Toxicity and bioavailability of mercury in spiked sediments on *Hyalella curvispina* Shoemaker, 1942

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Abstract: Mercury is a non-essential toxic heavy metal often found in bottom freshwater sediments. The aim of the present study was to evaluate the influence of the organic matter (OM) on the toxicity and bioavailability of mercury in sediment samples on the amphipod *Hyalella curvispina*, assessing survival and growth under chronic exposure. Species sensitivity in water-only tests was LC-50 of 96 h 0.025 mg/L. Results indicate that sediments with OM did not induce lethality under concentrations of mercury up to 10 mg/kg dw. On the contrary, exposed organisms to sediments without OM were significantly affected at half of that metal concentration. Sublethal effects were evident at 3 mg/kg. Presence and proportion of OM in sediment is clearly influencing mercury bioavailability, affecting toxicity in a different level according to the end point being assessed.

Keywords: mercury; bioavailability; *Hyalella curvispina*; organic matter; spiked sediments; sediment toxicity test; chronic exposure; lethality; sublethal effects; median lethal concentrations; CL-50; sediment quality guidelines; freshwater environment.

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1 Introduction

Within the group of heavy metals, mercury has been found to be non-essential to biota and there is no metabolic function associated with it. It is highly toxic to organisms, with a very slow kinetic of elimination and a high potential of bioaccumulation (Watras et al., 1998; De Marco et al., 2006). Although chemical speciation of mercury determines its toxicity, species in natural environments are difficult to quantify (Wiener et al., 2003). It can be found in aquatic environments in the elemental form, as inorganic or organic species. The most abundant in bottom sediment are inorganic forms of mercury (Mason and Lawrence, 1999), being the organic forms the most toxic ones (Bloom, 1992; Watras et al., 1998).

Mercury is accumulated in sediments by processes of sedimentation of suspended materials in aquatic environments and could be incorporated to the water column by dissolution equilibrium depending of pH, redox potential, organic matter, iron and manganese oxides or sulphide concentrations (Benoit et al., 1999; Zhong and Wang, 2006; Machado et al., 2008). Texture of sediment materials also regulates binding capacity of the metal (Boszke et al., 2004). Within the group of variables, organic matter and sulphide contents are the main factors controlling the distribution and bioavailability of the metal (Boszke et al., 2003). Zhong and Wang (2006) demonstrated under laboratory conditions with benthic invertebrate that the major bioavailable mercury fraction is associated with iron and manganese oxides, while the least bioavailable is bound to organic matter.

Although there are extensive reports on environmental factors that regulate the bioavailability of several metals such as copper, cadmium, nickel, lead and zinc, and their toxicity to benthic invertebrates supporting guideline criteria (Di Toro et al., 1991; Correia and Costa, 2000; Besser et al., 2005; USEPA, 2005; Roman et al., 2007), information is scarce in relation to mercury. Within this conceptual frame, the objective of the present research is reporting the results of a study on the acute (water only) and chronic toxicity and bioavailability of mercury in bottom sediments from freshwater systems to the amphipod *Hyalella curvispina*, in relation to sediment matrix variables and guideline levels.

2 Materials and methods

H. curvispina test organisms were obtained by sieving cultures maintained in our laboratory in dechlorinated tap water (hardness 220 mg L^{-1} $CaCO_3$, pH 8.2, conductivity 1.10 mS cm^{-1}) (Peluso et al., 2011). Two types of experiments were performed: water tests (acute test) and whole spiked sediment tests (chronic test).

Species sensitivity to mercury was assessed under acute exposure (96 h) in water-only tests with renewal, according USEPA (2000) method. Organisms were exposed in mercury solutions within the range 0.01–0.2 mg L^{-1} prepared in dechlorinated tap water. Testing dilutions were done from a stock solution of 1000 mg L^{-1} of mercury prepared from $HgCl_2$ (Anedra®). Treatments were done in triplicates in 500 ml polypropylene jars, with ten organisms per jar and 200 ml of testing solution. Mortality of amphipods was recorded at 24, 48, 72 and 96 h. The acceptability criterion of tests was having over 90% survival in negative controls.

