Differential effects on the toxicity and bioconcentration of hexavalent and trivalent chromium on the rotifer *Lecane papuana* (Rotifera: Monogononta)

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Abstract

Heavy metals are still a matter of concern as they might reach aquatic environments due to anthropogenic activities like metal-mechanic or mining, which are part of the main economic activities in different regions around the world. The exposure to chromium may be linked to discharges from automotive and tannery industries, representing a threat to aquatic biota. In the environment, chromium presents two stable forms, the trivalent (CrIII) and the hexavalent (CrVI), which differ on their chemical properties, and thus, on their bioavailability and effects on organisms exposed to this metal. Therefore, this work was aimed to assess the effects of both chromium III and VI on the demographic responses of the rotifer *Lecane papuana* and describe how the bioconcentration of chromium affects survival and fecundity. For this purpose, acute toxicity (48 h) and chronic toxicity test (alterations on the intrinsic growth rate) were carried out. Moreover, the bioconcentration factor (BCF) and metal body burden (MBB) were estimated after 24-h exposure to either CrIII or CrVI. Our results indicate that CrIII was less toxic according to their respective LC50 values (CrIII = 2.613 mg/L; CrVI = 0.177 mg/L). The intrinsic growth rate was significantly affected by CrIII while CrVI caused no significant changes but only at 0.0885 mg/L, which represents 0.5 of their respective LC50 value. Bioconcentration experiments demonstrated that *L. papuana* accumulated more CrIII before causing deleterious effects, although it was not as toxic as CrVI, which elicited toxic effects on *L. papuana* at concentrations of environmental concern.

Introduction

Pollution due to heavy metals is the result of discharges to the soil or water systems from diverse sources such as chemical manufacturing, metallurgy, and production refractory materials, among others. In the environment, chromium presents two main redox states, chromium (III) (CrIII) and chromium (VI) (CrVI) that which are the most stables forms, but differ on their bioavailabilities and mobilities, thus, their respective toxicities might be also modified (Aharchaou et al. 2017).

CrIII presents low solubility and low mobility, it forms complexes with organic matter in either soil or water environments, and presents certain inability to pass through cell membranes; thus, it might be considered as relatively innocuous. Nonetheless, its presence in water is due to the release of CrVI in wastewater and its biotransformation by both bacteria and eukaryotic cells; additionally, some other reduction processes take place in the environment like Fenton reactions with ferrous ions (FeII). Thereafter, in CrVI-contaminated waters the content of organic-CrIII complexes must be assessed since such complexes represent the main source for CrIII mobility, and thus, the intoxication of exposed biota. Nowadays, the information about fate and toxicity of CrIII and its organic complexes is still scarce (Chatterjee and Luo 2010). Nevertheless, CrIII plays an important role as an essential trace metal for the metabolism of diverse organisms (Bielicka et al. 2005).

CrVI exhibits higher solubility and mobility in comparison to CrIII, it can pass through biological membranes because of the active transport through the sulfate transporter (Borst-Pauwels 1981). It is considered the most toxic form of chromium due to its strong oxidant power that once CrVI has permeated the cells initiates complex mechanisms that involve different biochemical pathways and multiple targets (Rudolf and Červinka 2006). CrVI is reduced by enzymatic and non-enzymatic reactions to produce less reactive forms like CrIII and CrV; furthermore, such reduction produces reactive oxygen species (ROS) that alter the redox environment within the organisms and induce oxidative stress (Arzate-Cárdenas and Martínez-Jerónimo 2011).

Rotifers represent a very diverse group of zooplankton where at least 2000 species have been described, and play a significant role in water environments as the trophic link between algae and zooplanktivorous predators like fish or other invertebrates (Segers 2007). 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).

The genus *Lecane* (Family Lecanidae) is found in shallow waters, littoral areas, and eutrophic environments (Keppeler et al. 2010). According to De Manuel (1994), *Lecane* has been used in for biogeographic discussions and seemed to be endemic

in unexplored areas. Other uses of *Lecane* include their incorporation to biodigestors, mainly in activated sludge systems, to improve the quality of the process and removal of organic contaminants (Fiałkowska and Pajdak-Stós 2008). Furthermore, (Pérez-Legaspi and Rico-Martínez 2001) used three *Lecane* species (*L. hamata*, *L. luna*, and *L. quadridentata*) to assess the effect of several chemical compounds and suggested these rotifers as alternatives test species. (Klimek et al. 2013) also concluded that *Lecane inermis* could represent a better option in toxicological evaluations because is more sensitive than the conventionally used *Brachionus* test.

