

Toxicity of Zinc Dissolved In Saigon River to the Tropical Micro-Crustacean *Daphnia Lumholtzi*

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Abstract

In this study we evaluated the responses of the tropical micro-crustacean, *Daphnia lumholtzi*,

to zinc (Zn). For a more environmentally realistic assessment on the Zn toxicity, water sample collected from Saigon River was used as medium for the daphnids and Zn was spiked into field water for exposures. River water was physically and chemically measured and analyzed, and the sufficient water quality was confirmed prior to be used for the acute and chronic experiments. Metal concentrations in the test water were determined with an inductively coupled plasma/ mass spectrometry (ICP/MS). In the acute test implementation, Zn concentrations were from 67 – 360 µg/L. In the chronic experiments, the animals were incubated in control (filtered field water only) or filtered field water added with Zn (28, 46, and 75 µg/L) over the period of 14 days. The life history traits of daphnids including the survival, maturation, reproduction were daily observed. The results showed that the value of median lethal concentrations (48h-LC₅₀) was 136 µg Zn/L (95% CI = 107 – 165 µg Zn/L). Chronic exposures revealed that Zn caused the survival reduction, delayed maturity age and reproduction decrease. Our study suggested *D. lumholtzi* as a very good freshwater aquatic zooplankton for assessment on the toxicity of metals in tropics. Besides, the Vietnam guideline values regarding Zn should be re-considered and adjusted for the aquatic ecological protection.

Keywords: Acute toxicity, Chronic effects, *Daphnia lumholtzi*, Saigon River water, Zinc

1. Introduction

Trace metals are part of natural characteristics of aquatic and terrestrial environment. However, anthropogenic emissions (e.g. industrial and agricultural activities) have led to the metal concentration increase in aquatic environment. Metals are among the most common aquatic pollutants which are difficult to control (Tomasik and Warren, 1996). In aquatic ecosystem, many heavy metals such as zinc (Zn), copper (Cu), and nickel (Ni) among others at low concentrations are very important and essential for aquatic organisms (Wetzel, 2001). Nevertheless, these metals, at high concentrations, could intoxicate organisms such as phytoplankton, zooplankton and fish. In aquatic ecosystem, zooplanktons play a very important trophic position in the food chain and food web. Cladocerans, a group of zooplankton, consist of many genera and species (e.g. *Daphnia*) which are very sensitive to pollutants and widely used as good models for aquatic toxicological studies (Lampert, 2006; Shaw et al., 2008).

Numerous investigations on the toxicity of metals (e.g. Zn, Cd, Ni, Pb) on micro-crustaceans have been investigated. Naddy et al. (2015) conducted the acute test of Zn dissolved in artificial medium with the micro-crustacean *Ceriodaphnia dubia* and reported the 48h-LC₅₀ values of 61.8 – 130 µg/L. Vardia et al. (1998) and Chishty et al. (2012) reported the 48h-LC₅₀ values of *D. lumholtzi* tested with Zn dissolved in tap water were 2290 and 2300 µg/L, respectively. The median effective (immobilized) concentration (48h-EC₅₀) values of four different metals (Cd, Cu, Ni, Zn) to *Daphnia magna* were much different and the toxicity order of those metals to the tested species was Cd > Cu > Zn > Ni (Traudt et al., 2016). Besides, Cu was more toxic than some other metals such as Ni, Zn and chromium (Cr) to the two cladocerans, *Moina macropora* and *D. magna* (Biesinger and Christensen, 1972; Wong, 1992). Recently, Dao et al. (2016) reported the 48h-LC₅₀ of *D. lumholtzi* exposed to

Zn spiked into Mekong River water of 179.3 – 280.9 µg/L. In chronic toxicity of metals to micro-crustaceans, there have been more investigations on the effects of Cu and Ni than Zn. However, most previous studies agreed that the metals negatively affected on survivorship, maturity age and reproduction of micro-crustaceans (Winner and Farrell, 1976; Munzinger, 1994; Pane et al., 2004). Muysen et al. (2006) recorded the negative impacts of Zn (340 µg/L) on survival of *D. magna*. Dao et al. (2016) reported the Zn dissolved in Mekong River water at the concentrations of 50 – 56 µg/L impaired the life history traits of *D. lumholtzi* such as survival, maturity age, growth, fecundity and reproduction. Generally, toxicity of metals to *Daphnia* was studied considerably with species from temperate regions such as Europe and North American (e.g. *D. magna*, *D. pulex*, *D. parvula*, *D. ambigua*). However, investigations on the toxicity, especially chronic effects, of dissolved metals in field waters to the tropical micro-crustacean *D. lumholtzi* remained limited (Dao et al., 2016).

