International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:02, Issue:04

CHRONIC EFFECTS OF INDUSTRIAL WASTEWATER ON LIFE HISTORY TRAITS OF *Daphnia magna* UNDER THE LABORATORY CONDITIONS

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ABSTRACT

This study evaluated the chronic toxicity of wastewater from Nhon Trach Industrial Zone, in Southern Vietnam, to life history traits of *Daphnia magna.* Wastewater samples were collected at the out let of the industrial zone for heavy metal and toxicity bioassay. The animals were exposed to the wastewater at different concentrations 0, 10%, 50% and 100% (v/v) for 21 days under laboratory conditions. Survival, maturation, reproduction and negative appearance of *D. magna* were recorded daily while dry weight of the animal was measured by the end of experiments. The inductively coupled plasma/mass spectrometry analysis characterized many heavy metals with their concentrations within the guideline values of Vietnam water quality (QCVN) for discharge into nature. Results of toxicity tests showed that the wastewater quickly increased mortality, significantly delayed maturation and caused strong reduction on reproduction of *D. magna*. Dead embryos and neonates in the *Daphnia*'s shell were found in the wastewater exposures. Seriously, the phenomenon of *D. magna*'s organ disappeared after reproducing was recorded in wastewater incubation. To our knowledge, this is the first study on the toxicity of industrial wastewater to *D. magna*. Further investigations should be conducted for revising the QCVN to protect sensitive organisms in the aquatic ecosystem.

Keywords: *Daphnia magna*, industrial wastewater, chronic test, life history traits, malformation.

ISSN: 2455-6939

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1. INTRODUCTION

Industrial estates are established to fulfill the demand of the growing population in the world [1]. However, industrial wastewater is one of the important pollution sources of the aquatic environment [2]. The wastewater of industrial zones contained metals, organic compounds [3], inorganic complexes and other non-biodegradable substances [4]. These resulted in serious pollution problems in aquatic environment and caused negative effects on the ecosystem and community health [2]. Effluents from industrial zones could contain heavy metals which are very toxic to living organisms. Numerous investigations in the world showed that toxicity of metals has been studied extensively with standard organisms in western countries [5 – 11].

In nature, micro-crustaceans (e.g. *Daphnia*), one of the most diverse and important group of zooplankton, have the intermediate position in the freshwater food web, and play the important connector between primary production and other consumers [12]. In additional, *Daphnia* posses many ecological and biological characteristics and is very sensitive to toxins in aquatic environment. This animal has been used as a model organism for toxicity testing [13]. According to Ghazy and Fayed [14], the urban wastewater released from septic tanks had negative impacts on survival, growth and reproduction of *D. magna* at the concentrations of 13% (v/v). Besides, the mean survivorship proportion of *D. magna* exposed to the wastewater after primary, secondary and tertiary treatments were 29%, 76% and 100%, respectively [15].

So far, toxicological research and publications in term of bioassay from Vietnam have been scared and limited. The remarkable investigation conducted by Ngo et al. [16] showed that survival, maturation and reproduction of mother *D. magna* were negatively affected by both untreated and treated domestic wastewater of Hochiminh City. The study also reported the malformation of its offspring. For environmental protection, the technical regulation on metal concentrations in industrial wastewater was issued in Vietnam guideline values [17]. However, it has not been known whether there are negative impacts to organisms at levels of the concentrations specified. For partly answering this question, the toxicity of industrial wastewater released from Nhon Trach industrial zone, Dong Nai Province, to micro-crustacean *D. magna,* was implemented.

2. MATERIALS AND METHODS

2.1 Sample collection and test organisms

Nhon Trach industrial zone is located in Nhon Trach District, Dong Nai Province, Southern Vietnam. The industrial zone has an area of around 2,200 hectares and consists of multi-manufacturers such as mechanics, textile, food, paper and wood and other services of which the

ISSN: 2455-6939

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mechanics is the most dominant. The wastewater after treatment from Nhon Trach industrial zone is directly discharged into nature, the upstream of Thi Vai River [18].

