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Differential effects on the toxicity and bioconcentration of hexavalent and trivalent chromium on the rotifer *Lecane papuana* (Murray, 1913) (Monogononta: Lecanidae)

Efectos diferenciales en la toxicidad y bioconcentración de Cromo hexavalente y trivalente en el rotífero *Lecane papuana* (Murray, 1913) (Monogononta: Lecanidae)

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ABSTRACT

Background: While naturally occurring, heavy metals such as chromium, lead, and mercury also reach aquatic environments via anthropogenic activities, sometimes at alarming concentrations thereby altering the dynamics of the communities. Chromium, which is present in the discharge from automotive and tannery industries, occurs in two stable forms: trivalent (Cr III) and hexavalent (Cr VI). Because these forms differ in their chemical properties, their bioavailability differs and, as a result, so does their effects on organisms. **Goals:** The aim of our study was to assess effects of both Cr III and Cr VI on the rotifer *Lecane papuana* (Murray, 1913) by determining how these forms affect the demographic parameters of survival (l_x) and fecundity (m_x). **Methods:** we performed 48-h acute and 5-d chronic toxicity tests on both forms of chromium. In addition, we determined the bioconcentration factor and metal body burden after 24-h exposure to Cr III and Cr VI. According to their respective LC50 values our results indicate that Cr III was less toxic than Cr VI (Cr III = 2.613 mg/L; Cr VI = 0.177 mg/L). **Results**: Intrinsic growth rate was significantly affected by Cr III, while Cr VI caused no significant changes, but only at 0.0885 mg/L, a concentration representing 0.5 times of its LC_{50} value. Although Cr III was not as toxic as Cr VI, our bioconcentration experiments demonstrated that *L. papuana* accumulated more Cr III than Cr VI and did so at concentrations of environmental concern.

Keywords: alternative test organisms, bioconcentration factor (BCF), intrinsic growth rate, lethal median concentration (LC_{50}), metals.

RESUMEN

Antecedentes: Si bien, los metales pesados como el cromo, el plomo y el mercurio se encuentran de forma natural, también llegan a los ambientes acuáticos a través de actividades antropogénicas, a veces en concentraciones alarmantes, alterando así la dinámica de las comunidades. El cromo, que está presente en los vertidos de las industrias automotrices y curtiduría, se presenta en dos formas estables: trivalente (Cr III) y hexavalente (Cr VI). Debido a que éstas formas difieren en sus propiedades químicas, su biodisponibilidad es distinta, y como resultado, también los efectos sobre los organismos. **Objetivos:** El objetivo de nuestro estudio fue evaluar los efectos de Cr III y Cr VI en el rotífero *Lecane papuana* (Murray, 1913) determinando cómo estas formas afectan los parámetros demográficos de supervivencia (l_x) y fecundidad (m_x). **Métodos:** Realizamos pruebas de toxicidad aguda de 48-h y crónicas de 5-d en ambas formas de cromo. Además, se obtuvo el factor de bioconcentración y la carga corporal de metal después de 24 h de exposición a Cr III y Cr VI. **Resultados:** Respecto a los valores de CL50, nuestros resultados indican que Cr III fue menos tóxico que Cr VI (Cr III = 2.613 mg/L; Cr VI = 0.177 mg/L). La tasa intrínseca de crecimiento se vio significativamente afectada por Cr III, mientras que Cr VI no mostró cambios significativos, solo a 0.0885 mg/L cuya concentración representa 0.5X de su CL50. Aunque Cr III no fue tan tóxico como Cr VI, nuestros experimentos de bioconcentración demostraron que *L. papuana* acumuló más Cr III que Cr VI, y lo hizo en concentraciones ambientales relevantes.

Palabras clave: concentración letal media (CL50), factor de bioconcentración (BCF), metales, organismos de prueba alternativos, tasa intrínseca de crecimiento.

INTRODUCTION

Heavy metal pollution has developed in many countries due to unregulated or illegal discharges to the soil or water systems (He *et al.,* 2015). The sources of these include byproducts from chemical manufacturing (alloys, ornamental plating, green color glass, aluminum anodization, and oxidant agent in diverse chemical reactions), metallurgy (metal fabrication and industrial mining), and production of refractory materials (Jacobs and Testa, 2004). In the environment, chromium occurs in two stable redox states, chromium (III) (Cr III) and chromium (VI) (Cr VI). However, they differ in their bioavailability and mobility (Aharchaou *et al.,* 2017). While Cr III is present in the soil where it is available as a micronutrient for plants, Cr VI is more toxic and considered a human carcinogen (ATSDR, 2012).

Cr III has a low solubility and low mobility, forms complexes with organic matter in soils and waters, and is considered relatively innocuous (Fendorf, 1995)this paper describes surface reactions that influence Cr chemistry in soils. Specifically, retention reactions of Cr(III. Nonetheless, its presence in water results from the release of Cr VI in wastewater and its subsequent biotransformation by both bacteria and eukaryotic cells (Norseth, 1986). Thereafter, in Cr VI-contaminated waters the presence of organic-Cr III complexes must be assessed because such complexes represent the main source for Cr III mobility and thus, the intoxication of exposed biota. Currently, information about the fate and toxicity of Cr III and its organic complexes is limited (Chatterjee & Luo 2010). Nevertheless, while Cr III plays an important role as an essential trace metal in plant metabolism (Bielicka *et al.,* 2005), its role in animals remains controversial (Di Bona *et al.,* 2011; Vincent, 2017).