Toxicity of dissolved metals to micro-crustacean is regulated by the test conditions (De Schamphelaere and Janssen, 2002; Jo et al., 2010). The increase of pH and humic acid concentration in the test medium led to the decrease of toxicity of Zn to *D. magna* (Paulauskis and Winner, 1988). Naddy et al. (2015) reported a large variation on the values of 48h-LC₅₀ of *C. dubia* testing with Cd, Cu and Zn, under different hardness of the test medium. Similarly, dissolved metals (e.g. Cu, Ni) became more toxic when the hardness of water decreased (Taylor et al., 2016). Food availability also altered on the response of daphnids to metals and the fed daphnids got a higher tolerance to Cu than unfed daphnids (Dave, 1984).

Running through Hochiminh City Saigon River has largely contributed for the livelihood of so many local residents. Surface water from this river has been used as drinking water supply (Do-Hong et al., 2000). During the last decade, the rapid development of industrial activities up stream and along the Saigon River increases the threat to the water quality degradation of the river such metal contamination (Chanpiwat and Sthiannopkao, 2014). Consequently, environmental risks with metals in water of down stream Saigon River would be evoked. Hence, we questioned the potential toxicity affects on tropical micro-crustaceans in the near future. Also, we supposed that using field water for the toxicity tests would contribute a more realistic assessment of potential effects of metals than using artificial media (Dao et al., 2016). The aim of this study was to evaluate the acute and chronic effects of Zn spiked into Saigon River water on the tropical micro-crustacean, *Daphnia lumholtzi*.

2. Materials and Methods

2.1 Water Sampling

In September 2015, field water was collected at surface from Saigon River (Fig. 1). Water sample for toxicity tests were brought to the laboratory in the same day and prepared for the experiments. Field water was passed through the 0.45 µm filter (Sartorius, Germany), stored in plastic containers prior to the experiments. Sub-sample from the sampling site was taken for analyses of dissolved metal, alkalinity and hardness.

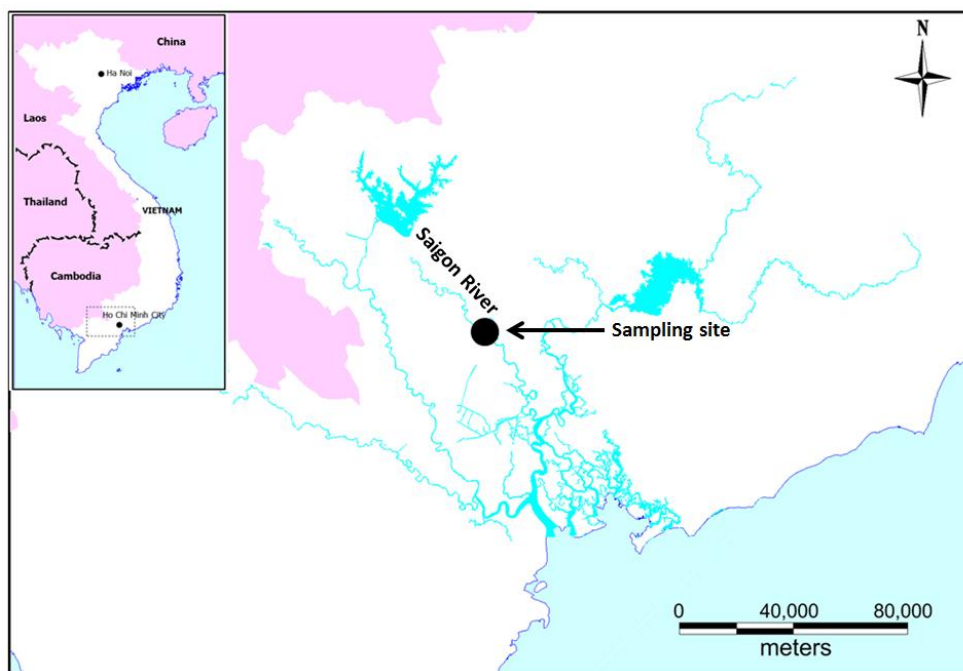


Figure 1. Saigon river in Vietnam with the sampling site, black round dot (coordinated with 10°58'31.2"N and 106°39'19.0"E), for the toxicity test.

