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Evaluation onto life cycle parameters of *Ceriodaphnia silvestrii* submitted to 36 days dietary copper exposure

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ABSTRACT

The present study aimed to investigate the response of several life history parameters (body length and age of primipara, duration of embryonic development, maximum body length, reproduction and survival) of the zooplankton *Ceriodaphnia silvestrii* while exposed to copper contaminated algae *Pseudokirchneriella subcapitata*. In order to evaluate chronic exposure on the animal's life history, long-term experimental design was used. Cladocerans were fed with a dietary copper concentration ranging from 3 to 68 fg Cu cell^{-1}. Low waterborne copper exposure (around 10^{-10} mol L^{-1} free Cu^{2+} ions) was kept in the experiments. The results showed that by exposure of cladocerans during 7 days to contaminated food with 68 fg Cu cell^{-1}, a significant reduction in neonate production, survival and body size were obtained. Inhibition on egg production of zooplankton at 38 fg Cu cell^{-1} were observed in 36 days chronic dietary copper exposure. The importance of entire life cycle study to better evaluate cladoceran responses to chronic dietary metal exposure was demonstrated.

1. Introduction

The release of metals into aquatic systems as a result of industrial processes has led to increasing concern about the effects of toxic metals in the environment. In aquatic environments, organisms are not exposed to high, acute toxic concentrations of metals, but usually to low concentrations for longer periods. This is characterized as chronic exposure of which organisms response are not fully understood. The majority of standardized chronic toxicity tests incorporate only a fraction of the test organism's life cycle. However, organisms in natural ecosystem may be exposed to metals at various stages of their life cycle or exposure may persist over their entire life cycle (Preston and Snell, 2001). In standardized chronic toxicity tests for aquatic invertebrates, survival and reproduction are the endpoints most often observed, however, analysis of these parameters alone is not enough for assessing the full ecological impacts of toxicants. Life history characteristics should be applied in ecotoxicological studies to better evaluate the impacts of metal pollution (Hanazato, 2001).

Traditionally, metal toxicity to aquatic organisms has been assigned to waterborne exposure via the dissolved water phase, whereas possible metal contributions in the particulate phase have been neglected. There is growing evidence that metals associated with food, referred as dietborne metals, may be readily assimilated by organisms and cause toxic effects (Balcaen et al., 2008). Some studies have demonstrated the importance of dietary uptake in metal accumulation and its resulting toxicity in freshwater animals. Guan and Wang (2004) showed that juveniles of *Daphnia magna* accumulated Cd and Zn feeding on algal cells exposed to metals. De Schamphelaere et al. (2004) found that Zn in algal cells inhibits reproduction of *Daphnia magna* with no effects on survival, growth or feeding rate. Geffard et al. (2008), using survival and reproduction rate of *D. magna* as toxic endpoints showed that daphnids exposed to waterborne Cd (50 μg L^{-1}) and dietary Cd (62 g Cd g^{-1} dry weight of the freshwater algae *Pseudokirchneriella subcapitata*) had inhibition on the number of newborns produced and no mortality was observed. According to the authors further research is needed for a better assessment of the importance of these two metal exposure routes to zooplankton. Recently, Rodgher et al. (2008) observed some modifications in life cycle parameters of the tropical cladoceran *Ceriodaphnia silvestrii* fed with 68 fg Cu cell^{-1} in partial chronic tests, but no effect on animals survival at 40 fg Cu cell^{-1} nor to the number of eggs produced per female at 38 fg Cu cell^{-1} were detected. These studies were based on 7 and 21 days cladoceran reproduction tests and allowed acquisition of partial information about possible processes that may be involved in the animals' response to dietary metal exposure.

Zooplanktonic organisms are sensitive bioindicators and have been widely used in freshwater ecotoxicology studies. Standard methods using *D. magna*, *Daphnia pulex* and *Ceriodaphnia dubia*

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has been recommended by major international organizations (USEPA, 1994; ASTM, 1992). However, there have been criticisms that the organisms accepted by regulation agencies and accepted protocols do not always reflect local taxa or site-specific conditions (Harmon et al., 2003). From the viewpoint of ecotoxicity testing, it is more desirable to use locally available organisms than non-indigenous ones.

The aim of this study was to evaluate the effects of Cu-contaminated microalgae *P. subcapitata* on life history parameters of the cladoceran *C. silvestrii* during its complete life cycle (36 days). *C. silvestrii* is a common neotropical cladoceran species (El-Moor-Loureiro, 1997) and it has been recommended as test organism in ecotoxicological investigations (ABNT, 2005). Test endpoints related to life history, such as survival, maximum body size, embryonic development, age and body lengths at primipara, and reproduction (mean number of eggs and neonates per female) were evaluated. We quantified free copper ions in test zooplankton media to infer about the contribution of waterborne exposure to dietary metal toxicity to the animals.