These two forms of Cr differ in their solubility and mobility. In comparison to Cr III, Cr VI exhibits higher solubility and greater mobility and it because of the active sulfate transporter it through pass through biological membranes (Pereira *et al.,* 2008). Besides Cr VI uptake via sulfate transporter, recent references highlight Cr VI-induced over-expression of several ABC transporters (Feng *et al.,* 2018). Cr VI is considered the most toxic form of chromium due to its strong oxidant power; once it has passed through the cell's membrane Cr VI initiates complex mechanisms involving different biochemical pathways and multiple targets (Rudolf & ervinka, 2006). Althought Cr VI is reduced by enzymatic and non-enzymatic reactions to produce less reactive forms like Cr III and CrV, such reduction reactions produce reactive oxygen species (ROS) that alter the redox environment within the organism, thereby inducing oxidative stress (Arzate-Cárdenas & Martínez-Jerónimo, 2011).

With at least 2000 species rotifers are a very diverse group of micrometazoans that play significant roles in aquatic ecosystems in both the microbial loop and classic food webs (Segers, 2007; Wallace *et al.,* 2015). Rotifers have been used for ecotoxicological studies because of their ease to culture and maintenance, their parthenogenetic mode of reproduction, their short life cycle and generation time, their relatively high growth rates, and their wide geographical distribution (Sarma *et al.,* 2006; Segers, 1996; Pérez-Legaspi & Rico-Martínez, 2001).

The genus *Lecane* (Family Lecanidae) is found in shallow waters, littoral areas, and eutrophic environments (Keppeler *et al.,* 2010). While many species of *Lecane* are cosmopolitan, depending on the zoogeographical region between 6.5 to 22% are endemic (De Manuel, 1994; Segers, 1996). Besides their geographic distribution some *Lecane* spp. are used in activated sludge systems, to improve the quality of the pro-

cess and removal of organic contaminants (Fiałkowska & Pajdak-Stós, 2008). Pérez-Legaspi & Rico-Martínez, (2001) used three *Lecane* species: *L. hamata* (Stokes, 1896), *L. luna* (Müller, 1776), and *L. quadridentata* (Ehrenberg, 1832), to assess the effect of several chemical compounds and suggested these rotifers as alternatives test species. Klimek *et al.,* (2013) have suggested that *Lecane inermis* (Bryce, 1892) could represent a better option in toxicological evaluations because is more sensitive than species used in the more commonly used *Brachionus* test.

The process of bioconcentration begins when a chemical is absorbed by an organism from the environment through its respiratory or dermal surfaces or through ingestion; concentration of the substance increases with subsequent uptake. The degree to which bioconcentration occurs over the concentration in the environment is expressed as the bioconcentration factor (BCF) and can only be measured under controlled conditions in which dietary intake of the chemical is not included (Arnot & Gobas, 2011). Acute and chronic toxicity tests are helpful to assess the impact of environmental pollutants. In the last few years, bioconcentration has been used to allow better assessment of toxicity together with toxicological tests (Gagneten *et al.,* 2009). Bioconcentration tests using rotifers as model organisms is advantageous because these invertebrates are easily cultured, their life cycles of are short, and their life history parameters are easily determined. Moreover, the stationary stage of analytes (like pesticides and semiochemicals) are reached in a few days (Rivera-Dávila *et al.,* 2021). Lethal Body Burden (LBB) is defined as the body burden in micrograms (μg) per body weight or mmol/kg at which a specific toxic effect is detected: e.g., mortality, reduction in reproduction, enzymatic inhibition. Some authors have applied LBB to evaluate toxicity of compounds and metals in rotifers (Hernández-Flores *et al.,* 2020; van Wezel *et al.,* 1995).

The maximum limits of chromium in water for the protection of aquatic biota and drinking water quality are set in specific guidelines. However, the Mexican regulations are set higher than those of several other countries (DOF, 2022). For instance, the U.S. Environmental protection agency has a standard of 0.1 mg/L (USEPA, 2008); in Canada and Europe drinking water guideline for chromium is a maximum acceptable concentration of 0.05 mg/L (Government of Canada, 2018; HBM4EU, 2020). The Australian Government (2011) has established 0.003 mg/L (for Cr III) and 0.001 mg/L (for Cr VI).

Given the many variations in international governmental norms and legislation dealing with Cr III and Cr VI in water, and taking into consideration the role of both Cr species affecting different trophic levels in freshwater ecosystems and the quality of drinking water in several regions of Mexico; this contribution set a goal of analyzing how Cr III and Cr VI affect the demographic response of *Lecane papuana* (a zooplanktonic native species of Mexico), using acute and chronic tests, and determined the Bioconcentration Factor (BCF) for both Cr chemical speciations.

