ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Toxicity Evaluation of Copper, Nickel, and Mixture on *Daphnia magna*

Tong Xuan Nguyen[1]*, Tuyen Thi Le[2], Thuy Thi Nguyen[3], and Khang Tang Phuc Luu[4]

[1] Institute for Environmental Science, Engineering and Management, Industrial University of Ho-Chi-Minh City, 700000, Vietnam
[2] Le Thanh Tong Highschool, 700000, Vietnam
[3] Institute for Environmental Science, Engineering and Management, Industrial University of Ho-Chi-Minh City, 700000, Vietnam
[4] Chiang Mai University, 50200, Thailand
* Correspondence: nguyenxuantong@iuh.edu.vn

Citation:
Toxicity evaluation of copper, nickel, and mixture on *daphnia magna*. *ASEAN J. Sci. Tech. Report*. **2024**, *27*(6), e254578. https://doi.org/10.55164/ajstr.v27i6.254578

Article history:
Received: June 17, 2024
Revised: September 23, 2024
Accepted: October 2, 2024
Available online: October 25, 2024

Abstract: This study conducted acute (48 hours) and chronic (21 days) toxicity tests of nickel (Ni^{2+}), copper (Cu^{2+}) ions, and a mixture of the Ni^{2+} - Cu^{2+} ions on *Daphnia magna* (*D. magna*) under M4 medium. The acute test results showed that the toxicity of Cu^{2+} was about 15 times higher than Ni^{2+}, demonstrated by the 50% effect concentration (EC_{50}) value of 185.2 $\mu g.L^{-1}$ and 2706.97 $\mu g.L^{-1}$. In addition, the results also illustrated that the mixture of the Cu^{2+} – Ni^{2+} ions was more toxic to *D. magna* than a single metal with an EC_{50} value of 175.22 $\mu g.L^{-1}$. Acute toxicity tests showed that the metal affected the viability of *D. Magna*, while the organism maturation and reproduction were affected under chronic exposure. Chronic test results showed that the toxicity of Cu^{2+} was higher than that of Ni^{2+} with EC_{50} values of 9.06 $\mu g.L^{-1}$ and 162.12 $\mu g.L^{-1}$; noticeably, the toxicity of the mixture of the two metals is higher than that of the single metal. In particular, Ni^{2+} at a concentration of 100 $\mu g.L^{-1}$ stimulated the maturation, survival, and reproduction of *D. magna*. but at higher concentrations (> 100 $\mu g Ni.L^{-1}$), Ni^{2+} would bind to Cu^{2+}, which exerted a more substantial effect on the test organism. This study initially evaluated the toxicity of Cu^{2+} and Ni^{2+} on microcrustaceans *D. magna*, which is the premise for further studies on the genotoxicity of heavy metals on microcrustaceans in general and *D. magna* in particular to ensure the quality of the ecosystem.

Keywords: *Daphnia magna*, acute, chronic, mixed metal toxicity, 50% effect concentration (EC_{50})

1. Introduction

Industrial, municipal, and municipal stormwater typically contains a mixture of metals such as copper (Cu^{2+}), nickel (Ni^{2+}), lead (Pb^{2+}), and zinc (Zn^{2+}) along with organic matters, all of which can be discharged directly or indirectly into aquatic systems [1, 2]. Consequently, aquatic ecosystems are contaminated by a mixture of chemicals, increasing worldwide concern [3]. Heavy metals such as Cu^{2+} and Ni^{2+} are often present in low concentrations in aquatic ecosystems, so they do not seriously affect human health. However, due to human impact, high concentrations of heavy metals have been detected in surface waters (lakes, lagoons, rivers and streams) [4-6] making artificial pathways the primart form of intrusion. Despite the known effects of heavy metals on humans and mammals, little is known regarding the modes of action of toxins in aquatic ecosystems.

In aquatic environments, metals rarely exist in isolation but rather as mixtures. Therefore, a broader perspective on water quality regulations is needed

better to understand the toxicity of metal mixtures to aquatic organisms. Numerous studies have demonstrated the relationship between water quality and metal toxicity [7, 8]. Water chemical parameters generally help organisms resist metal toxicity by forming complexes with metals or competing with metals for binding at toxic action sites in organisms [9]. However, the interactions between metal mixtures, water physicochemical parameters, and aquatic organisms are poorly understood. Previous reports have assessed the aquatic toxicity of metals using a variety of aquatic organisms like fish, protozoa, nematodes, and crustaceans [10]. Due to its sensitivity, water flea, *Daphnia magna* (*D. magna*), is a commonly used species for studying metal pollution and metal mixtures [11]. Moreover, *D. magna* is more sensitive than fish and other plankton [12–14]. As a crucial trophic level in the aquatic food chain, exposure of *D. magna* to heavy metals can result in harmful effects such as impaired growth, reduced reproductive capacity, and mortality [15]. Interestingly, most studies have concentrated on physiological characteristics such as death and reproduction, neglecting developmental endpoints [16, 17]. Winner and Farrell [18] tested the acute and chronic toxicity of Cu on four species of *Daphnia*, including *D. magna*, *D. pulex*, *D. parvula*, and *D. ambigua*. All four species had lower survival rates at Cu concentrations > 40 $\mu g.L^{-1}$. Additionally, Xiao and Peijnenburg [19] evaluated the toxicity risk of Cu metal nanoparticles on *D. magna*, revealing that the concentration causing a 50% effect on test organisms increased 12-fold when DOC levels rose from 0 to 10 $mg.L^{-1}$ in the exposure environment.

