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Effects of copper on the survival, hatching, and reproduction of a pulmonate snail (*Physa acuta*)

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HIGHLIGHTS

- *Physa acuta* is sensitive to copper (Cu) exposure with a 96 h LC_{50} of 23.8 μg L^{-1}.
- Cu exposure causes delay in embryonic development, deformity and lesion of embryos.
- Snails exposed at 12.5 and 25 μg L^{-1} Cu produce polynuclear eggs in one egg capsule.
- Cu exhibits potential genetic toxicity effect from multigenerational exposure.

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ABSTRACT

Acute and chronic bioassays provide essential basis for establishment of environmental quality standards. The effects of Cu on a pulmonate snail, *Physa acuta*, were investigated at a number of sublethal and lethal endpoints. Cu exposure suppressed movement and triggered an escape response in *P. acuta* at low and high concentrations, respectively, exerting acute toxic effects on adult snails exposed to a 96 h LC_{50} of 23.8 μg L^{-1}. Following 16 d exposure of Cu to the egg masses, successful hatching decreased with increasing Cu concentration. High Cu concentrations (12.5 and 25 μg L^{-1}) resulted in inhibition of eye and shell development at the veliger stage, and a deformed shell, abnormal eyes, and different morphological shapes with lesions and hemorrhages were observed after 9 days of exposure. A large number of eggs exposed to 2.5–25 μg L^{-1} Cu remained in the veliger and hippo stages for 2–7 days, with no further development. Results from reproduction tests showed that adult snails exposed to various Cu treatments produced more than three broods, with the total number of eggs ranging from 770 to 1,289, revealing little difference between the control and Cu-treated groups ($p > 0.05$). However, snails exposed to 12.5 and 25 μg L^{-1} Cu produced polynuclear eggs in one egg capsule. The hatching success rate and shell length of the filial generation were significantly reduced in a dose-dependent manner ($p < 0.05$). The shell length of newly hatched snails was shorter in the reproduction test than in the hatching test, indicating inherent Cu toxicity in the filial generation from the exposed parent strain. The present study provides essential data regarding Cu toxicity in pulmonate snail *P. acuta*.

1. Introduction

Copper (Cu) is not only an important nutrient for human health but also an essential transition metal ion for organisms due to its vital function in mitochondrial enzyme activity (Uriu-Adams and Keen, 2005; Mehta et al., 2006). In natural water bodies with minimal pollution, Cu concentrations are usually low, ranging only from about <0.1 to 4 μg L^{-1} (Gaillardet et al., 2007; Wilbers et al., 2014; Mebane et al., 2015). Gaillardet et al. (2007) estimated a global average for large rivers of about 1.5 μg L^{-1}, excluding extensively urbanized or industrialized basins. Copper concentrations in some large rivers and estuaries in China may also be similarly low (Zhang, 1995). However, in water bodies affected by industrial or urban discharges, Cu concentrations may be much higher, reaching at least 90 μg L^{-1} (Wong et al., 2007; Gao et al., 2015). In extremely polluted areas influenced by mine drainage, copper concentrations in streams may be much higher, reaching mg L^{-1} levels, with an

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extreme value of 37,620 μg L^{-1} being reported from the Odiel River, Spain (Olias et al., 2004; Mebane et al., 2015). Therefore, water pollution caused by Cu has attracted growing concerns due to its toxicity and ecological risk to aquatic organisms (Xia et al., 2012).

Many studies on the toxic effects of Cu have been conducted on aquatic organisms, including crustaceans, mollusks, invertebrates, and vertebrates, revealing different levels of sensitivity to Cu among aquatic organisms (Flemming and Trevors, 1989; Hsieh et al., 2004; Kwok et al., 2008; Cooper et al., 2009). Freshwater snails, as a typical representative of gastropods, may experience more stress when exposed to accidental pollution in ambient water, because they are less mobile than many other aquatic organisms, especially vertebrates (e.g., fish), which can move fast to escape the pollution area. Furthermore, widespread concern has been raised regarding gastropods, because they can accumulate substantial levels of trace metals during growth as a result of their high Ca requirements, resulting in a higher metal concentration in soft tissues than in ambient water (Ray, 1984; Ng et al., 2011). In long-term community exposures to Cu, pulmonate snails such as *Lymnaea* spp. and *Physa* sp. were the most sensitive invertebrates (Joachim et al., 2017). However, non-pulmonate snails may be even more sensitive to copper than *Physa* sp. (Besser et al., 2016). Pyatt et al. (2003) found that the freshwater snail *Lymnaea peregra* accumulated marked levels of Cu from the aquatic environment, with low Cu concentrations of 4 μg L^{-1}. However, under such exposure conditions, Cu has been shown to cause mortality in the gastropods *Pomacea paludosa* and *Lymnaea stagnalis* (Rogevich et al., 2008; Brix et al., 2011). Physiological abnormalities were found after an extended exposure time; for example, *Lymnaea luteola* L. exposed to 56 μg L^{-1} Cu for 7 weeks stopped feeding activity, leading to a significant decrease in reproductive capacity, and sublethal effects in terms of egg mass abnormalities were observed after exposure to 10 μg L^{-1} Cu (Khangarot and Das, 2010; Das and Khangarot, 2011). Gastropod exposure to Cu assessed at different endpoints corresponding to various life stages has provided baseline data for assessing the toxic effects of Cu on ecosystem health.

