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Research article

Evaluation of the freshwater copepod *Acanthocyclops americanus* (Marsh, 1983) (Cyclopidae) response to Cd, Cr, Cu, Hg, Mn, Ni and Pb

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Abstract: The toxic effect of cadmium, chromium, copper, mercury, manganese, nickel and lead on adult *Acanthocyclops americanus*copepods was evaluated to determine the sensitivity of this species to these metals. Toxicity tests were carried out to determine the LC_{50}, after which bioassays were carried out with environmentally relevant sublethal concentrations in order to measure oxidative damage to cell membranes as well as neurotoxic effects. Cadmium was the most toxic metal and manganese was the least harmful. Copper had the greatest oxidative effect (lipid peroxidation) and nickel had the least effect. A 3% to 79% drop was observed in acetylcholinesterase (AchE) activity, with copper causing the greatest inhibitory effect. *A. americanus* sensitivity to cadmium, manganese and lead was similar to that recorded for *Daphnia magna* neonates, but the copepods were less sensitive to chromium, copper, mercury and nickel. The response of *A. americanus* to exposure to metals makes it possible to propose it as a test organism to evaluate the presence and effect of these elements, particularly cadmium, manganese and lead, in toxicity studies, and possibly for monitoring purposes.

Keywords: *Acanthocyclops americanus*; metal toxicity; lipoperoxidation; neurotoxic effects; freshwater copepods

1. Introduction

The lacustrine catchment area of the Valley of Mexico presents high indices of disruption caused by various issues among which are the expansion of the urban area, the excessive exploitation of the water table, the desiccation of springs, the modification of riverbeds and the influx of untreated wastewater from industrial, domestic and agricultural activities [1,2]. All this has brought about drastic changes in the environmental conditions of rivers, creeks, ponds, dams and lakes, changes that affect the organisms that live there, decreasing their distribution and causing some species to be threatened and others to have disappeared [1,3].

High concentrations of contaminants, primarily metals and pesticides, have been found in the water systems of this region [4–9]. Metals are considered to be contaminants that cause serious problems to the environment since they cannot be removed efficiently through natural processes. Their removal requires physicochemical processes that are expensive, while they are highly persistent, accumulate and are biomagnified (e.g., As and Hg) in food chains. They are also toxic to aquatic organisms depending on their concentration [5,10,11].

High levels of Cd, Cr, Cu, Hg, Mn, Ni and Pb are found in the water of aquatic systems in the Valley of Mexico, including the catchment areas of the Lerma River where Cd, Cr, Cu, Hg, Ni and Pb concentrations vary from11.3 to 980 μg L^{-1} (Table 1) [4,5,7,12].

In the case of Xochimilco, the concentration of metals detected in the canals varies from0.071 to 9946.6 μg L^{-1} [6,9,13]. Average levels of Cr (1.93 mg L^{-1}), Ni (4.11 mg L^{-1}) and Pb (0.418 mg L^{-1}) detected in thenavigationcanals [9,13] are above those indicated by the NOM-001-SEMARNAT for the Protection of Aquatic Life (Cr = 0.5–1.0 mg L^{-1}, Ni = 2.0–4.0 mg $L^{-1}$and Pb = 0.2–0.4 mg L^{-1}) [14].

Table 1. Metal concentrations found in waters of aquatic systems in the Valley of Mexico.

Metals	Lerma River(μg/L)	Xochimilco (μg/L)	Sublethal bioassays Concentrations (μg /L)
Cd	11.3 ± 7.8	0.5 to 24.5	4.1
Cr	17.2 ± 8.0	54.1 to 331.5	331
Cu	119 ± 76	351.3 to 2198	90
Hg	118.5 ± 103.5	0.071 to 7.61	26
Mn	980 ± 440	1857 to 9946.6	35170.0
Ni	90 ± 12	89.4 to 8136.8	1660.0
Pb	116 ± 77	5.6 to 431.2	230
Reference	4, 5, 7, 12	6, 9, 13	This study

