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Monitoring Heavy Metal Bioaccumulation in Rivers Using Damselflies (Insecta: Odonata , Zygoptera) as Biological Indicator

(Memantau Bioakumulasi Logam Berat di dalam Sungai menggunakan Pepatung Jarum (Insecta: Odonata, Zygoptera) sebagai Penunjuk Biologi)

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ABSTRACT

Contamination by pollutants in freshwater ecosystem has been identified extensively in river, sediments, and freshwater biota. Pollutants may have incorporated into the sediments and accumulated in tissue of aquatic organisms which persist as difficult to degrade matter in upper trophic level. Therefore, few selected heavy metals were measured from the river sediment and tissue of damselflies larvae collected from the selected rivers using inductively coupled plasma optical emission spectrometry (ICP-OES). The results showed metals in damselflies tissue were higher than in the sediments. Mn and Zn were found in greatest concentrations both in sediment and damselflies 's tissue. Biota-sediment accumulation factors (BSAF) were computed based on these data, and it was discovered that all values of BSAF for Cd, Cu, Mn, and Zn were typically high (BSAF >1). In conclusion, the rivers contamination induced accumulation of heavy metal in the river sediments and damselflies larvae (*Pseudagrion microcephalum, Pruinosum fraseri,* and *Copera marginipes*). The highest concentration value was calculated as 29.23 for Cd in the *C. marginipes.* The high concentrations of this element in the insect body tissue has shown a trace of bioaccumulation and may pose biomagnification to organisms in the upper trophic level. The results of this study indicated that damselfly is reliable to become a bioindicator for heavy metals particularly pollution in the river.

Keywords: Aquatic insects; bioaccumulation; heavy metals; sediments

ABSTRAK

Pencemaran oleh bahan pencemar dalam ekosistem air tawar telah dikenal pasti secara meluas dalam sungai, sedimen dan biota air tawar. Bahan pencemar mungkin telah meresap ke dalam sedimen dan terkumpul di dalam tisu organisma akuatik yang berterusan sebagai sukar untuk diuraikan pada aras trofik teratas. Oleh itu, beberapa logam berat terpilih diukur daripada sedimen sungai dan tisu larva pepatung jarum yang dikumpul daripada sungai terpilih menggunakan spektrometri pelepasan optik plasma (ICP-OES) yang digabungkan secara induktif. Keputusan menunjukkan logam dalam tisu pepatung jarum lebih tinggi daripada dalam sedimen. Mn dan Zn didapati dalam kepekatan terbesar untuk sedimen dan tisu pepatung jarum. Faktor pengumpulan biota-sedimen (BSAF) telah dihitung berdasarkan data ini dan didapati bahawa semua nilai BSAF untuk Cd, Cu, Mn dan Zn adalah tinggi (BSAF >1). Kesimpulannya, pencemaran sungai menyebabkan pengumpulan logam berat dalam sedimen sungai dan larva pepatung jarum (*Pseudagrion microcephalum, Pruinosum fraseri* dan *Copera marginipes*). Nilai kepekatan tertinggi dihitung sebagai 29.23 untuk Cd dalam tisu *C. marginipes*. Kepekatan tinggi unsur ini dalam tisu badan serangga telah menunjukkan kesan bioakumulasi dan mungkin menimbulkan biopembesaran kepada organisma pada aras trofik atas. Hasil kajian ini menunjukkan bahawa pepatung jarum boleh digunakan sebagai penunjuk biologi bagi logam berat khususnya pencemaran di sungai.

