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HIGHLY POTENT TOXICITY OF NICKEL IN RIVER WATER TO *DAPHNIA LUMHOLTZI*

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ABSTRACT

Trace metals are essential for aquatic organisms at low concentrations but become toxic at certain concentrations. This study aimed to evaluate the acute and chronic toxicity of Ni dissolved in natural water of Saigon River in Vietnam to the tropical micro-crustacean, *Daphnia lumholtzi.* Filtered field water was physical and chemical characterization prior to the acute and chronic experiments. In the acute test, *D. lumholtzi* was exposed to Ni at the concentrations from 50 – 1000 µg/L. In the chronic experiments, the animals were incubated in control (filtered field water only) or filtered field water with Ni addition (final concentrations of 5, 12, and 196 µg/L) over the period of 14 days. The results showed that the values of median lethal concentrations (48h-LC_{50}) was 468 µg Ni/L (95% CI = 302 – 726 µg Ni/L). In chronic exposures, it was observed that Ni caused the strong survivorship decrease, maturation postponement, fecundity reduction and reproduction inhibition. The detrimental impacts of Ni on daphnids in natural water bodies would be more severe than those in the artificial medium in laboratory conditions. In addition, the Vietnam guideline values regarding Ni should be re-considered and adjusted to protect the aquatic ecosystem.

INTRODUCTION

Metals are natural chemicals however their occurrence, distribution and fate have been strongly changed under human activities (e.g. industrial and agricultural activities, mining operations) recently. In aquatic ecosystem, many trace metals (e.g. Cu, Ni, Zn) are essential for living organisms whereas some others are not (e.g. Hg, Ag). However, all metals induce toxicity when their concentrations go beyond a certain threshold level for organisms (Wetzel, 2001). Unlike other pollutants (e.g. organic compounds) dissolved metals are not degradable hence their toxicity quite constant in the environment (Walker *et al*., 1996). Therefore, metals are aquatic pollutants difficult to control (Tomasik and Warren, 1996). Zooplankton posses the intermediate position in aquatic 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). So far, there have been numerous investigations on the toxicity of metals to zooplankton but only a few studies on the acute effects of Ni on micro-crustaceans. Two daphnid species *Daphnia magna* and *Daphnia lumholtzi* were used for the acute test with Ni and the 48h-LC50 values ranged from 1068 – 1633 µg Ni/L for *D. magna* (Pane *et al*., 2003; Traudt *et al*., 2016) and 1026 – 1775 µg Ni/L for *D. lumholtzi* (Dao *et al*., 2015, 201X). Besides, Wong (1992) reported the 48h-LC50 value of 6480 µg Ni/L for another daphnid species *Moina macrocopa*. In chronic incubations, life span of *D. magna* was significantly reduced under exposure to 100 and 200 µg Ni L^{-1} (Munzinger, 1994). Besides, the same author proved that brood size, body length of adult *D. magna* and its accumulative offspring were decreased under the incubation with Ni at the concentrations from 40 – 200 µg/L. The chronic exposures of *D. magna* to Ni (40 – 160 µg/L) not only induced the negative impacts on the animals' body length, life span and reproduction but also inhibited the growth of their offspring although the progeny of

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pre-exposed daphnids were raised in Ni-free medium (Munzinger, 1994). Results from the investigation of Dao *et al*. (201X) showed that the concentrations of 6 µg Ni/L enhanced the body length of *D. lumholtzi* but from 46 – 302 µg Ni/L resulted in a strong mortality, reduced the body length, postponed the maturation, and lowered the fecundity. It is important to note that toxicity of dissolved metals to aquatic organisms such as micro-crustacean and fish was regulated by several environmental parameters such as pH, alkalinity, hardness and dissolved organic compounds among others (De Schamphelaere and Janssen, 2002; Ryan *et al*., 2009; Jo *et al*., 2010).

Generally, most of previous investigations on the chronic toxicity of metals to daphnids were implemented by spiking metals into artificial medium. However, investigations on the toxicity, especially chronic effects, of dissolved metals in field waters to the tropical micro-crustaceans (e.g. *D. lumholtzi*) remained limited. Additionally, it is important to note that the toxicity of trace metals became higher in field water due to other undetermined pollutants (Dao *et al*., 201X). As the matter of fact, it is more environmental realistic and ecological relevance to study the toxicity of pollutants like metals with natural water than with artificial medium. In this study we evaluated the detrimental impacts of Ni dissolved in natural water from Saigon River to *D. lumholtzi* and compared the negative effects with another previous study (Dao *et al*., 2015) in which the same species was acutely and chronically tested with Ni dissolved in an artificial medium, COMBO.

