INTRODUCTION

Aquatic ecosystems are the final sink for all potentially toxic chemicals in the environment via transfer from natural and/or anthropogenic sources. The increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity [1].

Cladoceran species are important aquatic organisms because they transfer energy and materials from primary producers to higher trophic feeders, such as fish. At the same time, they are also one of the most sensitive species to toxic chemicals [2]. Life table demographic responses of cladocerans are used for the assessment of water quality, including metal toxicity [3]. Most ecotoxicological works have considered continuous exposure of the test species to toxicants [4]. However, since the release of industrial effluents into natural waterbodies is pulsed and subsequently diluted, their impact on aquatic organisms remains mostly unknown [5]. Consequently, zooplankton that predominate in these altered systems, are in a constant physiological challenge as well as subjected to different levels of stresses during their life cycle [6, 7]. While Daphnia, mainly D. magna has received considerable importance as a test organism in ecotoxicological evaluations [8], in terms of sensitivity, Ceriodaphnia is comparable to daphnids [9].

Ceriodaphnia reticulata, littoral and limnetic, usually nearshore or in the warmer upper layers of water. Reproduction during most of the year is parthenogenetic, but when environmental conditions became unfavorable, males are produced and sexual reproduction occurs in C. reticulata, widespread usage in aquatic eco-toxicity laboratories.

The sensitivity of C. reticulata to toxic chemicals may differ from that of temperate Daphnia species. Investigating the impact of heavy metals on Ceriodaphnia species may be important for understanding the impact of toxic chemicals on the summer-temperate community with dominance of non-Daphnia species. I carried out acute toxicity tests of three heavy metals, copper (Cu), zinc (Zn), and cadmium (Cd), to examine how these metals affect the reproduction of C. reticulata and C. reticulata, widespread usage in aquatic eco-toxicity laboratories.
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Despite the important role of cladoceran species as the summer plankton in the temperate zone and a common species in tropical regions, few studies have investigated the sensitivity of cladoceran species to toxic chemicals. The sensitivity of *C. reticulata* to toxic chemicals may differ from that of temperate *Daphnia* species. Investigating the impact of heavy metals on *Ceriodaphnia* species may be important for understanding the impact of toxic chemicals on the summer-temperate community with dominance of non-*Daphnia* species. I carried out acute toxicity tests of three heavy metals, copper (Cu), zinc (Zn), and cadmium (Cd), to examine how these metals affect the survival of *C. reticulata*.

**MATERIALS AND METHODS**

The test species *C. reticulata* was originally isolated from the Hazar Lake (Elazığ/Turkey) and was cultured using the single celled green alga *Chlorella vulgaris*. The medium and food were replaced daily in the stock culture, with food being added at a concentration of approximately 0.3x10⁹ cells/mL. The stock culture was kept under constant laboratory conditions (23±1 °C, 16:8 h light:dark) for at least 1 month before the start of the bioassays. Bioassays were conducted for Cu, Zn, and Cd using the following test substances; CdCl₂·2½H₂O (CAS No. 10108642), CuSO₄·5H₂O (CAS No.7758998), and ZnSO₄·7H₂O (CAS No.773020). A stock solution of each chemical (5.000 mg/L) was prepared by dissolving 50 mg of the chemical in aged tap water to a final volume 10 mL. The test were conducted under the same laboratory conditions as those for the stock cultures (23±1 °C, 16:8 h light:dark) for at least 1 month before the start of the bioassays. Control and exposure treatments were prepared for each metal by diluting each stock solution of the chemical with aged tap water. Concentrations for Cu exposure were 60, 80, 105, 135, 178, and 230.5 µg/L; for Zn, 7, 10, 14, 18.5, 25.5, and 38 µg/L; for Zn, 95, 135.5, 190, 280, and 360.5 µg/L. Two glass beakers containing 30 mL of each solution were prepared for each treatment and the control concentrations for each metal (40 beakers in total). Five neonates or adult females of *Ceriodaphnia reticulata* were introduced into each beaker. Test individuals were not fed during the assays. Based on results of the tests, we determined 24- and 48- h median lethal concentration (LC₅₀) values with 95% confidence intervals (CI) using the Probit method.

**RESULTS AND DISCUSSION**

Acute toxicity tests indicated that lead at higher concentrations had a detrimental effect on the survival of *C. reticulata*. In this study, determined the impacts of Cu, Zn, and Cd *C. reticulata* survival (Table 1). The LC₅₀ values of Cu and Zn for *C. reticulata* were lower than those for *Daphnia magna*, and *Leptodora kindtii* one of the global Standard test organisms for toxic chemicals because it is relatively more sensitive to *C. reticulata* is more strongly influenced by Zn, and Cu than is *D. magna* and *L. kindtii*. In contrast, the LC₅₀ value of Cd for *C. reticulata* was more than or nearly comparable with that of *D. magna* and *L. kindtii* (Table 1), indicating that *C. reticulata* is less sensitive to Cd than is *D. magna* and *L. kindtii*.

