Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2020.25254

184 (2020) 80–85
April

Effects of water hardness on the toxicity of heavy metals to *Daphnia magna*

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Received 23 August 2019; Accepted 17 November 2019

ABSTRACT

This study assessed the relationship between the levels of heavy metal ion concentration vs. water hardness and the effects it had on the *Daphnia magna* acute toxicity test. Exposing *Daphnia magna* to heavy metal ions in different water hardness (100, 200, and 500 mg L^{-1} of $CaCO_3$) decreased the 24 h acute toxicity brought on by the heavy metals. The *Daphnia magna*, which were exposed to heavy metal ions, had varying tolerance responses that differed by levels of water hardness. Elevating the water hardness raised the survival rate of the *Daphnia magna*. This result suggests that mineral ions of hard water act mitigate the toxic effects of heavy metal ions through competition. In hard water containing 100 and 200 mg L^{-1} of $CaCO_3$, there was little difference in the response to the heavy metal ion toxicity. However, *Daphnia magna* exposed to water hardness of 500 mg L^{-1} of $CaCO_3$ showed a notable increase in survival rate. The water from the Gyeongan river was tested as well. The type of relationship between heavy metal ions and water hardness inducing ions appears to be one of mutual competition.

Keywords: Daphnia magna; Water hardness; Toxicity; Heavy metal; River; Monitoring

1. Introduction

Ever since the start of the industrial revolution, the amount of harmful chemical substances in the environment has been on the rise. In South Korea, about 400 different unnatural chemical compounds of environmental concern are reported to the Ministry of Environment every year. The concentration and number of pollutants continue to increase. There are toxic chemicals, environmental hormones, and heavy metals that can accumulate and induce symptoms like vomiting and diarrhea or worse problems like infertility and nervous and circulatory system damage.

All heavy metals have harmful effects on most organisms beyond certain levels of exposure and absorption [1]. Heavy metals play a significant role in ecotoxicology due to their constant presence [2]. To determine the scale of the harmful effects of heavy metal ions, the concentration of heavy metal ions in the system must be accurately measured. Common heavy metals found in our environment include copper, zinc, and lead. These are components of industrial wastewater. These heavy metals are managed through governmental standards. Heavy metal can release its toxicity only when present in water in an ionic state. The toxicity of heavy metals differs in accordance with change in precipitation,

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Presented at the 12th International Desalination Workshop (IDW2019), 28–30 August 2019, Jeju, Korea

which lowers or raises the hardness of the water. That is, the variation of precipitation can affect the degree of ion competition. In general, the higher the hardness of water, the lower the heavy metal-induced toxicity [3].

The toxic effects of heavy metals on *Daphnia magna* have been extensively studied by many scientists. Several studies have concluded that heavy metals adversely affect *D. magna*, in ways including changing food consumption levels [4], injuring reproductive organs [5], stunting their natural increase rate [6], and altering their digestive enzyme activity [7]. The U.S. EPA enacted the Standard Test Method for *D. magna* to manage and supervise aquatic ecosystems [8,9].

The biological effects of the heavy metal ions on the *D. magna* were assessed through the Acute Immobilization Test. Test methods were based on standard water quality test methods, and ToxCalc 5.0 was used to calculate no observable effect concentration (NOEC), lowest observable effect concentration (LOEC), median effective concentration (EC50), and toxic unit (TU).

2. Materials and methods

2.1. Materials

2.1.1. Daphnia magna

Daphnia magna are designated as international standard test species. *Daphnia magna* are freshwater invertebrate crustaceans that live in lakes and rivers. They lived for 40 d at 25°C and 20 d at 20°C. After hatching, they grow into adults after 6~10 d. The media where the crustaceans resided were changed every day and they were fed with the algae *Pseudokirchneriella subcapitata*. In the experiments, only the generations beyond third were used for the acute toxicity tests.