Kata kunci: Bioakumulasi; logam berat; sedimen; serangga akuatik

INTRODUCTION

The discharge of different pollutant into the aquatic environment is the factor of countless anthropogenic activities (Ekubo & Abowel 2011) including human action, accumulation of fertilisers from agricultural activities and waste from industrial that related with heavy metals or chemicals (Triquet-Amiard, Amiard & Rainbow 2013), which deteriorates the condition of aquatic ecosystems and damaging the quality of the environment. Aquatic environments are receiver for anthropogenic contamination including chemicals of heavy metals pollutants that can execrable the toxic actions causing damage to aquatic environment (Bhat et al. 2017; Hampel, Blasco & Segner 2015; Harguinteguy, Cirelli & Pignata 2014; Shahbaz et al. 2013). Once released to the aquatic environments, they are generally transported and bound to particulate matter, which eventually settle down and become incorporated into sediments and accumulated in the tissue of aquatic organisms (Briffa, Sinagra & Blundell 2020). As a result, many aquatic insect species such as Ephemeroptera, Trichoptera, and Odonata orders that serve as a link-up transfer for element accumulation from the sediments to tissue of different species of trophic stages (Addo-Bediako & Malakane 2020; Bouchelouche & Abdeslem 2019; Bozanic et al. 2018; Guimarães, Corbi & Giuliano 2019; Prommi & Payakka 2018). Heavy metals cannot decompose easily and quickly and can be transferred to the food chain through the process of bioaccumulation. Eventually heavy metals move to higher consumer levels with concentrations that exceed safety levels. This condition is feared to have caused chronic illness and serious threats to humans and other living organisms including aquatic insects such as dragonflies. Furthermore, metals that are readily dissolved in water and ingested by aquatic invertebrates, resulting in a wide variety of biological effects such as poisonous and harmful (Gheorghe et al. 2017). Low concentrations of certain metals for example, copper, manganese and zinc are necessary for living organisms, but 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. 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.

Iqra Azam et al. (2015) have found that metal and environmental pollution has harmed aquatic ecosystems, and a biomonitoring programme using aquatic insects is needed as a first alarm to avoid serious consequences in the future. Besides, biomonitoring had been applied as an alternative method to measure aquatic ecosystem health that involves the use of indicator organisms (Zaghloul et al. 2020). Aquatic insect was used to determine the water quality status and detect the contamination before it causes a decline in environmental health (Jones et al. 2010). Metal bioavailability, as defined by Gheorghe et al. (2017), is the selection of the total concentration of 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 & Luoma 2011). Metals uptake routes are through the permeable epidermis if they are absorbing, or through food absorption if they are particulate. In this sense, metal bioaccumulation may be compared to the trophic stage of the aquatic insect's 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.

Odonata or damselflies are good biological measures of river water quality because it can represent and indicate the condition of aquatic ecosystems, as their larvae resides in the water. Heavy metals can reach the aquatic food chain via trophic transfer through biomagnification and bioaccumulation of metals in predators, such as Damselflies larvae (Kraus et al. 2014). According to Villalobos-Jiménez, Alison and Christopher (2016) damselflies has been successfully used as a biological indicator for environmental pollution. Exploration of aquatic insect bio uptake of metals as another facet of their usefulness in biomonitoring is also something worth exploring because of its intriguing potential as bioindicators. The present study is a one piece of a comprehensive approach that relate the bioaccumulation of heavy metals towards Zygopteran larvae species such as *Pseudagrion microecphalum, Pruinosum fraseri, Ischnura senegalensis* (Family: Coenagrionidae) and *Copera marginipes* (Family: Platycnemididae). Therefore, this study is focusing on the effects of different contamination of heavy metals content in the sediment and tissue of Damselflies larvae; *Pseudagrion microcephalum* (Zygoptera: Coenagrionidae), *Pruinosum fraseri* (Zygoptera: Coenagrionidae) and *Copera marginipes* (Zygoptera: Platycnemididae). It is a very important finding that can contribute for monitoring heavy metal contamination in the rivers.

