DRAGONFLIES (INSECTA: ODONATA) AS HEAVY METAL BIOLOGICAL INDICATOR IN FRESHWATER ECOSYSTEM

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by

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LIST OF SYMBOLS

G	Gram
kg	Kilogram
L	Liter
m	Meter
mg	Miligram
mg/l	Miligram per liter
P	Significant
r	Correlation
µg	Microgram
µs/cm	MicroSiemens
%	Percentage
°C	Temperature in degree Celcius

LIST OF ABBREVIATIONS

AN	Ammonia Nitrogen
ANOVA	Analysis of Variance
APR	Ayer Puteh River
BOD	Biochemical Oxygen Demand
BSAF	Biota Sediment Accumulation Factor
CCME	Canadian Council of Ministers of the Environment
Cd	Cadmium
COD	Chemical Oxygen Demand
Cu	Copper
DO	Dissolved Oxygen
DOE	Department of Environment
Hg	Mercury
ISQG	Interim Freshwater Sediment Quality Guideline
KHTR	Kulim Hi Tech River
PEL	Probable Effect Level
Mn	Manganese
SPSS	Statistical package for Social Sciences
SR	Serdang River
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
USEPA	Environmental Protection Agency
WHO	World Health Organization

WQI	Water Quality Index
Zn	Zinc
pH	Potential hydrogen

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PEPATUNG (INSECTA: ODONATA) SEBAGAI PENUNJUK BIOLOGI LOGAM BERAT DALAM EKOSISTEM AIR TAWAR

ABSTRAK

Serangga akuatik merupakan penunjuk biologi yang berguna dalam pemantauan biologi sungai. Justeru itu, kajian ini dijalankan keatas larva pepatung dan tindak balas terhadap pendedahan logam berat yang terdapat di kebanyakan sungai-sungai. Empat spesis larva pepatung termasuk famili Coenagrionidae (*Pseudagrion microcephalum*, *Pruinsoum fraseri* dan *Ischnura senegalensis*) dan Platycnemidid (*Copera marginipes*) telah diambil daripada tiga sungai yang berbeza di Kedah. Berdasarkan Indeks Kualiti Air (WQI), ketiga-tiga sungai telah dikategorikan sebagai "sederhana bersih" (Kelas III) dan semua parameter persekitaran menunjukkan variasi ruang yang ketara dalam kalangan ketiga-tiga sungai ($F_{2,8} = 7.90$, $P = 0.00$) kecuali parameter suhu. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) telah digunakan untuk menganalisis empat jenis kepekatan logam berat (Cd, Mn, Zn and Cu) dalam air, sedimen dan larva Odonata. Analisis logam ini menunjukkan bahawa semua logam berat (Cd, Mn, Zn and Cu) terdapat di dalam air, sedimen, dan tisu larva Odonata. Walau bagaimanapun, kebanyakan kepekatan logam tinggi didapati paling besar pada tisu Odonata, khususnya Mn dan Zn. Analisis varians (ANOVA) menunjukkan bahawa semua kepekatan logam di *C. marginipes* secara statistik signifikan di kesemua tiga sungai berbanding dengan dua spesies lain yang dikaji. Ujian selanjutnya menggunakan analisis korelasi dan regresi mendapati bahawa pengumpulan logam Zn dan Mn dalam air dan sedimen telah mempengaruhi secara signifikan kandungan logam pada kedua-dua larva, *P. fraseri* dan *C. marginipes*, dengan nilai *r* yang signifikan pada $P < 0.05$. Namun, menurut nilai faktor pengumpulan sedimen biota (BSAF), semua larva Odonata menunjukkan hasil yang berbeza dengan kecenderungan bioakumulasi tinggi pada semua logam berat (BSAF > 1). Selain itu, analisis regresi juga menunjukkan bahawa COD, TSS, TDS, pH, dan kekonduksian mempengaruhi pengumpulan Cd, Mn, Zn dan Cu secara signifikan pada semua larva Odonata ($P < 0.05$). Untuk ketoksikan logam berat di dalam tetapan makmal, dua spesies Coenagrionidae larva; *P. microcephalum* dan *I. senegalensis* telah didedahkan kepada beberapa kepekatan (50, 100 and 200 mg/l) Cd, Mn dan Zn selama 7 hari bagi ujian kelangsungan hidup. Keputusan bioesei bagi Cd, Mn and Zn menunjukkan kesan toksik yang ketara terhadap daya tahan hidup untuk kedua-dua larva serangga *P. microcephalum* ($F_{11,180} = 14.50$, $P = 0.00$) dan *I. senegalensis* ($F_{11,180} = 15.10$, $P = 0.00$). Walau bagaimanapun, tiada perbezaan yang signifikan dalam larva yang masih hidup di antara kedua spesies tersebut pada semua logam berat setelah 7 hari tempoh pendedahan. Di antara kesemua logam berat tersebut, Cd mempunyai nilai LC_{50} dan LT_{50} yang terendah, menunjukkan bahawa ketoksikan tinggi Cd berlaku dalam tempoh masa yang singkat pada dua larva Odonata berbanding Mn and Zn..