2.1.1.1. Culture medium

The acute toxicity tests and culture methods were referenced from the U.S. EPA's standard methods. The constituents of the culture media are represented in Table 1. The temperature of the medium was 20°C ± 1°C. Levels of water hardness were maintained through the use of various ionic compounds (8 mg of KCl, 120 mg of $CaSO_4{\cdot}2H_2O$, 120 mg of $MgSO_4$, 192 mg of $NaHCO_3$). The conditions of the water were maintained in the following ways: hardness 160~180 mg L^{-1} as $CaCO_3$, alkalinity 110~120 mg L^{-1} as $CaCO_3$, pH 7.6~8.0, and dissolved oxygen (DO) 3.0+ mg L^{-1}. Aerations of culture water were used for DO concentrations above 80%. The humidity was kept at 50%. A cycle of 16 h light/8h dark with a light level of 1,000~2,000 lux was maintained. *D. magna* less than 24 h old were selected to be tested.

Table 1
Composition of artificial water for *Daphnia magna* culture (Korean Water Quality Standard Method)

Composition	Concentration (mg L^{-1})
$NaHCO_3$	192.0
$CaSO_4{\cdot}2H_2O$	120.0
$MgSO_4$	120.0
KCl	8.0

2.1.1.2. Food

The *Pseudokirchneriella subcapitata* algae were used as food for the *D. magna*. The medium for the culture used the F/2 medium culture, and vitamin stock solution was used for algae proliferation. The media used to culture the algae had the following conditions: pH of 7~8, the temperature of 25°C ± 1°C, DO concentration of 3 mg L^{-1}, and light level of about 4,000 lux. The photoperiod was 16 h/d, followed by 8 h of darkness. *Pseudokirchneriella subcapitata* were cultured for 4 d. Once fully cultured, the culture media were distributed into several 1 L bowls and then refrigerated for 4 d. After the algae settled, the supernatants were discarded and the *P. subcapitata* cells were concentrated to feed it to the *D. magna*.

2.1.2. Hard water

The purpose of this experiment was to see how different toxic effects of heavy metals were at different levels of water hardness. The different hardness of water was made using levels of $CaCO_3$ at 100, 200, and 500 mg L^{-1}. The hard water composition is shown in Table 2. The pH and DO levels were maintained similar to that of the culture media.

2.1.3. Heavy metal

The heavy metals used in this experiment are Cu, Zn, and Pb. Each stock solution was made of either $CuSO_4$ (99.99%, Sigma-Aldrich, USA), $ZnSO_4{\cdot}7H_2O$ (Sigma-Aldrich, USA), or $PbSO_4$ (98%, Sigma-Aldrich, USA). All water samples had their EC50 concentrations spiked to be twice that of standard solutions. Table 3 represents heavy metal concentration.

2.1.4. Sample site

The Gyeongan river is a major protected water source in South Korea. The length of the river is 49.5 km long and the area of the basin is 558.2 km^2 large. The basin of the river is relatively high in population density. Five sampling points were selected for this study, and each point is shown in Fig. 1. The water from the river is used for several purposes, including household use, agriculture, and industry. The selected sites were sampled in May of 2016. Three samples were collected from each site. These samples were then mixed to create one sample per site. All samples were collected using aseptic plastic bags. Certain characteristics of water quality (temperature, pH, DO, total dissolved solids (TDS)) were measured using a portable multimeter (YSI Professional Plus). All samples were stored in a refrigerator at 4°C ± 1°C. Table 4 represents sampling locations in this study.

2.2. Methods

2.2.1. Daphnia magna 24 h acute toxicity test

The acute toxicity test was conducted in ordnance with the U.S. EPA's Standard Methods. Heavy metals

Table 2
The hardness of artificial water each concentration for *Daphnia magna* (mg L^{-1})

Composition	100 mg L^{-1} as $CaCO_3$	200 mg L^{-1} as $CaCO_3$	500 mg L^{-1} as $CaCO_3$
$NaHCO_3$	115 mg L^{-1}	230 mg L^{-1}	690 mg L^{-1}
$CaSO_4 \cdot 2H_2O$	70 mg L^{-1}	140 mg L^{-1}	420 mg L^{-1}
$MgSO_4$	70 mg L^{-1}	140 mg L^{-1}	420 mg L^{-1}
KCl	4.8 mg L^{-1}	9.6 mg L^{-1}	28.8 mg L^{-1}

