




10-2018

Responses of *Daphnia Magna* to Chronic Exposure of Cadmium and Nickel Mixtures

Edgar Pérez
Loyola University Chicago

Tham C. Hoang
Loyola University Chicago, thoang@luc.edu

Follow this and additional works at: https://ecommons.luc.edu/ies_facpubs

 Part of the [Environmental Chemistry Commons](#), and the [Environmental Sciences Commons](#)

Author Manuscript

This is a pre-publication author manuscript of the final, published article.

Recommended Citation

Pérez, Edgar and Hoang, Tham C.. Responses of *Daphnia Magna* to Chronic Exposure of Cadmium and Nickel Mixtures. *Chemosphere*, 208, : 991-1001, 2018. Retrieved from Loyola eCommons, Institute of Environmental Sustainability: Faculty Publications and Other Works, <http://dx.doi.org/10.1016/j.chemosphere.2018.06.063>

This Article is brought to you for free and open access by the Faculty Publications at Loyola eCommons. It has been accepted for inclusion in Institute of Environmental Sustainability: Faculty Publications and Other Works by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.



This work is licensed under a [Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 License](#).
© Elsevier Ltd. 2018

Responses of *Daphnia magna* to chronic exposure of cadmium and nickel mixtures

Edgar Pérez

Tham C. Hoang*

thoang@luc.edu

Institute of Environmental Sustainability, Loyola University Chicago, 1032 W. Sheridan Road, Chicago IL, 60626, USA

*Corresponding author. 6349 N. Kenmore Ave., BVM 314, Chicago, IL 60660, USA.

Handling Editor: Jim Lazorchak

Abstract

The present study assessed the chronic toxicity of cadmium (Cd) and nickel (Ni) mixtures to *Daphnia magna*. Using a titration design, Ni concentrations of 20, 40, 80, 100, 120, 140, and 160 µg/L were tested alone and simultaneously titrated in increments against a constant concentration of 1.5 µg/L Cd. The results demonstrated that Cd at 1.5 µg/L was highly toxic to *D. magna*, and Ni alone concentrations ≥80 µg/L were toxic to *D. magna* survival, reproduction, and growth. No Ni alone concentration was found to induce a toxic effect on undeveloped embryos and the time to first brood. Only the Ni alone treatment containing 200 µg/L affected the reproductive rates of *D. magna*. For Cd

Ni mixtures, Ni concentrations of 20, 40, and 80 µg/L were found to strongly protect *D. magna* from Cd toxicity at the survival and growth endpoints, resulting in less-than-additive effects, but not on the reproductive endpoint. At higher concentrations, Ni exceeded the necessary concentration needed to protect *D. magna*, and appeared to contribute to the toxicity. Overall, the results of metal uptake support the competitive binding mechanism at the biotic ligand and explain the less-than-additive effects observed in the Cd

Ni mixtures concentration. The embryonic effects of Cd

Ni mixtures are not explained by the competitive binding mechanism at the biotic ligand. More research is needed to determine the mechanisms that produce embryonic impairment when cellular metals interact. Overall, the results of the present study are relevant for the development of improved environmental quality guidelines for metal mixtures.

Keywords: *Daphnia magna*; Metal toxicity; Metal uptake; Metal mixture toxicity; Reproductive toxicity; Growth toxicity; Embryonic development

1 Introduction

Heavy metals are commonly found as mixtures of multiple metals in aquatic environments, especially near metal mining and/or metal processing facilities. Aquatic communities near those facilities are usually exposed to metal mixtures. Of interest in the present study is the chronic toxicity of cadmium (Cd) and nickel (Ni) mixtures. Nickel is essential to many living organisms (e.g., rats, chicks, cows, goats, plants) at low concentrations. However, at high concentrations, Ni can produce toxic effects (ATSDR, 2005). The environmental concentration of Ni in surface waters have been reported to vary around the world. Eisler (1998) found a wide concentration range of 0.4–183,000 µg/L Ni while Balistrieri et al. (2015) found levels ranging from <1.5 to 1383 µg/L Ni near Sudbury, Canada.

Anthropogenic activities such as careless disposal of Ni/Cd batteries, electronic equipment, and electroplated items, etc. are believed to contribute to most of the Ni pollution in aquatic environments (Eisler, 1998). At concentrations in the upper end of these ranges, Ni produced toxic effect to *D. magna* as was found in the present study and reported by Eisler (1998). Cadmium on the other hand, is a trace non-essential metal that has no biological function and is exceptionally toxic to all aquatic life (Bodar et al., 1988a; US EPA, 2001). Similar to Ni pollution, Cd pollution of aquatic ecosystems is primarily due to human activities, such as from mining, refining, and in production of batteries, pigments, coatings, platings, plastics, and alloys (ATSDR, 2012). The environmental concentrations of Cd in the surface waters of Canada and USA have been reported to be between <0.02 and 15.4 µg/L Cd (ENVIRODAT, 1992; Balistrieri et al., 2015).

Studies on metal mixture toxicity have been conducted for decades (Meyer et al., 2015a) but most of the studies have examined the acute toxicity rather than chronic toxicity. According to review studies by Norwood et al.

(2003) and Vijver et al. (2011), only 35% and 9% of the mixture studies were for chronic toxicity, respectively. Since metals do not degrade in the natural environment as do organic compounds, the need for investigating chronic metal mixture toxicity is essential for understanding long-term metal exposures to organisms. The scientific understanding of metal mixture interactions and their effects on living organisms can lead to improved ecological risk assessments and to the development of water quality guidelines for metal mixtures.

Different types of metal mixture effects and many inconsistencies have been reported among metal mixture toxicity studies. Researchers have reported nonadditive (interactive) and additive (noninteractive) effects in evaluating the effects of Cd and Ni mixtures on freshwater fish, bacteria, algae, cyanobacteria, filamentous fungi, purple sea urchins, and across *Daphnia* species (Vandenbrouck et al., 2009; Traudt et al., 2016; Newton, 2015; Komjarova and Blust, 2008, 2009; Niyogi et al., 2015; Sato et al., 1986; Awasthi and Rai, 2004; Babich et al., 1986; Phillips et al., 2003). When studying metal mixture toxicity, the consistency of the organism health throughout the course of the study is very important. If both single-metal and metal mixture assays are not conducted concurrently, random sensitivity in organism responses may cloud the interpretation of the results; leading to misunderstanding of metal mixture effects (De Laender et al., 2009). For example, a truly additive response may be detected as non-additive (less-than-additive/more-than-additive) response, or vice versa. As a result, it was crucial that both assays in the present study (Ni alone and Cd Ni mixtures) were conducted simultaneously to avoid any misleading toxicity interpretations.

The objective of the present study is to characterize and differentiate the chronic effects on *D. magna* when simultaneously exposed to Ni alone, and a mixture of Cd and Ni using a titration experimental design proposed by Meyer et al. (2015b), and applied in our earlier study with Cd and Zn mixtures (Pérez and Hoang, 2017). The measured endpoints include: survival, growth, metal accumulation, and reproduction of *D. magna*.

2 Materials and methods

2.1 Test organisms

Daphnia magna used in this study were cultured in the Ecotoxicology and Risk Assessment Laboratory at the Loyola University Chicago's Institute of Environmental Sustainability (IES). *Daphnia magna* culture was maintained in 1 L glass beakers (30 individuals/beaker) filled with 0.9-1 L of reconstituted moderately hard water (MHW) made from 16 to 18 M Ω MilliQ water and certified grade chemicals (MgSO₄, CaSO₄·2H₂O, NaHCO₃, and KCl), as described in the U.S.EPA chronic toxicity testing method (US EPA, 2002).

The culture water for the test organisms was changed 3 times a week (Mondays, Wednesdays, and Fridays). Water quality, such as dissolved oxygen (DO), pH, and temperature were measured on the water change days. Hardness and alkalinity were measured weekly. Dissolved oxygen and temperature were measured using a model YSI 550A instrument, while pH was measured with an AP110 portable meter. Hardness and alkalinity were measured by titration methods (Methods 2320, 2340) (Eaton et al., 2005). Hardness was titrated against 0.01 M ethylenediaminetetraacetic acid. Alkalinity was titrated against 0.02 N H₂SO₄. The measured water quality parameters of the culture media water were as follows: hardness ranged from 80 to 84 mg/L as CaCO₃, alkalinity ranged from 55 to 61 mg/L as CaCO₃, pH ranged from 7.19 to 7.88, and dissolved oxygen (DO) ranged from 6.87 to 8.32 mg/L. Cultures were maintained at a temperature ranging from 21.2 to 25.6 °C and at a light:dark photoperiod of 16 h:8 h.