Wastewater samples from the obscure drain and main drain of the wastewater treatment system of Nhon Trach industrial zone, thereafter considered as the wastewater sample 1 and 2, respectively, were collected for toxicological investigation. The samples were transported to the laboratory of Department of Environmental Management and Technology, Institute of Tropical Biology, Vietnam Academy of Science and Technology, in the same day and stored at a temperature of – 70ºC prior to toxicity tests.

The test organism, *Daphnia magna,* provided by Microbiotests Inc (Belgium) was used for toxicity test. The animal was raised in ISO medium and fed with a green alga *Chlorella* sp. The organisms were incubated at 25 ± 1ºC, under a light intensity of around 1000 Lux and a photoperiod of 12h light and 12h dark [19,20].

2.2 Heavy metal characterization, experimental setup and data treatment

Sub-samples of wastewater were used for heavy metal analysis. The sub-samples were firstly passed through 0.45 µm filter (Sartorius, Germany) and acidified with saturated HNO_3 [19]. The heavy metals were characterized under an inductively coupled plasma/ mass spectrometry (ICP/MS – 9000 Shimadzu, Japan).

Prior to initiating the experiments, thirty female *D. magna* contain eggs were incubated in a 500 mL beaker and fed with *Chlorella* sp. Offspring from these female *D. magna* were used for toxicological bioassay. The animal was incubated in four different ratio of wastewater concentrations mixed with ISO medium (v/v), 0% (control), 10%, 50% and 100%. For each treatment, fifteen neonates (< 24h old) were randomly collected and individually transferred into a 50 mL beaker containing 20 mL test medium [20]. The organisms were fed with *Chlorella* sp. at the concentration of 1 mg C/L, approximately 140,000 cells/mL. Test media and food were renewed every two days. Survival, maturation, reproduction and negative appearance of *D. magna* were observed daily while dry mass of the animals was weighted at the end of the test. By the end of the experiment, alive *D. magna* was dried at 60ºC for 24h and weighted to determine its dry mass [22]. Water pH and dissolved oxygen of test media were measured with the Metrohm 744 and WTW Oxi 197 Meters, respectively, at the test initiation and termination.

2.3 Data analysis

Kruskal-Wallis test (Sigma Plot, version 12) was used to determine the significant difference between *Daphnia'*s maturation and dry mass from control and wastewater exposures.

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3. RESULTS

3.1 Concentrations of heavy metals in wastewater

The chemical analysis showed that the wastewater contained many heavy metals such as Al, As, Cr, Cu, Fe, Mn, Ni, Pb, Sb and Zn. The dissolved Al in the wastewater samples had highest concentration, up to 442 µg/L, compared to others whereas Pb had much lower concentrations, from 1 – 4 µg/L, in the wastewater. Generally, metal concentrations in sample 2 were higher than those in samples 1 (Table 1).

Table 1. Concentrations of dissolved heavy metals (µg/L) in the wastewater samples 1 and 2 from Nhon Trach industrial zone and the Vietnam guideline values 40:2011 (A) (QCVN) for discharge [17]. UDL: under detection level (1 µg/L); N/A: not available

Metals	Al	Cr	Mn	Fe	Ni	Cu	Zn	As	Sb	Pb
Sample 1	254	1	30	121	UDL	6	26	6	46	1
Sample 2	442	13	33	84	102	35	145	3	UDL	4
QCVN	N/A	100	500	1000	200	2000	3000	50	N/A	100

3.2 Chronic effects of wastewater on *Daphnia magna*

3.2.1. Effects on survival

The pH and dissolved oxygen measurement in the test media showed that pH valued from 7.1 – 8.5 and the dissolved oxygen was between 3.4 and 7.5 mg/L. These records revealed the suitable test conditions (in terms of pH and dissolved oxygen) for *Daphnia* well growing [19].

