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Copper Toxicity on Juveniles of *Hyalella pseudoazteca* González and Watling, 2003

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Abstract The aim of this work is to evaluate the toxic effect of water and sediment contaminated with Cu^{2+} on the survival and growth of juveniles of *Hyalella pseudoazteca*. The LC_{50} 96 h for Cu^{2+} in moderately hard water was 0.17 mg/L. The concentration of 100 mg Cu^{2+}/kg in sediment was found to have an inhibitory growth effect. This study provides information on the toxic effects of Cu^{2+} on a native benthic species occurring in the Pampean region, Argentina and contributes to validate, in this region, the interim use of Cu^{2+} values recommended by the sediment quality guidelines for the Northern hemisphere.

Keywords Spiked sediment bioassays · Amphipoda · *Hyalella pseudoazteca* · Copper toxicity

Freshwater sediments are known to play an important ecological role as intermediates in the exchange of chemicals among the particulate, dissolved and biological phases. The potential toxic effect of contaminated sediments on the associated biota is well documented for the Northern hemisphere, whereas studies involving representative species in continental aquatic ecosystems of South America are scarce. The direct extrapolation of results is inappropriate because ecosystem dynamics vary widely among regions. This fact reflects the necessity of assessing the toxicity of chemicals in sediments to native species (APHA 1995) by means of standardized protocols taking into account the distinctive features of both the test species and their environments. In particular, the spiked sediment technique is the most reliable for characterizing the response of benthic organisms to individual toxics (EC 1997).

The Cu^{2+} is usually present in sediments. Its consensus threshold effect level is 31.6 mg/kg dry weight in freshwater sediments (Burton 2002).

It is frequently used as a reference toxic in bioassays involving toxicological monitoring of sediments, mainly because there are no special hazards associated with its handling at the laboratory (EC 1995), the test organisms are sensitive to this metal and the literature provides information on copper toxicity for many species, which allows the comparison of results across studies (Kurbitz et al. 1995).

Hyalella pseudoazteca is a common species in South America, where it is representative of zoobenthic associations, and has a potential use in standardized assays (Giusto et al. in press).

The aim of this work is to assess the effect of Cu^{2+} in water and sediment on juveniles of *H. pseudoazteca*.

Materials and Methods

The amphipods *H. pseudoazteca* were collected from Las Flores Stream, where they are associated with macrophytes *Egeria densa* and *Ceratophyllum demersum*. Once back in the laboratory *H. pseudoazteca* individuals were kept in plastic containers with tap water and local plants before the beginning of the bioassays. The amphipods were fed with

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crushed fish food every 3 days. Once a week 50% of the water in each container was renewed and the food remains were removed. The culture was maintained in tap water (hardness: 80–90 mg CO_3Ca/L), under laboratory conditions at least for 15 days. Animals were acclimated to the experimental conditions. To achieve this objective, a pool of individuals shorter than 2.5 mm in length was selected from the culture tanks 10 days before the beginning of the assay; they were kept in moderately hard water (MHW) with the following chemical composition (mg/L): $NaHCO_3$, 96; $CaSO_4 \cdot 2H_2O$, 60; $MgSO_4$, 60; KCl, 4; pH, 7.4–7.8; hardness, 80–100 mg CO_3Ca/L (Weber 1993). The pool was placed into an experimental chamber under constant temperature of 23 ± 1°C and a photoperiod regime of 16 L:8 D.

The sensitivity of *H. pseudoazteca* to copper was assessed using a 96-h static toxicity test in MHW, following US-EPA guidelines (2000). Amphipods were exposed to concentrations of Cu^{2+} from 0 to 0.80 mg/L. Test solutions were prepared by dilution of a stock solution of 1,000 mg/L Cu^{2+} (as SO_4). The effective concentration of Cu^{2+} in water and tissues of surviving animals at the end of exposure time was determined by inductive plasma emission spectrometry.

The assays were performed in triplicate in polypropylene containers under the experimental conditions described above. Each replicate comprised 10 individuals per 160 mL of MHW. A piece of tulle of 5 cm^2 was added as substrate. The LC_{10} and LC_{50} (96 h) were calculated by Probit analysis using the program TOXSTAT-EPA.

