PRELIMINARY STUDY ON DETERMINATION OF MICROPLASTIC IN BIOLOGICAL CONSTRUCTION OF A FRESHWATER AQUATIC INSECT IN KELANTAN

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ABSTRAK

Mikroplastik terjejas secara global bukan sahaja kepada lautan, tetapi juga sistem air tawar yang selalu diremehkan oleh beberapa penyelidik. Walaupun begitu, kesan mikroplastik pada air tawar boleh membahayakan seperti di lautan. Kajian ini bertujuan untuk menentukan kewujudan mikroplastik dalam pembinaan biologi atau selongsong larva serangga akuatik di ekosistem air tawar Kelantan. Dua lokasi persampelan dipilih iaitu Sungai Galas dan Sungai Chegeh di daerah Gua Musang, Kelantan. Sebanyak 4 selongsong serangga kadis; *Nectopsyche sp.*, *Ylodes sp.*, *Apatania sp.* dan *Gumaga sp.* dikumpulkan secara manual di kedua-dua sungai dan dicerna secara kimia. Spektroskopi inframerah-transformasi mikro-Fourier (µFTIR) dilakukan untuk mengesan kehadiran mikroplastik dalam selongsong serangga kadis. Akibatnya, *Ylodes* sp. dijumpai di Sungai Galas mempunyai jumlah mikroplastik tertinggi yang tercatat dalam kes itu yang mengandungi sebatian selofan dan papan serpai. Kemudian, diikuti oleh *Nectopsyche sp.* dan *Gumaga sp.* Secara perbandingan, kes paling sedikit yang mengandungi mikroplastik adalah *Apatania sp.* yang kekurangan sebatian papan serpai dan lebih banyak mengandungi ubat dan alkohol. Oleh itu, sebatian mikroplastik dominan yang terdapat dalam semua selongsong adalah selofan. Walaupun begitu, mikroplastik pada pembinaan biologi mengurangkan kestabilan kes dan meletakkan larva dalam bahaya yang berkemungkinan dimakan oleh pemangsa. Oleh itu, kemungkinan hidup larva akan terhad.

Kata kunci: Mikroplastik, larva serangga akuatik, serangga kadis, µFTIR.

ABSTRACT

Microplastics are globally affected not only to the oceans or marine, but also freshwater systems that always been underestimated by some researcher. Nevertheless, the effect of microplastic in freshwater could be harm as in the oceans. This study was to determine the microplastic existence in the biological construction or casing of aquatic insects larvae in Kelantan freshwater ecosystem. Two sampling sites were selected namely, Sungai Galas and Sungai Chegeh in Gua Musang district, Kelantan. A total of 4 casing of caddisfly; *Nectopsyche sp.*, *Ylodes sp.*, *Apatania sp.* and *Gumaga sp.* were manually collected in both rivers and chemically digested. Micro-Fourier-transform infrared (µFTIR) spectroscopy were performed to detect the presence of microplastics in the cadisfly's casing. As a result, *Ylodes sp.* found in Sungai Galas has the highest amount of microplastic recorded in the case frankly containing cellophane and chipboard compound. Then, followed by *Nectopsyche sp.* and *Gumaga sp.* Comparatively, the least case that contained microplastic is *Apatania sp.* which lack of chipboard compound and consist of more drugs and alcohol instead. Hence, the dominance microplastic compound discovered within all the casing were cellophane. Nevertheless, microplastics at biological construction diminish case stability and putting larvae in danger of being eaten by predators. Therefore, the larvae's chances of survival will be limited.

Keywords: Microplastics, aquatic insects larvae, caddisfly, µFTIR.

