Microplastic ingestion of blood cockles (*Tegillarca granosa*) in Kuala Juru, Pulau Pinang

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Abstract

The marine plastic pollution is growing drastically all around the world yet the association of the discarded plastics with the marine organisms are poorly studied. This study is focused on the ingestion of microplastic in Blood Cockles (*Tegillarca granosa*) collected from Kuala Juru, Pulau Pinang. The aim of this study is to quantify the abundance of microplastic and to identify the type of microplastic found in the blood cockles. Total 50 blood cockles (*Tegillarca granosa*) were sampled along with surface water (n=5), bottom water (n=5) and sediment (n=5) for this study. Overall, 295 pieces of microplastics were found in blood cockles with an average of 5.9 (±0.62) microplastics in each blood cockle. The number of microplastics found in surface water, bottom water and the sediment was 134, 175 and 109 respectively. The microplastics were observed under MDSI-40X dissecting stereomicroscope and then analysed in SEM-EDX to confirm the presence of microplastic. The presence chemical components such as Carbon, Oxygen, Chlorine and Silicone in the sample analysed in the SEM-EDX affirms the occurrence of the microplastic. This study has uncovered the presence of microplastic in blood cockles and in the surrounding environment of blood cockles that is water and sediment. Since blood cockles are commonly consumed seafood in Malaysia, the contamination of microplastics is posing a threat to seafood safety and jeopardise human health due the potential toxicity of the microplastic.

Keywords: Blood cockles (*Tegillarca granosa*), Bottom water, Microplastic, Sediment, SEM-EDX, Surface water

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Introduction

The first synthetic plastic was discovered by Bakelite in 1907. Plastic was discovered though the revolution of polymer science and modern life, by inauguration of a numbered of polymers and plastic formulations for human usage, where many of which are found with high availability in the market till today (Shashoua, 2008). In recent years, the production of plastic have spiked the economy of the industry especially the chemical industry in the global economy due to the durability of plastic which is very flexible and also cheap. Plastics are used in massive scale in almost everywhere such as in food packing, clothing, transportation, shelter, medical, construction and leisure industries due to its durable capabilities. Plastics are highly used because of few factors such as lightweight, low cost, relatively unbreakable and extremely durable (Kumar *et al*., 2007). In addition, the inflation for the demand of plastic is anticipated to fourfold by the year 2050 with 20% total oil consumption and 15% of worldwide carbon budget.

Microplastic is fragmented plastic which measures less than 5mm while nanoplastics masures about 1-100 nm. The microplastic is originated either primarily as pre- production pellets, cosmetics, cleaning agents and personal care products or as secondarily from gradual fragmentation from larger piece of plastic. The disposal of plastic through anthropogenic activities degrades plastic into microplastics or nanoplastics after few years and persists in the environment (Barnes *et al*., 2009). The physical characteristic of microplastic such as relatively lower density and high buoyancy in the water encourages the global distribution of microplastic. In 2010, it was estimated 4.8-12.7million metric tons of plastic discarded in the marine environment and the number is expected to spike up to 100-250 million metric ton by year 2050 (Jambeck *et al*., 2015). Microplastic has become a global issue that has been concerning the marine organisms widely. The researches that have been conducted in the favour to get data to evaluate the risk assessment of the particular location and the organism involved (Pearce, 2001).

The consequences of plastic pollution are directly affecting the marine organisms. The discarded plastics such as plastic bags and fishing nets often entangles marine mammal to death. There are also a lot more cases of plastic ingestions in marine organisms as small as zooplankton to as big as whale. The vibrant colours and relatively small size of microplastic highly attracts the marine organisms to feed on microplastic. The microplastic and nanoplastic which mostly comes from the result of domestic runoff causes detrimental implications to the health of marine organisms and the ecosystem (Reichert *et al*., 2018). The ingested microplastic effects the organisms physically and chemically as the presence of additives and chemical substances in the microplastic is harmful. The marine organism that ingests microplastic undergoes satiation

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as the microplastic remains in the guts of the organisms and eventually reduces the energy supply to the organisms. Meanwhile, the ingestion of microplastic in phytoplankton decreases the absorption of chlorophyll (Long *et al*., 2017).

The distribution of microplastic is found in every levels of trophic, thus the accumulation of microplastic in the body is higher in the organisms which are at higher trophic level. In this case, the microplastic have accumulated in human body via food chain (Jamieson *et al*., 2019). The accumulation of microplastic in human body causes evident physical consequences such as inflammation, satiation, and laceration. Plastics is known to accumulate organic and inorganic contaminants such as polychlorinated biphenyl (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from the surrounding water. These deadly chemicals can cause destructive consequences such as cancer in human (Wang *et al*., 2017).

The presence of microplastic in commercial shellfish is highly concerning due to the effect it may impose in human health. Bivalves are widely consumed in Malaysia and the presence of microplastic is yet to be studied in Malaysia. In this study, the ingestion of microplastic in blood cockles (*Tegillarca granosa*) relation is being investigated. Along with blood cockles, sediment and water samples were also studied in order to identify the presence of the microplastic in the environment of the blood cockles and the blood cockles itself. The morphology of the microplastic found was grouped according to the shape of the microplastic present. The observation was carried out using digital microscope and stereo microscope. Then, the physical and chemical examination was made through scanning electron microscopy (SEM).

Methodology

Laboratory procedures were carried out at the teaching laboratory of Centre for Marine and Coastal Studies (CEMACS), Universiti Sains Malaysia. In this study, 50 cockles were collected from the sampling along with surface water sample, bottom water sample and sediment. In processing the samples before further microplastic observation and identification, several laboratory steps were measured: (1) Measurement of cockle's biometric parameters, (2) Isolating which including steps of (i) depuration, (ii) dissection and (iii) digestion, (3) Microplastic filtering**.**

Sampling collection

The sample collection was carried out in Kuala Juru (5°20'14.0''N 100°24'08.7''E), Penang on August 8th 2020. The samples collection comprises of Blood Cockles (n=50), *Tegillarca granosa* 1L of surface water sample (n=5), 1L of bottom water sample (n=5) and 200g of sediment (dry weight). The sample collection area was typically a mud bank near the mangrove forest. The sample location is located 1km away from Perai Free Industrial Area Zone. The location is also considered to have higher anthropogenic activities with

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higher rate of pollution that flows from the Sungai Juru. The source of pollution is mainly originated from the industrial area (Fig. 1).

