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Research Article

Assessment of Micro-Plastics in Guts Content of Fish in Semedang River-Kuching Using ATR-FTIR

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Abstract

Background and Objective

The impact of micro-plastic in the fresh water organism. And the aim of the study was to asses' micro-plastic in the guts of fresh water fish using Attenuated Total Reflectance-Fourier transform infrared (AIT-FTIR).

Material and Methods

Net and cast net used at 10 sampling stations. The sampling stations were divided equally between upper stream and downstream. The microplastic extracted from the fish gastrointestinal had been categorized into shapes by using light microscope and Fourier Transform Infrared Attenuated Total Reflectance was used to analysis the plastic samples.

Results and Discussions

The species Dermogenys pusillus, Mastacembelus maculatus, Puntius sealei, Rasbora myersi, Rasbora sumatrana with a stomach Weight (g) of 0.43 ±3.22, 0.82 ± 2.01, 0.52 ± 0.3, 0.31 ± 0.6 and 0.72 ± 4.31 respetively was observed to have microplastic; Fibers, Foams, Fragments and Beads within the range of 1-13 respectively in all the fish collected. This was revealed by the presence of some low density polymers materials from Attenuated Total Reflectance-Fourier transform infrared chromatogram as shown in figure a, b, c, d.

Conclusion

The microplastics in the marine environment has alerted the scientific world in this last few decades. However, a clear evidence of tissue accumulation and transfer of such microparticles in wild organisms is still lacking.

Introduction

In the recent years there has been an exponential increase in the usage of plastic and poor or in-proper disposal as well as fragmentation of this plastic polluting the ecosystem. The results from human activities, the debris of plastic called macro plastics are found on global scale in both populated and remote area [1]. This microplastic has have been observed in Marine, fresh water and terrestrial ecosystems due to its diverse nature, which comprise a wide variety of chemical compositions, densities, shapes and sizes, micro plastic less than 5mm are expected to have different distributional patterns. Study estimated that 5.25 trillion plastic particles (weighing 269,000 tons) are floating in the sea and more in the rivers and terrestrial ecosystem [2]. It had been reported that the micro plastic is abundance in several environmental matrices such as sediment, water and invertebrate species and such can be consumed by different array of marine organism such as protists, zooplankton annelids, echinodermis amphipods, fish etc., [3,4].

The microplastics contamination in freshwater habitat is drastically increasing. It can be categorized according to their application of manufacture called primary and secondary microplastics [5]. Microplastics also can be categorize in the form of shapes such as fibers, beads or fragments. Weak waste management system cause the disposal of plastic material release to the environment. The size and the physical appearance of microplastic mimically the food source for the fish, turtles, birds and bivalves [6]. The intake of the microplastics by fish are increasingly reported. The abundance of microplactics in aquatic ecosystem exposed the interaction between aquatic organisms with the microplastics as well as their feeding habits [6,7]. Initially, the density of microplastics is lower than the density of water give it the buoyancy charactristic. However, cross over the time, microplastics loss the bouyancy then sink from the water surface and settle into the sediments due to the development of algae on the surface of the microplastics.

As a result, the bottom feeder organism will indirectly consume the microplastic in the process of getting their food from the sediments. Once consumed, the microplastics bring up problems to organisms such as blockage of intestine and volatile organic pollutant leached from plasticizers. It has been reported that, microplastics released pesticides as part of the fragmentation due to exposure with sunlight and weathering process [7].

World organizations such as United Nations Environment Programme (UNEP) declare that the microplastic pollution become global issues and need to tackle holistically. Plastics are major materials for industrial process as well as domestics usage, hence good managements are needed to minimize the social and economics impacts. To avert the transfer of this macro-plastic from fish to human which invireable may cause cancer because of the presence of a compound phathalic accumulation which is most common in plastics [8].

