Acta Limnologica Brasiliensia

Publication of the Brazilian Association of Limnology

Original Article
Acta Limnologica Brasiliensia, 2022, vol. 34, e21
https://doi.org/10.1590/S2179-975X7821
ISSN 2179-975X on-line version

Acute toxicity of potentially toxic elements on ciliated protozoa from Lake Maracaibo (Venezuela)

Toxicidade aguda de elementos potencialmente tóxicos em protozoários ciliados do Lago de Maracaibo (Venezuela)

Julio César Marín-Leal[1]*, Neil José Rincón-Miquilena[2], Laugeny Chiquinquirá Díaz-Borrego[2] and María Carolina Pire-Sierra[3]

[1]Departamento de Ingeniería Sanitaria y Ambiental, Escuela de Ingeniería Civil, Facultad de Ingeniería, Universidad del Zulia, Maracaibo 4011-A, estado Zulia, Venezuela

[2]Laboratorio de Microorganismos Fotosintéticos, Departamento de Biología, Facultad Experimental de Ciencias, Universidad del Zulia, Maracaibo 4011-A, estado Zulia, Venezuela

[3]Programa de Ingeniería Agroindustrial, Decanato de Agronomía, Universidad Centroccidental Lisandro Alvarado, Barquisimeto, estado Lara, Venezuela

*e-mail: jmarin@fing.luz.edu.ve; julio.marin2789@gmail.com

Cite as: Marín-Leal JC, et al. Acute toxicity of potentially toxic elements on ciliated proto-zoa from Lake Maracaibo (Venezuela). *Acta Limnologica Brasiliensia*, 2022, vol. 34, e21.

Abstract: Aim: In this article the acute ecotoxicological effects of Cr(III), Cr(VI), Cd(II) and V(V) on ciliated protozoa isolated from Lake Maracaibo were evaluated, by estimating of the LC_{50} for an exposure time of 1-h and observations every 5 min. **Methods:** Isolations and cultures of ciliated protozoa were made from surface water samples to then carry out toxicity essays under static and controlled conditions, identifying cell immobility (death) as the endpoint. **Results:** The response of the ciliated protozoa made it possible to unequivocally determine the acute toxicity in presence of potentially toxic elements (PTE), with variable mortalities depending on the gender, the element tested and its concentration. The results obtained with *Euplotes* sp. indicate that protozoan is a sensitive biomonitor indicated for the biomonitoring of PTE contamination in Lake Maracaibo. **Conclusions:** The use of shorter exposure periods offers opportunities to show early toxicity effects on natural populations and to act in a timely manner (early warning systems) in contamination events by PTEs, as well as the development of sensitive and rapid biomonitoring methods for detection of these elements in the environment.

Keywords: *Euplotes* sp.; heavy metal; lethal concentration; short-term exposure; zooplankton.

Resumo: Objetivo: Neste artigo, os efeitos ecotoxicológicos agudos de Cr(III), Cr(VI), Cd(II) e V(V) foram avaliados em protozoários ciliados isolados do Lago de Maracaibo, estimando o CL_{50} para um tempo de 1 h exposição e observações a cada 5 min. **Métodos:** Isolamentos e culturas de protozoários ciliados foram feitos a partir de amostras de águas superficiais para então realizar testes de toxicidade em condições estáticas e controladas, identificando a imobilidade celular (morte) como critério de avaliação. **Resultados:** A resposta dos protozoários ciliados permitiu determinar inequivocamente a toxicidade aguda na presença de elementos potencialmente tóxicos (EPT), com mortalidades variáveis dependendo do gênero, do elemento testado e da sua concentração. Os resultados obtidos com *Euplotes* sp. indicam que o protozoário é um biomonitor sensível e indicado para o biomonitoramento de contaminação por EPT no Lago Maracaibo. **Conclusões:** O uso de períodos de exposição mais curtos oferece oportunidades de mostrar os efeitos da toxicidade precoce em populações

naturais e de atuar em tempo hábil (sistemas de alerta precoce) em eventos de contaminação de EPT, bem como o desenvolvimento de métodos de biomonitoramento sensíveis e rápidos para a detecção desses elementos no meio ambiente.

Palavras-chave: *Euplotes* sp.; metal pesado; concentração letal; exposição de curto prazo; zooplâncton.

