# Evaluation of Microplastics Ingested by Sea Cucumber *Stichopus Horrens* in Pulau Pangkor, Perak, Malaysia

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## Abstract

The widespread presence of microplastics has caused significant concerns on their potential effects on marine ecosystems. Microplastics are classified as plastic products of less than 5 mm in size and are known to be one of the most dangerous aquatic debris to marine species. Sea cucumbers are deposit-feeders living in sediment regions that may collect microplastics in low-energy environments. This research aims to evaluate the types of microplastic isolated from the intestine of *Stichopus horrens* in Malaysia. Sea cucumber samples collected from Pulau Pangkor, Malaysia were dissected, and their intestines were collected for digestion. Microplastics were extracted using NaOH and filtered using filter paper. Microplastic identification was conducted on the based on the physical characteristics (colour, shape, size) and chemical characterisation was evaluated using FTIR for polymer functional groups. A total of 1446 microplastics were found in *Stichopus horrens*. Among various types and colors, fibre (90%) and black (59%) were dominant amongst the various particles identified. The majority of microplastics sizes were 0.51 μm and 1–2 μm. Two polymer materials were identified, namely polyethylene and poly(methyl methacrylate). As a conclusion, the findings of the study will serve as primary data for pollution indicators in respective islands.


## Introduction

Marine environments are constantly under continuous exploitation of human activities. Contaminants such as pesticides, persistent organic pollutant (POP), hydrocarbons, heavy metals, plastics and microplastics can affect marine area. About 80 to 85% of marine litter comes from plastic (Gallo et al., 2018) and about 4.4 and 12.7 million tons of plastic were estimated to enter the aquatic ecosystem annually (Jungblut, 2020). Lightweight and corrosion-resistant plastics have become universal materials due to their strength. Global production of plastics was close to 360 million tonnes and China accounted for 30% of global production in 2018 (Plastics Europe, 2019). A numerous studies on the distribution and abundance of microplastics in Asia have been carried out. In particular, fragments, lines, films, foam, pellets, and microbeads cover a broad variety of microplastics (Khalik et al., 2018). In Malaysia, environmental data related to microplastic abundance has not been properly discussed. The plastic's abundance in the oceans is projected to increase between 100–250 million tonnes by 2025 without intervention (Hale et al., 2020). A significant number of plastic waste can stay in the water for a prolonged period of time, due to human activities, increasing quantities will continue to reach the oceans.

Microplastic pollution has been a worldwide issue in water and sediment (Cole et al., 2011; Van Cauwenberghe et al., 2015). Microplastics are characterised as particles smaller than 5 mm. it consists of primary microplastic (pellets or granules of microstructure and size) and secondary microplastic (macroplastic decomposition) sources (Law and Thompson, 2014). The common polymers in microplastics found in the environment are polypropylene (PP), polycarbonate (PC), polyethylene (PE), polyvinyl alcohol (PVA), polyvinyl chloride (PVC), polyamide (PA) and polypropylene (PP) (Ma et al., 2020). The effect of microplastic on marine biota is a concern because it contributes to the embroidery and ingestion that can be harmful to marine organisms. Microplastic sections are mostly from aquatic sources and particularly affected in the coastal environment of coral reefs by microplastic degradation (Auta et al., 2017).

In many different ecosystems, sea cucumbers are found, and are either suspension feeders or deposit feeders. Sea cucumbers are commonly found in low-energy habitats in organic-rich sediment regions that may absorb microplastics. Based on their feeding mode, sea cucumbers could be at risk of ingesting microplastics (Plee & Pomory, 2020). In the ecosystem, sea cucumbers are significant species that help fertilise the substratum by stirring the bottom of the lake. Microplastics are known to be eaten by sea cucumbers since these species do not select food (Sayogo et al., 2020). For instance, this study reported that the microplastics found in sea cucumber (*Holothuria sp.*) on Tidung Island and Bira Besar Island consisted of four types which were fibre, fragment, film and pellets. *Stichopus horrens* Selenka (SH), a type of sea cucumber from the Indo-Pacific, is a marine invertebrate of the phylum of Echinodermata that has been used in Asian traditional medicine since ancient times (Bakar et al., 2020). Since sea cucumber has been used as traditional medicine, the microplastics found in the intestine of the sea cucumber has the possibility to affect the quality of the medicine produced.

Currently, there are limited studies available to determine the presence of microplastic among marine filter feeders in Malaysia. Thus, this study was conducted to evaluate the occurrence of microplastic ingested by sea cucumber in Malaysia. This research is aligned with the goal of sustainable development of United Nations Development Programme (UNDP). Microplastics can accumulate toxins and harmful pollutants and release them. Plastic ingredients or toxic chemicals absorbed by plastics can build up over time and remain in the environment. These plastics will eventually reduce environmental sustainability and affect the ecosystem. Microplastics may be used to cause physical harm by blocking or destroying the intestines (Critchell & Hoogenboom, 2018). In several animals, some ingested microplastics decrease feeding ability, growth rate, reproduction and damage the immune system (Wright et al., 2013; Besseling et al., 2014; Avio et al., 2015; Sussarellu et al., 2016).

## Material & Methods

# Sample collection

Twenty sea cucumber *Stichopus horrens* samples were randomly collected from Pulau Pangkor, Malaysia. All samples were transported to the laboratory in airtight glass containers. The samples were kept in a -40°C freezer until further analysis.

