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Toxicity of Cadmium, Lead, Mercury and Methyl Parathion on *Euchlanis dilatata* Ehrenberg 1832 (Rotifera: Monogononta)

Juan Carlos Arias-Almeida · Roberto Rico-Martínez

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Abstract Acute toxicity tests with Cd, Pb, Hg, and methyl parathion were developed to compare the sensitivity of the rotifer *Euchlanis dilatata* with other model organisms used in aquatic ecotoxicology. Cd was the most toxic chemical (LC50 = 14.8 μg L^{-1}), while methyl parathion was the least toxic (LC50 = 864.2 μg L^{-1}). *E. dilatata* was more sensitive that other rotifer species, particularly of the genera *Brachionus* and *Lecane*. However, *E. dilatata* was less sensitive to mercury and methyl parathion than the crustacean, *Daphnia magna*. The high sensitivity of *E. dilatata* suggests that it may be an adequate benthic model to use in toxicity assessments of metal-contaminated sediments.

Keywords Aquatic toxicology · Metal toxicity · Pesticide toxicity · LC50 · Rotifer

Metals and pesticides are important chemical contaminants of aquatic environments. They are derived from mining, agro-industrial activities, and emissions from fossil fuel combustion (Fairbrother et al. 2007). They are responsible for adverse effects on biota due to their toxicity, persistence and bioavailability (DeForest et al. 2007), affecting biota's behavior, growth, and reproductive capacity (Fairbrother et al. 2007). Methyl parathion is widely used in Mexico and worldwide to eliminate domestic and agricultural pests (Sarma et al. 2001a; Sarma et al. 2001b). It is highly toxic to aquatic organisms and has been classified as extremely hazardous for the environment by the WHO (2004), and as a restricted-use pesticide by the U.S. Environmental Protection Agency (EPA) (Milam et al. 2004). Its toxicity to rotifers of the genera *Brachionus*, *Lecane* and *Asplanchna* have been documented with reference to the median lethal concentration (LC50) (Fernández-Casalderrey et al. 1992; Pérez-Legaspi et al. 2010). Metal pollution in aquatic ecosystems is also a critical problem in Mexico (Soto-Jiménez and Páez-Osuna 2001). Cadmium, lead, and mercury, are among the non-essential metals of greatest ecotoxicological importance (Castañé et al. 2003), being highly reported as causing impairment to water quality (Reiley 2007). Their adverse effects include alterations of physiological and biochemical processes in invertebrates (Sarma et al. 2000; Juárez-Franco et al. 2007) such as nerve impulse transmission and enzyme activity (Castañé et al. 2003). These metals can strongly compete with calcium, since they have a similar atomic ratio and the same valence (Reiley 2007).

The presence of these pollutants in the water–sediment interface and porewater (Salvadó et al. 2006), restricts the traditional use of planktonic invertebrates (*Daphnia magna*, *Brachionus calyciflorus*) as model organisms to assess sediment toxicity (Pérez-Legaspi and Rico-Martínez 2001). These species could be less affected than non-planktonic rotifers if toxicants have a tendency to settle (Sarma et al. 2001b). *Euchlanis dilatata* is a cosmopolitan, littoral and periphytic species, of large size and slow movement that lives in association with sediments (Segers

J. C. Arias-Almeida (✉)
Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, Delegación Coyoacán, 04510 México City, D.F., México
e-mail: jucaral0608@gmail.com

R. Rico-Martínez
Departamento de Química, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Avenida Universidad 940. Col. Ciudad Universitaria, 20131 Aguascalientes, México
e-mail: rrico@correo.uaa.mx

2008) and submerged vegetation (Walsh 1995). Due to its association with the habitat of the water–sediment interface, *E. dilatata* would appear to be a useful invertebrate species for assessing possible toxic effects of sediment-associated contaminants.

Lethality is a biologically important response with a direct impact on population size. For invertebrates, it is toxicologically estimated as the concentration where 50% mortality of test organisms occurs (LC50 value), usually at exposure times of 24 or 48 h (Milam et al. 2004). Data for rotifers consist primarily of LC50 values for metals, pesticides and herbicides (Sarma et al. 2001b). Snell and Janssen (1995) reviewed the lethal and chronic responses of rotifers exposed to metals and organic compounds. Exposure to cadmium, lead, and mercury has been evaluated with 24-h acute tests in *B. calyciflorus* (Janssen et al. 1994; Sarma et al. 2006; Kegley et al. 2010), *B. patulus* (Sarma et al. 2006; Garcia-Garcia et al. 2007), *B. macracanthus* (Nandini et al. 2007), and *Philodina acuticornis* (Kegley et al. 2010). Pérez-Legaspi and Rico-Martínez (2001) found differential sensitivity in three species of the genus *Lecane* exposed to six metals in 48-h acute tests.