In Sarawak, plastics debris was found in abundance along the beaches [8,9]. Kuching areas are surrounded by the Sarawak River and experience heavy downpour throughout the year. The rapid increasing of the population in Padawan, Kuching areas is one of the factors that give threat to the Sarawak River from the plastic debris. Micro plastics study in fishes of Semedang River is very limited. The presence of microplastics is being worldwide documented in water-column and sediment samples especially in urban area but not in remote area. This study was aimed to asses the presence of microplastics in fish stomach providing a new knowledge of microplastics distribution in remote marine ecosystem.

Materials and Method

Study Area and Sampling Stations

The study area was carried out at upper stream and down stream of Semedang river. There were 5 stations for every location. The distance for every stations of sampling about 1.0km.

Sampling Methods

Herbivores and omnivores species were collected by using fishing net and cast net at 10 sampling stations. The sampling stations were divided equally between upper stream and downstream. To avoid contamination, fish samples were wrapped using aluminum foil and frozen in -20°C. The length (cm), weight (g) and identification of the fishes were made before dissecting. All the dissecting tools made of steel and specimen stored in the glass container to avoid contamination. The microplastic in guts were isolated by using density flotation. The specimens were put on the glass petri dish for microscopic analysis.

Preparation Before Sampling

All the sampling apparatus will be clean by using pre-treated distilled water. Distilled water will be filter by using 0.45 micrometer fiber glass filter. Glass storage bottles will be use to preserve the samples.

Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR)

A Perkin Elmer FT-IR Spectrometer Spectrum Two Universal ATR was used to collect spectra from 4000cm^{-1} to 450cm^{-1} with a data interval of 1cm^{-1}. Resolution had been set at 4cm^{-1}. The ATR diamond crystal clean up with 70% ethanol and a background scan was performed between each sample. Each sample was compressed against the diamond to ensure good contact between sample and ATR crystal, as recommended by Perkin Elmer. Absorption bands identified by using a peak height algorithm within the Perkin Elmer software and compared to absorption bands of each polymer reported in the literature and obtained from in-house spectral library.

Results and Discussions

A total of 64 individuals fish and 5 selected species was collected. Table 1 shown the fish species with total length and weight. All fish collected known to be herbivorous feeding habits.

Table 1: *Total length, weight and number of individual species caught in Sungai Semedang*

Fish species	Number of individual species	Total length (cm) (min.-max.)	Stomach Weight (g)
Dermogenys pusillus	18	4.70-5.30	0.43 ± 3.22
Mastacembelus maculatus	10	9.30-9.83	0.82 ± 2.01
Puntius sealei	18	5.50-6.80	0.52 ± 0.3
Rasbora myersi	15	4.20-5.30	0.31 ± 0.6
Rasbora sumatrana	3	6.1-8.2	0.72 ± 4.31

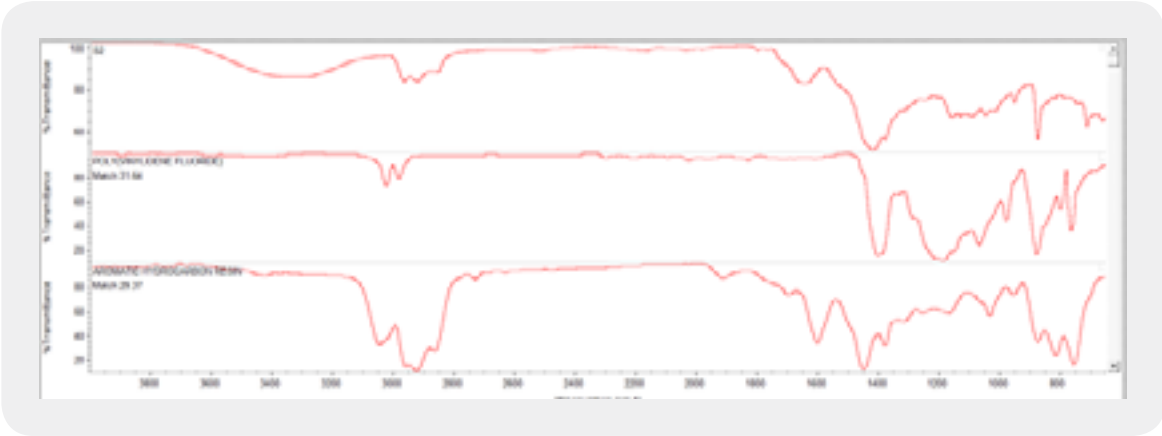
The microplastic extracted from the fish gastrointestinal had been categorized into shapes by using light microscope equiped with camera as was reported by Bessa *et al.* [10]. Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine pollution bulletin, 128,* 575-584. The occurance of microplastic in the fish collected, out the total number of 48 all were found to ingest microplastic with an average of 4.00±0.00mm. this ingestion of microplastic has becomewidespread phenimenon where the plastic were mistaken by the fishs as prey and instanly ingest it [11].

