Microplastic Pollution In Estuarine Ecosystem At Sungai Laloh, Pasir Putih

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Research Article

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Abstract

This paper presents the characteristics and trends of microplastic pollution in the estuarine ecosystem at Sungai Laloh, Pasir Putih. The sample collected were green mussels and sediment located at two different sampling point. A comparison of similar total weight was made for wild mussel and cultured mussel. The method involved the pre-treatment of green mussels and sediment sample, followed by digestion and analysis. In pre-treatment, the aquatic specimen and sediment undergo non-toxic density separation with the used of 1.2 kg/L sodium chloride (NaCl). The digestion was made by adding 35% (v/v) hydrogen peroxide (H_2O_2) and incubated for 24 to 48 hours by using incubator shaker at of 50°C and 80 rpm. Analysis was conducted by using stereomicroscope and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR FT-IR). In addition, SPSS statistical software was used to determine the significant difference between wild mussel and cultured mussel. Results show that microplastic fragment found abundant in mussel while microplastic fibers were found in sediment. The types of polymer microplastic found were polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), ethylene vinyl acetate (EVA) and nylon (all polyamides). In comparison based on percentage of abundance, cultured mussel was the most polluted with microplastic.

1.0 Introduction

Microplastic pollution is serious environmental problem of marine debris due to its difficulty to decompose which can be up to hundreds of years. Currently, airborne microplastic has been observed in atmosphere which includes indoor and outdoor environments. The microplastics can be through wind from the ocean and surrounding land areas. In 2017, the production of plastic reached 348 million metric tons. The production of plastic worldwide has continued to increase at approximately 3% each year. From the amount, only 3% of plastic used were recycled. Based on the trends, it is estimated that on 2050 approximately 67.8 million metric ton of plastics will be at the environment or landfill (Ebere et al., 2019). Microplastic can be recognized with size less than 5 mm in diameter (Dowarah et al., 2020; Wagner et al., 2014; Waite et al., 2018; Wu, 2020). Besides, this marine debris can be categorized based on its various sizes either nano, micro, meso, macro and megaplastics.

The smaller size of microplastic might be ingested by aquatic life which includes plankton, fish, mussel, seashell, crab and other animal that life on land. Plastic that is difficult to decompose cause harm to animal and human health as the food chain contained of the aquatic life (Han, 2020). It has been classified onto primary microplastic (personal care and cosmetic products) and secondary microplastic (fragmentation from chemical and biological interaction) (Defu et al., 2018). Some of the primary microplastic is microbead which originated from personal care. Microbeads usually have white and opaque colors (Hu et al., 2019). In the ocean and rivers, the microplastic fragment from the synthetic fiber usually come from fabric washing (Firdaus et al., 2020). Plastic which made up of polymer consisted of several types. The types of polymer microplastic are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), nylon (all polyamide), acrylonitrile butadiene styrene (ABS), cellulose acetate (CA), polycarbonate (PC), polyurethane (PU), polymethyl methacrylate (PMMA), polytetrafluoroethylene (PTFE) and polyethylene terephthalate (PET) (Cai et al., 2019; Defu et al., 2018; Jung et al., 2018). Previous study has focused on characterizing microplastic pollution in freshwater, ocean, terrestrial environment and wastewater treatment plant (WWTP) (Wang et al., 2020). However, there is lack of information on microplastic pollution at the estuarine ecosystem in Malaysia (Athey, 2020).

The estuary is an enclosed body of water that mixes freshwater and saltwater from the sea (Wu, 2020). It is also connected between land and sea. The classification of estuary consisted of harbors, inlets, bays, lagoons, wetlands and swamps. Estuarine play an important role as the nursery of the ocean. Besides, it provides rich feeding ground for fish and birds. The animals located at estuaries ecosystem includes fish, crab, sea birds, marine worms, skunks and shellfish (mussel). From the previous research, polypropylene (PP) and polyethylene (PE) bring an impact to ragworm and fish (Revel et al., 2020). However, there is lack of information on microplastic trends in shellfish or mussel. Mussel which known as bivalves is a valuable organism to determine the different levels of pollutants in the environment. In addition, mussel can be used as global bioindicator of microplastics (Dowarah et al., 2020). The mussel can act as a filter feeder which traps and accumulate the microplastic pollutant due to their low excretion rates (Su et al., 2018). Rather than aquatic life, sediment sample has been used to determine the microplastic pollutant due to its different density. Previous study show that from collected sediments, type of film microplastic found abundance at rivers strait of Johor (Shazani et al., 2018). However, the type of plastic ingested by marine might be different.

In this study, microplastic pollution in estuarine ecosystem was investigate at Sungai Laloh, Pasir Putih. The objective of this study are to (1) identify the trends of microplastic present in the mussel and sediment sample at estuarine of Sungai Laloh; (2) determine the characteristic of microplastic present based on color and size; (3) identify the functional group of polymer microplastic present; (4) compare abundance of microplastic pollution from similar total weight between wild mussel and cultured mussel. The scope of this study is covered on the mussels and sediments located within the research area from October to November 2020. In situ parameters were analysis for DO, pH, temperature, salinity, conductivity and turbidity. Lab analysis includes digestion, separation and identification using microscope and ATR FT-IR.

2.0 Methods

The samples collected were mussels and sediments. The methods involve three-step process which are pre-treatment, digestion and analyze by using high-end instruments. The characterization of microplastic present involves identification of size, color and their type. Besides, a comparison is made for wild and cultured mussel with amount approximately 350 g weight for each wet mussel from different sizes. Finally, correlation is made to determine the significant different of microplastic present in both mussels. All materials and methods used are described in Figure 1.

2.1 Sample collection

The sample was collected from two sampling point at estuarine ecosystem nearest to Sungai Laloh, Pasir Putih as show in Figure 2. The first point is located of cultured mussel with global positioning system (GPS) coordinate 1˚25’59.178” N 103˚55’44.238” E. The second point is located at GPS coordinate 1˚25’59.0” N 103˚54’25.1” E that locate wild mussel. The main activities within this area involve industrial activities and seafood restaurant. Before collection, safety precaution was taken by wear life jacket and suitable personal protective equipment (PPE). All types of plastic container for the storage of sample are avoided to prevent contamination. Initially, the water quality was measured by using YSI ProPlus. The parameters involved are dissolved oxygen (DO), pH, temperature, salinity, and conductivity. Turbidity was measured using portable turbidimeter HACH 2100Q. Next, 100 mussels of different size range were collected and stored in sampling box. For the sediment, 300 g of sample were collected using the Ekman dredge sediment sampler (Wu, 2020). The collected sample were stored at temperature 4℃. The sample was brought to the lab for sample preparation and undergo pre-treatment process. The next part was conducted in close area to prevent airborne microplastic contamination.