DRAGONFLIES (INSECTA: ODONATA) AS HEAVY METAL BIOLOGICAL INDICATOR IN FRESHWATER ECOSYSTEM

ABSTRACT

Aquatic insects are a useful biological indicator in river biomonitoring. Therefore, a this study was conducted on Odonata larvae and their responses to the heavy metals exposure found in most rivers. Four species of dragonflies larvae, including a Coenagrionid family (*Pseudagrion microcephalum*, *Pruinsoum fraseri* and *Ischnura senegalensis*) and Platycnemidid (*Copera marginipes*), were collected from three different rivers in Kedah. Based on the Water Quality Index (WQI), all the three rivers were categorized as "moderately clean" (Class III) and all the environmental parameters showed significant spatial variation among the three rivers ($F_{2,8}= 7.90$, $P= 0.00$) except temperature. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to analyze four heavy metals (Cd, Mn, Zn and Cd) concentrations in water, sediment and Odonata larvae. This metal analysis showed that all the metals were found in water, sediment, and the tissue of the Odonata larvae. However, most heavy metals concentrations were found greatest in Odonata tissue, specifically Mn and Zn. Analysis of variance (ANOVA) showed that all the concentration of metals in *C. marginipes* were statistically significant in all three rivers compared to the other two species studied. Further test using correlation and regression analysis found that the accumulation of Zn and Mn metals in water and sediment had significantly influenced the content of metals on both larvae, *P. fraseri* and *C. marginipes*, with a significant r-value at $P < 0.05$. However, according to the value of the biota sediment accumulation factor (BSAF), all Odonata larvae demonstrated different results with a

high tendency of bioaccumulation on all of the heavy metals (BSAF > 1). Beside that, regression analysis also showed that COD, TSS, TDS, pH, and conductivity significantly influenced the accumulation of Cd, Mn, Zn and Cu in all Odonata larvae ($P < 0.05$). For metal toxicity bioassay in the laboratory, two species of Coenagrionidae larvae; *P. microcephalum* and *I. senegalensis* were exposed to a different concentration (50, 100 and 200 mg/l) of Cd, Mn, and Zn for 7-day survival experiments. Bioassay results for Cd, Mn and Zn shows a significant toxicity effect towards the survivability of both larvae; *P. microcephalum* ($F_{11,180} = 14.50$, $P = 0.00$) and *I. senegalensis* ($F_{11,180} = 15.10$, $P = 0.00$) at all concentration. However, no significant difference in surviving larvae among the two species was observed in all heavy metals after 7 days of exposure. Among the heavy metals, Cd had the lowest LC_{50} and LT_{50} value, reflecting that the high toxicity of Cd took effect within a short period on both Odonata larvae compared to Mn and Zn.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Heavy metals and contamination, especially in aquatic ecosystems, have posed a significant environmental threat in recent decades (Ali *et al.*, 2013). According to Mani *et al.*, (2015), a persistent surge in metal pollution in aquatic ecosystems was primarily driven by urban development, agriculture, and industrialization, resulting in degradation of aquatic environments and threats to aquatic species' survival. Because of their long presence and deposition in sediments and living organisms' tissues, heavy metals from anthropogenic origins pose significant risks to the atmosphere and organisms (Lavoie *et al.*, 2013; Schertzinger *et al.*, 2018). Metals such as Cadmium (Cd), Manganese (Mn), Zinc (Zn), and Copper (Cu) may pose an increasing concern in aquatic environments due to their increased toxicity that caused a potential risk to live species for several years (Abdel-Baki *et al.*, 2011). This scenario is due to the bioavailability and bioaccumulation of heavy metals over time (Gaikwad and Kamble 2014). The accumulation of metals in sediments and aquatic organisms represents an unhealthy relationship in transferring metals to the upper trophic level in the food web (Gaikwad and Kamble, 2014). Pollutants like Mn and Zn are essential metals since they are cofactors for enzymes and components of the respiratory pigment (hemocyanin). However, higher concentrations of such metals, such as Cd, have a poisonous effect on cells. Also, their ions may have significant consequences for living beings' well-being at low concentrations (Despotović, 2013). According to the study conducted by Iqra Azam *et al.*, (2015), the influence of metal and environmental pollution has harmed aquatic ecosystems, and a biomonitoring program using aquatic insects is needed as a first alarm to avoid serious consequences in the future. Besides that, according to Zaghloul *et al.* (2020), biomonitoring methods had applied as an alternative method to measure aquatic ecosystem health that is linked to physical, chemical, and biological components that involve the use of indicator organisms or indicator groups. Besides, aquatic biomonitoring was often been used to assess water quality and detect contamination before it causes a decline in environmental and species health (Jones *et al.,* 2010). Odonates are a vital component of aquatic environments, where they can also be top predators, and their vulnerability to environmental factors makes them outstanding biological markers of environmental conditions (Balzan *et al.,* 2012; Bhandari *et al.,* 2016). Many studies also have indicated that Odonata is also sensitive to physical disturbance (Dolny *et al.,* 2012; Junior *et al.,* 2015). The association of Odonata with their habitats that include their useful importance within freshwater ecosystems, and their interrelation with other species and resources, make an overview of Odonata communities an important tool for characterizing and assessing the water assemblage through their function as indicators of ecosystem quality (Balzan *et al.,* 2012).

