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Research

Toxicity Assessment on Odonata Larvae Survivability in Monitoring Heavy Metal Contaminations

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ABSTRACT

The aquatic ecosystem has been suffering a continuous increase of metal contamination such as Cadmium (Cd), Zinc (Zn), and Manganese (Mn) due to their inadequate high potential to disturb the aquatic organism population. Meanwhile, some insects such as *Pseudagrion microcephalum* and *Ischnura senegalensis* can be used as biological indicators to determine stream health. Therefore, this study was conducted to determine the relationship between the heavy metal concentration and its effect on the survivability of two different species of damselfly larvae from the family Coenagrionidae; *Pseudagrion microcephalum* and *Ischnura senegalensis*. In this study, there is a significant effect of three heavy metal exposures on the survivability of *P.microcephalum* ($F_{11,180}$=14.50, *P*=0.00) and *I.senegalensis* ($F_{11,180}$=15.10, *P*=0.00). *Pseudagrion microcephalum* is more tolerable towards Mn ($F_{3,60}$=13.19, *P*=0.00) and Zn ($F_{3,60}$=16.07, *P*=0.00) at different concentrations compared to *I.senegalensis.* In the meantime, *I.senegalensis* was tolerable to Cd exposure. The LC_{50} value of Cd was much lower than other heavy metals. Besides, the LT_{50} value of Cd at 200 mg/L was the lowest on *P. microcephalum* (31 hr) and *I. senegalensis* (36 hr) compared to other heavy metals. Cd was the most toxic to *P.microcephalum* and *I.senegalensis* larvae followed by zinc and manganese (LC_{50} & LT_{50}=Cd > Zn > Mn). It is concluded that *I.senegalensis* was tolerant towards Cd, Mn, and Zn compared to *P.microcephalum* and Cd had the fastest-acting toxicity and significantly reduced the lethal time of mortality on both species.

Key words: Aquatic insects, aquatic water bodies, bioindicator, cadmium, manganese, zinc

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INTRODUCTION

The aquatic ecosystem supports a variety of species, including Odonata larvae, but most of the streams have been polluted due to a rise in heavy metal toxicity in the aquatic environment, such as Cadmium (Cd), Copper (Cu), and Zinc (Zn). Environmental developments are mostly a result of urbanization, agriculture, and industrialization, and they may have added substantially to the metal load in the lotic ecosystem (Mani *et al*., 2015) and have had an imperceptible impact as a result of the ineffectiveness of certain activities that have been used for various purposes, compounded by their proclivity to accumulate in biotic processes and accumulate in Odonata tissues and their increasing level is toxic to other trophic levels (Villalobos-Jiménez *et al*., 2016; Mebane *et al*., 2020). Odonata larvae (Insecta: Zygoptera) are common in freshwater ecosystems and can withstand a wide variety of chemical, physical, and biological environmental parameters (Villalobos-Jiménez *et al*., 2016). Furthermore, they are particularly vulnerable to various stressors and environmental changes. Odonata larvae are an excellent model organism and can be used as important bioindicators of any changes that have occurred in freshwater environments (Carvalho *et al*., 2013; Monteiro-Junior *et al*., 2013; 2014; Oliveira Junior *et al*., 2015; Hassal, 2015; Miguel *et al*., 2017; Tandin *et al*., 2020). As a result, Odonata larvae can be used as bioindicators in aquatic environments and can be used to measure current river conditions (Dorji & Nidup, 2020). Because they have such an important role in freshwater systems, odonate larvae are included in many environmental assessments including distribution, seasonal, environmental changes, and biological monitoring (Seidu *et al*., 2017; 2019; Oliveira Junior *et al*., 2019).

Unfortunately, the effect of heavy metal exposure toxicity on odonate survivability has never been put into consideration. Because of this, it was impossible to foresee the accumulation of heavy metals and their harmful effects on Odonata larvae. Heavy metal accumulation can cause disease, disturb the insects' development (Iqra Azam *et al*., 2015), create deformities (Di Veroli *et* al., 2013; Youbi *et al*., 2022), and mortality in aquatic organisms (Cadmus *et al*., 2020). This study was carried out to determine the heavy metal accumulation on Odonata larvae and to determine the survivability of zygopteran larvae; *P. microcephalum* and *I.senegalensis* (Family: Coenagrionidae) at a different level of heavy metal (Cd, Mn, & Zn) concentration to provide information of zygopteran larvae reliability as a biological indicator in aquatic environment.