All of *D. magna* in the control survived until the last day of experiment (Fig. 1). However, the *Daphnia*'s survival was decreased to 67 – 80% exposed to sample 1 (Fig. 1A). Differently, there was only 27% of *D. magna* alive after three weeks of incubation with 10% of sample 2. Seriously, the last animal in 50% and 100% of sample 2 died by the 8th and 6th days of experiment, respectively (Fig. 1B).

ISSN: 2455-6939

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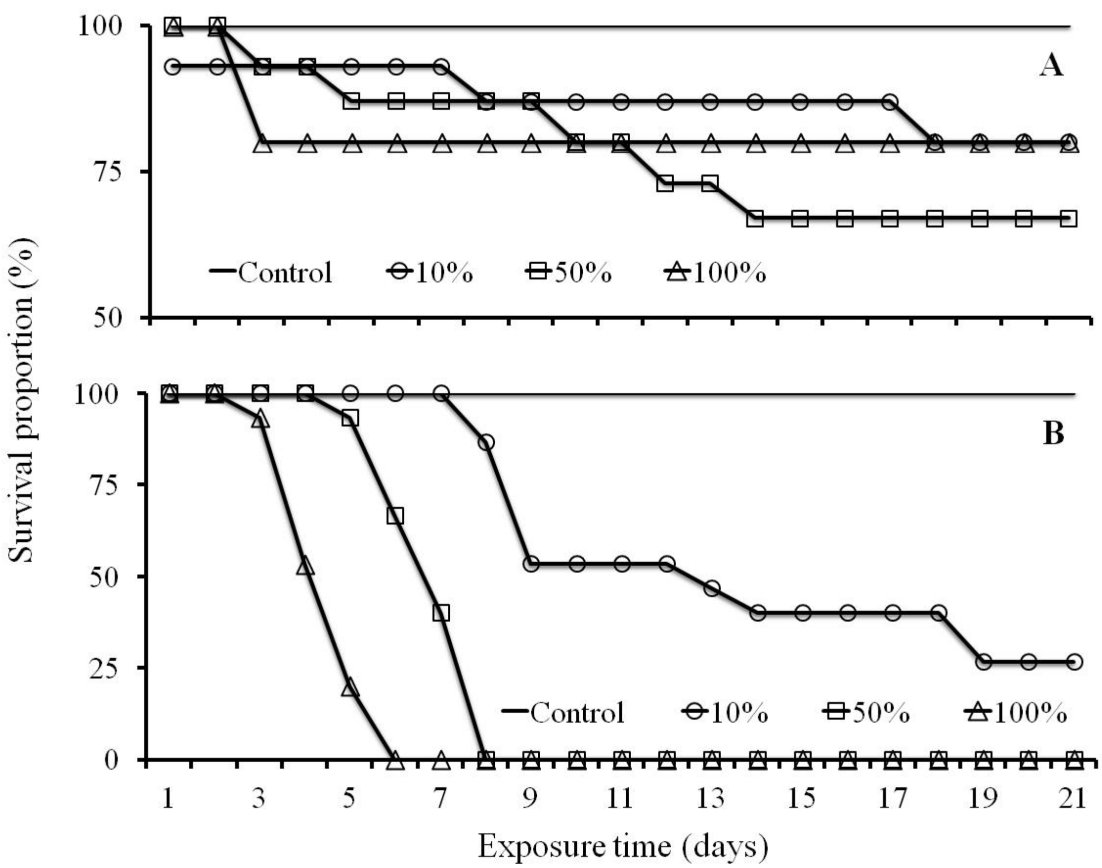

Figure 1. Survival proportion of *Daphnia magna* exposed to different concentrations (v/v) of (A) Sample 1 and (B) Sample 2 during 21 days (n = 15 at the beginning).

3.2.2. Effects on maturation and reproduction

Daphnia magna in control and the concentration of 10% wastewater reached their maturation at the age of approximately 4 days old. However, the animals exposed to 50% and 100% of sample 1 delayed their maturity ages (Fig. 2).