In parallel with acute exposure, there is a vital need for additional knowledge on the toxic effects of metal mixtures on aquatic organisms under chronic exposure conditions. However, the toxicity of some metals is known to be variable due to physicochemical factors in the environment, including pH, hardness, and DOC [20]. Therefore, a metal toxicity assessment must consider pH, hardness, and DOC. Understanding the mechanism of toxicity of metals in acute and chronic exposure, along with the effects on biological populations, allows scientists to make a more accurate risk assessment of metals. At the same time, this stimulates the development of more stringent water quality criteria for metal exposure. Therefore, this study was conducted to characterize and differentiate acute and chronic effects of Cu^{2+}, Ni^{2+}, and bimetallic mixtures on *D. magna* under laboratory conditions.

2. Materials and Methods

2.1 Test chemicals

The study used chemicals $NiCl_2$ (Ni^{2+}) và $CuCl_2.2H_2O$ (Cu^{2+}) (Merck, Germany), stored at 5°C to conduct the toxicity test of single metals and a mixture of Cu^{2+} – Ni^{2+}.

2.2 Expermental organism

D. magna was isolated and cultured at the laboratory of the Institute of Environmental Science, Engineering and Management - Industrial University of Ho Chi Minh City. Briefly, *D. magna* was grown in M4 medium according to ISO 6341:2012 at about 21 ± 1°C in a 16-8-hour light-dark cycle, light intensity 500 - 800 lux. *Chlorella vulgaris* was used as a food source, and 3 mL (concentration of 10^7 $cells.mL^{-1}$) of *Chlorella vulgaris* was added to each culture cup of adult *D. magna* three times a week. *D. magna* < 24 hours of birth were separated from the adult ones daily using plastic pipettes for toxicity tests.

2.3 Acute toxicity test

Set up the test in an M4 environment according to ISO 6341:2012, including a control sample (without chemicals added), the concentration range of Ni^{2+} (300, 600, 1200, 2400, and 3600 $\mu g.L^{-1}$) and Cu^{2+} (50, 100, 150, 200, and 250 $\mu g.L^{-1}$) were used to determine the 50% influence (EC_{50}) test individual range of each metal. Corresponding to each metal concentration, 9 *D. magna* were put into a beaker containing 40 mL of the medium prepared above. Each concentration is repeated three times. The number of immobilized/dead individuals after 48 hours of exposure was monitored and recorded.

The Ni^{2+} concentration range that caused mortality below and above 50% of *D. magna* individuals was 2400 $\mu g.L^{-1}$ and 3600 $\mu g.L^{-1}$, respectively. From the values just determined, the EC_{50} interval was subdivided into exposure concentrations of 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, and 3600 $\mu g.L^{-1}$. The range of Cu^{2+} concentrations causing mortality under and over 50% of *D. magna* was 150 $\mu g.L^{-1}$ and 250 $\mu g.L^{-1}$, respectively. From the values just determined, the EC_{50} interval was subdivided into an exposure concentration range of 40, 90, 130, 180, 220, 270, 310, and 360 $\mu g.L^{-1}$. The acute concentration mixture of Cu^{2+}–

Ni^{2+} was determined by using the concentration range of Ni^{2+} and choosing the EC_{50} value of Cu^{2+} as the fixed concentration. The experiment temperature was maintained at 21±1 °C. The medium pH, DO, hardness, and alkalinity were measured at the start and end of the test [21].

2.4 Chronic toxicity test

The experiment was set up and conducted similarly to that reported by Son, Chi [22], combined with the acute toxicity results. The experiment was conducted at different concentrations of Cu^{2+} (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 $\mu g.L^{-1}$), Ni^{2+} (0, 10, 20, 40, 80, 100, 120, 160, and 200 $\mu g.L^{-1}$); and the mixture of Cu^{2+} – Ni^{2+} were determined similarly to the acute toxicity test. Each trial batch was replicated with 9 D. magna three times and fed with *Chlorella vulgaris* every day [22] for 21 days of testing [23]. The endpoint of the chronic metal toxicity test for *D. magna* included survival (mortality rate), maturity (date of onset of egg-bearing), and fertility (number of offspring produced). The experiment temperature was maintained at 21±1 °C.

2.5 Data analysis

Experimental results were presented as the mean ± standard error. Sigma Plot 14.0 statistical software (Systat Software Inc., CA, USA) was used to create graphs. EC_{50} values were estimated through JMP Pro 16.0 software. At the same time, one-way ANOVA was used to evaluate the influence of Cu^{2+} and Ni^{2+} on the maturation of *D. magna* compared with the control. All statistical analyses were based on a significance level < 0.0001.

3. Results and Discussion

3.1 Physicochemical properties of the test medium

The M4 medium quality parameters were agreed upon during acute and chronic testings: temperature was 21 ± 1 °C; DO ranges from 7-8 $mg.L^{-1}$; pH was 8.2 ± 0.2; alkalinity was 90 $mgCaCO_3.L^{-1}$; hardness and DOC was 240 $mgCaCO_3.L^{-1}$ and 8.9 $mg.L^{-1}$, respectively. Similar to the present study, a pH range of 7.9 to 8.3 is considered favorable for the growth of *D. magna* [14]. Rodriguez and Arbildua [24] and Renzi and Blašković [25] also showed that high pH values, hardness, and dissolved substances can decrease the toxicity of metals to *D. magna*. Therefore, water physicochemical parameters such as hardness, pH, salinity, alkalinity, and DOC can modulate the toxicity of metals to aquatic organisms [26, 27].