MATERIALS AND METHODS

Collection of Odonata larvae

Three selected rivers in Kedah; Kulim Hi-Tech River, Serdang River, and Ayer Puteh River, Kedah were visited for the collection of Odonata larvae. The most consistently abundant species occurred in the selected rivers, *Pseudagrion microcephalum* and *Ischnura senegalensis* (Zygoptera: Coenagrionidae) were used in the experiment. All the zygopterans larvae for the bioassay experiment were collected using a D-frame aquatic net (mesh sieve 250 µm) among the leaf litters and aquatic vegetation. All the larvae were retained in the net and transferred to the plastic bags and stored in the ice storage during transportation to the laboratory and maintained in the laboratory of Aquatic Entomology Laboratory, School of Biological Sciences, Universiti Sains Malaysia. Specimens were identified through dichotomous keys followed by Theischinger and Endersby (2014). Only fourth instar larvae measured between 20 mm to 26 mm (Ilahi *et al*., 2020) were chosen for rearing and allowed to acclimate to room temperature (23 °C) in the river water.

Odonata larvae rearing

For rearing the zygopteran larvae in the laboratory, each polyethylene (PE) cup was cut at the sides to make two windows (3 × 5 cm), and each window was covered with a nylon screen (mesh size: 0.84 mm) (Ilahi *et al*., 2020; Toilet *et al*., 2009)". Larvae were placed into 30 regular polyethylene (PE) cups (240 mL: 8 cm × 9 cm × 6 cm). The entire polyethylene (PE) cup was placed inside the aquarium containers (30 cm × 20cm × 12 cm) (Figure 1). Each aquarium was filled to a depth of 15 cm with filtered water. The entire system was held in a laboratory room under the temperature of 27 ± 2 °C and a 12-hour light: 12-hour dark photoperiod regime. Larvae were fed two to three larvae mosquitoes of *Culex quinquefasciatus,* two to three times per week, before use in the experiment (Okude *et al*., 2017).

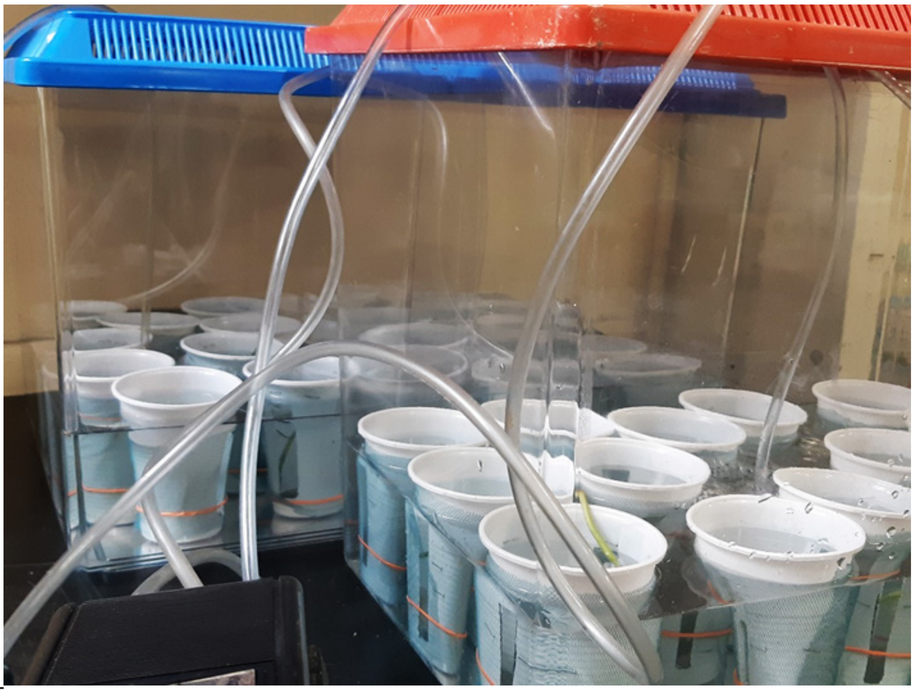

Fig. 1. Rearing Odonata larvae in 72 hour on a polyethylene cup inside an aquarium container under the temperature with oxygenated pump circulation.

