Ecotoxicology and Environmental Safety 162 (2018) 63–70

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Comparative study of the sensitivity of *Daphnia galeata* and *Daphnia magna* to heavy metals

Rongxue Cui[1], Jin Il Kwak[1], Youn-Joo An*

Department of Environmental Health Science, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea

ARTICLE INFO

Keywords:
Species entrapment
Daphnia galeata
Daphnia magna
Saponin
Heavy metal pollution
Aquatic ecotoxicology

ABSTRACT

Daphnia galeata and *Daphnia magna* belong to the family Daphniidae. *Daphnia galeata* has a smaller body size and longer helmet than *D. magna*. Although *D. galeata* is widely distributed in the northern hemisphere, it is not as commonly used in aquatic ecotoxicity tests as *D. magna*. There have been only few ecotoxicological studies on the toxicity of heavy metals, organic matter, and nanomaterials in *D. galeata*. Thus, there is a need to discover new test species and expand the number of currently known test species to elucidate species sensitivity to aquatic pollutants. We carried out a comparative study on the sensitivity of *D. magna* (which represents the test water flea species) and *D. galeata* to heavy metal toxicity. The acute toxicity values (EC50 and LC50) of 11 heavy metal species, including silver (Ag^{+}), arsenite (As^{3+}), cadmium (Cd^{2+}), chromate (Cr^{6+}), cupric (Cu^{2+}), ferrous (Fe^{2+}), mercury (Hg^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), lead (Pb^{2+}), and zinc (Zn^{2+}), in *D. galeata* and *D. magna* were compared by conducting acute toxicity assays and comparing the data with the available data. The age of the tested *Daphnia* individuals and the type of exposure medium were considered for more reliable comparison of species sensitivity. We observed that *D. galeata* was more sensitive to Ag^{+}, As^{3+}, Cr^{6+}, Fe^{2+}, Ni^{2+}, and Pb^{2+} than *D. magna*. The sensitivity to Cu^{2+}, Cd^{2+}, and Zn^{2+} was similar for *D. magna* and *D. galeata*. This study presents important aquatic toxicity and sensitivity data on *D. galeata*, which is not a widely used species in aquatic ecotoxicology studies. Our results recommend *D. galeata* as a suitable species for aquatic ecotoxicity tests because of its higher sensitivity.

1. Introduction

Daphnia galeata and *Daphnia magna*, which belong to the family Daphniidae, are pelagic daphinds that are widely distributed in the northern hemisphere (Petrusek, 2013). *Daphnia galeata* is smaller (adult size 1.0–2.5 mm) than *D. magna* (adult size 2.0–3.0 mm) (Cui et al., 2016; Koivisto et al., 1992). The size of neonates and adults of *D. galeata* is also smaller than those of *D. magna* (Fig. 1). As one of the representative aquatic invertebrates, water fleas play an important role in the food chain as a primary consumer in the aquatic ecosystem. Therefore, water fleas have been widely used for ecotoxicity assessment, and *D. magna* has been recommended as a test species in the OECD (OECD, 2012, 2004), US EPA (USEPA, 2002), and ISO (ISO, 2012) test guidelines. Compared with *D. magna*, *D. galeata* is not a widely used species in aquatic ecotoxicology studies and has not been studied extensively. There have been some studies on the toxicity of heavy metals (Bossuyt et al., 2005, 2004; Cui et al., 2017a, 2017b; De Schamphelaere et al., 2007; Koivisto et al., 1992; Marshall, 1979; Muyssen et al., 2005; NIER, 2001; Piscia et al., 2014; Ponti et al., 2010; Song et al., 2015; Vesela and Vijverberg, 2007; Völker et al., 2013; USEPA, 1976), organic chemicals (Day and Kaushik, 1987a, 1987b, 1987c; Liber et al., 1992; Liber and Solomon, 1994; Mano et al., 2010; Schroer et al., 2004; Shurin and Dodson, 1997; Stephenson et al., 1991; Tanaka and Nakanishi, 2001, 2002; van Wijngaarden et al., 2010), and nanomaterials (Cui et al., 2017a; Song et al., 2015; Völker et al., 2013) in *D. galeata*.

