



Integral Study of *Paramecium caudatum* Acute and Chronic Toxicity, Sites of Entry and Distribution, Bioconcentration and Body Burdens of Five Metals

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Abstract

An integral analysis of the acute and chronic toxicity, bioaccumulation, sites of entry, and distribution of four trace metals: copper, iron, lead, and nickel, and the non-trace metal mercury were performed in the ciliate *Paramecium caudatum*. Mercury was the fastest metal accumulated, and the most toxic. The sensitivity of *Paramecium caudatum* to the five metals tested (Cu, Fe, Hg, Ni, and Zn) falls in the range of other ciliate species. We observed similarities between the toxicity of the five metals to the ciliate *P. caudatum* with the rotifer *Euchlanis dilatata*: (a) Mercury was the most toxic metal in terms of acute and body burdens. (b) Acute values were very similar in both species, Hg as the most toxic and Fe as the less toxic, (c) the vacuole/ingestion chronic tests were more sensitive than growth inhibition chronic tests. These analyses would ideally help generate safer guidelines for protecting aquatic biota.

Keywords Metal toxicity · Ciliates · Aquatic toxicology · Toxicokinetics · Environmental toxicology

Protozoans are acellular organisms representing the most complex group of protists. Ciliates are protozoans characterized by the presence of organelles shaped like hair called cilia; freshwater ciliates have a size range between 50 µm and 3 mm; they reproduce asexually by binary fission and with duplication times ranging from 4 to 5 h up to 72 h (Nałęcz-Jawecki 2013). The ciliated protozoans are basic components of microplankton and microbenthos, where they play critical roles regulating the flow of abiotic and biotic molecules and energy from one trophic level to the next (Madoni and Romeo 2006). Ciliated protozoans are a prolific group in aquatic ecosystems where there is biological treatment, they eliminate most of the dispersed bacteria (Madoni et al. 1992). Some studies on water contaminated with heavy metals have shown changes in the population dynamics of protozoan communities (Miyoshi et al. 2003; Rehman et al. 2009; Gong et al. 2014). The structural and functional diversity of these protozoan communities allows an assessment of effects on the diversity of species and equilibrium in the

dynamics of the trophic cascade caused by toxicants. Therefore, the ciliate assay has become an important tool to detect environmental perturbations and to assess the trophic state of an ecosystem (Madoni and Romeo 2006).

The metals: Cu, Fe, Hg, Ni, and Zn have been reported in levels exceeding safe values worldwide. Thus, Contreras et al. (2004) at the River Haina in the Dominican Republic reported levels of Cu above 1 mg/L, and 220 mg/L of Fe. In Argentina at Pergamino Creek, levels of Cu (> 30 mg/L), Fe (> 1360 mg/L), and Zn (> 5.4 mg/L) have been reported (Reynoso and Andriulo 2009). Saeed and Shaker (2008) reported for the Northern Delta Rivers in Egypt, levels of Cu ranging from 0.002 to 0.68 mg/L, Fe ranged from 0.008 to 1.98 mg/L, and Zn ranged from 0.004 to 0.66 mg/L. In Mexico, there are reports of levels (mg/L) of (a) Cu ranging from 13 to 600 (Guzmán-Colis et al. 2011; Mancilla-Villa et al. 2012; Zarazúa et al. 2013; Dimas et al. 2015), (b) Fe ranging from 765 to 38,440 (Guzmán-Colis et al. 2011; Zarazúa et al. 2013; Quintana et al. 2015), (c) Hg ranging from 1 to 31.8 (Guzmán-Colis et al. 2011; Mancilla-Villa et al. 2012; Dimas et al. 2015), (d) Ni ranging from 2.2 to 1060 (Quintana et al. 2008, 2015; Mancilla-Villa et al. 2012; Flores et al. 2018), and (e) Zn ranging from 12.2 to 100 (Guzmán-Colis et al. 2011; Mancilla-Villa et al. 2012; Zarazúa et al. 2013; Flores et al. 2018).

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In Peru, Huaranga et al. (2012) reported levels of Cu, Fe and Zn above 1000 mg/L. In Poland mg/L levels of Cu (3250), Ni (9.4), and Zn (3.8) have been reported (Michalec et al. 2014). In the Ganges River in India mg/L levels of Cu (408), Fe (82,700), Hg (85), Ni (97) and Zn (4000) have been reported (Paul 2017).

Toxicity tests both acute and chronic complemented with data on bioconcentration and analysis of entry and distribution of toxicants become an integral analysis. Hernández-Flores et al. (2020) published a study of four trace metals (Cu, Fe, Ni, and Zn), and one non-trace (Hg) that enhanced our knowledge on the toxicity mechanisms inside the rotifer *Euchlanis dilatata*. Therefore, the objective of this study is to perform an integral analysis of the toxicity of four trace (Cu, Fe, Ni, and Zn), and one non-trace (Hg) metals in the ciliate *Paramecium caudatum* that includes: (a) acute and chronic (grow inhibition and inhibition of vacuole formation) toxicity tests, (b) determination of the bioconcentration factor, (c) analysis of entry and distribution of the five metals using the dye Phen Green™, (d) determination of acute and chronic body burdens.