2.2 Pre-treatment process

Initially, the size (length and width) and weight of mussel be measured by using vernier calipers and analytical balance as shown in Figure 3. The extraction of microplastic was made through density separation for mussels and sediment samples. In this process, the density of 1.2 kg/L saturated sodium chloride solution (NaCl) be used which also a non-toxic technique (Deng et al., 2020; Yulin et al., 2019). Eleven batches of wet mussel that has been removed from its shell was immersed and shake vigorously in separated 250 mL conical flask. The triplicate amount of 25 g sediment sample was taken for pre-treatment. In this method, less dense of microplastic cause it to float in the upper surface of solution. Usually, PP, PE, PET and nylon have density of 0.946 gcm^{-3}, 0.940 gcm^{-3}, 1.38 $gcm^{-3}$and 1.15 gcm^{-3} respectively (Quinn et al., 2017).

2.3 Digestion of sample

The sample was digested to breakdown the soft tissue. Previous study stated that digestion with hydrogen peroxide (H_2O_2) was the most effective technique (Waite et al., 2018; Yulin et al., 2019). However, this technique can destroy organics that present in the sample. A large amount of H_2O_2 was required in the digestion method. 100 mL of 35% v/v H_2O_2 was added into each sample. Then, it is placed inside an incubator shaker to be digested. The incubator is set at temperature 50 ºC and rotation speed 80 rpm for about 24 to 48 hours until the soft tissues breakdown completely. Then, the sample was filtered by using vacuum pump and Whatman GF/C (1.2 µm glass microfiber filters) as shown in Figure 4 (Alam et al., 2019).

2.4 Analysis technique

The details of the analysis method are explained in the next section.

2.4.1 Observe under stereomicroscope

A stereomicroscope HSZ 600 series as shown in Figure 5 was used in this study. It is used to identify and determine the characteristic of microplastic present by observe under the microscope. The characterization of microplastic involved identification the color and size (Zuo et al., 2020). This microscope can be used with turn on the up or bottom light which provided better light intensity of the image capture. The focusing knob be adjusted until clear image found. In this study, 12x to 15x optical magnification be used. The length of the microplastic present was measured by using i-Solution image analysis software.

2.4.2 Identification the functional group from ATR FT-IR absorption bands

A Perkin Elmer Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR FT-IR) used is shown in Figure 6 for wavelength 650 to 4000 cm-1. Initially, the ATR diamond was cleaned with acetone followed by background scan to remove contamination. Each of the selected sample was placed at the diamond surface and compressed with a force of 100 N to ensure good contact between sample and ATR diamond. The absorption bands obtained was compared with previous findings to identify types of polymer microplastic present. This technique is also known as a non-destructive method where the sample can be used again after analysis (Cai et al., 2019; Melissa et al., 2018; Waite et al., 2018).

2.5 Abundance of microplastic present in wild and cultured mussel

The data collected was performed statistical analysis by using SPSS software to determine the significant differences between microplastic abundance in the mussel. The sample is statistically significant if $p \leq 0.05$ (Zuo et al., 2020).

3.0 Results And Discussion

The water quality data for both sampling location is show in Table 1. The size of collected mussel was shown in Table 2. Estuarine is saline condition with mixing of fresh and saltwater. During summer, the salinity of estuarine increase as the freshwater flow reduces. In higher saline condition, the amount of oxygen can dissolve in water is decrease. Mussel is the common aquatic animal that live in estuarine ecosystem. In Malaysia, mussels can live in condition with temperature between 26°C to 32°C (Nishida et al., 2003).

From the data, sampling Point 2 have highest salinity and conductivity (29.19 ppt and 49701 µS/cm) compared to Point 1 as it is located far from freshwater body of Sungai Laloh. The suitable water salinity for mussel farming was reported between 27 ppt to 35 ppt. In addition, sampling Point 2 show highest amount of turbidity which was 3.40 NTU compared to Point 1. It’s also located nearest to industrial area in Pasir Gudang compared to Point 1 that is located at freshwater flow. From Table 2, the data obtained for mussel sample have difference size range. In this research, the cultured mussel was attached at the rope that tie with plastic container to make it float while wild mussel can be found attached to the bridge at nearest areas. The bigger size of mussel show that it has live more longer in the ecosystem, The growth of green mussel can be influenced by temperature, food source, salinity and competition for space. The high salinity condition and semi-enclosed bay was promote filtration rate of mussels (Tan, 2014).

Table 1
The water quality data measured from both sampling point.

Sampling Point 1: cultured mussel		**Sampling Point 2: wild mussel**	
Parameter	Result	Parameter	Result
DO	6.15 mg/L	DO	6.22 mg/L
pH	6.29	pH	6.97
Temperature	30°C	Temperature	30°C
Salinity	28.33 ppt	Salinity	29.19 ppt
Conductivity	48449 μs/cm	Conductivity	49701 μs/cm
Turbidity	2.35 NTU	Turbidity	3.40 NTU

Table 2
Size of collected mussels.

Sample no	**Cultured mussel**		**Wild mussel**	
	Width (cm)	**Length (cm)**	**Width (cm)**	**Length (cm)**
1	3.98±0.13	8.63±0.50	2.78±0.29	6.52±0.29
2	3.82±0.08	8.88±0.68	3.05±0.15	6.70±0.12
3	3.68±0.03	7.95±0.43	2.48±0.13	5.46±0.29
4	3.60±0.00	8.30±0.17	2.36±0.17	5.36±0.26
5	3.55±0.05	7.87±0.50	2.49±0.24	5.49±0.19
6	3.40±0.00	7.90±0.39	2.40±0.14	5.29±0.27
7	3.40±0.00	7.80±0.56	2.59±0.25	5.81±0.49
8	3.30±0.00	7.98±0.03	2.64±0.09	5.84±0.26
9	3.30±0.00	7.50±0.28	2.57±0.12	5.43±0.28
10	3.17±0.08	7.77±0.33	2.33±0.16	4.96±0.48
11	3.10±0.00	7.85±0.00	2.34±0.18	4.96±0.30
The data was recorded in mean±standard deviation.				

3.1 Trends of microplastic pollution at estuarine of Sungai Laloh

The microplastic observed was classified into threadlike and fragment as shows in the Figure 7. The trends of microplastic show similar between cultured and wild mussel. The most abundance in each mussel were from fragment microplastic. This might because of plastic debris have different density range where polypropylene (PP) and polyethylene (PE) have density <1.0 g/cm^3. Due to its low density cause it able to float in high water salinity. Some of the product from this polymer was plastic bottle which can break to fragment form. Mussels have more ability to feed fragment plastic that float on the surface of waters. Besides, fragment type was found abundant in wild fishes while threadlike was higher cage-cultured fishes at Setiu Wetland (Ibrahim et al., 2017)

However, the trends were vice versa for sediment sample. Thread like microplastic was most likely present in the sediment sample for both sampling location. In contrast, fibres made up from polyamides more denser in water with density of 1.38 g/cm^3 (Quinn et al., 2017). Polyamides is widely use in fabrication and textiles due its high durability and strength. The study of microplastic in sediment from Skudai and Tebrau river show 3 different shape of microplastic present. The shape includes fragment, threadlike and film (Shazani et al., 2018). The research on microplastic from urban estuary in Tasmania, Australia show that from sediment samples the fibres was detected most abundance total percentage of 87% (Willis et al., 2017). The detail image of microplastic observed under the microscope is shown in Figure 8.