Cultured *D. magna* were also fed daily with 6 mL algal suspension of *Raphidocelis subcapitata* at a concentration of 3 × 10⁷ cells/mL, and 3 mL YCT (a food suspension of yeast, cereal leaves and trout chow) at concentrations of 1.7-1.9 mg solids/L. The algae and YCT administered to the cultures were prepared in the Ecotoxicology and Risk Assessment Laboratory following the procedures outlined in the U.S.EPA chronic toxicity testing manual (US EPA, 2002).

2.2 Experimental design

A titration design experiment was used in this study to characterize the chronic toxicity of Cd and Ni mixtures on *D. magna*. The titration method was favored because of its advantage in precisely detecting graded changes in organism responses across a series of increasing metal concentrations (Meyer et al., 2015b). Based on previous Cd and Ni chronic toxicity tests in our laboratory (unpublished data), Cd

Ni mixtures were spiked with a constant nominal Cd concentration of 1.5 µg/L and titrated against a series of increasing Ni concentrations of 20, 40, 80, 100, 120, 140, and 160 µg/L. However, to accurately interpret mixture effects, a concurrent control consisting of MHW, a Cd alone treatment containing only Cd, and a Ni alone test consisting of the same Ni concentration series as those used in the Cd

Ni mixtures were also tested. Overall, the study was comprised of 2 toxicity tests (Ni alone, and Cd

Ni mixtures), each consisting of 7 treatments plus a control and a Cd alone treatment of 4 replicates each. Furthermore, to avoid the potential interference of variable brood sensitivity and neonate health, all neonate *D. magna* used in the present study were coming from the same brood (5th).

2.3 Test method and experimental procedures

In accordance with ASTM methods, a twenty-one day (21 d) chronic static renewal toxicity test was used in the present study (ASTM, 2012). Treatment replicates were comprised of 600 mL polypropylene cups containing 10 neonate *D. magna* (≤ 24 -h old) in 250 mL of test solution from day 0 to day 10, and 400 mL of test solution from day 11 to day 21 to compensate for *D. magna* growth. Tests were conducted in the Loyola IES aquatic toxicology testing room. The room was set at a temperature of 25 °C and a photoperiod of light:dark = 16 h:8 h. The exposure media for the test were prepared from stock solutions that were previously prepared from certified grade metal salts (CdSO₄ and ZnCl₂) every 48-h for renewal.

Due to the nature of the study, food rations were adjusted depending on the feeding rate of *D. magna*. Since *D. magna* were ≤ 24 -h old at test initiation, feeding rate was low but increased as the organisms grew into sexual maturity. Therefore, as the organisms aged, increasingly variable amounts of *R. subcapitata* at a concentration of 3×10^7 cells/mL and YCT of concentration 1.7-1.9 mg solids/L were fed to them (Table 1, Supplemental Data). This adjustment of the feeding rate also helped reduce the growth of unconsumed algae.

Mortality was recorded daily for both tests (Ni alone and Cd Ni mixtures) to determine survival effects of *D. magna*. An individual was considered dead if it was immobilized, and if no organ movements were observed after examination with a 6X Bausch and Lomb magnifier. All dead organisms were removed from the test chambers and discarded.

Once *D. magna* became sexually mature and began reproducing, reproductive performance was documented daily thereafter. All dead and alive neonates were separately counted, recorded, removed from the chambers, and disposed. The same method above was used if any aborted broods (embryos) were observed in the test chambers. However, the embryos were collected in a 30 mL polypropylene beaker for morphological screening with a Zeiss ZEN imaging software - model Stereo Discovery.v12. Morphological examination was conducted by comparing test embryos with control embryos obtained from a gravid adult *D. magna* from the laboratory culture.

At the end of the experiment, all surviving adults in each test chamber were collected for the analysis of dry weight and body metal accumulation. Twenty-four hours prior to the termination of the experiment, 50 mL polypropylene digestion tubes were labelled, placed in a drying oven, and dried at 60 °C overnight. After drying, the tubes were placed in a desiccator, allowed to acquire room temperature, and weighed prior to use with a Metro Toledo Balance Model XS64. At test termination, all surviving adults from each replicate were rinsed 3 times with DI water, and then transferred into a respectively labelled and weighed digestion tube. The digestion tube was then reweighed and the pooled wet weight of the surviving organisms was recorded. Subsequently, the organisms were dried in the same oven at 60 °C for 48-h and reweighed to determine the pooled dry weight. The average dry weight for each individual organism was obtained by dividing the pooled dry weight in each replicate by the number of surviving organisms in that replicate. Lastly, to determine body metal load, all surviving *D. magna* in each replicate were digested with concentrated nitric acid (HNO₃) as described in the U.S. EPA Method 3050B guidelines (US EPA, 1996). The digested solutions were used for metal analysis.

2.4 Water quality and chemical analyses

The physiochemical parameters of the water were measured on days 0, 7, 14, and 21 for both toxicity tests (Ni alone and Cd Ni mixtures). Dissolved oxygen, temperature, pH, hardness, and alkalinity were measured as described in the test organism's subsection above. Water samples for the analysis of total and dissolved metals and cations and anions (A/C) were also collected weekly and at the same time that water quality subsamples were taken for water quality measurement.

Approximately 14 mL of each sample type was collected in a 15 mL polypropylene sample vial. Using a sterile syringe, total metal samples were taken directly from the test water, while dissolved metal and A/C samples were filtered through Whatman polyvinylidene fluoride filters with a pore diameter of 0.45 µm. Total and dissolved metal samples were preserved with one drop of concentrated HNO₃ and refrigerated for later analysis. In addition, approximately 25 mL of sample water was filtered using the same filter type as mentioned above, and collected in a 30 mL amber glass bottle for later analysis of dissolved organic carbon (DOC).

A 300X NexION ICP-MS (Perkin Elmer) was used to measure the total, dissolved, and cation concentrations in the water samples. An 881 Compact Ion Chromatographer (Metrohm USA) was used to measure the concentration of anions. A TOC-L CSH analyzer (Shimadzu) was used to measure the DOC concentrations.

2.5 Endpoint calculation and data analyses

Reproductive rate per day was calculated as a ratio, where the number of neonates reproduced in a day were divided by the number of surviving adults on the same day. However, the proportion of dead neonates was calculated as a percentage, where the number of dead neonates observed in a day were divided by the total number of dead and alive neonates counted on the same day. Similarly, the proportion of undeveloped embryos was calculated as a percentage of the total number of aborted embryos in a day to the total number of dead and alive neonates + undeveloped embryos counted on the same day.

The data for both the Ni alone test and the Cd

Ni mixture test were analyzed for statistically significant differences between the control and their respective exposure treatments. However, for the mixture test, the data were also compared for statistically significant differences between Cd alone and the Cd

Ni mixtures.

A one-way ANOVA and a Tukey's HSD multiple comparisons test was used to detect treatment differences within each endpoint. Similarly, differences between the Ni alone treatments and their corresponding Cd Ni mixtures (e.g., T1 for Ni alone and T1 for Cd

Ni mixture) were also analyzed using the same statistical methods as stated above.

To test for the interaction effects of Cd and Ni, a two-way ANOVA was used, treating the concentration of Ni as a factor, and the metal exposure type as another factor (for example, *D. magna* were either exposed to Ni alone or to a corresponding mixture of Cd and Ni).

Data that did not meet the assumptions of the ANOVA's (homogenous variance and normal distribution) were transformed. An arcsine square root transformation was used in the analysis of 3 sets of data: mortality, percentage of dead neonates, and percentage of undeveloped embryos. No transformation was used in the analysis of total neonates (both tests), total neonate reproductive rates (both tests), Ni alone alive neonates, and Ni alone time to [first-st](#) brood data. However, a square root transformation was applied to the mixture alive neonate data and the alive neonate reproductive rates data (both tests). A Log transformation was used for the growth data of the Cd

Ni mixtures. The one-way and two-way ANOVAs were conducted with R-Program (Ver 3.1.1).

3 Results

3.1 Water chemistry

~~The average measured total and dissolved Ni and total Cd concentrations in both Ni alone and the Cd~~[The average measured total and dissolved Ni and total Cd concentrations in both Ni alone and the Cd](#)
Ni ~~mixture tests deviated within 10% from the nominal concentrations, except for the three highest treatments of~~[mixture tests deviated within 10% from the nominal concentrations, except for the three highest treatments of the](#) Ni alone test, at which the deviation was within 18% (Table 2, Supplementary data [\(change "data" to "Data" to be consistent with others\)](#)). Overall, the measured total and dissolved Ni concentrations in both tests were similar. The measured dissolved Cd concentrations were about half the measured total Cd. This difference suggests that Cd could be adsorbed on algae, DOC, and/or exoskeleton of *D. magna*. However, to be convenient, the nominal values are used to represent the treatment concentrations when the magnitude of the concentrations are not used for effect calculations.