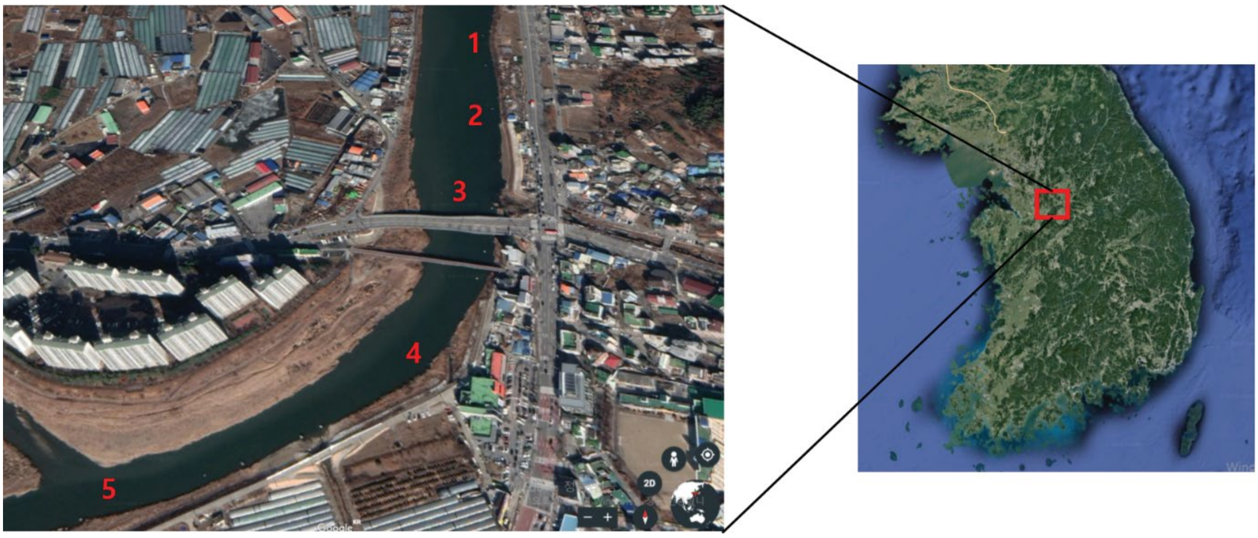

Fig. 1. A map of the Gyeongan river and sampling points with circle and numbers.

Table 3
100% concentration of test heavy metal for *Daphnia magna*

	Copper	Zinc	Lead
EC 50	1.0 mg L^{-1}	23.0 mg L^{-1}	1.0 mg L^{-1}

(Cu, Zn, Pb) were dissolved in the different hardness of water (100, 200, and 500 mg L^{-1} as $CaCO_3$). Serial dilutions were conducted for the samples (100%, 50%, 25%, 12.5%, and 6.25% and control). Each concentration solution was placed in a 50 mL beaker. The culture water was used as the control. Five neonates (less than 24 h old) were exposed to each beaker. After 24 h, the *D. magna* were immobilized. After both 24 h and 48 h, affected neonates were counted and had their EC50 values calculated with ToxCalc 5.0. The conditions of the experiment were kept the same as the conditions of the culture. Before and after every test, the pH and DO level of each water sample was measured. To guarantee the health of the *Daphnia magna*, a weekly QA/QC test was carried out using chromium (VI).

2.2.2. Water quality characteristics

The water quality characteristics required to be temporarily measured (temperature, pH, DO, TDS) were done so using a portable multimeter (YSI Professional Plus).

Table 4
Sampling locations performed in the study

Number	Site name	Latitude	Longitude
1	GA1	37°20′23.51″N	127°14′58,25″E
2	GA2	37°20′19.58″N	127°14′57.88″E
3	GA3	37°20′15.63″N	127°14′55.25″E
4	GA4	37°20′09.12″N	127°14′53.45″E
5	GA5	37°20′04.75″N	127°14′43.44″E

All samples were stored in a refrigerator at a temperature of 4°C ± 1°C refrigerator. The instrument (YSI) was calibrated before sampling.