Bioassay design for exposure of Cd, Zn, and Mn

A total of 360 fourth instar (180 instar larvae for *Pseudagrion microcephalum* & 180 instar larvae for *Ishcnura senegalensis*) were used for the bioassay experiment. Stock solutions of 10 g/L (100 mL) of Cd, Mn, and Zn were made from metal salts [Zinc chloride ($ZnCl_2$) metal salt; Cadmium chloride ($CdCl_2$) and Manganese (II) chloride tetrahydrate $Cl_2H_8MnO_4$)] which were dissolved in ultrapure water. Cadmium (Cd), Manganese (Mn), and Zinc (Zn) with metal at different concentrations (50 mg/L, 100 mg/L, and 200 mg/L) were prepared. For each experiment, three replicates of experimental containers with each having 10 individuals of 4th instar larvae were exposed to heavy metals, and one container served as a control group that only contained dechlorinated tap water. The procedure was conducted at room temperature of 27 ± 2 °C and 13:11 h (light: dark) for 24 h. Odonates were checked every day for dead larvae, as determined by lack of response to prodding. Both Odonata larvae; *P. microcephalum* and *I. senegalensis* were exposed to different types of heavy metal concentration and mortality was observed in time intervals of 24, 36, 48, 60, 72, 96, 120, 144, and 168 h. The larvae were not fed during the exposure.

Data analysis

Descriptive and statistical analysis consisting of (ANOVA) was used to compare the survivability of larvae among the heavy metals exposure at different levels of concentrations (50, 100, & 200 mg/L). Tukey's multiple comparisons (Post Hoc Test) were used to distinguish major variations between all metal treatments on two distinct species: *P. microcephalum* and *I.senegalensis.* In addition, probit analysis was used to determine the relative toxicity of heavy metals (cadmium, zinc, & manganese) towards both Odonata larvae. This is done by testing the Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}) mortality of both larvae under various concentrations of 50 mg/L, 100 mg/L, and 200 mg/L of each of the chemical treatments.

RESULTS

Survivability of Odonata larvae

The toxicity of Cd, Mn, and Zn at different concentrations (50 mg/L, 100 mg/L, and 200 mg/L) has affected the survivability of both species, *Pseudagrion microcephalum* and *Ischnura senegalensis* (Table 1). The percentage of survivability for both larvae decreased in Cd, Mn, and Zn from a concentration of 50 mg/L to 200 mg/L. The mean survivability of both larvae varies according to the concentration and metal element. Examination of Cd, Mn, and Zn intake showed a major toxicity impact on the survivability of *P.microcephalum* ($F_{11,180}$=14.50, *P*=0.00) and *I. senegalensis* ($F_{11,180}$=15.10, *P*=0.00) at 50 mg/L, 100 mg/L, and 200 mg/L concentrations. From the result, exposure of *I.senegalensis* larvae in 50 mg/L Cd showed high tolerability with a greater mean of survivability (5.88 ± 1.31) compared to *P. microcephalum* (4.85 ± 1.42). Exposure of Cd with 100 mg/L indicated that the toxicity actions of Cd towards both larvae species are similar by a notable decrease of number survivability. In other words, *P. microcephalum* was less tolerant towards Cd exposure compared *to I.senegalensis*. For Mn exposure, *P.microcephalum* (5.75 ± 1.42) and *I. senegalensis* (6.75 ± 1.44) were much tolerable compared to Cd with more than half of the total insect surviving in 50 mg/L of Mn. However, exposure to Mn at 200 mg/L signified that *I.senegalensis* was less tolerant (2.68 ± 1.24) and more sensitive compared to *P.microcephalum* (2.94 ± 1.27). As the Zn concentration increased to 200 mg/L, *I.senegalensis (*$F_{3,60}$=18.63, *P*=0.00) was less tolerant compared to *P.microcephalum* (($F_{3,60}$ =16.07, *P*=0.00), and the mean of survivability for *I. senegalensis* are gradually decrease and consistent with the result from the similar exposure of manganese in 200 mg/L.