In control, there were from 5 – 18 neonates per clutch reproduced by the mother *D. magna*. The total neonates from control during 21 days of experiment were 644 (Table 2). In the sample 1, the numbers of accumulative offspring were between 284 – 430 (Table 2). Especially, in the exposure to 50%, there were 2 female *D. magna* which matured but they didn't reproduce during the experiment.

In the incubation to 10% of wastewater of sample 2, total neonates were 166 and most of neonates were either weak or dead after born. In addition, in the concentrations of 50% wastewater, only one adult *D. magna* reproduced 1 neonate. There was no offspring from 100%

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of sample 2 because all *D. magna* died before they matured consequently no reproduction (Table 2).

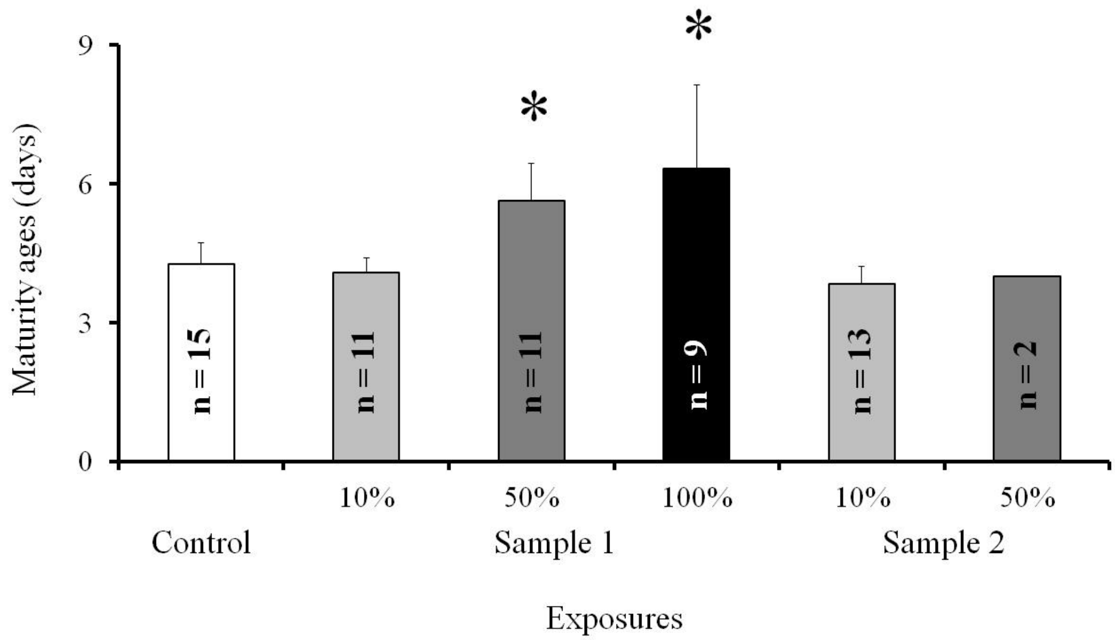

Figure 2. Maturity ages of *Daphnia magna* from control and exposures to two wastewater samples (asterisks indicated statistically significant differences from control; *, $p < 0.001$, Kruskal-Wallis test)

Table 2. Accumulative offspring of *Daphnia magna* during the experiments

	Control	Sample 1			Sample 2		
		10%	50%	100%	10%	50%	100%
Accumulative offspring	644	355	284	430	166	1	0

In addition malformation of embryos and neonate from adult *D. magna* incubated in wastewater were observed (Fig. 3). Dead embryos were found at concentration of 10% and 50% wastewater while the dead neonates in a shell of female *Daphnia* were found in 100% wastewater exposure in sample 1 (Fig. 3). Besides, the concentration of 100% wastewater also caused one of the antennas of the neonate was inactive, whereas the other was active normally by microscopic observation. Another morphological effect was also observed at concentration of 10% wastewater in the sample 2 such as the neonates could not move normally and having a weak body.