For that reason, studies by Silva *et al.,* (2010) and Zaghloul *et al.,* (2020) summarized several factors that mark Odonata as a well-suited bioindicator including the fact that they are abundant and tolerate a wide range of chemical, physical and biological conditions in a freshwater ecosystem. They are an excellent model organism for studying terrestrial and river environments because they are vulnerable to a variety of stressors, including toxins (Ferreras-Romero *et al.,* 2009; Abdul *et al.,* 2017) and temperature fluctuations (Hassall and Thompson, 2008; Clausnitzer *et al.,* 2009; Chaves *et al.,* 2015), and thus can be used to measure river environmental conditions. They also can be used as bioindicators in both water and terrestrial environments as they are aquatic during larvae and adults as terrestrial (Zaghloul *et al.*, 2020). Furthermore, some Odonata larvae responses can signal a risk of heavy metal toxicity in the early stages of pollution (Gremyatchikh *et al.*, 2009), and tracking changes at the individual level is more comprehensive than monitoring changes at the population level for purposes of the aquatic environment biomonitoring (Al-Shami *et al.*, 2013).

Studies on heavy metals with Odonata as a biological indicator has been widely reported from other countries related to a different type of environmental pollution (Corbi *et al.*, 2008, 2010; 2011; Girgin *et al.*, 2010; Nasirian *et al.*, 2017; Simon *et al.*, 2017; Guimarães *et al.*, 2019; Addo-Bediako *et al.*, 2020; Girardin *et al.*, 2020). Besides that, in Malaysia, Odonata's larvae have recently been incorporated as heavy metal bioindicator tools in the rivers (Al-Shami *et al.*, 2014; Suhaila *et al.*, 2016). Out of all the Odonata larvae, several genera, including *Copera*, *Ischnura* and *Pseudagrion*, are the most tolerant and can survive within a specific range of metals contamination (Abdul *et al.*, 2017). Besides, changes in water quality parameters with different levels of metals content in both water and sediment might affect the biology and survivability of Odonata larvae in freshwater ecosystems. Hence, this study is one piece of comprehensive approach experiments to provide a clearer view and information regarding the effect of metals contamination on the biology of Odonata larvae species such as *Pseudagrion microcephalum*, *Pruinosum fraseri*, *Ischnura senegalensis* (Family: Coenagrionidae) and *Copera marginipes* (Family: Platycnemididae). Therefore, this research can produce valuable knowledge on analyzing metals contamination and exposure related to water, sediment and Odonata tissue. This biological water quality analysis would help researchers to have better understand on the importance of using aquatic insects as an early warning indication for heavy metal pollution and complement the current chemical water quality control strategy to have better water resources management in the future. Thus, this study was conducted on the aquatic insects related to heavy metal contamination with the following objectives:

1.2 Objectives

1) To determine the water quality status and heavy metal content in water.

2) To investigate the influence of heavy metals content between water and sediment with Odonata tissue.

3) To study the effect of heavy metals exposure on Odonata larvae survivability.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metals toxicity in freshwater ecosystems

Heavy metal was defined as a group of metals and metalloid elements with relatively high weight and density compared to other forms of water. Heavy metals are also toxic even at low-level concentrations (Tchounwou *et al.,* 2012), while Hazrat Ali *et al.,* (2019) stated that heavy metals are naturally occurring metals that contain an atomic number greater than 20 and an elemental mass greater than 5 g cm^3. In addition, heavy metals, a natural constituent that exist in the ecosystem, are a source for water contaminations, pollution and can be the potential to become more toxic to the environment. All these metals are generally termed heavy metals that include Copper (Cu), Cadmium (Cd), Manganese (Mn) and Zinc (Zn). This category of metals has not only been known for its high density but, most importantly, for its adverse effects on the freshwater ecosystem and living organisms (Rezania *et al.,* 2016).

Heavy metal contamination in freshwater or river environments can be a major problem with significant environmental and biological consequences. According to Zhang *et al.,* (2016) heavy metals are continuously released in aquatic systems because of different anthropogenic and anthropic sources. Industrialization and urbanization, for example, have contaminated the atmosphere, and their rates of mobilization and transportation in the environment have risen dramatically over time. Furthermore, metals are readily dissolved in water and ingested by aquatic organisms such as aquatic invertebrates, resulting in a wide variety of biological effects ranging from important for living organisms to poisonous and harmful (Gheorghe *et al.,* 2017). Low concentrations of certain metals were very necessary for living organisms, for example, micronutrients (copper, manganese and zinc) and macronutrients (Calcium, Sodium and phosphorus). Meanwhile, high concentrations of these heavy metals can have detrimental effects on organisms' development, metabolism, and reproduction, with implications for freshwater environments' entire trophic stage and trophic network. Heavy metals are very notable and important environmental contaminants, according to Nagajyoti *et al.*, (2010) and Jaishankar *et al.*, (2013). Their toxicity is an issue of growing importance for ecological and environmental basis. Thus, the heavy metals may reach a toxic concentration level that can potentially damage the ecological environment (Jaishankar *et al.,* 2014). Heavy metals contamination and bioaccumulation may have an overwhelming impact on the receiver environment's ecological balance, food chain, and aquatic organisms' diversity.