# Sample Preparation

Samples of *Stichopus horrens* were thawed at 25–27°C and washed using distilled water. The measurement of weight (g) and length (cm) were recorded before the removal of gastrointestinal tract (GIT) of *Stichopus horrens*. All samples were dissected separately and the gastrointestinal tract was separated from the top of the oesophagus to the anus, which was then digested and placed individually in a 250 mL glass beaker containing 95% ethanol solution before further identification. The digested GITs were weighed individually prior to tissue digestion (Hossain et al, 2019).

# Isolation of microplastic from gastrointestinal tracts (GIT)

The digestion of GIT was carried out according to the Karami et al. (2017) method. A total of 200 ml of sodium hydroxide (NaOH) was applied to each 250 ml glass beaker containing 4 to 5 g GIT and were incubated for 72 h at 40°C. The digestate was then vacuum filtered through filter paper (pore size 11 µm). The filter paper was soaked in 10 to 15 mL of 4.4 M sodium iodide (NaI) (density: 1.5 g/mL), sonicated at 50 Hz for 5 min and agitated on an orbital shaker for 5 min at 200 rpm to isolate the high-density particles. The solution was centrifuged at 500 ×g for 2 min. The microplastics containing microplastics was vacuum filtered through filter membrane (pore size 1.2 µm). This method was repeated once more to ensure full isolation of microplastics (Karami et al, 2017).

# Microscopic identification and particles number

The microplastics were sorted and photographed by using a dissecting microscope (Olympus SZX7, Olympus Corp. Tokyo, Japan) and comparison microscope (Leica FS CB). Both microscopes were used to observe the maximum length, colour, and shape (threadlike, fragment or film). ImageJ software was used to measure the length of each microplastic. Microplastics were defined as particles which displayed the characteristic characteristics of synthetic polymers, i.e. mouldable objects with consistent thickness and colour that did not break when pressed with stainless forceps. A hot metal tip was applied on the object for confirmation of the presence of microplastics. The identified microplastics were placed on petri dish that was covered with filter paper (Ibrahim et al., 2017). Total number of microplastics that were found in a tissue sample was expressed as the number of particles per unit mass of the tissue sample with particles/g dry weight unit.

# Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared spectroscopy (FTIR) (NICOLET iS50 FT-IR, Thermo Fisher Scientific, USA) was used to analyse the functional groups correlated with polymer chemical properties. Prior to analysis, the environment spectrum was recorded as blank using attenuated total reflection (ATR) in the mid-IR range of 4000–650 $cm^{-1}$ and the microplastic particles were analysed (Karami et al, 2017). To gain the actual spectrum of microplastic particles, the blank spectrum was deducted. The sample was examined using the FTIR spectrum of previous studies (Käppler et al.,2016; Jung et al.,2018) and the INHART Laboratory, IIUM library search (OMNIC software).

# Quality assurance and quality control

Glassware, metal instruments and medical gloves were used in throughout the experiment to treat the samples, and the dissecting tools were rinsed with sterile water three times before application. During the trial, the participants wore cotton laboratory coats. All liquid reagents (Sodium hydroxide (NaOH) solution) were supplied with distilled water.

# Results And Discussion

### Characteristics of microplastics in Stichopus horrens

A total of 20 samples of sea cucumber *Stichopus horrens* were contaminated with 1446 microplastics. An approximate 4–5 g *Stichopus horrens* intestine contained 60–70 microplastics. With length range between 0.2 to 7.0 μm and average length of 0.5 to 2 μm, the microplastic length included four-length sizes; 0.5–1 μm (34.79%), 1–2 μm (27.73%), 2–5 μm (13.97%) and < 0.5 μm (23.51%).

Figure 1 exhibits the typical colours and shapes of microplastics found in *Stichopus horrens* intestine while Fig. 2 depicts percentage of microplastic colours. The rank of microplastic percentage according to their colour was as followed: black/grey (59.13%) > blue (27.04%) > red (9.9%) > green (0.96%) > transparent/ white (0.9%) > pink/ purple (0.83%) > brown (0.76%) > yellow/ orange (0.48%). Results were presented in Fig. 4. In terms of shape, the majority of the microplastics were filaments (90.87%), followed by fragments (8.23%) and film (0.9%) (Fig. 3).

The ingestion of microplastics by *Stichopus horrens* was first reported on the island of Malaysia, in particular, and in the Asian tropics, in general. The results of this study could be predicted from visual analysis, since the colour shown implies that different polymer types may be present. There are several types of polymers in *Stichopus horrens*, based on Fig. 1, by looking at the different colours of microplastic particles according to the classification of previous studies (Ibrahim et al., 2016).

Figure 2 shows the colour distribution of the microplastic particles isolated from the gastrointestinal tract of *Stichopus horrens*. The most abundant microplastic particles found in this sample were black particles, accompanied by blue and red particles. Figure 1 displays the picture of microplastics captured under a comparison microscope. The findings showed that black and blue fragments were the majority of plastic products in *Stichopus horrens*, accounting for 59.13% and 27.04%, respectively (Fig. 2). Black and blue particles were found in all samples suggesting fishing activities that may lead to the high concentration of transparent microplastics in marine ecosystems (Stolte et al., 2015).