MATERIALS AND METHODS

SAMPLING SITES

Three sampling sites; Sungai Serdang (SR), Sungai Kulim Hi-Tech (KHTR), and Sungai Ayer Puteh (APR) were selected based on different human activities and pollutant sources in the surrounding areas that received by these rivers. Sungai Kulim Hi-Tech (KHTP) is located in an industrial park which is the most important for high technology enterprises industry and was surrounded by fields and factories. For the microhabitat characteristics, the river substrates consist of sand and *Juncus* sp. (grasses) grows along the river bank while the water surfaces were directly exposed to the sunlight. Sungai Ayer Puteh (APR) and Sungai Serdang (SR) was confluence from various impact of human activities on the surrounding area specifically by the agricultural activities like oil palm plantation and rubber plantation activity. In certain areas, the river's substrate is sandy and alluvial clay. This river flows through both urban and rural areas, collecting anthropogenic waste from both. The unrestricted usage of chemicals in fertilisers and pesticides has had a negative impact on river water health and nutrient soil depletion.

INSECT SAMPLING

Sediment sample was collected in triplicate from the three selected rivers and was brought back to the laboratory. The Damselflies larvae were sampled using aquatic net with 40 × 30 cm frame size of about 0.25 mm mesh (Cezario et al. 2020; Mendes, Benone & Juen 2019). The frame was equipped with a handle of about 1.5 m long. The net was drag on the vegetation along the river bank and all the larvae were placed in plastic bag and brought to the laboratory insulated for sorting process. The specimens were identified in the laboratory under a dissecting microscope Olympus CX41 (Olympus, Tokyo, Japan) following keys and description by Cham (2012) and Theischinger and Endersby (2014). The following three Damselflies larvae species occurred in the three selected rivers: *Pseudagrion microcephalum, Pruinsoum fraseri* (Odonata : Coenagrionidae), *Copera* marginipes (Odonata : Platycnemididae. Before that, damselflies larvae were washed with water and cleaned with a small brush. At this time, live damselflies larvae were moved into a cooler and were stored at -18 °C until sample processing (Addo-Bediako & Malakane 2020; Bozanic et al. 2018; Girardin, Grung & Meland 2020; Guimarães, Corbi & Giuliano 2019; Simon et al. 2017). Until performing the heavy metal analysis, all instruments and containers were soaked in 10% HNO_3 and thoroughly rinsed with double distilled water (USEPA 2019).

DETERMINATION OF HEAVY METAL IN RIVER SEDIMENT AND IN TISSUE OF DAMSELFLIES LARVAE

Sediment samples were dried at 110 °C for 24 h to ensure a consistent weight. After cooling, the sample was passed through a 0.5 m steel sieve and stored in a storage bag for acid digestion. All the methods for determination of heavy metals content in the sediment and damselflies tissue was followed and modified based from Addo-Bediako and Malakane (2020) and Guimarães, Corbi and Giuliano (2019). After that, all sediment samples and frozen damselflies samples were lyophilized and homogenized with a pestle and mortar. Approximately 1.0 g of sediments and 0.10 g dry weight of larvae each were placed in 100 mL beakers, to which 10 mL concentration of Nitric acid (HNO_3) and 30 mL concentration of Hydrochloric acid (HCl) in the form of aqua regia (mixture of nitric acid and hydrochloric acid in a molar ratio of 1:3) and 5.0 mL of distilled water was added. Next, the samples were boiled until the temperature reached 90 ºC or the yellow-orange fuming (Aqua-regia) disappear. After cooling, the digested samples were purified using filter papers and stored in 100 mL beakers. The contents of the beakers were moved to 100 mL volumetric flasks after the filter papers (filter Whatman 42) were washed with 20 mL distilled water. Metal concentrations (mg/kg) were measured using an inductively-coupled plasma optical emission spectroscope (ICP-OES) in the final analysis. The detection limits for selected heavy metals were: (Cd-0.0002 mg/L; Mn-0.0005 mg/L; Zn-0.0004 mg/L, Cu-0.001 mg/L).