2. Materials and methods

2.1. P. subcapitata and C. silvestrii cultures

Cells of *P. subcapitata* and neonates of *C. silvestrii* were obtained from cultures maintained at the Plankton Laboratory at Universidade Federal de São Carlos (SP, Brazil).

Stock cultures of the cladoceran *C. silvestrii* were kept under controlled conditions of temperature (25 ± 2 °C) and light:dark cycle (12:12 h) using reconstituted water as culture media (pH 7.2, conductivity 160 μS cm^{-1} and hardness 42 mg L^{-1} of $CaCO_3$) that was changed every other day. Reconstituted water was prepared by adding $CaSO_4.2H_2O$ (1.2×10^{-4} mol L^{-1}), KCl (3×10^{-5} mol L^{-1}), $NaHCO_3$ (6×10^{-4} mol L^{-1}) and $MgSO_4.7H_2O$ (4×10^{-4} mol L^{-1}) according to ABNT (2005); its free Cu^{2+} ions concentration was 1×10^{-10} mol L^{-1}.

Stock cultures of the green algae *P. subcapitata* were kept in *Oligo* culture media described in AFNOR (1980), which was buffered with 500 mg L^{-1} of tris-hydroxymethyl aminomethane ($NH_2C(CH_2OH)_3$, J.T. Baker) at pH 6.8–7.0 and contained no EDTA. Culture media were sterilized by autoclaving at 121 °C for 15 min. Cultures were grown in 2 L Erlenmeyer flasks containing 1 L of medium, which was inoculated with 1×10^5 cells mL^{-1} from an exponentially growing culture and kept under controlled environmental conditions (100 μE m^{-2} s^{-2} in 12:12 h light/dark cycle at 24 ± 2 °C). Exponentially growing *P. subcapitata* cells were used as food for the zooplankton, which were fed every other day with 1×10^5 algal cells mL^{-1}. Besides this, the organisms were fed with a suspension of yeast and commercial fish food (Vitormonio®) in accordance with the procedures established by ABNT (2005).

2.2. Algal exposure to copper

P. subcapitata cells in exponential growth phase were exposed during 96 h to 8×10^{-8} (algal control); 2.5×10^{-7}, 5.0×10^{-7} and 1.0×10^{-6} mol L^{-1} total added copper concentrations and kept under the same controlled conditions as used for stock algal cultures (light intensity, light/dark cycle and temperature). Copper was furnished as $Cu(NO_3)_2.3H_2O$ (Carlo Erba) titrimetric solution. Copper stock solution (10^{-3} mol L^{-1}) was prepared using deionized water that was filter sterilized using 0.22 μm pore size cellulose acetate membrane filters (Schleicher and Schüll). The lowest copper concentration used corresponds to that in algal controls, e.g., originally present as nutrient in *Oligo* culture medium. Exponentially growing algal cells were inoculated into each experimental flask to provide initial cell densities of approximately 5×10^4 cells mL^{-1}. The experiments were performed with three replicates in 1000 mL polycarbonate Erlenmeyer flasks containing 600 mL of culture medium (ABNT, 2005). Values of pH in experimental media were measured throughout and kept within 6.8–7.0; free Cu^{2+} ions at the beginning of the algal experiments were quantified. After 96 h exposure to copper, algal cells were centrifuged at 1000 rpm for 10 min (FANEM Excelsa, model 206 PM, Brazil), washed three times with sterile culture media, suspended in reconstituted water and given as food to the zooplankton. Algal suspensions were stored in polyethylene bottles in the dark at 4 °C during the chronic toxicity test with zooplankton (De Schamphelaere et al., 2004).

2.3. Zooplankton toxicity

Neonates of *C. silvestrii* were kept in reconstituted water and exposed to dietary copper concentrations of 3.0 fg Cu cell^{-1} (6×10^{-9} g Cu day^{-1}), 38 fg Cu cell^{-1} (7.6×10^{-8} g Cu day^{-1}), 46 fg Cu cell^{-1} (9.2×10^{-8} g Cu day^{-1}) and 68 fg Cu cell^{-1} (1.3×10^{-7} g Cu day^{-1}). Ten neonates of *C. silvestrii* aged less than 24 h were added to 20 mL of reconstituted water and fed daily with 10^5 cell mL^{-1} Cu-contaminated *P. subcapitata* cells in polycarbonate cups. Ten replicates, each containing one neonate were prepared per treatment and the animals were monitored during 36 days, one complete life cycle. The reconstituted water used for the zooplankton toxicity tests, as well as the tests conditions, were the same as those used to keep the animals stock culture. Test media were gently stirred using a fine Pasteur pipette twice a day to ensure algal cell suspension and availability to the animals. The experiments were ended when individuals of each replicate died. Test media was monitored for water quality (free Cu^{2+} ions, pH, conductivity and hardness) every other day. Body length and age at first reproduction (primipara), duration of embryonic development, number of eggs and live neonates per female, survival of adults and animal's maximum (adult) body length were recorded. Body length measurement and number of eggs counting were performed using a micrometer eyepiece fitted to a Leica MZ6 stereomicroscope.