1.0 INTRODUCTION

Freshwater habitats globally, including lakes and waterways, are filled by microplastic debris (Eerkes-Medrano et al., 2015). Microplastic is a tiny particle of plastics and roughly defined as smaller than 5 millimeters. It also too small to be filtered in water treatment facility. It can be anything from tiny shards broken off larger items to the microbeads developed for use in cosmetic products. Microplastics also can be found in a wide range of items including cosmetics, synthetic garments, plastic bags and bottles. Many of these products are easily disposed of in the environment (Rogers K., 2020). For example, microbeads which is a form of microplastic are extremely small bits of manufactured polyethylene plastic used as exfoliants in health and beauty products such as cleansers and toothpastes. These microscopic particles easily bypass water filtering systems and end up in the water system posing a risk to aquatic life (National Ocean Service, 2021). Any plastic pieces or particles that are already 5.0 mm in size or smaller before entering the environment are considered primary microplastics such as microbeads in personal care products, pellets used in feedstock or plastic manufacture, scrubbers used in abrasive cleaning agents and plastic powder used for moulding. In contrast, secondary microplastics are microplastics that form when larger plastic products degrade into smaller plastic fragments after being exposed to the water system. This occurs as a result of mismanaged trash such as discarded plastic bags, photodegrading and other weathering processes (wave action, wind abrasion and ultraviolet radiation from sunlight) as well as inadvertent losses such as fishing nets (Boucher and Friot, 2017). Their creation has increasing rapidly in the last few decades and expected to be increasing over year (PlasticEurope, 2015). It is anticipated to keep growing which doubled the current production rates by 2050 (Geyer et al., 2017).

There are many studies and researchers has been putting attention towards the presence of microplastic in oceans and marine instead of freshwater. Only recently has a study on microplastic in freshwater been published, confirming the existence of microplastic lakes, reservoir and freshwater (Eerkes-Medrano et al. 2015; Li et al., 2018). These studies also claimed that the effect of microplastic in freshwater could be harm as in the oceans. The potential hazards to aquatic species from microplastic ingestion and assimilation are among the primary concerns about microplastic presence (Browne et al., 2008). Microplastic particles can be assembled in aquatic sediments and come into contact with epibenthic organism such caddisfly larvae. Microplastic then can be incorporated into caddisfly case (Windsor et al., 2019). In that case, this study is to determine the microplastic in biological construction of freshwater aquatic insect. Biological construction is the protection build by the species itself using biotic and abiotic material around their environment. For instance, larvae of caddisfly species (*Lepidostoma basale*). Larvae caddisfly might start building cases or known as larval case to protect themselves from predation (Boyero et al., 2006). In addition, microplastics may be incorporated into the structure built by the species (Ehlers et al., 2019). An observation from latest study by Ehlers et al (2020), during their constructing emergency or movable cases, caddisfly larvae aggressively gather PET and PVC microplastic. A higher amount of PVC and PET microplastic particles in case may causes the harm and reduce the stability of the case thus the case cracking by predators will easily occur. Nevertheless, the studies from Ehlers et al. (2019) hypothesized that microplastics would be fixed in the larval cases, which are constructed from sediment grains (Skuja, 2010) since the freshwater sediments are a sink for microplastic. Microplastic in biological construction of aquatic insect can increase the larva's visibility in habitats due to the less plastic incorporated in the sediment where the larva built its case. This is because microplastics have a range of different and often have bright colours (Skuja, 2010) and a high amount of colourful microplastic incorporated may attract the predators' attention such as trout and larger fish to feed on the biological construction and along with the larvae (Elliot, 1967). As consequence, fish that ingested the insect's biological construction that contained microplastic which will thereby enter the food chain and cause fish to have inflammatory reactions (Lu et al., 2016). Thus, a freshwater systems study is important as to the one in microplastics studied in marine system.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was conducted earlier on March 2021. The biological construction or larval case of aquatic insects were manually collected from Sungai Galas (5.08^o N, 102.07^o E) and Sungai Chegeh (5.10^o N, 101.97^o E) located in Gua Musang district. Sungai Galas (Figure 1) is a stream that has an elevation of 27 metres meanwhile Sungai Chegeh (Figure 2) is a stream that has an elevation of 132 metres and situated nearby to Gua Cha. The larval case of Nectopsyche sp. and Ylodes sp. were collected from Sungai Galas whereas Apatania sp. and Gumaga sp. were collected from Sungai Nenggiri respectively. All samples were kept in plastic container along with the sands, rocks and other materials nearby to be filtered in the laboratory later.