Whole spiked sediment tests were carried using two types of solid matrixes: a laboratory-formulated sediment and a stream sediment from the region. The formulated sediment was prepared according protocol 218 of the OECD (2004) with following size particle composition: 75% quartz sand, 20% kaolinite and 5% sphagnum moss peat and calcium carbonate. Sand was previously sieved through #35 and #60 mesh (ASTM) in series to obtain a size range <500 μm and <250 μm, respectively, thoroughly washed with tap water and finally rinsed with distilled water and then dried at room temperature. Moss peat was dried to ambient air and added as powder <1 mm. The formulated sediment was kept dry ready to be used in testing. The tested stream sediment was obtained from a fairly uncontaminated water stream, A° Juan Blanco (35º8´ S and 57º26´ W) that had been previously used as a reference sediment (Peluso et al., 2011) and characterised by Ronco et al. (2008). Before testing, dried sediments were rehydrated to 30% water. Additions of mercury were done from a stock solution with concentration of 1 g L^{-1} prepared from $HgCl_2$ (Anedra®) in distilled water. For each tested concentration, a volume of stock solution was added on the wet sediment to obtain the corresponding dilution, manually mixing for homogenisation. According to Simpson et al. (2004) recommendations, after mercury incorporation the spiked sediment was kept during a week at the same light and temperature conditions as those used in toxicity testing.

Whole sediment tests were done on formulated sediment with no organic matter (FS1), formulated sediment with 3.5 (±0.05)% organic matter (FS2), and stream sediment containing 12 (±0.1)% organic matter (SJB). Six mercury concentrations were tested and a control with no mercury addition in each treatment. Seven replicates were used for each concentration; 100 ml of sediment and 175 ml of overlying water were placed in each replicate, with ten individuals each. Test amphipods (7–14 days old) were previously

separated and fed with fish food (McShullet®) and boiled lettuce ad libitum. Exposure was conducted for 21 days at 21°C on a 16:8 light: dark photoperiod. At the start of the exposure, about 20 amphipods were taken and kept in a solution of 4% formaldehyde for measurement of length. One-third of the overlaying water was renewed every five days with the addition of food during exposure. Measured end points were survival and growth (length). On the 21st day of the exposure, sediments in each beaker were sieved through a #50 sieve (300 μm opening). Surviving amphipods were counted and preserved for later length measurements. Performance criteria for the control sediment required 80% survival.

At the beginning and end of testing, following parameters were measured in the overlying water: dissolved oxygen, pH and conductivity with sensors (Lutron® YK-200PDO, YK-2001PH and YK-200PCT, respectively), ammonia (commercial kit Aquamerck®), hardness and alkalinity by titration methods (Methods 2340C and 2320, APHA, 1998). Two of the seven replicates were used for chemical analysis of sediment and overlying water at the beginning and end of testing. In sediments were characterised pH (APHA, 1998), loss on ignition in a muffle furnace at 550°C to estimate organic matter proportion, and mercury concentration. Water samples were added with nitric acid for preservation and kept refrigerated until analysis (APHA 1998). Sediment samples were acid digested according method 7471B (USEPA, 1996). Mercury analysis were performed by cold vapour atomic absorption spectrometry (method 3112, APHA, 1998) using a Varian SpectraAA3000 with a hydride generator. Certified standard was from AccuStandard, Inc. (1000 mg L^{-1} standard stock solutions, traceable to the National Institute of Standards and Technology, USA).

Toxicity data were checked for normality and homoscedasticity assumptions with Shapiro-Wilk's and Bartlett's tests, respectively. Water-only toxicity tests were analysed by Probit analysis and LC values and the 95% confidence interval were calculated from estimated dose-response curves using the Probit Analysis Program, version 1.5 (USEPA, 1999). In sediments tests, the amphipod survival and growth (length) were compared by the One-Way Analysis of Variance (ANOVA), followed by Dunnet's test. Percent survival data were arcsine-transformed, and length data were log-transformed before analysis (Zar, 2010). Level of significance used was $p < 0.05$. All data were calculated using measured mercury concentrations in tested samples. Statistical analyses were done using STATISTICA (Stat Soft. Inc., version 7).

3 Results

The LC_{50} and LC_{10} obtained values as a function of time for juveniles of *H. curvispina* exposed to mercury in water-only test are given in Table 1, being the first reports for the species according searched literature.

