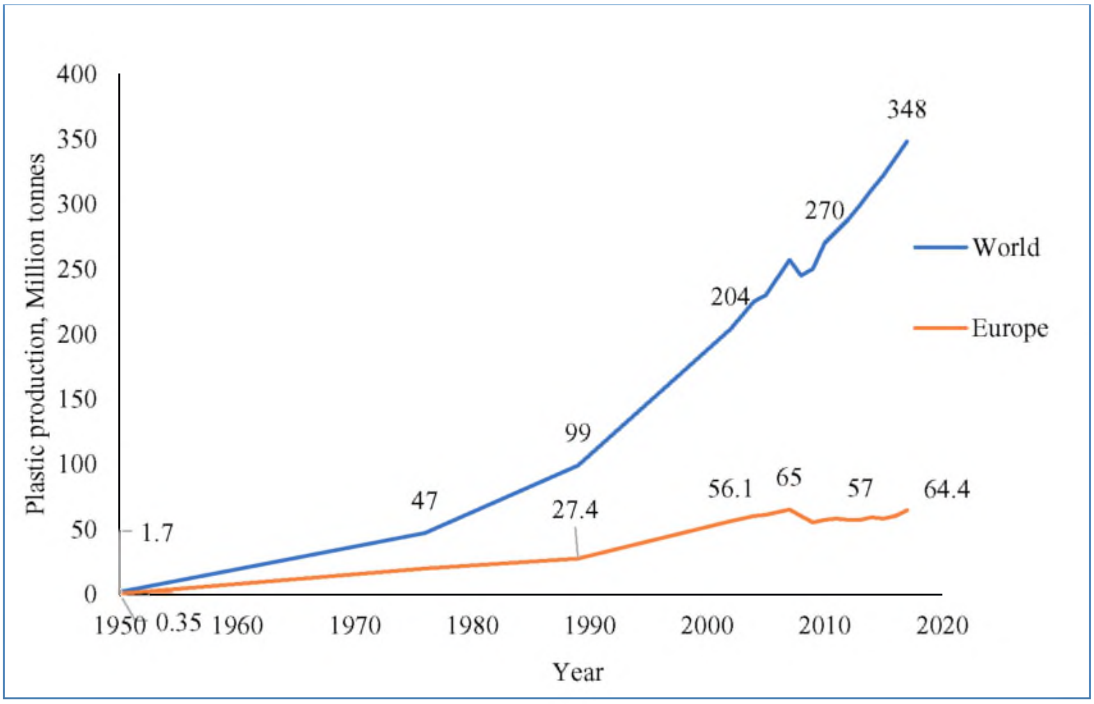

Figure 1.1 The increase in plastic production around the world from 1950-2017 (Plastics Europe, 2018)

The production of plastics grows rapidly each year due to industrial demands and their unique characteristics such as low cost, low density, and high durability in most industries, especially in the packaging industry. Being the most abundant and persistent debris found in marine (Cauwenberghe et al., 2015), plastics have a long lifespan and accumulate in the environment (Andrady, 2011; Cole et al., 2011; Galgani et al., 2013; Wright et al., 2013) despite the movement and campaign to reduce the use of plastics, which have been recognized as a threat to the marine ecosystems due to their abundance.

Based on estimations, 1.15 to 2.41 million tonnes of plastics are released into the marine environment annually (Lebreton et al., 2017). Thus, the alarming number of plastics and their persistent characteristics has led to environmental concerns (Paterson, 2019). As mentioned by previous researchers, consumer packaging is made from one-third of plastic production with 10% of municipal waste (Andrady, 2011), which mostly ends up in landfills and remains there for a long time (Barnes et al., 2009). Meanwhile, the remaining 90% are usually recycled or are not handled properly, which may end up in the environment via several routes. In general, plastics in all sizes, from meters to micrometers, are found in the environment (Barnes et al., 2009).