Microplastics collected from the gastrointestinal tract (GIT) of *Stichopus horrens* were classified into three shapes; fibre, fragment and film. The distribution of these three types of microplastics in all the samples were shown in Fig. 3. Fibre was the majority shape found in this species. The dominant type of plastic debris contained in this sample, accompanied by fibre, fragments and film. In the digestive organs of sea cucumbers, the type of microplastic that is easily transported by waves and reaches the digestive tract accumulates, resulting in digestive tract blockages from the absorption of plastic utensils known as fibre (Taylor et al., 2016; Mohsen et al., 2020; Sayogo et al., 2019; Iwalaye et al., 2020). Besides, the abundance of fibre was possibly attributed to the common usage of fishing equipment, as plastic fibres were important raw materials for fishing nets and lines (Zhao et al., 2018). Fibre type comes from monofilament fragmentation (single fibre) from fishing nets, ropes, synthesis cloth or clothing fibres. In addition, fragments were also found in gastrointestinal tract (GIT) of *Stichopus horrens*. Fragments come from plastic objects that can be degraded for a long time such as bottles, buckets, or other objects made of PVC. The source of the fragments could be the decomposition of large plastics, such as plastic utensils, cleaning items and packaging materials (Kyrikou et al., 2011). A type of microplastic that originates from the manufacturing of plastic products and plastic waste is film (Sayogo et al., 2019).

Another common measurement parameter for microplastics is size, although there is currently no uniform standard for measuring the size of microplastics. It was not unexpected that the number of microplastics, less than 1 mm, was high, because they were similar to previous research (Zhu et al., 2019; Zhang et al., 2020). Microplastics with smaller sizes (0.5 to 2 mm) have been found more often than other sizes, as seen in Fig. 4. Many previous studies have also reported a similar finding with smaller particles in a dominant position (Van Cauwenberghe et al., 2013; Mohamed Nor & Obbard, 2014; Van Cauwenberghe et al., 2015). Thompson (2015) reported that the small size of microplastic was probably due to the degradation of large microplastics. Small particles with large specific surface areas may be less resilient than large particles. Moreover, the accumulation rate often increases with the continuous degradation of plastic particles. As Zhang et al. (2020) proposed, the distribution of microplastics in size was directly linked to their rate of formation and degradation. The changes in this rate, are currently not clear and require further analysis.

# FTIR Analysis

FTIR spectrum of possible microplastic presence in *Stichopus horrens* is shown in Fig. 5 and Fig. 6. Spectral characteristics of polyethylene (PE) with chemical formula of $(C_2H_4)_n$ were peaks with wavenumbers 2916.14 $cm^{-1}$ – 2917.14 $cm^{-1}$, 1457.13 $cm^{-1}$ – 1471.78 $cm^{-1}$, 717.29 $cm^{-1}$ – 718.14 $cm^{-1}$ as shown in Fig. 5. Spectral characteristics of polymethyl methacrylate (PMMA) with chemical formula of $(C_5O_2H_8)$n were peaks with wavenumbers 2959.11 $cm^{-1}$, 1730.85 $cm^{-1}$, 1453.33 $cm^{-1}$, 1395.27 $cm^{-1}$, 1230.35 $cm^{-1,}$ and 1152.54 $cm^{-1}$ as shown in Fig. 6.

The FTIR spectra associated with microplastics are shown in Fig. 5 and Fig. 6. The collected microplastic particles identified by FTIR spectroscopy analysis proposed that the microplastics found in gastrointestinal tract *Stichopus horrens* were mostly polyethylene (PE) (Fig. 5) and poly(methyl methacrylate) (PMMA) (Fig. 6). During this study, polyethylene FTIR spectra carrying all major peaks linked to the alkyl functional group were observed as seen in Fig. 5. Polyethylene has a similar finding by Jung et al. (2018) which plastic pieces ingested by Pacific sea turtles accounted for 96% were identified by ATR FT-IR as low density polyethylene (LDPE) and high density polyethylene (HDPE). Besides, the microplastic found in wild *Scapharca cornea* bivalves were polyethylene (PE) (Ibrahim et al., 2016). In Fig. 5, at peaks of 2916.14 $cm^{-1}$ to 917.14 $cm^{-1}$ the strong absorption band of C-$H_2$ stretching can be observed. At peak of 1457.13 $cm^{-1}$ to 1471.78 $cm^{-1}$ the C-$H_2$ bending deformation was shown. In the occurrence of a very strong peak at 717.29 $cm^{-1}$ to 718.14 $cm^{-1}$, the rocking deformation of the ethylene linkage was shown. The peaks around 1734 $cm^{-1}$ on a polyethylene sample are indicative of oxidised content and peak at 1715 $cm^{-1}$ associated with the formation of a ketone group (Khalik et al., 2018). It was thought to have arisen due to solar radiation, biological processes or thermal oxidation. Polyethylene is abrasion prone and is commonly used in fishing products. As one of the generic incomes for the local population in Pulau Pangkor is fishery activities, this material is not surprisingly found. The most prevalent isolated plastic polymer was polyethylene in the marine environment. This could probably be due to high polyethylene demand and manufacturing that lead to the disposal of this plastic polymer in aquatic ecosystems (PlasticsEurope, 2017). Polyethylene is one of the most commonly used engineering plastics. The uses of polyethylene range from plastic bags, pipes and bottles (Kasirajan & Ngouajio, 2012). For CH and $CH_2$ groups, polyethylene has distinct absorption bands. For positive identification, peaks with wave numbers 2850 to 2960 $cm^{-1}$, 1450 to 1470 $cm^{-1}$ and 720 to 730 $cm^1$ must be present (Ng & Obbard, 2006).