The average and standard deviation for temperature, DO, pH, hardness, and alkalinity in the Ni alone test were 25.4 ± 0.30 °C (n = 26), 7.4 ± 0.50 mg/L (n = 26), 7.8 ± 0.24 (n = 26), 90.8 ± 5.81 mg/L as CaCO₃ (n = 12), and 62.3 ± 3.93 mg/L as CaCO₃ (n = 12), respectively. For the Cd

Ni mixtures test on the other hand, the same measured parameters were 25.3 ± 0.49 °C (n = 26), 7.6 ± 0.31 mg/L (n = 26), 7.9 ± 0.11 (n = 26), 93.7 ± 8.48 mg/L as CaCO₃ (n = 12), and 63.1 ± 4.23 mg/L as CaCO₃ (n = 12), respectively.

Dissolved organic carbon ~~(DOC)~~ was also measured in the present study although it was not added to the test treatments. The average concentration of DOC was 6.05 mg/L for the Ni alone test and 6.07 mg/L for the Cd Ni mixture test (Table 3, Supplemental Data). These DOC concentrations were likely coming from the fed diet of *D. magna* (YCT and algae). Our DOC analysis on the YCT samples confirmed this suspicion.

The treatment average concentration ranges (and test average concentration) of Cl⁻, PO₄³⁻, and SO₄²⁻, for the Ni alone test were 3.2-5.2 (4.1), 0.5-0.6 (0.5), and 195.1-202.2 (197.5) mg/L, respectively (Table 4, Supplemental Data). Those same anions in the Cd

Ni mixture test were 3.5-5.2 (4.1), 0.6-0.7 (0.6), and 195.1-201.9 (195.2) mg/L, respectively. The average concentration ranges (and test average concentration) of Na⁺, K⁺, Ca²⁺, and Mg²⁺ were 26.7-33.8 (29.1), 1.6-2.1 (1.8), 11.5-14.2 (12.5), and 11.1-13.6 (12.1) mg/L for the Ni alone test, and 28.9-36.9 (33.8), 1.8-2.2 (2.0), 12.0-15.4 (14.0), and 11.8-15.2 (13.9) mg/L for Cd

Ni mixture test, respectively (Table 4, Supplemental Data). There was no significant difference in the average concentration of each ion between the two tests.

3.2 Survival

In comparison to Cd alone, the average 21-d cumulative mortality percentages were significantly lower in the Cd

Ni mixtures containing 20, 40, 80, or 100 µg/L Ni ($p < 0.01$), but not in the other Cd

Ni mixtures (Fig. 1). These results demonstrate that when Ni is present at concentrations ≤ 100 µg/L in a mixture with 1.5 µg/L Cd, mortality substantially decreased, peaking at the Cd

Ni mixture containing 20 µg/L Ni (Fig. 1). The cumulative percent mortality of the Cd

Ni mixture containing 20 µg/L Ni (2.5%) was not significantly different from the control (0%) (Fig. 1). The low percent mortality of the Cd

Ni mixtures containing 20, 40, 80, or 100 µg/L Ni indicate that at these concentrations, Ni protected *D. magna* from Cd toxicity - evidence of a less-than-additive effect. However, at Ni concentrations ≥ 120 µg/L, the protective effect by Ni was no longer observed as mortality between those treatments and Cd alone were not significantly different (Fig. 1). Ni accumulation likely exceeded the threshold for Ni-caused toxicity to *D. magna* and thereby caused most or all of the observed toxicity.

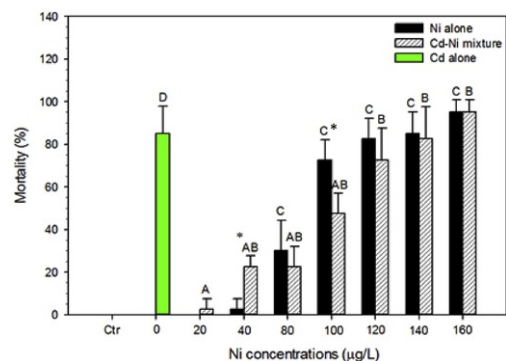


Fig. 1 21-d cumulative mortality of *D. magna* due to exposure to Cd alone, Ni alone, and Cd

Ni mixtures containing constant Cd concentration of 1.5 µg/L and varied Ni concentrations. A: significant differences between Cd alone and Cd

Ni mixtures ($p < 0.01$). B: significant differences between control and Cd

Ni mixtures ($p < 0.01$). C: significant differences between control and Ni alone ($p < 0.001$). D: significant difference between control and Cd alone ($p < 0.001$). *: significant differences between a Ni alone and its corresponding Cd

Ni mixture ($p < 0.05$). Error bars represent standard deviations.

("Cd-Ni" in the figure title should be one word and in the same line) alt-text: Fig. 1

In comparing the cumulative percent mortality between Ni alone treatments and their corresponding Cd

Ni mixtures, significant differences were found for treatments containing 40 and 100 µg/L Ni ($p < 0.05$) (Fig. 1). At a concentration of 20 or 40 µg/L Ni alone, there was no significant difference in mortality in comparison to the control (Fig. 1). Therefore, the toxicity observed in the Cd

Ni mixture containing 40 µg/L Ni was likely due to Cd exposure. At 80, 120, 140, and 160 µg/L Ni, the mortality of Ni alone and Cd

Ni mixture was significantly higher than the control's mortality but there was no significant difference between Ni alone and its corresponding mixture ($p < 0.05$) (Fig. 1). Therefore, the mortality observed in these Cd

Ni mixtures was mostly due to Ni or both Cd and Ni exposure (Fig. 1).

3.3 Reproduction

3.3.1 Time to first brood, total neonates, live neonates, and reproductive rates

The average time to first brood in Cd alone was significantly shorter than the time to first brood in the Cd

Ni mixtures containing 20, 40, 140, and 160 µg/L Ni ($p < 0.05$) ((here and in the entire paper, if "Fig. #" is in blue, all the number should be blue, such as letter "I" in this figure should be blue also.) Fig. 2I). The time to first brood for Cd alone was significantly

shorter (8 days on average) in comparison to the control (9.3 days on average) (Fig. 2I). With respect to Ni alone, none of the treatments were found to hold a significantly longer or shorter time to first brood in comparison to the control (Fig. 2I). At 160 µg/L Ni, the time to first brood of Ni alone was significantly shorter than the time to first brood of its corresponding mixture. This suggests that the effect on time to first brood seen in this mixture was likely due to Cd exposure, because the time to first brood in that Ni alone treatment was not significantly different from the control (Fig. 2I).

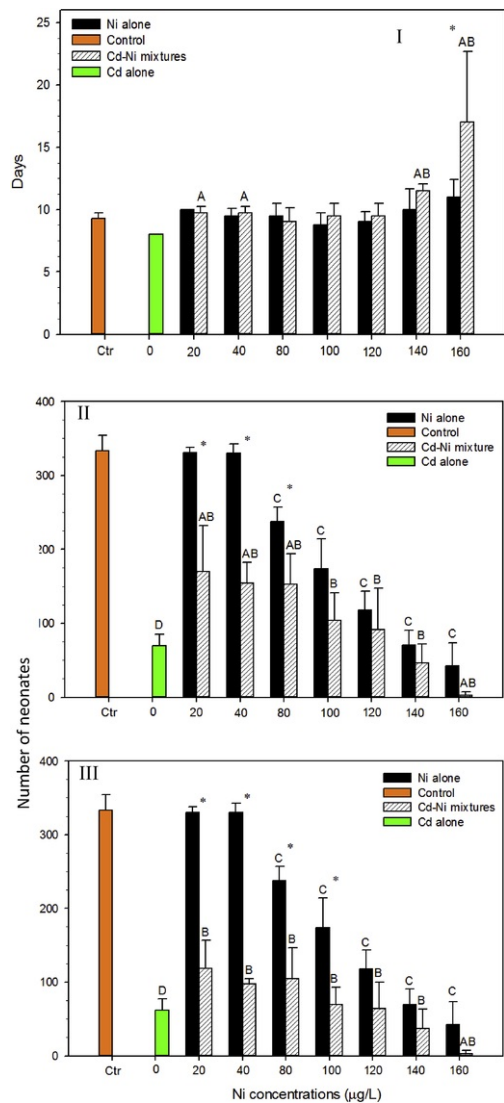


Fig. 2 Time to first brood (I), total dead and live neonates (II) and live neonates (III) reproduced over 21- d of exposure to Cd alone, Ni alone, and Cd

Ni mixtures containing constant Cd concentration of 1.5 µg/L and varied Ni concentrations. A: significant differences between Cd alone and Cd

Ni mixtures ($p < 0.05$). B: significant differences between control and Cd

Ni mixtures ($p < 0.001$). C: significant differences between control and Ni alone ($p < 0.001$). D: significant difference between control and Cd alone ($p < 0.001$). *: significant differences between a Ni alone and its corresponding Cd

Ni mixture ($p < 0.05$). Error bars represent standard deviations.

alt-text: Fig. 2

The mixtures containing 20, 40, and 80 $\mu\text{g/L}$ Ni reproduced a significantly higher number of total neonates in comparison to the total number of neonates produced in Cd alone ($p < 0.05$) (Fig. 2II). This is evidence of a less-than-additive effect. At Ni concentrations of 100, 120, and 140 $\mu\text{g/L}$, there was no significant reproductive effect between Cd Ni mixture and Cd alone (Fig. 2II). Nickel at these concentrations likely exceeded the necessary concentration needed to protect *D. magna* from chronic reproductive effect of Cd and became toxic to the organisms, especially at 160 $\mu\text{g/L}$ Ni, at which the number of total neonates in the Cd Ni mixture was significantly lower than that in Cd alone ($p < 0.05$) (Fig. 2II).