In assays conducted on a solid matrix, animals were exposed to increasing concentrations of Cu^{2+} added to whole sediment, which was collected 22 days before the beginning of the assays. The sediment was dredged from the bottom of Las Flores Stream. About 25 kg of sediment were taken up to a depth of 10 cm from three sites located 3 m apart.

The sediment was first homogenized by hand and cooled for 24 h; then, vegetation and macrofauna were removed and the material was homogenized once more. Three subsamples were taken and analyzed for chemical composition, pollutant content, dry weight/wet weight (DW/WW) ratio (Pasteris et al. 2003), humidity, organic matter content and granulometric composition by the Robinson pipette method (Lopretto and Tell 1995).

The nominal test concentrations assayed were: 0 (control), 10, 50 and 100 mg Cu^{2+}/kg dry sediment. The 10 g/L copper stock solution used to spike the sediments was prepared by dissolving reagent grade $CuSO_4$ in distilled water. Spiking was performed by mixing the required weight of wet sediments (corresponding approximately to 1.5 L), with 2 L of MHW and the required volume of Cu^{2+} stock solution in a 4-L jar. The contents of each jar were manually stirred for 5 min, using a stainless steel spoon. Jars were then placed in a refrigerator at 4°C for 14 days. Stirring was repeated every 3 days (Pasteris et al. 2003).

Twenty-four hours before the beginning of the spiked bioassay, the supernatants from the control and treatments were discarded and the remaining material was divided into five replicates, each one with 100 mL of treated sediment and 175 mL of MHW. The replicates were placed in the experimental chamber under controlled conditions as described above, covered with glass plates to minimize evaporation, and allowed to equilibrate for 24 h.

At the initial time of the assay, 10 individuals randomly selected from the pool subjected to acclimation were added to each replicate. The average length and dry weight were determined from 40 randomly selected individuals just before the beginning of the assay. Amphipods were exposed for 10 days, during which they were periodically fed with crushed fish food until the last 48 h of the assay.

The effect parameters considered at the end of the bioassay were survival and growth (length and biomass). The length from the base of the first antenna to the tip of the telson was measured using digital caliper (to the nearest 0.01 mm) under a stereoscopic microscope. The biomass of the amphipods surviving each treatment was expressed as dry weight obtained after drying at 60°C for at least 24 h.

The following parameters were determined for MHW (at the beginning of the bioassay) and for the overlying water (at the end of the bioassay): dissolved oxygen concentration (±0.1 mg/L), pH (±0.01 mg/L), hardness (±1 mg $CaCO_3$/L), and NH_4^+ concentration (±0.01 mg NH_4/L). The concentration of Cu^{2+} was determined in the overlying water at the end of the bioassay, and in the sediment at the beginning and end of the bioassay by atomic absorption spectrophotometry using direct aspiration into an air-acetylene flame (Method 3111B, APHA 1995). All the reagents were analytical grade. In addition, some subsamples of the control and the treated sediments were examined under microscope to assess the presence of organisms.

The Shapiro–Wilks and the Levene's tests were used to test normal distribution and homogeneity of variances, respectively. The statistical significance of differences between treatments for survival and growth was analyzed with the Kruskal–Wallis test, or one-way ANOVA followed by multiple comparisons or Tukey's test, respectively (Zar 1999). The statistical analysis was made with the Infostat Program. The significance level was set at $p < 0.05$.

Results and Discussion

The LC_{10} and LC_{50} values in function of time for juveniles exposed to Cu^{2+} in MHW are shown in Table 1. The LC_{50} value is within the range reported for *H. azteca* (Milani et al. 2003; Borgman and Norwood 1997) and *H. curvispina* (García et al. 2007). Table 2 shows the values of Cu^{2+} in tissue (whole animal) measured in survivors exposed for 96 h

Table 1 LC_{50}, LC_{10} and confidence limits

Time (h)	LC_{50}	Confidence limits	LC_{10}	Confidence limits
24	>0.40	–	<0.05	–
48	0.31	0.16–0.26	0.10	0.67–0.14
72	0.21	0.25–0.38	0.69	0.39–0.98
96	0.17	0.13–0.21	0.58	0.13–0.22

Table 2 Nominal concentrations (NC) and effective concentrations (EC) of copper at the final time of the assay in mg Cu^{2+}/L and copper uptake by surviving individuals (mg Cu^{2+}/g dry weight)

NC	0.0	0.05	0.10	0.20	0.40	0.60	0.80
EC	<0.02	0.05	0.12	0.17	0.34	0.50	0.75
Uptake	0.17	0.35	0.44	0.52	0.75	–	–

to increasing concentrations of Cu^{2+}, between 0 and 0.40 mg/L (nominal values). The basal level of Cu^{2+} in control animals was 0.17 Cu^{2+} mg/g body mass, which is substantially higher than those reported for *H. azteca* (Shuhaimi-Othman and Pascoe 2007; Borgmann and Norwood 1997). Under the experimental conditions, the incorporation of Cu^{2+} showed a positive trend in relation to concentration.