3.2 Acute toxicity of metals to D. magna

The mortality rate in *D. magna* after 48 hours of exposure increased with the test metal concentration (Figure 1).

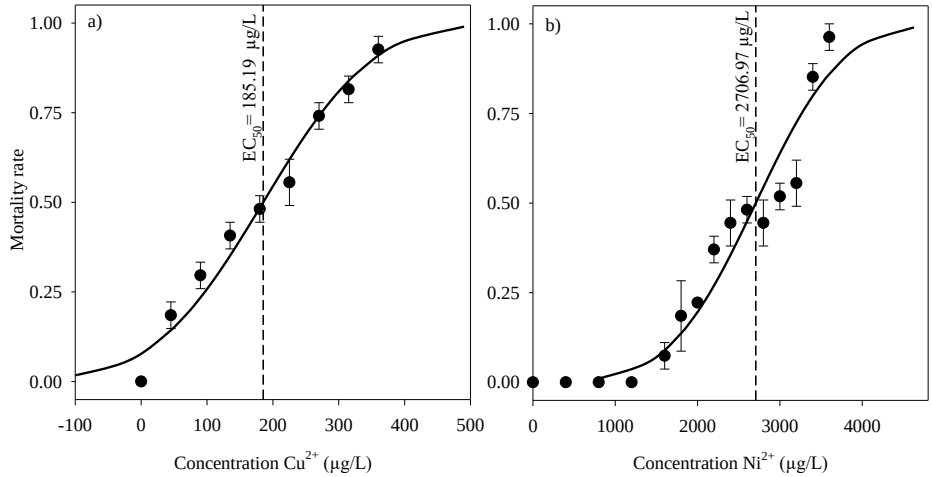

Figure 1. The mortality rate of *D. magna* upon exposure to a) Cu^{2+} and b) Ni^{2+} for 48 hours; n = 3, error bar: ± SE.

The mortality rate of *D. magna* due to Cu^{2+} reached 50% (EC_{50}) when the Cu^{2+} concentration increased to 185.19 $\mu g.L^{-1}$ and peaked at the estimated concentration of 490.29 $\mu g.L^{-1}$ (100%), which demonstrated that

the viability of the test organism was inversely proportional to the experiment with Cu^{2+} concentration (Figure 1a). Similar to the toxicity of Cu^{2+}, the mortality rate of *D. magna* also increased gradually with the concentration of Ni^{2+} metal exposure. At the concentration of 2706.97 µgNi.L^{-1}, it reached 50% and the highest at 4627.95 µgNi.L^{-1} (96%) after 48 hours (Figure 1b). The evaluation results showed that Cu^{2+} was more toxic to *D. magna* than Ni^{2+} (the lower the EC_{50}, the higher the toxicity) ($p < 0.0001$) (Figure 1).

The EC_{50} value of Cu^{2+} and Ni^{2+} in this present study was 13 and 650 µg.L^{-1}, respectively (hardness was 240 mg$CaCO_3$.L^{-1}) was higher (lower toxicity) reported by Meyer and Ranville [28] for Cu^{2+} (103 µg.L^{-1}) (hardness < 100 mg$CaCO_3$.L^{-1}), and for both Cu^{2+} and Ni^{2+} metals of Negin and Pedram [29] were 0.667 and 50.06 µg.L^{-1} (hardness of 88 mg$CaCO_3$.L^{-1}), Okamoto, Yamamuro [30] were 13 and 650 µg/L (hardness of 45 – 240 mg$CaCO_3$.L^{-1}), Lari, Gauthier [31] were 34.5 and 1502.5 µg.L^{-1}, respectively (hardness < 100 mg$CaCO_3$.L^{-1}). This demonstrates that metal toxicity increases as water hardness decreases, leading to variations in EC_{50} results between studies [32]. At the same time, from the tests and acute toxicity studies of the two metals, the toxicity of Cu^{2+} was higher than that of Ni^{2+} after 48 hours of exposure to *D. magna*. The results of the acute toxicity test of the mixture of Cu^{2+} – Ni^{2+} with a fixed concentration of 185.19 µgCu.L^{-1} are shown in Figure 2. EC_{50} of the mixture (175.22 µg.L^{-1}) was about 15 times lower when compared with Ni^{2+} (2706.97 µg.L^{-1}) and about 1 µg.L^{-1} compared with Cu^{2+} (185.19 µg.L^{-1}), indicating that the toxicity of mixture of Cu^{2+} - Ni^{2+} mixture (with 185.19 µgCu.L^{-1}) was higher than single Ni^{2+} and Cu^{2+} (Figures 1, 2).