The shapes observed from the microscope are fragments, foams, fibers and beads. The extraction of microplastics from wild fish species highlighted their presence in gastrointestinal tracts of the fish samples are shown in Table 2. The *P. Sealei* showed the highest amount of particles (23 particles), followed by *R. Mearsi* (12 particles), D. Pusillus (7 particles), *M.maculatus* (6 particles) and *M. Sumatrana* (4 particles). Thus, this study contributes with baseline data on the ingestion of microplastic by the fishs obtained in the Semedang River- Kuching.

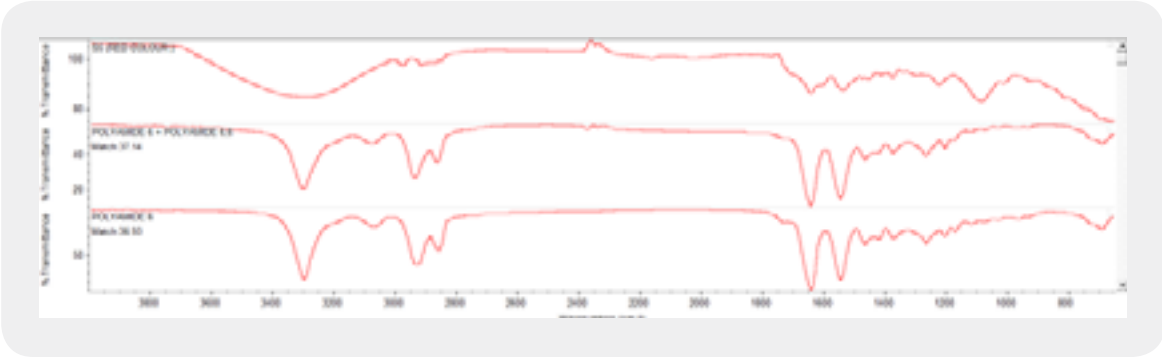
Table 2: *Shape and number of microplastic found in the gut content of the fish*

	Microplastic shape			
Fish species	**Fibers**	**Foams**	**Fragments**	**Beads**
Puntius sealei	13	5	5	-
Rasbora myersi	8	1	3	-
Dermogenys pusillus	7	1	3	1
Mastacembelus maculatus	6	3	9	2
Rasbora sumatrana	4	-	3	1
Sub-total	43	10	23	4
Total	80			

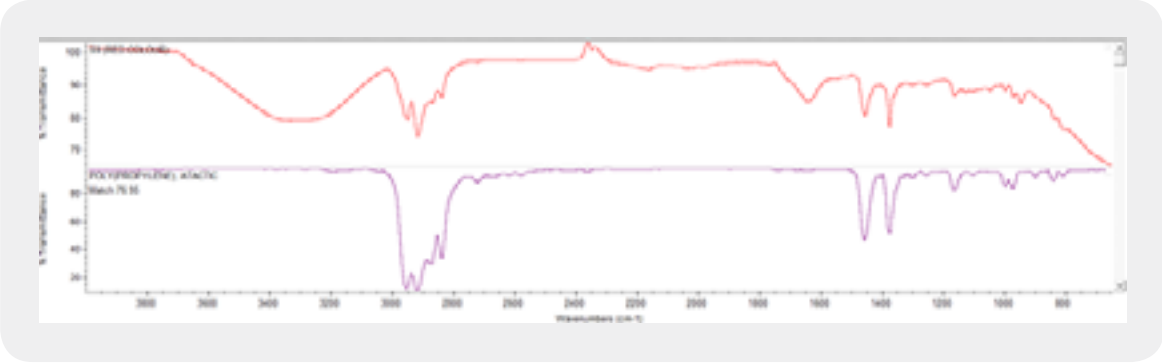
The FT-IR characterization support by the Polymers Library database, revealed the presence of some low density polymers (e.g. polyethylene) and high density particles (e.g. PET) in the fish stomach. Figure 1 represent the FT-IR spectrum of materials extracted from fish stomach.