When comparing the total number of neonates between Ni alone and its corresponding Cd Ni mixture, there was a significantly higher number of total neonates in Ni alone compared to Cd Ni mixture at Ni concentrations of 20, 40, and 80 $\mu\text{g/L}$ ($p < 0.05$) (Fig. 2II). This signifies that the effect observed in those Cd Ni mixtures was mostly due to Cd exposure since the effects of Ni alone were not significantly different from the control except at 80 $\mu\text{g/L}$. However, since there was no significant difference in the total number of neonates between Ni alone and its corresponding Cd Ni mixture at higher Ni concentrations, the reproductive effects observed in the Cd Ni mixtures containing 100, 120, 140, and 160 $\mu\text{g/L}$ Ni were mostly due to Ni exposure or both Ni and Cd exposure (Fig. 2II).

Dissimilarly, none of the Cd Ni mixtures were found to contain a significantly higher number of live neonates when compared to Cd alone (Fig. 2III). This suggested that Ni protective effects were not apparent, or possibly completely absent when only live neonates were considered in analysis. In addition, at 20 and 40 $\mu\text{g/L}$ Ni alone, the total number of live neonates did not significantly differ from the control (Fig. 2III). The effect in the total number of live neonate observed in those Cd Ni mixtures was mostly due to Cd exposure. Similarly, the results obtained for the overall reproductive rate (Fig. 3I), and the live neonate reproductive rate (Fig. 3II) showed that none of the Cd Ni mixtures had a significantly higher or lower reproductive rate in comparison to Cd alone. Hence, the results suggested that Ni didoes not protect adult *D. magna* from the reproductive effect of chronic Cd exposure. Overall, unlike the survival results, a less-than-additive effect was not observed on the reproductive endpoint of *D. magna* even when Ni concentration was sufficient, except when both dead and live neonates are collectively considered (Fig. 2II).

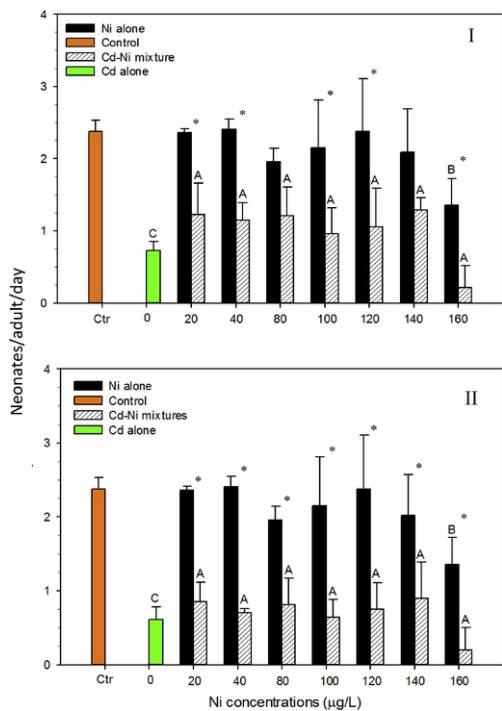


Fig. 3 Overall dead and live (I) and live (II) neonate reproductive rates of *D. magna* when exposed to Cd alone, Ni alone, and Cd

Ni mixtures containing constant Cd concentration of 1.5 µg/L and varied Ni concentrations. A: significant differences between control and Cd

Ni mixtures ($p < 0.01$). B: significant differences between control and Ni alone ($p < 0.05$). C: significant difference between control and Cd alone ($p < 0.001$). *: significant differences between a Ni alone and its corresponding Cd

Ni mixture ($p < 0.05$). Error bars represent standard deviations.

("Cd-Ni" in the figure title should be one word and in the same line) alt-text: Fig. 3

For the reproductive rate, there was no statistically significant difference in the overall and live neonate reproductive rates between Cd alone and the Cd

Ni mixtures (Fig. 3I and 3II). The overall and live neonate reproductive rates of Cd alone were significantly lower than those of the control. In addition, Ni alone treatments containing 20, 40, 100, 120, and 160 µg/L had significantly higher overall reproductive rates than their corresponding Cd

Ni mixtures ($p < 0.05$) (Fig. 3I). These results suggest that the effects observed in those mixtures were likely due to Cd exposure. Nickel alone treatment containing 160 µg/L was found to have a significantly lower overall reproductive rate ($p < 0.05$) (Fig. 3I), and live neonate reproductive rate ($p < 0.05$) (Fig. 3II) in comparison to the control. These results indicate that at 160 µg/L, Ni likely exceeded the threshold and became toxic to the organisms. The overall and live neonate reproductive rates in the Cd

Ni mixture containing 160 µg/L Ni were significantly lower than those in the 160 µg/L Ni alone treatment, suggesting the effects in this mixture were due to both Cd and Ni exposure.

3.3.2 Total dead neonates and undeveloped embryos

The percentage of dead neonates was significantly higher in the Cd

Ni mixtures containing 20, 40, 80, 100, and 120 µg/L Ni in comparison to the Cd alone treatment ($p < 0.05$) (Fig. 4I). This difference was not observed for other Cd

Ni mixtures (Fig. 4I). These results revealed that *D. magna* in the Cd

Ni mixtures containing 20, 40, 80, 100, and 120 µg/L Ni were able to increase reproductive effort in comparison to Cd alone (Fig. 4I). However, Ni did not seem to provide protective effects to the neonates in those Cd Ni mixtures (Fig. 4I) as evident by the higher percentage of dead neonates (Fig. 4I).

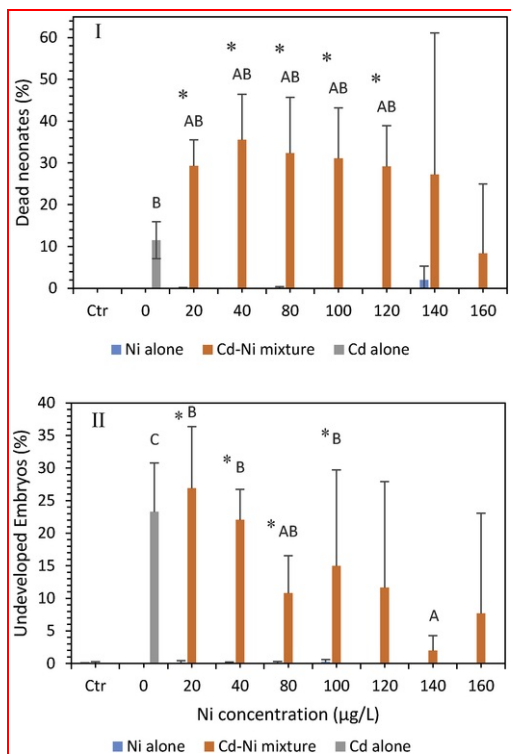


Fig. 4 Total dead neonates reproduced (I) and undeveloped embryos observed (II) over 21-d of exposure to Cd alone, Ni alone, and Cd

Ni mixtures containing constant Cd concentration of 1.5 µg/L and varied Ni concentrations. A: significant differences between Cd alone and Cd

Ni mixtures ($p < 0.05$). B: significant differences between control and Cd

Ni mixtures ($p < 0.001$). C: significant difference between control and Cd alone ($p < 0.001$). *: significant differences between a Ni alone and its corresponding Cd

Ni mixtures ($p < 0.001$). Error bars represent standard deviations.