Metals may cause adverse effects in aquatic organisms depending on the concentration and the exposure time. In elevated concentrations, they cause the death of sensitive organisms. In sublethal exposures, they bring about changes in enzymatic activities at the biochemical level [15]. They cause changes in physiological rates (feeding rate, excretion rate and growth rate), and upheavals in the behaviour, reproduction and survival of neonates and juveniles [11,15]. They also lead to the generation of Reactive Oxygen Species (ROS) that cause oxidative stress in aquatic organisms such as fish, molluscs and crustaceans [16,17]. ROS act on cellular macromolecules such as lipids, proteins and DNA. Enzymatic activity and the structural lipids of cell membranes are the main targets of metals [18]. Prolonged oxidative stress leads to aging, diseases and even cellular death due to necrosis and/or apoptosis [19].

Moreover, acetylcholinesterases (AChE) are enzymes in the esterases group whose function is the hydrolysis of the acetylcholine neurotransmitter (ACh). These enzymes are essential in controlling the transmission of nerve impulses that run from nerve fibers to muscle cells, autonomous ganglia and the central nervous system. Prior studies have demonstrated that metals may be neurotoxic to aquatic species because they affect AChE activity, causing changes in mobility and behaviour that compromise the survival of organisms [20,21].

*Acanthocyclops americanus*is a copepod species of the order Cyclopoida. These organisms are 0.44 to 1.12 mm in length and are part of the zooplankton community in freshwater aquatic systems [22,23]. They are important from the ecological point of view because they are food for fish, crustaceans and other predatory specimens like axolotls [23].

Apart from Mexico, *A. americanus* is found in Spain, the United States and France. In Mexico, the presence of populations of this species has been reported for the lacustrine catchment area of the Valley of Mexico [23], while Enríquez and assistants (2011) recently found them in the lake at Huetzalin, Xochimilco [22].

Studies on the effect of contaminants on native organisms are very few and its harmful effects are unknown in many of the species present in subtropical and tropical climates [24]. Since no studies have been carried out on *A. americanus*sensitivity to diverse xenobiotics, an evaluation of the toxicity, oxidative damage and neurotoxic effect of cadmium, chrome, copper, mercury, manganese, nickel and lead on adult*A. americanus*copepods was carried out in this study to determine the response of the species to each metal, and whether *A. americanus* is more sensitive to these elements compared with other copepod species and neonates of *Daphnia magna*, a species that is used in Mexico as a test organism to evaluate the toxicity of effluents that are discharged into natural systems [25].

2. Materials and method

2.1. Test organisms

*Acanthocyclops americanus*adults (985 ± 30 μm) were obtained from water samples collected from semi-permanent pools located at the CIBAC (Cuemanco Biological and Fish Farm Research Center). The organisms were grown in the laboratory, in two-litre glass jars (pyrex) with reconstituted water [26], for four months, under the following conditions: temperature 22 ± 2 ℃, pH 7.1, total hardness 165 ± 5 mg L^{-1} and dissolved oxygen > 7 mg L^{-1}. The copepods were fed every third day a mixture of microalgae (*Monraphidium* sp. 1 x 10^6 cel/mL), pulverised fish feed (Tetramin 0.1 g/L) and rotifers (*Brachionus* sp. 160 ± 20 organisms/mL) [22].

2.2. Chemicals

The metals were prepared as standard solutions [27,28] with deionized water and the following salts: $CdCl_2$, (Baker, 99% purity), $K_2Cr_2O_7$ (Merk, 99.5%), $CuSO_4$ (Baker, 99.9%), $HgCl_2$ (Merk, 99.98%), $MnCl_2 4H_2O$ (Baker, 99.99%), $NiCl_2$ (Merk, 99.5%) and $Pb(NO_3)_2$ (Baker, 99%). The standard solutions and the tests were prepared the day the bioassays began.