3.2 Physical characterization of microplastic

The observed microplastic were characterize based on color and size. The details are explained in the following section.

3.2.1 Color

The microplastics are categorized to 7 color which are black, white, red, blue, yellow, green and other (Yusof et al.,2017). As show in Figure 9, the red color is the most abundance in mussel with percentages of 51.22% and 44.71%. The second color present abundance on microplastic for cultured and wild mussel sample was blue with percentage of 23.47% and 27.40% each. In comparison, green color shows lowest percentage in cultured mussel while white color was absent in wild mussel. In sediment sample, both locations show white color microplastic was most abundant followed by red with percentages of 28.57%; 33.33% and 17.14%; 25% respectively. Yellow color has the lowest percentage of 2.86% for cultured location while black and yellow was absent in wild location.

Black colour microplastic was found in abundant cultured and wild *L. calcarifer* of Setiu Wetlands (Yusof et al.,2017). Coloured microplastic was reported the most found in three urban estuaries in China (Minjiang, Jiaojiang and Oujiang) followed by black or transparent colour. White colour plastic has recorded the lowest fraction. Colour plastics was cheap compared to colourless or clear plastics. Usually, colour plastics is added into the bulk of resins for be moulded and form new materials. Some of predator can mistake ingest food which actually microplastic due to its colour. Besides, coloured plastics was detected in mammals and reptiles include birds, fishs and turtles (Zhao, Zhu and Li, 2015).

3.2.2 Size

Microplastics have the size of less than 5 mm. Table 3 show the details size of microplastic found. From the data, the mussel and sediment not only size in micro range but also bigger than that (>5 mm). The biggest size of threadlike and fragment plastic was found in cultured mussel with length 11.05 mm and 11.71 mm respectively. The shortest length was identified around 0.1 mm for threadlike) and 0.02 mm for fragment in wild mussels. However, most of the sample present of microplastic size. In North Sea, the size plastic between 0.2 to 4.8 mm found in fish with percentage 2.6% from 1,203 fish (Zhao et al., 2015). The abundant of large microplastic was identified in beaches at Bu-An, Korea with amount 12,180 particles/m^2 (Lee et al., 2015).

Table 3
Size of microplastics in the samples.

	Size range (mm)			
	Sampling Point 1		Sampling Point 2	
	Cultured mussel	Sediment	Wild mussel	Sediment
Threadlike:				
Black	0.32 – 6.47	0.68 – 2.05	0.10 – 10.00	–
White	1.22 – 10.09	0.69 – 3.22	–	1.03 – 10.04
Red	0.77 – 7.05	0.76 – 1.71	1.55	–
Blue	0.20 – 7.90	0.68	0.32 – 2.66	2.33
Yellow	2.21 – 10.48	–	–	–
Green	1.50 – 11.05	1.07	1.38	2.56 – 5.59
Other	0.32 – 2.64	–	0.46	0.75
Fragment:				
Red	0.02 – 2.65	0.05 – 0.39	0.02 – 2.14	0.08 – 0.34
Blue	0.03 – 3.81	0.10 – 0.19	0.11 – 0.44	–
Clear/ film	1.54 – 11.71	2.48 – 3.61	–	0.62 – 1.47

3.3 Polymer microplastic present in estuarine ecosystem

Five (5) selected microplastics from different physical characteristic were selected for performed ATR FT-IR as show in Figure 10. The result of absorption bands was compared with previous findings as shown in Table 4 to determine the type of polymer present. The polymer found were polypropylene (PP), polyethylene (PP), polyethylene terephthalate (PET), ethylene vinyl acetate (EVA) and nylon (all polyamides). PP, PE, polyvinylchloride (PVC) and polytetrafluoroethylene (PTFE) was found in urban estuaries at China (Zhao et al., 2015).

3.4 The most polluted mussel

Comparison of abundance microplastic percentage in mussel was made to identify the most polluted sample as show in the Figure 11. The cultured mussels were collected on 26th October 2020 while the wild mussels were collected on 15th November 2020. In comparison, culture has a highest percentage of microplastic present with amount 70% compared to wild mussel with only 30%. Therefore, the cultured mussel that located at estuarine of Sungai Laloh was the most polluted. This might affect by waters flow from freshwater region and the use materials for cultured purpose.

Besides, previous findings on 2016 have shown that this area was also contaminated with heavy metal. Based on the accumulation of heavy metal in sediment that located within this cultured area, the concentration of copper (Cu) present was 146.5 μg/g and the highest compared to other point. In addition, zinc (Zn) concentration present was almost similar to the point that has highest concentration with amount 520.2 μg/g (Farah et al., 2016). In addition, *P. viridis* from Kampung Pasir Putih has been found accumulate with heavy metal. The analysis on mussels was conducted to identify the concentration of 4 heavy metals which are lead (Pb), cadmium (Cd), copper (Cu) and mercury (Hg). Result show that Pb and Cd have exceeded the maximum allowable limit of Food Act 1983 Regulations 2013 with concentration 25.10-38.60 μg/g and 9.10-13.00 μg/g (Mahat et al., 2018).

3.5 The correlation of microplastic in mussel at estuarine of Sungai Laloh

The statistical analysis was used to identify linear regression of the sample from both sampling point. Parametric analysis (paired T-test) was used to identify any difference between the mussel sample. Based on the evaluation, data obtained from sampling Point 1 for cultured mussel was normally distributed with significant value to $p > 0.05$. In addition, data obtained from sampling Point 2 show an almost similar trend and normally distributed ($p > 0.05$). However, based on the T-test the cultured and wild mussel have slightly no significant different ($p > 0.05$).

4.0 Conclusion

In this study, microplastics were found present in mussel and sediment sample at the estuarine ecosystem of Sungai Laloh, Pasir Putih. It can be concluded that

1) Trends of microplastic in this ecosystem show that fragment microplastic was most abundant in mussel while threadlike microplastic was most abundant in sediment with percentage of >50% each.

2) Red color microplastic presented higher in mussel while for sediment the white color is the most abundant. Most size of plastics particle or marine debris in the sample was in microplastic.
3) Microplastic that cause pollution to this ecosystem were from polymer group of polypropylenes (PP), polyethylene (PE), Polyethylene terephthalate (PET), ethylene vinyl acetate (EVA) and nylon.
4) In comparison, cultured mussel was more polluted with 70% of microplastic present while wild mussel was only 30%.
5) Microplastic in cultured and wild mussels have no significant difference ($p>0.05$).

Recommendation

The study only provided data on microplastics pollution at one Point location for each mussel causing limited data presented. Due to time constraint during movement control order (MCO), the scope of present study may cause underestimation of the extent microplastic contamination at the estuarine ecosystem of Sungai Laloh, Pasir Putih. The following recommendations can be considered for future studies:

1) Select the same sizes of cultured and wild mussel to improve data accuracy and reduce bias. Besides, dry mussels can be used.

