Hyalella curvispina (AMPHIPODA) AS A TEST ORGANISM IN LABORATORY TOXICITY TESTING OF ENVIRONMENTAL SAMPLES

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ABSTRACT

An intercalibration exercise using blind-liquid samples containing Cr^{+6} and Zn^{+2} as well as environmental sediment samples was carried out between two laboratories using *H. curvispina* as test organism. For liquid samples, LC_{50} 96-h values were in the same order of magnitude for both metals in each laboratory. The tested sediments in each laboratory included a control sediment, two heavily contaminated sediments (Riachuelo and Oeste Canal) and a moderately contaminated sediment (Lujan River). In the whole-sediment tests, an acceptable level of survival for the controls was obtained by both laboratories. Contaminated sediment samples exhibited high toxicity in both laboratories, while moderately contaminated sediment samples did not exhibit lethality, being survival >80%; nevertheless, growth was significantly lower compared with negative controls in test organisms exposed to sediments of this stream. This study provides relevant information for the validation of *H. curvispina* as a test organism in sediment monitoring studies at regional level.

KEYWORDS: *Hyalella curvispina*, bottom sediments, acute toxicity test, toxicity assessment, heavy metals

1. INTRODUCTION

Sediments are ecologically important because they mediate chemical exchange among the particulate, dissolved and biological phases. Sediments provide a valuable indication of overall environmental contamination, hence the relevance of conducting ambient sediment toxicity tests within the frame of risk assessment programs [1, 2]. Information from chemical analysis of contaminant levels should be complemented with that referred to toxicological quality [3]. In recent years, important efforts to determine sediment ecotoxicity have been made by scientists and authorities. Thus, many bioassays have been developed with benthic species. Amphipods are usually one of the most sensitive *taxa* to toxic substances in acute tests. *Hyalella* spp. are generally chosen due to their short life span and because they can be easily cultured in laboratory [4, 5]. There are freshwater standardized assays for *Hyalella azteca* (Crustacea, Amphipoda) and *Chironomus tentans* (Chironomidae, Insecta) [6].

Recently, efforts have began in Argentina towards developing toxicity tests to assess biological effects of sediments containing contaminants using representative species of the region, being *H. curvispina* one of the most used [7-9] due to its taxonomic closeness to *H. azteca* and to its abundance in freshwater bodies of the Pampas plains of the country. Recently, it has been informed that another native amphipod, *H. pseudoazteca*, was also found to be appropriate as test organism for sediment toxicity bioassays [10]. The present study has been carried out within the frame of an interlaboratory intercalibration exercise of toxicity tests with Neotropical species for monitoring contaminated sediments. Development and validation of sediment toxicity tests at a local level represent a contribution to the implementation of effective tools to assess sediment ecotoxicity in environmental management strategies. This study was conducted between two laboratories (A: CIMA, B: PRODEA) with reference toxicants and environmental sediment samples, using *H. curvispina* as test organism with water-only and whole-sediment tests, respectively.

2. MATERIALS AND METHODS

An intercalibration exercise was conducted, in which both laboratories received two blind-liquid samples, and five environmental sediment samples. For all assays, *H. cur-*

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vispina species were obtained from laboratory cultures. Dechlorinated tap water was used for cultures (Laboratory A: hardness 220 mg/L $CaCO_3$, pH 8.2, conductivity 1.10 mS/cm; Laboratory B: hardness 80 mg/L $CaCO_3$, pH 8.5 and conductivity 0.86 mS/cm). The amphipods were fed with fish food and boiled lettuce every three days. The cultures and tests were done in chambers with constant photoperiod (16L: 8D h) and temperature (20±1 °C). Test amphipods were juveniles (3-4 mm length), obtained by gently siphoning the culture media onto a nylon net.