In the spiked sediment assay the Cu^{2+} levels in overlying water at the end of the assay were lower than LC_{10} values for the test species (Table 1). Consequently, it is expected that the effect observed in tests involving copper-spiked sediment is mainly due to the interaction between the animals and the sediment.

The sediment from Las Flores Stream was selected as control in previous studies involving a whole-sediment toxicity test (Giusto et al. in press; Ronco et al. *enviado*) because of its low level of chemical contamination and granulometric composition, which minimizes the release of heavy metals into the water column. These attributes allow the assessment of the toxicity of sediment-associated metals to benthic organisms. The sample sediment used was characterized by the total organic matter (4.9%), humidity (48%), DW/WW ratio (0.52) and granulometry (sand: 47.0%; clay: 36.4% and loam: 17.4%). The humidity value is within the usual range of the sampling site and there is a relatively high organic matter content. The granulometric analysis indicates that it might be classified as loamy sediment (MESL 2001), which is capable of retaining heavy metals.

Table 3 Nominal concentrations (NC) and effective concentrations (EC), of copper at initial and final times in sediment and water

NC (mg Cu^{2+}/kg)	EC initial (mg Cu^{2+}/kg)	EC final (mg Cu^{2+}/kg)	EC final (mg Cu^{2+}/L)
Control	13.5	14.1	<0.05
10	15.1	19.9	<0.05
50	53.8	49.7	<0.05
100	95.5	106.6	<0.05

The nominal and effective values of Cu^{2+} in sediment and overlying water for the control and treated groups are shown in Table 3. The effective Cu^{2+} concentration in the spiked sediment was close to the nominal one and remained stable throughout the assay period, thus confirming that the exposure level to the toxic was almost constant. The Cu^{2+} concentration in the control sediment was within the range occurring naturally in the earth's crust and below the tolerance limits for this metal (CEQG 2002).

Table 4 shows the parameter values obtained for MHW and the overlying water at initial and final time, respectively. There was a significant increase in hardness and pH for all cases, probably due to the release of salts and electrolytes initially present in the sediment. Likewise, both the control and treated groups showed a significant increase in DO and NH_4^+ concentrations. The microscopic examination of the sediment showed the presence of algae, suggesting that the increase in DO was due to photosynthesis.

Figure 1 shows the average survival percentages and standard deviations for the treatments at final time. Only the amphipods exposed to 100 mg Cu^{2+}/kg (nominal value) showed a significant decrease in survival compared to the control ($p < 0.05$). Borgman and Norwood (1997) reported that *H. azteca* survival exceeded 90% when exposed during 1 week to sediments containing between 74 and 1,868 mg Cu^{2+}/kg. In the present work, *H. pseudoazteca* was found to have a higher sensitivity than *H. azteca* exposed to similar concentrations. However, the degree of copper effect on sediment reported in the literature is highly variable, possibly due to different assay conditions, matrix composition and diluting water composition, among other parameters.

Growth was evaluated by the comparison with the length of the initial group. The length of the control and treated groups exposed to 10 and 50 mg Cu^{2+}/kg showed a

Table 4 Physico-chemical characterization of the overlying water at final time of the assay (mean values ± SD; n = 5). MHW: dilution media

	MHW	Control mg Cu^{2+}/kg	10 mg Cu^{2+}/kg	50 mg Cu^{2+}/kg	100 mg Cu^{2+}/kg
pH	7.44	9.00 ± 0.23	8.69 ± 0.20	8.69 ± 0.25	8.54 ± 0.18
DO (mg/L)	5.3	10.7 ± 1.1	10.1 ± 1.2	11.3 ± 1.1	13.7 ± 2.2
NH_4^+ (mg/L)	<0.03	0.18 ± 0.04	0.06 ± 0.02	0.13 ± 0.60	0.18 ± 0.09
Hardness (mg CO_3Ca/L)	90.2	85.9 ± 5.5	113.1 ± 6.7	103.0 ± 15.7	120.4 ± 7.9