Materials and Methods

Selected *P. caudatum* specimens were cultivated in Petri dishes with Sonneborn medium (Sonneborn 1970). Acute tests were performed according to the protocol of El-Bassat et al. (2012) with slight modifications. Test started with washes of organisms with EPA medium (U.S. EPA 1992) to remove excess of culture medium. Then, 10 organisms were placed in each well in a 24-well polystyrene plate (Corning Co. USA). The ranges of the nominal concentrations used for each metal were: 10, 11, 12, 14, 16 µg/L for Cu, 1000, 1500, 2000, 2500, 3000 µg/L for Fe, 1, 5, 7, 10, 25 µg/L for Hg, 300, 400, 500, 600, 700 µg/L for Ni, and 500, 750, 1000, 1250, 2000 µg/L for Zn. Each well was taken to a final 1 mL.

Growth inhibition tests were performed according to the protocol of Miyoshi et al. (2003) with small changes. Tests started by placing five organisms in each well of a 24-well polystyrene plate (Corning Co. USA). The ranges of the nominal concentrations used for each metal were: 40, 60, 80, 100, 120 µg/L for Cu, 750, 1250, 1750, 2250, 2750 µg/L for Fe, 4.07, 8.14, 16.28, 32.56, and 65.12 µg/L for Hg, 10, 50, 100, 150, 200 µg/L for Ni, and 300, 475, 650, 825, 1000 µg/L for Zn. We added Sonneborn medium at 1 g/L at the start of the test. The total volume in the well was 2 mL. Then, the plate was placed in a bioclimatic chamber with a 16:8 light:darkness cycle at 25°C for 96 h. At the end of the 96 h exposure time the total number of organisms was counted in each well to obtain the percentage of inhibition of the population applying the following formula:

$$\%I = \left(\frac{N_c - N_t}{N_c} \right) 100$$

where % I is Percentage of inhibition, N is the total number of *P. caudatum* organisms alive after 96 h, t is treatment, c is control

We used the protocol of Kryuchkova et al. (2016) for the vacuole formation test with slight modifications. The test consisted of measuring the percentage of formation inhibition of digestive vacuoles in *P. caudatum* exposed to different concentrations of metal (Cu, Fe, Hg, Ni and Zn). We used fluorescent polystyrene microspheres of 1.01 µm of diameter (Dragon Green, Bangs Laboratories Inc., USA). The test started by placing 50 organisms per well in a 96-well 300 µL polystyrene plate (Corning Co. USA). The range of concentrations used for each metal were: 1, 3, 5, 7, 9 µg/L for Cu, 600, 900, 1200, 1500, 1800 µg/L for Fe, 1, 2.5, 4, 5.5, 7 µg/L for Hg, 100, 200, 300, 400, 500 µg/L for Ni, and 22, 55, 110, 550, 600 µg/L for Zn. Metal concentration was prepared in a 200 µL volume. Organisms were incubated in a bioclimatic chamber for 1 h at 25°C. Then, we added 1×10^6 microspheres/mL. The plate was slightly shaken and then incubated for 30 min in the dark at 25°C. Ingestion was interrupted by adding 40 µL of glutaraldehyde at 2.5% (v/v). Organisms were washed three times to eliminate microspheres in the solution. Then, they were placed at the center of a slide with masking tape strips at the edges to avoid the cover-glass crushing the organisms. Later, organisms were observed with a fluorescence microscope (Leica Co., Germany) with an excitation spectrum of 450 to 490 nm and an emission barrier at 515 nm, with a DC100 photographic chamber (Leica Co., Germany), coupled to an image analysis program (Kodak Co., USA). A total of 30 organisms were examined for each treatment.

Phen Green™ SK (PG) diacetate was purchased from Molecular Probes. The substrate was prepared as follows: 1 mg of PG was dissolved in 495 µL of distilled water and stored at 4°C at the dark until used. PG has an excitation maximum at 507/532 nm, and fluorescence is extinguished when interacting with metallic ions (Petrat et al. 1999; Shingles et al. 2004; Illing et al. 2012). Fifty organisms were placed in a well of a 96-well plate (Corning Co. USA), and we added 200 µL of the solution with metal or negative control. The substrate concentration was 71.5 nM. We evaluated two incubation times (1 and 24 h). Organisms exposed to metal were incubated at 25°C in darkness. Then, organisms were washed with EPA medium to remove metal and transfer to another well with 200 µL solution without metal. Then, we added 2 µL of PG, and incubated this sample at 25°C for 15 min (Petrat et al. 1999). At the end of exposure time, we added 50 µL of glutaraldehyde at 5% (v/v). Fluorescence was reported as in the inhibition of vacuoles formation tests.