Acute, and chronic toxicity tests are helpful to assess the impact of environmental pollutants, as the bioassays with zooplankton to evaluate the effects of contaminated sediments or dissolved phase. However, because of the great concentration capacity of the

accumulator organisms, heavy metals may easily be measured, even when they are so diluted in the water that they cannot be analyzed by the commonly used methods (Gagneten et al. 2009). Thus, bioconcentration becomes helpful, which is a process by a chemical substance is absorbed by an organism from the environment through its respiratory and dermal surfaces; the degree to which bioconcentration occurs 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 and Gobas 2006). Other concept that involves bioconcentration is the Lethal Body Burden (LBB), which is defined as μg per body weight or mmol/kg , which exerts a specific toxic effect (mortality, reduction in reproduction). Some authors have applied it to evaluate toxicity of different compounds and metals in rotifers. (Hernández-Flores et al. 2020; Van Wezel et al. 1995)

Therefore, this work was aimed to investigate how CrIII and CrVI affect demographic responses of *Lecane papuana* in acute and intrinsic growth rate test protocols, moreover measuring the capacity of the freshwater rotifer to accumulate Cr from water, to know the BCF in order to evaluate its potential as a biomonitor, and the likely relationship between chromium biococentration and the alterations on reproduction and survival of the exposed populations.

Material And Methods

Lecane papuana was originally collected at El Ocote, Aguascalientes (21.464°N, 102.313°W), Mexico (Saucedo-Ríos et al. 2017), and kept in laboratory conditions for more than five years prior to the beginning of the experiments. Briefly, 60 to 80 parthenogenetic females were placed in Petri dishes with moderately hard reconstituted water (MHRW) (USEPA 2002), fed on the green alga *Nannochloropsis oculata* (strain LB2194 of the University of Texas Collection) at 10^6 cells/mL, and cultured in a bioclimatic chamber (Revco Scientific, Inc.) at 25 ± 2 °C. Rotifer test organisms were obtained by daily separation of amictic eggs, which were placed in Petri dishes with MHRW without food supplementation, and kept at 25 ± 2 °C until hatching.

Acute toxicity tests with *L. papuana*

Potassium Dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and Chromium (III) potassium sulfate dodecahydrate ($\text{KCr}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) both ACS grade $\geq 98\%$, were acquired from Fisher Chemical, and they were used as CrVI and CrIII speciations respectively; all calculations were done taking into account the molecular weight of chromium to have the real chromium concentrations. A standard solution (1000 mg/L) of each chromium speciation was made with deionized water; later this standard solution was used to prepare the different concentration solutions along the study.

At least five chromium concentrations in the definitive acute test were tested to estimate the respective lethal median concentration (LC50). Ten neonates (< 24 h) were placed in a well of 24-well polystyrene plate (Costar Inc.) with a total volume of 1 mL, consisting of five CrIII and CrVI concentrations and the control, which had no chromium but only MHRW. Then, rotifers were transported to a bioclimatic chamber (Revco Scientific, Inc.), without food, for 48 h with a photoperiod of 16:8 h (light: darkness), at 25 ± 2 °C. At the end of the exposure time the number of dead or immobilized animals was

recorded. As a criterion of test acceptance, mortality in the controls was less than 10%. The LC50 values were estimated with the "drc" package in RStudio.

Chronic toxicity tests with *L. papuana*

Five different concentrations (0.5, 0.25, 0.125, 0.62 and 0.031 of the corresponding LC₅₀) were assessed. Five neonates of less than 24-h old were placed in 2 mL of EPA medium (negative control). Test conditions were the same used for culture and maintenance of rotifers. Eight replicates per concentration were used. All experiments were conducted in 24-well polystyrene plates (Costar Co., USA). At the end of five days (incubation time), the number of individuals per well was counted and used to estimate the intrinsic rate of population increase, r , using the formula:

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

where N_t is the final number of individuals, N_o is the initial number of rotifers, $\ln$ is the natural logarithm, and t is the exposure period (5 d).

Biomass determination

Ten thousand non-ovigerous adult females of *L. papuana* were separated ($n = 3$), rinsed with deionized water, and dried at 60 °C (Fisher Scientific, Isotemp® 500 Series) until constant weight. Then, the microtubes were weighted in an analytical balance. 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.