During this study, FTIR spectra of poly(methyl methacrylate) (PMMA) carrying all major peaks linked to the carboxylic ester functional group and similar to the PMMA spectra reported by Jung et al.(2018) were observed in their research. In Fig. 6, the strong C-H stretching absorption bands were observed at peak of 2959.11 $cm^{-1}$ showing intense absorption bands at peak 1730.85 $cm^{-1}$, associated with carbonyl group stretching (C = O), belonging to the PMMA polymer. The C-$H_2$ bending deformation was shown at peak 1453.53 $cm^{-1}$. Other absorption bands were observed at peak 1395.27 $cm^{-1}$ (C-$H_3$ bending vibration), 1230.35 $cm^{-1}$ and 1152.54 $cm^{-1}$ (C-O stretching, respectively). In products such as illuminated lights, shatterproof windows, aircraft canopies, the automotive industry (car windows, light fixtures and rear lamps), contact lenses, dental restoration, road lines and acrylic panels, PMMA is a translucent and rigid material that is often used as a replacement for glass (bathtubs) (Ali et al., 2015). There were no previous study on PMMA in any marine organisms.

# Conclusion

As a conclusion, microplastics were isolated and identified from the intestine of sea cucumber *Stichopus horrens*. The main polymer composition found in the gastrointestinal tract sea cucumber studies are polyethylene and poly(methyl methacrylate). The result reported in this study is the first evidence of sea cucumber contaminated by microplastics in Malaysia. The findings of the study will serve as primary data for pollution indicators in respective islands. They can also be the basis for authority and lawmaker in promoting sustainable ecosystems. Besides, the higher number of microplastics are leading to concern as they consist of potential impact on environmental health. It can also be as study of toxicity of microplastics towards the marine animals and human.

# Declarations

### Ethics approval and consent to participate:

This study does not require ethical approval.

**Consent for publication:**

All the authors give their consent for the publication to the journal.

**Availability of data and materials:**

The data and materials are available via the corresponding author.

**Competing interests:**

The authors declare that all authors and this study have no competing interest.

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**Authors' contributions:**

Miftahul Jannah and Nurzafirah Mazlan contributed to study conception and design. Materials preparation were performed by Nurzafirah Mazlan. Data collection were performed by Nurzafirah Mazlan, Miftahul Jannah, Jemimah Shalom, Muhamad Shirwan and Safaa Najah Saud. Data analysis were performed by Miftahul Jannah and Nurzafirah Mazlan. The first draft of the manuscript was written by Miftahul Jannah. Nurzafirah Mazlan and Muhamad Shirwan commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Figures