2.2.3. Water hardness analysis

Water hardness was measured with a modified ethylenediaminetetraaceticacid titrimetric method set forth by the US EPA (19th Edition 1995). A change in color from wine red to blue marked the endpoint of the titration. The volume of the titrant was recorded for use in hardness calculation.

2.2.4. Heavy metal analysis

The heavy metals analyzed in this study were copper, zinc, lead, iron, cadmium, and magnesium. The five samples were stored at 4°C ± 1°C and analyzed with inductively coupled plasma (ICP) atomic emission spectroscopy (Thermo Scientific, iCAP 7000 Series, USA).

2.3. Toxicity calculation

2.3.1. EC50 estimation

The EC50 value showed 50% of *D. magna* was either immobilized or had their reproduction rate decreased by 50%. The dose-response relationship was used to find the EC50 value. Test substance responses were calculated by linear interpolation, and ToxCalc 5.0 estimated the 95% confidence limits of the EC50 values.

2.3.2. NOEC and LOEC

The NOEC classification was for *Daphnia magna* groups that had no swimming inhibitions observed, while the LOEC classification was for groups that were immobilized.

3. Results and discussion

3.1. Acute toxicity test

The Acute toxicity test is a short-period test that assesses the effects of toxic materials on aquatic organisms [10]. The acute toxicity tests of copper, zinc, and lead on *D. magna* were performed at different water hardness concentrations (100, 200 and 500 mg L^{-1} as $CaCO_3$) with bioassay methods to calculate EC50 values and TU. The toxicity of heavy metals was found to be more profound in soft water than in hard water. The ions of heavy metals are more soluble in soft waters and these dissolved ions cause greater harmful effects to organisms that reside in these waters [11,12] demonstrated that an increase in water hardness from 50 to 400 mg L^{-1} as $CaCO_3$ results in a decrease in toxic effects of copper to catfish (*Ictalurus punctatus*). They indicated that as water hardness increased, catfish survival rate rose from 10% at soft water (10 mg L^{-1} as $CaCO_3$) to 95% at very hard water (400 mg L^{-1} as $CaCO_3$). Water hardness can influence the degree of toxicity that heavy metal ions have on aquatic organisms [13]. In this experiment, as water hardness increased, *D. magna* survival rates improved significantly for all heavy metal ions. Heavy metal toxicity in soft water (100 and 200 mg L^{-1} as $CaCO_3$) was found to be much higher than in hard water (500 mg L^{-1} as $CaCO_3$). Water hardness weakens metal toxicity by saturating gill surface binding sites. The calcium and magnesium ions prevent the heavy metal ions from binding to the sites [14–16]. The mobility rates of the *D. magna* for the three water hardness after the after a 24 exposure to copper, zinc, and lead are shown in Figs. 2–4, respectively. The EC50 concentration of each heavy metal increased in accordance with the hardness of the water. Copper was found to be the most toxic of the heavy metals to the *D. magna* while zinc was the least toxic. Toxicity was the lowest for all three ions when in hard water (500 mg L^{-1} as $CaCO_3$). The results of this study conclude that hard water ions compete with dissolved heavy ions to lower their toxic