Lethal Concentration (LC_{50}) of Cd, Mn and Zn on *P.microcephalum* and *I.senegalensis*

Cd, Mn, and Zn toxicity on *P.microcephalum* and *I.senegalensis* was evaluated and viewed in the form of median lethal concentration (LC_{50}) after seven days of exposure (Table 2). Both larvae displayed less resistance/tolerance as the concentration of various heavy metals increased from 50 mg/L to 200 mg/L. For Cd exposure, the LC_{50} value shown *by P.microcephalum* (LT_{50} =367.87 mg/L) was higher than *I.senegalensis* (LT_{50} =348.22 mg/L). High Cd concentrations with increasing exposure time resulted in different median lethal concentration (LC_{50}) values for both larvae. The LC_{50} of Cd for *P. microcephalum* (23.07 mg/L) was much lower than *for I. senegalensis* (25.12 mg/L). For Zn exposure, *P.microcephalum* was more tolerant and insensitive to Zn toxicity than *I. senegalensis*. For instance, the LC_{50} values of both species decreased with time from 425.40 mg/L to 24.01 mg/L and 420.45 mg/L to 22.25 mg/L for *I. senegalensis* and *P. microcephalum.* LC_{50} of Mn for *I.senegalensis* at 24 hr was slightly higher than *P. microcephalum*, however, after 120 hr, the result is different, with LC_{50} Mn for *P. microcephalum* (LT_{50} =36.66 mg/L) significantly higher than LC_{50} for *I.senegalensis* (24.01 mg/L).

Lethal Time

(LT_{50}) of Cd, Mn and Zn on *P. microcephalum* and *I. senegalensis*

The value of LT_{50} for each heavy metal is shown in Table 3. LT_{50} is the ability of the tested insecticide to kill 50% of the tested population and from the results, Cd was the fastest-acting heavy metal compared to other heavy metals followed by Zn and Mn. At 50 mg/L Cd, half of the populations of *P. microcephalum* and *I. senegalensis* were killed after 80 and 105 hr, respectively, and the lethal time (LT_{50}) was shortened as the Cd concentration was increased to 200 mg/L. *Ishnura senegalensis* was found tolerated to Cd compared to *P.microcephalum* as the mortality of *P. microcephalum* was greater. However, the lethal time (LT_{50}) for Zn on *I.senegalensis* was lower on 50 mg/L (LT_{50} =91.90), 100 mg/L (LT_{50} =54.00) and 200 mg/L (LT_{50} =38.21) compared to *P.microcephalum* as 50 mg/L (LT_{50} =99.92), 100 mg/L (LT_{50} =70.12) and 200 mg/L (LT_{50} =44.76). The results showed that LT_{50} of Mn at 200 mg/L on *I.senegalensis* (24.82 hr) was much lower than *P. microcephalum* (51.65 hr). All the results related to Lethal Time (LT_{50}) specified that the Cd had the fastest mode of action for toxicity induction and took less time to kill the *P. microcephalum* compared to *I. senegalensis*. However, Zn and Mn are acting toxicity of heavy metals and also take a longer time to kill *P.microcephalum* compared to *I. senegalensis*.

Table 1. Mean survivability (±SE) for *P.microcephalum* and *I.senegalensis* larvae exposed to different concentrations (50 mg/L, 100mg/L, and 200 mg/L) of cadmium (Cd), manganese (Mn), and zinc (Zn)