("Cd-Ni" in the figure titles should be one word and in the same line) alt-text: Fig. 4

None of the Ni alone treatments had a significantly higher percentage of dead neonates in comparison to the control (Fig. 4I). However, Cd alone and Cd

Ni mixtures containing 20, 40, 80, 100, and 120 µg/L Ni were found to have a significantly higher percentage of dead neonates in comparison to the control (Fig. 4I). Since the percentage of dead neonates observed in the Ni alone treatments containing 20, 40, 80, 100, and 120 µg/L were significantly lower than their corresponding Cd

Ni mixtures ($p < 0.001$) (Fig. 4I), the effects observed in those Cd

Ni mixtures were likely due to Cd exposure. However, the effects observed in the Cd

Ni mixtures containing ≥ 140 µg/L Ni were mostly due to Cd exposure or both Ni and Cd exposure.

Only the Cd

Ni mixtures containing 80 or 140 µg/L Ni had a significantly lower percentage of undeveloped embryos in comparison to Cd alone ($p < 0.01$) (Fig. 4II). No significant differences in the percentage of undeveloped embryos was apparent between Cd alone and the other Cd

Ni mixtures. These results indicate that the Cd

Ni mixtures with 20 or 40 µg/L Ni did not provide protective effects to *D. magna* embryos (Fig. 4II).

The percentage of undeveloped embryos observed in all Ni alone treatments were not significantly different from the control (Fig. 4II). However, the percentage of undeveloped embryos observed in Cd alone, and the Cd Ni mixtures containing 20, 40, 80, and 100 µg/L Ni were significantly higher in comparison to the control ($p < 0.001$) (Fig. 4II). These results indicate that the embryotoxicity observed in those Cd Ni mixtures was likely due to Cd exposure.

The results from morphological examination revealed developmental obstructions and morphological alterations in embryos exposed to Cd Ni mixtures. Obstructions included: less developed blastomeres as seen in Fig. 5B compared to Fig. 5A, less head development as seen in Fig. 5D and F compared to Fig. 5C and E or no further embryo development as seen in Fig. 5G compared to Fig. 5H. The effects could be either halted mitotic cell division (cleavage) or disrupted cellular arrangement and organization which likely prevented the development of an embryo.

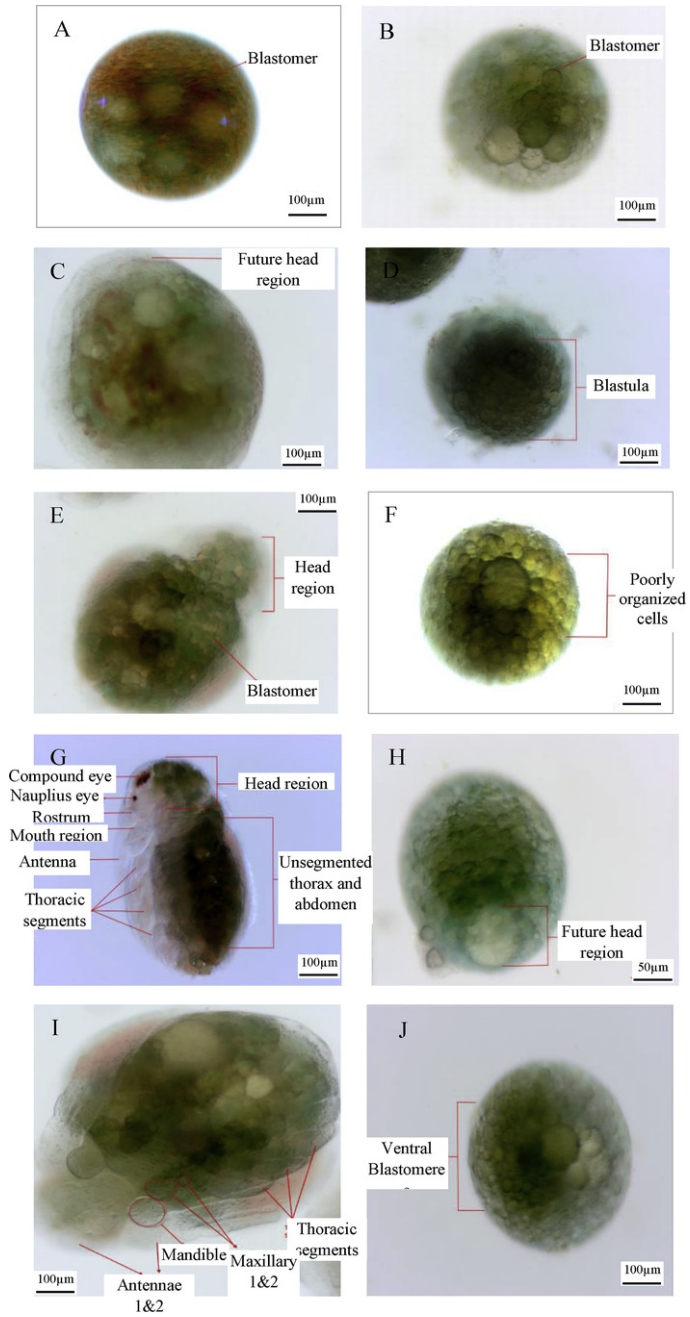


Fig. 5 Embryo development of *D. magna* over 10 stages. A: Control embryo in stage 2 of development (early cleavage), B: Embryo in stage 2 of development (early cleavage) of Cd alone, C: Control embryo in stage 7.3 of development (Postnaupliar segments, ventral view), D: Embryo in stage 2 of development (late cleavage) for Cd

Ni mixture with 140 µg/L Ni, E: Control embryo in stage 9 of development (appearance of compound and nauplius eye, dorsal view), F: Embryo in stage 3 of development (gastrulation) for Cd

Ni mixture with 120 µg/L Ni, G: Control embryo in stage 9 of development (lateral view), H: Embryo in stage 5 of development (head formation, anterodorsal view) for Cd

Ni mixture with 20 µg/L Ni, I: Control embryo in stage 10 of development (hook-shaped abdomen, ventral view), J: Embryo in stage 3 of development (gastrulation, anteroventral view) for Cd

Ni mixture with 40 µg/L Ni.

alt-text: Fig. 5 ("Cd-Ni" in the figure titles should be one word and in the same line)

3.4 Body weight

The average dry weights of the surviving *D. magna* in Cd

Ni mixtures containing 20, 40, 80, or 100 µg/L Ni were found to be significantly higher than the dry weight of the surviving *D. magna* in Cd alone ($p < 0.05$) (Fig. 6). These results indicate that Ni at those concentrations were sufficient to protect *D. magna* from the growth effect of Cd. At higher Ni concentrations, the dry weights of the surviving *D. magna* in Cd alone and Cd

Ni mixtures were not significantly different (Fig. 6). This suggests that the concentration of Ni in those mixtures exceeded the necessary concentration needed to protect *D. magna* from chronic Cd growth effect.

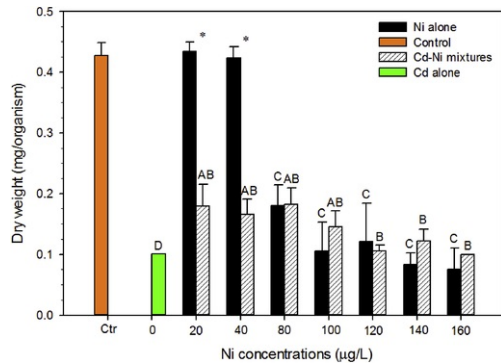


Fig. 6 Dry weight of surviving adults at test termination. A: significant differences between Cd alone and Cd

Ni mixtures ($p < 0.05$). B: significant differences between control and Cd

Ni mixtures ($p < 0.001$). C: significant differences between control and Ni alone ($p < 0.001$). D: significant difference between control and Cd alone ($p < 0.001$). *significant differences between a Ni alone and its corresponding Cd

Ni mixture ($p < 0.001$). Error bars represent standard deviations.

alt-text: Fig. 6 ("Cd-Ni" in the figure titles should be one word and in the same line)

The dry weights of the surviving *D. magna* in the Ni alone treatments containing 20 and 40 µg/L Ni were significantly higher in comparison to their corresponding Cd

Ni mixtures ($p < 0.001$) (Fig. 6). Plus, the dry weights of the surviving *D. magna* in those same Ni alone treatments were not significantly different from the control (Fig. 6). Therefore, the growth effects manifested in the Cd

Ni mixtures containing 20 and 40 µg/L Ni were most likely due to Cd exposure (Fig. 6). However, the dry weights of the surviving *D. magna* in all other Ni alone treatments containing ≥ 80 µg/L were not found to be significantly higher than their corresponding Cd

Ni mixture (Fig. 6), but they were significantly lower than the control ($p < 0.001$) (Fig. 6). These results suggest that the growth effects observed in Cd

Ni mixtures containing ≥ 80 µg/L was mostly due to Ni exposure.