Chromium quantification in rotifers and MHRW

Six hundred rotifers were placed in 2 mL of either CrIII or CrVI solutions. The concentrations tested correspond to the LC50, LC10 and LC1 for every metal speciation. Negative control consisted of organisms cultured in MHRW without metals. All experiments consisted of four replicates ($n = 4$). Then, rotifers were transported to a bioclimatic chamber (Revco Scientific, Inc.), without food supplementation, for 24 h with a photoperiod of 16:8 h (light: darkness), at $25 \pm 2^\circ\text{C}$. After exposure, rotifers were collected in Petri dishes and carefully rinsed with deionized water to eliminate the chromium excess. Then, each sample of rotifers was placed in a microtube with 1 mL of deionized water and 500 µL of nitric acid (65%), at 4 °C, until their analysis.

Chromium was quantified in: rotifers exposed to every metal speciation, MHRW, and MHRW with either CrIII or CrVI at the same concentrations tested. Atomic absorption spectrophotometry was performed with an Analyst 800 Spectrometer (Perkin Elmer, Norwalk, CT) and the following settings: Transversely heated graphite furnace, longitudinal Zeeman-effect background correction, and a AS-60 autosampler. Chromium was quantified according to the Mexican norm NOM-117-SSA1-1994 (DOF 1994). Briefly, the Cr analysis methodology was performed with 5 points of a calibration curve, with a minimum r^2 of 0.995. With a percentage of variability less than 5% (% RSD) and a maximum standard deviation of 5%, analyzing reagent blanks and fortified and replicated samples, with a variation less than 20% of the analyte. The detection limit was 0.372 µg/L.

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

$$q = (C_o - C_t) * V / W$$

Where: C_0 , initial chromium concentration in the medium ($\mu\text{g/L}$); C_t , metal concentration at time t ($\mu\text{g/L}$); V , total volume of sample in liters (L) and W dry weight of rotifers in grams (g).

The bioconcentration factor (BCF) was determined by dividing the concentration of chromium accumulated in the whole body of *L. papuana* and the metal concentration in the solution.

Moreover, dry weight of each rotifer was used to calculate the metal body burden ($\mu\text{g/individual}$) and metal concentration per unit body weight ($\mu\text{g/g}$). We calculate the Lethal Body Burden (LBB) (mmol/kg) with the following formula:

$$\text{LBB} = (\text{LC50})(\text{BCF}) \text{ (McCarty 1986; Waykar and Shinde 2011).}$$

Results

The data of the acute toxicity tests, showed that *L. papuana* is more tolerant to CrIII than it is to CrVI, as the LC50 for CrIII is 15-fold higher than the respective value for the hexavalent chromium (Table 1).

Table 1
Toxicity values obtained from the acute toxicity test with chromium III and VI on *L. papuana*.

Chromium species	LC50, mg/L (CI 95%)	LC10, mg/L (CI 95%)	LC1, mg/L (CI 95%)	r^2
CrIII	2.613 (2.13–3.10)	0.1028 (0.061–0.143)	0.0117 (0.0005 – 0.0004)	0.92
CrVI	0.177 (0.13–0.23)	0.0788 (0.041–0.116)	0.0132 (0.0044–0.0264)	0.96
LC, Lethal concentration; 50, 10, and 1, represent the percentage of dead individuals at the corresponding concentration after 48-h exposure; CI 95%, confidence interval at 95% probability for the corresponding LC values; r^2 , coefficient of determination of the linear regression.				

Chronic toxicity tests were based on the corresponding LC50 values for both chromium species (was used 0.5, 0.25, 0.125, 0.62 and 0.031 of each LC50) and were obtained their respective intrinsic growth rates. CrIII affects *L. papuana* significantly at the concentration of 0.163 mg/L (0.5 of its respective LC50); CrVI affects significantly too, at the concentration of 0.0885 mg/L (0.62 of its respective LC50) (Fig. 1).

The results of the bioconcentration analysis showed that from LC10 (0.1028 mg/L) with CrIII exist significant difference comparing it with the control (Fig. 2), which means that CrIII is bioconcentrating in the organism, and is affecting it (intrinsic growth rate) (Fig. 1); Regarding to CrVI, can be seen that is bioconcentrating since CL50 (0.177 mg/L), however according to the Intrinsic growth rate is affecting the rotifer significantly at a lower concentration (0.0885 mg/L) which is a half of the respective CL50.