Most plastic polymers found in the environment are polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) (Andrady & Neal, 2009; Andrady, 2017) due to their various applications. They can also be found in different levels of the water column due to their density. Generally, polyethylene and polypropylene are buoyant and have high mobility due to water currents and wind; hence, most of them will be permanently trapped or stranded in a location that cannot further move them with physical processes such as tidal and biofouling that can cause an increase in the density. Besides, polyethylene and polypropylene debris are abundantly found in the marine environment (Xu et al., 2020) or remote areas (Wang et al., 2018; Lusher, 2015; Nakashima et al., 2012). The denser fragment of PVC and PET is also readily settling out of suspension in the marine environment. Nonetheless, due to persistency character, all plastics that end up in the marine environment from years ago, either transported, degraded, fragmented, fouled, or deposited, are presumed to exist until now (Thompson, 2015).

Plastics particles with a size less than 5 mm are classified as microplastics (Thompson, 2015; GESAMP, 2019), which have been considered as pollutants of high concern (Kögel et al., 2020; Kroon et al., 2020). Evidently, microplastics are widely found in marine sediments or water columns (Guo et al., 2020; Kik & Sici, 2020; Peng et al., 2020). In general, there are a few forms of microplastics detected in the environment such as fiber, pellets, beads, and fragments.

Primary microplastics are those introduced directly into the environment, mostly from proposed products, wastes from manufacturing processes, or derivatives from the erosion and tearing of large plastic products such as tires, wheels, and boards. On the other hand, plastic production uses powder or pellets as raw materials; therefore, these materials might accidentally end up in the environment during accidental release (Dris et al., 2016; Gasperi et al., 2019; Marnane et al., 2006; Vandermeersch et al., 2015), shipping, or cleaning machinery through shot blasting (Cole et al., 2011). These materials exist typically as resin pellets (Rocha-Santos & Duarte, 2015; Waller et al., 2017), microbeads (Yurtsever, 2019), microfiber (Mark Anthony Browne et al., 2011), and other forms.

The degradation of macro or mesoplastics in the environment under the physical, chemical, and biological forces with a size less than 5 mm is called secondary microplastics (Zhang et al., 2016; Thompson, 2015; Rocha-Santos & Duarte, 2015; Waller et al., 2017). These processes include heat, mechanical forces, ultraviolet (UV) light, oxidation, or biodegradation (Rillig et al., 2017). Macroplastics do not only impact the natural system but also the range of organisms in the environment through ingestions for large organisms and entanglement for smaller ones, especially birds and fish (Phuong et al., 2016; Compa et al., 2018; Provencher et al., 2018; Horn et al., 2019).

In addition, macroplastics potentially degrade and break down into smaller fragments based on their rate of degradation that is controlled by several environmental factors. As such, the carbon in the polymer can be transformed into carbon dioxide and incorporated into marine biomass, while complete mineralization is achieved when the polymer is transformed from the organic carbon (Andrady, 1994; Eubeler et al., 2009). Compared to microplastics, the chemical and physical effects of macroplastic debris are well-known globally. Previously, researchers have only focused on the contaminants released from plastics but not the ability of the plastics to absorb harmful contaminants from the environment.

Microplastics have been found in different water sources such as wastewater treatment plants, freshwater, and marine (Rezania et al., 2018). Previous studies have successfully identified that microplastic debris may exceed 100,000 items/m^2 in water surface and 100,000 pellets/m in beach sediment (Eerkes-Medrano, Thompson, & Aldridge, 2015; Cauwenberghe et al., 2015). According to previous researchers, microplastics are also suggested as a long-term sink in sediments (Cozar et al., 2014; Imhof et al., 2017; Coppock et al., 2017). The density of seawater is around 1.020 to 1.029 gcm^{-3}; hence, plastics with a density higher than seawater will sink and potentially accumulate in the sediment. However, if the plastics' density is lower than the seawater density, they tend to float in the water or the surface column (Cauwenberghe et al., 2015).