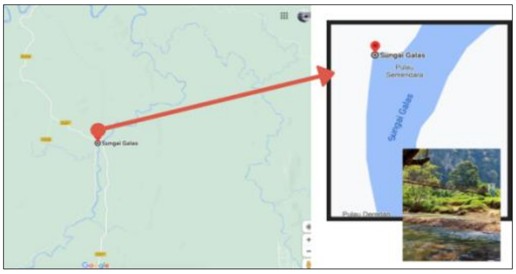

Figure 1: Map of the study area showing the sampling site in Sungai Galas, Gua Musang, Kelantan.

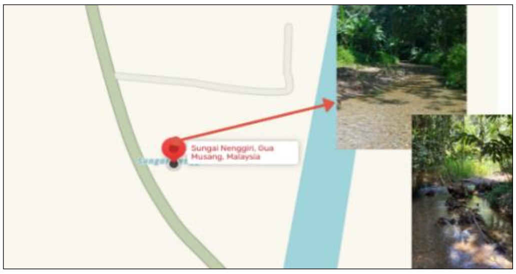

Figure 2: Map of the study area showing the sampling site in Sungai Chegeh, Gua Musang, Kelantan.

2.2 Casing Collection and Identification

The cases were randomly collected all-together with sediment and freshwater which approximately 3km from the littoral/sub-littoral sections of each river using aquatic frame net that was dipped in the shallow river. Sampling effort was deployed over a stretch of approximately 100 metre along the river bank. Then, the samples were kept in plastic containers with 14.25 cm length, 12.5 cm width and 24.5 cm height. Since it was impossible to collect for larval cases only due to the small sizes and barely to be appeared, so the cases were collected along with other sediment and freshwater to be filtered in the lab later. Then, the filtering process were carefully done by using filter net with small sizes of hole in order to filter sands and rocks in the plastic container. This is because it will be much easier to look up for the larval cases. The process was constantly done until the cases found. Note that the cases were collected using dropper instead of using forceps since it will harm the morphology of the cases. The cases then were placed in universal bottles and need to be rinsed off with ultrapure water. To avoid any airborne microplastic contamination of the larvae, cotton clothes were worn during the sampling collection. Then, after the filtering process, the cases were identified in the laboratory under a stereo microscope referring to Hinchliffe and Palmer (2010) article and John. C (2004), Freshwater Invertebrates of the Malaysian Region (Insect: Trichoptera) that have listed the types of portable cases of different species with morphological species. Identifying phases were done thorough by observing and emphasizing the general information and external morphology such as body colours and morphology of the aquatic insect larvae.

2.3 Preparation of Larval Cases

To remove any particles that adhered to the case surface, all cases were gently rinsed in ultrapure water and put it in individual glass of Petri. Next, the wet weight of the cases were measured straight away use an analytical balance (XS205 Dual-Range Analytical Balance) before immediately transferred into the fume cupboard. All laboratory surfaces and glassware were cleaned using 70% ethanol and ultrapure water before starting any laboratory work to prevent microplastic contamination. Furthermore, to prevent microplastic cross-contamination between the caddisfly larval cases, the forceps has also been rinsed between samples. Finally, all the petri dishes were immediately covered with aluminium foil to prevent any airborne microplastic contamination.