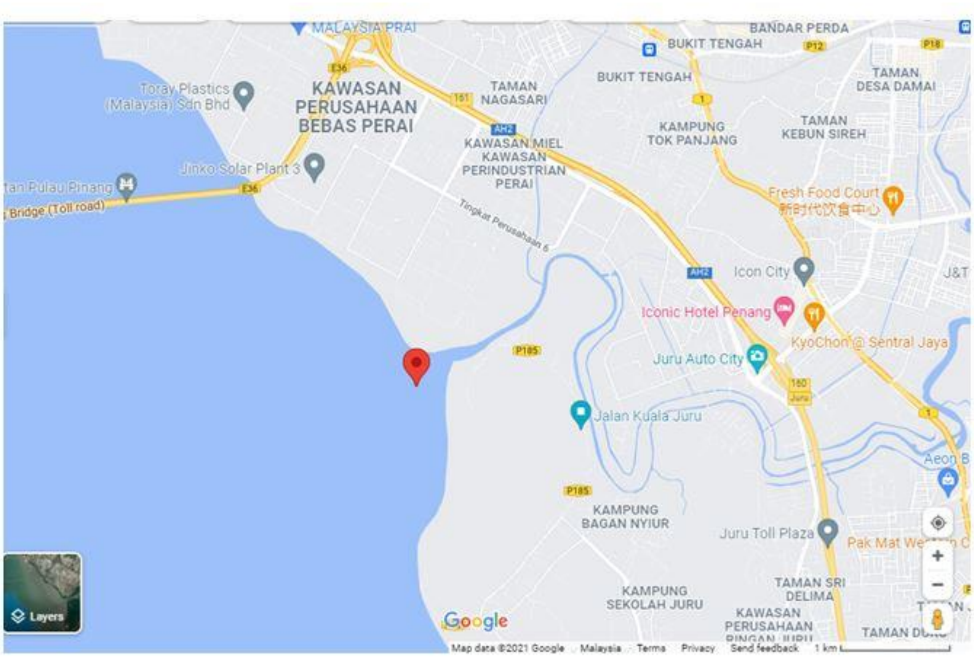

Figure 1: The map of the sampling location

Biometric measurements

Sample biometric parameters were obtained by measuring their total shell lengths and total wet weight. The total length was measured from the anterior of the shell to the posterior of the shell.

Experiment design for blood cockles

Cleaning

To ensure the study primarily focus on cockles consumption of microplastics, the cockles were washed by filtered distilled water so the external adhered microplastics or other debris could be removed. This process is to ensure only microplastics retain within the tissues of the organism studied (Lusher *et al*., 2017).

Dissection

The laboratory equipment used in the experiment was rinsed with filtered distilled water to prevent any form of contamination of microplastic from surroundings. The cockles were dissected using dissecting forceps and immediately weighed on electronic scale. Then, each sample was transferred into individual 200ml glass jar with lid covered.

Digestion

10% Potassium Hydroxide (KOH) was prepared using KOH and filtered distilled water. Then, 75ml of 10% KOH was transferred into the glass jar containing cockle samples. KOH acts as a base to denature protein and hydrolyse chemical bonds. Thus, it digests the

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cockles and leaves the inorganic materials behind. The solution was left in room temperature until complete digestion was observed.

Filtering

The samples were taken out from the incubator after 91 hours of digestion. Clear digestant was observed indicated that completed digestion was done. All 25 blood cockles obtained clear digestant and proceed with direct vacuum filtration process using Whatman GF/C glass microfibre filter membrane (pore size: 1.2 μm). All filters were kept individually in a clean petri dish until further process.

Experiment design for water sample

Digesting

First, the water sample was poured through the stacked 0.3-mm stainless steel mesh sieves. The solids were transferred and collected in the 0.3-mm sieves to a clean beaker with distilled water. Then, the beaker was incubated at 60 °C for 8 hours, until completely dry. Next, the dried sample was added with 200 mL 10% potassium hydroxide (KOH) and kept in room temperature until the organic materials completely digests.

Filtering

Both surface water sample and bottom water sample obtained clear digestant and proceed with direct vacuum filtration process using Whatman GF/C glass microfibre filter membrane (pore size: 1.2 μm). All filters were kept individually in a clean petri dish until further process.

Experiment design for sediment sample

Drying

The sediment samples collected were sieved using 1.17mm sieve to remove the unwanted big particles. Then the sediments were dried in the oven in 65°C until it completely dries. After completely drying, 200g of the dried sediment samples were transferred into glass jar and kept in a dark condition.

Density separation

The microplastics from each sediment sample were extracted using a density separation method. First of all, a concentrated zinc chloride (ZnCl) stock solution with 1.5 g/mL density was prepared. Next, the 200 g of sediment was mixed with 250 mL of KI and stirred thoroughly. The solution was observed until a clear column between the meniscus and the denser than the KI solution's sample which would be settled down to the bottom of the beaker.

The supernatant was then collected and poured through the stacked 0.3-mm stainless steel mesh sieves. The solids were transferred and collected in the 0.3-mm sieves to a clean beaker with distilled water. Then, the beaker was incubated at 60 °C until completely dry. Next, the dried sample was added with 200 mL 10% potassium hydroxide (KOH) and incubated in an oven at 60 °C for 30 mins to remove the organic matter mixed in the sample. The aqueous solution was stirred at room temperature for 10 minutes.

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Filtering

After stirring, the solution was poured into beaker and left it to settle overnight. Then, the liquid was filtered with Whatman GF/C glass microfibre filter membrane (pore size: 1.2 µm). All filters were kept individually in a clean petri dish until further process.

Microplastic analyses

The filter papers were observed under MDSI-40X dissecting stereomicroscope using suitable magnification to search before undergoing physical and chemical observation under scanning electron microscope SEM-EDX. The quality of the microstructure element (chemical characteristics) was determined using the Energy-Dispersive Spectroscopy (EDX) analysis.