The Table 2 shows that the accumulated chromium and metal body burden (MBB), both are higher for CrIII than those values corresponding to CrVI (1.6 fold times in both measurements), Nevertheless CrIII has the lowest BCF (9.4-fold lower than that for CrVI) in spite of accumulate more in the rotifer. The lethal body burden was higher for CrIII, which means that the rotifer can tolerate higher concentrations and accumulate more of this speciation than that of CrVI.

Table 2
Bioconcentration of chromium speciations (CrIII and CrVI) in *Lecane papuana* after 24-h exposure. N = 4.

Cr speciation	Metal Body Burden (ug of Cr/rotifer)	Accumulated Cr concentration in <i>L.papuana</i> (ug/g)	BCF	LBB mmol/kg DW	LOEC _{chronic} mg/L	NOEC _{chronic} mg/L	MATC	ACRr
III	1.71x10 ⁻⁴ ± 3.15x10 ⁻⁶	3420 ± 62.93	1308.84041	65.76	0.163	0.0816	0.1153	22.66
VI	1.089x10 ⁻⁴ ± 8.97x10 ⁻⁶	2177 ± 179.38	12299.435	41.86	0.0885	0.04425	0.06257	2.828
ACR = LC50/MATC _{chronic} ; Bioconcentration factor (BCF); lethal body burden (LBB), chronic lowest observed effect concentration (LOEC); Maximum allowed toxic concentration (MATC); chronic no effect observed concentration (NOEC). All data in the table represent the mean ± SD, N = 4), except for the Dry Weight (DW) (mean ± SD, N = 3). DW = 50 ± 5.5 ng/rotifer								

Discussion

This contribution assessed the CrIII and CrVI acute and chronic effects on *L. papuana*, including acute and lethal body burdens and bioconcentrations factors to aid answering questions on the fate and potential environmental effects of chromium in non-target organisms. Our results suggest that *L. papuana* presents similar sensitivity to toxicants than other model organisms like the cladocerans, moreover offers the advantage to assess the effect of contaminants in the sediment-water interphase, preferably on subtropical and tropical environments (Garza-León et al. 2017)

Acute toxicity tests.

Hexavalent chromium (CrVI) caused toxicity at a lower concentration than the trivalent chromium (CrIII) (Table 1). CrVI passes easily through the biological membranes of cells and can be actively transported into the cell by the sulfate transporter, and already inside of them generates reduced intermediates of chromium that along with hydrogen peroxide (H₂O₂) generate reactive oxygen species (ROS) via Fenton and Haber-Weiss reactions, which can cause oxidative damage (Ercal and Gurer 2001), moreover could be reduced by enzymatic and non-enzymatic reactions in the cells and interact with some endogenous reductants, such as glutathione (GSH), cysteine, or nucleotides. (Arzate-Cárdenas and Martínez-Jerónimo 2011). On the other hand, these mechanisms mentioned above do not occur with CrIII, which is not very permeable into the cells, thus it is accumulated at the cation-binding sites of the cell membrane. Is toxic due to its high capacity to form complexes with proteins and organic compounds, but not as CrVI (Albert 1997; Dayan and Paine 2001; Gagneten and Imhof 2009)

As observed in Table 3, CrVI is 46.9-fold more toxic for *L. papuana* than for *B. calyciflorus*; it was 4,425-fold more toxic for *L. quadridentata* than for *L. papuana*. In addition, cladocerans as *Daphnia exilis*, *Daphnia magna* and *Daphnia pulex* exhibited LC50 values for CrVI very close to what we found for *L. papuana*. All these tests were carried out under the similar conditions as those used in this work (only varying temperature), which could mean that *Lecane papuana* is as sensitive as organisms used in worldwide normative use (i.e. *Daphnia magna*) (Martínez-Jerónimo et al. 2008), at least to assess the effect of metal ions like CrIII and CrVI. Moreover, it has been described that this rotifer species is also susceptible to the effects of pesticides at environmental concern concentrations (Garza-León et al. 2017).

Table 3
Comparisons of LC50 values for chromium in other invertebrates and one fish species.