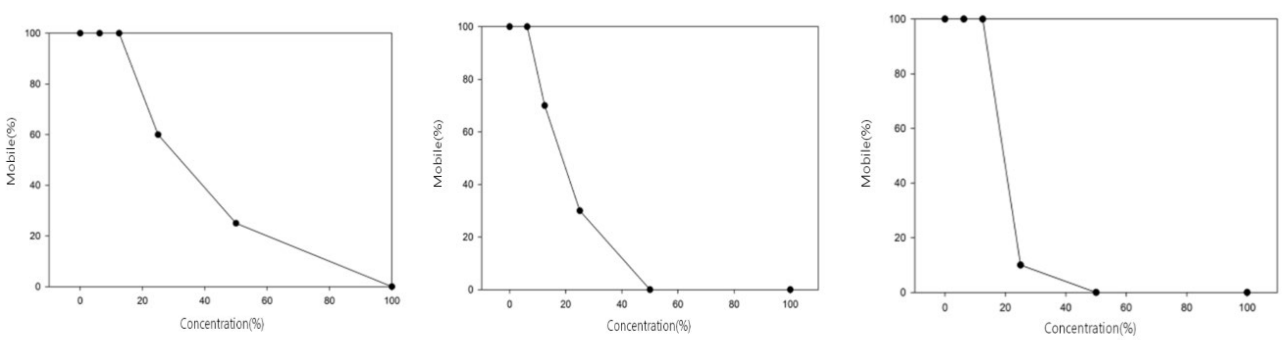

Fig. 2. Percentage mobile of *D. magna* after 24 h exposure to copper at three different water hardness (100, 200, and 500 mg L^{-1} as $CaCO_3$).

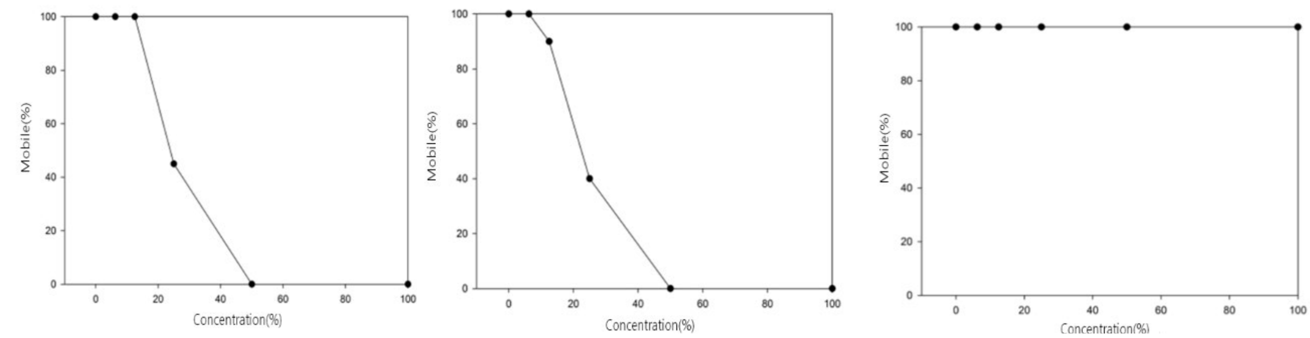

Fig. 3. Percentage mobile of *D. magna* after 24 h exposure to zinc at three different water hardness (100, 200, and 500 mg L^{-1} as $CaCO_3$).

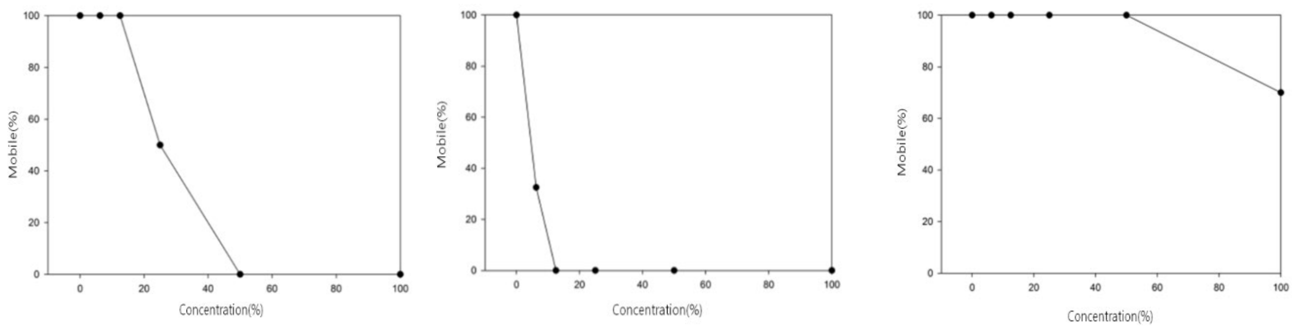

Fig. 4. Percentage mobile of *D. magna* after 24 h exposure to lead at three different water hardness (100, 200, and 500 mg L^{-1} as $CaCO_3$).