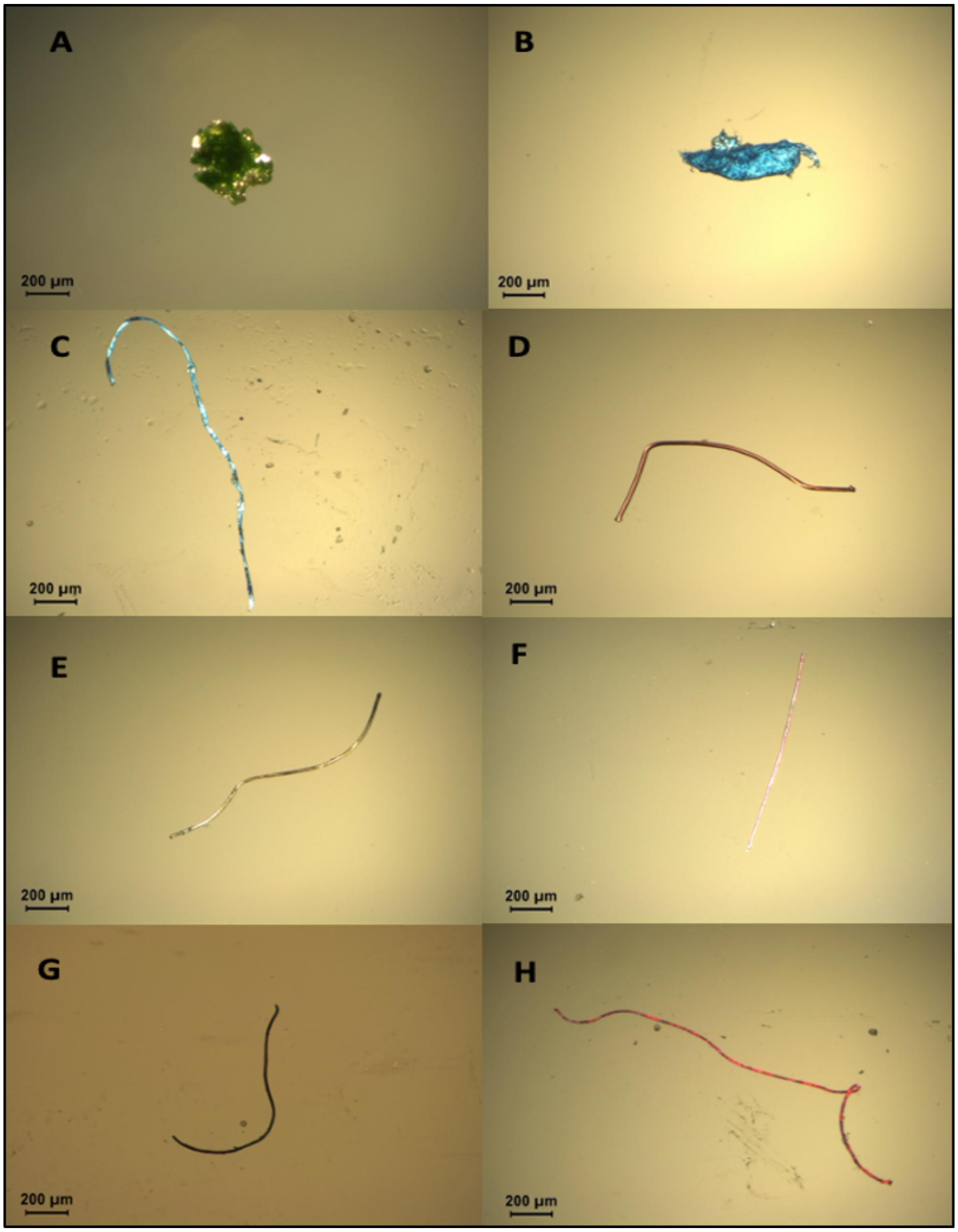



**Figure 1**

The colours and shapes of microplastic isolated from the gastrointestinal tract (GIT) of sea cucumber, Stichopus horrens a) green fragment b) blue fragment c) blue filament, d) brown filament, e) white filament, f) pink filament, g) black filament, and h) red filament. [Scale bars: 200 µm]

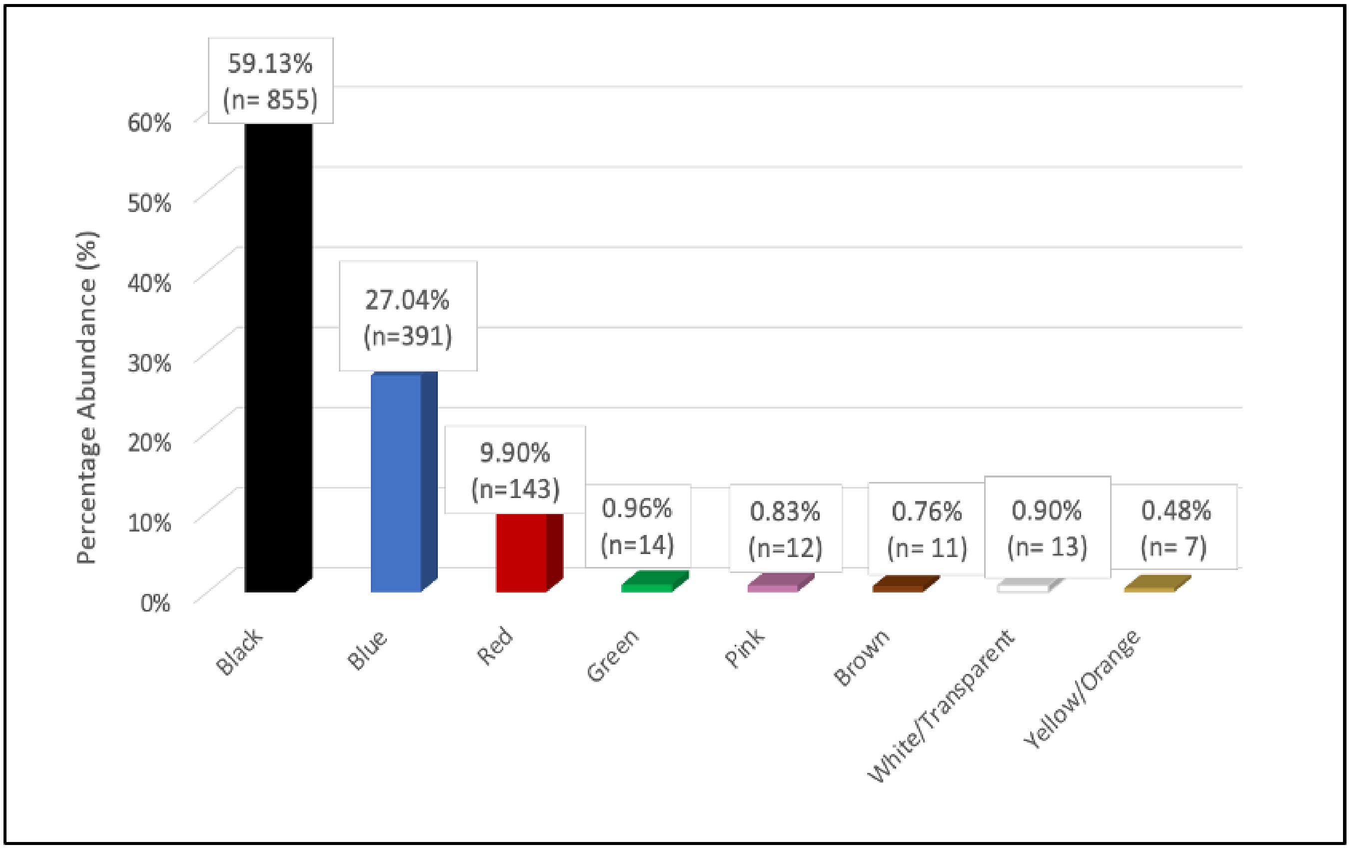



Figure 2

Percentage abundance (%) of microplastic based on colours isolated from gastrointestinal tract (GIT) of Stichopus horrens.