MATERIALS AND METHODS

Field water was collected at surface from Saigon River in March 2015 (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 on dissolved metal, alkalinity and hardness. The filtered water sample was acidified with concentrated HNO_3 (Merck) and used for dissolved metal characterization (APHA, 2005) with an inductively coupled plasma/ mass spectrometry (ICP/MS – Agilent 7500, USA). The ICP-MS operating conditions and parameters for metal analysis in sample are presented in Table 1. The total hardness of filtered river water was determined based on concentrations of Ca^{2+} and Mg^{2+} and the alkalinity was determined by titration method (APHA, 2005).

Test organisms

The test micro-crustacean, *Daphnia lumholtzi*, 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 ^oC 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, cerrophyl and trout chow digestion), prepared according to the U.S. Environmental Protection Agency Method (US EPA, 2002).

Toxicity testing

For control experiments, *D. lumholtzi* was incubated in the filtered field water while metal treatments were implemented by spiking Ni (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. Tests were conducted at 27 ± 1 ^oC with a light dark cycle of 12h light: 12h dark, and the light intensity of around 1000 Lux, in the Laboratory of Environmental Toxicology. The neonates of *D. lumholtzi* (age ≤ 24 h) were used for testing. Transferring organisms into test chambers initiated the test. Organisms were fed before but during the tests (US EPA, 2002). Each test included a control (field-collected water) and five treatment concentrations of Ni. 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. Sub-water samples were collected from each treatment when the test terminated for analysis of dissolved Ni. The sub-samples were acidified to pH 2 with saturated HNO_3 (Merck) prior to analysis. Dissolved Ni characterization was performed by ICP-MS (as described above). For the acute test, Ni concentrations of 50, 250, 500, 750 and 1000 µg/L were used. 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 5, 12 and196 µg Ni/L were chosen for the chronic exposures. When the tests ended, the medium was filtered and acidified (APHA. 2005) and the concentrations of Ni in the exposures were determined by ICP/MS. 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 2^{nd} – 3^{rd} clutch were individually incubated in 50 mL polypropylene beaker containing 20 mL filtered field water. For each treatment (control or Ni exposure), 15 replicates were prepared. The food and test conditions were the same as the rearing conditions mentioned above (see Test organism). 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).

Data analysis

Median lethal concentrations with 95% confidence intervals (95% CIs) were calculated by Toxcalc Program (Tidepool Scientific LLC. USA). Kruskal Wallis test (Sigma Plot, version 12) was applied for calculation on the significant difference of the maturation and fecundity of *D. lumholtzi* in control and Ni exposures.

RESULTS

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 including Al, B, Ba, Fe, Mn, Mo and Pb (Table 2). Among the found metals, Al (305 µg/L) and Pb (4 µg/L) were those may induce or contribute to the negative influences to aquatic organisms.

The physical and chemical measurement showed that the pH and dissolved oxygen of field water and test water from Saigon River was slightly varied from 6.78 – 6.91 and 6.48 – 6.61 mg/L, respectively (Table 2). The hardness of Saigon River water used for the tests was 29 mg/L (as $CaCO_3$). However, the alkalinity of the test water from the river varied from 9 – 15 mg/L (as $CaCO_3$) due to the Ni addition.

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.

Table 2. Dissolved metal concentrations (µg/L) and physical characteristics of filtered field water from Saigon River used for the test. BDL, below detection limits of the ICP/MS, 1 µg/L; N/A, not available. *, submitted manuscript to Environmental Management and Sustainable Development

Metals/ physical parameters	Bui et al. (2016)	Dao et al.*	This study
Ag	< 30	BDL	BDL
Al	BDL	111	350
As	BDL	BDL	BDL
B	BDL	BDL	64
Ba	BDL	BDL	111
Cd	BDL	BDL	BDL
Cr	BDL	BDL	BDL
Cu	BDL	2	BDL
Fe	N/A	279	460
Mn	N/A	43	33
Mo	N/A	BDL	28
Ni	BDL	BDL	BDL
Pb	BDL	BDL	4
Se	< 160	BDL	BDL
Zn	BDL	5	BDL
pH	6.62 – 7.88	7.07 – 7.31	6.78 – 6.91
Dissolved oxygen (mg L^{-1})	N/A	6.2	6.48 – 6.61
Alkalinity (mg $CaCO_3$ L^{-1})	18	15	9 - 15
Hardness (mg $CaCO_3$ L^{-1})	45	29	29