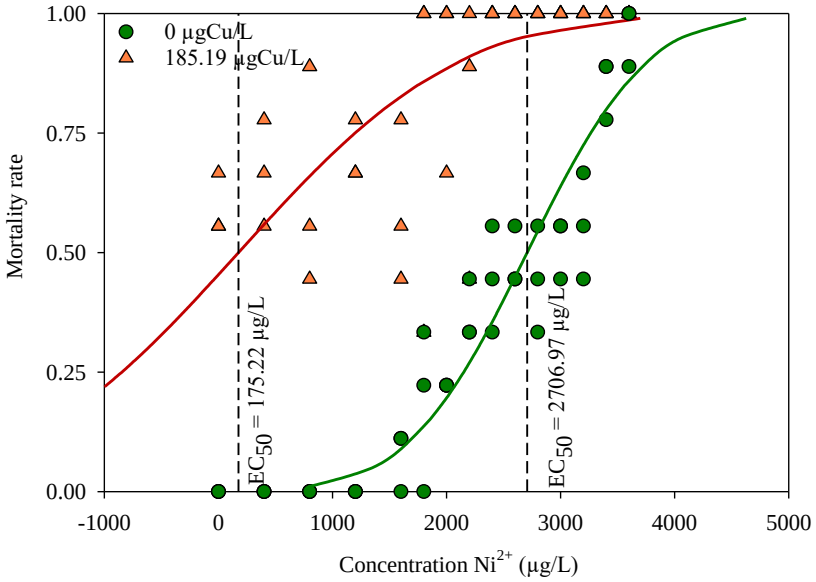

Figure 2. The mortality rate of *D. magna* in 48 hours of exposure to mixtures of Cu^{2+} – Ni^{2+}; n = 3, error bar: ± SE.

3.3 Chronic toxicity of metals to D. magna

3.3.1 Effect on the survivability of D. magna

After chronic exposure (21 days) to Cu^{2+}, the EC_{50} was 9.06 µg/L (Figure 3a). For Ni^{2+}, the EC_{50} obtained was 162.12 µg/L (Figure 3b). Evaluation results showed that the toxicity of *D. magna* with Cu^{2+} was higher than with Ni^{2+} under the same time and test conditions.

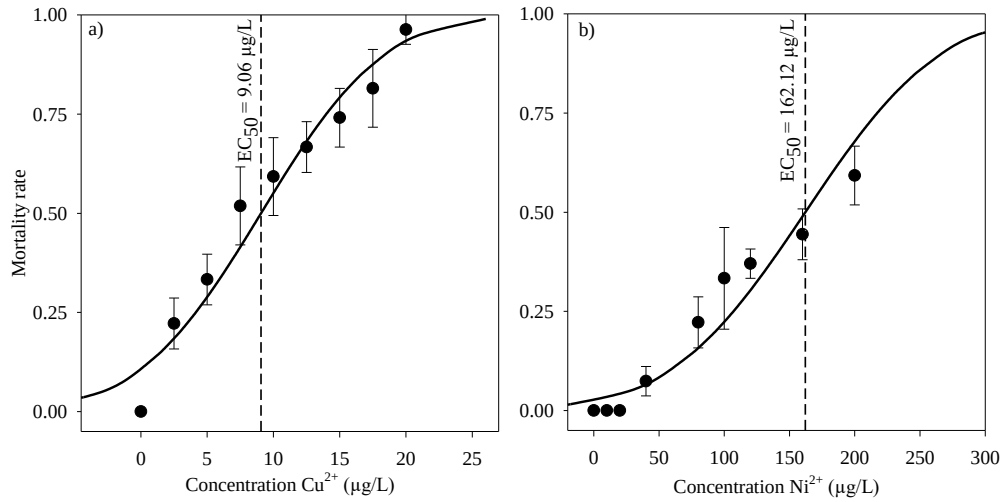

Figure 3. The mortality rate of *D. magna* upon exposure to a) Cu^{2+} and b) Ni^{2+} for 21 days; n = 3, error bar: ± SE.

In the chronic toxicity test of the mixture of Cu^{2+} – Ni^{2+}, the mortality rate of *D. magna* by metal concentration was shown in Figure 4.

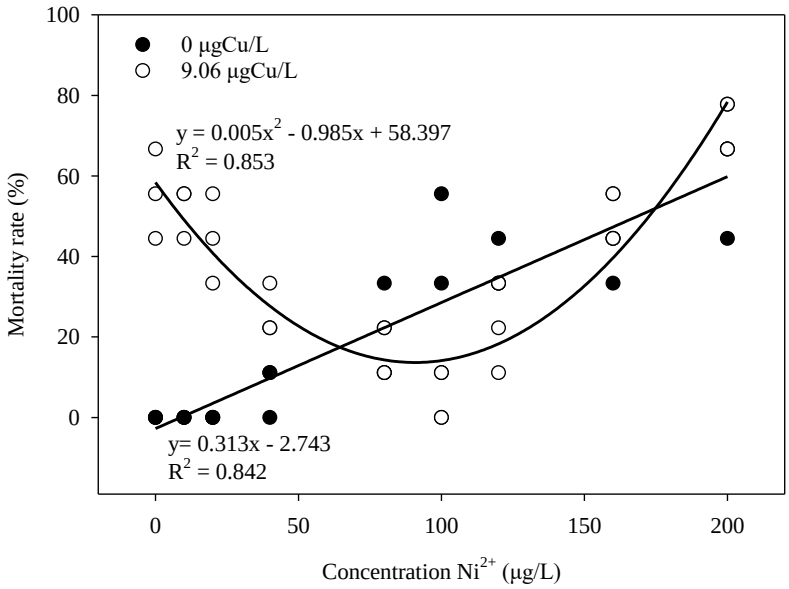

Figure 4. The mortality rate of *D. magna* in 21 days when exposed to Ni^{2+} and the mixture of Cu^{2+} – Ni^{2+}; n = 3, error bar: ± SE.