2.2 Physical and Chemical Characterization of the Field Water Sample

The filtered water sample was acidified with concentrated HNO₃ (65%, Merck) and used for dissolved metal characterization (APHA, 2005) with an inductively coupled plasma/ mass spectrometry (ICP/MS – Agilent 7500, USA). All the interested metals in water samples were analyzed. The ICP-MS operating conditions and parameters for metal analysis in sample are presented in Table 1. For analysis, calibration solutions were analyzed followed by an initial calibration blank to control carry-over. Immediately following the initial calibration blank, an initial calibration verification which was prepared from a different source of analyses, was analyzed. The calibration verification standard should be a mid-range calibration standard. The percent recovery of the initial calibration verification standard should be 90-110% for each element being determined.

Table 1. ICP-MS operating conditions and parameters for metal analysis

Operating conditions	
Spray chamber	Scott double pass
Nebulizer pump (rps)	0.1
RF power (W)	1550
RF Matching (V)	0.2
Sample depth (mm)	8
Torch-H (mm)	0.2
Torch-V (mm)	0.2
Plasma gas flow rate (L/min)	15

Carrier gas (Ar) flow rate	0.9 (optimized daily)
Measurement parameters	
Scanning mode	Peak hopping
Resolution (amu)	0.7
Readings/replicate	1
No. of replicates	3
Internal standard	⁴⁵ Sc
Isotopes – Collision gas (He)	²⁷ Al, ⁵² Cr, ⁵⁵ Mn, ⁵⁶ Fe, ⁵⁹ Co, ⁶⁰ Ni, ⁶³ Cu, ⁶⁶ Zn ⁷⁵ As, ⁷⁸ Se, ⁹⁵ Mo.
Isotopes – No gas	¹⁰⁷ Ag, ¹¹¹ Cd, ¹³⁷ Ba, ²⁰⁸ Pb.

The total hardness of filtered river water was determined based on concentrations of Ca²⁺ and Mg²⁺ and the alkalinity was determined by titration method (APHA, 2005).

2.3 Toxicity Test

The test micro-crustacean, *Daphnia lumholtzi* (Fig. 2), was collected from a fish pond in Northern Vietnam (Bui et al., 2016) and has been well maintained in the Laboratory of Environmental Toxicology, Institute for Environment and Resources, Vietnam National University – Hochiminh City, for more than 2 year. *Daphnia lumholtzi* was raised in COMBO medium (Kilham et al., 1998), at 27 ± 1 °C with a photoperiod of 12h light and 12h dark and under the light intensity of around 1000 Lux. The micro-crustacean was fed with a mixture of green alga (*Chlorella* sp.) which was cultured in COMBO medium and YCT (yeast, cerrophyll and trout chow digestion), prepared according to the U.S. Environmental Protection Agency Method (US EPA, 2002).

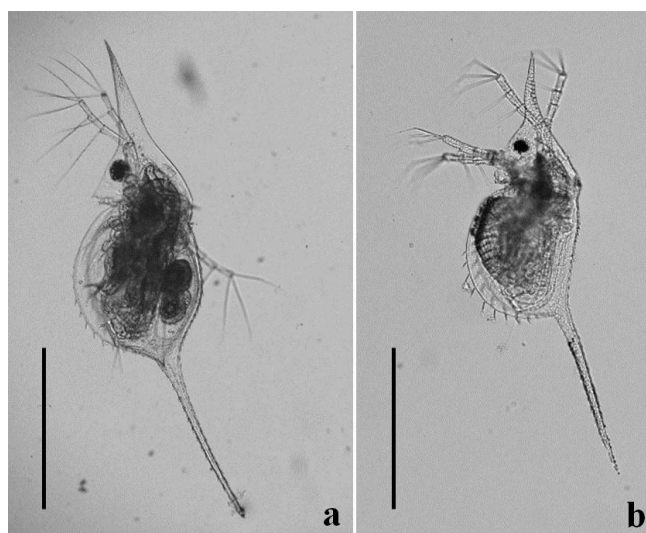


Figure 2. *Daphnia lumholtzi* from Vietnam; a, adult *D. lumholtzi* at its maturity age (scale bar = 500 µm) and b, new born *D. lumholtzi* (scale bar = 200 µm).

For control experiments, *D. lumholtzi* was incubated in the filtered field water while metal treatments were implemented by spiking Zn (stock solution of 1000 ppm, Merck) into the filtered field water prior to the test initiation.