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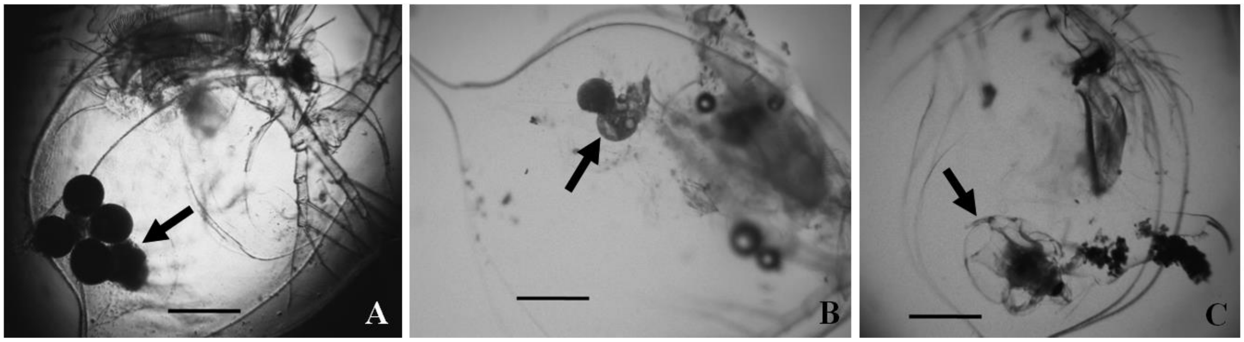

Figure 3. Morphological effects or malformation *D. magna* exposed to wastewater. A and B: dead embryos, and C: a dead neonate in shells of mother *Daphnia*. Scale bars = 0.5 mm.

3.2.3. Effects on growth

The average dry mass of one adult *D. magna* (21 days old) in the control was approximately 0.3 mg. Similar dry mass of *D. magna* from the high wastewater concentration exposures was also recorded (Fig. 4). The lowest wastewater concentration (10%) caused a reduction on the *Daphnia* dry mass.