Modified from Hernández-Ruiz et al. (2016)

Species	LC50 values mg/ L	Source
<i>Brachionus calyciflorus</i> (Monogononta: Brachionidae)	8.3 (48 h) CrVI	(Snell and Moffat, 1992)
<i>Brachionus calyciflorus</i> Gainesville strain (Monogononta: Brachionidae)	1.051 (24 h) Cr III	Hernández-Ruiz et al. (2016)
<i>Brachionus calyciflorus</i> San Pancho strain (Monogononta: Brachionidae)	0.64 (24 h) Cr III	Hernández-Ruiz et al. (2016)
<i>Brachionus calyciflorus</i> Gainesville strain (Monogononta: Brachionidae)	4×10^{-6} (24 h) Cr VI	Hernández-Ruiz et al. (2016)
<i>Brachionus calyciflorus</i> . San Pancho strain (Monogononta: Brachionidae)	4×10^{-6} (24 h) Cr VI	Hernández-Ruiz et al. (2016)
<i>Cnesterodon decemmaculatus</i> (Cyprinodontiformes: Poeciliidae)	35.1 (24 h)	(Vera-Candioti et al. 2011)
	27.5 (48 h)	
	24.0 (72 h)	
	21.4 (96 h)	
<i>Daphnia exilis</i> (Anomopoda: Daphniidae)	0.1170 (48 h)	(Martínez-Jerónimo et al. 2008)
<i>Daphnia magna</i> (Anomopoda: Daphniidae)	0.015 (24 h) Cr VI	(Canadian Council of Ministers of the Environment, 1999)
<i>Daphnia magna</i> (Anomopoda: Daphniidae)	0.2076 (48 h) Cr VI	(Martínez-Jerónimo et al. 2006)
<i>Daphnia pulex</i> (Anomopoda: Daphniidae)	0.13 (48 h) CrVI	(Velandia-Guauque and Montañez- Cardoso, 2010)
<i>Lecane hamata</i> (Monogononta: Lecanidae)	4.41 (48 h) total Cr	(Pérez-Legaspi and Rico-Martínez, 2001)
<i>Lecane luna</i> (Monogononta: Lecanidae)	3.26 (48 h) total Cr	(Pérez-Legaspi and Rico-Martínez, 2001)
<i>Lecane papuana</i> (Monogononta: Lecanidae)	2.613 (48 h) CrIII	This study
<i>Lecane papuana</i> (Monogononta: Lecanidae)	0.177 (48 h) CrVI	This study
<i>Lecane quadridentata</i> (Monogononta: Lecanidae)	4.50 (48 h) total Cr	(Pérez-Legaspi and Rico-Martínez, 2001)
<i>Lecane quadridentata</i> (Monogononta: Lecanidae)	1.279 (24 h) Cr III	Hernández-Ruiz et al. (2016)
<i>Lecane quadridentata</i> (Monogononta: Lecanidae)	4.7×10^{-5} (24 h) Cr VI	Hernández-Ruiz et al. (2016)
<i>Procambarus clarkia</i> (Decapoda: Cambaridae)	500 (96-h LC40)	(Canadian Council of Ministers of the Environment, 1999)
<i>Simocephalus vetulus</i> (Anomopoda: Daphniidae)	0.015 (24 h) Cr VI	(Canadian Council of Ministers of the Environment, 1999)

Trivalent chromium was 2.5, 4 and 2-fold more toxic to *B. calyciflorus* (Gainesville strain), *B. calyciflorus* (San Pancho strain) and *L. quadridentata*, respectively, in comparison to the effects elicited on *L. papuana* (Table 3). As observed, CrIII promoted lower toxicity than CrVI, which can be related to some molecular processes that were explained in previous paragraphs. Furthermore, *L. papuana* is more resistant to both chromium speciations than *B. calyciflorus* and *L. quadridentata*. Saucedo-Ríos et al. (2017) have pointed out the different sensitivities to metal ions within the family Lecanidae, thus, observed that *L. papuana* might be the more tolerant species to some toxic compounds.

On the other hand, taking into account that total chromium was used in *Lecanidae*: *L. luna*, *L. hamata* and *L. quadridentata*; and all values are above *L. papuana* CrIII LC50, been the last one 1.24, 1.68, and 1.72-fold more toxic respectively, we can affirm that these species are more resistant than *L. papuana*; thus the wide *Lecanidae* family seems to vary slightly in acute chromium toxicity among species.