Results

Identification of microplastic

The study investigated on the occurrence of microplastic <1.2 µm in blood cockles and its environment which comprises of surface water, bottom water and sediment. The microplastic was classified into four categories in this experiment to make easier identification. The four types were film, fibre, fragment and pallet. Each type has specific shapes and comes in various colours. The abundance of each type of microplastic was shown in the table (Fig. 2).

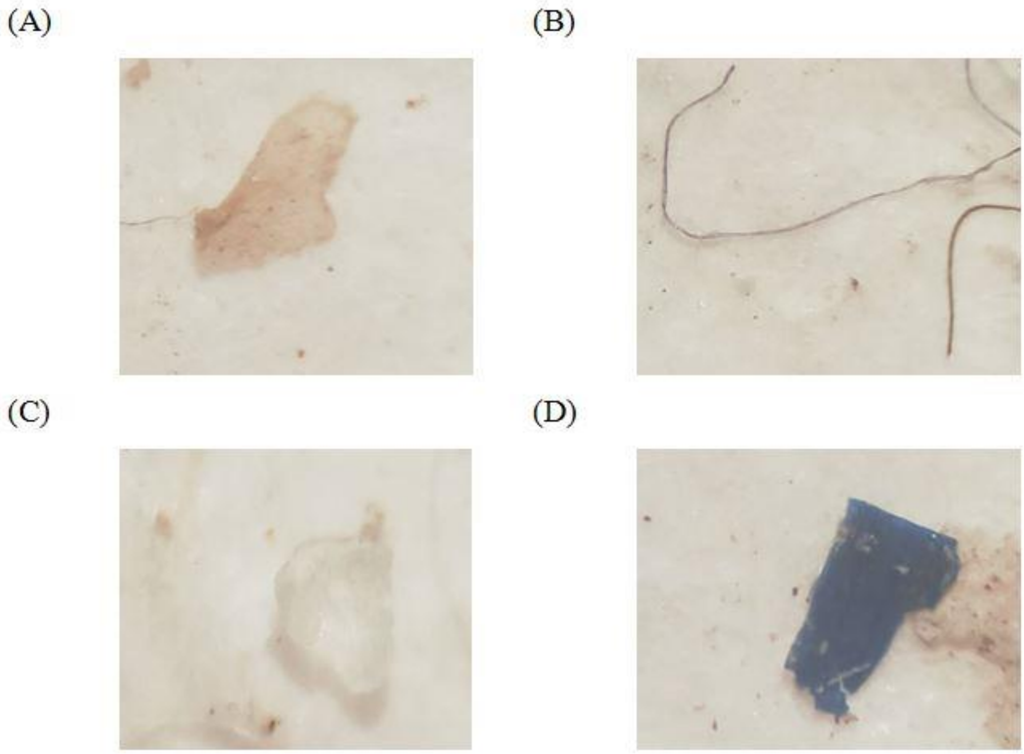

Figure 2: Images of different microplastic morphological types found in all samples by using stereo microscope. Particles identified as (a) film (b) fibre (c) pellet (d) fragment

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Microplastic in blood cockles (Tegillarca granosa)

Table 1: Microplastic classification by morphological types in Blood cockles (*Tegillarca granosa*) from Kuala Juru

	Mean	N	Std Deviation	Total number of MP found
Pallet	0.08	50	± 0.27	4
Fibre	4.68	50	± 3.94	233
Fragment	0.48	50	± 0.95	24
Film	0.66	50	± 0.75	33

The presence of microplastic in blood cockles is 100% in total 50 blood cockles (FO=100%). The total microplastic (size<5mm) found in the cockles were 294 pieces which were classified into four different categories such as pallet, fibre, fragment and film (Fig. 3).

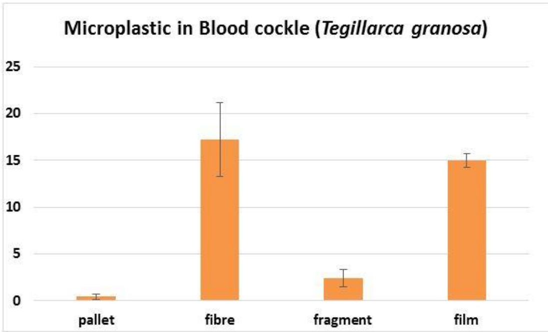

Figure 3: Microplastic classification by morphological types in Blood cockles (*Tegillarca granosa*) from Kuala Juru

Among four different types of microplastics identified, the highest type of microplastic found was fibre with an of average 4.68 (±0.27) per individual of blood cockles. While the lowest type of microplastics ingested by blood cockles was pallet with total 4 pallets in blood cockles obsserved (n=50). The mean value of fragment and film ingested by a blood cockle is 0.48 (±0.95) and 0.66 (±0.75), respectively.

Microplastic in bottom water sample

Table 2: Microplastic classification by morphological types in Bottom water sample from Kuala Juru

	Mean	N	Std Deviation	Total number of MP found
Pallet	0.4	5	± 0.55	2
Fibre	17.2	5	± 5.26	86
Fragment	2.4	5	± 1.52	12
Film	15.0	5	± 2.74	75

The bottom water sample (n=5) was collected near the seafloor at the average depth of 0.92m. The water collected were analysed for the presence of 4 different types of microplastic (size<5mm) which categorized as pallet, fibre, fragment and film. The abundance of microplastic found in the bottom water sample was 175 pieces (n=5) (Fig. 4).

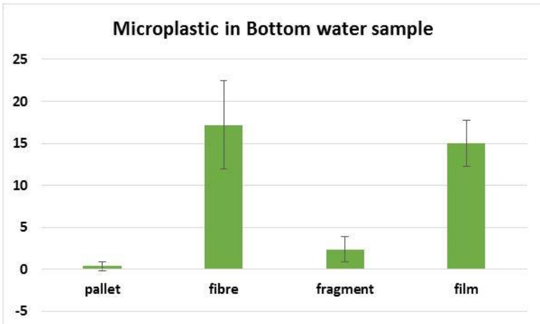

Figure 4: Microplastic classification by morphological types in bottom water sample from Kuala Juru

Out of four different types of microplastic classified, fibre have contained the most with a mean of 17.2 (±5.26) per replicate. The abundance of the microplastic is followed by film, fragment and pallet with a mean value of 15.0 (±2.74), 2.4 (±1.52) and 0.4 (±0.55) per replicate, respectively.