Corbi *et al.* (2010) and Hazrat Ali *et al.,* (2019) summarized that vertical (trophic level) and horizontal (diet at any given trophic level) locations on the web of foods may be regarded as key for determining the metal concentrations in tissues of various species types when transferred through macroinvertebrate food webs. Furthermore, food chains symbolize the relationships amongst organisms and the contamination can directly affect all organisms (Corbi *et al.,* 2010). Therefore, heavy metals can reach the aquatic food chain via trophic transfer through biomagnification and bioaccumulation of metals in predators, including Odonata larvae (Kraus *et al.,* 2014). Thus, metals have two impacts on the aquatic insects related to the food chain in freshwater ecosystems. This is because (1) the toxic effect from metal exposure on aquatic insects could alter the nutrient transport, where a reduction in emerging adults or metamorphosis will reduce the nutrient flux to the terrestrial environment (Kraus *et al.*, 2014; 2019). (2) Aquatic systems could also act as a source of contaminants by emerging aquatic insects transporting metals that they have accumulated during their larval stage, exposing terrestrial insectivores to heavy metal toxicity from the aquatic ecosystems. (3) High level of metals toxicity was ranked among the priority metals in environmental health and considered systemic toxicants that cause considerable tissue damage and affect the entire tissue rather than a specific site even at lower levels of metal exposure (Tchounwou *et al.,* 2012). In general, Hazrat Ali *et al.,* (2019) summarize that the biomagnification of these metals in food chains can enhance a greater risk for organisms at higher trophic levels in the food chains that can directly pose a health risk to these organisms (tertiary consumer) or their human consumers. Therefore, heavy metal toxicity on aquatic organisms is an integral part of environmental research because they have different toxicity and bioaccumulation properties through the food chain (Velma *et al.*, 2010).

2.2 Description of heavy metals (Cd, Mn, Zn and Cu) characteristics

The essential element is the elements required by living organisms for any metabolism function for organisms like growth, development, biochemical and physiological function (Hazrat Ali *et al.,* 2019). Non-essential components, on the other hand, have no established biological role in an organism's body and can become toxins that pose a danger to other species in the ecosystem. For example, Mn, Cu and Zn are essential heavy metals, are also highly poisonous if present in high amounts of concentrations (Sauliute and Svecevicius, 2015). According to Gao *et al.,* (2013) the study on the essentials of heavy metals and toxicity was significant because the essential heavy metals like Mn, Zn and Cu were categorized as a narrow window between their essentiality and toxicity. As a result, certain metals are among the most environmentally hazardous heavy metals (Gao *et al.,* 2013). Meanwhile, the four environmentally most hazardous heavy metals and metalloids like Cd and Hg are generally classified as non-essential elements. Cd was the most toxic and hazardous metal (Dissanayake and Chandrajith 2009). The route of Cd exposure releases to the ecosystem can be observed from a natural occurrence and anthropogenic activities from humans. Anthropogenic activity releases are mainly from the mobilization of Cd impurities in unprocessed materials such as phosphate minerals and fossil fuels finally been releases by manufacturing disposed of and incineration process of products intentionally into the water (UNEP, 2010). When Cd has been introduced to freshwaters ecosystems, a great amount of the metal precipitates and resides in the bottom of sediments. Thus, sediment may be a significant source for Cd to precipitated or settled in the aquatic environment (Dissanayake and Chandrajith, 2009). However, the effects of Cd on aquatic organisms such as aquatic insects and other macroinvertebrates can be directly or indirectly affect the populations and ecosystems.

Manganese (Mn) is a naturally occurring mineral found in rock, soil, and water, and Mn is present in the entire world, accounting for around 0.1% of the Earth's crusts (WHO, 2004). The primary component of Mn on sediments, according to WHO (2004), are atmospheric precipitation, wash-off from plant and other waste, leaching from plant tissues, and excretion of material such as leaves, dead plant and animal material. Furthermore, Mn was generated from various sources, including urban wastewater discharges, sewage sludge, mining and mineral processing, pollution from manufacturing operations such as steel and aluminium manufacture, and fossil fuel combustion. Mn in water can dissociate into Mn^{2+} which is significantly bioaccumulated by aquatic biota at lower trophic levels towards the upper trophic level. However, Ben Shahar *et al.,* (2018) reported that the uptake of Mn by aquatic invertebrates including aquatic insects was influenced by several environmental parameters such as water temperature, pH, dissolved oxygen and salinity. High concentrations of Mn were influenced by water temperature, pH, and salinity related to hydrogen ions in water.

Zn (Zn) is an essential element that serves as a structural component and has unique properties needed by aquatic organisms (Tahmina Hoq *et al.,* 2014). Zn is required for many biological processes, including protein, carbohydrate, lipid metabolism, and more advanced functions. Not only that, but Zn also plays a significant role in the immune system, neurotransmission, and cell signaling in insects. The persistence of Zn toxicity in the environment is aggravated because Zn cannot be directly destroyed but is only transform on a reduction form like oxidation state or organic complex (Tahmina Hoq *et al.,* 2014). Zn bioaccumulation represents the amount of metal absorbed by the organism by various ingestion routes, how the metals are dispersed among different tissues such as gills and the degree to which the metal is stored in each tissue type over different exposure periods. Thus, the accumulation of Zn has attained a severe issue causing a toxicity stage for an aquatic species. However, Zn in specific concentrations is very needed by freshwater organisms for growth. Still, if the concentration was over accumulation, Zn would become more hazardous to exposed organisms that consume high-rate Zn metals, whether directly or indirectly through the food chain in freshwater ecosystems.

Copper is a trace metal that is essential micronutrient for the regular growth and cellular respiration for living organism (Padrilah *et al.*, 2018).). Copper also acts as a catalytic co-factor for at least 12 major proteins and 30 different enzymes (Ajani and Akpoilih, 2010) responsible for countless metabolic processes required to sustain life. However, if this element is utilised above its limit, it can transform into continuous metal compounds that can build in water and disrupt the biological system (Padrilah *et al.,* 2018). Copper toxicity occurs when copper enters the cells and attaches to proteins and nucleic acids, causing normal cellular function to be disrupted. Within cells, copper may shift between Cu^{2+} and Cu^{1+} oxidation states, allowing it to precipitate in the Fenton reaction and generate free radicals such as the extremely damaging hydroxyl radical (Balamurugan and Schaffner, 2006). As a result, copper is a non-biodegradable substance that cannot be broken down once it begins cellular functions. However, it can be be easily absorbed and bioaccumulated in organs and cellular processes. (Ajani and Akpoilih, 2010).