In addition, the maximum allowable limits of chromium for protection of aquatic biota are around 0.5 mg/L (NOM-001-ECOL-1996 1996), which represents an excess of chromium concentration about 3-fold higher than the LC50 values recorded for *L. papuana* and some other native species that are more sensitive to this metal. In addition, the maximum limits of chromium in guidelines for the protection of aquatic biota or drinking water quality in several countries are lower than the Mexican regulations, which may pose a threat to some species in this region; The Environmental protection agency has a standard of 0.1 mg/L (USEPA 2008); in Canada and Europa the drinking water guideline for chromium is a maximum acceptable concentration of 0.05 mg/L (Government of Canada 2018; HBM4EU 2020). All of them includes all forms of chromium; for freshwater guidelines, Australian government has established 0.003 mg/L (for CrIII) and 0.001 mg/L (for CrVI). As can be seen these international values mentioned above are more in concordance with the acute sensitivity of the rotifer even some orders of magnitude below the Mexican Law.

Chronic toxicity test.

The intrinsic growth rate of *Lecane papuana* was significantly affected by chromium concentrations used (Fig. 1). *Lecane papuana* was affected significantly from 0.163 and 0.0885 mg/L (LOEC values of CrIII and CrVI respectively); Snell and Moffat (1992) carried out a study with similar conditions as ours, with CrVI in *Brachionus calyciflorus*, Determining an LOEC of 3.2 mg/L, resulting thus 36-fold more toxic for *L. papuana* than *B. calyciflorus*.

Other chronic studies have been carried out with other zooplankton genera, like the one made by Hermens et al. (1984), with *D. magna*, using CrVI, they found a LOEC value under 0.27 mg/L, being then 3-fold more toxic for *L. papuana* than *D. magna*, similar comparison could be made with the study of Wong and Pak (2004) where they report same LOEC (at least 0.27 mg/L) over the copepod *Mesocyclops pehpeiensis* (Also more toxic for the rotifer).

The chronic studies over intrinsic growth rate in general with chromium are variated, because the Genus and species level diversity in zooplacton is tremendous, leaving big variations in taxa, that have morphological differences (Segers 2007) that makes vary results on toxicity.

Bioconcentration tests

The criterion to classify a chemical substance as “bioaccumulative” requires the BCF to be comprised between 1000 and 5000; thus, chromium in *L. papuana* can be classified as “very bioaccumulative” because of its high BCF, which was higher than 5000. The CrIII presented a BCF of 1308.84, which is about ten-fold lower than the one obtained for CrVI (BCF = 12,299.44). Thus, there is a significant difference in the accumulation rate between these two metal speciations.

According to the Table 4, we can compare some studies carried out with CrVI with ours; the fish *Puntius sarana* was exposed to 2.19 mg/L (about 12-fold times higher than the concentration used in *L. papuana*) with food supplementation; the concentration of CrVI found in the fish indicates that the rotifer is over 21-fold more bioaccumulative than the fish. Moreover the highest bioaccumulation of CrVI found on the fish *Catla catla* (exposed to 2.19 mg/L) was over 2.7-fold times

smaller than what we found with *L. papuana* (Table 4). The fish *Mugil Cephalus* bioaccumulated at least three times less CrVI with at least 56-fold times higher concentration of exposition (160 mg/L), regarding to *L. Papuana*; thus we could infer that the rotifer bioaccumulate more CrVI than some freshwater fishes.

Table 4
Bioconcentration Factor for Chromium VI in different taxa.

Species	Accumulated Cr concentration (µg/g)	Source
<i>Argyrodiaptomus falcifer</i> (Calanoida: Diaptomidae)	50 (2 d) CrVI	(Gagneten et al. 2009)
<i>Catla catla</i> (Cypriniformes: Cyprinidae)	800 (7 d) CrVI	(Sanyal et al. 2017)
<i>Daphnia magna</i> (Anomopoda: Daphniidae)	80 (2 d) CrVI	(Gagneten et al. 2009)
<i>Hyalella Azteca</i> (Amphipoda: Hyalellidae)	134.59 (4 w) CrVI	(Norwood et al. 2005)
<i>Lecane papuana</i> (Monogononta: Lecanidae)	2177 (24 h) CrVI	This study
<i>Mugil Cephalus</i> (Mugiliformes: Mugilidae)	700 (96 h) CrVI	(Rajkumar and Tennyson, 2013)
<i>Puntius sarana</i> (Cypriniformes: Cyprinidae)	100 (21 d) CrVI	(Sanyal et al. 2017)
<i>Zilchiopsis collastinensis</i> (Decapoda: Trichodactylidae)	200 (14 d) CrVI	(Gagneten and Imhof, 2009)