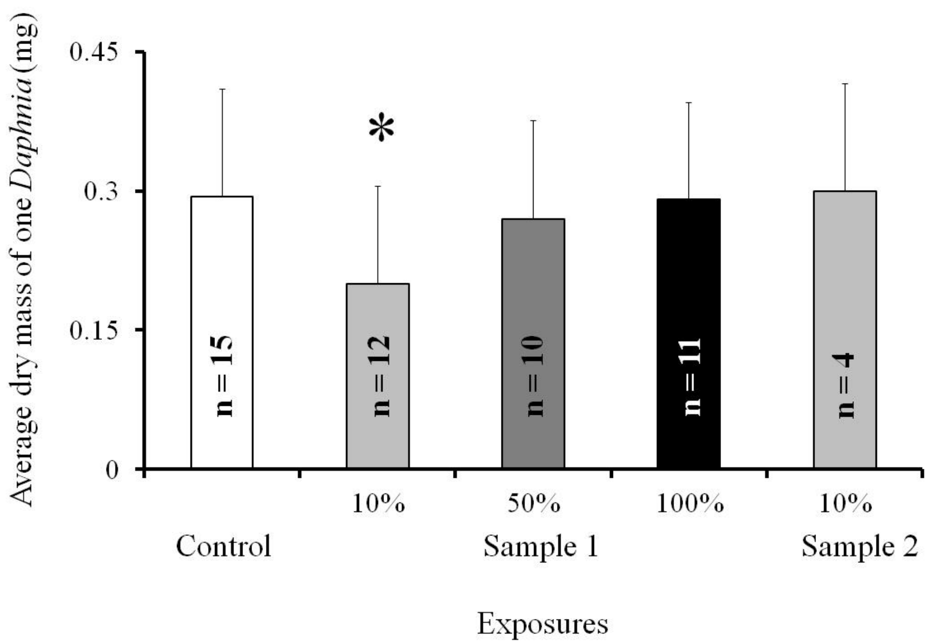

***Figure 4*. Average dry mass (mg) of one *Daphnia magna* from control and exposures to two wastewater samples.**

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4. DISCUSSION

4.1 Concentrations of heavy metals in wastewater

The heavy metal concentrations in the two samples were quite different and interestingly the concentrations in wastewater the main drain were much higher than those in the obscure drain. This might be the wastewater from the drains had different sources of effluents which needs further monitoring to confirmed. Dao et al. [18] reported some heavy metals in wastewater from Nhon Trach industrial zone such as Cu, Cr, Zn and Cd with concentrations of each metal from 4 – 33 µg/L. Hence the metal concentrations in the current study were within the range of those previously recorded. However, the metal concentrations in the two wastewater samples in this study were within the Vietnam guideline values, QCVN [17].

4.2 Chronic effects of wastewater on *Daphnia magna*

4.2.1. Effects on survival

Negative effects on survivorship of *D. magna* in this study should be related to the heavy metal concentrations in the wastewater [23, 24]. Cr (5µg/L; 15 µg/L; 100 µg/L) and Ni (250 µg/L) had slight impact on survival of *Daphnia* with 21 days test [3, 25, 26]. This agrees with a previous study, in which after 3 weeks exposure to Zn concentrations of 80, 115, and 170 µg/L survivorship of daphnids was reduced to 91%, 86% and 74%, respectively [27]. However, Pane et al. [28] reported that no effect on survival of *D. magna* was observed in 21 µg Ni/L but 10% mortality occurred at 42 µg Ni/L and survival dropped linearly with time at 85 µg Ni/L to only 30% of control levels by the end of the 21 days test. Moreover, Cu at the concentration of 20 µg/L was already potent toxic to survivorship of *Daphnia* [25, 26]. Therefore, the higher mortality of *D. magna* exposed to the wastewater sample 2 in this study should be closely related to the high Cu concentration and/ or the high Ni concentration in the wastewater (see Table 1). Our results could also comparable with results of Ghazy and Fayed [14]. These researchers observed that the survival of *D. magna* decreased gradually with increasing the concentrations of raw wastewater released from waste stabilization pond in a domestic sewage treatment plant in India. They found that 60% of *D. magna* incubated in the 19% wastewater could survive for 21 days. However, these researchers focused on the biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total dissolved solid (TSS) as main causes of the suvival reduction of *D. magna*. The industrial solid waste such as sludge from textile treatment plant, metal mechanic treatment plant and pulp and paper treatment plant after dissolved with distilled water as test solution adversely affected toward survivorship of *D. magna* at concentration of 12.5% and 25% (textile treatment plant), 50% and 100% (pulp and paper treatment plant) and no statistically significant effect at any levels of concentrations (metal mechanic treatment plant)

ISSN: 2455-6939

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[29]. Heavy metals in the component of industrial solid waste such as Pb, As, Cr (textile treatment plant); Cr, Fe, Mn, Zn (metal mechanic treatment plant) and As, Pb, Cr (pulp and paper treatment plant) (which also found in wastewater samples of our study) could be the root of issues which predicted in our study. The concentrations of 0.27 µL/L and 0.68 µL/L of Nif Brook water in Izmir, Turkey which receiving chemical discharges from industrial, municipal, and agricultural sources reduced to 75% and 70% of survival of *D. magna*, respectively [30]. These also supported the results of our study.

4.2.2. Effects on maturation and reproduction

The total number of neonates of *D. magna* increased gradually with increasing the concentration of mixture of wastewater (sources from industry, urban, and agriculture) [30] while the results of our study showed that the concentrations of wastewater were in inverse ratio to the total reproduction of *D. magna* (except the concentration of 100% in sample 1). Daphnids fed Cu-contaminated-algae exhibited a significant reduction, up to 50%, of total reproduction [31]. Besides, *D. magna* chronically exposed to Cu (20 µg/L or higher) was negatively affected on its fecundity (number of neonates per female) [25]. This strongly supported results of our study.