Regarding methyl parathion, some studies have indicated a lethal sensitivity range between 636 μg L^{-1} for *B. angularis* (Gama-Flores et al. 2004) and 8.8 mg L^{-1} for *B. patulus* (Sarma et al. 2001a). Pérez-Legaspi et al. (2010) estimated a 48-h LC50 value of 9.5 mg L^{-1} for this pesticide with the benthic rotifer *Lecane quadridentata*. LC50 values have been obtained for several species of rotifers with emphasis on the genera *Brachionus* and *Lecane*. Since the LC50 value is a relevant index of direct environmental stress and a starting point for ecotoxicological tests, it is important to expand the number of rotifer species in which LC50 values are calculated.

The aim of this study was to compare the lethal sensitivity of the freshwater rotifer *E. dilatata* by exposure to the pesticide methyl parathion and the metals cadmium, lead, and mercury with others model invertebrates used in aquatic ecotoxicology, and to evaluate the potential use of this rotifer as a bioindicator organism for determining toxicity in the water–sediment interface area.

Materials and Methods

The freshwater rotifer *E. dilatata* was collected in a reservoir (21°44′13.7″ N, 102°21′3.3″ W) in the city of Aguascalientes, Mexico. Cultures were established and maintained in Petri dishes with EPA medium (96 mg $NaHCO_3$, 60 mg $CaSO_4.2H_20$, 60 mg $MgSO_4$ and 4 mg KCl per liter of deionized water, US EPA 2002), which is synthetic moderately hard water (80–100 mg $CaCO_3$ L^{-1}, pH 7.5), at a temperature of 25 ± 2°C and a photoperiod of 16:8 h of light:darkness in a bioclimatic chamber (Revco Scientific, Asheville, NC, USA). Cultures were fed every two days with the microalgae *Nannochloris oculata* (1×10^6 cell mL^{-1}, UTEX strain LB2194) grown in Bold's Basal Medium (Nichols 1973). Deionized water was obtained from a Water Pro System (Labconco Co., Kansas City, USA) at 18 MΩ. The hatching percentage of amictic eggs at 24-h under the culture conditions mentioned above was always higher than 95% (cultures in log phase growth), which ensured the availability of neonates for testing. The toxicants evaluated were reference chemicals of the highest purity available: mercury chloride (99.8%, Sigma Co., USA), methyl parathion (1,000 μg mL^{-1} dissolved in acetone, Supelco Co., USA), and atomic absorption standards of cadmium and lead (dissolved in 1% HNO_3, Sigma Co., USA). Exposure concentrations were prepared in EPA medium and then stored at 4°C in the darkness. Methyl parathion was previously dissolved in acetone (J.T. Baker Co., USA) before preparation of stock solutions.

Acute toxicity tests followed the protocol developed by Pérez-Legaspi and Rico-Martínez (2001) with small modifications. Briefly, neonate females less than 24-h-old hatched from amictic eggs were placed in each well of 24-well polystyrene plates (Costar Co., USA) and filled with a test volume of 1 mL. This was a static test of 48-h, without feeding or renewal of the medium. Plates were sealed with transparent adhesive tape to prevent loss of the toxicant and/or of the solvent due to volatilization. We performed range-finding tests for each toxicant and a negative control. Mortality was recorded as immobilized organisms or without motion in the mastax after mechanical stimulation during 20 s. The definitive tests included the following concentrations: $Cd(NO_3)_2$: 0.1, 0.5, 1.0, 50, 100, 250, 500 μg L^{-1}; $Pb(NO_3)_2$: 0.1, 0.5, 50, 100, 250, 1,000, 2,500 μg L^{-1}; $HgCl_2$: 10, 25, 50, 150, 250, 500, 1,000 μg L^{-1}; Methyl parathion: 0.1, 1.0, 5.0, 50, 100, 500, 1,500 μg L^{-1}. Fifty test animals (10 per well, five replicates) were used for each negative control and toxicant concentration. Tests were considered valid only when mortality in the negative control was less than 10%. We included solvent controls of deionized water and 1% HNO_3 for the metal tests, and the highest acetone concentration used for each methyl parathion test to monitor the toxicity due to solvents. Mortality data were analyzed with DL50 software (S.B.I.-I.R.C.T. Montpellier, France), which estimates the LC50 values with Probit Analysis and performs a chi-square to test the validity of the linearity of the regression ($p < 0.05$). It also calculates the 95% confidence limits, the coefficients of determination, and the regression line equation. We used one-way analysis of variance (ANOVA) with Statistica 7.0 software (Statsoft Inc., 2004) to establish significant differences between treatments and controls, and to obtain NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values using Duncan's test.