2) Conduct a long period for sampling to ensure actual data presentation on the extend microplastic pollution.

3) Select more sampling stations to improve data accuracy.

Declarations

Statements and Declarations

The authors declare that no funds, grants or other supportive were received during the preparation of this paper.

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Tables

Tables 3 & 4 are available in the Supplementary Files section.

Figures

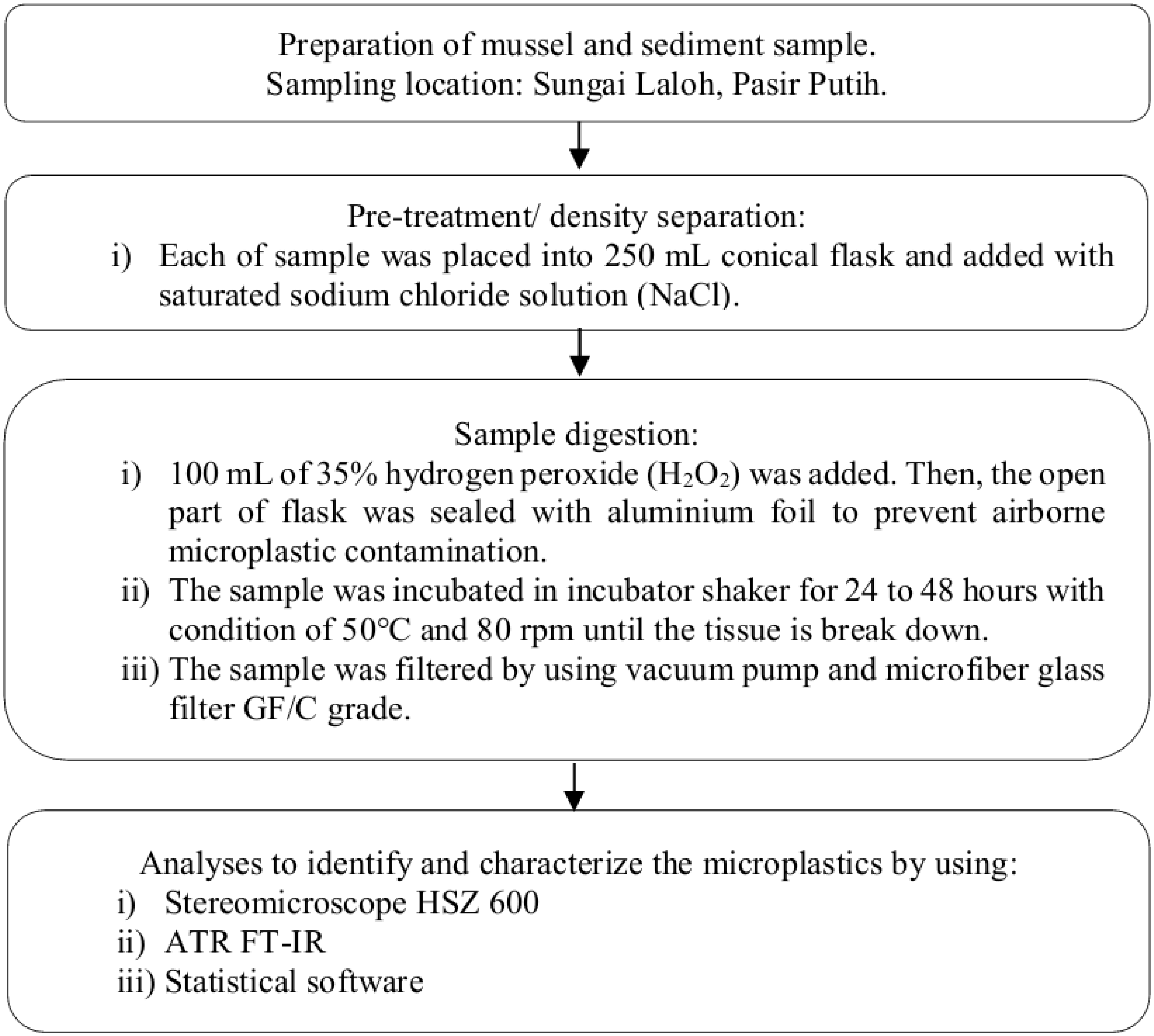


Figure 1

Summary process of research methodology.