Heavy Metal Concentration (mg/L)	*P. microcephalum*	*I. senegalensis*
	Cadmium	
Control	9.94 ± 0.06^{a}	9.94 ± 0.06^{a}
Cadmium 50 mg/L	4.85 ± 1.42^{b}	5.88 ± 1.31^{b}
Cadmium 100 mg/L	2.81 ± 1.17^{ab}	2.94 ± 1.22^{c}
Cadmium 200 mg/L	1.81 ± 1.00^{ab}	2.06 ± 1.16^{cd}
	Manganese	
Control	9.94 ± 0.06^{a}	9.94 ± 0.06^{a}
Manganese 50 mg/L	5.75 ± 1.42^{b}	6.75 ± 1.44^{b}
Manganese 100 mg/L	4.06 ± 1.51^{ab}	5.00 ± 1.51^{ab}
Manganese 200 mg/L	2.94 ± 1.27^{ab}	2.68 ± 1.24^{ab}
	Zinc	
Control	9.94 ± 0.06^{a}	9.94 ± 0.06^{a}
Zinc 50 mg/L	5.75 ± 1.38^{b}	6.14 ± 1.53^{b}
Zinc 100 mg/L	4.19 ± 1.35^{ab}	4.29 ± 1.32^{ab}
Zinc 200 mg/L	2.50 ± 1.19^{ab}	2.26 ± 1.11^{ab}

Different letters indicate significant differences in survivability of both species among concentration at $P<0.05$, one-way ANOVA followed by Tukey's HSD test.

DISCUSSION

Heavy metals like Cd, Zn, and Mn are highly harmful to aquatic organisms and they also bioaccumulate in the tissues of Odonata larvae (Villalobos-Jiménez *et al*., 2016; Ilahi *et al*., 2020). The bioassay results have confirmed the toxicity value against both species, reaching a 90% mortality rate for both larvae. High mortality of *P. microcephalum* was recorded compared to *I. senegalensis* and the results were contradicted by Tollet *et al*. (2009) and Ilahi *et al*. (2020). Studies by Tollet *et al*. (2009) and Ilahi *et al*., (2020) indicated that several larvae from Odonata species like *Pachydiplax longipennis, Trithemis aurora, and Pantala flavescens* (Odonata: Libellulidae), *Enallagma simplicicollis, and Ischnura elegans* (Odonata: *Coenagrionidae*) had no appreciable mortality under 100 mg/L of Cd concentrations. The maximum percentage of larvae that slightly survived at the highest concentration (200 mg/L) of Cd can be seen in *I. senegalensis* species. Various Coenagrionidae genera have different tolerances and susceptibility to Cd toxicity. Tchounwou *et al*., (2012) have reported that Cd high toxicity and rapid adsorption have negative effects on living organisms when exposed for long periods. Insects ingest Cd from polluted water or the atmosphere, accumulating elevated levels in their bodies (Zhang *et al*., 2011; Li *et al*., 2018) which can suppress the growth, survivability, and development of these insects (Sang *et al*., 2018). Due to metamorphosis and detoxification mechanisms, the toxicity stressors on certain insects, including Odonata larvae, do not consistently absorb and digest Cd throughout their life cycle (Rivero *et al*., 2001). In other words, all zygopteran larvae from the Coenagrionidae family have a moderate to high tolerance value, making them a sensitive invertebrate taxonomic community (Tollet *et al*., 2009; Ilahi *et al*., 2020). Based on the lethal time (LT_{50}) *P.microcephalum* was more tolerant and insensitive to high concentrations of Zn for more than 38 hours indicating that Zn has a mild to low toxicity action (Ilahi *et al*., 2020). According to Girgin *et al*. (2010), there is a positive correlation between *Sympecma fusca* (Odonata: Lestidae) with a high concentration of Zn (>10 mg/L) and this specified that *Sympecma* sp. was less sensitive and more tolerable compared to other odonates species, especially anisoptera larvae. In comparison with other aquatic insects order, which is ephemeropteran larvae, Brinkman *et al*.

Table 2. Lethal Concentrations (LC_{50}) of cadmium, zinc, and manganese (mg/L) with *Pseudagrion microcephalum* and *Ischnura senegalensis* larvae at different exposure periods (hr).