MATERIALS AND METHODS

Culture of test organisms*. Lecane papuana* was originally collected at El Ocote, Aguascalientes (21.464'N, 102.313'W), Mexico (Saucedo-Ríos *et al.,* 2017), and cultured in the laboratory for more than five years prior to the beginning of the experiments. To start experiments we placed 60 to 80 parthenogenetic females in Petri dishes (~ 70 mm

diameter) filled with ~ 50 mLs of moderately hard, reconstituted water (MHRW) (USEPA, 2002). Stock cultures were maintained in a bioclimatic chamber (Revco Scientific, Inc.) at 25 ± 2 ºC. Rotifers to be used in the toxicity tests were obtained by removing amictic embryos from the stock culture and placing them into Petri dishes with MHRW, without food supplementation, and kept in a bioclimatic chamber at 25 ± 2 ºC.

Acute toxicity tests. ACS grade ≥98% potassium dichromate ($K_2Cr_2O_7$) and chromium (III) potassium sulfate dodecahydrate ($KCr(SO_4)_2 \cdot 12H_2O$) (Fisher Chemical, Hampton NH) were used as Cr VI and Cr III species, respectively. A standard solution (1000 mg/L) of each was made with deionized water; later these standard solutions were used in our experiments. pH was monitored throughout the experiments to ensure that Cr III and Cr VI species were maintained during the experiment.

To do these experiments we placed, 10 neonates (<24 h old) into the wells of a 24-well polystyrene plate (Corning Inc. New York) prefilled with the appropriate solution of MHRW. Each well had a total volume of 1 mL. All treatments were replicated four times. These plates were incubated in a bioclimatic chamber (Revco Scientific, Inc.), without food, for 48 h with a photoperiod of 16:8 h (light: dark), at 25 ± 2ºC. Following exposure, the number of dead or immobilized animals was recorded. We only accepted experimental runs where mortality in the control plates was less than 10%. The LC_{50} values were estimated with the *dcr* package in R.

Chronic toxicity tests. Briefly, eight replicates at six concentrations (0.0, 3.125, 6.25, 12.5, 25, and 50% of the corresponding acute LC_{50}) were used to assess chronic toxicity. For each replicate, five neonates of < 24-h old were placed in 2 mL of EPA medium. All experiments were conducted in 24-well polystyrene plates (Costar Co., USA). After five days, the number of individuals per well was counted and used to estimate the intrinsic rate of population increase, *r*, as follows:

$$r = \frac{ln\left(\frac{N_t}{N_o}\right)}{t},$$

where N_t is the final number of individuals, N_0 is the initial number of rotifers, *Ln* is the natural logarithm, and *t* is the exposure period (5 d). Statistical analyses were performed using GraphPad Prism version 6.0.0

Biomass determination. Ten thousand non-ovigerous, adult females of *L. papuana* were separated (3X), rinsed with deionized water, and dried at 60°C (Fisher Scientific, Isotemp® 500 Series; Waltham, MA) in microtubes until we recorded a constant mass using an analytical balance (Chyo, model JK-200, Japan, ± 0.001 g). The difference in the dry weight of the empty microtube with the microtube with the rotifer biomass is the dry weight expressed as nanograms per individual.

Bioconcentration determinations. We placed 600 rotifers in small Petri dishes (1 mm diameter) with 2 mL of either Cr III or Cr VI solution without food. The concentrations tested correspond to the LC_{50}, LC_{10} and LC_1 for every metal species (n = 4). Rotifers cultured in MHRW without metals served as a negative control. These Petri dishes were placed into a bioclimatic chamber (Revco Scientific, Inc.) at 25 ± 2ºC for 24 h with a photoperiod of 16:8 h (light: dark). After this exposure, rotifers were collected in Petri dishes and carefully rinsed with deionized water to eliminate excess chromium. Then the rotifers were placed in an microtube with 1 mL of deionized water and 500 µL of nitric acid (65%), at 4°C, until analysis.

We quantified chromium in rotifers exposed to all metal concentrations tested in our experiments. Exposure concentrations of the acute toxicity tests were also analyzed with three replicates to obtain actual concentrations instead of nominal concentrations. We performed atomic absorption spectrophotometry with an Analyst 800 Spectrometer (Perkin Elmer, Norwalk, CT) at three settings: (1) Transversely heated graphite furnace, (2) longitudinal Zeeman-effect background correction, and (3) AS-60 autosampler. Chromium was quantified according to the Mexican normative NOM-117-SSA1-1994 (DOF, 1995). To do this we performed Cr analysis methodology with 5 points of a calibration curve, with a minimum r^2 of 0.995. The percentage variability was less than 5% (% RSD) and the maximum standard deviation was 5%, analyzing reagent blanks and fortified and replicated samples, with a variation less than 20 % of the analyte. The detection limit of this method was 0.372 µg/L. In all cases blanks were below detection limits.

The accumulated amount (q) of chromium (µg/g dry weight) was calculated according to Hernández-Flores *et al.,* (2020):

$$q = \frac{(C_o - C_t) * V}{W}$$

Where: C_o, initial chromium concentration in the medium (µg/L); C_t, metal concentration at time t (µg/L); *V*, total volume of sample in liters (L) and *W* dry weight of rotifers in grams (g).

The Bioconcentration Factor (BCF) (dimensionless) was determined according to the following formulas:

$$BCF = \frac{q}{c_0},$$

$$MBB = \frac{q}{n},$$

$$LBB = (LC_{50}) \cdot (BCF).$$

RESULTS

Acute and chronic toxicity tests. Results of the acute toxicity tests showed that *L. papuana* is more tolerant to Cr III than it is to Cr VI; the LC_{50} for Cr III was 15x higher than the respective value for Cr VI (Table 1). Actual exposure concentrations are similar to nominal concentrations (98.75% of similarity, n = 4 for three concentrations tested at beginning of acute exposure). Table 2 shows LC_{50} of several invertebrate species.