3.5 Metal accumulation effects

The measured Cd concentrations in the tissues of the surviving daphnids exposed to Cd alone and to Cd

Ni mixtures were significantly higher than the measured Cd concentration in control organisms (Fig. 7). When concentration in the mixture exposure media increased, Ni concentrations gradually increased in the tissues of the

surviving daphnids, fitting the trend of a linear function (Fig. 7). Subsequently, a gradual decrease in Cd tissue concentrations followed, fitting the trend of a linear function (Fig. 7). These results provide a strong evidence of a competitive accumulation of Ni and Cd.

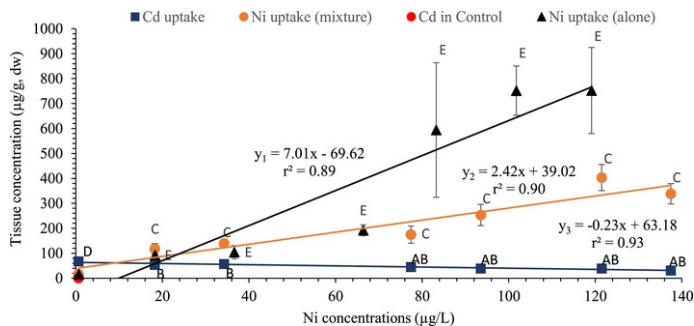


Fig. 7 Relationship between water metal concentration and body concentration of surviving daphnids exposed to Ni alone and Cd

Ni mixture over 21 days. A: significant differences between Cd alone and Cd

Ni mixtures ($p < 0.05$). B: significant differences between control and Cd

Ni mixtures ($p < 0.05$). C: significant differences between control and Cd

Ni mixtures ($p < 0.05$). D: significant difference between control and Cd alone ($p < 0.001$). E: significant differences between Ni alone and control. Error bars represent standard deviations. y_1 = body Ni concentration for Ni alone exposure. y_2 = body concentration for Cd

Ni mixture exposure. y_3 = body Cd concentration for Cd

Ni mixture exposure.

alt-text: Fig. 7

The results obtained from tissue analysis of the surviving daphnids in the Cd

Ni mixtures support the assumption that metals compete for binding sites on the biotic ligand. The organisms exposed to Cd alone accumulated significantly more Cd than did the organisms exposed to a mixture of Cd and Ni ($p < 0.05$), except the mixtures containing 20 and 40 $\mu\text{g/L}$ Ni (Fig. 7). Only the daphnids in the Cd

Ni mixtures containing $\geq 80 \mu\text{g/L}$ Ni were found to accumulate significantly less Cd in the tissues than the surviving daphnids exposed to Cd alone. These results again enforce a strong evidence of a competitive accumulation effect which results in a less-than-additive effect in Cd

Ni mixtures at Ni concentrations less than 80 $\mu\text{g/L}$.

4 Discussion

In general, most results of the present study are consistent with other literature studies. Mixtures of Cd and Ni have shown less-than-additive toxicity on the survival of *D. magna* (Traudt et al., 2016), uptake in *D. magna* (Komjarova and Blust, 2008), uptake in the zebra fish *Danio rerio* (Komjarova and Blust, 2009), growth of the freshwater bacteria *Nitrosomonas europaea* (Sato et al., 1986), and adsorption capacity of the freshwater algae *Chlorella vulgaris* (Awasthi and Rai, 2004). However, at high Ni concentrations (i.e., 100–160 $\mu\text{g/L}$) in a mixture with Cd, Ni became toxic to the organisms. The less-than-additive or joint toxic effect in the Cd

Ni mixtures only occurred at optimal (non-toxic) concentrations of Ni (20–80 $\mu\text{g/L}$). Since water quality (e.g., hardness, alkalinity, pH, DOC) has a strong influence on Ni toxicity to aquatic organisms (Hoang et al., 2004), the optimal Ni concentrations discussed herein refer to the moderately hard water conditions used in the present study. The discussion below focuses on the link between competitive accumulation and less-than-additive effects, early reproduction and possible detoxification mechanisms, and effect on embryo development.

4.1 Less-than-additive effect and competitive metal accumulation

Overall, results of the present study indicate that at Ni concentrations $\leq 80 \mu\text{g/L}$, the chronic effect of Cd and Ni mixtures was antagonistic (less-than-additive). When the Ni concentration was not too high to produce toxic

effects (when present alone), Ni appeared to protect the organisms from the chronic toxicity of Cd. Similarly, studies on *D. magna* have shown less-than-additive toxicity that is believed to be mediated by a competitive binding mechanism at biotic ligands (BL) (Meyer et al., 2015a; Santore and Ryan, 2015). The mechanisms driving such metal mixture interactions are not clearly understood. Metals are believed to interact with a BL based on its affinity to the BL. A metal with the strongest affinity for a BL will occupy more binding sites and be accumulated more in an organism than metals with less affinity.

In the present study, we found that as Ni concentration gradually increased in the test media of the mixtures, body Cd concentration progressively decreased in *D. magna*, as the Ni concentration increased (Fig. 7). This finding supports the competitive binding mechanism of metals at the BL and agrees with the results published by Komjarova and Blust (2008). Our earlier chronic exposure study with Cd

Zn mixtures and *D. magna* also found similar results to the findings of the present study (Pérez and Hoang, 2017). Meyer et al. (2015b) also reported less-than-additive acute toxicity of Cd and Zn mixtures to *D. magna* at low Zn concentrations but Zn appeared to contribute to the toxicity at high Zn concentrations.

However, the decrease in body Cd concentration in *D. magna* when increasing water Ni concentration, especially at low Ni concentrations, does not clearly explain the less-than-additive effects observed in the Cd Ni mixtures. For example, *D. magna* exposed to Cd

Ni mixtures containing 20 or 40 µg/L Ni had significantly higher body Cd concentrations compared to the control, and similar body Cd concentrations compared to Cd alone (Fig. 7). Nevertheless, Ni produced protective effects against Cd toxicity on survival of *D. magna*, causing a decrease in mortality (Fig. 1). The no significant difference in body Cd concentrations in those mixture and Cd alone suggests that Ni did not completely block Cd binding to the BL. As a result, Cd still entered the organisms and induced toxic effects. For instance, mortality in the mixtures did not completely decrease to 0% (mixture treatments 20, 40, 80 µg/L Ni, Fig. 1), and the reproductive rates of the mixtures were not significantly higher than the reproductive rate of Cd alone (20, 40, 80 µg/L Ni, Fig. 3I and 3II). In addition, more dead neonates (20-120 µg/L, Fig. 4I) were found in the mixtures than in the Cd alone treatment.

In mixtures containing higher concentrations of Ni (≥120 µg/L), no protective Ni effect was observed. This suggests that Ni exceeded the necessary concentration needed to produce protective effects and appeared to contribute to the toxicity.

4.2 Early reproduction, food consumption, and possible detoxification mechanism

It is interesting that *D. magna* in the Cd alone treatment reproduced neonates earlier than the control *D. magna* (Fig. 2I). Furthermore, it is also interesting that the percentage of undeveloped embryos in the Ni alone treatments with 20-100 µg/L (Fig. 4II), and the percentage of dead neonates in the Ni alone treatments with 20-120 µg/L (Fig. 4I) were significantly lower than their corresponding Cd

Ni mixtures (Fig. 4I and 4II). Our earlier study on Cd

Zn mixtures and *D. magna* found similar results (Pérez and Hoang, 2017).

A recent review by Jezierska et al. (2009) on metal toxicity to fish embryos showed that metals can affect embryo development and result in premature hatching, delay in the hatching process, or to deformations and death of the larvae. Although the premature release of the embryos from the brood pouch of *D. magna* due to toxic metal stress is a viable explanation for our results, the question of why the organisms reproduced neonates earlier under the Cd alone exposure is still unanswered. Yu and Wang (2002) reported that up to 67% and 47% of selenium (Se) uptake by *D. magna* from the aqueous phase and dietary phase, respectively, were transferred to the offspring; while for Cd, neonate release only represented a small fraction of Cd loss when the organisms were exposed to the aqueous and dietary phases. Another study by Tsui and Wang (2004) also revealed that Hg (11-15%) and MeHg (32-41%) were eliminated from the mothers via maternal transfer after dietary exposures. Similarly, Cazan and Klerks (2015) reported that Cu and Cd were transferred to the offspring of mosquitofish mothers. In addition, *D. magna* have been reported to produce a greater number of smaller sized neonates when exposed to Cd at low concentrations (i.e., ≤5 µg/L) (Bodar et al., 1988b). Thus, the question(s) of whether a significant portion of accumulated Se, Cd, Cu, or Hg was transferred to the offspring and earlier reproduction with a higher percentage of dead neonates, a higher percentage of undeveloped embryos, and/or a higher reproductive rate with smaller neonate served as a detoxification mechanism are still unanswered. Further studies are needed to answer this question.