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Microplastic in surface water sample

Table 3: Microplastic classification by morphological types in surface water sample from Kuala Juru

	Mean	N	Std Deviation	Total number of MP found
Pallet	0.08	5	± 0.84	4
Fibre	22.4	5	± 10.97	112
Fragment	1.00	5	± 1.00	5
Film	2.6	5	± 1.14	13

The surface water sample (n=5) was collected near the top most surface of water using. The water collected were analysed for the presence of 4 different types of microplastic (size<5mm) which categorized as pallet, fibre, fragment and film. The abundance of microplastic found in the bottom water sample was 134 pieces (n=5) (Fig. 5).

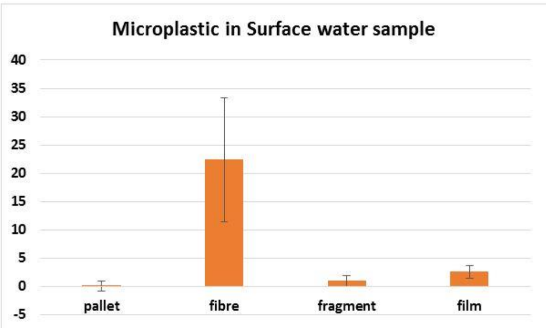

Figure 5: Microplastic classification by morphological types in surface water sample from Kuala Juru.

The microplastic abundance in surface water sample was dominated by fibre with a mean value of 22.4 (±10.97) per replicate. The other three types were very infreqeunt with a mean of 0.08 (±0.84), 1.00 (±1.00) and 2.6 (±1.14) per replicate for pallet, fragment and film, respectively.

Microplastic in sediment

Table 4: Microplastic classification by morphological types in sediment sample from Kuala Juru

	Mean	N	Std Deviation	Total number of MP found
Pallet	7.6	5	± 5.81	38
Fibre	6.8	5	± 4.32	34
Fragment	10.6	5	± 7.67	53
Film	3.4	5	± 3.97	17

The sediment sample (n=5) was collected at the muddy sea bed at depth of 0.92m using ponar grab sampler. The sediment collected were analysed for the presence of 4 different categories of microplastic (size<5mm) which categorized as pallet, fibre, fragment and film. The abundance of microplastic found in the bottom water sample was 109 pieces (n=5) (Fig. 6).

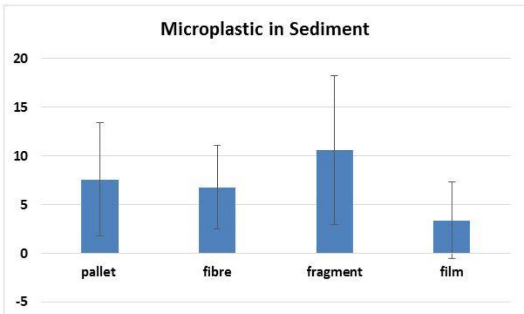

Figure 6: Microplastic classification by morphological types in sediment sample from Kuala Juru.

The microplastic abundance in sediment sample was dominated by fragment with a mean value of 10.6 (±7.67) per replicate. The other three types were very infreqeunt with a mean of 7.6 (±5.81), 6.8 (±4.32) and 3.4 (±3.97) per replicate for pallet, fibre and film, respectively.

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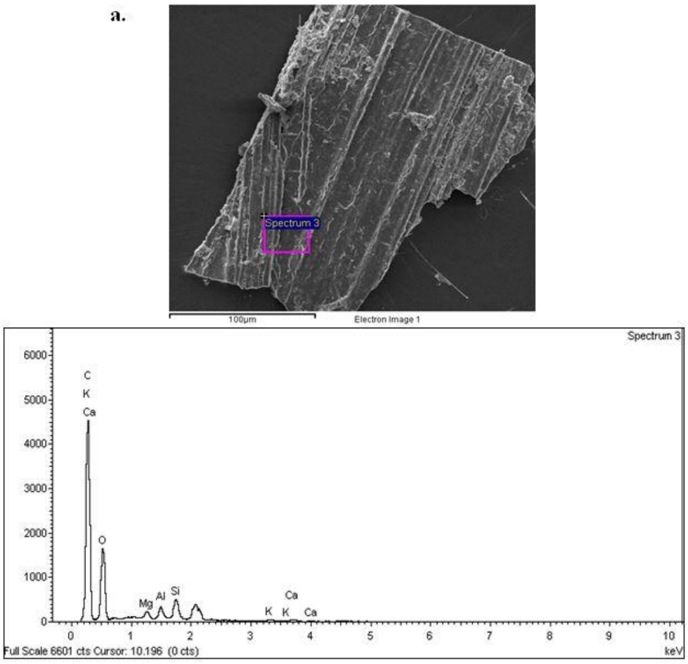
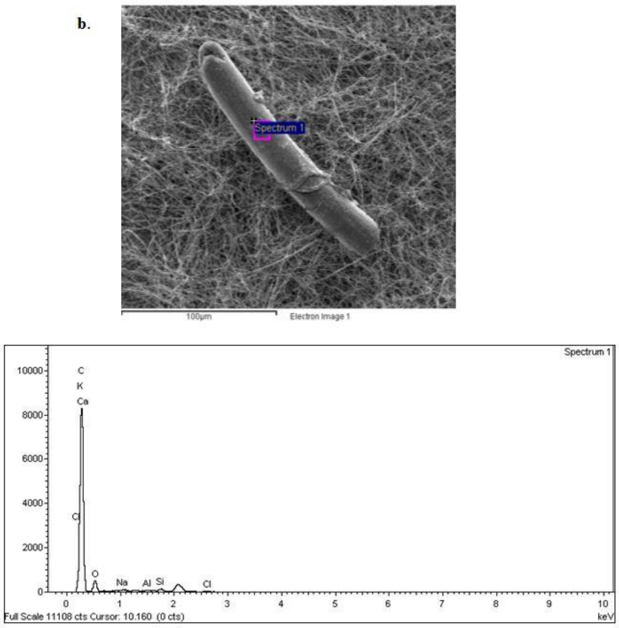
SEM-EDX imaging of microplastic

The SEM-EDX imaging shows the physical and chemical properties of the microplastic identified. The SEM shows the physical properties that includes the condition of the microplastic which is degraded with porous and rough surface. The EDX shows the chemical properties that includes elements such as Carbon, Oxygen, Chlorine and Silicone. The elements present in the EDX analysis supports the identification of the sample which is microplastic.

a.



b.