STATISTICAL ANALYSIS

Descriptive statistics, Analysis of Variance (ANOVA), Pearson correlation and regression analysis were made on recorded data by testing significance difference at $P<0.05$ using SPSS 26.0. Values of metal concentration in sediment, and in larvae at the sampling sites were compared statistically using one-way ANOVA followed by the Post Hoc t- test (Tukey's t- test) for significant difference ($P<0.05$). In addition, Pearson's correlation and regression analysis were used when calculating the significant correlations between the content of heavy metals in the sediment and in tissue of different damselflies larvae species. In order to measure metal bioaccumulation in aquatic fauna, the bioaccumulation sediment factor (BSAF) was measured as the ratio of the heavy metal concentration in the body to the heavy metal concentration in the environment. 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). It is considered biota-sediment accumulation factor (BSAF) when metal concentrations in organisms are larger than the concentrations of the aquatic sediment. The BSAF is described by the following formula:

BSAF = metal in organism (mg/kg)/metal in sediment (mg/kg)

(Nenciu et al. 2016, 2014; Ziyaadini, Yousefiyanpour & Ghasemzadeh 2017).

According to BSAF values, organisms can be classified into three categories; BSAF>2, the organism is macro-concentrator,
1<BSAF<2, the organism is micro-concentrator,
BSAF<1, the organism is de-concentrator and releasing the metal in sediment.

Results

At all three rivers, zinc (48.50±3.67 mg/kg) exhibited greatest concentration in sediment at KHTR (Table 1) while Cd recorded lowest concentration (0.13±0.00) at APR. In addition, all heavy metal readings recorded from selected rivers showed low readings below the standard level of EPA guidelines (Table 2). It was found that, all heavy metals studied were reported in the three species of damselflies tissues and Mn recorded the highest readings compared to other heavy metals (Table 3). *Copera marginipes* accumulated great amount of all heavy metals in all selected rivers with great amount of Mn (204.5±2.5 mg/kg) at APR. For *Pruinosum fraseri*, Zn was found highest (161.5±7.5 mg/kg) in their tissues compared to the other two species. Besides, *P. microcephalum* accumulated great amount of Mn at KHTR with 125±5.00 mg/kg. All concentration of the heavy metals showed no significant differences among the predatory insects *P. microcephalum* (F=0.167, P=0.222), *Pruinosum fraseri* (F=0.212, P=0.812) and *Copera marginipes* (F=2.984; P=0.081). BSAF for *P. microcephalum, P. fraseri,* and *C. marginipes* at all rivers were calculated and results show more than 1 (BSAF > 1) (Table 4) meaning bioaccumulation do happen in those damselflies. All damselflies species recorded highest BSAF for Cd and least for Zn. Besides, *C. marginipes* recorded highest BSAF value for Cd (29.23) followed by Mn (12.78), Zn (10.41) and Cu (10.67). Bioaccumulation value of Mn for *P. microcephalum* was greater in KHTR with 5.1 compared to APR (4.81) and SR (1.72). The bioaccumulation of Cd for *P. microcephalum* was also great at APR with 10.8. Interestingly, *C. marginipes* at APR also showed a great value of BSAF compared with other rivers. This can be observed when there was a high bioaccumulation in *C. marginipes* was recorded for all heavy metals at APR except for Cu content (Cd=29.23; Mn=12.78; Zn=10.41). However, the value of BSAF of *C. marginipes* for Mn and Zn was low at SR (Mn=1.81; Zn=2.85) and KHTR (Mn=1.45; Zn=2.34). Meanwhile, *P. microcephalum* and *P. fraseri, C. marginipes* also produced high BSAF value in relation with Cu concentration at KHTR (Cu=10.67) followed by APR (Cu=4.3) and SR (Cu=3.92). As in general, all the three species can act as macroceoncentrator (more than BSAF>2) which can efficiently accumulate heavy metals (Petrova, Yurukova & Velcheva 2013; Ziyaadini, Yousefiyanpour & Ghasemzadeh 2017).