Table 3. The values of 48h median lethal concentrations (48h-LC_{50}) Ni for daphnid species

Species	Metals	48h-LC_{50}	Test water	Sources
Daphnia lumholtzi	Ni (µg L^{-1})	1775	Artificial medium	Dao et al., 2015
Daphnia magna	Ni (µg L^{-1})	1068	Tap water	Pane et al., 2003
Daphnia magna	Ni (µg L^{-1})	1633	Artificial medium	Traudt et al., 2016
Daphnia lumholtzi	Ni (µg L^{-1})	1026 - 1516	Mekong River,	Dao et al., 201X
Moina macrocopa	Ni (µg L^{-1})	6480	Aquarium	Wong, 1992
Daphnia lumholtzi	Ni (µg L^{-1})	468 (95% CI: 302 – 726)	Saigon River	This study

Table 4. The accumulative offspring *Daphnia lumholtzi* during the experiment

Control	5 µg Ni/L	12 µg Ni/L	196 µg Ni/L
714	578	475	24

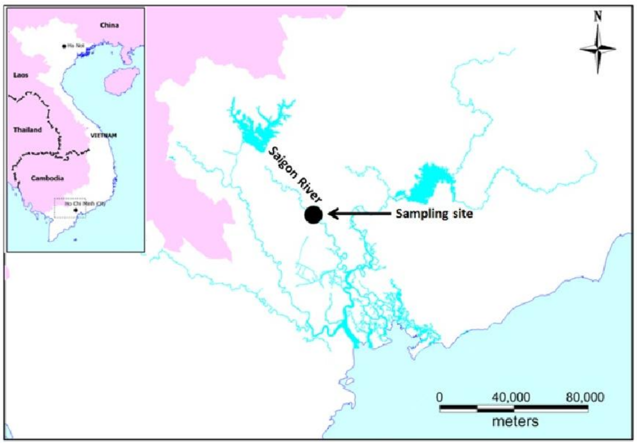

Figure 1. Saigon River in Vietnam with the sampling site (coordinated with 10°58'31.2"N and 106°39'19.0"E) for the toxicity test

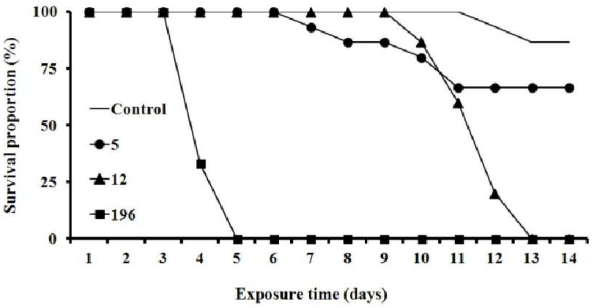

Figure 2. Survival proportion of *Daphnia lumholtzi* exposed to Ni spiked into filtered water from Saigon River

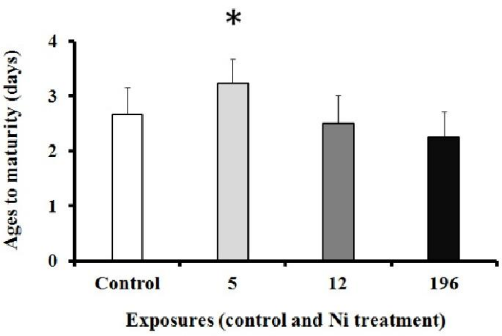

Figure 3. Maturity age of *Daphnia lumholtzi* (mean value ± SD) exposed to Ni spiked into filtered water from Saigon River. Asterisk indicates significant difference between control and exposures by Kruskal Wallis test (*, P < 0.05).

Acute effects of metals on *Daphnia lumholtzi*

The result of acute test showed that the 48h-LC_{50} was 468 µg Ni/L (95% CI = 302 – 726 µg Ni/L; Table 3).