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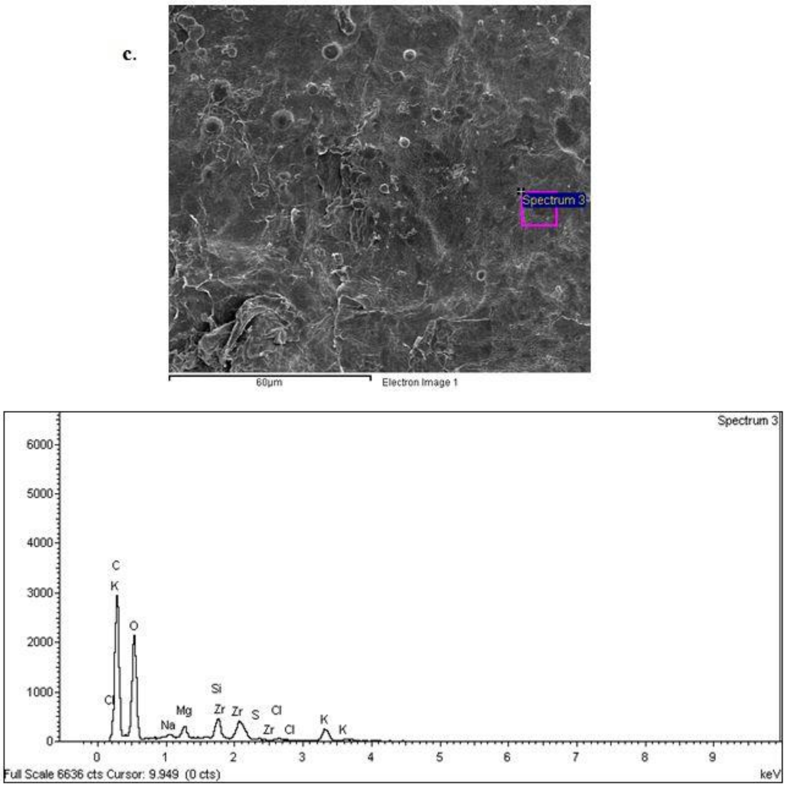
c.



d.

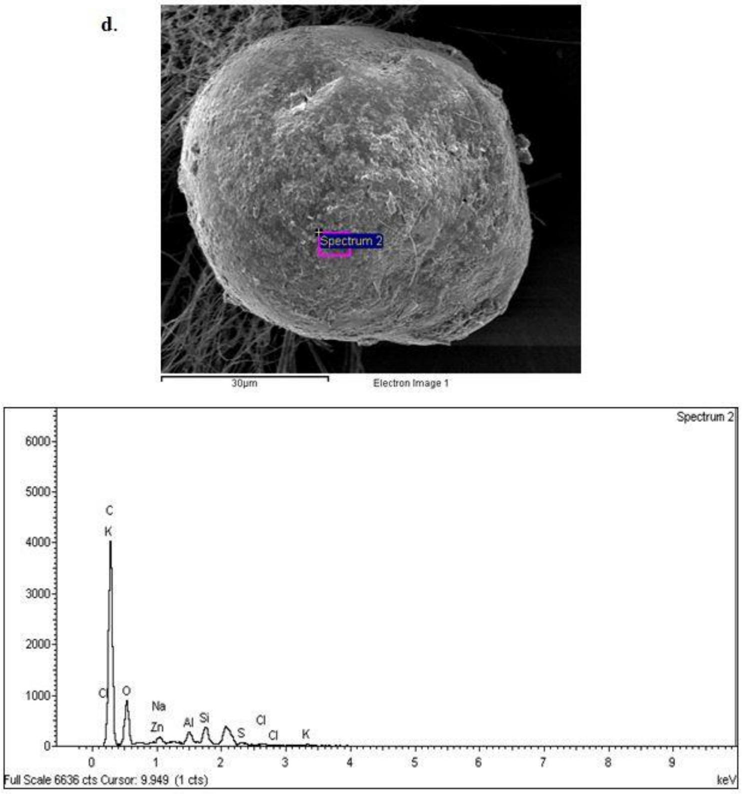


Figure 7: The SEM-EDX imaging of microplastic studied. Particles identified as (a) fragment (b) fibre (c) film and (d) pallet from surface water, bottom water, blood cockle and sediment, respectively.

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Discussion

Microplastic in sea water

The ocean is vast opened with millions of lives living harmoniously. At the same time, the ocean is now heavily polluted due to human activities. Microplastic from different types and shapes have been spotted in the river, sea shores, sedimentary habitats, deep sea and even in living organisms including humans. The overproduction of plastic and mismanagement of waste have contributed to this pollution. Moreover, the COVID-19 outbreak have contributed more plastic pollution as the demand for face mask and health supplies surged. From the remotest island to the busiest city, microplastic pollution in the ocean is alarming.

In this study, the sampling location was at Kuala Juru, Penang which is at the mouth of heavily polluted Juru River. According to Department of Environment, the Juru River is classified as the most polluted river where the water is isn't safe for drinking even after boiling. The water quality index of the river is at 63 and 62. This indicates the water quality of the river is poor and not advisable for drinking (Toriman *et al*., 2011). The sampling location which located at the mouth of the river is great candidate to investigate the presence of microplastic in the environment and its biota.