It was reported that the age at first brood was 7 days for daphnids in the control and raw wastewater concentrations of 13 and 14%, while the time to the first brood increased at higher wastewater concentrations, 13 days at 19% [14]. Heavy metals such as Cu and Cr caused a delay on maturation of *D. magna* [25, 26, 32] which was, again, confirmed by the result in this study. However, according to the study of De Schamphelaere et al. [31] and Luciana et al. [25], the time to first brood of daphnids was not affected by exposure to Cu while the second and third broods were significantly delayed by 0.7 and 1.5 days, respectively (maybe the number of molts declined). Moreover, Cr also did not effect negatively on the time to first brood at the lower concentration, 25 µg/L [25]. On the contrary, the time to the first brood increased gradually when increasing the concentrations of Cr from 13 – 23 µg/L [32]. The concentration of 85 µg Ni/L caused a delay on maturation of *D. magna* [28]. We found that the concentrations of heavy metals in sample 2 was much higher than those in sample 1 (see Table 1) but the effect on the time to first brood was not observed while those in sample 1 was observed. We proposed that the high concentrations of heavy metals in sample 2 should be related to the rapid dead or the inhibition of *D. magna* in early developmental stages, consequently indirect effect to the time to first reproduction.

The dead embryos in this study were similar to another observation reported elsewhere in which *D. magna* was exposed to a natural toxins, microcystins at the concentrations of 5 – 50 µg/L [22] and Cd at the concentrations of 60 – 80 µg/L [33]. On the other hand, parthenogenetic eggs of *D. magna* exposed to 60 – 80 µg/L of Cd were retracted the yolk mass as well as the complete disruption of cell arrangement, developmental defects of embryos and a caudal spine malformation [33]. The metals Cr, Zn and Ni also caused the dead of embryos of zebrafish [23] while Cr, As and Ni (include Al) caused the dead of embryos of the freshwater snail (*Radix quadrasi*) at the high concentrations and the malformation of embryos at the low concentrations [34]. The river contained heavy metals, specially Cu, Pb and Cr, due to industrial activities created negative effects on *Dicrotendipes simpsoni* (Diptera: Chironomidae) such as some deformations of head part at a extremely low concentrations [24] which also recorded in our study. We assumed that some heavy metals had the similar mechanism of toxicity to daphnids which needs further investigations at cellular levels.

4.2.3. Effects on growth

This record was controversial to a previous study where dry mass of *Daphnia* was decreased with increase of exposed wastewater concentration [14]. However, our result was in line with the investigation of Tran et al. [26] where low Cu concentration reduced the *Daphnia* dry mass but higher Cu concentration did not. On the other hand, Daphnids were fed with the algal cells exposed to 500 µg Cu/L to be significantly adversely effected to dry weight after 21 days [31]. It was reported that the concentrations of Cu at 20 µg/L and Cr at 15 µg/L caused a decline of the number of molts, consequently inhibiting the growth of *D. magna* [25]. Similar results were also recorded that the average weight of adults females *D. magna* decreased gradually with increasing the concentration of Cr from 13 – 23 µg/L [32]. During the experimental stage, we found that most of daphnids in sample 2 did not grow as well as reproduction due to the molts were inhibited and finally died. Although, the results of our study about dry mass showed that no effect was on *D. magna* but contrarily, heavy metals in sample 2 should be responsible for growth inhibition of *D. magna*.

5. CONCLUSIONS

The present study found that wastewater from the industrial zone in Southern Vietnam adversely impacted on life history traits of *D. magna* including survival reduction, maturation postponement and reproduction inhibition. Additionally, wastewater also caused severely morphological effects on the embryos and neonates of *D. magna*. Although concentrations of heavy metals in wastewater recording in the Vietnam environmental quality guidelines (QCVN), the potent toxicity of wastewater was clearly showed. Therefore, further investigations on toxicity of wastewater on other aquatic organisms as well as the interactions of heavy metals in streams of wastewater combined with regularly monitoring on chemical characteristics of the wastewater should be implemented to fully evaluate the toxicity of wastewater released from the Nhon Trach Industrial Zone and to adjust the QCVN for environmental and ecological protection.

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