Chronic effects of metals on life history traits of *Daphnia lumholtzi*

Effects on survival

After 14 days of incubation, the survival proportion of *D. lumholtzi* in the control was 87% while that in the exposure to 5 µg Ni/L was 67% (Fig. 2). In the incubation with 12 µg Ni/L the first daphnids died at the 10th day of experiment then sharply reduced afterward, and the last daphnid died on the 13th day of experiment. Seriously, none of the daphnids in the 196 µg Ni/L treatment was alive after 5 days of the test.

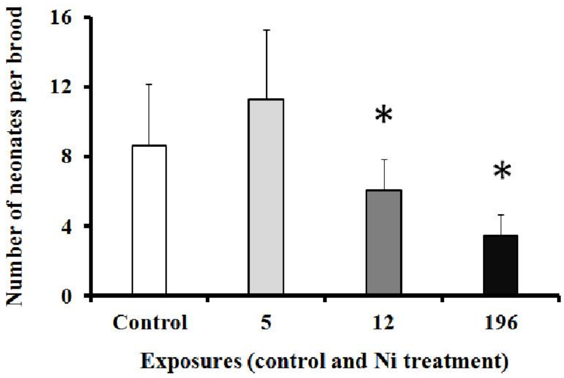

Figure 4. Fecundity of *Daphnia lumholtzi* exposed to Ni spiked into filtered water from Saigon River. Asterisk indicates significant difference between control and exposures by Kruskal Wallis test (*, P < 0.05)

Effects on maturation

The ages of the animals in the control and the two higher Ni concentration exposures were from 2.25 – 2.7 days and not statistically different (Fig. 3). However, the exposure to the lowest Ni concentration in our study, 5 µg Ni/L, resulted in the postponement of the maturation of the daphnids with the ages of around 3.2 days old.

Effects on fecundity and reproduction

The fecundity of the *D. lumholtzi* in the four incubations was different. In the control, the mean brood size of the daphnids was around 8.6 neonates (Fig. 4). However, it was around 11, 6 and 3.4 neonates in treatments of 5, 12 and 196 µg Ni/L, respectively. The accumulative neonates from the control were highest 714 (Table 4). However, those from the exposures to Ni at the concentrations of 5, 12 and 196 µg/L were 578, 475 and 24, respectively.

DISCUSSION

Chemical and physical characteristics

Generally, concentrations of some trace metals from Saigon River in the current study were a little higher than in previous investigations. For example, Al and Fe concentrations were more than double than those in a previous sampling time (Dao *et al*. submitted manuscript to Environmental Management and Sustainable Development). Additionally, the metals B, Ba, Mo and Pb were detected in the present study but not in previous investigations (Table 2). The contamination of trace metals in this study would contribute to side effects of field water to micro-crustaceans especially during chronic exposures. Both pH and DO in the test water were within the range for the well growth of micro-crustaceans (APHA, 2005). The pH of water in this study was similar to that in the study of Bui *et al*.

(2016; Table 2), but lower than the pH range from many other tropical rivers and lakes (7.03 – 8.62; Villavicencio *et al*., 2005; Dao *et al.,* 201X). The hardness and alkalinity from Saigon River in this study, like the two previous investigations (Table 2) could be classified into the soft water (Villavicencio *et al*., 2005; Naddy *et al*., 2015) which would enhance the toxicity of dissolved metal to daphnids. Besides, the pH, DO and alkalinity of Saigon River in our study are comparable with those from the COMBO medium (Dao *et al*., 2015).

Acute test

The 48h-LC50 value for *D. lumholtzi* exposed to Ni in this study water was much lower than that in previous investigation (Table 3). The difference in the 48h-LC50 values of the daphnids to Ni (Table 3) could be partly due to the different sensitivity of daphnid species as reported elsewhere (Dao *et al*., 2010; Dao *et al*., 201X). As the same species exposed to Ni, the 48h-LC50 tested with Mekong River (Dao *et al*., 201X) was from two – three times higher than the 48h-LC50 tested with Saigon River in our study. This should be closely related to the higher hardness (79 – 87 mg/L) and alkalinity (64 – 68 mg/L) from Mekong River than Saigon River (alkalinity of 9 – 15 mg/L; hardness of 29 mg/L) because the toxicity of metals increased with decrease of these two physical parameters (De Schamphelaere and Janssen, 2002). However, the large gap of 48h-LC50 values (Table 3) of the same species (*D. lumholtzi*) exposed to the same metal (Ni) in similar pH and alkalinity, and favorable DO from a previous (Dao *et al*., 2015) and current studies should be appointed to the major contribution of the other trace metals (e.g. Al, Pb) in natural water from Saigon River (Table 2). Traudt *et al.* (2016) noted the combined effects of different metals (e.g. Ni, Cd, Cu and Zn) to *D. magna* which could be the very reasonable explanation to the enhanced and very potent toxicity of Ni to *D. lumholtzi* when the metal was spiked into river water.