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2.3 Heavy metal bioavailability and bioaccumulation to aquatic organisms

Metals are difficult to break down into less toxic compounds and making metals lack biodegradability. The biodegradability of metals in the environment has caused the metals to continuously redistributed throughout the water column after being introduced into the aquatic environment and directly accumulated in sediments or consumed by biota (Fremion *et al.*, 2016). The sediments are a long-term source of impurity or pollution to the environment due to metal dissociation, absorption and remobilization processes. This process can be supported by the statement from Gheorghe *et al.*, (2017) that specify the metal accumulation in sediments occurs as a result of metal compound precipitation, which is linked to the physical and chemical conditions that exist between the sediment and the river's water column (Equeenuddin *et al.*, 2013). As the content of metals in the sediment increases, an extensive amount of metal bioavailability resides in the sediment.

Metal bioavailability, as defined by Gheorghe *et al.*, (2017), is the selection of the total concentration of the metal that has the potential to be absorbed in the body of organisms and directly combined into metabolic processes. The fraction of metals absorbed interacts with receptors and physiological sites in the body's metabolism, causing toxic effects directly (Rainbow and Luoma, 2011). Several factors control the bioavailability of metals; (1) they are biology aspect (metal assimilation and development stage), (2) metal chemistry (distribution of metals in sediment and suspended matters) (Roosa *et al.*, 2014), (3) physical and chemical factors (pH, temperature, salinity and total suspended solids) (Fu *et al.*, 2014; Bonnail *et al.*, 2016). The proportions of water and food consumed by insects for a given metal would be determined by the bioavailable metal concentration associated with each source and the process and rate by which the metal reaches the insect bodies. According to

Gheorghe *et al.,* (2017) metal bioavailability regulates the amount of metal accumulates in aquatic life. If they are particulate, direct absorption of metals routes is through the permeable epidermis or through food absorption. In this sense, metal bioaccumulation may be compared to the trophic stage of the aquatic insects' studies and metal bioaccumulation could occur in a cascading phase with higher concentrations in species at higher trophic levels, such as fishes and other vertebrates. In assessing water quality and environmental problems, the monitoring approach of toxicity and bioaccumulation of metals in aquatic biota or sediment is critical for various ecological monitoring (Schafer *et al.,* 2015).

Gheorghe *et al.,* (2017) stated that the pollutants of heavy metals could be uptake by an organism in different ways such as directly from the ingestion of particles from the environment (food or sediment particle ingestion) and indirectly accumulation that can occur through water via epidermis and gills. Then, the heavy metals were transported inside the cells through protein and biological membranes that allow ions to pass through the channel pore when an organism absorbs toxic chemicals faster than the chemical was metabolized (Ashish and Amitabh, 2014). Understanding the bioaccumulation process is vital because persistent pollutants like heavy metals exposed in high concentrations could increase the potential toxic risk by bioaccumulation in the ecosystem. In addition to this, there are many studies related to heavy metals contamination with aquatic insects in freshwater ecosystems including mayfly (*Baetis pavidus* and *Ephemera danica*), aquatic beetles *(Hydroglyphus pusillus* and *Laccophilus minutus*) and caddisfly larvae (*Hydropsyche angustipennis*) (Burghelea *et al.,* 2011; Tszydel *et al.,* 2016; Bouchelouche *et al.,* 2019; Bozanic *et al.,* 2019; Mebane *et al.,* 2020; Lidman *et al.,* 2020). However, Odonata larvae have been used extensively for biomonitoring in many countries related to the accumulation of heavy metals. For example, a study by Corbi and Trivinho-Strixino, (2008); Corbi *et al.,* (2010) shows that the bioaccumulation of Cd, Cu, Mn and Zn from the sediment to some species of aquatic insects like *Dasythemis* sp., and *Erythemis* sp.,(Odonata: Libellulidae) was impacted from fertilizers residue near the sugar cane cultivation. In addition, Iqra Azam *et al.,* (2015) used many insect species in an industrial environment, including *Crocothemis servilia* (Odonata: Libellulidae), because of increased metal concentrations due to bioaccumulation of Cu, Cd, and Zn within the insects' bodies. Simon *et al.,* (2017) reported that there is an accumulation of heavy metals based on trace element concentrations of Cu, Mn, Pb and Zn between sediment and *Gomphus flavipes* (Odonata: Gomphidae) larvae. A recent observation from Addo-Bediako *et al.,* (2020) showed a tendency for bioaccumulation for almost all of the selected elements in the insect because the content of heavy metals was significantly higher in the tissue of insects than in the sediments. Based on previous research, understanding the bioaccumulation mechanism is critical because persistent contaminants such as metals can increase the potential toxic danger in the environment through bioaccumulation, resulting in a long-term impact that cannot be measured through laboratory toxicity tests (Iordache, 2009, Iordache *et al.,* 2015). According to Iordache (2009), the amplification of metal concentrations in ecological food chains is dependent on the form of metal and the food chain. Metal residues in polluted environments will bioaccumulate in aquatic ecosystems, causing harm to aquatic flora and fauna (Hasan *et al.,* 2016).