According to Table 5, relationship between Zn content in sediments and Damselflies larvae was significant for *P. fraseri* and *C. marginipes* at (P<0.05) with different R^2 adjusted value; (42%) and (20%), respectively. The relationships between Mn concentration and water with *P. fraseri* and *C. marginipes* are significant at (P<0.05). A weak correlation of Mn accumulation between sediment and water with *P. fraseri* can be observed based on the regression equation for Mn accumulation in water sample Y (Mn metal) = -0.538 (Mn damselflies) + 0.002 (Mn sediment) that had a value of negative correlation which only increases with 0.538 when the accumulation of Mn in *P. fraseri* variables decreases. Influence involving Cd concentration between sediment and damselflies larvae only can be seen in *C. marginipes* because these species have a significant intercept at (P<0.05) with a low R^2 adjusted value (23%). By looking at the regression equation, significant negative correlation Y (Cd metals) = -0.309 (Mn damselflies) + 0.049x (Mn sediment) had been observed when there is an increasing number of Cd accumulation by 0.309 when the accumulation of Cd in *C. marginipes* variables decreases by one. The regression equation of metals accumulation between water and *P. microcephalum* indicated that there is a small incline number of Cd accumulation by 0.110 when the accumulation of Cd in *P. microcephalum* variables decreases by one.

TABLE 1. Mean ± standard error of heavy metals contents (mg/kg) in sediment at all sampling sites

Rivers/Metals	Cadmium	Manganese	Zinc	Copper
Sungai Kulim High Tech (KHTR)	0.15±0.02	24.50±1.22	48.50±3.67	4.50±0.41
Sungai Ayer Puteh (APR)	0.13±0.00	16.00±3.27	18.50±4.49	5.00±1.63
Sungai Serdang (SR)	0.33±0.03	39.00±1.00	27.00±2.00	6.50±2.50

TABLE 2. Heavy metal concentration standard in freshwater ecosystems according to CCME (μg/L) and EPA (mg/kg) guidelines

Metals	Mean of heavy metals sediment* (mg/kg)	CCME- protection of aquatic life (μg/l)		EPA guidelines (mg/kg)		
		ISQG	PEL	Not polluted	Moderately polluted	Heavily polluted
Cadmium	0.20	0.6	3.5	N/A	<6	>6
Manganese	26.5	N/A	N/A	<300	300-500	>500
Zinc	31.3	123	315	<90	90-200	>200
Copper	5.3	35.7	197	< 25	25-50	>50

CCME - Canadian Council of Ministers and Environment, ISQG – Interim Freshwater Sediment Quality Guideline, PEL – Probable Effect Level, EPA – Environmental Protection Agency, * Mean of heavy metals sediment were pooled from three rivers

TABLE 3. Mean (± SE) of heavy metals concentration in tissue of Damselflies larvae (*P. microcephalum*, *P. fraseri,* and *C. marginipes*) (mg/kg) at all selected rivers

Damselflies larvae species	Site	Type of heavy metals concentrations (mg/kg)			
		Cadmium	Manganese	Zinc	Copper
Pseudagrion	Sungai Kulim High Tech (KHTR)	1.30 ± 0.40	125.0 ± 5.0	81.5 ± 6.50	24.5 ± 3.50
microcephalum	Sungai Ayer Puteh (APR)	1.41 ± 0.10	77.0 ± 1.0	80.5 ± 0.50	25.5 ±1.50
	Sungai Serdang (SR)	1.12 ± 0.37	67.0 ± 1.0	58.5 ± 7.5	18.5 ± 4.5
Pruinosum	Sungai Kulim High Tech (KHTR)	0.41 ± 0.09	61.0 ± 7.0	81.5 ± 7.50	37.0 ± 7.0
fraseri	Sungai Ayer Puteh (APR)	2.0 ± 0.00	137.5 ± 3.50	161.5 ± 7.50	35.0 ± 5.0
	Sungai Serdang (SR)	1.8 ± 0.20	58.5 ± 1.50	90.5 ± 5.50	33.0 ± 6.0
Copera	Sungai Kulim High Tech (KHTR)	1.55 ± 0.25	35.5 ± 4.50	113.5 ± 3.50	48 ± 2.0
marginipes	Sungai Ayer Puteh (APR)	3.8 ± 0.30	204.5 ± 2.50	192.5 ± 2.50	21.5 ± 1.50
	Sungai Serdang (SR)	1.3 ± 0.10	74.5 ± 2.50	77.0 ± 7.0	25.5 ± 0.50