Chronic toxicity exposure concentrations were based on the corresponding LC_{50} values for both chromium species (3.125, 6.25, 12.5, 25, and 50% of each LC_{50}) and we obtained their respective intrinsic growth rates. Cr III affects *L. papuana* significantly at 0.163 mg/L (6.25% of its respective LC_{50}); Cr VI affects significantly at 0.0885 mg/L (50% of its respective LC_{50}) (Figure 1).

Bioconcentration determinations. In metal-exposed rotifers the content of Cr III was significantly higher (LC_{10}, 0.1028 mg/L) when compared to controls (Figure 2). Exposure to 0.163 mg/L (6.25% of the LC_{50}) of Cr III negatively affected the intrinsic growth rate of *L. papuana* (Figure 1). Rotifers exposed to Cr VI bioconcentrated this metal at 0.177 mg/L (the respective LC_{50}) (Figure 2). Fecundity was significantly affected when Cr VI reached 0.0885 mg/L (Figure 1), which corresponds to the 50% of the respective LC_{50}.

Table 3 presents results of Cr content in rotifers exposed to Cr, determining the respective BCF and LBB for both redox forms. These data show that *L. papuana* accumulated higher amounts of Cr III and that the LBB for Cr III was higher (1.6X) than that of Cr VI. The BCF for Cr III was 9.4-fold lower than that of Cr VI.

DISCUSSION

This contribution assessed the acute and chronic effects of Cr III and Cr VI in the rotifer *L. papuana* including acute and chronic body burdens (LBB and CBB) and bioconcentration factors (BFC) to make progress in the understanding on the fate and potential environmental effects of chromium in non-target freshwater organisms.

We found *L. papuana* to be a good model to assess the toxicity of Cr III and Cr VI and as a monitor of chromium bioaccumulation. Our results in the acute and chronic toxicity tests indicate that *L. papuana* presents similar sensitivity to toxicants as Cladoceras but offers the advantage

to assess the effect of contaminants in the sediment-water interphase (Garza-León *et al.*, 2017).

Acute toxicity tests. As expected from published research, Cr VI caused toxicity at a lower concentration than Cr III (Table 1). This is because Cr VI passes through cell membranes and can be actively transported into the cell by the sulfate transporter. Once inside of a cell, Cr VI generates reduced intermediates of chromium that with hydrogen peroxide (H_2O_2) generate reactive oxygen species (ROS) via Fenton and Haber-Weiss reactions. The result of that metabolism is substantial oxidative damage (Ercal *et al.*, 2001). Moreover, Cr VI can be reduced by enzymatic and non-enzymatic reactions and then interact with some endogenous reductants, such as glutathione (GSH), cysteine, or nucleotides (Arzate-Cárdenas & Martínez-Jerónimo, 2011). However, cells are not very permeable to Cr III and these mechanisms do not occur in the presence of Cr III. Cr III is accumulated at cation-binding sites of the cell membrane. While Cr III is toxic due to its capacity to form complexes with proteins and organic compounds, it is not as toxic as Cr VI (Albert, 1997; Dayan & Paine, 2001; Gagneten & Imhof, 2009).

Table 1. Toxicity values obtained from the acute toxicity test with *Lecane papuana*

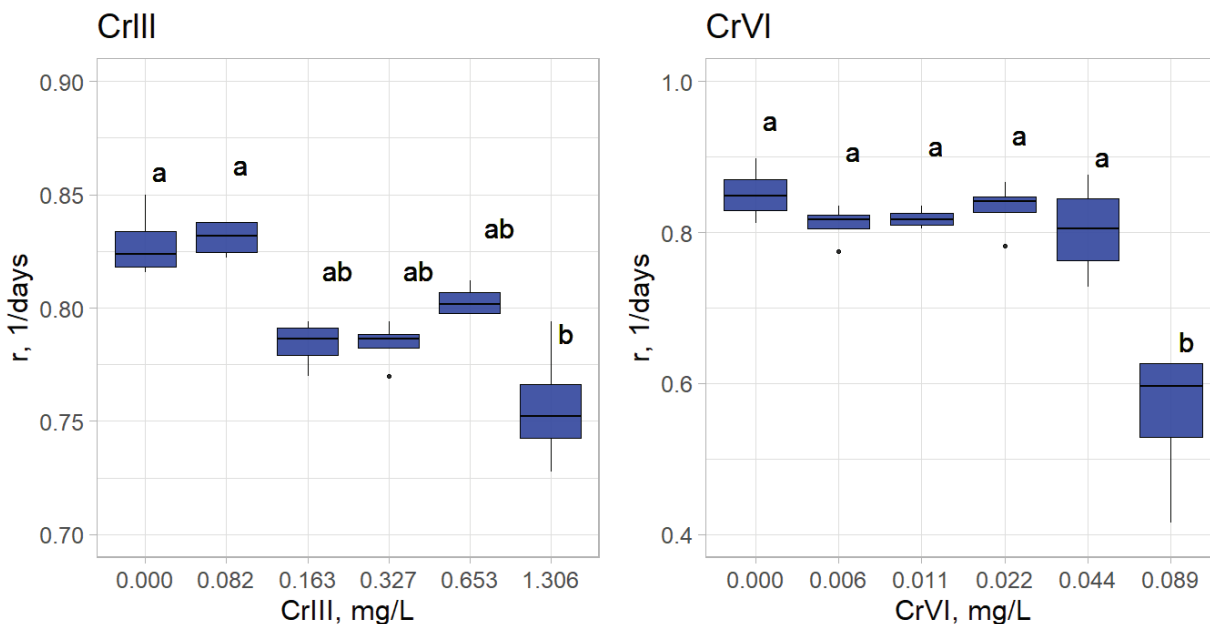| Chromium species	LC$_{50}$, mg/L (CI 95%)	LC$_{10}$, mg/L (CI 95%)	LC$_1$, mg/L (CI 95%)	r^2
Cr III	2.613 (2.13-3.10)	0.1028 (0.061-0.143)	0.0117 (0.0005- 0.0004)	0.92
Cr VI	0.177 (0.13-0.23)	0.0788 (0.041-0.116)	0.0132 (0.0044- 0.0264)	0.96