The 48-h static nonrenewal acute toxicity tests were conducted according to the US EPA

methods (US EPA, 2002) with a minor modification to temperature and light and temperature conditions for tropical species as mentioned above. The neonates of *D. lumholtzi* (age ≤ 24 h) were used for testing. Organisms were fed before exposure but starved during the tests (US EPA, 2002). Each test included a control (field-collected water) and five treatment concentrations of Zn (67, 113, 179, 309, and 360 $\mu\text{g/L}$). Four replicates per treatment with 10 organisms per replicate were used and the organisms were incubated in 50 mL polypropylene beakers containing 40 mL test water. When the test terminated, sub-water samples were collected from each treatment for analysis of dissolved Zn. The sub-samples were acidified to pH 2 with saturated HNO_3 (Merck) prior to analysis. Dissolved Zn analysis was performed by ICP-MS (as described above). The record of mortality and removal of dead organisms from the test beakers were performed daily. Mortality was the test end point and the data were used to determine median lethal concentrations (LC_{50}). Based on the 48h- LC_{50} values from the acute tests, the concentrations of 28, 46 and 75 $\mu\text{g Zn/L}$ were chosen for the chronic exposures.

Chronic tests were performed according to the APHA (2005) and Dao et al. (2010) with minor modification on the temperature and light: dark cycle for tropical organisms. Briefly, neonates of *D. lumholtzi* less than 24 h from 2nd – 3rd clutch were individually incubated in 50 mL polypropylene beaker containing 20 mL filtered river water. For each treatment (control or Zn exposure), 15 replicates were prepared. The food and test conditions were the same as the rearing conditions mentioned above. Media and food for daphnids were renewed 3 times per week. The life history traits of daphnids including the dead, maturation, reproduction were daily observed. The chronic tests lasted for 14 days. Water temperature (WTW Oxi197i multi-detector), dissolved oxygen (DO, WTW 350i), and pH (Metrohm 744) were measured at test initiation and termination (for acute test) or medium renewal (chronic test).

2.4 Data Analysis

Median lethal concentration with 95% confidence intervals (95% CIs) was calculated by the Probit (USA). Shapiro-Wilk test (Sigma Plot, version 12) was applied for calculation of the significant difference of the maturation of *D. lumholtzi* in control and Zn exposures.

3. Results

3.1 Physical and Chemical Characteristics of Field Water From Saigon River

The ICP/MS analysis showed that filtered field water from Saigon River contained several metals with quite low concentrations (2 $\mu\text{g/L}$ of Cu, 5 $\mu\text{g/L}$ of Zn), except Al (111 $\mu\text{g/L}$), Mn (43 $\mu\text{g/L}$) and Fe (279 $\mu\text{g/L}$, Table 2).

Table 2. Dissolved metal concentrations ($\mu\text{g/L}$) in filtered field water from Saigon River. BDL, below detection limits of the ICP/MS, 1 $\mu\text{g/L}$.

Al	Ag	As	Au	Be	B	Ba	Co	Cr	Cu
111	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2
Fe	Mn	Ni	Pb	Sr	Se	Sb	V	Ti	Zn
279	43	BDL	BDL	BDL	BDL	BDL	BDL	BDL	5

The physical and chemical measurement showed that the pH and dissolved oxygen of field water from Saigon River was 7.07 and 6.2 mg/L, respectively (Table 3). The pH in the test water (control and Zn exposures) slightly varied from 7.03 – 7.31. The hardness and alkalinity of Saigon river water used for the tests were 29 and 15 mg/L (as CaCO₃), respectively.

Table 3. Physical and chemical characteristics of the field water from Saigon River and the test water during the experiments.

Parameters	Saigon River
pH (in the field water)	7.07
pH (in the test water after metal addition)	7.07 – 7.31
Dissolved oxygen in the test water (mg L ⁻¹)	6.2
Hardness (mg/L as CaCO ₃)	29
Alkalinity (mg/L as CaCO ₃)	15

3.2 Acute and Chronic Effects of Zn on *Daphnia Lumholtzi*

The result of acute test showed that the 48h-LC₅₀ was 136 µg Zn/L (95% CI = 107 – 165 µg Zn/L). In chronic exposures, the survival proportion of *D. lumholtzi* in the control was 80% after 14 days of incubation while the survival rate of the animals in the exposures to 28 and 46 µg Zn/L was 60 and 46%, respectively (Fig. 3). However, the first *D. lumholtzi* in the exposure to 75 µg Zn/L started to die after 4 days of incubation then the survival of the animals in this treatment sharply decreased afterward. The last *D. lumholtzi* died on the 9th day of experiment (Fig. 3).