Metal concentrations were measured by atomic absorption using the recommended technique (APHA 2005): Graphite Furnace for Fe, Ni and Zn (Method 3030E), Cu was determined by Flame Air-Acetilene (Method 3113B), and Hg by Hydride Generator (Method 3112B). We used a Perkin-Elmer AAnalyst for all determinations. Ten thousand *P. caudatum* organisms were exposed in a volume of 30 mL with each metal solution and incubated at 25°C with a 16:8 h light:darkness photoperiod for 24 h. After exposure, organisms were washed with EPA medium through centrifugation at 10,000 rpm. Then, samples were placed in Falcon tubes where the sample was washed with deionized water and HNO₃ Instra (70%). Each sample was analyzed in triplicate. The amount accumulated of metal (q) (µg/g dry weight) was calculated according to Volesky and Holand (1995) modified by Arunakumara and Zhang (2008):

$$q = (C_0 - C_t) V / W$$

where C₀ is the metal concentration in the medium (µg/L), C_t is the metal concentration at time “t” (µg/L), V is the total volume of the sample (L), W is the dry weight of *P. caudatum* organisms (g)

Bio concentration factor (BCF) was determined by dividing the concentration of metal accumulated in the body of *P. caudatum*, by the concentration of the metal in the solution.

Results

The measured concentrations of acute and chronic exposure to metals were determined by atomic absorption. The values obtained were: 94.1 ± 0.07%, 91.55 ± 0.085%, 81.4 ± 0.06%, 71.2 ± 0.28%, 92.4 ± 0.16% (mean ± 1 SD, n = 3) of the nominal values for Cu, Fe, Hg, Ni, and Zn respectively. The limits of detection and quantitation for each toxicant are included in the Supplementary Table 1.

The results of the acute and chronic toxicity tests for the five metals are shown in Table 1. In all tests NOEC < LC₁₀ < LOEC. In all tests R² was higher than 0.76 and the variation coefficient (VC) percentage was lower than 21%. The metals with higher toxicity were Hg and Cu, and Fe has lowest toxicity. The order of toxicity was Hg > Cu > Ni > Zn > Fe.

Nickel was the most toxic metal that inhibited the growth of *P. caudatum*, followed by Cu; Fe was the least toxic metal regarding growth inhibition (Table 1). In the case of vacuole formation inhibition, the most toxic metal was Hg followed by Cu and Fe was the least toxic (Table 1). The EC₅₀ values of the percentage of growth inhibition were less sensitive than those of the percentage of vacuole formation inhibition. Figure 1 shows a typical sample of the vacuole formation inhibition test. A greater

Table 1 Results of lineal regression (LC₁₀, LC₅₀, 95%C. L., R², and %VC), and ANOVA (NOEC and LOEC) of the acute and chronic toxicity tests with *Paramecium caudatum*. N = 5. 95%C.L. = 95% Confidence Limits of the LC₅₀ values.

Values	Cu	Fe	Hg	Ni	Zn
Acute					
NOEC	9.4	915.5	0.81	214	462
LC10	10.2	1220	2.9	277	480
LOEC	10.3	1373	4.1	285	693
LC ₅₀	11.7	1666.5	5.6	349.6	742
95%C.L	11.5–12	1567–1773	5.33–5.95	332–368	714–771
R ²	0.93	0.85	0.97	0.82	0.97
%V.C	1.83	4.3	4.15	0.49	4.98
Chronic %GI					
NOEC	19	687	4.07	7	214
LC10	29	708	5.48	7.2	255
LOEC	38	1144	8.14	36	338
EC ₅₀	72.9	1647	36.63	66.9	547
95%C.L	58–87	1256–2038	11.3–62.0	49–85	373–721
R ²	0.90	0.88	0.64	0.92	0.78
%V.C	4.7	1.64	11.98	5.87	4.52
Chronic % VFI					
NOEC	0.94	549	0.8	71	20
LC10	1.07	473	1.4	74	26
LOEC	2.82	824	2	142	51
EC ₅₀	4.72	1268	3.8	239	327
95%C.L	4–5.5	1008–1527	3–5	201–277	180–475
R ²	0.96	0.89	0.85	0.95	0.80
%V.C	6.79	4.54	4.55	3.72	3.05

%GI percentage of grow inhibition, %VFI percentage of vacuole formation inhibition, %V.C. percentage of the variation coefficient of the regression

All values are in µg/L

quantity of florescent microspheres is shown in the negative control of *P. caudatum* when compared with organisms exposed to metals for one hour (Fig. 1). The least vacuole formation is observed in organisms exposed to Hg (Fig. 1).

The dry weight of *P. caudatum* was 0.166 ± 0.026 µg for every individual (mean ± one SD, n = 5). The Bio concentration Factors (BCF) of Cu, Fe, Hg, Ni, and Zn are shown in Table 2. Mercury had the highest value followed by Fe, Zn, Cu and Ni. The accumulated concentration of each metal had the following order: Fe > Zn > Ni > Cu > Hg (Table 2). Mercury and Cu had the lowest LBB and Fe the highest. Regarding CBB in the case of vacuole formation inhibition Cu was the most toxic metal and for growth inhibition, Ni was the most toxic (Table 2). We calculated the Acute to Chronic Ratio (ACR).

