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Occurrence Of Microplastics in Immature Aquatic Insects of Gua Musang Tributaries in Kelantan

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Abstract. Microplastics (MPs) are known for being one of the environmental pollution sources. Nevertheless, the study of accumulation of MPs in living entity particularly in immature insect larvae that serve as food resource in aquatic food chain, is still scarce. This study aimed to determine the presence of microplastics in the freshwater immature aquatic insects comprising of caddisfly (Trichoptera) and dragonfly (Odonata) larvae sampled from two tributaries of Gua Musang namely Sungai Chegeh and Sungai Galas. The collected samples were analysed by using FTIR (Fourier Transform Infrared) spectroscopy and referred to the Spectral Libraries of OMNIC Specta software. Two types of MPs were found within the immature aquatic insect, namely cellophane and chipboard. The MPs particles existed in the form of which were mostly ingested by immature aquatic insects may pose a threat to an aquatic food chain within both Sungai Galas and Sungai Chegeh of Gua Musang, Kelantan. The occurrence of microplastics recorded in these rivers as well as in the immature aquatic insects could be a reflection of microplastic pollution in the respective Gua Musang tributaries in Kelantan.

1. Introduction

Microplastics are small pieces of debris or trash, which plastic particles smaller than 5 mm (0.2 inch) in size. There are two types of microplastics which primary microplastics and secondary microplastics. Primary and secondary forms of microplastics have their own categorization [1]. For the size range in the micrometre measurement, primary microplastics are directly generated and probably to be flow over from industrial or domestic drainage pathways and release into the wastewater treatment channels [2]. A significant number of synthetic fibres are also released into wastewater through laundry of washing machines. As a result of meso-plastics (> 5.0 mm) and macro-plastic (greater than a few centimetres) litter fragmentation, secondary microplastics are produced due to continuous UV light exposure, weathering process and physical abrasion [2]. Indeed, plastic is a UV susceptible material and its outdoor lifetime tends to decrease because oxidative reactions can be initiated by UV radiation, leading to degradation. Mechanical degradation, which occurs when resistant material is torn into smaller particles by resistance forces that occur during transportation through the diverse habitats of the environment, is another crucial factor for secondary microplastic [3]. There is, however, a link between the typology of microplastics and their position in water systems, with primary microplastics likely to be more concentrated near wastewater run-off sites [4].

Microplastics are one of the emerging concerns among other freshwater contaminants [5] which may severely impact to aquatic organisms particularly on immature aquatic insects. Aquatic insects are essential ecological roles in both land and water environment as primary consumers, detritivores, pollinators and predators. From that, immature aquatic insects are also important in maintaining the ecology cycles. The ecology of various groups is thoroughly studied due to their roles as bio-indicators or disease vectors. The freshwater aquatic insects have been mostly overlooked as a focus of diversification, despite their uneven contribution to global biodiversity [6]. Some of aquatic insects such as caddisflies, mayflies, stoneflies, beetles, dragonflies, true flies and some moths are among the groups of insects which mostly their larvae phases represented in streams. These immature aquatic insects play a crucial role in linking the food web in freshwater ecosystem between producers and higher consumers [7].

Microplastics have been known for widely invaded the both aquatic environments, however the knowledge or study is particularly remaining inadequate for freshwater ecosystem. It includes the studying of microplastics in freshwater ecosystem which is least studied. Not only less studied, the properties of microplastics can be quite diverse. Furthermore, [8] demonstrated that, aquatic freshwater insects or immature infected with microplastics are then likely to be eaten or ingested by aquatic living species. For instance, immature aquatic insect is one of food sources for the fish. Hence, it will affect the health and growth of the fish and other aquatic organisms. [9] had stated that, once ingested, microplastics can affect aquatic organisms in various ways, regardless how small the aquatic organisms that ingested the particles.

The study of the microplastics in immature aquatic insects in Malaysia is scarce particularly in Kelantan. The recent study of microplastics in Kelantan river was related to distribution of microplastics in surface water and sediment bay [10] but not within the living entity particularly in aquatic insects that serve as food source in aquatic food chain. In that case, this study was conducted in order to determine the occurrence of the microplastics in the immature aquatic insects of Gua Musang tributaries where, for instance, the presence of microplastics in the digestive system has the potential to impair nutrient uptake and hinder the development, reproduction, and survival of aquatic species (particularly aquatic insects) as well as the use of resources [11].

2. Methodology

2.1 Sampling site

Figure 1. Map of the study area showing the sampling site in Sungai Galas, Gua Musang, Kelantan.

Figure 2. Map of the study area showing the sampling site in Sungai Chegeh, Gua Musang, Kelantan.