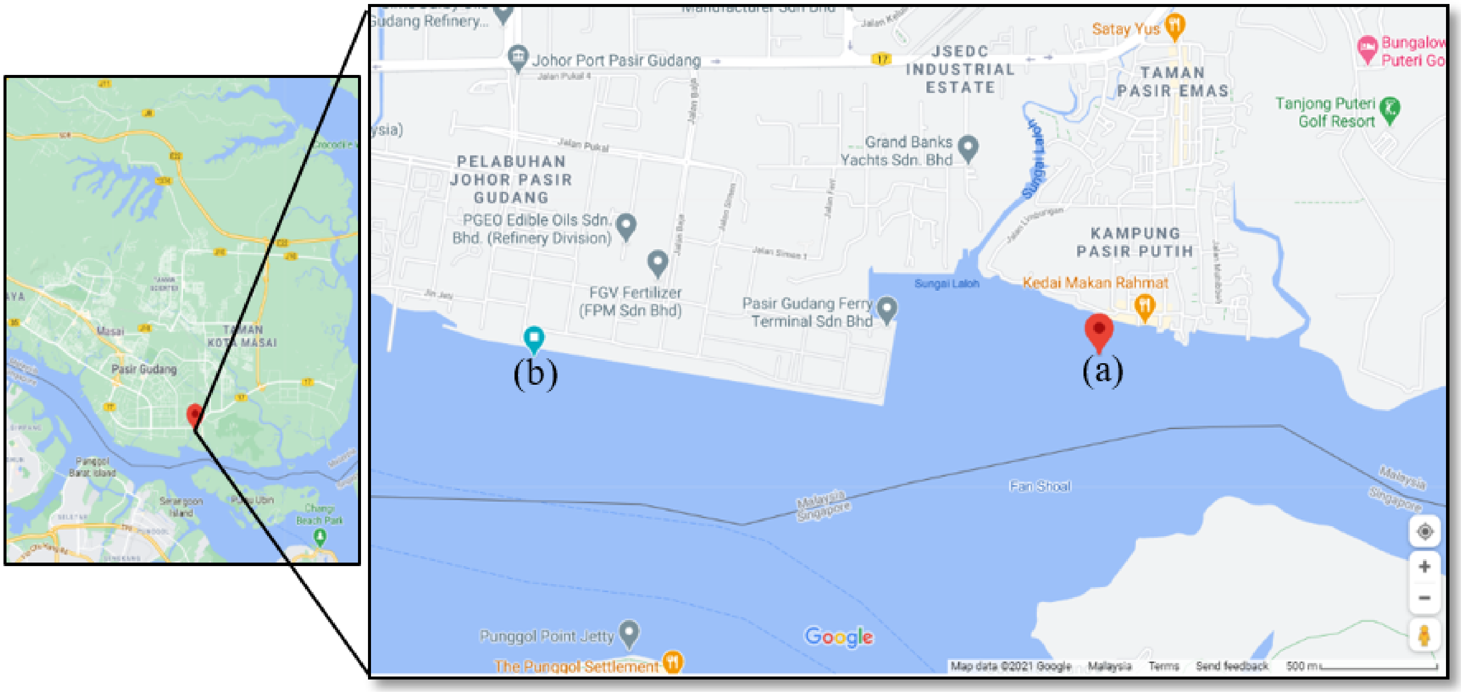
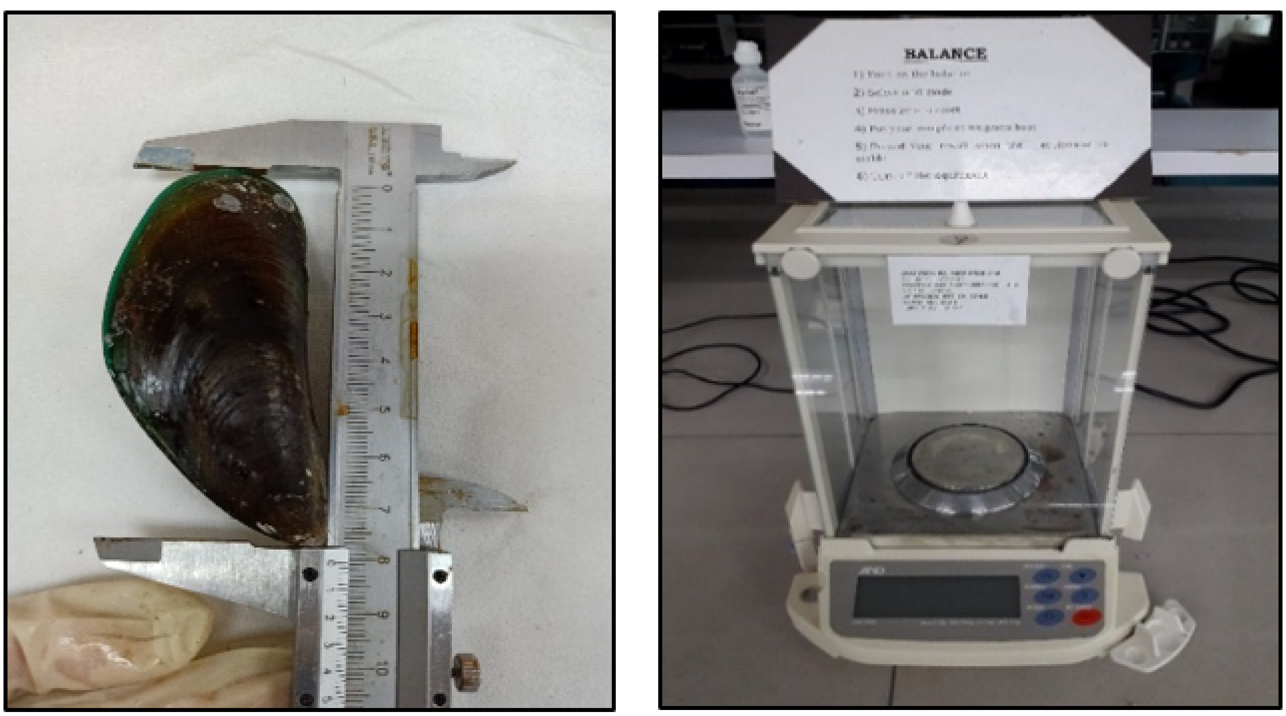


Figure 2

The location of sampling point at estuarine ecosystem near to Sungai Laloh, (a) Point 1 (cultured mussel) and (b) Point 2 (wild mussel).



(a) (b)

Figure 3

(a) Vernier calipers to measure the size of mussel and (b) analytical balance to measure the weight of mussel.

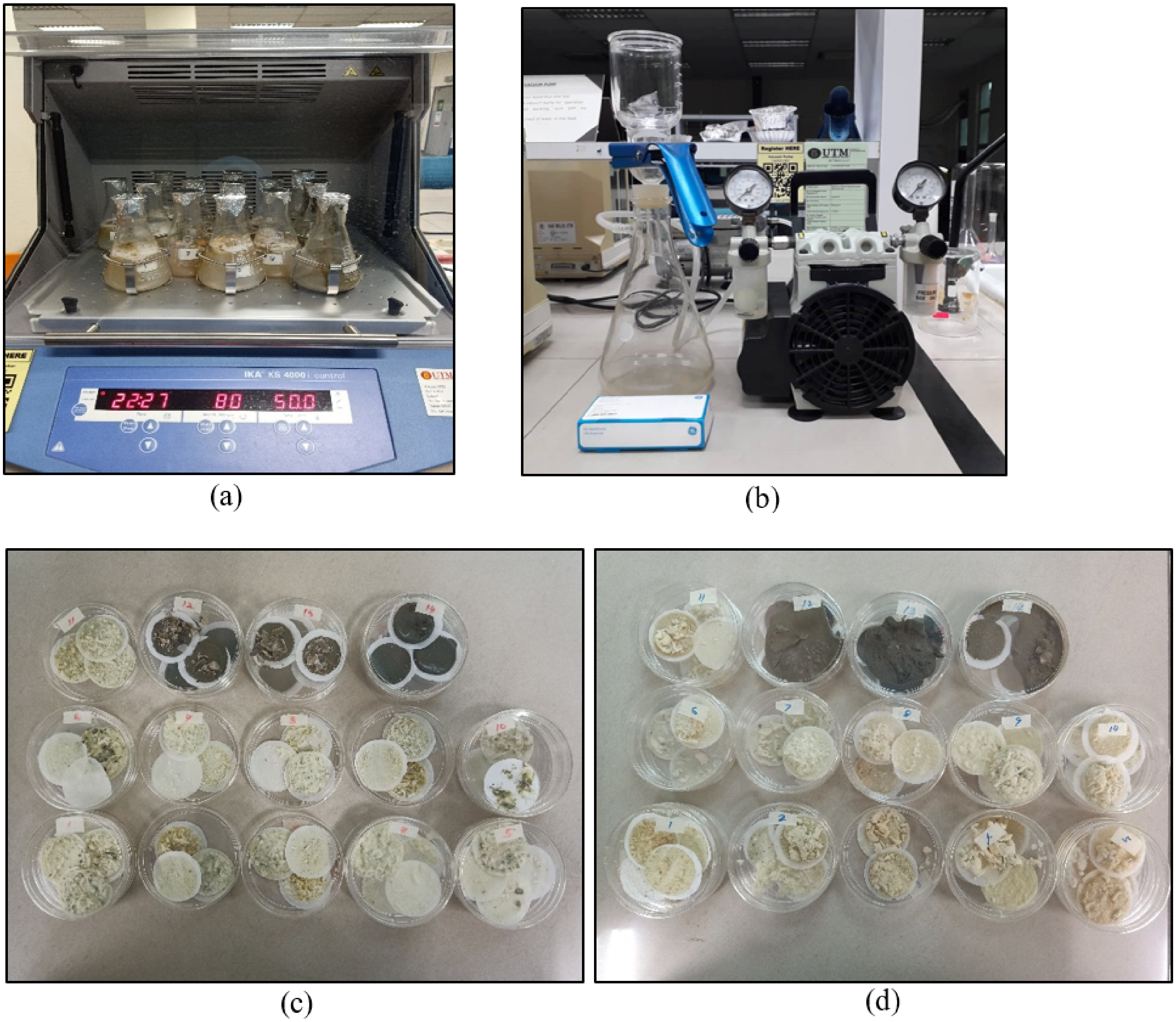


Figure 4

(a) Incubator shaker used for sample digestion, (b) materials and apparatus used in the filtration process, (c) all replicate sample of wild mussels and sediment placed in petri dish after be filtered and (d) cultured mussels and sediment sample after be filtered.

