Lethal effects of five metals on the freshwater rotifers *Asplanchna brigthwellii* and *Brachionus calyciflorus*

Efectos letales de cinco metales en los rotíferos dulceacuícolas *Asplanchna brigthwellii* y *Brachionus calyciflorus*

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Santos-Medrano G. E and R. Rico-Martínez. 2013. Lethal effects of five metals on the freshwater rotifers *Asplanchna brigthwellii* and *Brachionus calyciflorus*. *Hidrobiológic*a 23 (1): 82-86.

ABSTRACT

Acute toxicity tests of five metals (aluminum, cadmium, iron, lead, zinc) were performed to determine LC50 values in two species of freshwater rotifers: *Asplanchna brigthwellii* and its prey *Brachionus calyciflorus*. We conducted the tests using neonates less than 24 hr-old, each test consisted of five replicates, negative control and five metal concentrations (Al, Cd, Fe, Pb, Zn). We found that the prey rotifer *B. calyciflorus* was more sensitive to Al, Cd, Pb and Fe than the predator rotifer *A. brightwellii*. For both rotifers Cd was the most toxic of the five metals. It was established that the strain of *B. calyciflorus* studied is sensitive when compared with other *B. calyciflorus* strains and other species and genera of the family Brachionidae. In the other hand, LC50 values of *A. brigthwellii* are compared with rotifer and copepod predators.

Key words: Acute toxicity, aquatic toxicology, LC50, metal toxicity, trophic interactions.

RESUMEN

Se realizaron pruebas de toxicidad aguda con cinco metales (aluminio, cadmio, hierro, plomo, zinc) para determinar los valores CL50 en dos especies de rotíferos dulceacuícolas: *Asplanchna brigthwellii* y su presa *Brachionus calyciflorus*. Para las pruebas se usaron neonatos menores de 24 horas, cada prueba consistió de cinco replicas, un control negativo y cinco concentraciones (Al, Cd, Fe, Pb, Zn). Se determinó que el rotífero *B. calyciflorus* fue más sensible al Al, Cd, Pb y Fe que su rotífero depredador *A. brigthwellii*. Para ambas especies el Cd fue el metal más tóxico de los cinco evaluados. Se comprobó que la cepa estudiada de *B. calyciflorus* es sensible al compararla con otras cepas de *B. calyciflorus* y otros géneros y especies de la familia Brachionidae. También se compararon los valores de CL50 del rotífero *A. brigthwellii* con otros rotíferos y copépodos depredadores.

Palabras clave: CL50, interacciones tróficas, toxicidad aguda, toxicidad de metales, toxicología acuática.

INTRODUCTION

The assessment of metals effects in water is important due to their high toxicity and persistence, and rapid uptake by organisms. Metals are difficult to eliminate in the environment, since organisms incorporate them into their tissues and transferred to predators (Förstner & Prosi, 1979). Toxicity is proportional to the amount of metal absorbed by aquatic organisms. A metal dissolved in ionic form can be absorbed more easily than in its elemental form, while the reduced form increases the likely metal toxicity due to

oxidation and retention in different organs. Usually metals are not removed from aquatic ecosystems by natural processes because they are not biodegradable (Förstner & Wittmann, 1981). Metals tend to form associations with minerals (carbonates, sulfates) as well as with organic substances, by phenomena of ion exchange, adsorption, chelation, formation of chemical combinations, etc., and therefore accumulate in the environment, mainly in the sediments of rivers and lakes (Förstner & Wittmann, 1981; Dekov *et al.*, 1998). Metals are indicators of the ecological quality of water because of their high toxicity and bioaccumulative behavior (Purves, 1985). Rotifers are common in freshwater zooplankton communities. Species of the genus *Brachionus* are sensitive to different toxicants (metals and organic compounds), consequently they can be used as standard toxicity test organisms. On the other hand, *Asplanchna* is an important predator on smaller zooplankton; rotifers, ciliates and cladocerans (Wallace *et al.*, 2006). Both genera are important organisms in freshwater food webs.