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2.2 Immature larvae sampling

Freshwater aquatic insect larvae samples were collected from two tributaries of Gua Musang namely Sungai Chegeh and Sungai Galas on February 2021 for seven (7) consecutive days. The samples were collected using Surber frame net that was dipped in the shallow river. Sampling effort was arranged over a stretch of about 100 metres along the riverbank. Ten sampling points were established within each of the sampling sites with an interval of one metre.

Some of the samples have already been extracted on-the-spot at the sampling site, some of them were brought back to laboratory for further extraction process in order to save time for sampling plus in this pandemic situation. The pre-extracted samples in the field were preserved using 70% ethanol. Other samples were kept in container which we took up along with the river water and sediments (sand, rocks, fallen and leaves). The samples were kept in 28 mL universal glass bottles.

2.3 Identification of immature larvae

The identification of the larvae was done in the laboratory and up to Order level. All of the collected freshwater aquatic insects were identified by referring to the Freshwater Invertebrates of the Malaysian Region book [12]. Identification was done thoroughly observing and emphasizing the general information and external morphology such as body colours and morphology of the aquatic insect larvae. The aquatic insect larvae were identified under stereomicroscope.

2.4 Digestion of Microplastics

Prior to the digestion process, the samples were rinsed with ultra-pure distilled water in order to remove any possible laboratory contaminant. Firstly, samples were transferred straight away into 100 mL conical flask. There were ten (10) conical flasks which represented each sample. Every process that involved with chemicals was done in the laminar flow cabinet. Firstly, 20 ml each of KOH (10 M) and H_2O_2 (30% v/v) with KOH: H_2O_2 ratio was added to every conical flask filled with sample, and afterward fully covered the conical flask with parafilm. Potassium hydroxide (KOH, 10 M) is an alkaline, that hydrolyse chemical bonds and denature protein in which it is useful in removing the biological material. KOH is also enable to digest the fish tissues, as well as the esophages, stomach and intestines. The hydrogen peroxide (H_2O_2) is an oxidizing agent, which an effective digestant [13].

After that, samples were put into a laboratory shaker (Incubators WIS-20 Shaking incubator bench-top orbital motion) and keep it shaking for 120 hours (5) days. The shaker was set up with temperature of 26.9 ℃ and speed 100 rpm.

2.5 Filtering Digestion

The digested (digestion of aquatic insects larvae) sample was filtered using cellulose nitrate membrane filter paper with 0.2-μm pore size. The filter papers were accompanied with vacuum pump to reduce time consuming filtration, removing any air bubbles additional molecules that exponentially harder to eliminate (VAC AERO International, 2016). Chemical agents were used to filter the following digestion to preserve any recalcitrant compounds (e.g., undigested tissue, inorganic residue, microplastics). To filter all the digested materials, we used cellulose nitrate membrane filter paper which was 0.2 μm pore size. Larger pore size makes faster filtering simpler, but it can result in smaller plastics being lost. Plus, we also used the vacuum pump to speed up the filtration process.

After that, each filter paper was put into petri dish and been kept in the killing jar that were filled with silica gel to be dried at room temperature for six (6) days. Then, it was analysed by using the micro-Fourier-transform infrared (μFTIR) spectroscope.

2.6 Microplastics Determination

Microplastics were analysed in the form of solid that can be found on the cellulose nitrate membrane filter papers. There was no specific or projected spot on the filter to analyse the microplastics which the scanning of microplastics was done randomly on the surface of filter papers. In particular, the collected

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IR spectra where its chemical conformation of polymer particles was identified by comparing with the reference spectra database.

2.7 Micro-Fourier-Transform Infrared (μFTIR) Spectroscopy Analysis

A FTIR was achieved by using an Attenuated Transverse reflection (ATR) unit where it is a technique where material was placed immediately on top of a crystal (or diamond), and an IR beam passed through the crystal into the material's bottom surface before reflecting and being detected.

The Nicolet TM iNTM10 FTIR system (Thermo Fisher Scientific, USA) used for the -FTIR analysis has a single-element mercury cadmium telluride detector. Any suspected microplastic particles or compound was analysed under the micro-Fourier-transform infrared (μFTIR) spectroscope. FTIR spectroscopy was one of the methods used for identification of microplastics.

The measurements were executed in attenuated total reflectance (μATR) mode with 32 co-added scans per replicate with the wavelength of 4000–500 cm^{-1} at a spectral resolution of 4 cm^{-1}. For microplastic (especially microplastic polymer) identification was done by using the OMNIC software package. The identification and interpretation of data were processed by Thermo Scientific™ OMNIC™ Specta™ software and compared with spectral libraries (including Aldrich Polymers, Hummel Polymer and Additives, Polymer Additives and Plasticizers, Sprouse Polymer Additives, and Synthetic Fibers by Microscope).