1.2 Research Background

Since the 1970s, plastics have been incidentally ingested by organisms such as fish (Wieczorek et al., 2018; Lv et al., 2019), invertebrates (Windsor et al., 2019; Horn et al., 2019), turtles (Nicolau et al., 2016), and seabirds (Basto et al., 2019). Thus, plastic ingestion may affect organisms physically or chemically (Mattsson et al., 2018; Mattsson et al., 2017). In terms of physical impact, organisms such as seabird will experience suppressed feeding activity when the plastic pellets in the gizzard indicate no fresh food is reaching the proventriculus; thus, this blocks the movement of food through the digestive tract and might reduce its appetite or change its food hunting behaviour (Rochman et al., 2014; Law, 2017), thereby causing histopathological alterations in intestines, changes in behaviour and lipid metabolism, and potentially translocation to the liver (Jovanović, 2017).

In the case of microplastics, a study has found that seabass larvae ingest polyethylene microbeads that went through the digestive tract during fish fed diet despite its high egestion behavior (Mazurais et al., 2015). Meanwhile, in a highly contaminated area, microplastics with co-contaminants might be ingested by zooplankton and then fish larvae (Mazurais et al., 2015; Moira et al., 2015; Khan et al., 2017). Thus, large predators with complex digestive tracts such as crustaceans, ctenophores, and medusae, or vertebrates such as fishes might also ingest the fish larvae even before the egestion process (Cole et al., 2013; Carlos et al., 2018). Consequently, the bioaccumulation process could harm organisms, especially the top predators because the microplastic co-contaminants have a potential for biomagnification (Teuten et al., 2009). Figure 1.2 shows potential pathway for the transport of microplastics & their biological interactions in environment.

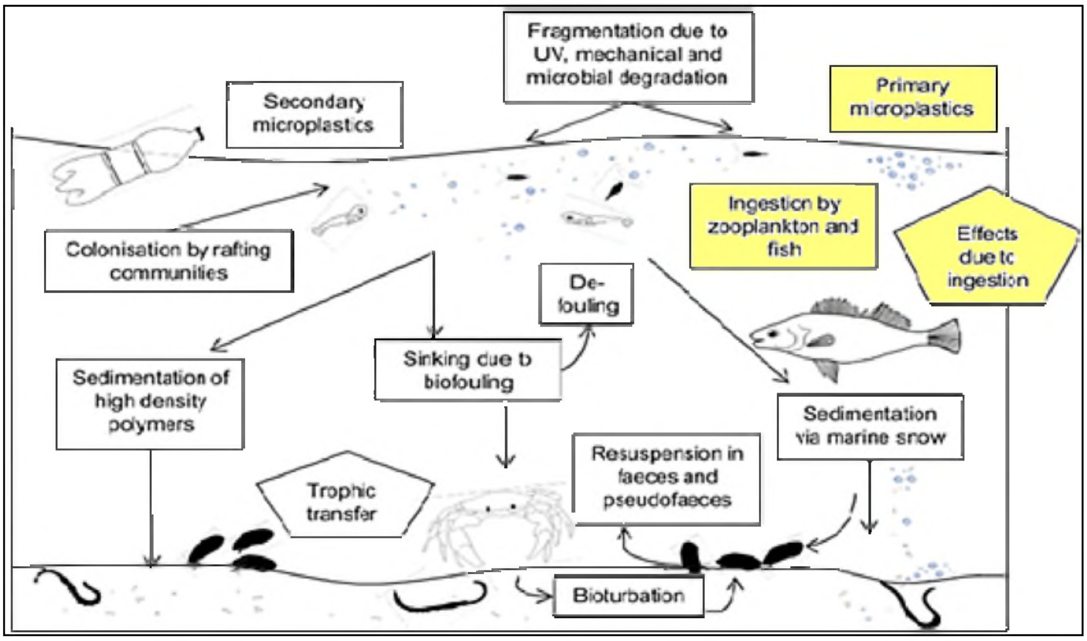

Figure 1.2 Potential pathway for the transport of microplastics & their biological interactions (adapted from Wright et al., 2013)

Research has been paying attention to investigating the impacts of toxic chemicals, especially on the relationship involving microplastic debris. Microplastics might act as a source or sink of toxic chemicals (Cole et al., 2011) since they have the ability to adsorb persistent, bioaccumulative, and toxic substances (PBTs) such as heavy metals, polychlorinated biphenyls (PCBs), and dioxins from water or sediment. These PBTs may then be released when the microplastics are ingested by aquatic life (Rochman et al., 2014; Karami et al., 2017), and the accumulation of microplastics is likely ingested by a wider range of organisms, thus raising concern in the marine ecosystem.