2.3.1 Biota-sediment accumulation factors (BSAF)

According to the American Society for Testing and Materials (ASTM, 2010), bioaccumulation is the net amount accumulation of a chemical by a specific organism that can accumulate metals through all respiration, ingestion, or direct contact with contaminated water and sediment. Bioaccumulation was described by the United States Geological Survey Toxic Substances Hydrology Program (USGS) as the biological absorption of heavy metals at a higher concentration than occurs in the surrounding medium (water or sediment). To be specific, bioaccumulation is the deposition of a contaminant of heavy metals in an organism because of uptake from the ambient abiotic environment and the organism's food or diet (Hazrat Ali *et al.*, 2019). On the other hand, an earlier study by Burkhard *et al.*, (2003; 2005) observed that BSAF is the ratio of a substance’s concentration in the tissue of an aquatic organism (lipid content) with a concentration in the sediment (organic carbon). Aside from that, several other researchers have used the term biota-sediments accumulation factor (BSAF) and measured it using the equation below:

$$\text{BSAF (Biota Sediments Accumulation Factor)} =$$

$$\frac{\text{Metal concentration in biota (Mass of metals per kg of biota/dry weight)}}{\text{Metal concentration in sediment (Mass of metals per kg of sediment/dry weight)}}$$

(Nenciu *et al.*, 2014; Ziyaadini *et al.*, 2017)

No analysis or recommendation can be used as a threshold value for the Biota-Sediment Bioaccumulation Factor (BSAF), since BSAF values are often dependent on the physical-chemical properties of both the metals and the sediment, as well as the lipid quality of the organism that the chemical bioaccumulates into (Nenciu *et al.*, 2014). However, based on the calculated values above, Ziyaadini *et al.*, (2017) stated that different types of species in water (only marine) environment can be classified into three basic groups, such as macro-concentrator (BSAF > 2), micro-concentrator (1 < BSAF) or de-concentrator (BSAF < 1). This group was created based on toxicity that organisms can directly absorb from the atmosphere by intake or absorption of heavy metals, and accumulation happens when the toxicity of a harmful metal is absorbed at a higher rate than it is metabolized (Nenciu *et al.*, 2016).

2.3.2 Effect of environmental parameter with the bioaccumulation of metals into Odonata larvae

Toxicology's fundamental states that any element can be poisonous, and the relationship of dose-response in the bioaccumulation phase can determine the degree of toxicity. Thus, the toxicity of metals can depend on the exposure dose and chemical and physical factors on metals bioavailability and accumulation. Knowledge of the environmental factor that affects the bioaccumulation of heavy metals in water is essential in determining their potential toxicity and mobility in the environment (Magalhaes *et al.,* 2015). Firstly, pH is an important factor that influences the action and behavior of metals' mobility in the environment (Magalhaes *et al.*, 2015). Low availability of metals will occur when the pH value is around pH 6.5 to 7.0. The degree of hydrolysis, the combination of chemical processes, aggregation and precipitation, and proton competition for available molecules are all affected by pH (Magalhaes *et al.*, 2015). Low pH stimulates and weakens dissociation on metals, enhancing their solubility and, as a result, their toxicity. In contrast, alkaline pH causes metals to precipitate as oxides and hydroxides, making them less bioavailable to the sediment, less toxic, and promoting adsorption and precipitation (Ma *et al.,* 2016).

According to Li *et al.,* (2013) high water temperature in freshwater ecosystems will increase the release rates of heavy metals both in water and sediment that caused the carbonates and hydroxides to disband at a faster rate. As a result, the metal release rate of the water-soluble fraction and other fractions from the sediments into the overlying water increased in freshwater environments. This evidence was supported by Li *et al., (*2013) which indicated that the release rate of heavy metal was more significant at high temperatures than low temperatures. With increases in electrical conductivity, which is connect to anion and cation, the adsorption content of heavy metals on sediment also decreases gradually. Therefore, the overall absorption of heavy metals in the water from the sediment would be reduced due to increased competition among heavy metals and certain other cations (Katip *et al.,* 2012). In addition, dissolved oxygen (DO) also has a role in determining the contents of heavy metals in sediments (Huang *et al.,* 2017). Therefore, the metals released from sediment into the water are higher when the amount of dissolved oxygen increases. Thus, it could be because it contains high organic matter contents (decaying plants and animals) and is under the high oxygen content (aerobic) condition. Besides, the oxidation of organic compound rate is higher than that under the anaerobic condition can cause high rates of metals release. From that, the release of heavy metals will become more improve and seen to be higher with higher of DO value (Li *et al.,* 2013).