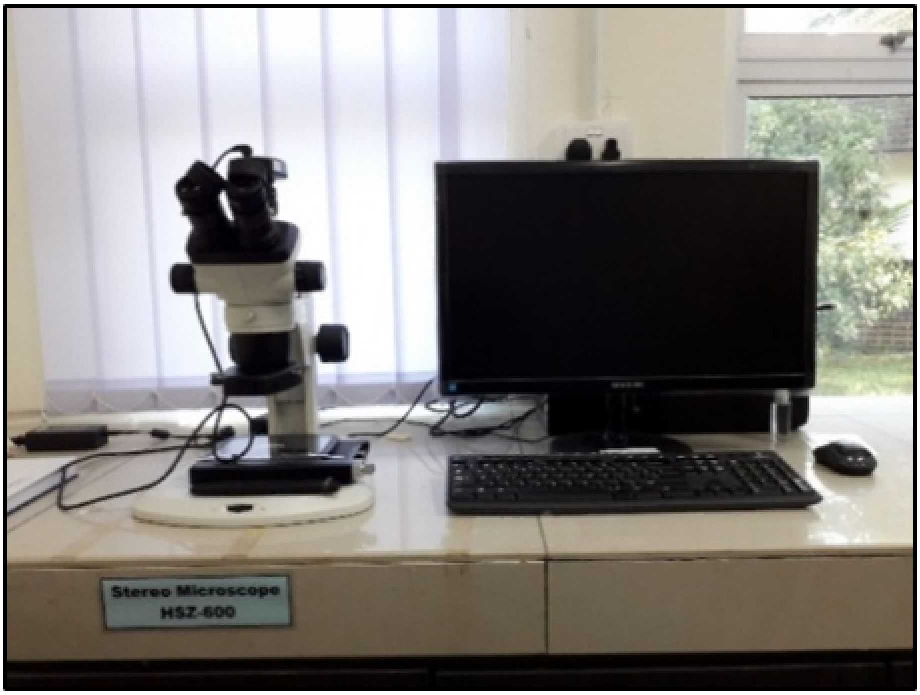


Figure 5

Stereomicroscope HSZ 600 Series.

Figure 6

Perkin Elmer Frontier ATR FT-IR.

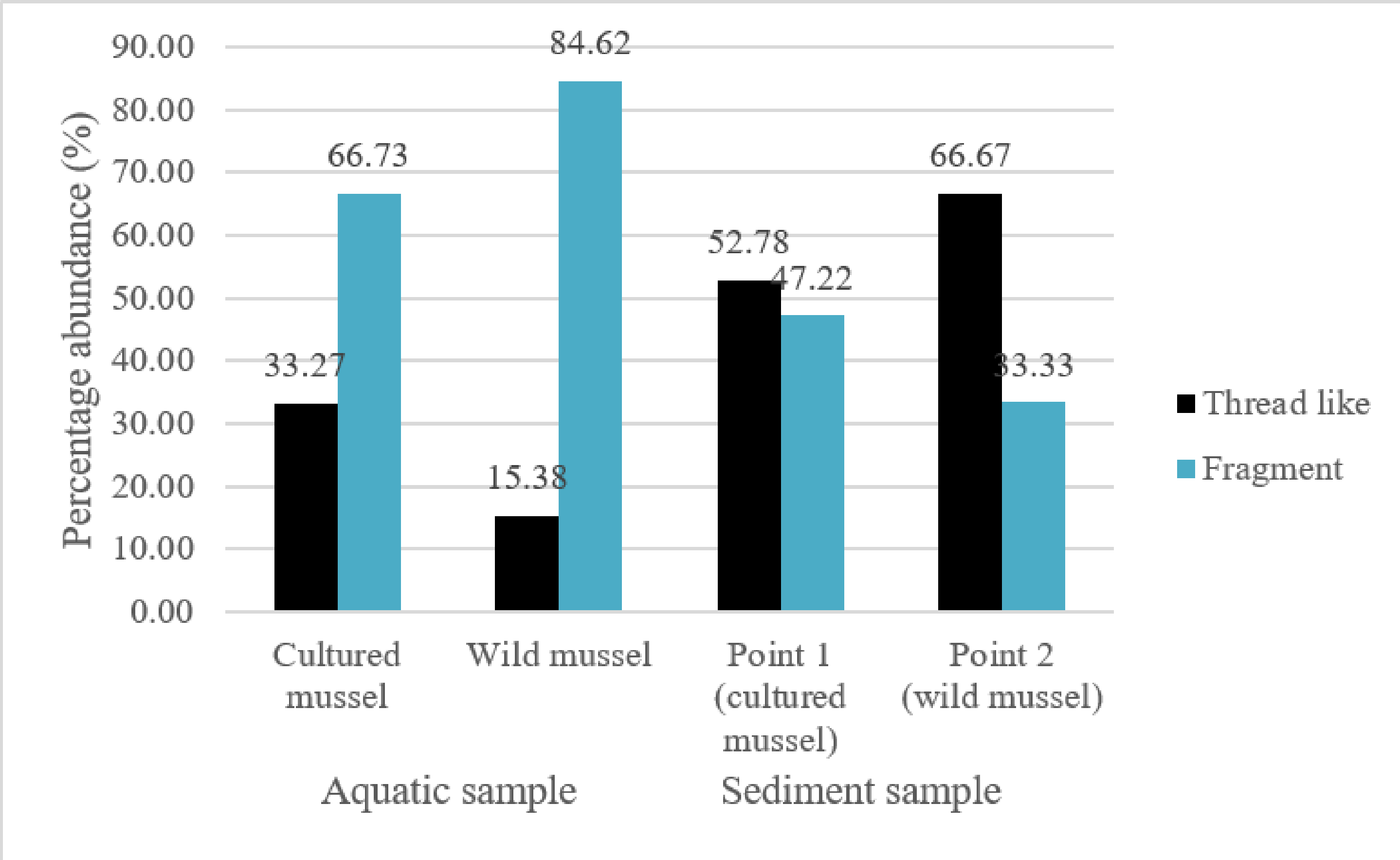


Figure 7

Trends of microplastic found in mussels and sediments.