Actual exposure concentrations of metals were determined through digestion with HNO_3/H_2SO_4 and atomic absorption spectrophotometry, following the recommended analytical method (WPCF, AWWA, APHA 1989): graphite furnace for cadmium (3030E) and lead (3113B), and hydride generation for mercury (3112B). The detection limits with the AAnalyst 100 Spectrometer (PerkinElmer, Inc.) for cadmium, lead, and mercury were 0.05, 1.50, and 0.30 μg L^{-1}, respectively. To determinate actual values of methyl parathion, we used High Performance Liquid Chromatography with a VARIAN Chromatograph model CP3800 (CP-Sil5 column). The analytical method was 8321A (US EPA 1989) with a detection limit of 0.10 μg L^{-1}.

Results and Discussion

Although the LC50 value is used as an endpoint to evaluate the effects of anthropogenic toxicants in aquatic environments, there is scarce information on 48-h standardized tests that use rotifer neonates hatched from asexual eggs. Most of the available information on LC50 values in rotifers comes from 24-h standardized tests with neonates hatched from cysts, especially of the genus *Brachionus* (Snell and Janssen 1995; Preston and Snell 2001; Huang et al. 2007; Snell and Hicks 2009; Kyriakopoulou et al. 2009). In this study, the acute response of *E. dilatata* varied widely amongst the studied test chemicals, with a high sensitivity to cadmium, followed by an intermediate sensitivity for lead and mercury chloride, and a low sensitivity for methyl parathion. The 48-h LC50 values, estimated with analytically measured concentrations, ranged from 14.8 to 864.2 μg L^{-1} (Table 1). The coefficients of determination (r^2) varied between 0.74 ($HgCl_2$) to 0.97 ($Pb[NO_3]_2$), which indicates a good adjustment of the mortality data (probit units) in response to the logarithm ($\log_{10}$) of the actual concentrations for each toxicant assessed (Table 1). The mean percentage mortality for deionized water, acetone and 1% HNO_3 was 1.7 ± 4.1, 3.3 ± 5.2, and 1.7 ± 4.1 (mean ± one SD, n = 6), respectively. The mortality recorded in each solvent control was less than 10%, which validates the results of the acute toxicity tests. The recovery efficiency for cadmium, lead, and mercury chloride was 82.1 ± 7.1%, 90.5 ± 1.8%, and 74.4 ± 8.5% (mean ± one SD, n = 6), respectively. For methyl parathion, we obtained a recovery efficiency of 93.8 ± 13.5% (n = 10).

The 48-h LC50 values of *E. dilatata* were usually lower than the LC50 values for other rotifer species. For instance, the LC50 value of cadmium for *E. dilatata* is lower than the LC50 values for *B. calyciflorus* and *B. plicatilis*, which ranged between 0.81 and 1.30 mg L^{-1} and between 39.0 and 56.8 mg L^{-1}, respectively (Snell and Janssen 1995). Also, other species were reported to be less sensitive to cadmium than *E. dilatata*, such as *B. patulus* (0.09 mg L^{-1}, Sarma et al. 2000), *B. macracanthus* (0.19 mg L^{-1}, Nandini et al. 2007), and *B. havanaensis* (0.41 mg L^{-1}, Juárez-Franco et al. 2007). The value obtained with our test species compares well with that of McDaniel and Snell (1999), who reported a 24-h LC50 value of 10 μg L^{-1} for *E. dilatata* using a similar test protocol. Our test strain had a higher sensitivity to cadmium when compared to other species studied by those authors, ranging from 2-fold (*Trichocerca pusilla*, 30 μg L^{-1}) to 18-fold (*B. calyciflorus*, 270 μg L^{-1}). Regarding Pb, *B. plicatilis* and *B. calyciflorus* with LC50 > 4.0 mg L^{-1} (Snell and Janssen 1995) and *B. patulus* with LC50 of 6.15 mg L^{-1} (Garcia-Garcia et al. 2007), were less sensitive than *E. dilatata* (Table 1). Cadmium and lead LC50 values of *E. dilatata* are lower than those of three *Lecane* species (Pérez-Legaspi and Rico-Martínez 2001). *E. dilatata* is 4-fold more sensitive than *L. luna* for lead and 15-fold more sensitive than *L. hamata* for cadmium. For mercury chloride, *E. dilatata* showed a moderate sensitivity. Some species such as *B. calyciflorus* and *B. plicatilis* have LC50 values around 60 μg L^{-1} (Snell and Janssen 1995), while *L. quadridentata* and *L. hamata* are 3 to 11-fold less sensitive (Pérez-Legaspi and Rico-Martínez 2001) than *E. dilatata.* These results indicate existence of interspecific variation in the sensitivity to some toxicants, even among organisms that share the same habitat. It also suggests