The mortality rate of *D. magna* after 21 days of exposure to Cu^{2+} - Ni^{2+} mixture increased high at concentrations of 10 and 20 µgNi.L^{-1}, but when the concentration of Ni^{2+} in the mixture increased (80 - 120 µgNi.L^{-1}), the mortality rate of *D. magna* tended to decrease significantly and was comparable to the control concentration (Figure 4). However, when the Ni^{2+} concentration increased rapidly from 160-200 µgNi.L^{-1}, *D. magna* reacted strongly with the metal, causing higher mortality than the single Ni^{2+} concentrations.

The results of the current study align with the observation that the chemical interactions of organisms in water exposed to high levels of toxicants result in more significant toxicity in Cu^{2+} - Ni^{2+} mixtures [12]. However, once the protective concentration threshold for D. magna is exceeded, Ni^{2+} combines with Cu^{2+} to produce a more pronounced toxic effect on the test organisms than individual metals. Deleebeeck and De Schamphelaere [33] used *D. magna* to evaluate Ni^{2+} toxicity and showed that the EC_{50} value after 21 days of exposure was 23 µg.L^{-1} at low hardness and EC_{50} from 59 – 365 µgNi.L^{-1} at medium hardness from moderate to high. This was consistent with the current research results with high hardness (240 mg$CaCO_3$.L^{-1}), with EC_{50}

value reaching 162.1 μg.L^{-1}. Nam and Son [34] evaluated the chronic toxicity of Ni^{2+} at a concentration of 5, 65, and 254 μg.L^{-1} to *D. lumholtzi* had a mortality rate of 20, 0, and 27.3%, indicating that *D. magna* was more sensitive than *D. lumholtzi* to exposure.

3.3.2 Effect on the maturation of D. magna

The chronic toxicity assessment of Cu^{2+}, Ni^{2+}, and bimetallic mixtures on *D. magna* maturation was shown in detail in Figure 5.

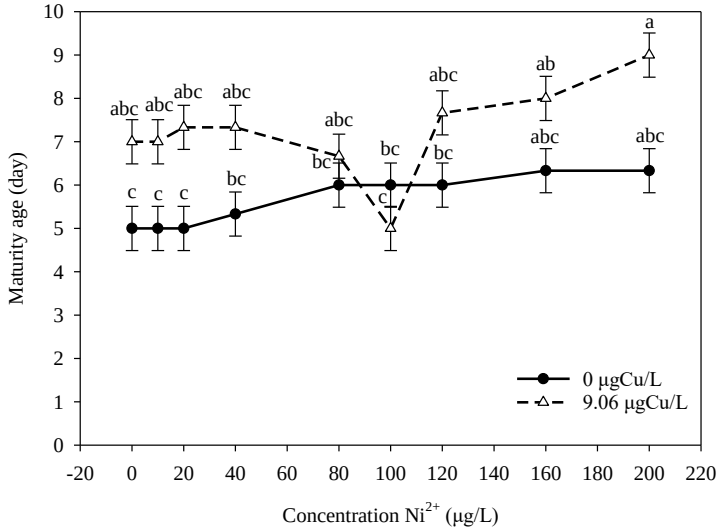

Figure 5. Maturity age of *D. magna* after 21 days when exposed to Ni^{2+} and the mixture of Cu^{2+} - Ni^{2+}; n = 3, error bar: ± SE.

The first *D. magna* matured on day 5 at control concentrations (0 μgCu.L^{-1}) and concentrations of 10 – 40 μgNi.L^{-1}, with no difference in statistical significance ($p < 0.05$) (Figure 5). When the concentration of Ni^{2+} increased from 80-200 μgNi/L, the adult age of *D. magna* showed signs of increasing by 1 day. In particular, in the Cu^{2+} – Ni^{2+} mixture (9.06 μgCu.L^{-1}) at the first three concentrations, maturation occurred on day 7 (Figure 5). As the concentration in the mixture increased (80-100 μgNi.L^{-1}), the maturation age of *D. magna* was on day 5. Nevertheless, at concentrations of 120, 160, and 200 μg.L^{-1}, the maturity date of *D. magna* tended to last up to 8 and 9 days (Figure 5). Up to this limit, the combination of Ni^{2+} and Cu^{2+} strongly affected the maturation.

The results of the present study showed that the first individual *D. magna* matured at day 5 of age (similar to the circadian cycle of *Daphnia*) in control and four concentrations: 10, 20, 40, and 80 μgNi.L^{-1} (Figure 5); which was higher than that of Nam and Son [34], reporting a concentration of 0, 5, 65, and 254 μgNi.L^{-1} of mature *D. lumholtzi* on day 4.

3.3.3 Effect on the fertility of D. magna

In a single Ni^{2+} chronic toxicity test (0 μgCu.L^{-1}), after 21 days of exposure, the number of *D. magna* juveniles decreased with the increase in Ni^{2+} concentration ($p < 0.0001$) (Figure 6). For the mixture of Cu^{2+} - Ni^{2+} (9.06 μgCu.L^{-1} and 0 - 100 μgNi.L^{-1}), the amount of *D. magna* produced gradually increased but tended to decrease sharply from the concentration of 120 - 200 μgNi.L^{-1} ($p < 0.0001$) (Figure 6).