TABLE 4. Biota sediment accumulation factor (BSAF) for damselflies (*P. microcephalum, P. fraseri,* and *C. marginipes*) larvae from three selected rivers

Site	Damselflies larvae	Type of Heavy Metals (BSAF value)			
Sungai Kulim Hi Tech (KHTR)		Cadmium	Manganese	Zinc	Copper
	P. microcephalum	8.97	5.10	1.68	5.44
	P. fraseri	2.83	2.49	1.68	8.22
	C. marginipes	10.69	1.45	2.34	10.67
Sungai Ayer Puteh (APR)		Cadmium	Manganese	Zinc	Copper
	P. microcephalum	10.81	4.81	4.35	5.10
	P. fraseri	15.39	8.59	8.73	7.00
	C. marginipes	29.23	12.78	10.41	4.30
Sungai Serdang (SR)		Cadmium	Manganese	Zinc	Copper
	P. microcephalum	3.68	1.72	2.17	2.85
	P. fraseri	5.54	1.50	3.35	5.08
	C. marginipes	4.00	1.91	2.85	3.92

TABLE 5. Regression analysis between heavy metals contents in sediments and tissue of Damselflies larvae

Heavy metals in sediment	Damselflies species	R	Regression equation	Intercept P (significant)	R square (R^2)	R^2 adjusted
Zinc	*P. microcephalum*	0.209	$Y=14.34+0.23x$	0.74	0.04	0.195
	P. fraseri	0.729	$Y=-60.98+0.27x$	0.014*	0.53	0.415
	C. marginipes	0.464	$Y=-47.64+0.24x$	0.046*	0.22	0.210
Manganese	*P. microcephalum*	0.300	$Y=-36.87+0.12x$	0.10	0.09	0.137
	P. fraseri	0.790	$Y=-44.5+0.21x$	0.004*	0.62	0.530
	C. marginipes	0.597	$Y=-35.00+6.08x$	0.007*	0.36	0.195
Copper	*P. microcephalum*	0.029	$Y=-5.63+0.01x$	0.34	0.00	0.249
	P. fraseri	0.116	$Y=4.00+0.0x$	0.53	0.01	0.233
	C. marginipes	0.202	$Y=-6.45+0.04x$	0.09	0.04	0.199
Cadmium	*P. microcephalum*	0.164	$Y=-0.23+0.02x$	0.08	0.03	0.216
	P. fraseri	0.355	$Y=0.14+0.05x$	0.22	0.13	0.092
	C. marginipes	0.620	$Y=-0.31+0.05x$	0.016*	0.38	0.230

DISCUSSION

The concentration of all heavy metals including Zn, Cu, Cd, and Mn were found in the sediment at all three selected rivers; KHTR, APR, and SR. Comparing the concentration of all heavy metals with the metal standard by Canadian Council of Ministers and Environment (CCME) guidelines, all the content of metals concentration in sediment from the three rivers were approximately above the limit of quantification. However, according to United States Environmental Protection Agency (USEPA) (Table 2), metals concentration in this study were much lower and the river sediments is considered not polluted. The concentration of Zn and Mn was found to be the greatest in all the three selected rivers followed by Cd and Cu. This trend of metal accumulation in the sediment was correspondent with the accumulation of metals content in water. For example, the concentration for Zn and Mn in both sediment and water were greatest compare to Cu and Cd. Thus, the concentrations of Zn and Mn in sediment, on the other hand, were significantly higher because sediment acts as both a sink and a source of heavy metals (Huang et al. 2020). After entering rivers, most heavy metals rapidly deposit into the sediment and are considerably more concentrated in the sediment than in the water body of riverine systems (Liu et al. 2018). Not only that, observation from Reda and Ayu (2016) also showed that the sediments may serve as a metal puddle that desorb and suspend the metals so that the sediment can release metals to the water body that could potentially adversely affect the aquatic ecosystems (Huang et al. 2020; Kouidri et al. 2016).