2.3. Toxicity tests

The adult copepods (917 ± 74 μm) obtained from the laboratory cultures were exposed to five nominal concentrations of each metal (for the tests with Cd, Cr, Cu and Hg: 0.1, 0.5, 1.0, 5.0 and 10 mg L^{-1}, and for the bioassays with Mn, Ni and Pb: 1, 10, 25, 50 and 100 mg L^{-1}). One control was left with no toxic substance. Twenty organisms were placed in crystal glasses (pyrex) with 50 mL of each test solution (in triplicate). The conditions during the bioassays were as follows: temperature 22 ± 2 ℃, pH 7.1, total hardness 160 ± 5 mg L^{-1}, photoperiod 12 hrs light/12 hr darkness and dissolved oxygen > 7 mg L^{-1}. The copepods were not fed during the test trials. Each bioassay was repeated a minimum of three times.The number of immobile organisms in each test was recorded after 24 and 48 hours of exposure [28,29]. The LC_{50} (Lethal Concentration 50) was determined with the data obtained following the Probit method via the EPA Probit software. The calculation to compare the LC_{50} and its confidence intervals was carried out using the statistics described in APHA (1994) [28] to evaluate the statistical significance of the differences recorded among the different treatments:

$$f_{1,2} = antilog\sqrt{(log f_1)^2 + (log\ f_2)^2}$$

$$f_{1,2} = 1.96\ SD/LC50_{1,2}$$

where:

SD =Standard deviation
LC_{50} = Lethal concentration 50
f = Confidence limit factor
f_1 = Upper endpoint of the 95% confidence interval
f_2 = Lower endpoint of the 95% confidence interval

2.4. Sublethal bioassays

A sublethal assay was then carried out for 8 days, with a change in the test solutions every 48 hours. The organisms were exposed to the LC_1 of each metal (table 1). Fifty copepods were exposed to the metals (in triplicate). At days 2, 4 and 8 of exposure, a random sample of 15 organisms was taken from each replica and put together to create a composite sample (n = 45) (in triplicate). These samples were used to determine the degree of lipid peroxidation and acetylcholinesterase (AchE) activity in copepod tissues to evaluate the oxidative and neurotoxic effect of these metals.

2.5. Determination of biochemical parameters

2.5.1. Lipid peroxidation analysis

The degree of lipid peroxidation was determined in a thiobarbituric reactive species (TBARS) assay. This method is based on the reaction of thiobarbituric acid (TBA) and the subproducts formed by the effect of free radicals (ROS) on cell membranes [30].

The composite samples were homogenised in a 3 mL phosphate buffer (0.02 M, pH 7.2) using a stainless steel homogeniser in an ice bath at 4 ℃.

One mL of each homogenate was incubated at 90 ℃ in a mixture of thiobarbituric acid (0.5%), trichloroacetic acid (15%) and hydrochloric acid (0.25 M) for 20 minutes. Three replicates per

treatment were prepared. The samples were then centrifuged and analysed in a spectrophotometer (Genesis) at 535 nm. The concentration of TBARS was calculated with an extinction coefficient of 1.56 x 10^5 M/cm, and the results were expressed as nmol TBARS/mg protein.

2.5.2. AChE activity analysis

AChE enzyme activity was measured following the Ellman et al. (1961) method, modified for microplates. One mL of each homogenate was centrifuged at 5000 g and 4 ℃ for 10 minutes, and the supernatant was collected to determine AChE activity.

A volume of 0.05 mL of supernatant and 0.25 mL of reaction mixture (DTNB 10 mM, phosphate buffer pH 7.2 and acetylcholine 0.075 M) was placed in a multi-well plate (96 wells).

Activities were measured at 405 nm in an ELISA reader (Multiskan Spectrum). The reaction was monitored for 20 minutes. AchE activity was expressed as the amount of enzyme that catalysed the hydrolysis of 1 nmol of acetylcholine per minute per milligram of protein (nmol/min/mg protein) [31,32].

The concentration of protein in the samples was evaluated following the Bradford method, with 100 μL of each homogenate and 1 mL of the Bradford reactive. The samples were read in a spectrophotometer (Genesis) at 535 nm. A calibration curve was used with bovine serum albumin as a standard [33].

2.6. Statistical analyses

The Tbars level and AchE activity data obtained in the bioassays with the metals were analysed with a Kolmogorov-Smirnov test to determine normality. Later, the data were analysed with a two-way ANOVA test considering the type of metal and the exposure time as factors. A multiple comparison was then carried out with a Bonferroni test to determine the statistical importance of the differences observed between the control and the different treatments with metals and exposure times. A significance level of <0.05 was considered for the analyses. All statistical analyses were run with the NCSS v 97 software for Windows [34].