Because of the occasional entrapment of *D. galeata* in the water surface film (Cui et al., 2017b), it is difficult to study some water flea species belonging to the genera *Daphnia* and *Bosmina*. Therefore, in our previous study (Cui et al., 2017b), we proposed the use of natural surfactants (saponin) to prevent the entrapment of *D. galeata* and confirmed the applicability of saponin in ecotoxicity testing. Saponin used in the present study was a natural surfactant extracted from *Quillaja* bark. It is known that surfactants are amphiphilic compounds, which can reduce surface tension and increase solubility and interfacial tension (Mulligan et al., 2001). Furthermore, it has been reported that saponin enhances the desorption of metals from soils (Cao et al., 2013; Gusiatin and Klimiuk, 2012).

* Corresponding author.
E-mail address: anyjoo@konkuk.ac.kr (Y.-J. An).
[1] Equal contribution to this work.

https://doi.org/10.1016/j.ecoenv.2018.06.054
Received 5 March 2018; Received in revised form 10 June 2018; Accepted 18 June 2018
0147-6513/

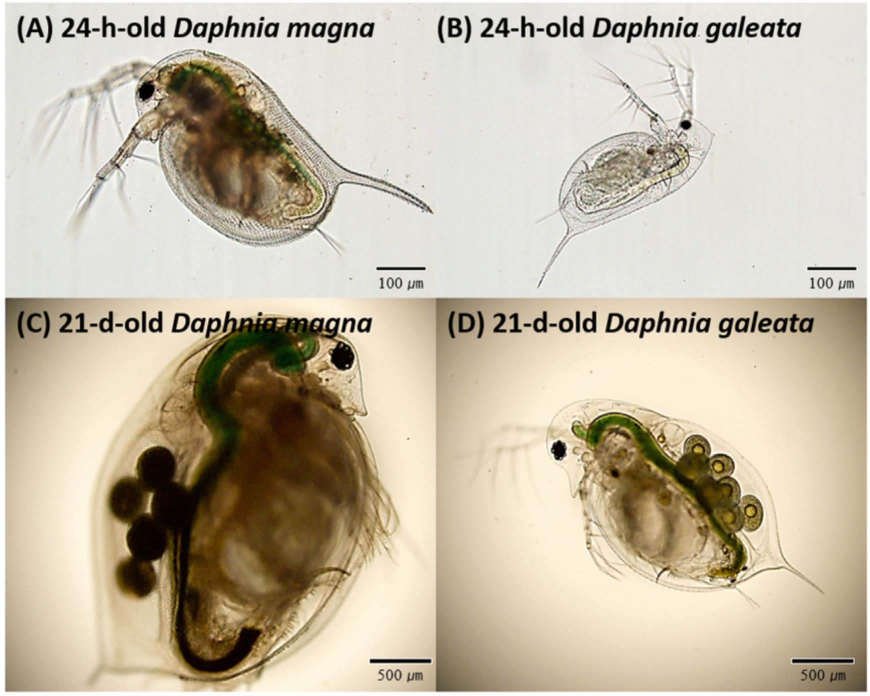

Fig. 1. Microscopy images of (A) 24-h-old *Daphnia magna*, (B) 24-h-old *Daphnia galeata*, (C) 21-d-old *D. magna,* and (D) 21-d-old *D. galeata*.

The objective of this study was to compare the sensitivity of *D. galeata* and *D. magna* to toxic chemicals. Eleven heavy metals, including silver (Ag^{+}), arsenic (III) (As^{3+}), cadmium (Cd^{2+}), chromium (VI) (Cr^{6+}), copper (Cu^{2+}), iron (Fe^{2+}), mercury (Hg^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), lead (Pb^{2+}), and zinc (Zn^{2+}), were selected as model species. Heavy metals have been widely used in our daily life and are released into the environment through various routes (Boyd, 2010). Currently, there are many countries that have water quality standard or guidance for heavy metals, such as USA (USEPA, 2018), European Unions (EC, 2008), and Canada (CCME, 2003). In this study, the sensitivity of *D. galeata* and *D. magna* to these chemicals was compared by performing acute assay and comparing the data with the available data. For an accurate comparison, we considered (1) the age of the tested *Daphnia* and (2) the type of test media. We performed acute assay with > 24-h-old *D. galeata* neonates in a moderately hard water (MHW) medium.