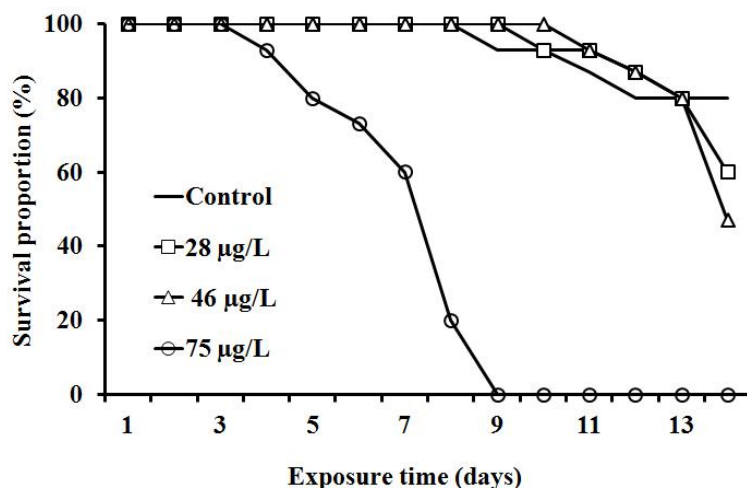


Figure 3. Survival proportion of *Daphnia lumholtzi* exposed to Zn spiked into filtered water from Saigon River

The maturation of *D. lumholtzi* from the chronic tests was showed in the Figure 4. The animals in the control and the two lower Zn concentration exposures reached their maturity age at approximately 3 days old. However, exposed the highest Zn concentration, 75 µg/L,

the *D. lumholtzi* matured at around 4 days old, significantly delayed its maturation compared to the control.

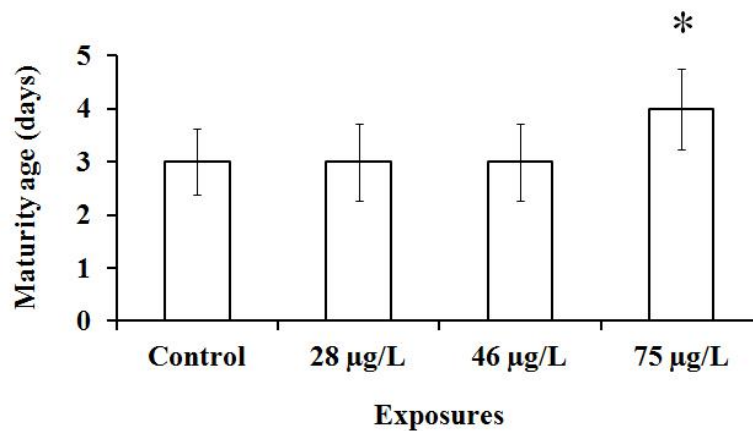


Figure 4. Maturity age of *Daphnia lumholtzi* (mean value \pm SD) exposed to Zn spiked into filtered water from Saigon River. Asterisk indicates significant difference between control and exposures by Shapiko-Wilk test (*, $P < 0.01$).

The accumulative neonates from the control were 329. However, those from the exposures to Zn at the concentrations of 28, 46 and 75 µg/L were 335, 279 and 38, respectively (Table 4).

Table 4. The total accumulated offspring *Daphnia lumholtzi* in the exposures during 14 days of experiment.

Control	28 µg Zn/L	46 µg Zn/L	75 µg Zn/L
329	335	279	38

4. Discussion

Chemical and physical characteristics

Generally, the trace metals of field water from Saigon River in the current study were quantitatively more or less similar to that from the same location in a previous investigation (Bui et al., 2016). Besides, the concentrations of some found trace metals (e.g. Cu, Zn) in Saigon River in our study were comparable to those from Mekong River (Dao et al., 2016). Hence, based on the metal characteristics the filtered water from Saigon River would be suitable for using as the control medium for testing with the micro-crustacean *D. lumholtzi*.

Both pH and DO in the test water were favorable for the living and growth of daphnids (APHA, 2005). The pH of water in this study was slightly higher than that from Saigon and Dongnai Rivers in a previous study (6.62 – 6.92; Bui et al., 2016), but similar to the pH range from Mekong River or many other tropical rivers and lakes (7.03 – 8.62; Villavicencio et al., 2005; Dao et al., 2016). The hardness and alkalinity from Saigon River used for the current study were not high and could be classified into the soft water as described in previous investigations (Villavicencio et al., 2005; Naddy et al., 2015). The soft water would enhance the toxicity of dissolved metal to daphnids. The pH, alkalinity and hardness of the test water should regulate the toxicity of dissolved metals in our acute and chronic tests.