Recent studies have found that microplastics might be mobilized from digestive organ to other internal systems and trophic transferred from prey to predator (Dawson et al., 2018), and it has also been proven that toxic pollutants such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), dioxin, and heavy metals tend to adsorb onto microbeads and yield higher concentration compared to water and sediments (Brennecke, Duarte, Paiva, Caçador, & Canning-Clode, 2016). Therefore, the presence of microbeads in the marine ecosystem poses a threat to food safety. Current research suggests that, despite the significant uncertainty and complexity in the kinetics and thermodynamics of the interaction, plastic debris also appears to act as a vehicle, transferring PBTs from the water to the food web, thus snowballing risk to the entire marine food web including humans. Due to the extremely long lifetime of plastics and PBTs in the ocean, prevention strategies are, therefore, crucial to minimize these risks (Engler, 2012; Turner, 2016).

Microbeads are classified as a primary source for microplastic pollution since the size of microbeads is in the range of microplastics (Thompson, 2004). Microbeads are manufactured and designed as demanded such as facial cleansers (Fendall & Sewell, 2009; Brennecke et al., 2016; Boucher et al., 2016). These materials are also widely used in cleaning products, printer toner, and abrasive media for plastic blasting, textile printing, and automotive molding (Pettipas et al., 2016). Subsequently, after treated and discharged, microbeads tend to accumulate, persist, and potentially act as vectors for contaminants in the environment (Imhof et al., 2016; Brennecke et al., 2016; Smith, 2018) and poses a threat to food safety.

The main concern is that, when organisms ingest plastic-co-contaminants, the bound contaminants will likely be released due to chemical or physical conditions in the avian gizzard or the digestive tract of the organisms. To date, research on the ingestion effect of adsorbed heavy metals on microplastics in the marine organism is still lacking. The adsorption of metals to microplastics raises the potential for chemical transfer to marine animals that falsely ingests microplastics, which is identified as a "vector effect" (Syberg et al., 2015; Ory et al., 2018).

As plastics become vectors due to their ability to deploy and concentrate contaminants through ingestion to organisms (Khan et al., 2015), other than bioaccumulation, the contaminants also allow for biomagnification within the environment system, which starts from the low trophic predators until the top predators (Graham & Thompson, 2009). As such, various trophic level organisms that ingest microplastics might affect the vast ecosystem through the biomagnification of microplastic co-contaminants or microplastics alone. While the focus is directed towards the persistent organic pollutants (POPs) possibility concerning microplastics that carry heavy metals and ingested by the organism; therefore, it is important to investigate some organisms that appear to not only be unintentionally ingesting but also selectively consuming the floating microplastics (Ory et al., 2018; Hall et al., 2015).

The term "heavy metals" could be explained as the elements with high density and toxic to the environments even at low concentrations (Carolin et al., 2017). Heavy metals have been a major threat to the environment due to their flexibility, accumulation, endurance, and non-biodegradable characteristics (Raval et al., 2016). Usually, heavy metal pollution is caused by discharging untreated wastewater from industries such as pesticides, tanneries, metal plating industries, or mining operations.

Some heavy metals have become the main concern in the existing environment such as cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, silver, and zinc (Bhattacharyya & Gupta, 2008; Turner & Millward, 2002). The concentrations of heavy metal ions in plastic particles are significantly higher than in the water column, which makes them likely to become toxic. As stated by Holmes (2013), heavy metals adsorbed to plastic pellets are highly bioavailable; therefore, toxic elements are expected to be extracted by the acidic digestive tract environment (Holmes & Thompson, 2014). As such, the ability of heavy metals to release back to the marine environment is more readily in a soluble form (Holmes et al., 2012).