Table 5
EC50, TU, NOEC, LOEC value obtained from 24 h-acute immobilization tests of each hardness-heavy metal for *Daphnia magna*

Hardness-metal	EC50 (%)	TU	NOEC (%)	LOEC (%)
100-Cu	20.2	4.95	12.5	25
100-Zn	24.864	4.02	12.5	25
100-Pb	23.9	4.18	12.5	25
200-Cu	23.864	4.19	12.5	25
200-Zn	45	2.22	25	50
200-Pb	54.545	1.83	25	50
500-Cu	40.625	2.46	25	50
500-Zn	100	1	100	100
500-Pb	100	1	50	100

effects. The absorption of calcium and magnesium ions by the cell membrane makes it stable, thus reducing the flow of heavy metal ions into the cell membrane. EC50, TU, NOEC, LOEC values are shown in Table 5.

3.2. Physico-chemical properties and toxicity evaluations of the Gyeongan river

Water samples from the Gyeongan river were collected to study the relationship between water hardness and heavy metal toxicity in natural ecosystems. Five sampling sites were selected. Obtained water samples underwent various physical and chemical analysis experiments to find information on their heavy metal content, water hardness, etc. Toxicity evaluations with *Daphnia magna* were carried out. The *Daphnia magna* survived unchanged in the samples. Therefore, the Gyeongan river was given a TU value of ‘0’. ICP was used to analyze heavy metal content. The results of the ICP are shown in Table 6.

4. Conclusion

Heavy metals of wastewater that contaminate bodies of water can give adverse effects on organisms that rely on them. To test the relationship between the toxicity of heavy metals and the hardness of water, biotoxicity tests on *Daphnia magna* were performed with different heavy metal types and water hardness. The following results were concluded:

- The toxicity of heavy metals decreased as the hardness of water increased.
- The harder the water of a system is, the more ions there are that can compete with and mitigate the harmful effects of heavy metals.
- Based on the results of the present study it is concluded that the toxicity of several heavy metals decreased with increasing water hardness. Acute toxicity tests are important steps in establishing appropriate water-quality criteria and standards. If such tests are to be ecologically relevant, the important sensitive test animals should be used. However, except for daphnids and certain species of fish, little effort was made to do so.
- In the natural environment, Ca and Mg are present at much higher concentrations than the heavy metals. Therefore, by competing with heavy metals and blocking their access to aquatic organisms, Ca and Mg levels are important considerations with respect to the toxic effects of heavy metals upon biota in aquatic systems

Table 6
Analysis of heavy metal materials from Gyeongancheon

Location	Hardness (mg L^{-1} as $CaCO_3$)	Mg (mg L^{-1})	Fe (mg L^{-1})	Zn (mg L^{-1})	Cu (mg L^{-1})	Cd (mg L^{-1})	Pb (mg L^{-1})
37°20′23.51″N 127°14′58,25″E	120	8.13	0.02	0.01	0.01	0.02	0.17
37°20′19.58″N 127°14′57.88″E	120	8.12	0.01	0.01	0.01	0.01	0.17
37°20′15.63″N 127°14′15.25″E	115	8.15	0.03	0.02	0.01	0.01	0.15
37°20′09.12″N 127°14′53.45″E	120	8.13	0.02	0.01	0.02	0.01	0.13
37°20′04.75″N 127°14′43.44″E	117	8.12	0.03	0.02	0.01	0.02	0.15

The Gyeongan river had a water hardness of 120 mg L^{-1} as $CaCO_3$. In a time of severe drought and no introduction of heavy metal substances, the heavy metal toxicity effects would be low.

Acknowledgments

This work was supported by Korea Environment Industry & Technology Institute (KEITI) through Industrial Facilities & Infrastructure Research Program, funded by Korea Ministry of Environment (MOE) (RE201901109 and Hankuk University of foreign studies research fund of 2019.

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