3. Results and Discussion

The immature aquatic insects captured in this study were the larvae of caddisfly (Trichoptera) and dragonfly (Odonata). Figure 3 and 4 shows that both sampling sites possess quite similar in shape of spectra (based from the graph) although there were slightly differed in the peak high. In Figures 3 and 4, the red spectrum indicates representative spectra that was compared with quite similar spectra (other than red colour spectrum) of reference polymer library.

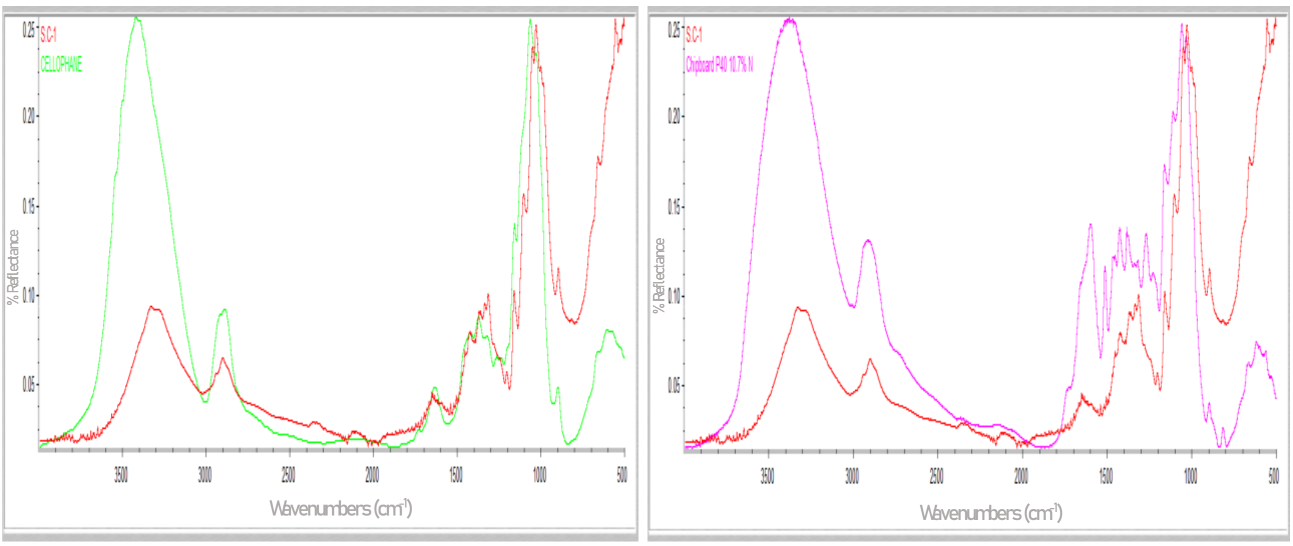

Figure 3. Two microplastic types namely cellophane and chipboard were detected in Sungai Chegeh, Gua Musang, Kelantan.

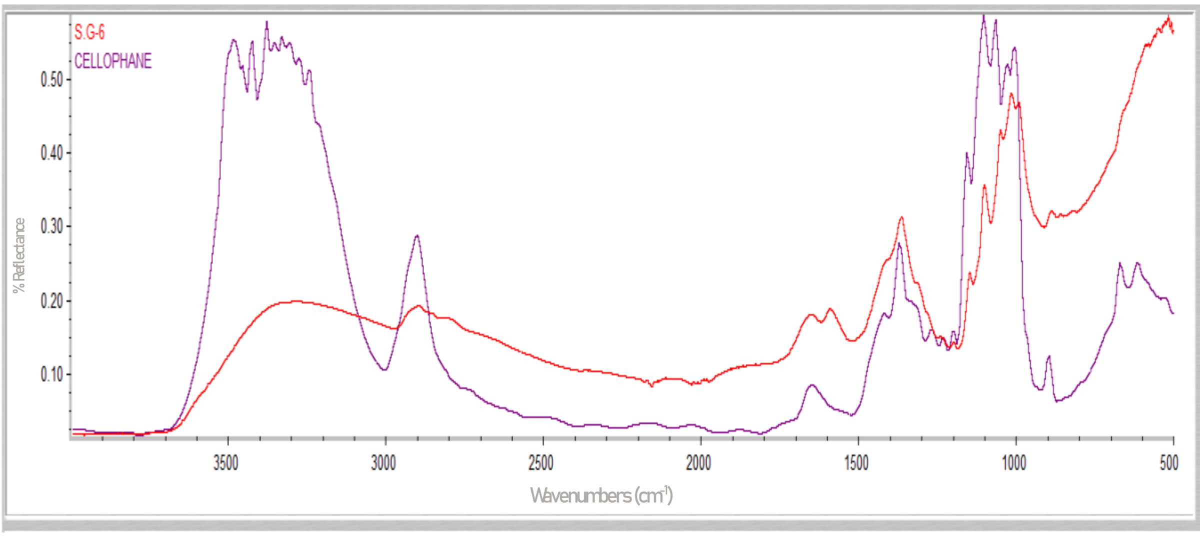

Figure 4. One type of microplastic type namely cellophane was detected in Sungai Galas, Gua Musang, Kelantan.