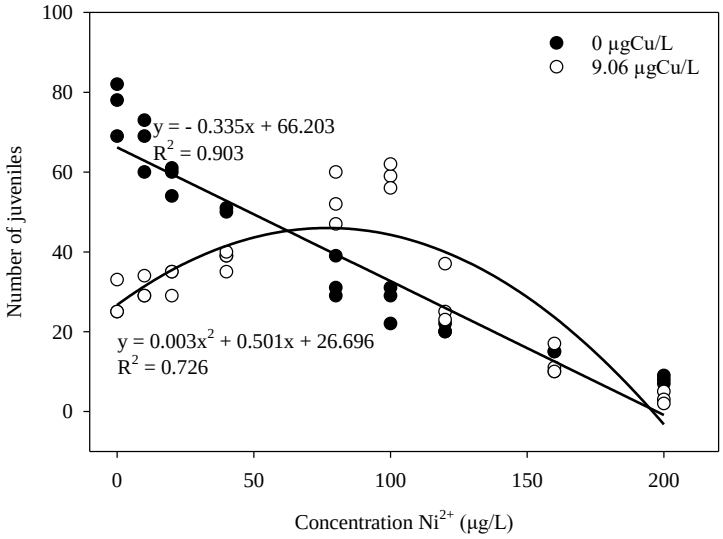

Figure 6. The number of juveniles *D. magna* after 21 days of exposure to Ni^{2+} and the mixture of Cu^{2+} - Ni^{2+}; n = 3, error bar: ± SE.

In the trial with single Ni^{2+} (0 µgCu.L^{-1}), the number of *D. magna* juveniles gradually decreased as the Ni^{2+} concentration increased. No statistically significant difference was observed between the number of juveniles in control at the concentration of 10, 20 µgNi.L^{-1} (Figure 6). Besides, the number of juveniles at Ni^{2+} concentrations of 10 and 20 µg.L^{-1} was higher than in the mixture. However, at 40 – 200 µgNi.L-1 concentration, the number gradually decreased with the lowest value of 8 juveniles (200 µgNi.L^{-1}) (Figure 6). For the chronic toxicity test of the Cu^{2+} – Ni^{2+} mixture (containing 80 and 100 µgNi.L^{-1}), the amount of *D. magna* produced was significantly higher than the control concentration($p < 0.0001$) (Figure 6). At the same time, the research results showed that the amount of *D. magna* generated in the mixture of Cu^{2+} - Ni^{2+} was higher than that of the single Ni^{2+}. However, when the Ni^{2+} concentration was at 120-200 µg.L^{-1}, the number of *D. magna* juveniles decreased gradually to the lowest level of 3 juveniles. Taylor reported the fertility of Daphnia under the chronic effects of Cu2+ and Ni2+, which had a higher number of juveniles > 40 individuals than in the current study [26]. The results demonstrated that different environments and crustaceans directly affected the toxicity of metals to test organisms. Notably, *D. magna* can switch from female to male under stress conditions and continue normal development without reproducing [36]. This phenomenon helps explain the differences in reproductive outcomes observed in *D. magna* following metal exposure. In addition, Pane and Smith [37] provided evidence that Mg^{2+} antagonism was a mechanism of acute and chronic toxicity of Ni^{2+} in water to *D. magna*. For Cu^{2+}, the effects observed on *D. magna* showed the simultaneous involvement of several toxic mechanisms, such as increased metabolic time. This process decreased energy intake (through inhibiting enzyme activity and digestion), reproductive inhibition (via inhibition of cell formation), and molting [38].

4. Conclusions

The results of toxicity assessment on microcrustaceans *D. magna* after 48 hours of exposure to metals in the M4 medium showed that Cu^{2+} was more toxic than Ni^{2+}. When combining two metals, the toxicity of the mixture increased as the concentration of Ni^{2+} increased, meaning that Ni^{2+} had no protective effects on *D. magna* from Cu^{2+} toxicity but combined to create more toxicity compared to Cu^{2+} with a single metal. Chronic toxicity of Cu^{2+} and Ni^{2+} affected the survival, maturation, and reproduction of *D. magna*. In particular, the presence of Cu^{2+} in water at a concentration of 20 µg.L^{-1} caused more deaths than 95% of tested *D. magna* individuals. When combined, the chronic toxicity of the two metals Cu^{2+} – Ni^{2+} was higher than that of the corresponding single metal. However, the protective effect of Ni^{2+} on *D. magna* could be observed at sufficient concentrations (80, 100, and 120 µgNi.L^{-1}); Cu^{2+} in the mixture increased toxicity to the organism expressed through the degree of influence on survival, maturation, and reproduction. Therefore, further studies are needed to fully understand the effects related to gene expression and the accumulated metal content in *D. magna* after acute and chronic exposure.

5. Acknowledgements

I would like to thank the Institute of Environmental Science, Engineering and Management - Industrial University of Ho Chi Minh City, Vietnamand the students for their enthusiastic support during the research.