Fig. 1 Vacuole formation inhibition test with *Paramecium caudatum*. Digestive vacuoles are the rounded structures in green. Bar = 20 μm. N = 30. Photographs show the highest concentration used in each test, both at bright and dark microscope fluorescent light

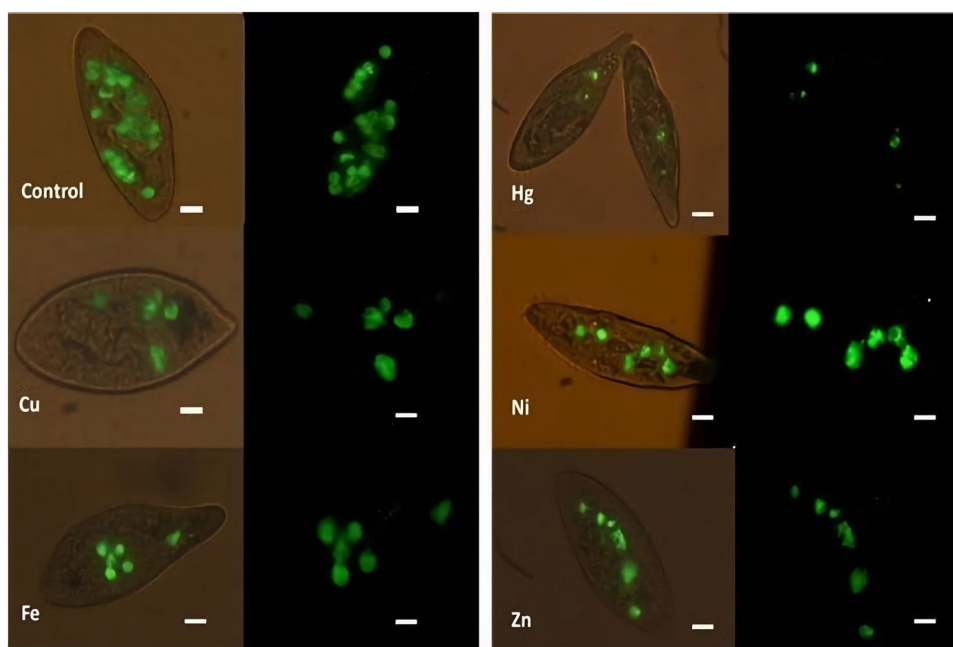


Table 2 Bio concentration Factors (BCF), Lethal Body Burden (LBB), Chronic Body Burden for vacuole formation inhibition (CBB_{VFI}), Chronic Body Burden for growth inhibition (CBB_{GI}), and

metal concentration accumulated in *Paramecium caudatum* after 24 h exposure to the acute LOEC concentration of each metal

	Metal Body Burden (μg metal for individual)	BCF	LBB (μg/g DW)	CBB _{VFI} (μg/g DW)	CBB _{GI} (μg/g DW)	ACR _{VFI}	ACR _{GI}
Cu	$1.94 \times 10^{-9} \pm 7.6 \times 10^{-12}$	7791	91.1 ± 0.2	36.8 ± 0.08	567.9 ± 1.2	7.20	0.44
Fe	$3.7 \times 10^{-7} \pm 6.2 \times 10^{-10}$	11,181	18,633 ± 55	14,177 ± 42.4	18,415 ± 55	2.48	1.87
Hg	$6.18 \times 10^{-11} \pm 5.68 \times 10^{-12}$	15,944	89.3 ± 1	60.6 ± 0.7	5835.6 ± 67.6	4.43	0.91
Ni	$1.23 \times 10^{-8} \pm 9.2 \times 10^{-11}$	4044	1413.8 ± 9	966.5 ± 6.1	270.5 ± 1.7	3.48	22.03
Zn	$1.35 \times 10^{-7} \pm 2.4 \times 10^{-9}$	8248	6120 ± 59.3	2697 ± 26.1	4511.6 ± 43.7	23.2	2.76

ACR_{VFI} acute to chronic ratio for vacuole inhibition formation, ACR_{GI} acute to chronic ratio for growth inhibition. Dw dry weight (mean ± one SD, n = 5)

For all other values (mean ± one SD, n = 3)

Regarding the vacuole formation chronic test, in the case of Cu, Fe, and Zn, there was a greater formation of vacuoles in the 1 to 24 h period; but this effect was not observed in Ni.

The acute-to-chronic (ACR) values in Table 2, show that Zn shows the highest ACR value for the vacuole inhibition tests, while Ni showed the highest ACR values for the growth inhibition tests. In contrast, Cu, Fe, and Hg showed low ACR values suggesting that LC₅₀ values are high for Cu and Hg in comparison to chronic values; in contrast for Fe both LC₅₀ and EC₅₀ values are high and close (Tables 1 and 2).