	Cadmium LC_{50} (95% CL) (Lower limit – Upper limit)		Zinc LC_{50} (95% CL) (Lower limit – Upper limit)		Manganese LC_{50} (95% CL) (Lower limit – Upper limit)	
Hours	*P. microcephalum*	*I. senegalensis*	*P. microcephalum*	*I. senegalensis*	*P. microcephalum*	*I. senegalensis*
24	367.87 (*)	348.22 (*)	420.45 (*)	425.40 (*)	1238 (*)	524.16 (*)
36	144.81 (97.85 - 341.56)	196.99 (128.85-360.44)	289.85 (*)	206.76 (139.47- 288.79)	218.83 (*)	231.34 (*)
48	85.57 (46.28 - 102.98)	109.295 (63.86- 213.39)	146.69 (89.41- 234.28)	117.82 (76.53 - 213.21)	202.26 (134.16 - 571.58)	187.35 (127.75 - 521.70)
72	58.97 (26.49 - 81.71)	66.91 (40.75 - 41.87)	83.05 (40.34 - 135.96)	72.05 (47.46 - 98.99)	117.66 (79.31 - 190.94)	116.64 (84.72 - 160.72)
96	35.12 (*)	52.54 (21.53 - 70.22)	58.87 (18.34 - 85.03)	49.44 (1.923 - 74.54)	72.05 (47.46 - 98.99)	74.37 (48.91 - 103.01)
120	23.66 (*)	46.17 (*)	36.66 (*)	24.01 (*)	51.14 (*)	48.37 (2.28 - 71.70)
144	23.07 (*)	25.12(*)	22.25 (*)	18.35 (*)	26.84(*)	23.66 (*)
168	**	**	**	**	**	**

LC_{50} = 50% Lethal concentration, CL = Confidence Limit, * The 95% confidence limits were not available. ** Lethal concentrations (LC_{50}) were not estimated as all 100% of Odonata larvae mortality was recorded. Note: Mean results of two replicates from 3 different experiments with 10 larvae per treatment.

Table 3. Lethal Time (LT_{50}) of cadmium, zinc, and manganese (50 mg/L, 100 mg/L, and 200 mg/) on *Pseudagrion microcephalum* and *Ischnura senegalensis*.

Concentration (mg/L)	Cadmium LT_{50} (95%CL) (Lower limit – Upper limit)		Zinc LT_{50} (95%CL) (Lower limit – Upper limit)		Manganese LT_{50} (95%CL) (Lower limit – Upper limit)	
	P. microcephalum	*I. senegalensis*	*P. microcephalum*	*I. senegalensis*	*P. microcephalum*	*I. senegalensis*
50	80 (70.73–89.73)^{a}	105 (95.11–123.17)^{a}	99.92 (86.38–106.67)^{a}	91.9 (80.12–101.21)^{a}	112.13 (96.50–121.87)^{a}	115.18 (102.23-129.80)^{a}
100	42 (35.74–48.95)^{ab}	49 (37.44–59.67)^{b}	70.12 (61.82–76.96)^{ab}	54 (45.12–62.92)^{b}	71.22 (60.91–81.50)^{b}	79.31 (65.30–86.15)^{b}
200	31 (26.34–35.44)^{ab}	36 (31.81–40.93)^{ab}	44.76 (39.78–52.17)^{ab}	38.21 (37.09–48.73)^{ab}	51.65 (44.73–59.73)^{ab}	42.82 (36.14–47.31)^{ab}

LT_{50} = 50% Lethal Time, CL = Confidence Limit, Note: Mean results of two replicates from 3 different experiments with 10 larvae per treatment. Different letters indicate significant differences in Lethal Time (LT_{50}) of both species among concentration at $P < 0.05$, one-way ANOVA followed by Tukey's HSD test.