The presence of microplastic was analysed in two different layers of water which is surface water and bottom water. Different layers of water distinguishes the density of the microplastic discovered as the lighter weight microplastic tends to be found more in surface water while denser microplastic in bottom water. The total number of microplastic pieces found in the surface and bottom water is 134 and 175 (n=5). The occurrence of microplastic also 100% in all replicates. In addition, several studies that were carried out on the microplastic in sea water have resulted in 100% occurrence in every location sampled (Alfaro-Núñez *et al*., 2020). The fibre was dominating among other types of microplastic found. Microplastic fibre is also known as the most dominating type of microplastic in microplastic pollution with 91% (Murphy *et al*., 2016). The microplastic fibres are originally manufactured in industries such as carpeting, textiles, fishing nets, ropes, and upholstery (Koelmans *et al*., 2019). The microplastic fibre is dominating in surface water compared to other types of microplastic with 83.5% prevalence due physical characteristic of it. Microplastic fibre is less dense compared to other type of microplastic. Thus, it can be transported easily on the surface water.

In this study, microplastic in bottom water is mainly consists of fibre and film with 49.1% and 42.8% prevalence respectively. The presence of microplastic has been reported in every part of the ocean including the hadal zones (6000-11000m depth). The different densities of the microplastic have become the reason of the transportation of microplastic in different columns of water (Koelmans et al., 2017). The bottom water which was sampled at depth of 0.9 m evidently have

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different amount of microplastic of different shapes. When the amount of microplastics compared between surface and bottom water, the presence of microplastic film is higher in bottom water. In contrast to the result obtained, Kooi and Koelmans (2019) have stated the distribution of microplastic was dominated by fibers and fragments in the water. The low density polymers such as Polyethylene and Polypropylene are the most common in the surface water (Erni-Cassola *et al*., 2019). The presence of microplastic in the sea water will directly affect the marine organisms. Marine organisms accidentally ingests microplastic as it is highly available in the environment.

Microplastic in sediment

Microplastic is distributed all-over throughout the sediments of both marine and freshwater such as in trenches, lagoons, estuaries, seas, lakes and rivers (Shruthy and Ramasamy, 2017). According to ter Halle *et al.* (2016), the most frequent observation of microplastics were in the form of fragment, microbeads and microfibers. The fragment is basically broken pieces of larger plastic as the larger pieces of plastic can degrade due to mechanical and biological actions. The microbeads are normally manufactured in the cosmetic industry as abrasives (Mason *et al*., 2016). These microbeads are originally smaller in size compared to other type of microplastics. Meanwhile, the microfibers are the fragmented fabrics and ropes (Browne *et al*., 2011).

In this study, the microplastics observed in the sediment were categorized as fibre, fragment, film and pallet. In contrast to water sample, the fragment dominated all other microplastics in the sediment sample. According to Erni-Cassola *et al.* (2019), plastic such as polyamide, polyester and acrylic (PPandA) are denser than seawater, thus found more on the bottom part of the sea. PPandA dominated in the bottom part of the sea (77%) along with chlorinated PE (Bergmann *et al*., 2017). Moreover, the microplastic particles in the sediments were in some case originated from the fecal waste as the marine organisms ingests microplastic. This process transports lower density microplastic such as Polyethylene and Polypropylene to end in the sediment (Cole *et al*., 2016). The presence of microplastic in the sediment contributes to the ingestion of microplastic in marine organisms.

Microplastic in blood cockles (Tegillarca granosa)

The microplastic ingestion in marine organisms has been widely reported and quantified in previous studies (Erickson *et al*., 2014). Studies on the microplastic fragmentation have discovered the degradation of plastic in the marine environment often affects the marine organisms directly. The ingested microplastic and nanoplastic bio accumulates in the guts of the marine organisms (Haward, 2018). Study by Mcneish *et al*. (2018) states that 85% of fish had microplastic in their digestive tissues with an average of approximately 13 particles fish−1 with fibers the

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dominant microplastic. Anthropogenic litter has been found in 25–28% of fish collected from markets prepared for human consumption (seafood) in the USA and Indonesia ranging from 0.3–5.9 particles fish−1 58. These studies suggest microplastic abundance in fish could vary across a gradient of aquatic habitats (Rochman *et al*., 2015).

In this research, blood cockle (*Tegillarca granosa*) is studied to determine the ingestion of microplastic. The presence of microplastic was with 100% occurrence in every individual cockle (n=50) with a mean of 5.9 (±0.62) microplastics in each blood cockle. Although, the research about microplastic in blood cockles is not studied previously, there were few researches studied on the ingestion of microplastic in bivalves. Previous study by Ding *et al*. (2021) have reported the abundance of microplastic in four species of bivalves ranging between 0.5 to 3.3 items per individual. Compared to Ding *et al*. (2021), the microplastic ingestion in blood cockles from Kuala Juru is higher. However, another study carried on the oysters and razor clams resulted in 0.95±0.77 and 8.84±0.45 microplastic pieces per individual respectively (Baechler *et al*., 2019).

In addition, the microplastic fibre was mainly dominated in the samples analysed by Baechler *et al.* (2019) with over 99% prevalence (n=3026) while the other microplastics such as fragments (n=12), beads (n=5), films (n=5) and unknown (n=3) only represent less than 1% of the findings. The presence of microplastic in the blood cockles was 79.3% (n=50). The presence of the microplastic in the environment that is seawater and sediment contributes to the ingestion of microplastic in blood cockles.

The ingestion of plastic components risks the marine organisms as it can cause sub-lethal consequences such as declined reproductivity outcome and fatality (Fossi *et al*., 2014). In cockles, the microplastic will directly affect humans as cockles are eaten directly without removing its guts. Thus, the bio accumulation of microplastic will occur in humans.

Microplastic analysis in SEM-EDX

The microplastic is widely observed under visual microscope for identification and quantification of microplastic. Unfortunately, the identification of microplastic using microscope alone is not valid as overestimation or underestimation of microplastic counts can occur. This is because in visual microscope, the microplastic can only be identified through the shape and size. Thus, additional analysis method is required to determine the presence of microplastic. In this study, SEM-EDX was used to identify the physical and chemical properties of the microplastic.

In this study, SEM-EDX was analysed on film from blood cockles, pallet from sediment, fibre from bottom water and fragment from surface water. The physical properties of the microplastic under SEM shows the condition of microplastic identified. Microplastic in the marine environment is either

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originally manufactured as microplastic or a larger piece of plastic fragmented into microplastic. The surface of the fragmented microplastic is porous as it has undergone degradation. Weathering, biological degradation and chemical actions are among the degradation undergone by microplastic in the marine environment (Shahnawaz *et al*., 2019).