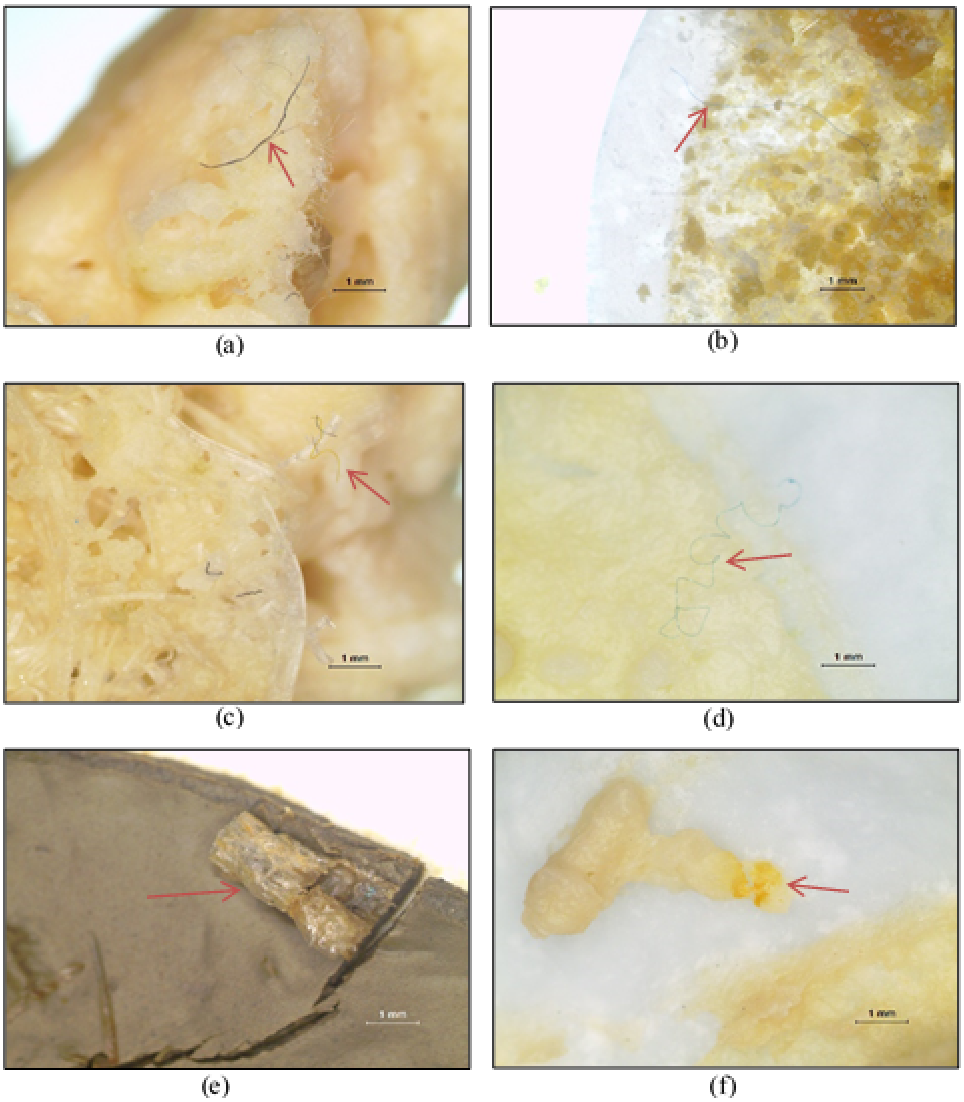


Figure 8

Red arrow show the threadlike microplastic(a) black (b) blue (c) yellow (d) green and fragment microplastic (e) clear/ film (f) yellow.

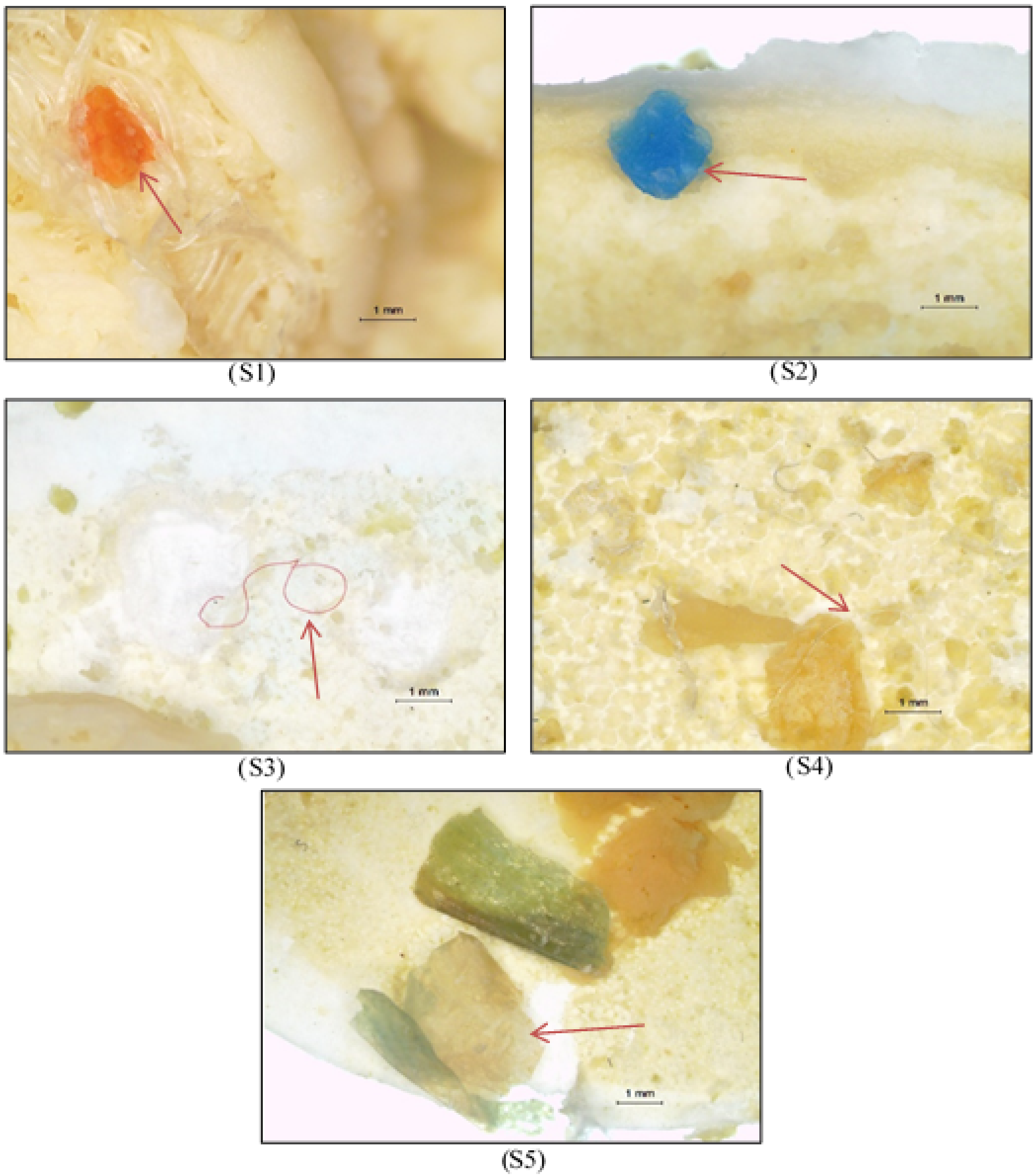


Figure 9

Characterization of microplastic based on color.