3. Results

3.1. Toxicity tests

The data obtained from the lethality bioassays indicated that the most toxic metal for *A. americanus* adults was cadmium and the least harmful was manganese (Figure 1). Cadmium was 77 times more toxic than chromium, 49 times more toxic than copper, 17 times more toxic than mercury, 1725 times more toxic than manganese, 513 times more toxic than nickel, and 1546 times more toxic than lead.

LC_{50} values (48 hours) indicated that the toxicity of the metals was, from the most toxic to the least: Cd > Hg > Cu > Cr > Ni > Pb > Mn.

The analysis of the LC_{50} dataand its confidence intervals indicated no significant differences in Mn and Pb toxicity ($P < 0.05$). However, significant differences were observed in the responses to Cd, Cr, Cu, Hg and Ni ($P < 0.05$) (Figure 1).

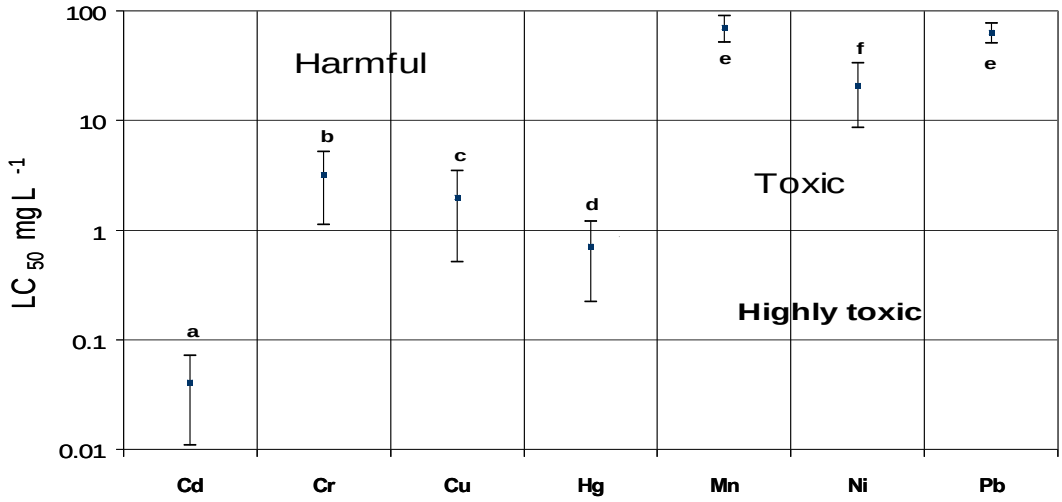

Figure 1. Median lethal concentration (LC_{50}, 48 h) values and confidence limits ($P < 0.05$) for *Acanthocyclops americanus* copepods exposed to Cd, Cr, Cu, Hg, Mn Ni and Pb. (Different letters indicate significant differences among different metal treatments ($P < 0.05$).)

3.2. Sublethal bioassays

3.2.1. Lipid peroxidation analysis

The mortality rate in the sublethal bioassaysvaried from 0 to 4%, values that lie within acceptable limits for toxicity tests [26–28].

It is important to mention that the Cd, Cr, Cu, Hg, Ni and Pb concentrations used in the sublethal bioassays are environmentally relevant. This is because they fall within the concentration intervals that are reported for water in the aquatic systems of the Valley of Mexico (Table 1).

The average data on the degree of lipid peroxidation recorded in the metal assays ranged from 2.07 ± 0.34 nM Tbars mg^{-1} to 22.23 ± 5.47 nM Tbars mg^{-1} (Figure 2). The metal that caused the greatest oxidative effect on the copepods was Cu and the metal with the least effect was Ni. In the case of the tests carried out with Cu, Cr, Hg, Mn and Pb, a direct relationship was observed between the concentration of Tbars and the exposure time, as the degree of lipid peroxidation increased with exposure time ($P < 0.05$) (Figure 2). In most cases, significant differences were observed in relation to the control group, however, no significant differences were observed at days 2, 4 and 8 of exposure in the bioassays with Ni ($P < 0.05$). Similarly, no significant differences were recorded in relation to the control group after 2 days of exposure in the tests with Cd and Mn ($P < 0.05$) (Figure 2).