The EDX analysis of microplastic shows chemical elements present in the microplastic particles identified. Carbon (C) represented as the highest percentage for all four microplastic tested in this sample. The presence of Carbon indicates the microplastic identified as Carbon is one of the main chemical element of plastic. Next, the presence of Potassium (K) also found in the samples analysed. The presence of Potassium (K) is basically the deposition of chemical, KOH on the surface of the microplastic as KOH was used to digests the organic materials in the sample (O'Donovan *et al*., 2018).

The sample (a), which is a fragment have chemical elements such as C, K, Ca, O, Si, Mg and Al. The presence of Carbon and Oxygen mainly shows the fragment is a Polyethylene which ages less than 10 years. The presence of Carbon and Oxygen in sample (b) fibre shows the fibre is typically a polyethylene terephthalate (PET). PET is a plastic material that used in the textile industry (Wang *et al*., 2017). For the sample (c) is a film with chemical elements such as C, K, Cl, O, Na, Mg, Si and Zr. The presence of Carbon and Oxygen indicates that the microplastic is Polyethylene (PE). Lastly, the sample (d) which is a pallet have chemical elements such as C, K, Cl, O, Na, Zn, Al and Si. The presence of C and O indicates, its Polypropylene (PP). The chemical elements in found less than 5% in EDX analysis suggests that they were either trace materials or common additives in the plastic (Canopoli *et al*., 2020).

Conclusion

In conclusion, the presence of microplastic is proven in the Blood Cockles (*Tegillarca granosa*) from Kuala Juru, Penang. The microplastic sizes <5mm observed in all samples with different types of microplastics such as film, fragment, pallet and fibre. The most common microplastic that was observed is microfiber. This presence of microplastic in all samples suggests the study location is potential for further studies. As stated in the previous chapters of this dissertation, there is still a large research gap in microplastic ingestion studies due to unsynchronized protocols from sampling methods to lab processing of sample organisms. Thus, it is critical to employ a standardized protocol so that more representative and precise data regarding microplastic ingestion of marine fishes at a global scale can be obtained.

Malaysia was ranked at the eighth worst nation for plastic pollution globally (Chen et al., 2021). Not only in Malaysia, plastic pollution actually a huge environmental challenge which is faced by all the countries in the world (Iroegbu et al., 2021). Thus, this study suggests more severe measures should

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be implemented at local, national and international stages to control and curb the issue. Also, stricter laws and regulations as well as international agreements accompanying with updated scientific data should be applied across developed and developing countries to improve the waste management systems. Awareness programmes or education outreach are the basic steps to increase public awareness so that the public could have more understanding about plastic pollution and might be more willing to reduce their plastic usage in daily life. Awareness efforts could urge for participations of the public, corporates, media and tourism in ameliorating microplastic pollution issue. Thus, we as a part of the environment should always play a role to implement the principles of 6R's in our daily lives, which are 'reduce, reuse, recycle, remanufacture, recover and redesign' Nagalingam and Sev (2009) to decrease plastic waste and pollution in the marine environment.

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References

Alfaro-Núñez, A., Astorga, D., Cáceres-Farías, L., Bastidas, L., Villegas, C.S., Macay, K.C. and Christensen, J.H., 2020. Microplastic pollution in seawater and marine organisms across the tropical eastern Pacific and Galápagos. https://doi.org/10.21203/rs.3.rs-83558/v1

Baechler, B., Granek, E., Hunter, M. and Conn, K., 2019. Microplastic concentrations in two Oregon bivalve species: Spatial, temporal, and species variability. Environmental Science and Management Datasets. https://doi.org/10.15760/esm-data.1

Barnes, D.K.A., Galgani, F., Thompson, R.C. and Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(**1526**), 1985–1998. DOI: 10.1098/rstb.2008.0205

Bergmann, M., Wirzberger, V., Krumpen, T., Laurenz, C., Primpke, S., Tekman, M.B. and Gerdts, G., 2017. High quantities of microplastic in arctic deep-sea sediments from the Hausgarten Observatory. *High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN Observatory*. https://doi.org/https://doi.org/10.1021/acs.est.7b03331

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R., 2011. Accumulation of microplastic on Shorelines Woldwide: Sources and sinks. Environmental Science & Technology, 45(21), 9175–9179. https://doi.org/10.1021/es201811s

[DOI: 10.18331/SFS2022.9.1.8]

Canopoli, L., Coulon, F. and Wagland, S.T., 2020. Degradation of excavated polyethylene and polypropylene waste from landfill. *Science of The Total Environment*, 698, 134125. https://doi.org/10.1016/j.scitotenv.2019.134125

Chen, H. L., Nath, T. K., Chong, S., Foo, V., Gibbins, C., & Lechner, A. M., 2021. The Plastic Waste Problem in Malaysia: Management, recycling and disposal of local and Global Plastic Waste. SN Applied Sciences, 3(4). https://doi.org/10.1007/s42452-021-04234-y

Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C. and Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. *Environmental Science and Technology*, 50(**6**), 3239–3246. https://doi.org/10.1021/acs.est.5b05905

Ding, J., Sun, C., He, C., Li, J., Ju, P. and Li, F., 2021. Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution. *Science of the Total Environment*, 782, 146830. https://doi.org/10.1016/j.scitotenv.2021.146830

Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G. and Reisser, J., 2014. Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS ONE*, 9(**12**). https://doi.org/10.1371/journal.pone.0111913

Erni-Cassola, G., Zadjelovic, V., Gibson, M.I. and Christie-Oleza, J.A., 2019. Distribution of plastic polymer types in the marine environment; a meta-analysis. *Journal of Hazardous Materials*, 369, 691–698. https://doi.org/10.1016/j.jhazmat.2019.02.067

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E. and amp; Clò, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (Cetorhinus Maximus) and fin whale (Balaenoptera physalus). *Marine Environmental Research*, 100, 17–24. https://doi.org/10.1016/j.marenvres.2014.02.002

Haward, M., 2018. Plastic pollution of the world's seas and Oceans as a contemporary challenge in Ocean Governance. *Nature Communications*, 9(**1**). https://doi.org/10.1038/s41467-018-03104-3

Iroegbu, A. O., Ray, S. S., Mbarane, V., Bordado, J. C., & Sardinha, J. P., 2021. Plastic pollution: A perspective on matters arising: Challenges and opportunities. ACS Omega, 6(30), 19343–19355.