Acute test

The 48h-LC₅₀ value for *D. lumholtzi* exposed to Zn dissolved in Saigon River from this study water was lower than that in Mekong River (Dao et al., 2016). So toxicity of Zn became more potent in Saigon River compared to Mekong River water. The pH, alkalinity and hardness of Saigon River water were lower than those of Mekong River water and that should be the major root of the two different acute test results of our study and investigation of Dao et al. (2016). This supports the report of De Schampelaere and Janssen (2002) and Jo et al. (2010).

The values of 48h-LC₅₀ of *D. magna* and *Ceriodaphnia.dubia* to Zn were 928 and 102 – 130 µg/L, respectively (Naddy et al., 2015; Traudt et al., 2016) at moderately hard water (in the test with *D. magna*) and hard water (in the test with *C. dubia*) revealed that *D. lumholtzi* was more sensitive to Zn than *D. magna* but less sensitivity to Zn than *C. dubia*. This record is in line with previous investigation revealing the high sensitivity of *D. lumholtzi* to trace metals (Dao et al., 2016).

Chronic test

The high survival rate of *D. lumholtzi* in control (80%) after 14 days of experiment met the requirement of APHA (2005). However, the much lower survival rate in the exposures to 28 and 46 µg Zn/L (Fig. 3) supported the previous study in which *D. lumholtzi* was incubated in Mekong River water containing 50 µg Zn/L (Dao et al., 2016). Seriously the exposure to 75 µg Zn/L caused the collapse of the daphnid population within 9 days of treatment. The Vietnam Environmental Protection Agency issued the allowed Zn concentration in the industrial wastewater of 3000 µg/L (QCVN 40:2011) which is far higher than the test concentrations of Zn used in our study. Therefore, the Vietnam guideline values regarding Zn concentration in wastewater should be re-considered and adjusted to protect the aquatic organism in nature.

Acharya et al. (2006) raised *D. lumholtzi* in water from Ohio River and observed the age to first clutch of the animals of around 7 days old. The maturity age of *D. lumholtzi* incubated in field water from Saigon River of around 3 days old which is similar to study result with Mekong River water of Dao et al. (2016). Hence, the daphnids raised in Saigon River water seemed to reach its maturation a little earlier than those in Ohio River water. Ebert (1992) hypothesized that the maturation of daphnids is closely related to threshold size and the animals' maturation could only be initiated at a certain body size. Properly, the experiment conditions, food availability and quality could strongly regulate the development or growth consequently maturation of daphnids. The response of *D. lumholtzi* to Zn at the concentration of 75 µg/L in our study is in line with previous investigations in which *D. lumholtzi* exposed to 50 – 56 µg Zn/L and *D. obtusa* shortly exposing to Cr delayed the maturation and time to first reproduction (Coniglio and Baudo, 1989; Dao et al., 2016).

Zinc at low concentrations should be an essential trace element for the living and growth of micro-crustacean (Kilham et al., 1998). However, the metals at high concentration could induce the impairment on the respiratory function of *D. magna* (Pane et al., 2003) and inhibit

the sodium uptake leading to the osmotic imbalance in aquatic animals (Grosell et al., 2002) leading to the energy cost for maintain consequently fecundity reduction. This helped to explain the strong inhibition on reproduction in the exposure to 46 and 75 $\mu\text{g Zn/L}$ (Table 4) which was ever reported elsewhere (Dao et al., 2016).

5. Conclusions

Saigon River water, at the time of sampling, had a sufficient quality regarding metals contamination for growth and development of micro-crustaceans. The effect of Zn on survival of *D. lumholtzi* in acute and chronic test revealed the high sensitive of this animal to Zn. Chronic exposures caused the maturity postponement and reproduction inhibition of the *D. lumholtzi*. The results of this study are confident proofs for the reconsideration and adjustment of the Vietnam guideline values related to Zn concentration in wastewater for the aquatic ecosystem protection. The negative effects of Zn on *D. lumholtzi* may be useful for extrapolation/ prediction the potential toxicity of the trace metal to micro-crustaceans *in situ* in the future. Further investigations on the toxicity of Zn to other tropical organisms and combined effects of Zn and other pollutants in river water on *D. lumholtzi* are suggested.

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