Toxicity tests using rotifers are becoming an interesting alternative to traditional methods using cladocerans. These tests measure various parameters such as mortality, reproduction, behavior, physiology, biochemistry or molecular biology, and microcosms (Snell & Janssen, 1995). As a starting point of a broader research it has been proposed to assess the toxicity in a battery of freshwater organisms of different trophic levels and to determine the 24-h LC50 (median lethal concentration) values of aluminum, cadmium, iron, lead and zinc in the freshwater predator rotifer *Asplanchna brigthwellii* (Gosse 1850) and its rotifer prey *Brachionus calyciflorus,* Pallas 1766 (Gilbert, 1967). Our hypothesis states than since *B. calyciflorus* is located in a lower trophic level it should be more sensitive to toxicant effects of metals than *A. brigthwellii* Gosse 1850 the predator of *B. calyciflorus.*

MATERIALS AND METHODS

Asplanchna brightwellii females carrying embryos were placed in a 24-well polystyrene plate (one female per well to avoid cannibalism), then incubated at 25 °C, photoperiod 16:8 (l:d) in absence of food. Neonates less than 24-hr-old were collected next day. Ten neonate females were placed in bottles of 20 ml total volume; with a test volume of 5 ml. On the other hand, *Brachionus calyciflorus* neonates less than 24-hr-old hatching from cysts were placed in a 24-well polystyrene plate (ten neonates per well) with a test volume of 1 ml. For both species, each test consisted of five replicates, negative control EPA medium pH 7.5 (USEPA, 1985), and five metal concentrations (atomic absorption standards of aluminum, cadmium, iron, lead, zinc from Sigma-Aldrich Co). All metals were in 2% nitric acid solution). In order to derive LC50 for all metals, we performed a range-finding test. For the definitive test, five toxicant concentrations are chosen covering the 0 and 100% mortality concentration range (Table 1) determined in the range-finding test. The animals were Incubated for 24 hours at 25 °C, photoperiod 16:8 (l:d) in absence of food. At the end of incubation, dead animals were counted using a stereomicroscope. A one-way analysis or variance (ANOVA) and Duncan's test were calculated to compare mortality percentages for each toxicant concentration to that of the control. From these data the NOEC (No observed effect concentration) and LOEC (Lowest observed effect concentration) values were calculated. The LC50 values were calculated using regression between probit units and the logarithm of each toxicant concentration using the software Statistica 6.0 (StatSoft Inc., 2001).

RESULTS

The range of LC50 values for *A. brightwellii* were between 0.146-0.358. Cadmium was the most toxic of the five metals investigated with a LC50 value of 0.146. On the other hand, Fe was the least toxic metal with a LC50 value of 0.358 (Table 2).The highest NOEC values was 0.5 (Fe) and the lowest 0.1 (Al and Cd). The highest LOEC values was 1.0 (Fe) and the lowest 0.25 (Cd) (Table 2). The range of LC50 for *B. calyciflorus* were 0.094-0.324. Cadmium was again the most toxic metal and zinc was the least toxic one. The highest NOEC was Pb (0.25) and the lowest Al (0.01) (Table 3). The highest LOEC was Fe (1.0) and the lowest Al (0.05). All values were in mgl^{-1}.

We found that *B. calyciflorus* was more sensitive to all metals, except Zn, than *A. brightwellii* when the LC50 values were

Table 1. Metal nominal concentrations for each rotifer toxicity test.

Metal	*B. calicyflorus*	*A. brightwellii*
Al	0.01, 0.05, 0.25, 1.0, 1.75	0.1, 0.5, 1.0, 1.5, 2.0
Cd	0.5, 0.10, 0.25, 0.50, 0.75	0.1, 0.25, 0.50, 0.75, 1.0
Fe	0.10, 1.0, 1.75, 2.50, 3.0	0.5, 1.0, 2.0, 2.5, 3.0
Pb	0.25, 0.75, 1.0, 1.5, 2.0	0.5, 1.5, 2.0, 2.5, 3.0
Zn	0.05, 0.50, 1.25, 2.50, 3.5	0.5, 1.0, 1.5, 2.0, 2.5

All values are in mgl^{-1}.

Table 2. Analysis of the acute toxicity test performed on the rotifer *Asplanchna brightwelii* with five metals.

Metal	LC50	NOEC	LOEC	r^2	CV%	CL
Cd	0.146	0.1	0.25	0.70	44.29	0.076-0.280
Al	0.174	0.1	0.5	0.76	43.68	0.089-0.338
Zn	0.222	0.5	1.0	0.68	38.96	0.099-0.498
Pb	0.318	<0.5	0.5	0.70	39.46	0.144-0.706
Fe	0.358	0.5	1.0	0.66	44.89	0.159-0.810

LC50 = Lethal concentration where 50% of animals die. NOEC = No Observed Effect Concentration. LOEC = Lowest Observed Effect Concentration. r^2 = *C*oefficient of determination. CV = Coefficients of variation. CL = 95% Confidence limits for the LC50 values. $p > 0.05$. All values are in mgl^{-1}.