Table 1 Results of *H. curvispina* water-only toxicity test. LC_{50}, LC_{10} and 95% confidence limits (data given as mg Hg L^{-1})

Time (h)	*LC_{50}*	*Confidence limits*	*LC_{10}*	*Confidence limits*
24	0.089	–	0.036	–
48	0.046	0.039–0.054	0.015	0.011–0.020
72	0.032	0.027–0.037	0.012	0.009–0.016
96	0.025	0.022–0.029	0.012	0.009–0.015

Table 2 Sediment test: mercury concentrations measured in the sediment (mg Hg Kg^{-1}) and overlying water (mg Hg L^{-1}) at initial (Ti) and final time (Tf)

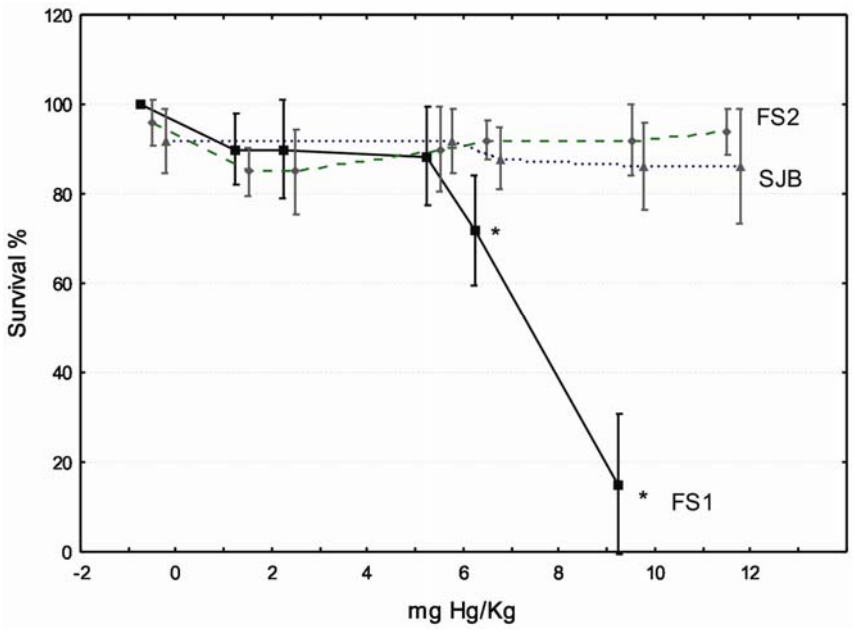
Nominal concentration	*control*		*1.7*		*3.0*		*6.0*		*7.5*		*9.6*		*12*	
Sediment	*Ti*	*Tf*	*Ti*	*Tf*	*Ti*	*Tf*	*Ti*	*Tf*	*Ti*	*Tf*	*Ti*	*Tf*	*Ti*	*Tf*
FS1	<0.05	<0.05	1.7	1.0	2.9	2.0	5.5	3.5	6.4	4.0	9.0	6.5	11.9	9.1
FS2	<0.05	<0.05	1.6	1.3	2.4	1.7	5.6	4.5	6.4	5.6	9.3	7.8	11.8	9.5
SJB	<0.05	<0.05	–	–	–	–	4.5	4.0	6.8	5.2	8.2	7.0	10.2	8.5
Water														
FS1	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	0.008	<0.001	0.008	<0.001	0.010	<0.001	0.010
FS2	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	0.005	<0.001	0.005	<0.001	0.008	<0.001	0.008
SJB	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	0.003	<0.001	0.003	<0.001	0.005	<0.001	0.008

Mercury concentrations measured in sediments at initial and final time of chronic tests are detailed in Table 2. The percentages of recovery were within 83.0–98.5% of the nominal concentrations. Mercury concentration in negative control was below detection limits (<0.05 mg Hg kg^{-1}). At the end of exposure, the average concentration of the metal decreased in 20% and 50%, respectively, for sediments with and without OM respect to initial time. Results indicate that sediments with OM (both, formulated and natural) bind more strongly mercury during the 21 days of testing.

The concentration values of pH and conductivity of the overlaying water during the experiments remained constant according to the measurements at the beginning and end of time. The DO slightly decreased, though remained above the recommended levels over 2.5 mg L^{-1} (USEPA, 2000). Ammonium concentrations ranged between 0.4 and 0.6 mg L^{-1} along 21 days of testing in all treatments. Water hardness was higher at the end of testing, possibly related to biological activity in the system and/or dissolution of calcium and magnesium along the testing period. Concentrations of mercury in overlaying water at initial time were below 0.001 mg L^{-1} and detectable after 21 days (see Table 2).