Author Contributions: Conceptualization, Tong Xuan Nguyen; methodology, Tong Xuan Nguyen and Thuy Thi Nguyen; software, Tuyen Thi Le; investigation, Tong Xuan Nguyen and Thuy Thi Nguyen; writing—review and editing, Tong Xuan Nguyen and Khang Tang Phuc Luu. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Gobeil, C.; Rondeau, B.; Beudin, L. Contribution of municipal effluents to metal fluxes in the St. Lawrence river. *Environmental Science & Technology*, **2005**, *39*, 456–464. https://doi.org/10.1021/es049335x

[2] Birch, G.F.; Matthai, C.; Fazeli, M.S.; Suh, J.Y. Efficiency of constructed wetlands in removing contaminants from stormwater. *Wetlands*, **2004**, *24*, 459–466. https://doi.org/10.1672/0277-5212(2004)024[0459:EOACWI]2.0.CO;2

[3] Shaw, J.R.; Dempsey, T.D.; Chen, C.Y.; Hamilton, J.W.; Folt, C.L. Comparative toxicity of cadmium, zinc and mixtures of cadmium and zinc to daphnids. *Environmental Toxicology and Chemistry*, **2006**, *25*, 182–189. https://doi.org/10.1897/05-243r.1

[4] Mokarram, M.; Saber, A.; Sheykh, V. Effects of heavy metal contamination on river water quality due to the release of industrial effluents. *Journal of Cleaner Production*, **2020**, 123380. https://doi.org/10.1016/j.jclepro.2020.123380

[5] Muhammad, S.; Ahmad, K. Heavy metal contamination in water and fish of the Hunza River and its tributaries in Gilgit-Baltistan: Evaluation of potential risks and provenance. *Environmental Technology & Innovation*, **2020**, *20*, 101159. https://doi.org/10.1016/j.eti.2020.101159

[6] Tomno, R.M.; Nzeve, J.K.; Mailu, S.N.; Shitanda, D.; Waswa, F. Heavy metal contamination of water, soil and vegetables in urban streams in Machakos municipality, Kenya. *Scientific African*, **2020**, *9*, e00539. https://doi.org/10.1016/j.sciaf.2020.e00539

[7] Bury, N.; Shaw, J.; Glover, C.; Hogstrand, C. Derivation of a toxicitybased model to predict how water chemistry influences silver toxicity to invertebrates. *Comparative Biochemistry and Physiology Part C*, **2002**, *133*, 259–270. https://doi.org/10.1016/s1532-0456(02)00096-0

[8] Meyer, J.; Clearwater, S.; Doser, T.; Rogaczewski, M.; Hansen, J. Effects of Water Chemistry on Bioavailability and Toxicity of Waterborne Cadmium, Copper, Nickel, Lead, and Zinc to Freshwater Organisms. *SETAC, Pensacola, FL, USA*, **2007**.

[9] Paquin, P.; Gorsuch, J.; Apte, S.; Batley, G.; Bowles, K.; Campbell, P.; Delos, C. The biotic ligand model: A historical overview. *Comparative Biochemistry and Physiology Part C*, **2002**, *133*, 3–35.

[10] Rajput, V.D.; Minkina, T.M.; Behal, A.; Sushkova, S.N.; Mandzhieva, S.; Singh, R.; Gorovtsov, A. Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: A review. *Environmental Nanotechnology, Monitoring & Management*, **2018**, *9*, 76–84. https://doi.org/10.1016/j.enmm.2017.12.006

[11] Okamoto, A.; Yamamuro, M.; Tatarazako, N. Acute toxicity of 50 metals to *Daphnia magna. Journal of Applied Toxicology*, **2015**, *35*(7), 824-830.

[12] Traudt, E.M.; Ranville, J.F.; Smith, S.A.; Meyer, J.S. A test of the additivity of acute toxicity of binary-metal mixtures of ni with Cd, Cu, and Zn to *Daphnia magna*, using the inflection point of the concentration-response curves. *Environmental Toxicology and Chemistry*, **2016**, *35*(7), 1843–1851. https://doi.org/10.1002/etc.3342

[13] Pérez, E.; Hoang, T.C. Responses of *Daphnia magna* to chronic exposure of cadmium and nickel mixtures. *Chemosphere*, **2018**. https://doi.org/10.1016/j.chemosphere.2018.06.063

[14] Cui, R.; Kwak, J.I.; An, Y.-J. Comparative study of the sensitivity of *Daphnia galeata* and *Daphnia magna* to heavy metals. *Ecotoxicology and Environmental Safety*, **2018**, *162*, 63–70. https://doi.org/10.1016/j.ecoenv.2018.06.054

[15] Paylar, B.; Asnake, S.; Sjöberg, V.; Ragnvaldsson, D.; Jass, J.; Olsson, P. Infu- ence of water hardness on zinc toxicity in *Daphnia magna. Journal of Applied Toxicology*, **2022**, *42*(9), 1510–1523. https://doi.org/10.1002/jat.4319

[16] Paylar, B.; Bezabhe, Y.; Mangu, J.; Thamke, V.; Igwaran, A.; Modig, C. Assessing organism diferences in mixed metal sensitivity. *Science of the Total Environment*, **2023**, *905*, 167340. https://doi.org/10.1016/j.scitotenv.2023.167340

[17] Jankowski, M.; Fairbairn, D.; Baller, J.; Westerhof, B.; Schoenfuss, H. Using the Daphnia magna Transcriptome to Distinguish Water Source: Wetland and Stormwater Case Studies. *Environmental Toxicology and Chemistry*, **2022**, *41*(9), 2107–2123. https://doi.org/10.1002/etc.5392

[18] Winner, R.W.; Farrell, M.P. Acute and chronic toxicity of copper to four species of *Daphnia. Journal of the Fisheries Research Board of Canada*, **1976**, *33*(8), 1685–1691. https://doi.org/10.1139/f76-215

[19] Xiao, Y.; Peijnenburg, W.J.G.M.; Chen, G.; Vijver, M.G. Toxicity of copper nanoparticles to Daphnia magna under different exposure conditions. *Science of The Total Environment*, **2016**, 563-564, 81–88. https://doi.org/10.1016/j.scitotenv.2016.04.104

[20] De Schamphelaere, K.A.C.; Janssen, C.R. Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to *Daphnia magna. Environmental Toxicology and Chemistry*, **2004**, *23*(5), 1115–1122. https://doi.org/10.1016/j.aquatox.2007.01.002

[21] APHA, *Standard methods for the examination of water and wastewater, Washington*. **2012**.