2.4 Larval Case Oxidation

In case of disintegration, all rinsed larval cases were moved to the individual glass beakers in which each of the case was immersed in 2.3 ml of hydrogen peroxide solution (30 % H_2O_2) and 2 ml of potassium hydroxide, KOH (10M). Potassium hydroxide (KOH, 10 M) is an alkali, known as a strong base that hydrolyze chemical bonds and denature protein in which it is useful in removing the biological material. The hydrogen peroxide (H_2O_2) is an oxidizing agent, which an effective digestant (Lusher et al., 2017). In that case, 30% of H_2O_2 is the best method and solution to disintegrate them and remove any organic substances present. This is because H_2O_2 is the ideal reagent to remove approximately 50% of biological matter, whereas another 50% illustrated an obvious reaction such discoloured and transparent (Nuelle et al., 2014). The beakers were then coated with parafilm and thereafter shaken on a laboratory shaker (Edmund Buhler 7400 Tübingen) for 120 hours. After that, the drying process was done using vacuum pump to filter the disintegration cases from the solution. Each sample was poured onto the cellulose membrane filter with 0.2-µm pore size. These filters were used to enable µFTIR measurements in transmission mode during further microplastic analyses. After all, each cellulose membrane filter was then kept in a relaxing jar or desiccator to maintain its humidity.

2.5 µFTIR Analyses of Microplastics found in Biological Construction

All microplastics found in the cases were being analysed by using a Hyperion 2000 µFTIR microscope fitted with a mercury-cadmium telluride detector (Bruker) in a wavenumber range between 4000 and 600 cm^{-1}. Whilst the measurement was performed in attenuated total reflectance (µATR) mode with 32 co-added scans and a spectral resolution of 4 cm^{-1}. The software used for microplastic identification was ThermoFisher Scientific. At every stage of the analysis, all the apparatus used were also cleaned and rinsed off with 70% ethanol.

3.0 RESULTS

There were only four species of biological construction of freshwater aquatic insect were successfully collected and identified under binocular microscope throughout this study (Figure 3). Comparatively, *Ylodes sp.* (Leptocerinae) was the smallest cases in this study based on wet body weight which approximately 1 mg. The wet body weight for *Nectopsyche sp.* (Leptocerinae) with 2mg and *Apatania sp.* (Apataniidae) was 3 mg. Whereas, the biggest among all cases were *Gumaga sp.* (Sericostomatidae) and its weight was 11 mg. The types of portable cases of *Nectopsyche sp.*, *Apatania sp.*, and *Gumaga sp.* are categorized as sand grain and they are normally in oblongated shape. Whilst, *Ylodes sp.* is spiral wall shape and random type. The physically almost looks like a wood. Besides, *Nectopsyche sp.* and *Ylodes sp.* were collected from Sungai Galas while *Apatania sp.*, and *Gumaga sp.* were collected from Sungai Chegeh.

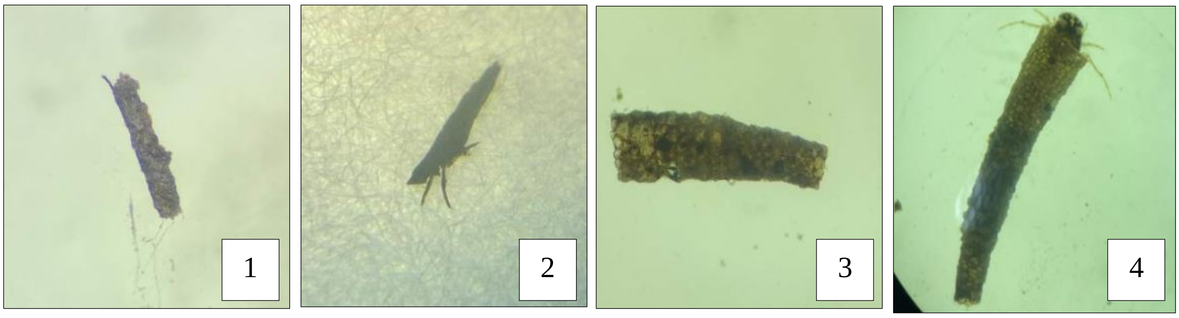

Figure 3: Biological construction of freshwater aquatic insect collected at Sungai Galas and Sungai Chegeh, Gua Musang. 1-*Nectopsyche sp.* (Leptocerinae); 2- *Ylodes sp.* (Leptocerinae); 3- *Apatania sp.* (Apataniidae); 4- *Gumaga sp.* (Sericostomatidae). Identification of cases under stereo microscope according to John.C (2004) and Hinchliffe and Palmer (2010).