The experiments with Phen Green™ dye at 1 h were performed with the LC₅₀ value of each metal, and the 24 h exposure was performed at the LOEC value of each metal. We observed no significant difference for Fe, Hg, Ni, and

Zn, between both treatments (1 h LC₅₀ vs. 24 h LOEC). In the case of Cu, we observed more fluorescence at 24 h (Fig. 2). Our observations suggest that Cu was the slowest metal to accumulate after 24 h exposure, Fe, Ni, and Zn showed an accumulation similar between 1 and 24 h, and the metal that accumulated faster was Hg (Fig. 2). The assimilation of the Phen Green™ dye allowed us to observe changes in individual *Paramecium* organisms as the metals enter the individuals. However, there was no clear pattern or tendency. One of the problems that we observed is that Phen Green™ dye interacts with calcium vesicles of the *Paramecium* coloring them. This interaction might be difficult to estimate the accumulation or any other potential adverse effect caused by metal exposure.

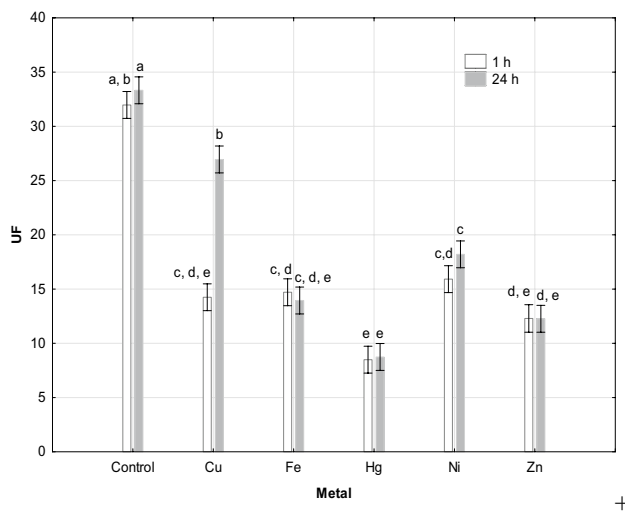


Fig. 2 Comparison between the means of the fluorescent units obtained between the two treatments with the dye Phen Green in *Paramecium caudatum* (30 min and 1 h metal exposure). N=30. Mean ± one SE. $p < 0.05$

Discussion

This integral study of toxicity of five metals in *P. caudatum* is the second published by our working team related to freshwater zooplanktonic organisms. The first publication made a similar analysis of the rotifer *Euchlanis dilatata* (Hernández-Flores et al. 2020). Our goal is to better understand the mechanisms of metal toxicity and analyze the similarities and differences, hopefully finding some trends and generalizations. In fact, in both zooplanktonic species, the non-trace metal Hg was the most toxic metal both in terms of acute toxicity and LBB (see Tables 1 and 2; see Hernández-Flores et al. 2020). The order of acute toxicity (LC_{50} values) was strikingly similar: $Hg > Cu > Ni > Zn > Fe$ in this analysis vs $Hg = Cu > Zn > Ni > Fe$ in Hernández-Flores et al. (2020). When comparing the LC_{10} values Hg was the most toxic for *Paramecium*, but Cu was more toxic for the rotifer (Hernández-Flores et al. 2020). In terms of CBB_{VFI} *P. caudatum* was more sensible to Cu, and for CBB_{GI} was Ni (Table 2). In that regard, Hg was the most toxic metal for rotifer in both chronic tests (Hernández-Flores et al. 2020). The mechanism of metal toxicity observed in *P. caudatum* through acute and chronic values suggests that the ciliate reduces its rate of vacuole inhibition at low Cu and Hg concentrations (Table 1), and the effect of reproduction inhibition is observed at higher concentrations that sometimes are higher than the LC_{50} values (Table 1). This is remarkably like what we found in the rotifer *Euchlanis dilatata* where ingestion inhibition was a sensitive endpoint and reproduction inhibition was affected very close to LC_{50} values (Hernández-Flores et al. 2020). Shakoory et al. (2004),

Madoni and Romeo (2006) and Rehman et al. (2009) have reported protozoans resistant to metals in wastewater and reservoirs contaminated by metals. The use of Phen Green™ (PG) dye allows the determination of the speed of metal concentration. After 1 h of exposure we determined the following trend: $Hg > Fe = Ni = Zn = Cu$. At 24 h exposure the trend was: $Hg > Fe = Ni = Zn > Cu$. Several studies reported that ciliates are very sensitive to Cu and its bioaccumulation is less efficient than that in other microorganisms (Díaz et al. 2006; Abraham et al. 2017). In contrast, Hg was the metal that accumulated fastest and the most toxic. Soto et al. (2018) found that protists incorporate Hg passively from the dissolved phase and actively through consumption and/or join to the picoplankton carrying Hg. The metals Fe, Ni, and Zn bioaccumulated at about the same speed at both exposure times (1 and 24 h); Fe and Zn were the least toxic. The tolerance of ciliates to Zn might be in part explained by the presence of metallothioneins (MTs) that can be induced by Zn (Díaz et al. 2006; Somasundaram et al. 2018). In presence of metals, the expression of MTs genes increase to detoxify (Martín-González et al. 2006; Somasundaram et al. 2018).