Chronic test

The survivorship of *D. lumholtzi* in control (87%) by the end of the chronic test was within the acceptance according to the guide of APHA (2005). However, the strong decease of the survival of daphnids in Ni treatments (5, 12, 196 µg/L) revealed the serious toxicity of the metal in river water characteristics. In the experiment with COMBO medium, negative effect of Ni on daphnids survival was slightly at the concentrations from 65 – 750 µg Ni/L (Dao *et al*., 2015). Hence the toxicity of Ni spiked into field water was more potent than in artificial medium. Besides, Dao *et al*. (201X) reported the survival effect of Ni (dissolved in Mekong River water) on *D. lumholtzi* was mild at the concentrations of 5 – 59 µg/L. However, the current study showed the serious impact with 100% mortality of daphnids caused by 12 µg Ni/L (Fig. 2). Therefore, again the water characteristics especially other pollutants in the Saigon River water (e.g. Al, Pb) should be the important and major elements enhanced on the toxicity of Ni in our study. Further investigations on the combined effects of trace metals in artificial medium on daphnids are suggested. The Vietnam Environmental Protection Agency issued the allowed Ni concentrations of 100 and 500 µg/L in the surface and effluent, respectively (QCVN 40:2011), which are far higher than the test concentrations of Ni used for our study. Therefore, the Vietnam guideline values regarding Ni concentration in surface and wastewater should be re-considered and adjusted for the aquatic ecosystem protection.

Dao *et al*. (2015) reported that *D. lumholtzi* in COMBO medium matured at the age of around 4 days old. Additionally, 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. We observed the maturity age of *D. lumholtzi* in the control of our study was around 2.7 days old which is quite earlier than the previous records (Acharya *et al*., 2006; Dao *et al*., 2015). This is probably due to the consequence of the different test media and also the pollutant contamination in field water used for the test. However, the current observation is similar to another result in *which D. lumholtzi* was also raised in natural water collected in the same location but different collecting time from Saigon River (Dao *et al*., submitted manuscript to Environmental Management and Sustainable Development). The postponement of maturation of daphnids exposed to 5 µg Ni/L in current study (Fig. 3) is in line with previous study (Dao *et al*., 201X). Nickel is an essential trace element for aquatic animals including daphnids. However, trace metals at high concentration could induce the impairment on the respiratory function of daphnids (*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 fecundity and reproduction in the exposures to 12 and 196 µg Ni/L (Fig. 4; Table 4) which was ever reported elsewhere (Dao *et al.,* 2015, 201X). However, it is important to note that fecundity of *D. lumholtzi* was enhanced at low Ni concentrations (5 – 46 µg/L) and inhibited at high Ni concentrations (225 – 302 µg/L; Dao *et al*., 201X). Seriously, the significant decrease of fecundity of *D. lumholtzi* in the current study was already happened at the concentration of 12 µg Ni/L (Fig. 4). Therefore, the hypothesis of combined effects of other pollutants in field water (e.g. Al, Pb) is now supported again. Anyway, this needs further investigations with artificial medium and purified chemicals for clarification.

Conclusion

The acute and chronic effects of Ni dissolved in natural water from Saigon River were observed. There was strong effect on survival of *D. lumholtzi* in Ni exposures. Life history traits of the daphnids such as maturation, fecundity and reproduction were adversely affected in the chronic incubation with Ni addition. Contamination of trace metals such as Al and Pb was found in Saigon River water. This led to the more potent toxicity of Ni in the incubations with the river water. Therefore, the detrimental impacts of trace metals on daphnids in natural water bodies would be more severe than those in the artificial medium in laboratory conditions. Besides, the results recommended the reconsideration and adjustment of the Vietnam guideline values related to Ni in effluents for the aquatic ecosystem protection. The combined effects of trace metals on daphnids in artificial medium are suggested.

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