3.2.2. AChE activity analysis

Regarding AchE activity, significant differences were observed in relation to the control group in thetests with Cd, Cr, Cu, Hg and Pb, for all evaluation times ($P < 0.05$) (Figure 3). The AchE

activity values recorded in these tests were lower than those recorded for the control group, indicating thatthese metals may inhibit AchE activity. This varied from 15% to 79%. The metal that caused the greatest inhibitory effect was Cu (Figure 3). In the case of the tests with the copepods exposed to Mn, AchE activity was observed to be similar to that recorded for the control group 2 days after beginning the bioassay. However, a 36% and 34% decrease in the activity of this enzyme was observed after 4 and 8 days of exposure to this metal, respectively. Ni caused no inhibitory effect on AchE activity (Figure 3).

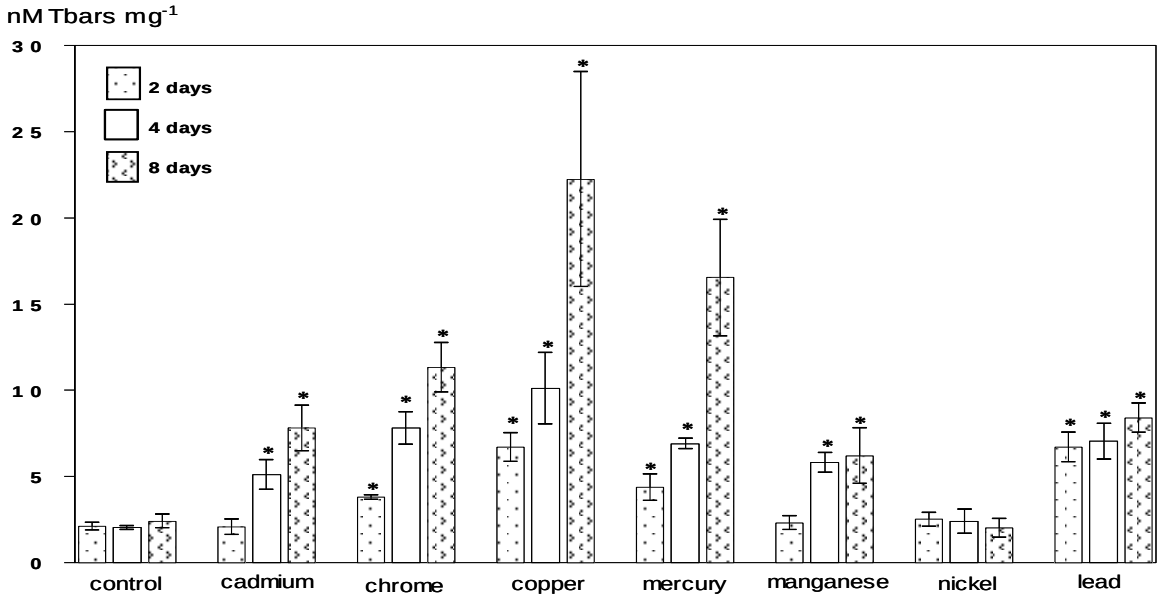

Figure 2. Levels of lipid peroxidation measured as Tbars (nM mg^{-1}) (mean ± standard deviation) in tissues of the copepod *Acanthocyclops americanus* exposed to Cd, Cr, Cu, Hg, Mn, Ni and Pb. (*Significant differences ($P < 0.05$) among the exposed organisms and the control group (Bonferroni test).)