Likewise, the concentration of all metals in damselflies tissue was varied and high concentration of Mn in *C. marginipes* is as expected because of the high bioavaibility of Mn in sediment at the rivers. Simon et al. (2017) also indicated Mn in dragonfly, *Gomphus flavipes* (Anisoptera: Gomphidae) larvae with maximum value of 13.1 mg/kg. This was also supported by Guimarães, Corbi and Giuliano (2019), which observed similar result from Damselflies larvae, and Bouchelouche and Abdeslem (2019) on mayfly, *Ephemera danica* (Ephemeridae) under influence of a trout farm outlet waters. In addition, Ben-Shahar (2018) has discovered that aquatic insects rapidly absorb and accumulate Mn and other divalent metal ions in their environments that are highly reactive with other elements which often have a direct negative effect on their fitness. Several experiments have discovered that aquatic insects rapidly absorb and accumulate Mn and other divalent metal ions (Dittman & Buchwalter 2010) in their surroundings (Poteat, Díaz-Jaramillo & Buchwalter 2012), which can have a significant detrimental impact on their health and affect the fitness costs in the terrestrial adult phase (Krång & Rosenqvist 2006; Oweson et al. 2008). The accumulation of Mn in *C. marginipes* larvae in this study was higher compared to other metals maybe due to the good absorption of this metal into its tissue. Dittman and Buchwalter (2010) have reported that Mn oxides formed on the integument of several insects can provide a mechanism for the larvae to alter the heavy metals uptake and adsorption.

Concomitantly, *P. fraseri* accumulated high amount of Zn at the three selected rivers. Damselflies larvae were much interrelated with the metal content and bioavaibility in the sediment (Simon et al. 2019). Iqra Azam et al. (2015) described larvae of *Crocothemis servilia, Erythrodiplax* sp. and *Tramea* sp. (Odonata: Libellulidae) accumulated high amounts of Zn from disturbed rivers. Similar results also found by Guimarães, Corbi and Giuliano (2019) which observed the high content of Zn accumulated by damselflies larvae compared to other aquatic insects. Previous study from Simon et al. (2017) noticed that *Gomphus flavipes* (Odonata: Gomphidae) accumulated high amount of Zn that are related with river sediment that contain high concentration of Zn.

This study reported *P. microcephalum, P. fraseri,* and *C. marginipes* accumulated high concentration of heavy metals probably with the aid of detoxification mechanism. Luo et al. (2020) stated that the role of Metallothioneins (MTs) in insects are very essential in chelating, regulating heavy metal concentrations in cells, and detoxification of toxic heavy metals even in high concentrations. Lavilla et al. (2010) demonstrated the absorption of Zn was associated with the inner tissue of dragonfly larvae, while, Mn are only adsorbed into external body parts. Lavilla et al. (2010) also showed that *Cordulegaster boltoni* (Family: Cordulegastridae) and *Onychogomphus uncatus* (Family: Gomphidae) exhibit significant correlation values between Cd–Cu and Cd–Zn which suggests there is a relationship between the accumulation and regulation of the three elements. The amounts of Cd, Mn, Zn and Cu were observed to be higher in the insect tissue than in the sediment for the composition of heavy metals.

The value of biota sediment accumulation factor (BSAF) for all heavy metals in this study showed more than 1 which indicate that the transfer efficiency of heavy metals accumulation from sediment into Damselflies larvae is high and these three selected species are considered as macro-concentrator. Therefore, it can assure that damselflies tissue are efficient in having bioaccumulation process. For aquatic insects, the concentrations of Cd, Mn, Zn and Cu could change with the size, life cycle stages and bioaccumulation patterns (Cid et al. 2010), and, many studies have shown that the free metallic ion in the sediment is the most bioavailable form and there were Cu, Cd, and Zn (Wojtkowska, Bogacki & Witeska 2016) that could be an important source for bioaccumulation in aquatic organisms' especially insects (Rosado, Usero & Morillo 2016). In this study, bioaccumulation of Cd in *C. marginipes* (BSAF=29.23), *P. fraseri* (BSAF=15.39), and *P. microcephalum* (BSAF=10.81) was high. This results were dissimilar from the previous studies (Guimarães, Corbi & Giuliano 2019; Nasirian & Irvine 2017) as they reported bioaccumulation of Cd was not detected in sediment and damselflies larvae. However, study by Addo-Bediako and Malakane (2020) showed that the bioaccumulation sediment factor (BSAF) for Cd was greater (BSAF=16) at Blyde River, South Africa that caused by industrial effluents and indirect ground surface drainage from agricultural lands, as well as the release of municipal waste.