Table 1 Acute toxicity (48-h, n = 5) of metals and methyl parathion on the freshwater rotifer *Euchlanis dilatata*

Toxicant	NOEC	LOEC	LC50	CL95	r^2	Regression equation
Cd	0.1	0.5	14.8	5.7–37.7	0.95	$y = 0.6478x + 4.3124$
Hg	10.0	25.0	122.0	88.5–167.4	0.74	$y = 0.6239x + 3.5162$
Pb	0.1	0.5	35.3	13.5–91.4	0.97	$y = 0.5508x + 4.2545$
Methyl parathion	1.0	5.0	864.2	449.8–1,646	0.84	$y = 0.5296x + 3.4696$

NOEC No Observed Effect Concentration, *LOEC* Lowest Observed Effect Concentration, *LC50* Lethal concentration for 50% tested organisms, *CL95* Confidence limits 95% for LC50 value, r^2 Coefficient of determination, *y* Probit units, *x* decimal logarithm of actual concentrations. All values in μg L^{-1}

that *E. dilatata* may be a suitable test organism for assessing the presence of metals in the littoral zone and water–sediment interface of aquatic environments.

Our LC50 value for methyl parathion is more sensitive than that estimated for *B. calyciflorus* (29.19 mg L^{-1}, Fernández-Casalderrey et al. 1992), *B. patulus* (24-h LC50: 8.8–10.4 mg L^{-1} depending on food density, Sarma et al. 2001a) or *L. quadridentata* (48-h LC50: 9.5 mg L^{-1}, Pérez-Legaspi et al. 2010). Our *E. dilatata* LC50 value (864.2 µg L^{-1}) was less sensitive than that for *B. angularis* of 636 µg L^{-1} (Gama-Flores et al. 2004). These results could indicate an intermediate lethal sensitivity of *E. dilatata* among rotifers for this pesticide.

Daphnia magna is a cladoceran that is widely used in ecotoxicological studies in the U.S. and Canada. However, it has never been found in any reservoir of Mexico. Most of the freshwater reservoirs of Mexico are found in tropical areas where the mean annual temperature is 25°C or higher, and the *D. magna* toxicity tests are performed at lower temperatures (15–20°C). These temperatures do not reflect the natural conditions of Mexican aquatic ecosystems (Martínez-Jerónimo and Muñoz-Mejía 2007). In a comparison of cadmium sensitivity between *E. dilatata* and *D. magna* or *D. pulex* (Rico-Martínez et al. 2000), the rotifer was 12-fold more sensitive than the two cladoceran species. However, the cladoceran *Simocephalus vetulus* was almost as sensitive to Cd (30 µg L^{-1}) as *E. dilatata* (14.8 µg L^{-1}). In the case of mercury, *D. magna* has a 24-h LC50 of 12 µg L^{-1}, which is lower than that for *E. dilatata* (Table 1), confirming reports that describe this cladoceran as one of the most sensitive species to Hg (Atici et al. 2008). Similarly, others authors have found cladocerans to be more sensitive than *E. dilatata* to methyl parathion. Mangas-Ramírez et al. (2004) obtained a 48-h LC50 value of 12.3 µg L^{-1} for *D. magna*, and 24-h LC50 values of 0.37 and 50 µg L^{-1} for *Simocephalus* sp. and *Moina macrocopa*, respectively.

Although LC50 values allow the determination of the relative sensitivity to toxicants among test species, the concentrations found in *E. dilatata* must not be assumed as constants. Many biotic factors, such as age, nutritional state, metabolism, and history of exposure influence such values. Abiotic factors, such as temperature, pH, photoperiod, and hardness may also influence the obtained LC50 values. Therefore, LC50 values and their confidence limits are only valid for that particular species (or strain) and the exposure time assessed under the specific conditions utilized. *E. dilatata* showed greater sensitivity to the lethal effects of cadmium and lead than the aquatic invertebrate species traditionally used in toxicity testing. Its sensitivity towards mercury chloride and methyl parathion was moderate when compared to other cladoceran and rotifer species. The results of our study suggest that *E. dilatata* may serve as a sensitive test organism for toxicological assessment of water–sediment interface where metals and pesticides are present.

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