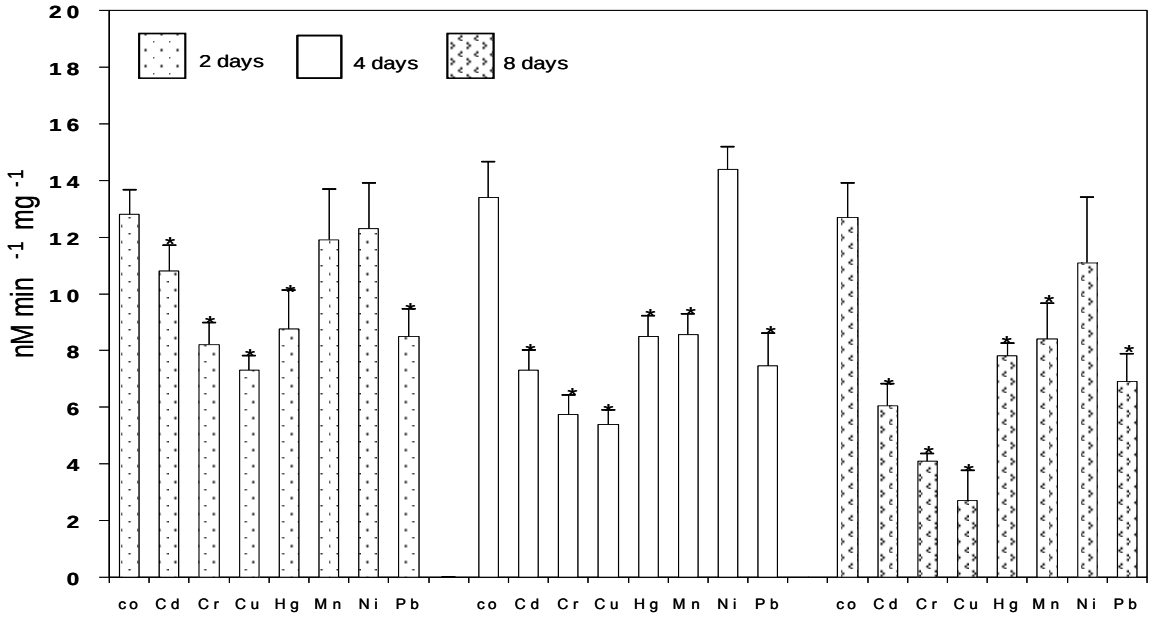

Figure 3. Changes in AchE enzyme activity (nM min^{-1} mg^{-1}) (mean ± standard deviation) in *Acanthocyclops americanus* exposed to Cd, Cr, Cu, Hg, Mn, Ni and Pb. (*Significant differences ($P < 0.05$) among the exposed organisms and the control group (Bonferroni test).

4. Discussion

Most of the studies that have evaluated the toxic effect of different contaminants on copepods have involved species that live in marine systems. The species that are most frequently used in toxicological evaluations are *Acartia tonsa* and *Tigriopus japonicus* [35–37]. Very few studies have taken place with freshwater species, however, this research proves that freshwater copepods are quite sensitive to metals [38–40].

When we compared the response of*A. Americanus* adults to the different metals, as has been done for other species of freshwater copepods like *Cyclops abyssorum* and *Eudiaptomus padanus* that live in oligotrophic environments [41], we observed that they are more sensitive to Cd, Cr, Cu and Hg (Table 2) and less sensitive to Ni and Pb (Table 2). Also, *A. americanus* is less sensitive to Cr, Cu and Ni compared with *Mesocyclops pehpeiensis*, whereas *Tripocyclops prasinus mexicanus* and *Cyclops* sp. are less sensitive to Cd, Cr and Cu than *A. americanus* [42–44](Table 2). In addition, the LC_{50} values obtained in the tests with Cd and Cr indicated that *A. americanus* is more sensitive to these metals than species of marine copepods like *Acartia tonsa*,*Tisbe holothuriae*, *Tigriopus japonicas*, *Tigriopus fulvus*, *Tisbe battagliai* and *Tigriopus brevicornis* [45–51]

Moreover, our results indicate that *A. americanus* is sensitive to Cd, Mn and Pb, as are *Daphnia magna* neonates. When comparing the sensitivity of *A. americanus* adults with that of *D. magna* neonates [45,52,53], a species that is used worldwide to evaluate toxicity in samples of water, elutriation and leaching, it is evident that *A. americanus* adults are less sensitive to Cr, Cu, Hg and Ni (Table 2). The LC_{50} recorded for *A. americanus* exposed to Cd was 0.041 ± 0.03 mg L^{-1}, a value that is close to that which is tolerated by *D. magna* neonates (0.054 mg L^{-1} (0.039 to 0.069 mg L^{-1}) [52,53].