[DOI: 10.18331/SFS2022.9.1.8]

https://doi.org/10.1021/acsomega.1c02760

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Law, K.L., 2015. Plastic waste inputs from land into the ocean. *Science*, 347(**6223**), 768–771. DOI: 10.1126/science.1260352

Jamieson, A.J., Brooks, L.S., Reid, W.D., Piertney, S.B., Narayanaswamy, B.E. and Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Royal Society Open Science*, 6(**2**), 180667. https://doi.org/10.1098/rsos.180667

Koelmans, A.A., Besseling, E., Foekema, E., Kooi, M., Mintenig, S., Ossendorp, B.C., Redondo-Hasselerharm, P.E., Verschoor, A., van Wezel, A.P. and amp; Scheffer, M., 2017. Risks of plastic debris: Unravelling fact, opinion, perception, and belief. Environmental Science *and* Technology, 51(**20**), 11513–11519. https://doi.org/10.1021/acs.est.7b02219

Koelmans, A. A., Mohamed Nor, N. H., Hermsen, E., Kooi, M., Mintenig, S. M., & De France, J., 2019. Microplastics in freshwaters and drinking water: Critical Review and assessment of Data Quality. Water Research, 155, 410–422. https://doi.org/10.1016/j.watres.2019.02.054

Kooi, M. and Koelmans, A.A., 2019. Simplifying microplastic via continuous probability distributions for size, shape, and density. *Simplifying Microplastic via Continuous Probability Distributions for Size, Shape, and Density*. https://doi.org/10.1021/acs.estlett.9b00379

Kumar S., Hatha, A.A.M., Christi, K.S., 2007. Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *International Journal of Tropical Biology*, 55 (**3-4**), 2007, 777-786.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Paul-Pont, I. and Soudant, P., 2017. On the Potential Role of Phytoplankton Aggregates in Microplastic Sedimentation. *Fate and Impact of Microplastics in Marine Ecosystems*, 71. DOI: 10.1016/b978-0-12-812271-6.00069-7

Lusher, A.L., Welden, N.A., Sobral, P. and Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods*, 9(**9**), 1346-1360. DOI:10.1039/c6ay02415g

Mason, S. A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., & Rogers, D. L., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environmental Pollution, 218, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056

McNeish, R.E., Kim, L.H., Barrett, H.A., Mason, S.A., Kelly, J.J. and

Hoellein, T.J., 2018. Microplastic in riverine fish is connected to species traits. *Scientific Reports*, 8(**1**). https://doi.org/10.1038/s41598-018-29980-9

Murphy, F., Ewins, C., Carbonnier, F. and Quinn, B., 2016. Wastewater treatment works (WWTW) as a source of microplastics in the aquatic environment. *Environmental Science and Technology*, 50(**11**), 5800–5808. https://doi.org/10.1021/acs.est.5b05416

Nagalingam, Sev., 2012. A framework of product recovery to improve sustainability in manufacturing. Advances in Mechanical Engineering. 2. 41-47.

O'Donovan, S., Mestre, N.C., Abel, S., Fonseca, T.G., Carteny, C.C., Cormier, B., Keiter, S.H. and amp; Bebianno, M.J., 2018. Ecotoxicological effects of chemical contaminants adsorbed to microplastics in the clam scrobicularia plana. *Frontiers in Marine Science*, 5. https://doi.org/10.3389/fmars.2018.00143

Pearce, J.B., 2001. A sea of troubles, UNEP Joint Group of Experts on the scientific aspects of marine pollution (GESAMP). report study gesamp no. 70. 35 pages, 2001. protecting the oceans from land-based activities. UNEP Joint Group of Experts on the scientific aspects of marine pollution (GESAMP). report study gesamp no. 71. 162 pages, 2001. *Marine Pollution Bulletin*, 42(**11**), 1194–1195. https://doi.org/10.1016/s0025-326x(01)00152-7

Reichert, J., Arnold, A. L., Hoogenboom, M. O., Schubert, P., & Wilke, T., 2019. Impacts of microplastics on growth and health of hermatypic corals are species-specific. *Environmental Pollution*, *254*, 113074. https://doi.org/10.1016/j.envpol.2019.113074

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., The, F.C., Werorilangi, S. and The, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific Reports*, 5(**1**). DOI:10.1038/srep14340

Shahnawaz, M., Sangale, M.K. and Ade, A.B., 2019. Analysis of the plastic degradation products. *Bioremediation Technology for Plastic Waste*, 93–101. https://doi.org/10.1007/978-981-13-7492-0_9

Shashoua, Y., 2008. Conservation of plastics. *Conservation of Plastics: Materials Science, Degradation and Preservation*. https://doi.org/10.4324/9780080878782

Sruthy, S. and Ramasamy, E.V., 2017. Microplastic pollution in Vembanad Lake, Kerala, India: The first report of microplastics in Lake and estuarine sediments in India. *Environmental Pollution*, 222, 315–322.

https://doi.org/10.1016/j.envpol.2016.12.038

Ter Halle, A., Ladirat, L., Gendre, X., Goudouneche, D., Pusineri, C., Routaboul, C., Tenailleau, C., Duployer, B. and Perez, E., 2016. Understanding the fragmentation pattern of marine plastic debris. *Environmental Science and Technology*, 50(**11**), 5668–5675. https://doi.org/10.1021/acs.est.6b00594

Toriman, M.E., Hashim, N., Hassan, A.J., Mokhtar, M., Juahir, H., Gasim, M.B. and Abdullah, M.P., 2011. Study on the impact of tidal effects on water quality modelling of Juru River, Malaysia. *Asian Journal of Scientific Research*, 4(**2**), 129–138. https://doi.org/10.3923/ajsr.2011.129.138

Wang, Z.M., Wagner, J., Ghosal, S., Bedi, G. and Wall, S., 2017. SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and Fish Guts. *Science of The Total Environment*, 603-604, 616–626. https://doi.org/10.1016/j.scitotenv.2017.06.047

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