The relationships between heavy metal concentrations in predator species and water are highlighted by low R^2 adjusted values. The highlighted R^2 adjusted values between the Mn content in water samples in both species were much higher compared to other heavy metals with more than 70% of the variability. This value indicated that more than 70% of the variance in Mn can be predicted from *P. fraseri* and *C. marginipes* which have a strong association with Mn content in water and in sediments. Thus, a negative correlation indicates a low content of Mn in sediment and water that highly impacted the increased of Mn in the tissue of Damselflies larvae. These results were supported by the study done by Addo-Bediako and Malakane (2020) that observed a similar trend with Mn concentration on insect predator species. Mn was efficiently transferred and bioaccumulated to upper trophic levels even though the Mn content in the sediment was low. Therefore, it is assuring that Damselflies tissue is efficient in having a bioaccumulation process of this metal. Likewise, high concentration of Zn transference from larvae to adult will gave huge impact on the metamorphosis process and survivability rate of the larvae. For example, study done by Wesner et al. (2014) indicated that high mortality rate during metamorphosis in *Centroptilum triangulifer* (Family: Baetidae) may be a key mechanism explaining the rivers metal contamination can impact terrestrial communities by reducing the ability of the aquatic insect emergence to completing the metamorphosis.

This findings hypothesized there was a possible absorption of metal transfer through the ectoderm of the insect to the cuticle (ecdysis) during the exposure period as the detoxification mechanism (Buchwalter et al. 2007; César dos Santos Lima et al. 2019) and it tends to be more prominent for Mn (Dittman & Buchwalter 2010). Golovanova (2008) reported that ions of Mn were being absorbed in large amounts by the insect body surfaces, which are not threatening and bind to the cuticle of aquatic insect. Meanwhile, Cd, Cu, and Zn will accumulate within cells and provide immense of toxicity towards the larvae (Golovanova 2008), predominantly in the cytosol, where they may subsequently perform their toxic action. Studies show that certain odonate species can deliberately resist consuming Mn and have developed mechanisms for efficient dietary Mn excretion and sequestration in unique body parts (Kula et al. 2014; Rokytova et al. 2004). If the concentration of heavy metals keeps increasing in longer exposure period, they can readily enter the food chains via their accumulation because some heavy metal is water soluble. Prolonged exposure to the mineral is also poisonous and harmful to the fitness of most insects, causing changes in eating habits, fertility, immunity, and overall survival (de Barros et al. 2017; Kula et al. 2014; Martinek, Kula & Hedbávný 2018; Olsén 2011). In general, *P. microcephalum* and *I. senegalensis* larvae are generally resistant to elevated levels of heavy metal toxicity in aqueous medium.

Conclusions

Heavy metal exposure had caused a localised and statistically important rise in Cd, Mn, Zn, and Cu concentrations in sediment as well as in aquatic insect, Damselflies . Different species of damselflies larvae had significant correlation related with accumulation on different type of heavy metal contents from the sediment. The metal concentrations accumulated in all three species of damselflies larvae related to the heavy metals content in water and sediment were as follows: Zn > Mn > Cu > Cd. The process of accumulation between water and sediment with damselflies tissue can be observed and Cd, Mn, Zn and Cu, were greatest on the Damselflies tissue. Besides, Mn was efficiently transferred from sediment into damselflies larvae and the highest BSAF value for the three species was more than 1. Based on BSAF, the *P. microcephalum*, *P. fraseri,* and *C. marginipes* are macro-concentrator for Cd, Mn, Zn and Cu.

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