Ciliates exposed to metal lose their morphological integrity acquiring a circular shape (Pudpong and Chantangsi 2015), and their movements are slower (Wanick et al. 2008). Ciliates can show intracellular vacuolization, morphological deformities, and cellular rupture after metal accumulation (Pudpong and Chantangsi 2015; Madoni and Romeo 2006). By using PG we observed vacuolization of *P. caudatum* cytoplasm exposed to Fe for 1 and 24 h. In general, we observed vacuole formation after exposure of *P. caudatum* to all five metals tested. The accumulation of metals enclosed by membranes is present in numerous phyla from protozoans to mammals (Martín-González et al. 2006; Abraham et al. 2017).

A comparison of *P. caudatum* LC_{50} values for Cu (Table 1) with other ciliate species shows that *P. caudatum* is orders of magnitude more sensitive than *Pseudourostyla* sp. and *Tetmemena* sp. (Abraham et al. 2017); and more sensitive than *Euplotes* sp., *Notohymena* sp., and *Breslauides* sp. (Abraham et al. 2017). For tolerant species like *Colpoda steinii* and *Colpoda elongata* this difference is higher (Díaz et al. 2006). Regarding Ni, *Spirostomum teres* (Madoni 2000) is twofold more sensitive than *P. caudatum* (Table 1). *Paramecium caudatum* is more sensitive than other ciliates like *Pseudourostyla* sp., which is 14-fold less sensitive, and *Euplotes patella* which is 20-fold less sensitive (Abraham et al. 2017). Regarding Zn, *P. caudatum* is 100, 167, and 194-fold more sensitive than *Pseudourostyla* sp., *Euplotes* sp. and *Tetmemena* sp., respectively (Abraham et al. 2017).

Fluorescence microscopy studies in ciliates has revealed that metal accumulation is one of the defense mechanisms of ciliates (Gutiérrez et al. 2003). Martín-González et al.

(2006) detected Cd and Zn bioaccumulation in *Drepanomonas revoluta*, *Uronema nigricans* and *Euplotes sp.* through fluorescence microscopy and electron transmission microscopy. Dias and Lima (2002) used dyes extracted from viability/cytotoxicity kits to observe morphological changes in *T. pyriformis* exposed to Cu and Zn.

Comparison of bioconcentration factors (BCFs), lethal (LBBs) and chronic body burdens (CBBs) and acute to chronic ratios (ACRs) between *P. caudatum* (Table 2), and the rotifer *Euchlanis dilatata* (Hernández-Flores et al. 2020) for the same five metals, shows some interesting trends. Regarding BCFs the rotifer values ranged from 54.98 for Ni to 3186 for Zn while for *P. caudatum* range is 4044 for Ni to 15,944 for Hg. There is more bioaccumulation in the ciliate. However, LBBs range of the rotifer was lower (7.35 to 321.79) than that of *P. caudatum* (89.4 to 18,633). As expected CBBs of *P. caudatum* are higher (36.8 to 14,177) than those of *E. dilatata* (0.065 to 599.86). Remarkably, the ACRs between both species are quite similar 0.91 to 23.2 in the ciliate and 0.99 to 92.4 in the rotifer.

In conclusion, we observed similarities between the toxicity of the five metals to the ciliate *P. caudatum* when compared to the rotifer *Euchlanis dilatata*: (1) The non-trace metal Hg was the most toxic metal in terms of LC50 and LBBs to both zooplanktonic organisms. (2) The order of toxicity (LC50 values) was very similar in both species with Hg as the most toxic and Fe as the least toxic. (3) Vacuole/ingestion chronic tests were more sensitive in both species than growth inhibition chronic tests. Mercury was the fastest metal accumulated in the ciliate. The sensitivity of *Paramecium caudatum* to the five metals tested (Cu, Fe, Hg, Ni, and Zn) falls in the range of other ciliate species reported in the scientific literature.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00128-023-03768-8>.

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Declarations

Conflict of interest The authors state that they do not have competing interests.