Likewise, *A. americanus* presented a similar sensitivity to that of *D. magna* neonates to manganese and lead [45] (Table 2).

Species of the genus *Acanthocyclops* have been proposed as water quality indicators in cases of contamination by metals. The species *A. balcanicus* and *A. venustus*that inhabit the Aries River in Romania have been proposed as indicator organisms for water with high concentrations of aluminum, copper and zinc associated with particulate and suspended solids. However, these metals, in dissolved form, are very toxic to these species [54]. *Acanthocyclops trajani*, a species that inhabits the Nile River, has been proposed as a species that is sensitive to water contaminated with metals [55,56]. Krupa (2007) proposed*A. rubustus*, along with other species of Cyclopoida, as an indicator of mesotrophy [57]. In addition, this researcher found a highincidence of deformed *A. rubustus*males in sites where elevated concentrations of metals were recorded in the Shardarinskoe Lake, Kazakhstan. Finally, Gagneten and Paggi (2009) recorded Cyclopoida, including *A. robustus*, as more sensitive to metals than rotifers in the Salado River, Argentina [58].

Table 2. Median Lethal 50 values determined for other copepods species in similar test conditions

SPECIES	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Reference
	LC_{50} 48 hours ppm							
Cyclops abyssorum	3.8 ±2.5	10 ±2.1	2.5 ±0.5	2.2 ±1.1	nd	15 ±10.5	5.5 ±2.2	41
Eudiaptomus padanus	0.55 ±0.22	10.1 ±1.8	0.5 ±0.15	0.85 ±0.17	.	3.6 ±1.1	4.0 ±2.4	41
Mesocyclops pehpeiensis	-	0.51 ±0.15	0.075 ±0.046		-	1.191 ±0.47	-	42
Cyclops sp.	-	10.47	-	0.60	-	-	-	44
Tripocyclops prasinus mexicanus	2.23 ±1.7	-	2.11 ±0.79	0.199 ±0.156	-	-	-	43
Daphnia magna	0.054 ±0.015	0.77 ±0.25	0.086 ±0.03	0.0186 ±0.0098	61.2 ±9.8	5.1 ±4.5	57.1 ±15.5	45, 52
Marine copepods								
Acartia tonsa	0.337 ±0.12	11.9 ±2.7	0.064 ±0.01	0.019 ±0.008	-	5.0 ±1.9	-	45
Tisbe holothuriae	0.97	8.14	0.08	-	-	-	-	46
Tigriopus japonicus	25.2 ±7.39		3.9 ±1.97	-	-	-	-	47
Tigriopus japonicus	12.1 ±3.1	-	-	-	-	17.7 ±5.1	-	48
Tigriopus fulvus	12.36 ±4.3	128.16 ±14.9	-	0.52 ±0.15	-	-	-	49
Tisbe battagliai	0.340	-	0.088	-	-	-	-	50
Tigriopus brevicornis	0.048 ±0.021	-	0.150 ±0.085	-	-	-	-	51
Acanthocyclops americanus	0.041 ±0.03	3.16 ±2.05	2.004 ±1.5	0.712 ±0.49	70.74 ±19.05	21.04 ±12.4	63.40 ±13.005	This study

The toxicity of metals recorded for *A. americanus*,according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), was as follows: Cd and Hg were highly toxic, Cr and Cu were toxic, and Mn, Ni and Pb were harmful to this species (i. highly toxic: $EC_{50} \leq 1$ mg L^{-1}; ii. toxic: EC_{50}:1 mg $L^{-1} \leq 10$ mg L^{-1}; iii. harmful to aquatic organisms: EC_{50} 10 mg $L^{-1} \leq 100$ mg L^{-1}; iv. non-toxic: $EC_{50} > 100$ mg L^{-1}) [59].