2. Materials and methods

2.1. Test species and chemicals

Daphnia galeata individuals were collected from Ilgam Lake (Seoul, South Korea) and maintained in modified MHW (USEPA, 2002) (21 °C, 16-h light:8-h dark period). The neonates (< 24- h old) were used in the 48-h acute assay according to the OECD test guideline (OECD, 2004). For the comparison of sensitivity to heavy metals, 11 heavy metals (Ag^{+}, As^{3+}, Cd^{2+}, Cr^{6+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) were selected as test chemicals. Five heavy metals (As^{3+}, Cd^{2+}, Fe^{2+}, Mn^{2+}, and Pb^{2+}) were tested in this study. $CdCl_2$ (99%, Sigma Aldrich, USA), As_2O_3 (Samchun Pure Chemical, South Korea), $FeSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$ (Junsei Chemical Co., Ltd., Japan), and Pb $(NO_3)_2$ (Kanto Chemical Co., Inc., Japan) were purchased from the respective companies. The data pertaining to other heavy metals (Ag^{+}, Cr^{6+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, As^{3+}, and Cd^{2+}) were obtained from our previous studies (Cui et al., 2017b, 2017a). $AgNO_3$, $K_2Cr_2O_7$, $CuCl_2{\cdot}2H_2O$, $HgCl_2$ $NiCl_2$, and $ZnCl_2$ were procured from Sigma Aldrich (USA). As^{3+} and Cd^{2+} with different salts and purity levels were tested (Cui et al., 2017b), using $NaAsO_2$ (Fluka Chemical Corp, USA) and $CdCl_2$ (99.9%, Sigma Aldrich, USA). In addition, *Quillaja saponaria* saponin (*Quillaja* saponin) (Sigma Aldrich, USA) was used in this study to prevent the surface film entrapment of *D. galeata*.

2.2. Acute assay for Daphnia galeata

The acute assay with five heavy metals (As^{3+}, Cd^{2+}, Fe^{2+}, Mn^{2+}, and Pb^{2+}) for *D. galeata* was based on the OECD TG 202 method (OECD, 2004). In the present study, modified MHW (USEPA, 2002) was used to prevent surface film entrapment (Cui et al., 2017b). Based on the results of a previous study, the test concentration of each heavy metal was prepared using 1 mg/L saponin in MHW (Cui et al., 2017b). The test concentrations were 0.6–3 mg/L for As^{3+}, 0.01–0.2 mg/L for Cd^{2+}, 0.1–1 mg/L for Pb^{2+}, 10–60 mg/L for Mn^{2+}, and 20–40 mg/L for Fe^{2+}. With Pb^{2+} and Fe^{2+}, we found white and reddish-brown precipitation, respectively, in the MHW. Therefore, Pb^{2+} test solutions were prepared using MHW without $NaHCO_3$. Fe^{2+} test solutions were prepared using MHW and passed through a 0.45-μm syringe membrane filter after 24 h. The filtered solution containing dissolved Fe^{2+}, not solid suspension of Fe^{2+}, was used for the acute assay of Fe^{2+} and also analyzed by the ICP-AES (Horiba, Ultima Expert, Fe detection limit = 0.005 mg/L)

Ten milliliters of each test solution were added into 35-mL glass vials and five individuals were placed in each vial. After 24 and 48 h exposure at 21 °C, 16 h light and 8 h dark period, with 6–12 replicates, the entrapment-related immobilization (EI), entrapment-related mortality (EM), toxicity-related immobilization (TI), and toxicity-related mortality (TM) were evaluated. The EI and EM can be determined with the live or dead organisms trapped in the surface film, respectively. Although we used 1 mg/L saponin to prevent the entrapment of *D. galeata*, according to our previous study (Cui et al., 2017b), 3 ± 7% and 1 ± 5% of negligible entrapment effects were observed at 24 and 48 h with 1 mg/L saponin, respectively. In addition, high concentrations of toxic compounds can also cause entrapment phenomenon (Jonsson and Baun, 2003). Therefore, we also measured entrapment-related effects at 24 and 48 h. The TI and TM can be determined with live or dead organisms in the test medium.