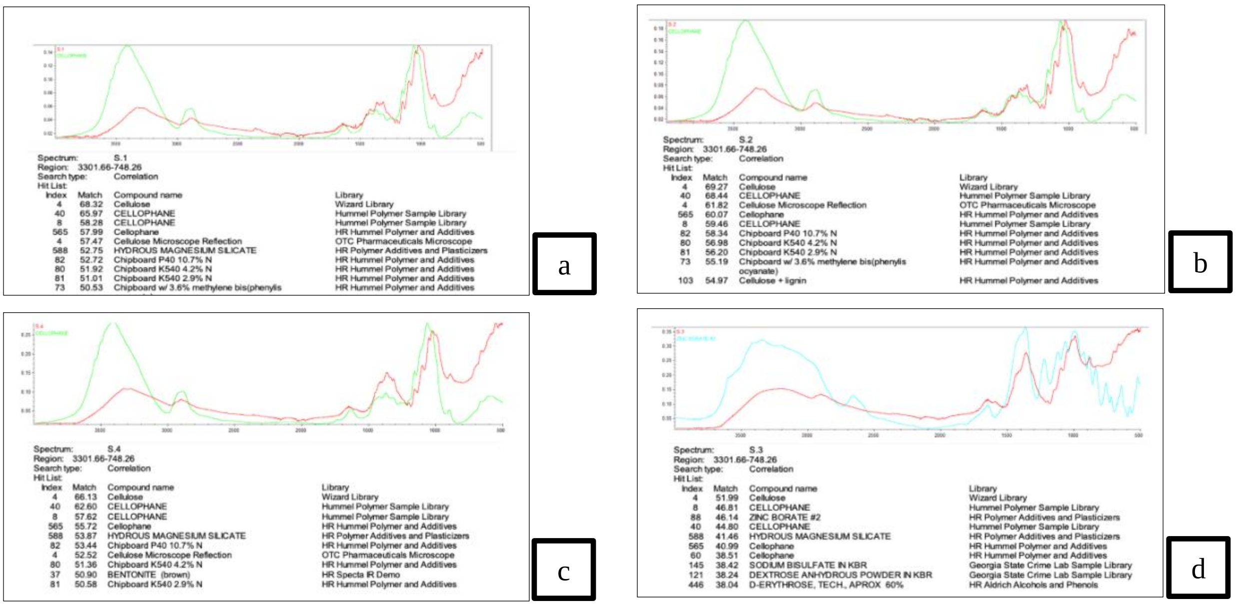

Figure 4: µFTIR absorbance graph analysis that indicate the presence of microplastics with the compound name. a-Sample 1; b- Sample 2; c- Sample 3 and d- Sample 4.

In Figure 4, µFTIR spectroscopy revealed that the dominance microplastic contained in all four biological constructions were cellophane. Another compound that found in cases and claimed as microplastic were cellulose-based, chipboard and zink borate. Furthermore, zinc borate is considered as microplastic because of its purpose that primarily used as flame retardant in plastics, paper and textiles. Thus, it was believed that the existence of zinc borate are the causes for the microplastic to be there either. *Apatania sp.* is the only cases that consist of the zinc borate. Based on FTIR absorbance graph, Ylodes sp. has the highest amount of microplastic detected in the case which contained more chipboard compound. Then, followed by *Nectopsyche sp.* and *Gumaga sp.* Comparatively, the least case that contained microplastic is *Apatania sp.* which lack of chipboard compound and consist of more drugs and alcohol instead. However, the species detected to have zinc borate.

3.0 DISCUSSION

In this study, the microplastic were determined and detected using µFTIR spectroscopy in each biological construction of different species. Overall, all the biological construction consists of cellophane which categorized as plastic that tear off into the smaller particles called microplastic. Thus, at smaller size, microplastic particles can be easily digested and absorbed by biological construction. Cellophane is a polymer that has a cellulose base material and normally used as packaging especially for food (Hisano, 2017) as it is flexible and transparent plastic film. It also used in glass, coatings for paper, photographic films and clothing (McKeen, 2019). Moreover, according to Britannica (2019), cellulose films are made up of synthetic polymers such as polyethylene (PE), polyvinylidene chloride (PVDC) and polyethylene terephthalate (PET). Besides, to protect it from becoming too brittle for packaging purposes, various glycol combinations, such as propylene, ethylene, or triethylene glycol, are generally utilized as the plasticizer (Barry, 2017). The FTIR analysis revealed that a large percentage of microplastic were made of PP and PE which are the most widely employed (Zhang et al., 2016).