The use of biochemical indices to identify sublethal effects produced by xenobiotics has currently increased. These indices are called biomarkers [60,61]. The degree of lipid peroxidation and AchE activity have been proposed as biomarkers that are effective in identifying damage that may be either reversible or permanent, depending on the concentration and the exposure time to metals and other xenobiotics [61,62]. Publications on biomarker evaluations in copepods are scarce, and most have been carried out on marine species.

The results obtained in this study indicate that Cd, Cr, Cu, Hg, Mn and Pb significantly increased lipid peroxidation in copepod tissues after 8 days of exposure. This shows that these metals caused oxidative stress, as the degree of lipid peroxidation indicated that the generation of ROS overcame the antioxidant defenses, causing cellular membranes to be altered [63].

The lipid peroxidation levels recorded in this study demonstrated that the most deleterious metal was Cu (22.23 $\pm$ 5.56 nM Tbars mg^{-1}; control = 2.4 $\pm$ 0.65 nM Tbarsmg^{-1}). Prior studies carried out by Bo-Mi et al. (2014) on the copepod *Tigriopus japonicus* also recorded Cu with a highly oxidative effect at concentrations of 0.01 and 0.1 mg L^{-1} [37]. Likewise, we observed that Cd induces lipid peroxidation. This was previously reported by Wang and Wang (2009) who tested concentrations of 80.01 to 0.1 mg L^{-1} and reported that the degree of lipid peroxidation was positively correlated with Cd concentration in assays with *T. japonicus* [64]. Likewise, Bo-Mi et al. (2014) observed that Cd induced the generation of ROS in *T. japonicus* tissues [37].

Our results indicate that Ni did not cause any rise in lipid peroxidation levels in *A. americanus*. This agrees with previous reports by Wang and Wang (2010) on tests with *T. japonicus* [64].

Another biochemical response evaluated in this study was the activity of the AchE enzyme. The tests with *A. americanus* showed that Cd, Cr, Cu, Hg, Mn and Pb had an inhibitory effect on the activity of this enzyme, with average inhibition percentages of 38%, 54%, 60%, 36%, 26% and 41% respectively, making it possible to consider these metals as neurotoxic agents.

Studies on aquatic organisms have shown that a 20% decrease in AchE activity affects important physiological functions such as feeding and swimming [66]. When inhibitions greater than 25% occur, the life expectancy of the affected organisms is reduced [67,68].

Nickel did not decrease AchE activity after 8 days of exposure in the tests with *A. americanus*, as Wang and Wang (2010) recorded for the marine copepod *Tigriopus japonicus*when exposed to Ni [65].

Finally, according to the results obtained in this study, the Cd, Cu and Hg concentrations recorded for the Lerma River basin, and those of Cd, Cr, Cu and Ni recorded for the Xochimilco canals, constitute a risk to *A. americanus*, since they are greater than the concentrations tested inthe sublethal bioassays with *A. americanus* that had an oxidative and neurotoxic effect on these organisms.

It should also be mentioned that the LC_{50} values for Cd and Cu recorded in this study (LC_{50} Cd = 0.041 $\pm$ 0.03 mg L^{-1}; Cu = 2.004 $\pm$ 1.5 mg L^{-1}) are lower than the values established as Maximum Permissible Limits in the NOM-001-SEMARNAT for the Protection of Aquatic Life (Cd = 0.1 to 0.2 mg L^{-1}; Cu = 4 to 6 mg L^{-1}) [14]. This may be considered a possible risk to organisms that come in contact with discharges that contain these metals. Thus, it is important to continue carrying out studies to evaluate the degree of the effects on the *A. americanus* populations in the short and long terms.

5. Conclusion

A. americanus is an organism with greater sensitivity to Cd and Cr metals compared to other freshwater and marine copepods species. *A.americanus* may be proposed as a test organism to evaluate the presence and effect of metals, especially Cd, Mn and Pb, in toxicity studies, as well as for monitoring purposes. However, more studies need to be done to propose this species as an indicator organism.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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