(2007) made a laboratory test to evaluate the sensitivity of *Rhithrogena hageni* (Family: Heptageniidae), towards Zn and, the 96-hour lethal concentrations for Cd and Zn exposures were 50.5 mg/L (Brinkman *et al*., 2007). That finding is similar to this study for Zn exposure on *P.microcephalum* (58.87 mg/L) and *I.senegalenis (*49.44 mg/L). This indicated that both aquatic insects are tolerant to high concentrations of Zn. The high tolerance of aquatic insects is related to the presence of metallothioneins in their body for detoxifying mechanisms. According to Khati *et al*. (2012), Zn binds to the cytosol metallothioneins in the midgut of many insect species. However, it can be toxic at high concentrations. Metallothioneins are short, cysteine-rich peptides. For heavy-metal homeostasis and detoxification, metallothioneins can bind heavy metals and protect the cells from the damage caused by heavy metal free radicals, according to recent research by Luo *et al*. (2020), several organisms including aquatic invertebrates have evolved intricate strategies to detoxify and excrete heavy metals. Although Zn is an essential element and is usually tightly regulated in organisms (Kim *et al*., 2012), there are indications that Zn is more toxic for aquatic insects compared to lead (Brix *et al*., 2011; Zheng *et al*., 2015). Moreover, Zn is potentially toxic when its concentration in an organism seriously exceeds physiological limits and has detrimental effects on the development and survival of many aquatic organisms when reaching a threshold (Sfakianakis, 2015). For Mn exposure, *I.senegalensis* larvae survived at the highest concentration (200 mg/L).

Manganese exposure, unlike Cd and Zn, had the least toxic activity on both Odonata species. Even though Mn exposures are tolerable to Odonata larvae, Ali *et al*. (2019) found that the induction of toxic against different species is distinctive, especially in the case of Odonata species. César dos Santos Lima *et al*. (2019) experimented on *Tramea cophysa* (Family: Libellulidae) and found Mn to be the least toxic examined for the genus probably the Mn is transferred to the insect cuticle during the exposure period as the detoxification mechanism (César dos Santos Lima, *et al*., 2019). In line with this, aquatic insects have been reported to be relatively tolerant to Mn as compared to other metals (Harford *et al*., 2015). One potential reason for those results is that invertebrates may pass ingested metals via the ectoderm to the cuticle during molting (ecdysis), which could serve as a detoxification mechanism and increase tolerant towards heavy metals (Buchwalter *et al*., 2007; Dittman & Buchwalter, 2010). Golovanova (2008) reported that ions of manganese metals were being absorbed in large amounts by the insect body surface and only bind to the cuticle of aquatic insects which are less toxicity compared to Cadmium, copper, and zinc which accumulate within cells (Golovanova, 2008), predominantly in the cytosol, where they may subsequently perform their toxic action. Even though the absorption of manganese was important for the insect's mechanism, the presence of manganese is often water-soluble and highly dissociated with ion forms that may enter the food chains via their accumulation in freshwater environments. Not surprisingly, several studies have found that aquatic insects exhibit rapid uptake and tissue accumulation of Mn present in their environment (Poteat *et al.*, 2012), which often has a direct negative effect on their fitness (Oweson *et al*., 2008). Manganese (Mn) also plays a part in the development and behavior of insects through various molecular and physiological processes. Even though manganese is essential for life, prolonged exposure to the mineral is also poisonous and harmful to the fitness of most insects, affecting feeding habits, fertility, immunity, and overall survival (de Barros *et al*., 2017; Martinek *et al*., 2018; Ben-Shahar, 2018).

CONCLUSION

As a conclusion, the pattern of the response of both *P.microcephalum* and *I. senegalensis* (Family: Coenagrionidae) showed differences in the LC_{50} values and LT_{50} values. The toxicity test revealed the rate of mortality increased with the increase in concentrations but with shorter LT_{50} values from cadmium, manganese, and zinc. From this study, it is demonstrated that the three metals were ranked in order of toxicity for concentration (LC_{50} =Cd> Zn ≥ Mn) and different periods (LT_{50} = Cd > Zn ≥ Mn) towards both zygopteran larvae. Survivability of both odonate larvae was able to survive up to 200 mg/L concentration in Cd, Mn, and Zn. *Ischnura senegalensis* was found more tolerant towards Cd, Mn, and Zn compared to *P. microcephalum.* These studies are beneficial to build a database regarding the toxicity test of metals in Odonata larvae at certain metal concentrations which may serve as effective biological monitors of heavy metal pollution and this would be a useful tool for preventative and remedial interventions as well as for the development of a freshwater metal monitoring program, particularly in waterways affected by human activities and agricultural.

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ETHICAL STATEMENT

Please write the ethical statement. If no ethical approval is required, please state "Not applicable"

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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