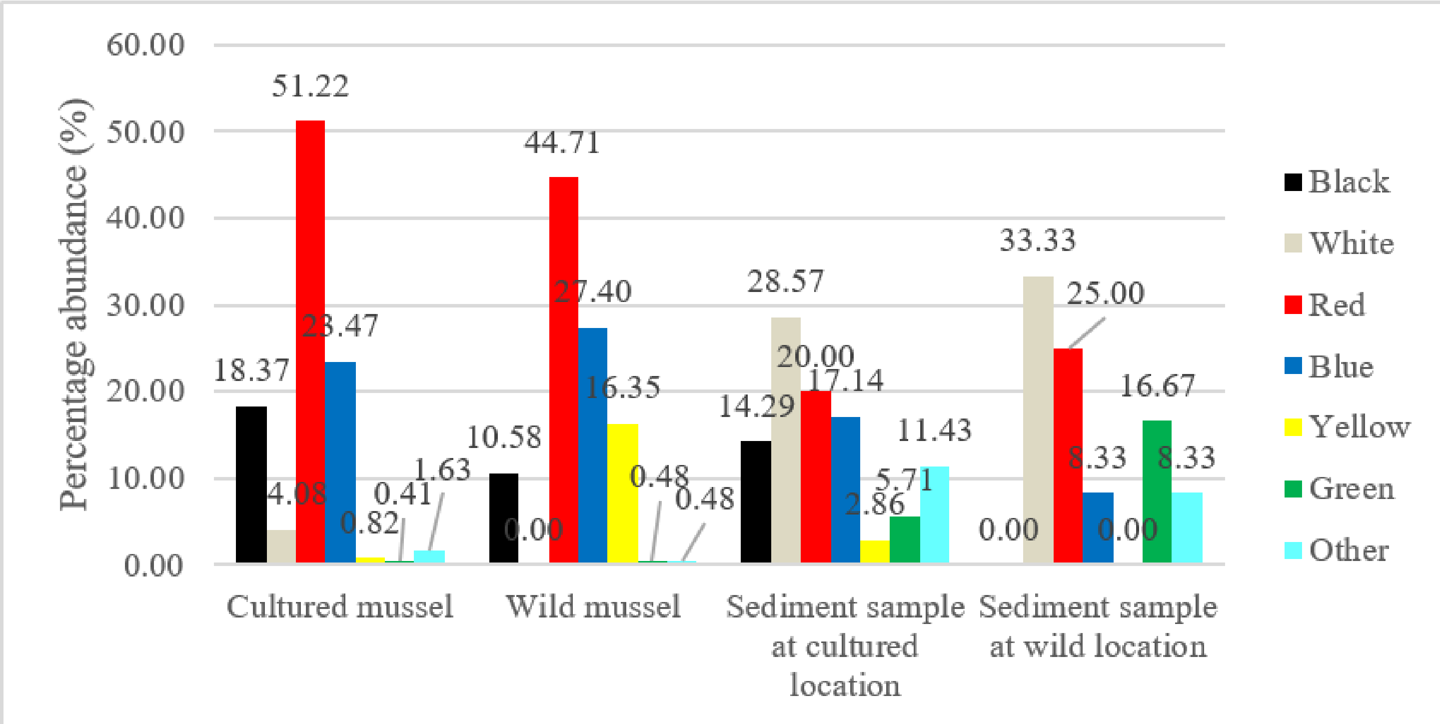


Figure 10

Red arrow show the selected microplastic for obtained ATR FT-IR result (S1) red fragment, (S2) blue fragment, (S3) red thread, (S4) clear threadlike and (S5) film/ clear fragment.

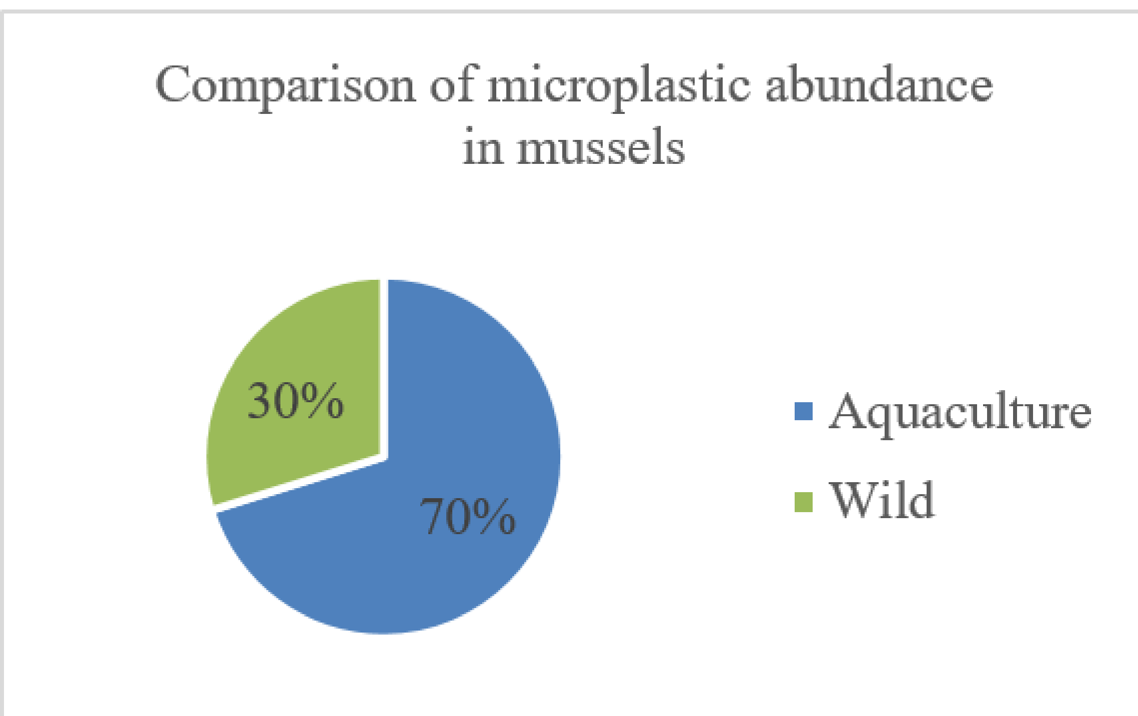


Figure 11

Percentage abundance of microplastic in mussel sample.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table45.docx