Statistical analyses by ANOVA followed by Dunnet's test showed significant differences in survival ($p < 0.05$) with the control group in the two last concentrations of mercury in FS1 sediment (Figure 1). The FS2 and SJB sediments did not show effect on survival. The FS1 NOEC and LOEC values (referred to measured mercury concentration) were 5.5 and 6.4 mg kg^{-1}, respectively. No lethality was observed with the mercury spiked sediments with OM; hence the estimation of those end points was not possible, being for this case the highest tested concentration twofold the NOEC value with no OM.

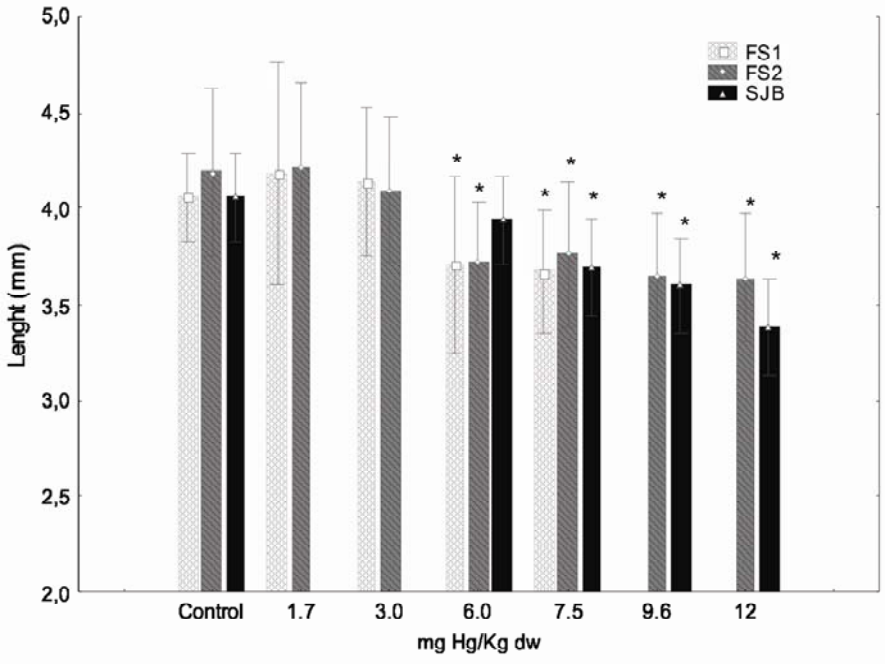
Figure 1 *H. curvispina* survival as a function of mercury concentration in sediment for three treatments (see online version for colours)



Note: *Significant differences ($p < 0.05$) with respect to control.

The growth of chronically exposed organisms was evaluated by comparing the length of the initial group in each treatment with that recorded at the end of the exposure. Figure 2 shows the growth of the amphipods exposed to the three tested sediments. Control organisms in all treatments showed an acceptable growth (between 79 and 90%) after 21 days according growth control curves determined in our laboratory (Peluso, 2011). Organisms exposed to FS1 showed a comparable increase in length at 1.7 and 3.0 mg Hg kg^{-1}. At concentrations greater than 3 mg Hg kg^{-1}, the growth was reduced by 20% with respect to controls and to those exposed to sediments with lower Hg concentrations. Under exposure to SF2 with Hg concentration higher than 1.7 mg kg^{-1} the length of test organisms showed a significant inhibition in growth rate between 25 and 30% relative to controls. Furthermore, in the Spiked Stream Sediment (SJB), the amphipods exposed to 5.8 and 8.2 mg Hg kg^{-1} exhibited a 15–18% growth inhibition, while the highest tested concentration induced a 33% inhibition.

Figure 2 *H. curvispina* growth (mean length values ± SD) exposed to different concentrations of mercury in sediment treatments (see online version for colours)



Note: *Significant differences ($p < 0.05$) with respect to control.