[22] Son, D.T.; Chi, D.H.L.; Wiegand, C. Chronic effects of cyanobacterial toxins on *Daphnia magna* and their offspring. **2010**, *55*(7), 1244–1254. https://doi.org/10.1016/j.toxicon.2010.01.014

[23] ASTM, A., *E384: Standard test method for knoop and vickers hardness of materials*. **2012**.

[24] Rodriguez, P.; Arbildua, J., *Copper acute and chronic toxicity to D. magna: Sensitivity at three different hardness at pH 6.3 (MES buffered) in the presence of 2 mg/L DOC*. 2012: Report submitted to the International Copper Association (ICA).

[25] Renzi, M.; Blašković, A. Ecotoxicity of nano-metal oxides: A case study on *daphnia magna. Ecotoxicology*, **2019**, *28*(19), 878–889. https://doi.org/10.1007/s10646-019-02085-3

[26] Pereira, C.M.S.; Blust, R.; De Schamphelaere, K.A.C. Effect of temperature on nickel uptake and elimination in *Daphnia magna. Environmental Toxicology and Chemistry*, **2019**, *38*, 784–793. https://doi.org/10.1002/etc.4352

[27] Mano, H.; Shinohara, N. Acute toxicity of nickel to *Daphnia magna*: Validation of bioavailability models in Japanese rivers. *Water, Air, & Soil Pollution*, **2020**, *231*(9), 459. https://doi.org/10.1007/s11270-021-05180-6

[28] Meyer, J.S.; Ranville, J.F.; Pontasch, M.; Gorsuch, J.W.; Adams, W.J. Acute toxicity of binary and ternary mixtures of Cd, Cu, and Zn to *Daphnia magna. Environmental Toxicology and Chemistry*, **2015**, *34*(4), 799–808. https://doi.org/10.1002/etc.2787

[29] Negin, K.; Pedram, M. Evaluation of acute toxicity of copper and nickel on *Daphnia magna. Journal of Wetland Ecobiology*, **2018**, *10*(36), 77-87.

[30] Okamoto, A.; Yamamuro, M.; Tatarazako, N. Acute toxicity of 50 metals to *Daphnia magna. Journal of Applied Toxicology*, **2015**, *35*(7), 824–830. https://doi.org/10.1002/jat.3078

[31] Lari, E.; Gauthier, P.; Mohaddes, E.; Pyle, G.G. Interactive toxicity of Ni, Zn, Cu, and Cd on *Daphnia magna* at lethal and sub-lethal concentrations. *Journal of Hazardous Materials*, **2017**, *334*, 21–28. https://doi.org/10.1016/j.jhazmat.2017.03.060

[32] Jo, H.; Son, J.; Cho, K.; Jung, J. Combined effects of water quality parameters on mixture toxicity of copper and chromium toward Daphnia magna. *Chemosphere*, **2010**, *81*, 1301–1307. https://doi.org/10.1016/j.chemosphere.2010.08.037

[33] Deleebeeck, N.M.E.; De Schamphelaere, K.A.C.; Janssen, C.R. A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magn*a in artificial and natural waters. *Environmental Toxicology and Chemistry*, **2008**, *27*(10), 2097. https://doi.org/10.1897/07-579.1

[34] Nam, L.V.; Son, D.T. Highly potent toxicity of nickel in river water to *Daphnia lumholtzi. International Journal of Development Research*, **2016**, *6*(9), 9526-9531.

[35] Taylor, N.S.; Kirwan, J.A., .; Johnson, C.; Yan, N.D.; Viant, M.R.; Gunn, J.M.; McGeer, J.C. Predicting chronic copper and nickel reproductive toxicity to *Daphnia pulex*-pulicaria from whole-animal metabolic profiles. *Environmental Pollution*, **2016**, *212*, 325–329. https://doi.org/10.1016/j.envpol.2016.01.074

[36] Thorp, J.; Covich, A. Ecology and Classification of North American Freshwater Invertebrates, 2nd ed. *Harcourt Science and Technology, San Diego, CA, USA*, **2001**.

[37] Pane, E.F.; Smith, C.; McGeer, J.C.; Wood, C.M. Mechanisms of acute and chronic waterborne nickel toxicity in the freshwater cladoceran, *Daphnia magna. Environmental Science & Technology*, **2003**, *37*(19), 4382–4389. https://doi.org/10.1021/es034317l

[38] De Schamphelaere, K.; Forrez, I.; Dierckens, K.; Sorgeloos, P.; Janssen, C. Chronic toxicity of dietary copper to *Daphnia magna. Aquatic Toxciology*, **2007**, *81*(4), 409–418. https://doi.org/10.1897/02-593