Figure 1. Intrinsic growth rates (r) of *Lecane papuana* exposed to five different concentrations of Cr III and Cr VI. Significant differences were established through one-way ANOVA and Bonferroni's multiple comparison tests. Different letters above the boxes indicate significant differences (*P*<0.05). (N = 4). Statistical analyses were performed with the packages *agricolae* and *ggplot2* in R.
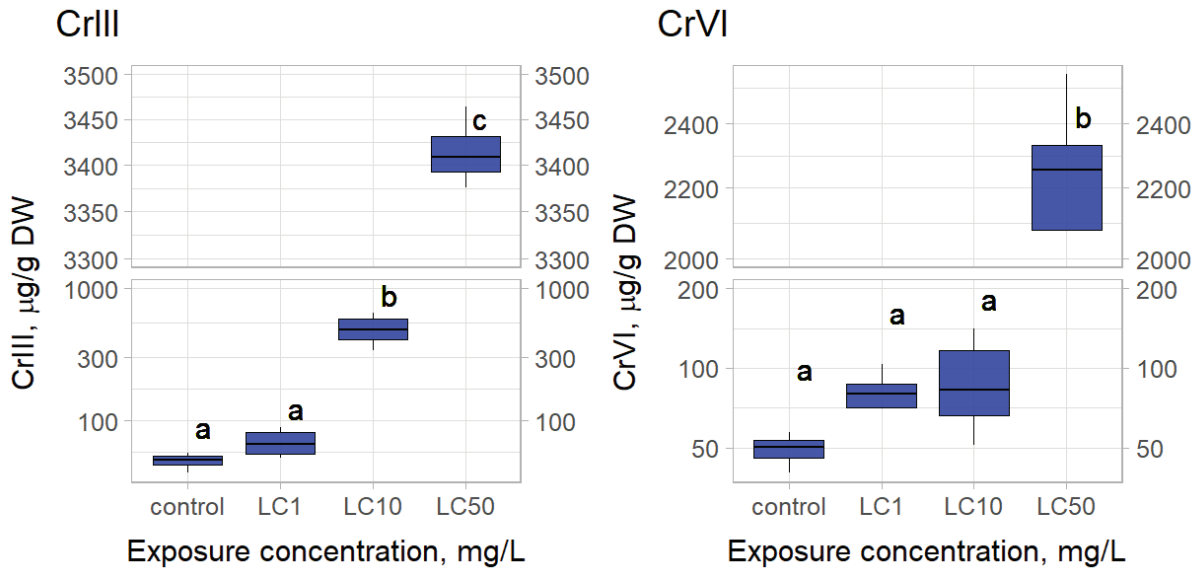
Figure 2. Results of bioconcentration on *Lecane papuana* exposed to three different concentrations of chromium. For Cr III: LC1, LC10 and LC50 correspond to 0.012, 0.103, and 2.613 mg/L, respectively. For Cr VI: LC1, LC10 and LC50 correspond to 0.013, 0.0.079, and 0.177 mg/L, respectively. Significant differences were established through one-way ANOVA and Bonferroni's multiple comparison tests. Different letters above the boxes indicate significant differences (*P*<0.05). (N = 4). Statistical analyses were performed with the packages *agricolae* and *ggplot2* in R.

As observed in Table 2, Cr VI is more toxic for *L. papuana* than for *Brachionus calyciflorus* (Pallas, 1766) (a 48h). However, Cr VI is more toxic for *L. quadridentata* than for *L. papuana*. Cr III is more toxic to *B. calyciflorus* and *L. quadridentata*, in comparison to the effects elicited on *L. papuana* (Table 2). As observed, Cr III promoted lower toxicity than Cr VI.

Several authors have found differences in chromium susceptibility within the family Lecanidae (Saucedo-Ríos *et al.,* 2017) and in comparison to Brachionidae (Sarma *et al.,* 2006).