Daphnia magna in the Cd alone treatment did not routinely consume much of the algae fed to them. Consequently, lower reproductive rates and body weights were found in the Cd alone treatment compared to the control (Fig. 3I and 3II, 6). At the end of the experiment, the average body weight of *D. magna* exposed to Cd alone decreased by 76.7% in comparison to control organisms (Fig. 6). Similarly, a decrease of 40% in body weight was reported for *D. magna* exposed to 1 and 5 µg/L Cd for 14 days, compared to the control (Bodar et al., 1988a). In addition, in a separate study, Bodar et al. (1988b) also reported that Cd directly disrupted feeding behavior and digestive mechanisms, which resulted in altered metabolism that led to reduced food consumption. Elnabaraway et al. (1986) demonstrated that after 14 days of exposure to a Cd concentration of 2.5 µg/L, fecundity of *D. magna* was significantly reduced. Recent studies using embryonic exposure methods also demonstrated adverse effects on *D. magna* egg development and survival when exposed to higher Cd concentrations such as LC50 of 11 µg/L Cd (Sobral et al., 2001) or 60 µg/L Cd (Djekoun et al., 2015). While water quality was not reported by Sobral et al. (2001), the higher water hardness level (230-245 mg/L as CaCO₃) used by Djekoun et al. (2015) might explain the higher effect concentration of their study compared to

the present study.

4.3 Effects on embryo development

The present study suggests that the effects on embryo development was likely due to Cd exposure because the percentages of undeveloped embryos in all Ni alone treatments were less than 0.83% (Fig. 4II), and the percentage of aborted embryos in the Cd

Ni mixtures containing 20–100 µg/L Ni were significantly higher than their corresponding Ni alone pairs (Fig. 4II).

Jezierska et al. (2009) found that dissolved waterborne metals can exert direct effects on developing embryos or they can accumulate in the gonads of fish and deleteriously affect gamete viability and production. Since the coating (egg shell) on the embryo does not fully protect the egg during the swelling phase, metals can penetrate and accumulate in the developing egg. In fact, metals can affect various developmental processes during embryonic development, leading to reduced brood quantity and quality (as observed in the Cd alone treatment), developmental defects (body malformations, anomalies during organogenesis), and death. For example, a recent acute toxicity study found that *D. magna* embryos experienced severe morphological defects after exposure to 60, 80, or 100 µg/L Cd (Djekoun et al., 2015), resulting in neonates with caudal spine malformations, poorly developed carapaces, and no antennas or eyes. Although the study used a Cd concentration that was between 1 and 2 orders of magnitude higher than the concentration of Cd used in the present study, it can be seen that with the water chemistry of the present study (lower hardness than the hardness used in the study above, 245 mg/L as CaCO₃), 1.5 µg/L Cd with chronic exposure is sufficient to cause embryonic deformities.

To further understand the effect of Cd on embryonic development at different stages, we used the defined developmental stages by Mittmann et al. (2014) as a reference to compare with our results. Embryos produced from Cd alone and the Cd

Ni mixtures demonstrated morphological deformities, such as the mitotic shutdown of early or late cleavage (Fig. 5B and D), disruption of cellular differentiation and gastrulation (Fig. 5F and J), or inhibition from development beyond stage 5 (Fig. 5H); unlike control embryos, which displayed normal cleavage and a higher degree of faithful cellular arrangement, differentiation, and organization (Fig. 5A, C, 5E, 5G, 5I).

Our results further showed that embryos exposed to Cd alone only underwent early cleavage (8 cell stage to morula) (Fig. 5B), while embryos exposed to the Cd Ni mixtures either progressed to a blastula (Fig. 5D), underwent gastrulation (Fig. 5F and J), or developed to stage 5 (Fig. 5H). Although some embryos were able to form a blastula, the blastomeres were somewhat disorganized, and idly arranged into a splotch of cells (Fig. 5F). This indicated some degree of protective Ni effects against Cd toxicity.

Cadmium is believed to induce deletion mutations, interact with reactive oxygen species, disrupt biochemical signaling, and interfere with DNA repair (Filipic and Hei, 2004). In the present study, Cd likely disrupted the communication of cellular signals that were essential to the successful development of *D. magna* embryos. Or, it could have acted as a genotoxic agent that led to the premature death of the developing embryos. In addition, Cd is also known to inhibit Ca uptake, disrupt Ca homeostasis, and result in altered Ca-dependent cellular signaling. Since cytoplasmic Ca signaling is known to regulate genes associated with cellular differentiation, growth, and apoptosis, disruption of those biochemical pathways would have led to deleterious effects such as DNA damage, malignant cellular growth, and transcriptional abnormalities (Kasprzak and Salnikow, 2007).

5 Conclusions and suggestions

In the moderately hard water conditions, Ni at concentrations ranging from 20 to 80 µg/L protected *D. magna* from the chronic toxicity of Cd, resulting in less-than-additive effects on survival and growth, but not reproduction. Therefore, Cd

Ni mixtures seem to behave both interactively and noninteractively in a concentration dependent manner, and at different endpoints of analysis. It is evident that studies examining metal mixtures need to examine multiple endpoints in order to obtain a holistic idea of the interactive or noninteractive toxicity of the metals in question. At concentrations that were higher than the optimal range (>80 µg/L) Ni appeared to contribute to the toxic effects of Cd. In general, when water Ni concentration increased, body Ni concentration increased and body Cd concentrations decreased. These results can be explained by a competitive binding mechanism at the BL and support the observed toxic effects.

Cadmium affected the embryonic development of *D. magna* even in the presence of Ni, except at concentrations ≥ 120 µg/L Ni. This embryonic effect is not explained by the competitive binding mechanism at the BL, where metals first enter the organisms from the environment. Another interactive mechanism of cellular metals is needed to explain the embryonic impairment. Similarly, the question of whether breeding adult *D. magna* transfer toxic metals to their offspring as a means of detoxification is still unanswered. Additional studies are warranted to address these questions. Overall, the results of the present study are useful for the development of improved environmental quality guidelines for metal mixtures.

Acknowledgement

We are grateful to the scholarship provided by the Loyola Institute of Environmental Sustainability. We acknowledge the assistances of Erik Reynolds, Anthony Signa, Tien Trinh, Sylvia Sadowicz, Tam Nguyen, and Ritesh Kashyap in conducting the experiments. **(We would like to insert this acknowledgement here "We would like to thank Rodney Dale for allowing us to use his microscope for the morphological examination.")**We would also like to thank Martin Berg for his assistance with data analysis and Stephen Mitten for contributing valuable comments and suggestions.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2018.06.063>.