Table 3. Analysis of the acute toxicity test performed on the rotifer *Brachionus calyciflorus* with five metals.

Metal	LC50	NOEC	LOEC	r^2	CV%	CL
Cd	0.094	0.10	0.25	0.70	52.68	0.051-0.174
Al	0.105	0.01	0.05	0.79	46.25	0.056-0.197
Pb	0.248	0.25	0.75	0.67	81.45	0.117-0.530
Fe	0.232	0.10	1.0	0.77	42.89	0.114-0.471
Zn	0.324	0.05	0.5	0.71	45.96	0.149-0.702

LC50 = Lethal concentration where 50% of animals die. NOEC = No Observed Effect Concentration. LOEC = Lowest Observed Effect Concentration. r^2 = coefficient of determination. CV = Coefficients of variation. CL = 95% Confidence limits for the LC50 values. $p > 0.05$. All values are in mgl^{-1}.

compared (compare Tables 2 and 3). Regarding NOEC values *B. calyciflorus* was more sensitive to all metals than *A. brightwellii*. When LOEC values were compared *B. calyciflorus* was more sensitive to Al, Cd and Zn than *A. brightwellii*. There were the same LOEC values for Fe, and *A. brightwellii* was more sensitive than *B. calyciflorus* for Pb (Compare Tables 2 and 3).

DISSCUSION

We found enough data on the literature to compare the sensitivity (24-h LC50) of *B. calyciflorus* with other species of the family *Brachionidae* (Table 4). For Al *B. calyciflorus* (0.105 mgl^{-1}, this work) was more sensitive than the strain used by Snell *et al.* (1991b) (>3.0 mgl^{-1}), the same effect was observed with Pb (*B. calyciflorus* 0.77 mg l^{-1}, this work), (Snell *et al.,* 1991a), *B. plicatilis* Müller, 1786 (4.0 mgl^{-1} Snell *et al*., 1991b) and *B. patulus* Müller, 1776 (6.15 mgl^{-1} García-García *et al*., 2007) (Table 4).

Cadmium was the metal that showed greater variation in the LC50 values in the family *Brachionidae* with a range of 0.09 mgl^{-1} (*B. patulus*, Sarma *et al*., 2006) –39 mgl^{-1} (*B. plicatilis*, Snell *et al.,* 1991a). In addition LC50 values of Zn (*B. calicyflorus* 0.324 mgl^{-1}, this work) was more sensitive than others strains of *B. calyciflorus*, 1.3 mgl^{-1} (Snell *et al.* 1991b), 1.32 mgl^{-1} (Couillard *et al*., 1989), and 1.67 mgl^{-1} (Nelson & Rolin, 1998) (Table 4).

Anuraeopsis fissa Gosse, 1851 was the most sensitive to Zn (0.31 mgl^{-1}) its CL50 value being similar to that of *B. calyciflorus* (0.324 mgl^{-1}) used in this work (Table 4).

We found enough data on the literature to compare the sensitivity (24-h LC50 and EC50) of *A. brightwellii* with other copepod or rotifer predator species preying on rotifers (Table 5). *A. brightwellii* (0.174 mgl^{-1}, this work) was more sensitive to Al than *Acanthocyclops vernalis* Fischer, 1853 (Havens, 1991; Al LC50 value = 0.54 mgl^{-1}) and *Mesocyclops edax* Forbes 1891 (Havens, 1991) with a very close value (0.58 mgl^{-1}) (Table 5). The toxic effects of Cd were LC50 value for *A. brightwellii* (0.146 mgl^{-1}) and *Megacyclops viridis* Jurine, 1820 (LC50 0.0005 mgl^{-1}, Braginskij & Shcherban, 1979) have a difference of 292-fold lower than *A. brightwellii* (this work, see table 5) otherwise, it showed a value very close to *Eurytemora affinis* Poppe, 1880(LC50 0.13 mgl^{-1}) . The EC50 value (0.05 mgl^{-1}) of the copepod *T. prasinus mexicanus* Kiefer, 1938 (Lalande & Pinel-Alloul, 1986) was 4.44-fold lower than the LC50 (0.222 mgl^{-1}) value for *A. brigthwellii* for Zn (Table 5).

Table 4. Differences in sensitivity among results for the rotifer *Brachionus calyciflorus* compared with another species of the family *Brachionidae*.