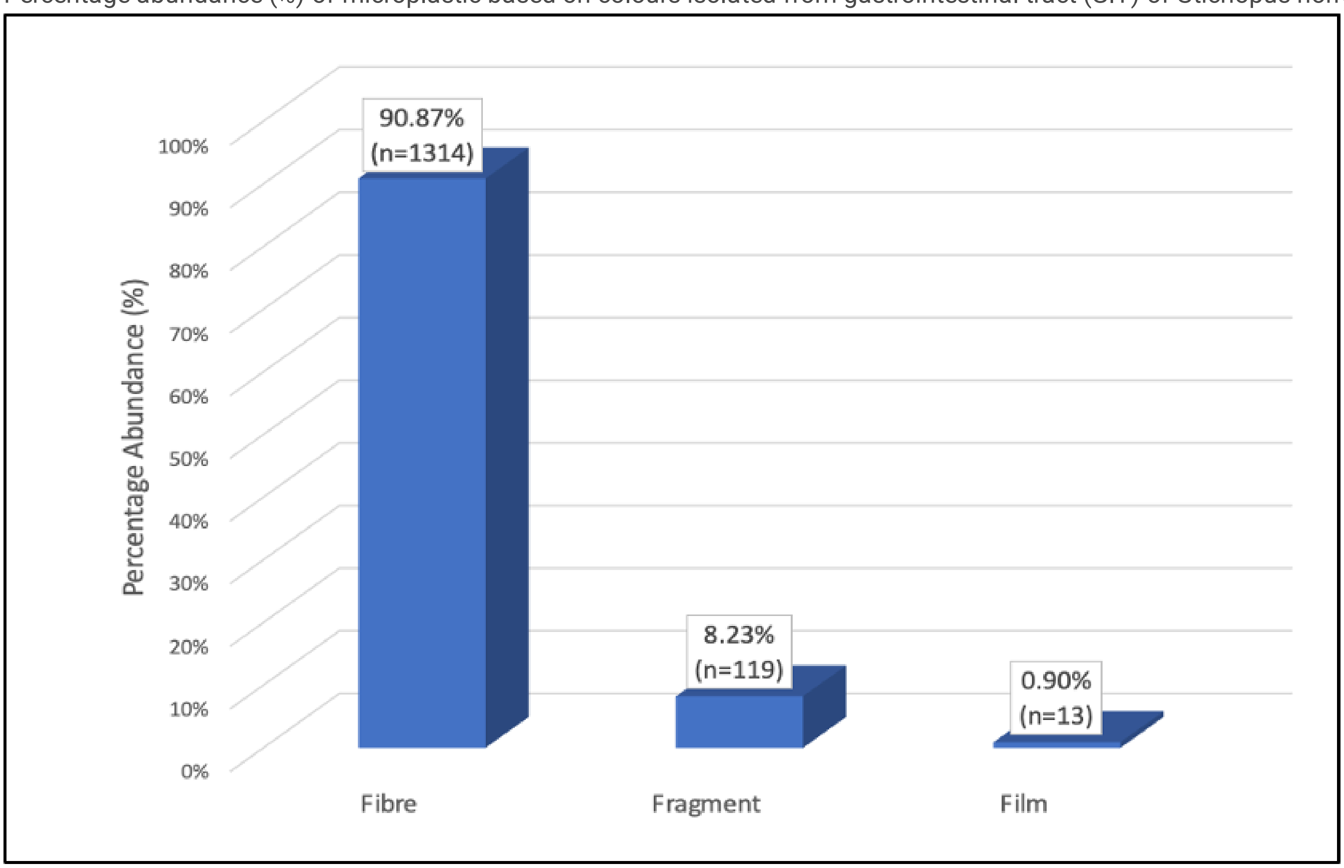



Figure 3

Percentage abundance (%) of microplastic based on shapes isolated from gastrointestinal tract (GIT) of Stichopus horrens.