4 Discussion

Concerning sensitivity to mercury, Borgmann et al. (2005) reported a *Hyalella azteca* LC_{50} of 0.002 mg Hg L^{-1} after seven days exposure in moderately hard water. In our study, the 96 h exposure LC_{50} for *H. curvispina* was 1 order magnitude higher. Possibly the difference of time exposure and water type could be an explanation of variation between measured sensitivity. Reish (1993) reported LC_{50} values of 0.02 and

0.08 mg Hg L^{-1} for two *Gammaridae* species closer to the 0.025 mg Hg L^{-1} obtained for *H. curvispina*. A comparison of *H. curvispina* sensitivity with other representative organisms of the water column such as cladocerans (*Daphnia magna*) (Fargasova, 1994), copepods (*Acartia sp.*) and amphipods (Reish, 1993; Annicchiarico et al., 2007) indicates closer sensitivity values to mercury.

The survival of *H. curvispina* under chronic exposure does not seem to be affected by mercury concentrations in sediments containing OM; however, sediments without OM induced a significant decrease in the survival of the amphipods at the highest tested concentration. On the other hand, significant decreases in the growth rate of organisms exposed to similar concentrations of mercury in sediments with the lowest OM content and without OM (LOEC 5.6 and 5.5 mg Hg kg^{-1}, respectively) were detected. A similar effect was observed at higher concentrations in SJB with higher OM content (LOEC 6.8 mg Hg kg^{-1}) (Figure 2).

The main factor controlling mercury bioavailability in the sediment phases is associated with organic complexes (Zhong and Wang, 2006). According the experimental design done in our experiments, treatments with formulated sediment only differ in the organic matter content; hence we could assume that this is the factor influencing mercury bioavailability to organisms. Our results are in agreement with recommendations from the literature that when testing toxicity to this metal it should always be taken into account the OM content in sediments (Ankley et al., 1996; Chapman et al., 1998; Besser et al., 2003). For example, Winger et al. (1993) did not observe toxic effects testing sediments from the Brunswick Estuary with 24.7 mg Hg kg^{-1} on *H. azteca* under 10 days exposure. Those sediments contained high concentrations of OM and sulphides. Also, studies with mercury contaminated sediments (0.3–4.6 mg kg^{-1}) from Lavaca Bay showed the absence of adverse effects on the amphipod *Leptocheirus sp*. after 28 days exposure (Robinson et al., 1997). Sferra et al. (1999) did not detect toxic effects on *H. azteca* and *Leptocheirus plumulosus* exposed to sediments from the Calcasieu Estuary containing up to 4.1 mg Hg kg-^{1} and 3.4% OM after 10 days exposure. Although, sublethal effects are detected at lower concentrations, even when testing in sediments with OM. Bundschuh et al. (2011) reported a decrease in feeding rate of the amphipod *H. azteca* exposed to sediments containing 10 mg Hg kg^{-1}dw and 10% OM. This is in agreement with our sublethal effects on growth observe on *H. curvispina*.

Several studies have reported mercury concentrations in aquatic environments of Argentina in samples of water, sediments and biota (mollusks, fish and mammals) (Marcovecchio and Moreno, 1993; Lomniczi et al., 2004; Ribeiro Guevara et al., 2005; SMA-DS, 2006). Concentrations of Hg in polluted aquatic environments reached levels between 0.51 and 7.00 mg Hg kg^{-1}dw (Ronco et al., 2001; Arribére et al., 2003; SMA-DC, 2006; Ronco et al., 2008). Those concentrations are over the international sediment quality guideline levels, such as the Threshold Effect Level (TEL) and Probable Effect Level (PEL), with values of 0.17 and 0.48 mg kg^{-1}, respectively (CEQG, 2002); consensus levels 0.18 mg kg^{-1} TEC and 1.06 mg kg^{-1} PEC (MacDonald et al., 2000), and the reference guideline according to the Dutch regulation of 0.3 mg kg^{-1} (IADC/CEDA, 1997).

Literature reports together with our results suggest levels of protection within a range of 1.6–24.7 mg Hg kg^{-1}, depending on the sediment characteristics and as assessed according toxic end points. Although, amphipods such as *H. azteca* bioaccumulate mercury with bioaccumulation factor (BAF) of the order of tenfold (Borgmann et al., 1993); Lawrence and Mason (2001) detected values near one for the species *L.*

plumulosus in sediments with high content of organic matter. These last authors also report a decrease in the BAF with the increment of OM content, pointing to necessary information on the processes of bioaccumulation and food chain transfer in aquatic ecosystems for a broad spectrum of risks assessment of mercury pollution.

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