Based on the µFTIR result shown in Figure 2 above, each of the biological construction were comprise of cellulose compound. Cellulose are not considered as microplastic as it is naturally organic compound that usually take up from the larvae to build their cases. For instance, caddisfly larvae built the cases by collecting the different biotic (Sheath et al., 1995) and abiotic materials (Okano et al., 2012). Frankly, cellulose is an organic compound that generally synthesized by living organisms (plants) ranging from the bacteria until forest trees (Inder et al., 2001). Therefore, during the cases construction, the larvae are intentionally taking up the cellulose to build the cases as it is the organic compound that existed nearby. Other than that, three species of biological construction; *Nectopsyche sp.*, *Ylodes sp.* and *Gumaga sp.* were detected to have chipboard compound. Chipboard was considered as microplastic since it is a type of polymer used widely in construction and furniture particularly (Nanvaee et al., 2009). As aforementioned, *Apatania sp.* is the least larval case species that contained microplastics. A zinc borate and cellophane were determined in *Apatania sp.* However, *Apatania sp.* contained various of alcohol and drug substance such as sodium bisulfate, dextrose anhydrous powder and D-erythrose. Dextrose anhydrous powder and D-erythrose are listed in Georgia State Crime Lab Sample Library as drug substance. The samples were secured as pure drug standards from the U.S. Drug Enforcement Administration. Dextrose Anhydrous is commonly utilized in food manufacturing as a nutrition supplement and a sweetener. Besides, sodium bisulfate is primarily used in metal finishing, cleaning products (John. T et al., 2005) and in swimming pools and hot tubs in order to reduce the pH of the water for efficient chlorination. Whereas in food is used as a food additive. Therefore, it can be concluded that these subtances contained in *Apatania sp.* due to the food waste streaming on the river. Overall, the microplastic contained in the biological construction were not the same. This is probably because of the limited number of specimens from only one species tested in our study may explain why we didn't detect more similarities between microplastics in larval cases. The microplastics are definitely harm the biological construction or the cases of aquatic insect due to the fact that plastics frequently leak hazardous chemicals and it may disrupt and weaken the stability of case species. For example, PVC contain phthalates which is high plasticiser (Hermabessiere et al., 2017) that are toxic to aquatic organisms (Capolupo et al., 2020). Thus, microplastic will affect the growth of the aquatic insect. In previous lab experiments, it has been proved that microplastics ingestion will affect the growth, survival and emergence (Ziajahromi et al., 2018) in Chironomied larvae.

4.0 CONCLUSION

Cellophane are the compound and polymer that predominant by all the four species of biological construction. The µFTIR spectroscopy result as shown above, *Ylodes sp.* contained the highest amount of microplastic detected by the FTIR followed by *Nectopsyche sp.* > *Gumaga sp.* > *Apatania sp.* Apart from that, each of the species were detected to have some cellulose compound which the cases are believed to uptake the cellulose while constructing the cases since cellulose is an organic compound. Larvae built the cases by collecting the different biotic such leaves and abiotic materials such sediment grains. Some species use both; biotic and abiotic (Hansell, 1972). In a nutshell, microplastics at biological construction diminish case stability and putting larvae in danger of being eaten by predators. As a result, the larvae's chances of survival will be limited (camouflage and respiration). Moreover, fish that ate the insect's biological construction, which contained microplastic, will enter the food chain and causing inflammatory reactions in the fish (Lu et al., 2016). Hence, the insect also will be negatively impacted by the microplastic in the ecosystem unintentionally.

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