Recognizing that there are species-specific, pollutant and polymer specific, as well as experimental differences between the studies, varied results demonstrated that the impact of microplastics on the uptake and accumulation of pollutants is far from consistent. One of the key determinants may be whether pollutants and microplastics encounter each other before organism exposure or whether or not they are introduced as a co-exposure.

To date, scientists have discovered a new pathway for heavy metal pollution by a carrier vector effect with microplastics as the vector (Kalčíková et al., 2017; Bradney et al., 2019; Hodson et al., 2017). For example, Vedolin et al. (2018) demonstrated the ability of microplastics to adsorb heavy metals as the concentration of the adsorbed heavy metals in the collected pellets were higher than the original particles. Following the ban by the US, UK, Canada, and other countries, it is crucial to investigate the ability of pristine polyethylene microbeads to adsorb heavy metals such as cadmium, chromium, and lead. Thus, this study focuses on the adsorption of heavy metals to the microbeads to prove the vector effect of heavy metals on the marine vertebrates.

1.3 Problem Statement

There have been fewer studies about ingestions of microbeads from cosmetic usage regardless of their occurrences in wastewater treatment plants (Rezania et al., 2018). In Malaysia, the only study of microbeads occurrence was conducted by Praveena et al. (2018). It is estimated that about 0.199 trillion microbeads particles from facial and personal care products were reported to enter the wastewater treatment plants (WWTPs) in Malaysia per day; hence, this problem is at an alarming point and needs sustainable solutions (Praveena et al., 2018). However, the data on possibility ingestion effect on microbeads to organism in Malaysia was still scarce.

Studies have mostly proven the ability of a general type of microplastics to adsorb heavy metals from the aqueous environment (Tchounwou et al., 2014; Boucher et al., 2016; Alomar et al., 2017; Peng et al., 2018; Munier & Bendell, 2018; Prunier et al., 2019). Specifically, studies on microbeads in the cosmetic industry and their ability to sorb contaminants are scarce.

Based on the literature review, microbeads have the potential to be a vector for heavy metals in the marine environment (Bayo et al., 2017; Zon et al., 2018). As the microbeads have the ability to carry hazardous ions from the domestic wastewater system into the marine environment, this increases the potential of heavy metal ions to pollute the water system and interact with marine animals. These phenomena increase the mobility of heavy metals into the water system. As the mobility of the heavy metals increases, the marine animals are highly likely to consume the heavy metal ions, thus transferring the pollution into the food web up to the final consumers, which are the humans. Therefore, this study will focus on the adsorption and desorption abilities of microbeads onto heavy metal ions.

This study was focused on juvenile seabass. To date, there is no study preformed in evaluating heavy metals with microbeads effects on the uptake of juvenile seabass species specifically *Lates calcarifer*. Juvenile fish is predominantly exposed to pollutions in the environment, especially heavy metal ions (Morán et al., 2018). Therefore, any changes in this stage will positively affect the growth of the fish.

On the other hand, the bio-accumulation of heavy metal ions would cause a significant effect on the tissue growth for some period; thus, the polluted organs may affect the consumers. The early monitoring of the pollution is vital, especially in the juvenile animal class so the initial prevention acts can be planned and executed to save the environment. Therefore, further investigation was carried out for the effect of adsorbed microbeads with heavy metals into juvenile seabass as a marine organism model in different exposure condition. This experiment is crucial to prove the vector effect of microbeads and identify the pollution level in the vital organs of juvenile seabass.

1.4 Objectives

This study aims to clarify the interactions between polyethylene microbeads and heavy metals in the environment and to determine the possibility of polyethylene microbeads to become heavy metal vectors for juvenile seabass, also known as seabass. As such, the following objectives are addressed in this study:

1. To determine the adsorption ability of polyethylene microbeads in cadmium, chromium, and lead using a batch approach.
2. To determine the kinetic and isotherm characteristics for the adsorption of cadmium, chromium, and lead onto polyethylene microbeads.
3. To quantify the uptake of cadmium, chromium, and lead within gastrointestinal tracts, gills, and skin of juvenile seabass.
4. To determine the effect of polyethylene microbeads on seabass via direct and preloaded exposure with cadmium, chromium, and lead.