Based on the FTIR tests, there were two (2) types of polymers discovered namely, cellophane and chipboard. Each type of polymer provides numerous uses and benefits for various purposes particularly to human’s need and undoubtedly came from different sources. Cellophane was found to be the most dominant polymer in both tributaries. Immature aquatic insects of Sungai Chegeh were recorded with the occurrence of cellophane and chipboard compounds, meanwhile only immature aquatic insects of Sungai Galas were recorded with cellophane only. The findings indicate the microplastic ingested by immature aquatic insects of Sungai Chegeh was over 50% as compared to the microplastic ingested by immature aquatic insects in Sungai Galas. In the proximity of Sungai Chegeh, it was observed that several houses belong to indigenous people were built along the riverbank. Since there are community-living present, there might be domestic waste released into the river.

Cellophane is widely used in food packaging products, labels, and photographic films, coatings for paper, glass and clothing [14]. Based from [15] study, the release of microfibres during the washing process of clothing was impacted by certain textile materials, such as the type of fibres making up the yarns and their twist. The mechanical and chemical stressors that synthetic textiles experience throughout a washing procedure are the principal causes of the release of microplastics from such garments possibly by local community that live nearby the rivers. Incidentally, its presence in both rivers and this has been potentially related to direct clothing washing in rivers which are commonly practised by many locals in the vicinity, based on the site observation.

Meanwhile, the occurrence of chipboard is most probably due to anthropogenic activities such as housing construction and furniture manufacturing [14]. In this study, chipboards were predicted to originate from the wood debris. The wood debris can be naturally produced or released from human activities. The wood debris also consist of cellulose materials since it is a part of plants. Table 3.1, indicates three (3) samples of Sungai Chegeh consisted of chipboard. The occurrence of two types of microplastics recorded in the immature aquatic insects between two rivers do not reflect the entire microplactics contamination for the entire tributaries. As for Sungai Galas, it is under the protection of Department of Irrigation and Drainage, Gua Musang (Jabatan Pengairan dan Saliran Negeri Kelantan Jajahan Gua Musang). It also is located nearby Gua Madu, a recreational area. By virtue of persistent monitoring by the authorities, the cleanliness of Sungai Galas that adjacent to Gua Madu is well kept and less contaminated with microplastics as compared to Sungai Chegeh. Most of the residents in Sungai Chegeh are indigenous people. Lack of awareness among them on the usage of plastics and cleanliness of river, contribute to the microplastic contamination in Sungai Chegeh.

In river systems, primary or secondary MPs could end up in any of the following ways: Firstly, they might be carried by the water mass into nearby seas or lakes.; Secondly, they might adhere to the riverbed like other suspended materials if the flow velocity is insufficient to maintain their suspension and lastly, they might easily be consumed by aquatic organisms as previously conveyed in few previous studies where one of them was [16] study. For instance, aquatic organisms that accumulate MPs face the threat of their populations declining due to decreased food intake, weight loss, slowed development and energy depletion. [17]. Since MPs act as vectors for such transfer, ingested MPs by immature aquatic insects may also cause the transmission of hydrophobic and persistent organic pollutants (such as polychlorinated biphenyls, dioxins, and dichlorodiphenyltrichloroethane) to higher trophic levels in the food chain [18]. Due to their physiological and ecotoxicological effects once ingested by immature aquatic insects, MPs may pose a threat to an aquatic food chain within both Sungai Galas and Sungai Chegeh of Gua Musang, Kelantan.

4. Conclusion

This study indicates the occurrence of microplastics within freshwater immature aquatic insect, emphasising a potential risk to freshwater ecosystems. Based from the findings, the abundance of microplastics in immature aquatic insects conjured up that the river was polluted with microplastics namely cellophane and chipboard. The MPs particles existed in the form of which were mostly ingested by immature aquatic insects may pose a threat to an aquatic food chain within both Sungai Galas and Sungai Chegeh of Gua Musang, Kelantan. Further studies are needed to relate between the target organisms with plastics sources and processes.

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