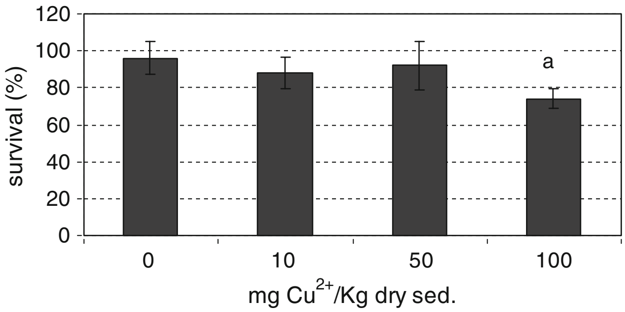

Fig. 1 Relationship between copper concentration in the sediment and number of survivors after 10 days of exposure

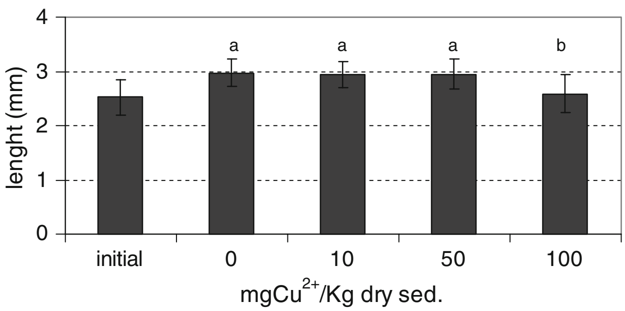

Fig. 2 Relationship between sediment copper concentration and mean length of individuals after 10 days of exposure

significant increase of between 17% and 18% with respect to the initial group, whereas the group exposed to 100 mg Cu^{2+}/kg showed a significant decrease in growth rate, with an increase in length of only 2.7% with respect to the initial group (Fig. 2). The biomass of the control group (163.7 mg/ind.) and the groups treated with 10 and 50 mg Cu^{2+}/kg (147.9 and 162.4 mg/ind., respectively) increased between 70% and 90% with respect to the initial group (0.086 mg/ind.), whereas that of the group exposed to 100 mg Cu^{2+}/kg (129.2 mg/ind.) increased 50%. These results indicate an important growth inhibition in the group exposed to 100 mg Cu^{2+}/kg. The decrease in biomass may be due to different factors, such as decreased feeding, increased feces production and molt arrest (Borgmann and Norwood 1997), but the decrease in body length is likely to be linked to molt arrest. For *H. azteca* chronically exposed to copper-spiked sediment, Milani et al. (2003) recorded a 25% growth inhibitory concentration of 76 mg Cu^{2+}/kg. Despite the differences in experimental conditions between their work and ours, this result suggests that both species have a similar sensitivity.

During the past 20 years, most of the numerous sediment quality guidelines (SQGs) being developed to assist regulators in dealing with contaminated sediments were based on data obtained from North America (Burton 2002).

The specific applicability of the SQGs to a particular geographical region is uncertain, due to differences in geography (e.g. temperature, salinity, geological formation), in species composition and sensitivity, and in pollutant bioavailability. This reflects the necessity of baseline data for local species.

Traditionally, sediment contamination has been determined by assessing the bulk chemical concentrations of individual compounds and often comparing them with background or reference values. In general terms there are two threshold levels, one below which effects rarely occur [e.g. the lowest effect level (LEL), threshold effect level (TEL), minimal effects threshold (MET), effects range low (ERL) with a value of consensus level (TEC)], and one above which effects are likely to occur [e.g. the toxic effect threshold (TET), severe effect level (SEL), probable effect level (PEL), effect range median (ERM) with a value of consensus level (PEC)] (Burton 2002). In the present work, the Cu^{2+} added to the sediment of Las Flores Stream exerted toxic effects on *H. pseudoazteca* at intermediate values between TET (86 mg Cu/kg) and SEL (110 mg Cu/kg) (Burton 2002).

This work provides information on the toxicological effects of Cu^{2+} on a benthic native species in sediments from the Pampean region in Argentina, and contributes to validate the interim use in this region of the consensus-based SQGs for Cu^{2+} developed in the Northern hemisphere.

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