References

- Abraham JS, Sripoorna S, Choudhary A, Toteja R, Gupta R, Makhija S, Warren A (2017) Assessment of heavy metal toxicity in four species of freshwater ciliates (*Spirotrichea:Ciliophora*) from Delhi, India. *Curr Sci* 113:2141–2150. <https://doi.org/10.18520/cs/v113/i11/2141-2150>
- APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington
- Arunakumara KK, Zhang X (2008) Heavy metal bioaccumulation and toxicity with special reference to microalgae. *J Ocean Univ China* 7:60–64. <https://doi.org/10.1007/s11802-008-0060-y>
- Contreras Pérez JB, Mendoza Gómez CL, Gómez A (2004) Determinación de metales pesados en aguas y sedimentos del Río Haina. *Cienc Soc* 29:38–71. <https://doi.org/10.22206/cys.2004.v29i1.pp38-71>
- Dias N, Lima N (2002) A comparative study using a fluorescence-based and a direct-count assay to determine cytotoxicity in *Tetrahymena pyriformis*. *Res Microbiol* 153:313–322. [https://doi.org/10.1016/s0923-2508\(02\)01326-8](https://doi.org/10.1016/s0923-2508(02)01326-8)
- Díaz S, Martín-González A, Carlos Gutiérrez J (2006) Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. *Environ Int* 32:711–717. <https://doi.org/10.1016/j.envint.2006.03.004>
- Dimas MJJ, Garza MND, Treviño DBM (2015) Índice de la calidad del agua y metales pesados del cauce aguas blancas del municipio de Acapulco Guerrero, México. *Rev Mexicana Cienc Agríc* 1:113–118
- El-Bassat R, Touliabah H, Harisa G (2012) Toxicity of four pharmaceuticals from different classes to isolated plankton species. *Afr J Aquat Sci* 37:71–80. <https://doi.org/10.2989/16085914.2012.666376>
- Flores CM, Del Angel E, Frías DM, Gómez AL (2018) Evaluación de parámetros fisicoquímicos y metales pesados en agua y sedimento superficial de la Laguna de las Ilusiones, Tabasco, México. *Tecnol y Cienc Del Agua* 9:39–57. <https://doi.org/10.24850/j-tyca-2018-02-02>
- Gong ZL, Chen Y, Yan Y, Pei SY, Wu D, Zhang M, Wang Q (2014) Toxicity of three heavy metal pollutants of the pharmaceutical wastewater to *Paramecium caudatum*. *Adv Mater Res* 937:571–577. <https://doi.org/10.4028/www.scientific.net/amr.937.571>
- Gutiérrez JC, Martín-González A, Díaz S, Ortega R (2003) Ciliates as a potential source of cellular and molecular biomarkers/biosensors for heavy metal pollution. *Eur J Protistol* 39:461–467. <https://doi.org/10.1078/0932-4739-00021>
- Guzmán-Colis G, Thalasso F, Ramírez-López EM, Rodríguez-Narciso S, Guerrero-Barrera AL, Avelar-González FJ (2011) Evaluación espacio-temporal de la calidad del agua del Río San Pedro en el Estado de Aguascalientes, México. *Rev Int Contam Ambie* 27:89–102
- Hernández-Flores S, Santos-Medrano GE, Rubio-Franchini I, Rico-Martínez R (2020) Evaluation of bioconcentration and toxicity of five metals in the freshwater rotifer *Euchlanis dilatata* Ehrenberg, 1832. *Environ Sci Pollut Res* 27:14058–14069. <https://doi.org/10.1007/s11356-020-07958-3>
- Huaranga MF, Méndez GE, Quilcat LV, Huaranga AF (2012) Contaminación por metales pesados en la Cuenca del Río Moche, 1980–2010, La Libertad-Perú. *Sci Agropecu* 3:235–247
- Illing AC, Shawki A, Cunningham CL, Mackenzie B (2012) Substrate profile and metal-ion selectivity of human divalent metal-ion transporter-1. *J Biol Chem* 287:30485–30496. <https://doi.org/10.1074/jbc.m112.364208>
- Kryuchkova M, Danilushkina A, Lvov Y, Fakhruilin R (2016) Evaluation of toxicity of nanoclays and graphene oxide in vivo: a *Paramecium caudatum* study. *Environ Sci: Nano* 3:442–452. <https://doi.org/10.1039/c5en00201j>
- Madoni P (2000) The acute toxicity of nickel to freshwater ciliates. *Environ Pollut* 109:53–59. [https://doi.org/10.1016/s0269-7491\(99\)00226-2](https://doi.org/10.1016/s0269-7491(99)00226-2)