In addition, cladocerans as *Daphnia exilis* Herrick 1895*, Daphnia magna* Straus 1820*,* and *Daphnia pulex* (Leydig, 1860), exhibited LC$_{50}$ values for Cr VI very close to those ones found for *L. papuana* (Tabla 2). All these tests were carried out in similar conditions to the ones employed in this work (except for the variation in temperature); which it might suggest that *L. papuana* is as sensitive as the freshwater model organisms used worldwide such as *Daphnia magna* (Martínez-Jerónimo *et al.,* 2008). Some authors have suggested that Neoartic cladocerans like *D. magna* are not good model species to predict the effects of contaminants in neotropical ecosystems (Gutierrez *et al.,* 2010)a representative calanoid copepod, we carried out two (acute and chronic. Moreover, it has been described that this rotifer species is also susceptible to the effects of pesticides at environmental concerning concentrations (Garza-León *et al.,* 2017).

For protection of aquatic biota, the maximum allowable limit of chromium is 0.5 mg/L. However, that level is at least 3 times higher than the LC$_{50}$ values recorded for *L. papuana*. Moreover, 0.5 mg/L is even higher for some other native species that are more sensitive to this metal. International guidelines report lower limits for chromium concentration in comparison to the Mexican normative. Nevertheless, these limits are

higher than concentrations that cause deleterious effects on rotifers. Thus, while substantial variation exists for acceptable chromium limits among countries, Mexican law is less protective. Our results add information to the need to establish lower protective values for Cr to better protect native freshwater species from acute toxicity.

Chronic toxicity test. The intrinsic population growth rate of *L. papuana* was significantly affected by exposure to Cr (Figure 1). *Lecane papuana* was affected significantly at 0.163 and 0.0885 mg/L, which correspond to LOEC values for Cr III and Cr VI, respectively. Snell & Moffat (1992) carried out a study with similar conditions as ours, in which *Brachionus calyciflorus* was exposed to Cr VI and obtained a LOEC of 3.2 mg/L; representing lower toxicity to *Brachionus* than for *L. papuana.*

Hermens *et al.,* (1984), found a chronic Cr VI EC50 value of 0.27 mg/L for *D. magna*. Wong & Pak, (2004) reported a EC50 of at least 0.268 mg/L for larval development inhibition in the copepod *Mesocyclops pehpeiensis* Hu 1943. Our value for Cr VI lethal effects was lower that both chronic values (Table 1). Gutierrez *et al.,* (2010) found EC50 values from 0.170 to 0.599 mg/L in the copepod *Notodiaptomus conifer* (Kiefer, 1936), for 48 and 24 h respectively. Planktonic organisms are more tolerant to Cr VI than *L. papuana.*

The effects of metals in lifetable parameters have been analyzed in many zooplanktonic species of rotifers and cladocerans (Sarma *et al.,* 2006). A decrease in peak population of a species due to stress may mainly result in (a) poor filtration, consumption or assimilation of the food and (b) reduced neonate production or higher rate of mortality (Sarma *et al.,* 2006).

Bioconcentration determinations. The criterion to classify a chemical substance as "bioaccumulative" requires the BCF to be comprised between 1000 and 5000 (Arnot & Gobas, 2011). Thus, Cr VI in *L. papua-*

na can be classified as "very bioaccumulative" because its BCF was higher than 5000. In contrast, Cr III has a BCF of 1,308.84, which is about 10X lower than one obtained for Cr VI (BCF = 12,299.44). Thus, there is a significant difference in the accumulation rate between Cr III and Cr VI. These results suggest that the patterns of accumulation of the two chromium chemical species and the mechanisms of toxicity are different (Rainbow, 2007)all of which have the potential to cause toxic effects. Subsequent tissue and body concentrations of accumulated trace metals show enormous variability across metals and invertebrate taxa. Accumulated metal concentrations are interpreted in terms of different trace metal accumulation patterns, dividing accumulated metals into two components — metabolically available metal and stored detoxified metal. Examples of different accumulation patterns are described from crustaceans but have a general applicability to all aquatic invertebrates. Toxicity does not depend on total accumulated metal concentration but is related to a threshold concentration of internal metabolically available metal. Toxicity ensues when the rate of metal uptake from all sources exceeds the combined rates of detoxification and excretion (if present. These differences are clear when we compare the concentrations used for each chemical species; while for Cr III we used 2.613 mg/L, for Cr VI we used a 15-fold lesser concentration (0.177 mg/L). Even so, the Cr VI BCF was an order of magnitude higher (10-fold). This behavior of chromium chemical species was already reported for other freshwater rotifer species like *L. quadridentata* and *B. calyciflorus*, where Cr VI was more efficiently bioconcentrated than Cr III (Hernández-Ruiz *et al.,* 2016). A comparison of bioconcentration studies shows that *L. papuana* bioconcentrated more Cr VI than freshwater fishes (Table 4).

The results of Bioconcentration of *L. papuana*, show that even at low concentrations Cr III is bioconcentrated in the organism (Figure 2). However, adverse effects are detected at a slightly higher concentration (see LOEC value in Figure 1). Regarding Cr VI, bioconcentration starts at LC_{50} value, but chronic effects are observed at very low concentrations at ½ of the LC_{50} value (see figures 1 and 2). However, we have to consider that acute tests only last 24h, while chronic tests last 5 days.