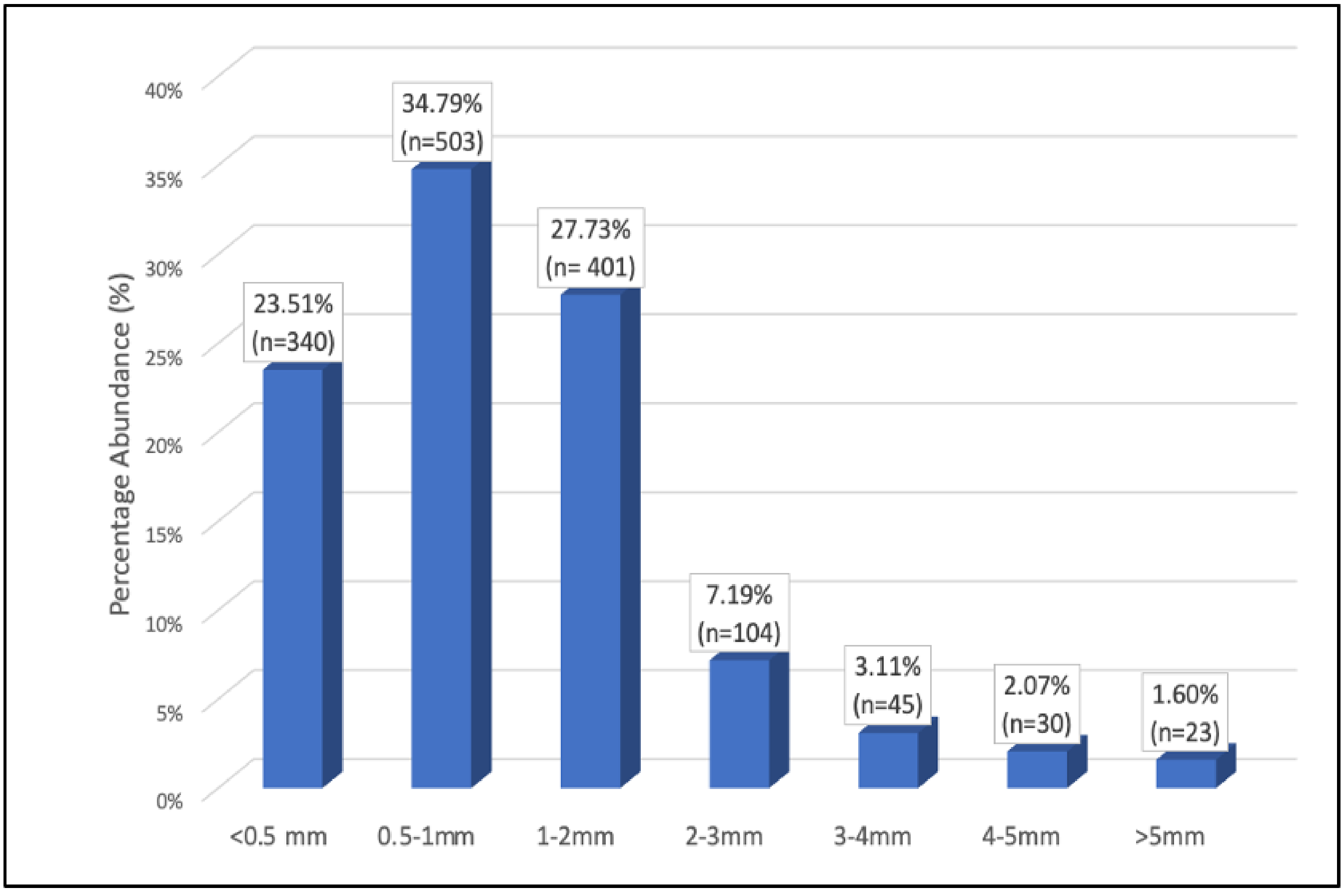



Figure 4

Percentage abundance (%) of microplastic based on shapes isolated from gastrointestinal tract (GIT) of Stichopus horrens.