1.5 Scopes

The following research scopes have been summarized to accomplish the research aims. The scopes are listed as follows:

a) Microbeads were used as a model of microplastics in this study. Primarily, it is the second-most obviously ingested type of microplastics and evidently found within every species of marine animals. Moreover, polyethylene microbeads have also been found in scrubs/peelings, shower/bath products, facial cleaners, toothpaste, bubble bath, lotions, and sunscreens. Besides, other types of polymer and wastewater discharge might be the ultimate ways to enter the aquatic ecosystem. The source of microbeads was obtained from a local private company that produces microbeads for cosmetic usage, purposely with a diameter of ~300 um.

b) Cadmium (Cd), chromium (Cr), and lead (Pb) were selected because they are common contaminants to the marine ecosystems (Yunus, 2020). In addition, these heavy metals are known in industrial, domestic, agricultural, medical, and technological applications and are widely distributed in the environment; hence, this has raised concerns over their toxicology to human health and the environment.

c) Juvenile seabass was collected from the Aquaculture Fisheries Research Institute, Johor Bahru with a size of 6-7 cm in length. While they are highly demanded food with a high market value in the aquaculture industry, they are also important for commercial purposes and game fish. Briefly, they are valuable both as recreational and commercial fish with a high, fairly stable price. Thus, juvenile seabass was chosen because it potentially ingests microplastics in the environment, mistaking the microplastics as prey or unable to distinguish between prey and food. Another vital factor for the selection of the juvenile class is its feeding behaviour. Even though recent studies have focused on the adult class, the bioaccumulation and distribution of heavy metals in the adult's organs and tissues are questionable since the adult diets are more selective, involving a wide range of animals. Therefore, the various sources or vectors of pollutants may be consumed by the adults. As a comparison, juveniles only have a plankto-phytophage diet, which only consumes diatoms, zooplankton, or green algae (Markovic, 2007). Since the size of microbeads is similar to plankton, the chance is higher for this proposed vector to be consumed by the test subjects.

1.6 Significance of the Study

Environmental and health consequences are the main concern of this study since there is a high potential for microbeads and heavy metals to bio-accumulate in organisms, thus climbing up to the top predator through the food chain. As such, the ability of microbeads to accumulate heavy metals higher than seawater has been proven in previous studies (Khan et al., 2015; Brennecke et al., 2016). In general, heavy metals adsorbed onto microbeads are highly available in the environment. Microbeads with heavy metals tend to be desorbed in organisms via acidic conditions in their digestive tracts after ingestion (Holmes & Thompson, 2014; Khan et al., 2017). Consequently, microbeads have become a crucial concern since they appear in small sizes and can easily be ingested by a broader range of marine organisms. Besides, the bioavailability of vector-heavy metal microbeads increases the possibility to transfer harmful chemicals to the food chain.

While varied research results have demonstrated microplastics and contaminants adsorption due to the ‘specific polymer adsorbed specific pollutant’ characteristic, the same situation has been predicted for different organisms for different microplastic-heavy metals intake. Thus, the issue should begin with finding the relation involving the presence of microbeads in heavy metal exposure, including the exposure condition that favours the vector effect of microbeads carrying heavy metals into juvenile seabass via ingestion. In Malaysia, a website called cleanmalaysia.com was established to create the awareness of microbeads pollutant as an effort to bring the light of environmental danger since 2016. As the occurrence of microbeads in the marine ecosystem poses a threat to food safety and results in the banning of microbeads by the US, UK, and Canada, it is essential to study the sorption of heavy metal pollutants on microbeads and their ingestion effects on marine organisms. Thus, raising the awareness of microbeads usage in cosmetic products and followed the banned action in the same way as other countries.

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