Due to the scarcity of data on Cr VI accumulation in invertebrates, we decided to compare our data with freshwater invertebrates and freshwater/coastal fishes (Table 4). In the fish *Puntius sarana* (Hamilton, 1822) exposed to 2.19 mg/L (12-fold higher than the exposure concentration for *L. papuana*) with supplemental food; the concentration of Cr VI found in the fish indicates that *L. papuana* bioconcentrated 21-fold more Cr VI than the fish. Bioacummulation of Cr VI in the fish *Catla catla* (Hamilton, 1822) (exposed to 2.19 mg/L) was 2.7-fold lower than *L. papuana* (Table 4). The fish *Mugil cephalus* (Linnaeus, 1758), bioaccumulated at least 3-fold less Cr VI with an exposure concentration at least 56-fold higher (160 mg/L) than *L. papuana*. Therefore, we can infer that the rotifer *L. papuana* bioconcentrates more Cr VI than these freshwater fishes. These differences can be explained by the presence of more sophisticated mechanisms to excrete Cr VI in fishes and the fact that the metabolism of small organisms is more accelerated with respect to larger organisms (Gutierrez *et al.,* 2010).

Table 2. Comparisons of values for chromium in other invertebrates

Species	values mg/ L	Source
Brachionus calyciflorus (Monogononta: Brachionidae)	0.64 - 1.051 (24 h) Cr III	Hernández-Ruiz *et al.* (2016)
Lecane quadridentata (Monogononta: Lecanidae)	4×10^{-6} (24 h) Cr VI	
	1.279 (24 h) Cr III	
	$4.7 \times 10_{-5}$ (24 h) Cr VI	
Lecane papuana (Monogononta: Lecanidae)	2.613 (48 h) Cr III	This study
	0.177 (48 h) Cr VI	
Brachionus calyciflorus (Monogononta: Brachionidae)	8.3 (48 h) Cr VI	Snell and Moffat (1992)
Daphnia exilis (Anomopoda: Daphniidae)	0.1170 (48 h) Cr VI	Martínez-Jerónimo *et al.* (2008)
Daphnia magna (Anomopoda: Daphniidae)	0.2076 (48 h) Cr VI	Martínez-Jerónimo *et al.* (2006)
Daphnia pulex (Anomopoda: Daphniidae)	0.13 (48 h) Cr VI	Velandia and Montañez (2010)
Lecane hamata (Monogononta: Lecanidae)	4.41 (48 h) total Cr	Pérez-Legaspi and Rico-Martínez (2001)
Lecane luna (Monogononta: Lecanidae)	3.26 (48 h) total Cr	
Lecane quadridentata (Monogononta: Lecanidae)	4.50 (48 h) total Cr	
Daphnia magna (Anomopoda: Daphniidae)	0.015 (24 h) Cr VI	CCME (1999)
Procambarus clarkia (Decapoda: Cambaridae)	500 (96 h) Cr VI	
Simocephalus vetulus (Anomopoda: Daphniidae)	0.015 (24 h) Cr VI	

Table 3. Bioconcentration, Body burdens, and chronic toxicity ("r" inhibition) of chromium in *Lecane papuana* after 24-h exposure to Cr III or Cr VI. n = 4.

Chromium species	(µg of Cr/rotifer)	Accumulated Cr concentration in *L. papuana* (µg/g)	BCF	LBB mmol/kg DW	EC_{50} "r"	$LOEC_{chronic}$ mg/L	$NOEC_{chronic}$ mg/L	MATC	ACRr
III	$1.71 \times 10^{-4} \pm 3.15 \times 10^{-6}$	3420 ± 62.93	1308.84	65.76	0.1181	0.163	0.0816	0.115	22.66
VI	$1.089 \times 10^{-4} \pm 8.97 \times 10^{-6}$	2177 ± 179.38	12299.44	41.86	0.1127	0.0885	0.04425	0.062	2.83

Hyalella azteca (Smith, 1874), bioaccumulated 16-fold lower concentration of Cr VI than what we found in *L. papuana* (Table 4), but this study was carried out in a period of 4 weeks of exposure (ours was 24 h) and the exposure concentration was 0.176 mg/L, almost the same concentration that we used (0.177 mg/L). The bioconcentration of chromium in *Daphnia magna* and *Argyrodiaptomus falcifer* (Daday, 1905) was approximately 20 and 10-fold lower (respectively) than that found in *L. papuana* (Table 4). In addition, the concentration to which *L. papuana* was exposed (0.177mg/L) was about the 50% of that used with those two species (0.350 mg/L); however, our study was carried out in 24 h and those mentioned above in two days. The crab *Zilchiopsis collastinensis* (Pretzmann, 1968), showed a bioconcentration 10-fold lower than the one found in *L. papuana*, which were exposed 14 and 5 days respectively (Table 4). Moreover, the crab was exposed to a concentration 28-fold times higher (5 mg/L) than what was used in *L. papuana*. Thus, comparison of bioconcentration and adverse effects of Cr among aquatic species suggests that some species had a strong ability to resist the Cr VI exposure and/or to remove it from the body, involving Glutathione S-transferases and metallothionein proteins in the detoxification process; antioxidant enzyme systems have been reported in cases of recovery from Cr VI damage (Yuan *et al.,* 2017). Similarly, previous studies with organisms of the Lecanidae family mention that these rotifers have a thick lorica that acts as a barrier against metal ions, which can be deposited in the lorica of *Lecane* rotifers; thus, bioconcentrating more chromium than other species (Table 4). The ecological niche occupied by species might have played a significant role in the accumulation; *Lecane* as a surface feeder and planktivorous species, can accumulate chromium from water, mud, debris, and detritus in addition to macrophytes and algae (Joadder, 2014). Binding of chromium in the sediment soil, depends on oxidation status, which is altered by pH, and microbial processes (Sanyal *et al.,* 2017). *Lecane papuana* due to its high sensitivity to Cr VI can be considered a biomonitor species; biomonitor species can indicate the presence of contaminants even when they are not detected in a specific environment (Gagneten &