- Madoni P, Romeo MG (2006) Acute toxicity of heavy metals towards freshwater ciliated protists. *Environ Pollut* 141:1–7. <https://doi.org/10.1016/j.envpol.2005.08.025>
- Madoni P, Esteban G, Gorbi G (1992) Acute toxicity of cadmium, copper, mercury, and zinc to ciliates from activated sludge plants. *Bull Environ Contam Toxicol* 49:900–905. <https://doi.org/10.1007/bf00203165>
- Mancilla-Villa OR, Ortega-Escobar HM, Ramírez-Ayala C, Uscanga-Mortera E, Ramos-Bello R, Reyes-Ortigoza AL (2012) Metales pesados totales y arsénico en el agua para riego de Puebla y Veracruz, México. *Rev Int Contam Ambient* 28:39–48
- Martín-González A, Díaz S, Borniquel S, Gallego A, Gutiérrez JC (2006) Cytotoxicity and bioaccumulation of heavy metals by ciliated protozoa isolated from urban wastewater treatment plants. *Res Microbiol* 157:108–118. <https://doi.org/10.1016/j.resmic.2005.06.005>
- Michalec BK, Lenart-Boroń AM, Cupak AK, Wałęga AS (2014) The evaluation of heavy metal content in water and sediments of small reservoirs in light of various environmental quality regulations. *J Environ Sci Health A* 49:827–832. <https://doi.org/10.1080/10934529.2014.882645>
- Miyoshi N, Kawano T, Tanaka M, Kadono T, Kosaka T, Kunimoto M, Takahashi T, Hosoya H (2003) Use of *Paramecium* species in bioassays for environmental risk management: determination of IC₅₀ values for water pollutants. *J Health Sci* 49:429–435. <https://doi.org/10.1248/jhs.49.429>
- Nałęcz-Jawecki G (2013) Protozoans in ecotoxicology. In: Féraud JF, Blaise C (eds) *Encyclopedia of aquatic ecotoxicology*. Springer, Dordrecht, pp 909–916
- Paul D (2017) Research on heavy metal pollution of river Ganga: a review. *Ann Agrar Sci* 15:278–286. <https://doi.org/10.1016/j.aasci.2017.04.001>
- Petrat F, Rauen U, De Groot H (1999) Determination of the chelatable iron pool of isolated rat hepatocytes by digital fluorescence microscopy using the fluorescent probe, phen green SK. *Hepatol* 29:1171–1179. <https://doi.org/10.1002/hep.510290435>
- Pudpong S, Chantangsi C (2015) Effects of four heavy metals on cell morphology and survival rate of the ciliate *Bresslauides* sp. *Trop Nat Hist* 15:117–125
- Quintana MEC, Sosa CM, Rubio AH, Puga TS, Quintana MG, Moreno M, Alcalá JJ (2008) Comportamiento de la Contaminación por Plomo, Níquel y Vanadio en la Cuenca del Río Conchos. *Rev Latinoam Recur Nat* 4:68–76
- Quintana MRM, Espinoza PJR, Frescas MAD, Pinedo CA (2015) La importancia de la evaluación de la calidad del agua en la Laguna de Bustillos, Chihuahua, México. *Papeles De Geografía* 6:187–203. <https://doi.org/10.6018/geografia/2016/255811>
- Rehman A, Shakoory FR, Shakoory AR (2009) Heavy metal uptake by *Euplotes mutabilis* and its possible use in bioremediation of industrial wastewater. *Bull Environ Contam Toxicol* 83:130–135. <https://doi.org/10.1007/s00128-009-9725-5>
- Reynoso L, Andruilo A (2009) Estado actual de la calidad del agua en la Cuenca del Arrollo Pergamino. *Estación Experimental Agropecuaria Pergamino*. Instituto nacional de Tecnología Agropecuaria. Argentina. <https://inta.gob.ar/documentos/estado-actual-de-la-calidad-del-agua-en-la-cuenca-del-arroyo-pergamino>. Accessed 10 Sept 2020
- Saeed SM, Shaker IM (2008) Assessment of heavy metals pollution in water and sediments and their effect on *Oreochromis niloticus* in the Northern Delta Lakes, Egypt. *Proceedings of the 8th International Symposium on Tilapia in Aquaculture*, Cairo, 12–14 October 2008, 475–490.
- Shakoory AR, RehmanRiaz-ul-Haq A (2004) Multiple metal resistance in the ciliate protozoan, *Vorticella microstoma*, isolated from industrial effluents and its potential in bioremediation of toxic wastes. *Bull Environ Contam Toxicol* 72:1046–1051. <https://doi.org/10.1007/s00128-004-0349-5>
- Shingles R, Wimmers LE, McCarty RE (2004) Copper transport across pea Thylakoid membranes. *Plant Physiol* 135:145–151. <https://doi.org/10.1104/pp.103.037895>
- Somasundaram S, Abraham JS, Maurya S, Makhija S, Gupta R, Toteja R (2018) Cellular and molecular basis of heavy metal-induced stress in ciliates. *Curr Sci* 114:1858–1865. <https://doi.org/10.18520/cs/v114/i09/1858-1865>
- Sonneborn TM (1970) Chapter 12: methods in paramecium research. In: Prescott DM (ed) *Methods in cell biology*. Academic Press, Cambridge, pp 241–339
- Soto CC, Gereá M, Queimaliños C, Ribeiro Guevara S, Diéguez MC (2018) Inorganic mercury (Hg²⁺) accumulation in autotrophic and mixotrophic planktonic protists: Implications for Hg trophodynamics in ultraoligotrophic andean patagonian lakes. *Chemosphere* 199:223–231. <https://doi.org/10.1016/j.chemosphere.2018.02.035>
- US EPA (1992) *Manual of guidelines for water reuse*. US Environmental Protection Agency 625/R-92/004, Center for Environmental Research Information, Cincinnati, p 247
- Volesky B, Holan ZR (1995) Biosorption of heavy metals. *Biotechnol Prog* 11:235–250. <https://doi.org/10.1021/bp00033a001>
- Wanick RC, Paiva TDS, de Carvalho CN, de Silva-Neto ID (2008) Acute toxicity of cadmium to freshwater ciliate *Paramecium bur-saria*. *Biociências* 16:104–109
- Zarazúa G, Ávila-Pérez P, Tejada S, Valdivia-Barrientos M, Zepeda-Gómez C, Macedo-Miranda G (2013) Evaluación de los metales pesados Cr, Mn, Fe, Cu, Zn y Pb en sombrero de agua (*Hydrocotyle ranunculoides*) del curso alto del Río Lerma, México. *Rev Int Contam Ambient* 29:17–24

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