Similarly, biochemical oxygen demand (BOD) is characterized as the biochemical splitting (degradation) of organic matter by microorganisms in the presence of oxygen required for their formation (Li *et al.,* 2013). However, the presence of some toxic metals like Cu, Zn and other heavy metals will influence the process and function of the organisms (Mittal and Ratra, 2000). Because of their toxicity, the presence of all of these metals in excessive concentrations can interfere with specific beneficial uses of water. Metal ion toxicity to microorganisms can be fatal or dangerous, depending on the type and concentration of the ion from heavy metals (Mittal and Ratra, 2000). Meanwhile, chemical oxygen demand (COD) is oxygen equivalent to the organic matter in water consumed by oxidation in strong oxidizing agents (Yao *et al.,* 2014). High decomposition of organic matter in the rivers may be ascribed to the high concentration of heavy inorganic metals like Cd and Zn (Mutlu *et al.,* 2016). Higher COD levels indicate more oxidizable organic content in the water sample that can lower dissolved oxygen levels and increase heavy metal distribution efficacy in the water or sediment (Mutlu *et al.*, 2016). Therefore, toxic metals are readily transported through suspended solids towards rivers. In contrast, inorganic chemicals are non-degradable because inorganic chemicals have the non-existence of hydrogen and oxygen to combine with other compounds (Liu *et al.*, 2015; Goa *et al.*, 2016). Thus, suspended sediments can play a role in metal mass flux and relocation, with suspended particles acting as vectors for potentially bioavailable metal species (Nasrabadi *et al.*, 2018).

TSS, or total suspended solids, are too large to get through the filter that separates them from the water. Dissolved solids refer to smaller particles as well as ionic compounds. High concentrations of suspended solids can settle out onto a river bottom and cover aquatic organisms, preventing sufficient oxygen transfer and resulting in the death of organisms. Many organic and inorganic pollutants absorb sediments so that the pollutant concentrations on the residues solids are high. From that, it can be transported along the river, resulting in the exposure of organisms to pollutants. Similar to ammonia, Abou-Eleala *et al.*, (2012) indicated that both molecular ammonia (NH3) and ammonia in the form of the ammonium ion (NH4+) occur in equilibrium as soluble ammonia. The relative concentration of both is affected by pH and temperature. Higher pH and temperature promote the production of higher ionization of ammonia, which causes increases in the concentration of ammonia ion toxicity (Abou-Eleala *et al.*, 2012).

2.4 Bioassay and toxicity studies related the implication of heavy metal exposure towards insect survivability

As stated by Krishnan (2019), the purpose of direct toxicity testing or assessment is to determine whether a compound or water sample has the potential to be toxic to biological organisms, including aquatic insects and if so to what extent (Luesch and Chapman, 2011). Besides that, toxicity testing has been widely used as (a tool to identify suitable organisms as a bioindicator and derive water quality standards for chemicals. Toxicity testing also is one of the essential tools for assessing the effect and fate of toxicants in aquatic ecosystems, including aquatic insects. In evaluating the safety of chemical substances and for regulatory purposes, it is necessary to have precise data on the chemical and its effects on organisms (Krishnan, 2019). Therefore, acute toxicity studies can provide fast and valuable information and indicate whether further toxicity studies should be conducted and help explain toxic effects (Luesch and Chapman, 2011).

Toxicity can be evaluated in whole organisms or using molecules or cells. The main advantage of toxicity testing is that it detects toxic compounds based on their biological activity. As such, it does not require prior knowledge of the toxicant to identify its presence, unlike chemical analysis (Blaise and Férard, 2005). The organisms are exposed to the chemicals or mixtures of interest and monitored for any sign of adverse health effects. This can be either a gross morphological effect (such as weight loss, visible lesions, death or survivability) or more subtle biochemical markers, whether biomarkers of exposure (an indicator of the internal dose) or biomarkers of effect (an indicator of a health effect, such as enzyme activity). The duration of the exposure depends on the type of toxicity monitored, from short-term acute effects (96h or less), sub-acute (a couple of days), sub-chronic (a couple of weeks) to the chronic impacts (a significant portion of the organism's life expectancy) (Leusch and Chapman, 2011). Since toxicity is based on the effect that a toxicant produces at a target site within an organism, establishing the relationship between the concentration of a substance at the target site, including tissue of aquatic insects and the subsequent toxic effect can provide a tool for predicting toxicity. The behavior of a single toxicant could not be fully understood without knowing the physical and biochemical properties of substances that can change (Mansouri *et al.,* 2011). According to Khaliq *et al.,* (2014), insects are rapid adaptive organisms and can tolerate an extreme environment, including high response resistance on the toxicity of heavy metals. Insects still can produce an abundance of offspring or new growth and have a short life cycle even though human interruption can disturb the insect population.

Anthropogenic (human activity and pollution) and natural environmental changes (climate change) are insatiable, affecting insects at various times. For example, both abiotic (temperature, water and light) and biotic (producer and consumer in trophic level, vegetative and agro-biodiversity and stress effector) stresses significantly influence the insects and their population distribution related to ecosystem and dynamics. Thus, in response to these factors, insects may prolong their metamorphic stages and survivability rate. Apart from that, research was done by Cordero–Rivera *et al,* (2019), which gathered a large dataset about survivability and insects' behavior, allowing a good understanding of their survivability strategies in any food web ecosystem. Damselflies (Odonata) which act as predator species, for example, are an essential component of the river food chain before emerging as adults (Stoks and Córdoba-Aguilar, 2012). This processes that can contribute to an energy flux from the river to the terrestrial environment (Stoks and Córdoba-Aguilar, 2012) for maintaining the survivability of the Odonata larvae. As a result, any adjustments or modifications, such as heavy metals, can impact their longevity, development, or physiology, including critical fatty acid composition. As a result, growth can affect food web structure and energy flow within surface waters and across habitat boundaries (Schulz *et al.*, 2015). Furthermore, the effect from urbanization, agricultural development and any other major factor can directly impact the damselflies aquatic habitats (Schulz *et al.*, 2015). Therefore, Odonata had several capabilities to maintain their survivability on the population throughout the environmental-stressors. According to Finotello *et al.,* (2017), Odonata can sequestrate their body and act as detoxification processes to eliminate the unwanted chemical body for survivability. For example, even in high concentrations of metals or pollution, Odonata is always consistent in capturing the prey and increasing the energy to actively forage and maintain tolerability, although energetically costly (Combes *et al.*, 2013).