Table 4. Bioconcentration Factor for Chromium (VI) in different taxa

Species	Accumulated Cr concentration (µg/g)	BCF	Exposure concentration (mg/L)	LC_{50}	Source
Argyrodiaptomus falcifer (Calanoida: Diaptomidae)	50 (2 d) Cr VI	231	0.350	NA	Gagneten *et al.* (2009)
	80 (2 d) Cr VI	281			
Daphnia magna (Anomopoda: Daphniidae)					
Catla catla (Cypriniformes: Cyprinidae)	800 (7 d) Cr VI	NA	2.19	105.87(24h); 51.41 (48h)	Sanyal *et al.* (2017)
	400 (7d) Cr VI			24.53(24h); 17.13(48h)	
Labeo bata (Cypriniformes: Cyprinidae)	100 (21 d) Cr VI			59.31(24h); 29.44(48h)	
Puntius sarana (Cypriniformes: Cyprinidae)					
Hyalella Azteca (Amphipoda: Hyalellidae)	134.59 (4 weeks) Cr VI	200	NA	NA	Norwood *et al.* (2006)
Lecane papuana (Monogononta: Lecanidae)	2,177 (24 h) Cr VI	12,299.44	0.177	0.177	This study
Mugil Cephalus (Mugiliformes: Mugilidae)	700 (96 h) Cr VI	NA	160	65.01 (96h)	Rajkumar and Tennyson (2013)
Zilchiopsis collastinensis (Decapoda: Trichodactylidae)	200 (14 d) Cr VI	766.9	2 and 5	NA	Gagneten and Imhof (2009)

Imhof, 2009). The sensitivity of an organism depends on several toxico-kinetic mechanism; bioaccumulation of a substance is the process that confers the organism with more resistance to adverse effects (Muggelberg *et al.,* 2017). In the case of *L. papuana* it has been documented elevated BCF´s (which explain its tolerance) when compared with other species and contaminants (Rivera-Dávila *et al.,* 2021).

The Maximum permissible levels of chromium in water of diverse uses in Mexico is at least 0.5 mg/L, and the values that we obtained for chronic LOEC and NOEC for Cr III were 0.163 and 0.0816 respectively, which are around 3 and 6 times lower than 0.5 mg/L in Mexican Regulations. The chronic LOEC for Cr VI was 0.0885 mg/L and the NOEC was 0.0443 mg/L, which are 5.6 and 11.3 times lower than the maximum concentration allowed in the Mexican Regulation. These findings mean that *L. papuana* could be harmed in chronic exposures to chromium, in spite of compliance with the current environmental legislation.

There are few studies of chromium carried out with aquatic invertebrates (including cladocerans and rotifers), and the differences in the ranges for acute and chronic values (such as LC_{50}) is great. There is a need to analyze these values and the protocols employed by different researchers. A first step is to use actual values instead of nominal ones (or at least indicate the percentage of similarity between actual and nominal values). The use of acute tests were acute values are obtain in absence of food somehow reduces the differences in ranges. The differences among species exposed to chromium could be due that this metal can act in different ways, in different species. Chromium has a role in glucose, fat and protein metabolism, participating in the insulin action. It links directly to macromolecules; fragments the DNA chains and acts in the peroxidation of lipids, generating free radicals and modifying the routes of cell signals. All these processes can contribute to the toxicity and carcinogenicity of chromium compounds (Gagneten & Imhof, 2009). Moreover, depuration of accumulated chromium in aquatic organisms differ markedly among the different taxa and the process generally has a complex pattern of elimination, showing large differences among metals and invertebrate groups (Rainbow, 2007).

CONCLUSION

In this research, acute, chronic, and bioconcentration toxicity tests were evaluated with the rotifer *L. papuana*, which is a sensitive organism to effects of both chromium species. We evaluated the susceptibility of this organism at concentrations that are of environmental concern; we discussed the acute rotifer sensitivity comparing our results with those of other aquatic invertebrate species. In general, *L. papuana* is one of the most sensitive species for both Cr III and Cr VI. In general, both chromium species showed high BFCs values. Our results support the idea that *L. papuana* is a suitable organism for toxicity assessment in tropical and subtropical environments, in acute, chronic, and bioconcentration tests. Maximum allowable chromium concentration in Mexican Regulations are higher than LC_{50} values obtained for this rotifer; and mostly above international values for protection of aquatic biota.

Acute and chronic effects on *L. papuana*, including acute and lethal body burdens and bioconcentrations factors aid answering questions on the fate, mechanisms of toxicity, and potential environmental effects of chromium in organisms.

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