Both laboratories received two blind-liquid samples, with Cr^{+6} and Zn^{+2}. Stock solutions of pure compounds were prepared from analytical reagents: zinc, $ZnSO_4$ (Baker®); chromium, $K_2Cr_2O_7$ (Analar®). The effective concentrations of metals were determined by atomic absorption spectrometry (Varian Spectra AA, air-acetylene flame) [11]. Traceable certified standards for the analysis of metals were from AccuStandard, Inc. (1000 mg/L standard stock solutions, traceable to National Institute of Standards and Technology, USA). In order to establish the range of concentrations, non-replicate preliminary tests were carried out for each sample with the following dilutions: 0 (control), 0.1, 1, 10, 50 and 100 % (v/v); definitive tests were done using 8-9 dilutions series between 0–2 % for Cr^{+6} solution and between 0-50% for Zn^{+2} solution. Water-only toxicity tests (acute, lethality) were carried out following USEPA standardized protocol [12]. In liquid samples tests, Moderate Hard Water (MHW) was used with the following composition (mg/L): $NaHCO_3$, 96; $CaSO_4$ $2H_2O$, 60; $MgSO_4$, 60; KCl, 4; pH, 7.4-7.8; hardness $CaCO_3$, 80-100) [13]. Three replicate test chambers (300-ml beakers containing 200 ml of test water and 10 test organisms) were used for each test. The number of dead organisms was registered at 96-h exposure. The following parameters were determined at the beginning and the end of all assays: DO, pH, hardness, ammonia and conductivity.

Tested sediments included two unpolluted controls and three contaminated samples from streams of the Pampas region of Argentina. The control sediments were from Juan Blanco and Las Flores streams (laboratories A and B, respectively). The tested sediments for each laboratory included a control, two heavily contaminated (Riachuelo and Canal Oeste) and a moderatly contaminated sample (Lujan River). Sampling point locations are shown in Figure 1. Description of sample sites and chemical information of sediments was previously reported [14]. Ten-day whole-sediment tests were conducted following a modified standardized protocol [12]. Five replicates were used for each sediment sample in laboratories A and B, 100 ml of sediment and 175 ml of overlying water were placed in each replicate, with 10 individuals each. Test organisms were previously separated and acclimatized to test conditions during 48 h, and fed with fish food and boiled lettuce. Test containers were placed in a culture chamber. Temperature and DO were determined daily. Conductivity, pH and hardness from the overlying water were also measured at the beginning and at the end of testing. Measured endpoints were survival and growth (length). Animal sub-samples (n=20) were taken for characterization of the initial group (length). Length measurement was done with a digital caliper (± 0.01 mm). Performance criteria for the control sediment required 80% survival.

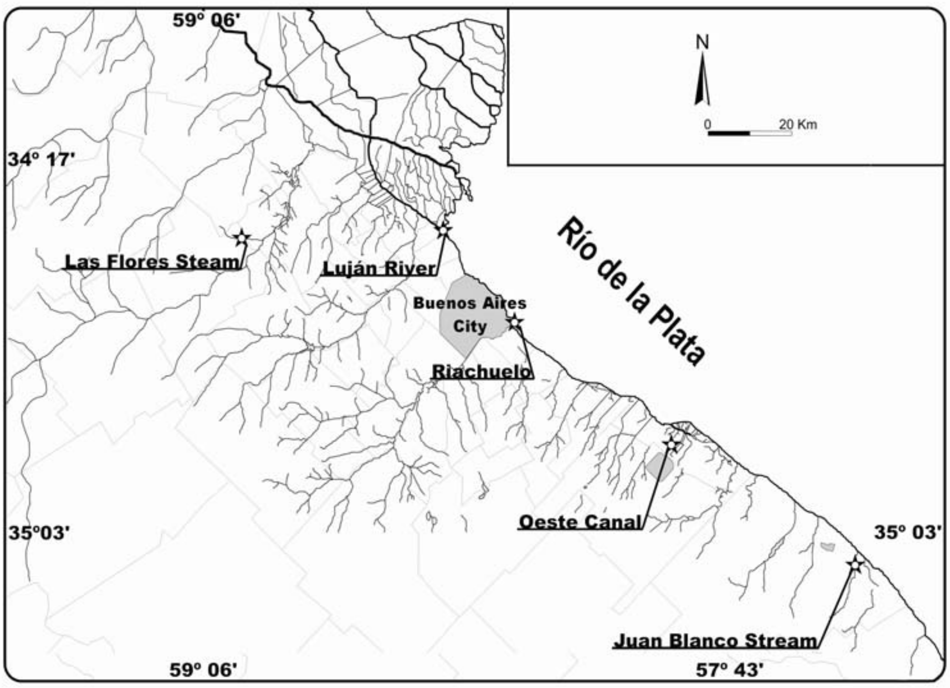

FIGURE 1 - Study area and sampling point locations.