There have been no reports addressing the acute toxicity of heavy metals to *C. reticulata*. These results clearly demonstrated that *C. reticulata* was more vulnerable to Cu and Zn from heavy metals than was *D. magna* and *L. kindtii* (Table 1). In *D. magna* and *L. kindtii*, the LC₅₀ values for Cu (120 µg/L [12]; 19.69 µg/L [13]) were six times higher than that in *C. reticulata* (19.7 µg/L) (Table 1). Similarly, in *D. magna* and *L. kindtii* the LC₅₀ values for Zn (1800 µg/L [14], 2332 µg/L [13]) were seven and nine times higher than that in *C. reticulata* (258.5 µg/L) (Table 1). Results indicate that than in *C. reticulata* is more strongly influenced by Zn and Cu than *D. magna* and *L. kindtii*. In contrast, the LC₅₀ values of Cd for *C. reticulata* was more than or nearly comparable with that of *D. magna* and *L. kindtii* (Table 1), indicating that *C. reticulata* is less sensitive to Cd than is *D. magna* and *L. kindtii*.

The main intake route of the soluble toxicants is through contact with body surface, and thus large cladocerans (subsequent lower –to- ratio) tent to be more tolerant to the chemicals than the small enes [15].

Freshwater zooplankton community is composed of cladocerans, copepods, and rotifers. Species sensitivity distributions of cladocerans and copepods to Cd, Cu, and Zn have been reported (Wong et al, 2009). The 5% hazardous concentrations (HC5) of cladocerans are 8.16 µg/L for Cd, 2.72 µg/L for Cu, and 30.8 µg/L for Zn. The HCS copepods are 19.6 µg/L for Cd, 23.4 µg/L for Cu, and 32.0 µg/L for Zn. These indicate that *C. reticulata* may show moderate sensitivities to Cd, Cu, and Zn in cladocerans species, but *C. reticulata* has a slightly lower sensitivity to Cu than copepod species. If *C. reticulata* is a prominent species in a zooplankton community, relatively low concentrations of Cu may affect the zooplankton community and functions such as the energy and material flow to higher trophic organisms due to the decrease in *C. reticulata*.

Although Zn is an essential heavy metal, it is required only in low concentrations for zooplankton. Thus, the cuticular structure and life span of cladocerans are affected if Zn is not available in sufficient quantity. For example, When the availability of Zn is < 25 µg per daphnid, reduced survival and reproduction has been reported for *D. magna* and *D. pulex* [16]. However, Zn is also toxic to a variety of aquatic organisms including both phyto- and zooplankton [17].

Freshwater zooplankton community is composed of cladocerans, copepods, and rotifers. Species sensitivity distributions of cladocerans and copepods to Cd, Cu,
and Zn have been reported [18]. The 55% hazardous concentrations (HC5) of cladocerans are 8.16 μg/L for Cd, 2.72 μg/L for Cu, and 30.8 μg/L for Zn. The HC5 of copepods are 19.6 μg/L for Cd, 23.4 μg/L for Cu, and 32.0 μg/L for Zn. These indicate that C. reticulata may show moderate sensitivities to Cd, Cu, and Zn in cladoceran species.

The presence of heavy metals has been reported in natural lakes. For example, Zn, Cu, and Cd in water of Lake Hazar, Demirköprü Dam Lake, and Avşar Lake [19, 20]. In Demirköprü Dam Lake, the concentration of Cu (0.02 mg/L) was higher than in other in Turkey, such as Hazar Lake, Avşar Lake, but was lower than the LC50 value for C. reticulata as were the concentrations of Cd (0.7 – 1 μg/L) and Zn (25 – 98 μg/L). The C. reticulata population may be affected not only by acute effects of heavy metals on individual mortality but also by chronic effects on the demography. Although in this study, I could not examine chronic effects of heavy metals on Diaphanosoma population, I could estimate roughly the chronic effects on Diaphanosoma population by dividing LC50 values obtained in my study by acute –to- chronic ratio [21, 22].

When the acute –to- chronic ratio was assumed to be a factor of 10 that is used in risk assessment [21], the estimated no –observed- effect concentrations (NOECs) of Cd, Cu, and Zn were 6.98, 1.04, and 17.41 μg/L, respectively. The estimated NOEC value of Cu was lower than the concentration in Hazar Lake, and Avşar Lake.

Table 1. Acute toxicities (24- and 48-h lethal concentration (LC50) values, μg/L) and 95% confidence intervals (CI) of cadmium (Cd), copper (Cu), and zinc (Zn) for C. reticulata, with values included for D. magna and L. kindtii as a reference species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Metal</th>
<th>24-h LC50 (95% CI)</th>
<th>48-h LC50 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. reticulata</td>
<td>Cd</td>
<td>183.2 (150.4-410.7)</td>
<td>82.1 (51-120.8)</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>18.7 (14.3-22.4)</td>
<td>12.7 (6.3-13.8)</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>258.5 (122.4-331.6)</td>
<td>182.1 (87.4-310.6)</td>
</tr>
<tr>
<td>D. magna</td>
<td>Cd</td>
<td>92°</td>
<td>43°</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>120°</td>
<td>24.93°</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>1800°</td>
<td>690°</td>
</tr>
<tr>
<td>L. kindtii</td>
<td>Cd</td>
<td>20.14f</td>
<td>Not determined</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>19.69f</td>
<td>Not determined</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>2332f</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Note: aLewis & Weber [24], bMilam CD et al. [12], cMcWilliam and Baird [25], dCairns et al. [14], eKhangarot et al. [23], fSakamoto & Ogmino [13]

CONCLUSION

C. reticulata population may be affected by a relatively lower concentration of Cu in temperate lakes. Further studies will be needed to evaluate the chronic effect of Cu on the C. reticulata population.

REFERENCES

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