Hyalella Azteca bioaccumulated 16-fold times smaller concentration of CrVI than what we found in *L. papuana* (Table 4), but this study was carried out in a period of 4 weeks of exposition (ours was five days) and the concentration of exposition was 0.176 mg/L, almost the same concentration that we used (0.177 mg/L). In *Daphnia magna* and *Argyrodiaptomus falcifer* were obtained bioconcentration values, being approximately 20 and 10 times smaller (respectively) than what we found in *L. papuana* (Table 4), in addition the concentration that *L. papuana* was exposed (0.177 mg/L) was almost a half of that used with these two species (0.350 mg/L). However, our study was carried out in five days and those mentioned above in two. The crab *Zilchiopsis collastinensis* showed a bioconcentration 10 times smaller of what we 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, certain species as mentioned above (e.g. Cypriniformes: Cyprinidae) had demonstrated to had strong ability to resist the CrVI exposure and to remove it from the body, involving Glutathione S-transferases and metallothionein proteins in the detoxification, moreover the capability of antioxidant enzyme systems recovery from the CrVI damage has been reported (Yuan et al. 2017); on the other hand previous studies with organisms of the Lecanidae family mention that these rotifers have a thick lorica that acts as a barrier against toxic substances, for example, with metal ions (negatively charged chromates), it has been observed that they can be deposited in the lorica of rotifers of this genus (Aguilar et al. 2019); and for this, they could be bioconcentrating more chromium than other species such as those mentioned in the Table 4. Not to mention that the ecological niche occupied by species might have played a significant role in the accumulation, some of them are surface feeders and planktivorous, probably accumulating chromium mostly from water; or omnivorous which diet is constituted by mud, debris, and detritus in addition to macrophytes and algae (Joadder 2014); indicating thus, that there is intricate interaction between chromium accumulation in water, sediments, aquatic plants or fishes, even varying between its tissues accumulation (gill, liver, etc) depending on environment exposition, or fluctuating by concentration modified for Binding of chromium in the sediment soil, depending on oxidation status, which is altered by pH, and microbial processes (Sanyal et al. 2017)

Then, as we mentioned above, the Maximum permissible levels of chromium in water of diverse uses in Mexico is at least 0.5 mg/L (NOM-001-ECOL-1996 1996), and the values that we obtained for chronic LOEC and NOEC for CrIII were 0.163 and 0.0816 respectively, which are around 3 and 6 times lower than 0.5 mg/L in the law; the corresponding values for CrVI were 0.0885 and 0.0443 respectively, 5.6 and 11.3 times lower. These findings mean that the rotifer could be harmed chronically in those environments that contain chromium, in spite of being in compliance with the rule.

There are very few studies of chromium carried out with rotifers and it is necessary to delve into them; in general, the comparison of some studies with Cr with other species, point the rotifer as more sensitive in acute, chronic and bioconcentration tests. The great differences between 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 ADN 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 and Imhof 2009), moreover depuration of accumulated Chromium in aquatic organisms differs 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)

Conclusions

In this work, acute, chronic and bioconcentration toxicity tests were evaluated with the rotifer *L. papuana*, which is a sensitive organism to the effects of the two chromium speciations, Susceptibility of this organism was evaluated at low concentrations, which are of environmental concern; was discussed the acute rotifer sensitivity comparing our results with those of other species tested, being *L. papuana* more sensitive in almost all the cases. Maximum permissible chromium levels in the Mexican regulations are above the LC50 value obtained for the rotifer; even above the international regulations. In general both chromium speciations showed bioconcentration in the rotifer, and even more than other species compared in all cases. Could be proposed this species as model organisms for toxicity assessment in tropical and subtropical environments, in acute, chronic, and bioconcentration tests, subject to studies that indicate otherwise.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Intrinsic growth rates (r) of *Lecane papuana* exposed to five different concentrations of the two chromium speciations studied :a) CrIII and b) CrVI. Significant differences were established through one-way ANOVA and Dunnett's multiple comparison tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (N = 4)

Figure 2

Results of bioconcentration on *L. papuana* exposed to three different concentrations (CL1, CL10 and CL50) of a) CrIII and b) CrVI determined by atomic absorption spectrophotometer. Significant differences were established through one-way ANOVA and Dunnett's multiple comparison tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant differences are observed with respect to the control in both cases. $N = 4$