For intercalibration with blind-liquid samples, significance of the regression and correlation coefficients and comparison of two linear regression equations were tested following Zar [15]. The LC_{50} were estimated by fitting data to a Probit model [16] using software from the USEPA [17]. For the whole-sediment test, the statistical significance between treatments and control for growth was analyzed with the t-test [13]; length data were log-transformed before analysis. The significance level was set at $p<0.05$. Statistical analysis of chemical data results and mortality data were performed using principal component analysis (PCA) [18].

3. RESULTS AND DISCUSSION

Both laboratories were made aware of the identity and nominal concentration of blind samples after the test was carried out: Cr^{+6} 50 mg/L and Zn^{+2} 25 mg/L. The values of physicochemical parameters recorded in the tests were within the acceptable values in both laboratories. The LC_{50} 96-h are shown in Table 1, with its respective 95% confidence limits. Control survival of >90% was found in both laboratories. Regression lines comparison shows significant differences in chromium slopes as well as those of zinc ($p = 0.0217$ and 0.0109, respectively). Differences between the two laboratories are not highly significant (α=0.01), as can be seen in LC_{50} values since they were of the same order of magnitude for both metals; this may be due to differences between the cultures of *H. curvispina* in each laboratory.

H. curvispina LC_{50}s 96-h shows the following trend: $Cd^{+2} > Hg^{+2} > Cu^{+2} > Cr^{+6} > Zn^{+2}$, [9, 19, 20]. Comparing *H. curvispina* sensitivity profile to metals with published data for *H. azteca* for Zn, *H. curvispina* and *H. azteca* varied in one order of magnitude [21, 22], as regards Cd, Hg, Cu and Cr, responses in both species are of the same order [22, 23]. Chromium and zinc LC_{50} values for *H. curvispina* were compared among several invertebrates from EPA AQUIRE data base [24]. *H. curvispina* was the most sensitive to both metals among other species, such as amphipods, chironomids (Cr: 1-4 ; Zn: 10-20 mg/L) and *Tubifex tubifex* (Cr: 2.9; Zn: 100 mg/L), whereas sensitivity values were one order of magnitude greater than those found for cladocerans, such as *Daphnia* spp. and *Ceriodaphnia dubia* for both metals.

TABLE 1 - Intercalibration LC_{50} values and 95% confidence limits.

	LC_{50} (mg/L)		Lower limit		Upper limit	
Lab	A	B	A	B	A	B
Chromium	0.20	0.55	0.15	0.47	0.28	0.65
Zinc	2.36	2.19	2.17	0.71	2.54	2.95

In the whole-sediment tests with environmental samples, acceptable control survival was met by the two laboratories. Sediment from Lujan River did not exhibit lethal effects, being survival of >80%; nevertheless, growth (length) was significantly lower in animals exposed to sediments from this stream (Laboratories A and B: p= 0.0001 and p= 0.001, respectively) compared with negative controls. In Riachuelo and Canal Oeste samples, no survival was observed. Table 2 shows survival and growth results whereas Table 3 shows physicochemical parameters measured at the end of testing; in both laboratories dissolved oxygen values remained higher than the required levels (>2.5 mg/L) [12]. Principal component analysis (PCA) (Fig. 1) based on chemical data from Ronco et al. [14] shows a clear differentiation between Canal Oeste and Riachuelo sediments as opposed to the rest. The group of Las Flores, Lujan River and Juan Blanco split from Riachuelo and Canal Oeste by component 1, mainly determined by the majority of the variables. Riachuelo and Canal Oeste are separated from each other by effect of component 2. The PCA did not include pesticides and hydrocarbons, since in the majority of samples these were below the detection limits, and therefore yielding a meaningless PCA. The heavy metal concentrations in the Riachuelo and Canal Oeste samples exceed the probable effect levels (PEL), values provided by the Canadian Council of Ministers of the Environment [25]; this is in agreement with the low survival observed for *H. curvispina.*

TABLE 2 - Survival and length (mean value ± SD; n = 40) for *H. curvispina* in 10-d whole-sediments tests in laboratories A and B.