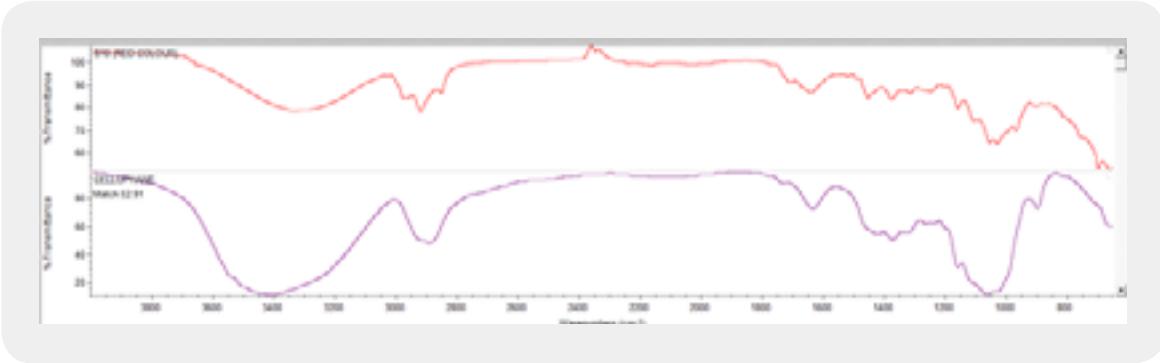
(a)



(b)



(c)



(d)

Figure 1: *Spectrum of common micro-plastics detected in fish stomach*

It is known that buoyancy of micro plastics can be influenced by factors like fouling which cause their sinking and accumulation in sediments. High density particles can be ingested by the fish through their preys as secondary ingestion. It has been recently pointed out an enormous loss of micro plastics from sea surface compared to expected rates of fragmentation suggesting the presence of mechanisms removing particles smaller than 4.75mm. the overall findings indicate that these fish found in Semedang River- Kuching are vulnerable to plastic ingestion. Studies of commercially targeted fresh water species have recorded similar rate of micro plastic [12]. This work agrees with the report of Jovanović, (2017) that this plastic fraction can be directly ingested by the organisms, stored in the sediments and/or indirectly transferred trough the trophic webs [13].

Conclusion

The result of this study provided a new knowledge regarding the distribution of microplastics in river of Semedang- Kuching. This indicate that all the fishes in this river are vulnerable to microplastic contermination of Fibers, Foams, Fragments and Beads which supports transfer of these particles from water column to sediments and benthic species. The results of this work support the hypothesis that this plastic fraction can be directly ingested by the organisms, stored in the sediments and/or indirectly transferred trough the trophic webs. The distribution of microplastics along food webs, and their capacity to penetrate the edible tissues, with potentials concerns for human consumers should also be considered. Future studies is required to determine the processes and pathways responsible for the release of this microlplastic into the enviroment and ecosystem as well as the ingestion of this microlplastic by species from transitional ecosystem which are the connection to land disposal.

Significance Statement

This study discovered that several organisms can ingest microplastics with potentially adverse effects on the digestive tract, respiratory system and locomotory appendages as well as transfering it to human. However, a clear evidence of tissue accumulation and transfer of such microparticles in wild organisms is still lacking, as such partially hampered by technical difficulties in isolation and characterization protocols from biological

samples. This study can be beneficial to enviromentalist and palstic company as well as plastic users to find a design a better option of plastic disposal to save guide the ecosystem.

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