Metal	Species	LC50	Source
Al	***B. calyciflorus***	**0.105**	**This work**
Al	*B. calyciflorus*	>3.0	(Snell *et al*., 1991b)
Cd	***B. calyciflorus***	**0.094**	**This work**
Cd	*B. calyciflorus*	1.3	(Snell *et al*., 1991b)
Cd	*B. plicatilis*	39	(Snell *et al*., 1991a)
Cd	*B. rubens*	0.81	(Snell & Persoone, 1989)
Cd	*B. macracanthus*	0.19	(Sarma *et al*., 2007a)
Cd	*B. havanaensis*	0.41	(Juárez-Franco *et al*., 2007)
Cd	*B. calyciflorus*	0.18	(Sarma *et al*., 2006)
Cd	*B. patulus*	0.09	(Sarma *et al*., 2006)
Pb	***B. calyciflorus***	**0.248**	**This work**
Pb	*B. calyciflorus*	> 4.0	(Snell *et al*., 1991a)
Pb	*B. plicatilis*	4	(Snell *et al*., 1991b)
Pb	*B. patulus*	6.15	(García-García *et al*., 2007)
Zn	***B. calyciflorus***	**0.324**	**This work**
Zn	*B. calyciflorus*	1.30	(Snell *et al*., 1991b)
Zn	*B. calyciflorus*	1.32	(Couillard *et al*., 1989)
Zn	*B. calyciflorus*	1.65	(Nelson & Rolin, 1998)
Zn	*B. havanaensis*	2.27	(Juárez-Franco *et al*., 2007)
Zn	*Anuraeopsis fissa*	0.31	(Sarma *et al*., 2007b)
Zn	*B. rubens*	0.55	(Sarma *et al*., 2007b)

All values are in mgl^{-1}. All bioassays were conducted at 24 h and absence of food.

The high toxicity of Al is influenced by the aqueous chemistry, and is extremely complex. The changes in the molecular form or concentration of Al are largely dependent on its pH. Other factors include complexes with ligands (Wauer *et al*., 2004). While Zn is an essential trace element for living organisms, it was more toxic than Pb to *A. brightwellii.*

When we compared rotifer predators, *A. brigthwellii* was more sensitive than *A. vernalis* (copepod) to Al. Our hypothesis was partially fulfilled; *B. calyciflorus* was more sensitive to four metals (Al, Cd, Fe, Pb) than *A. brigthwellii.*

Table 5. Differences in sensitivity among results for the rotifer *Asplanchna brightwellii* compared with another zooplankton copepod and rotifer predator species.

Metal	Specie	Toxicity endpoint	Source	Rotifer prey (source)
Al	***Ab***	**LC50 0.174**	**This work**	*Bc* (Gilbert, 1967)
Al	*Av*	LC50 0.54*	(Havens, 1991)	*Kc* (Ramos-Rodríguez & Conde-Porcuna, 2004)
Al	*Me*	LC50 0.58*	(Havens, 1991)	*Bc* (Williamson & Gilbert, 1980)
Cd	***Ab***	**LC50 0.146**	**This work**	*Bc* (Gilbert, 1967)
Cd	*Mv*	LC50 0.0005****	(Braginskij & Shcherban, 1979)	*P* sp. (Marten & Reid, 2007)
Cd	*Ea*	LC50 0.13**	(Sullivan, *et al*., 1983)	*Fl, Kc* (Yoshida *et al*., 2000)
Cd	*Tpm*	EC50 0.14***	(Lalande & Pinel-Alloul, 1986)	*Kc* (Stemberger & Gilbert, 1984)
Pb	***Ab***	**LC50 0.318**	**This work**	*Bc* (Gilbert, 1967)
Zn	***Ab***	**LC50 0.222**	**This work**	*Bc* (Gilbert, 1967)
Zn	*Tpm*	EC50 0.05***	(Lalande & Pinel-Alloul, 1986)	*Kc* (Stemberger & Gilbert, 1984)

Abbreviations: *Ab = A. brigthwellii. Av = Acantocyclops vernalis. Ai = A. intermedia. Bc = B. calyciflorus. Ea = Eurytemora affinis. Fl = Filinia longiseta. Kc = Keratella cochlearis*. (*Me = Mesocyclops edax. Mv = Megacyclops viridis. Psp = Philodina* sp. *Tpm = Tropocyclops prasinus mexicanus*. All values are in mgl^{-1}. * = 24h, ** = 96h, *** = 2 days,**** = 3 days.

ACKNOWLEDGMENTS

We thank to Dr. F. J. Flores-Tena for his comments and recommendations. Thanks to the National Council for Science and Technology for a grant (CONACYT Ref. 209263).

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Recibido: 9 de septiembre del 2011.

Aceptado: 07 de enero del 2013.