	Survival (%)		Length (mm)	
Lab	A	B	A	B
Controls	96	98	2.92 (± 0.40)	3.09 (± 0.21)
Río Luján	90	97	2.58 (± 0.37)	2.81 (± 0.22)
Riachuelo	0	0	-	-
Canal Oeste	0	0	-	-

TABLE 3 - Physicochemical parameters in overlying water at the end of exposure time in Laboratories A and B (mean values ± SD; n = 5).

	pH		Conductivity (mS/cm)		Hardness (mg/L $CaCO_3$)	
Site	*A*	*B*	*A*	*B*	*A*	*B*
Las Flores [a]	-	8.7 (±0.03)	-	1.37 (±0.01)	-	200
Juan Blanco [a]	8.1 (±0.04)	-	1.04 (±0.02)	-	180	-
Riachuelo	8.4 (±0.06)	8.5 (±0.09)	1.02 (±0.02)	1.17 (±0.05)	220	100
Canal Oeste	8.2 (± 0.04)	8.5 (± 0.1)	1.22 (±0.04)	1.27 (±0.05)	200	120
Río Luján	8.4 (± 0.10)	8.6 (± 0.06)	0.86 (±0.01)	0.85 (±0.04)	200	140

[a] reference-control samples

Lujan River sediment only induced sublethal effects (Table 2), possibly associated to the presence of pesticides. These values are comparable to those detected in sediments from streams near to areas with high agricultural activity [26]. These results suggest that sublethal endpoints should be included in sediment tests, providing further information by assessing sublethal endpoints in 10-d tests.

Also the 10-d tests could be used for the screening of toxicity of samples before long-term tests are conducted.

The toxicity testing protocols used in this study provided reliable results in agreement with detected pollutants in highly contaminated sediments, allowing the differentiation of those with low levels of contamination. The present study provides relevant information for the validation of *Hyalella curvispina* as a test organism in sediment monitoring studies at regional level.

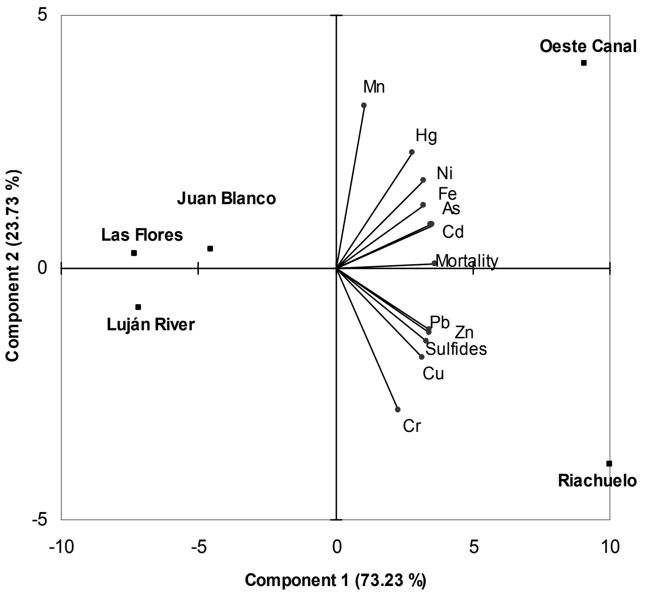

FIGURE 2 – Two-dimension plots from Principal Component Analysis (PCA), with two components explaining 96.96 % of the total variance.

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FEB/ Vol 20/ No 2/ 2011 – pages 372 - 376