Two controls were performed, a stock diet control (DC) that contained the food concentration normally used in the maintenance of the test cladoceran (1×10^5 cells mL^{-1} plus the suspension of yeast and fish food) and an experimental diet control (EC) containing 1×10^5 cells mL^{-1} of algae as food source. The DC was performed to verify that organisms' physiological integrity was kept under the laboratory conditions used (ABNT, 2005).

2.4. Metal analysis

Initial free Cu^{2+} ions concentrations in *P. subcapitata* contamination experiments were determined in culture filtrates. These were obtained by gentle vacuum filtration (24 mm Hg) of 100 mL algal culture through 0.45 μm pore size cellulose acetate membrane filters (Schleicher and Schüll). Because membrane filters are known to release organic materials and metals even after the passage of 1 L of water (Mart, 1979), the filters used were left in 1.0 mol L^{-1} HNO_3 for 24 h and then rinsed with deionized water immediately before use. Free copper ions were determined using an ANALION (model Cu-641, Brazil) copper ion selective electrode (ISE) in conjunction with a glass double-junction reference electrode (ANALION, model R-684, Brazil). Potential readings were obtained using a pH meter (ANALION 2000, model 608, Brazil). Ionic strength was adjusted to 0.1 mol L^{-1} using high-purity $NaNO_3$ (MicroSelect, Fluka, Switzerland); the same ionic strength was used for samples and calibration standards. Electrode calibration was performed using metal ion buffers performed according to Lombardi et al. (2007), which is a modification of that described in Jardim et al. (1986). A typical calibration curve for the ions selective electrode system is shown in Fig. 1. For each day of analysis, a new calibration was performed.

Total copper accumulated by *P. subcapitata* was obtained at the end of algal contamination experiments. Aliquots were filtered through previously acid washed 0.45 μm membrane filters that were then dried at 65 °C during 24 h. Copper determination was performed after acid digestion of the dried filter by adding 10 mL of 3.0 mol L^{-1} HNO_3/1.0 mol L^{-1} HCl (Ultrapure acids, J.T. Baker) as described in Lombardi et al. (2002). Total particulate copper determination was performed in the digested filters by differential pulse anodic stripping voltammetry (DP-ASV) using an EG&G Instruments Polarograph Analyzer (PAR model 394) with mercury electrode (SMDE EG&G PAR 303A). Standard additions technique was employed and the sample was scanned from −0.6 to 0.0 V, with a scan rate of 8 mV/s, 1–5 min deposition time, depending on copper concentration. Ten min initial high-purity nitrogen purge time was used.

The concentration of copper in algal cells was considered as the total amount of metal accumulated, which corresponds to that adsorbed onto algal surface and

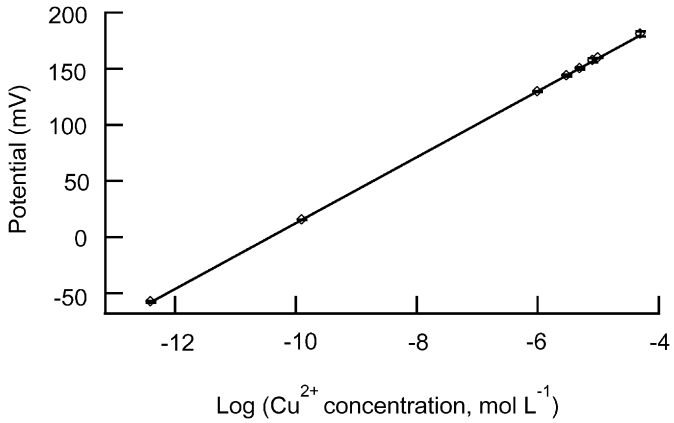

Fig. 1. Calibration curve for the Cu-ISE system. Free Cu^{2+} concentration (mol L^{-1}) plotted as function of potential readings (mV). Fit equation: $y = 306.6+29.39x$, $R = 0.999979$.