References

- Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Nickel, (Update)2005, Public Health Service, US Department of Health and Human Services; Atlanta, GA, USA.
- Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Cadmium (Update), 2012, Public Health Service, US Department of Health and Human Services; Atlanta, GA, USA.
- ASTM, Standard Guide for Conducting Daphnia Magna Life-cycle Toxicity Tests, E1193-97, 2012, ASTM International; West Conshohocken, PA, USA.
- Awasthi M. and Rai L.C., Adsorption of nickel, zinc, and cadmium by immobilized green algae and cyanobacteria: a comparative study, *Ann. Microbiol.* **54** (3), 2004, 257-267.
- Babich H., Shopsis C. and Borenfreund E., Cadmium-nickel toxicity interactions towards a bacterium, filamentous fungi, and a cultured mammalian cell line, *Bull. Environ. Contam. Toxicol.* **37** (1), 1986, 550-557.
- Balistreri L.S., Mebane C.A., Schmidt T.S. and Kellerk W., Expanding metal mixture toxicity models to natural stream and lake invertebrate communities, *Environ. Toxicol. Chem.* **34** (4), 2015, 761-776.
- Bodar C.W.M., Van Der Sluis I., Voogt P.A. and Zandee D.I., Effects of cadmium on consumption, assimilation and biochemical parameters of *Daphnia magna*: possible implications for reproduction, *Comp. Biochem. Physiol.* **90C** (2), 1988a, 341-346.
- Bodar C.W.M., Van Leeuwen C.J., Voogt P.A. and Zandee D.I., Effect of Cadmium on the reproduction strategy of *Daphnia magna*, *Aquat. Toxicol.* **12** (4), 1988b, 301-310.
- Cazan A.M. and Klerks P.L., Physiological effects and reduced tolerance following maternal metal exposure in the live-bearing fish *gambusia affinis*, *Environ. Toxicol. Chem.* **34** (6), 2015, 1337-1344.
- De Laender F., Janssen C.R. and De Schampelaere K.A.C., Non-simultaneous Ecotoxicity testing of single chemicals and their mixture results in erroneous conclusions about the joint action of the mixture, *Chemosphere* **76**, 2009, 428-432.
- Djekoun M., Bensoltane S., Bourechrouche A., Bourechrouche M. and Berrebah H., In-vitro toxicity of cadmium on the development of parthenogenetic eggs of a freshwater cladoceran: *Daphnia magna*, *J. Mater. Environ. Sci.* (4), 2015, 957-962.
- Eaton A.D., Clesceri L.S., Greenberg A.E. and Rice E.W., Standard Methods for the Examination of Water and Wastewater, Twenty-first Ed. American Public Health Association, 2005, United Book Press Inc; Baltimore, Maryland, USA.
- Eisler R., Nickel Hazards to Fish, Wildlife, and Invertebrates: a Synoptic Review, 1998, US Geological Survey, Biological Resources Division, Biological Science Report.
- Elnabaraway M.T., Welter A.N. and Robideau R.R., Relative sensitivity to three daphnid species to selected organic and inorganic chemicals, *Environ. Toxicol. Chem.* **5** (4), 1986, 393-398.
- ENVIRODAT, National Environmental Quality Data Bank Codes Dictionary, 1992, Inland Waters Directorate, Environment Canada; Ottawa, Water Quality Branch.
- Filipic M. and Hei T.K., Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage, *Mutat. Res. Fund Mol. M* **546** (1-2), 2004, 81-91.
- Hoang T.C., Tomasso J.R. and Klaine S.J., Influence of water quality and age on nickel toxicity to fathead minnows (*Pimephales promelas*), *Environ. Toxicol. Chem.* **23**, 2004, 86-92.
- Jeziarska B., Ługowska K. and Witeska M., The effects of heavy metal on the embryonic development of fish (a review), *Fish Physiol. Biochem.* **35** (4), 2009, 625-640.

- Kasprzak K.S. and Salnikow K., Nickel toxicity and carcinogenesis, In: Sigel A., Sigel H. and Sigel R.K.O., (Eds.), *Nickel and its Surprising Impact in Nature* **vol. 2**, 2007, John Wiley & Sons, Ltd; Chichester, UK, 621-645, <https://doi.org/10.1002/9780470028131.ch17>.
- Komjarova I. and Blust R., Multi-metal interactions between Cd, Cu, Ni, Pb, and Zn in water flea *Daphnia magna*, a stable isotope experiment, *Aquat. Toxicol.* **90**, 2008, 138-144.
- Komjarova I. and Blust R., Multi-metal interactions between Cd, Cu, Pb, and Zn uptake from water in the Zebrafish *Danio rerio*, *Environ. Sci. Technol.* **43**, 2009, 7225-7229.
- Meyer S.J., Farley J.K. and Garman R.E., Metal mixtures modeling evaluation project: 1. Background, *Environ. Toxicol. Chem.* **34** (4), 2015a, 726-740.
- Meyer S.J., Ranville F.J., Pontasch M., Gorsuch W.J. and Adams J.W., Acute toxicity of binary and ternary mixtures of Cd, Cu and Zn to *Daphnia magna*, *Environ. Toxicol. Chem.* **34** (4), 2015b, 799-808.
- Mittmann B., Ungerer P., Klann M., Stollewerk A. and Wolff C., Development and staging of the water flea *Daphnia magna* (Straus, 1820; Cladocera, Daphniidae) based on morphological landmarks, *EvoDevo* **5** (1), 2014, 1-20.
- Newton M.K., Toxicity of Binary Metal Mixtures to Freshwater Aquatic Organisms: Experimental Design, Acute and Chronic Effects, 2015, Dissertation Clemson University, Publication No. 3722428.
- Niyogi S., Nadella S.R. and Wood C.M., Interactive effects of waterborne metals in binary mixtures on short-term gill-metal binding and ion uptake in rainbow trout (*Oncorhynchus mykiss*), *Aquat. Toxicol.* **165**, 2015, 109-119.
- Norwood W.P., Borgmann U., Dixon D.G. and Wallace A., Effects of metal mixtures on aquatic biota: a review of observations and methods, *Hum. Ecol. Risk Assess.* **9**, 2003, 795-811.
- Pérez E. and Hoang T.C., Chronic toxicity of binary-metal mixtures of cadmium and zinc to *Daphnia magna*, *Environ. Toxicol. Chem.* **36** (10), 2017, 2739-2749.
- Phillips B.M., Nicely P.A., Hunt J.W., Anderson B.S., Tjeerdema R.S., Palmer S.E., Palmer F.H. and Puckett H.M., Toxicity of cadmium, copper, nickel, zinc mixtures to larval purple sea urchins (*Strongylocentrotus purpuratus*), *Bull Environ. Contam. Toxicol.* **70**, 2003, 592-599.
- Santore R.D. and Ryan A.C., Development and application of a multimetal multibiotic ligand model, *Environ. Toxicol. Chem.* **34**, 2015, 777-787.
- Sato C., Schnoor J.L. and McDonald D.B., Characterization of effects of copper, cadmium, and nickel on the growth of *Nitrosomonas europaea*, *Environ. Toxicol. Chem.* **5**, 1986, 403-416.
- Sobral O., Chastinet C., Nogueira A., Soares A.M.V.M., Gonialves F. and Ribeiro R., In vitro development of parthenogenetic eggs: a fast ecotoxicity test with *Daphnia magna*?, *Ecotoxicol. Environ. Saf.* **50**, 2001, 174-179.
- Traudt E.M., Ranville J.F., Smith S.A. and 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, *Environ. Toxicol. Chem.* **35**, 2016, 1843-1851.
- Tsui M.T.K. and Wang W.-X., Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*, *Environ. Sci. Technol.* **38.3**, 2004, 808-816.
- U.S. Environmental Protection Agency, Method 3050B: Acid Digestion of Sediments, Sludges, and Soils, Revision 2, 1996 <https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf>.
- U.S. Environmental Protection Agency, Update of Ambient Water Quality Criteria for Cadmium, EPA-822-r-01-001. Final Report 2001, Office of Water; Washington, DC.
- U.S. Environmental Protection Agency, Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-r-02-013 4th edition, 2002, Office of Water; Washington, DC.
- Vandenbrouck T., Soetaert A., Van der Ven K., Blust R. and De Coen W., Nickel and binary metal mixture responses in *Daphnia magna*: molecular fingerprints and (sub) organismal effects, *Aquat. Toxicol.* **92** (1), 2009, 18-29.
- Vijver M.G., Elliot E.G., Peijnenburg W.J.G.M. and De Snoo G.R., Response prediction for organisms water-exposed to metal mixtures: a meta-analysis, *Environ. Toxicol. Chem.* **30**, 2011, 1482-1487.
- Yu R.Q. and Wang W.-X., Trace metal assimilation and release budget in *Daphnia magna*, *Limnol. Oceanogr.* **47**, 2002, 495-504.

Appendix A. Supplementary data

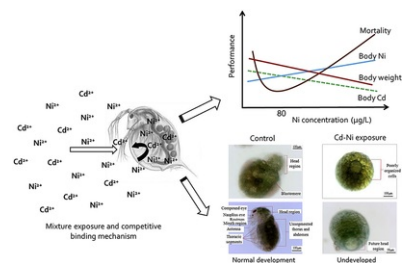
The following is the supplementary data related to this article:

[Multimedia Component 1](#)

Multimedia component 1

alt-text: Multimedia component 1

Graphical abstract



alt-text: Image 1

Highlights

- At low concentrations, Ni protected *Daphnia magna* from Cd toxicity.
- At high concentrations, Ni contributed to the toxicity of Cd.
- Protective effect of Ni was not clearly observed for reproductive endpoints.
- Cadmium affected embryotic development of *Daphnia magna* but Ni did not.
- Competitive metal uptake between Ni and Cd by *Daphnia magna* was observed.

Queries and Answers

Query: Please confirm that the provided email thoang@luc.edu is the correct address for official communication, else provide an alternate e-mail address to replace the existing one, because private e-mail addresses should not be used in articles as the address for communication.

Answer: correct

Query: Please note that author's telephone/fax numbers are not published in Journal articles due to the fact that articles are available online and in print for many years, whereas telephone/fax numbers are changeable and therefore not reliable in the long term.

Answer: agree

Query: Please confirm that given names and surnames have been identified correctly and are presented in the desired order and please carefully verify the spelling of all authors' names.

Answer: correct

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact p.das@elsevier.com immediately prior to returning your corrections.

Answer: This paper is for a regular issue