The pulmonate snail, *Physa acuta*, is regarded to be an early invader in Europe and is found in many regions of Asia, Australia, and South Africa (Appleton, 2003; Beckmann et al., 2006; Zalizniak et al., 2007a) due to its high adaptability to different environmental characteristics and a high reproductive capacity (Kefford and Nugegoda, 2005). Hence, a worldwide distribution of *P. acuta* could be achieved in the future, becoming a useful bioindicator of toxicants. In previous studies, *P. acuta* or its egg masses have been exposed to ionic solutions of different strengths (Kefford and Nugegoda, 2005; Zalizniak et al., 2007a, b), cadmium (Cd) (Piccinni et al., 1985; Cheung and Lam, 1998), organic compounds (Sanchez-Arguello et al., 2009; Ma et al., 2014), nanomaterials (Musee et al., 2010), and ionic liquids (Bernot et al., 2005; Li et al., 2014) in ambient water and the effects evaluated at various lethal, biochemical, developmental, and reproductive endpoints. However, little information regarding the toxic effects of Cu on movement, development, reproduction, and multigenerational exposure of *P. acuta* to Cu has been reported. To supplement essential data regarding Cu toxicity in gastropods, this study aimed to (1) assess the sensitivity of *P. acuta* to Cu at various endpoints and (2) to investigate the adverse impacts of multigenerational Cu exposure on populations of *P. acuta*.

2. Materials and methods

2.1. Snail culture

The *P. acuta* specimens used in this study were collected from creeks in Adelaide Hills, Australia and were acclimated in an in-house static culture. Approximately 150 adult snails were cultured in 15 L aquariums containing at least 10 L modified FETAX solution (MFS) (75 mg $MgSO_4 \cdot 7H_2O$, 96 mg $NaHCO_3$, 15 mg $CaCl_2 \cdot 2H_2O$, 400 mg NaCl, 30 mg KCl, and 60 mg $CaSO_4$ per liter deionized water), with sufficient aeration, a constant temperature of 21 ± 0.5 °C, and a photoperiod regime of 16 h light: 8 h dark. Snails were fed algae wafers (Hikari, Hayward, CA, USA) once a week after renewal of the culture medium.

2.2. Acute exposure of adult snails to Cu

Acute Cu exposure was evaluated over a 96 h period in adult snails, with a shell length of 11.6 ± 1.54 mm and dry weight of 65.8 ± 19.0 mg. The adult snails were fasted for 2 days before exposure to avoid Cu loss due to absorption by feces. The test was conducted under semi-static conditions without a food supply and aeration. The test solution was aerated and renewed on day 2 (48 h). Following a range-finding test, six concentrations of Cu (0, 5, 10, 20, 40, and 80 μg L^{-1}) were prepared from a stock solution (50 mg L^{-1} Cu as $CuSO_4 \cdot 5H_2O$) by diluting with the MFS to 600 mL in a pre-cleaned glass jar (1 L) and then left for 6 h to equilibrate. MFS was used as a negative control. Five snails were randomly collected and placed into a sealed polyethylene cage that was submerged in the solution to prevent the snails from escaping as a result of Cu stress. The test solutions were measured before and after renewal for pH, electrical conductivity (EC), and dissolved oxygen (DO), and approximately 10 mL of the water sample were filtered through a 0.45 μm syringe filter and stored at 4 °C prior to measurement of the Cu concentration by inductively coupled plasma-mass spectrometry (Agilent 7700, Santa Clara, CA, USA). All measurements were performed in quadruplicate at 25 ± 0.5 °C. Survival was evaluated at 24, 48, 72, and 96 h by gently touching the snail with a soft probe. Death was defined as a lack of response to stimulation, and dead snails were immediately removed from the jar.

2.3. Embryo toxicity test

Fifty adult snails reaching sexual maturity were collected from the aquarium cultures, and equal numbers were placed into 10 beakers (1 L) containing 900 mL MFS, a food supply, and sufficient aeration. Generally, snails produce egg masses (egg jelly) after acclimation for 1 or 2 days under "new" culture conditions. Then, 24-h-old egg masses, typically containing 30–100 egg capsules, were gently collected from the back of shells or the container walls. Eighteen egg masses with similar size were collected and placed into 18 polyethylene cups containing approximately 30 mL of the testing solution, where they sank to the bottom. The embryos were exposed to a range of Cu concentrations (0, 1, 2.5, 5, 12.5 and 25 μg L^{-1}) in triplicate under conditions identical to those described for the acute test. The exposure period was 16 days, and the test solutions were renewed every 2 days. Water samples were collected before and after each renewal for measurements of pH, EC, DO, and Cu concentration. Observations were made at various developmental stages of the egg embryos (morula, trochophore, veliger, and hippo stages at days 2, 5, 7, 9, and 12–16) using an inverted microscope (Olympus SZX9, Tokyo, Japan). Normal or abnormal development of embryogenesis at different stages was recorded and photographed. Coagulation of embryos (e.g., a whitish cloud) with no capsule rotation was defined as death, and both normal and abnormal egg embryonic development was regarded as survival. During the exposure period, the numbers of hatched snails per treatment were counted. The shell length of hatched snails was also determined using the inverted microscope.