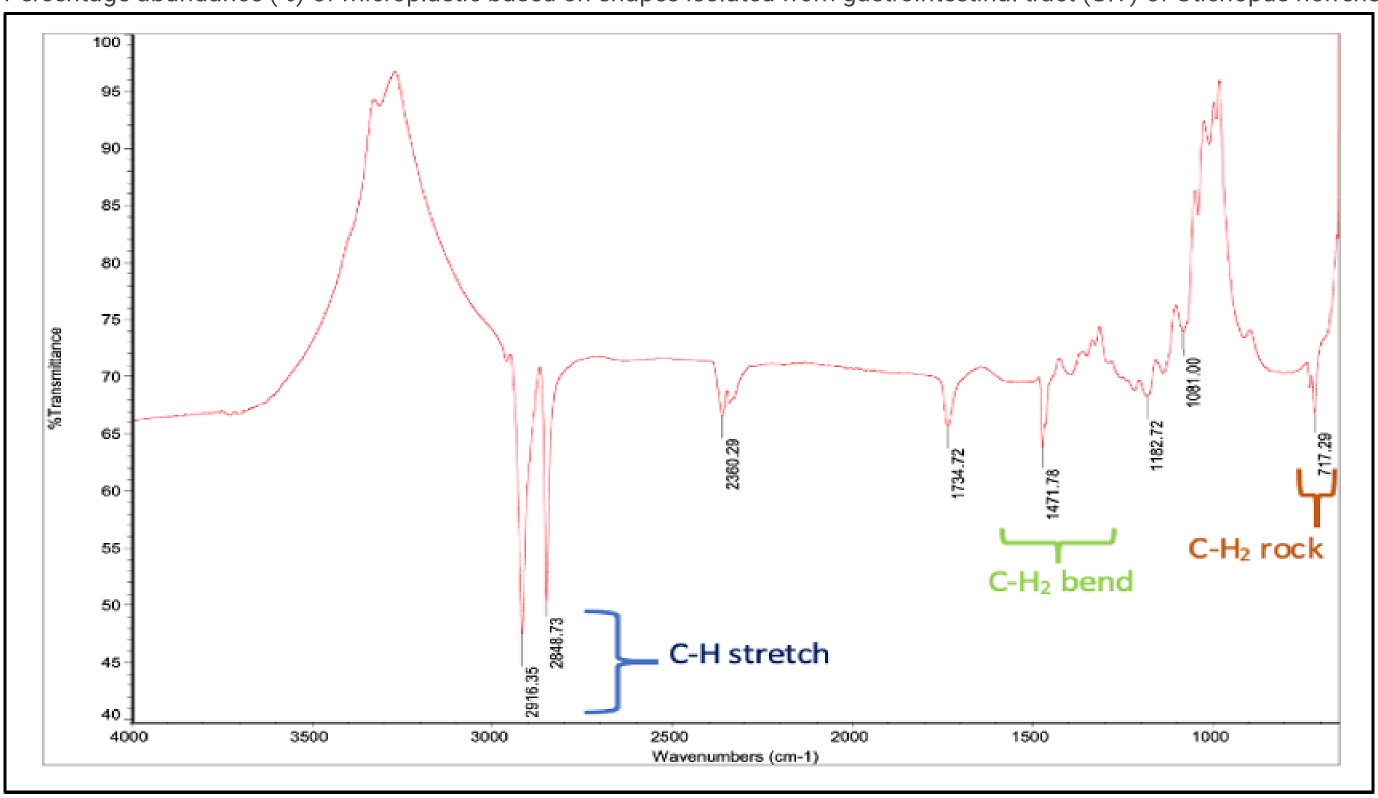
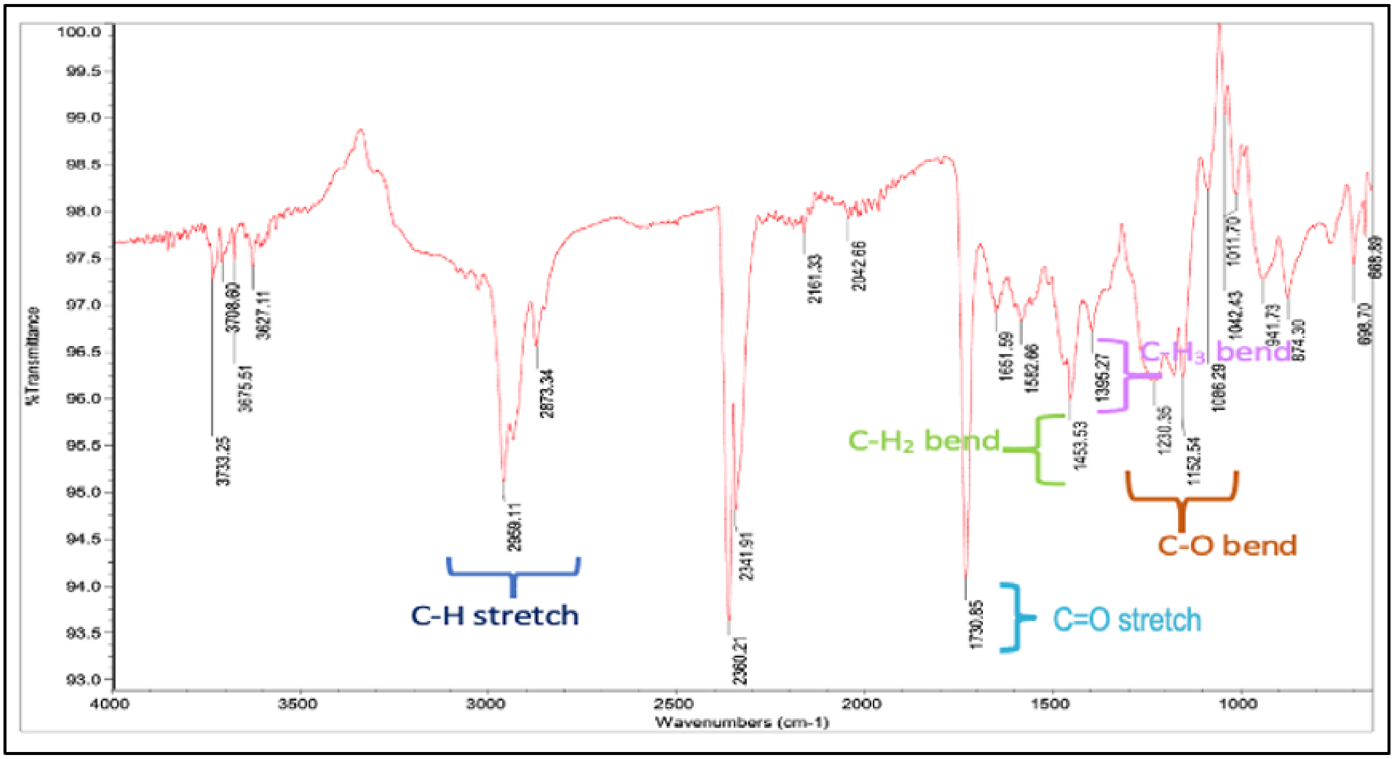



Figure 5

FTIR spectrum showing the functional group associated to polyethylene (PE) composition obtained from gastrointestinal tract (GIT) of Stichopus horrens.




## Figure 6

FTIR spectrum showing the functional group associated to polymethyl methacrylate (PMMA) composition obtained from gastrointestinal tract (GIT) of Stichopus horrens.