Similarly, to maintain their survivability, Odonata can also increase the number of protein and antioxidant enzymes that can be used as protection against its cellular damage (Combes *et al.*, 2013). Interestingly, it will contribute to the constant foraging activity for the high survivability rate of Odonata in finding prey even in high pollutants habitats. Finotello *et al.*, (2017), for example, found that the measured LC_{50} values declined over time as mortality increased, implying that the Odonata had recovered and moved on to detoxifying results. Besides that, Odonata can maintain the survival rate by sustaining the number of feeding in prey-predator interaction (Finotello *et al.*, 2017). Even under heavy metal exposure, the efficiency of Odonata preying is still unaffected. Contradict from that, He *et al.,* (2008) observed that the percentage of strikes against prey decreases because of heavy metals' toxic mode of action, which causes paralysis and the lack of the capacity to perform synchronized motions in exposed organisms.

Lastly, to maintain the survivability of heavy metal induction, reducing growth rate, including body length and lipid content, is very important to Odonata because they need higher energy requirements for detoxification and defence mechanisms under heavy metal exposure. Based on the study done by Finotello *et al.,* (2017), the fatty acids composition can allow a more detailed interpretation of the induction of heavy metals towards the physiological effects on the species. The study showed that the composition of fatty acid in damselflies was significantly shifted and experiencing a reproduce peak of high exposure fatty acids. Therefore, Odonata growth was directly affected by the heavy metals, but Odonata's survivability is still high under a stressful environment. Changes in a fatty acid composition may have consequences for damselflies at the individual stage, such as reduced growth and increased mortality and predators of damselflies, which may be affected by changes in the fatty acid composition of their prey. Furthermore, because environmental factors can influence the health and survivability of insects during their adult stages through carry-over effects, lower body mass and energy reserves at emergence have a negative impact on survival to maturation and reproduction in the terrestrial stage (Stoks and Córdoba-Aguilar, 2012). Related heavy metals concentrations in the field are typically minimal, implying that the bulk of the results are unlikely to occur in agricultural and industrialized streams due to heavy metals toxicity alone.

2.5 Odonata larvae as bioindicator of heavy metals

Heavy metal contamination is a major source of concern for river management. Heavy metal accumulation of waterways may be caused by anthropogenic activities such as drainage water from farming, recreational, and industrial areas. For example, when introduced into an aquatic system, the interaction with the variable of the water column can greatly modify the ecosystems of the environment, and they can be toxic from the degradation processes (Qu *et al.,* 2010). As a result, heavy metals assimilated by animals can interact with physical and chemical functions of the organism, such as reproductive factors and species survival, resulting in population and community structure changes (Qu *et al.,* 2010). In addition, Gray and Delaney (2008) also observed that the decline of aquatic insect species richness insects is caused by the high toxicity of heavy metals and density, growth and production. Furthermore, heavy metals can accumulate in insect's guts and tissues (Sola and Prat, 2006), via the food system and directly affect the predator-prey relationships in macroinvertebrate and aquatic ecosystems (Watanabe *et al.,* 2008).

Environmental consequences of particular high pollution were difficult to measure and identify even in many polluted areas and after long exposure. Therefore, Lorenzi *et al.,* (2008) stated that using an aquatic organism such as aquatic invertebrates will make it possible to be implemented in biomonitoring programs in freshwater and help to indicate the implications of pollutants in the environment specifically related to heavy metals study. Despite the Odonata's low diversity compared to other insect orders, the Odonata exhibit preferences for particular requirements and environments (Bybee *et al.,* 2016). They are also important ecologically since they are large predators and are recognized as strong indicators of the state of river ecosystems. According to Yule and Yong (2004), there are 342 designated species of Odonata in Malaysia, including 161 species of Zygoptera in ten families. Sabah, Sarawak, and Brunei have 239 species, with Peninsular Malaysia having 226. As been stated by Yule and Yong (2004), the family of Coenagrionidae from Zygoptera has the highest number of species (33 species) occurring in Peninsular Malaysia. Hence, by selecting Odonata larvae as tools for biomonitoring program, they have two obvious advantages: (1) It refers to a period (the larvae must have been in the water for at least several weeks) rather than a single chemical sample taken at a certain time that may or may not be indicative of conditions over a longer period. (2) It's low-cost and can be performed at any time of year, using either mature larvae or adults or both, depending on the time of year and the experiment's goals. Not only that, they are ubiquitous and relatively easy to be observed and identified.

They are extensively used as dependent variables which interconnected with the ecological conditions of the environment. Besides, Odonata larvae also interact with the environmental condition because they inhabit the sediments between rocks or leaves and are associated with macrophytes. Al-Shami *et al.,* (2013) supported this condition, which stated that Odonata larvae show a different distribution pattern at different spatial and temporal scales and in various chemical and physical settings. Furthermore, according to Souza *et al.*, (2015), changes in habitat composition caused by water contamination, such as heavy metals, significantly impact the diversity and distribution of Odonata larvae. This increases their potential to be applied as an indicator for environmental pollution in the aquatic ecosystem (Berquier *et al.*, 2016).