1. Introduction

The Lake Maracaibo (Venezuela) is a tropical hypereutrophic estuary surrounded by one of the areas of greatest population density of Venezuela, whose domestic wastewaters from coastal cities are discharged without any treatment or treated improperly (Marín-Leal et al., 2017). As well, wastewaters of many industries (e.g. petrochemical, metal-mechanic, gas processing, transportation of hydrocarbons, oil production, pharmaceutical, tanning, agricultural, mining, among others) installed along the coast are also dumped, which has accelerated eutrophication process (Corona, 2013; Rodríguez, 2000; Parra-Pardi, 1979). Additionally, in this ecosystem oil transportation and extraction operations are performed from the 1922, which has resulted in increasing concentrations and trophic transfer of potentially toxic elements (PTE), such as metals, metalloids and others, in sediments and aquatic food webs (Cáceres, 2012; Ávila et al., 2010; Colina de Vargas & Romero, 1992). Castro & Marín (2018) reported multiresistance patterns in heterotrophic bacteria from Lake Maracaibo, whose inhibitory effects were: Cr(VI): 200 - >5,000 mgCr(VI).L^{-1} >Cd(II): 75 - >5,000 mgCd(II).L^{-1} >Ni(II): 2,500 - >5,000 mgNi(II).L^{-1} >Cr(III): 3,750 - >5,000 mgCr(III).L^{-1} >Pb(II): >5,000 mgPb(II).L^{-1}, possibly resulting from exposure to increasing conditions of elemental contamination in this water body. In addition, for the clam *Polymesoda solida*, a mean lethal concentration (96-h LC_{50}) of 15.94 mgCr(VI).L^{-1} was found, also observing that the Cr content in this bivalve mollusk (>1.33 μgCr.g^{-1} dry weight) represents a potential risk for ecosystem and human health (Rojas et al., 2015). There are no reports on PTE toxicity in ciliated protozoa from Lake Maracaibo.

The potential of zooplanktonic organisms as bioindicators in toxicity tests has been well referenced (Vilas-Boas et al., 2020; Gomiero et al., 2012; Gomiero et al., 2013; Mortimer et al., 2010; Qing-Hua et al., 2008). The main advantage of using them in ecotoxicological research is to show toxic effects at individual level and its subsequent impact on higher levels of biological organization, such as population and community (Alayo & Iannacone, 2002). Protozoa play an important role in microbial food webs (microbial loop) of aquatic ecosystems and represent a link between the microbial food chain components and the classic food chain (Premke & Arndt, 2000). The microbial loop dynamics has been poorly studied in tropical aquatic ecosystems, particularly in the trophic levels of Bacteria and their natural predators. Planktonic bacteria use the dissolved organic matter from its surroundings, being preyed upon by ciliates and flagellates, which in turn are food for zooplanktonic macroinvertebrates. The food chain Bacteria-ciliates-flagellate can consume approximately 60-70% of the primary production in the water column, and can be seen as a parallel to conventional food web of grazing that occurs between phytoplankton, zooplankton and fish (El-Serehy et al., 2012; Faure et al., 2010). Gomiero et al. (2012) suggested that because of the short time and simplicity of the test procedures, *Euplotes crassus* is a promising and convenient bioindicator for evaluating the toxicity of different environmental matrixes like pore water, sediments and wastewaters-contaminated by inorganic and organic pollutants. Similarly, Maurya & Pandey (2020) have greatly highlighted the advantages of species of the ciliated protozoa *Tetrahymena* for toxicological and ecotoxicological studies, due to its wide distribution, ease of handling, greater sensitivity and ability to study the direct and indirect effects of chemical toxics at the biochemical biomarkers, individual, population and community levels.

Therefore, considering that the protozoa are important organisms for aquatic food web, their sensitivity to changes in environmental quality and the lack of ecotoxicological information in Lake Maracaibo, the present study aimed to evaluate the acute ecotoxicological effect of chromium [Cr(III) and Cr(VI)], cadmium [Cd(II)] and vanadium [V(V)] on ciliated protozoa isolated from water surface samples of Lake Maracaibo, by estimating the LC_{50} for an exposure time of 1-h and quantification of dead organisms every 5 min. We hypothesize that the tested PTEs cause an adverse toxic effect on their survival, possibly affecting the trophic relationships in this body of water in situations of anthropogenic chemical contamination. Those

elements were selected because to its appreciable magnitude in water, sediment and biota, as well as numerous anthropogenic sources of PTE in the hydrographic basin of this water body. The toxicity tests at short exposure times could serve as a practical and rapid tool to generate short-term results and, in this way, provide the opportunity to act on emergency situations or those that deserve immediate legal action. In this regard, certain investigations with exposure times of 1-h have been carried out, for example, to propose protozoan species as bioindicators of water quality (Nalecz-Jawecki et al., 1993) or to understand the ecological effects caused by stress in the presence of PTE (Blanck & Wängberg, 1988).

2. Materials and Methods

2.1. Study area

Lake Maracaibo is located in the West of Venezuela, Zulia state, between 70°30' and 73°24' W and 09°00' and 10°30' N (Figure 1). It is connected to the Gulf of Venezuela by a strait of 55 km long at North and fed by some 135 rivers, of which the most important are Catatumbo, Chama, Santa Ana and Escalante (South region). The lake has 13,210 km^2 of surface area, 95,923 km^2 of drainage, 1485.2 km of coastline and is one of the oldest on Earth. It has an altitude of 3 m and a maximum depth of 34 m (Rodríguez, 2000; Parra-Pardi, 1979).

2.2. Sampling

Zooplankton samples were collected at depth one meter in the water column, using a conical mesh (150 µm), on Western shore of the Maracaibo Strait, in the site Lake Park Vereda, with following geographical coordinates: 71º35'25.14" W and 10º40'10.57" N. Samples were placed in sterile glass containers of 350 mL and transferred to the laboratory in a cooler with ice.

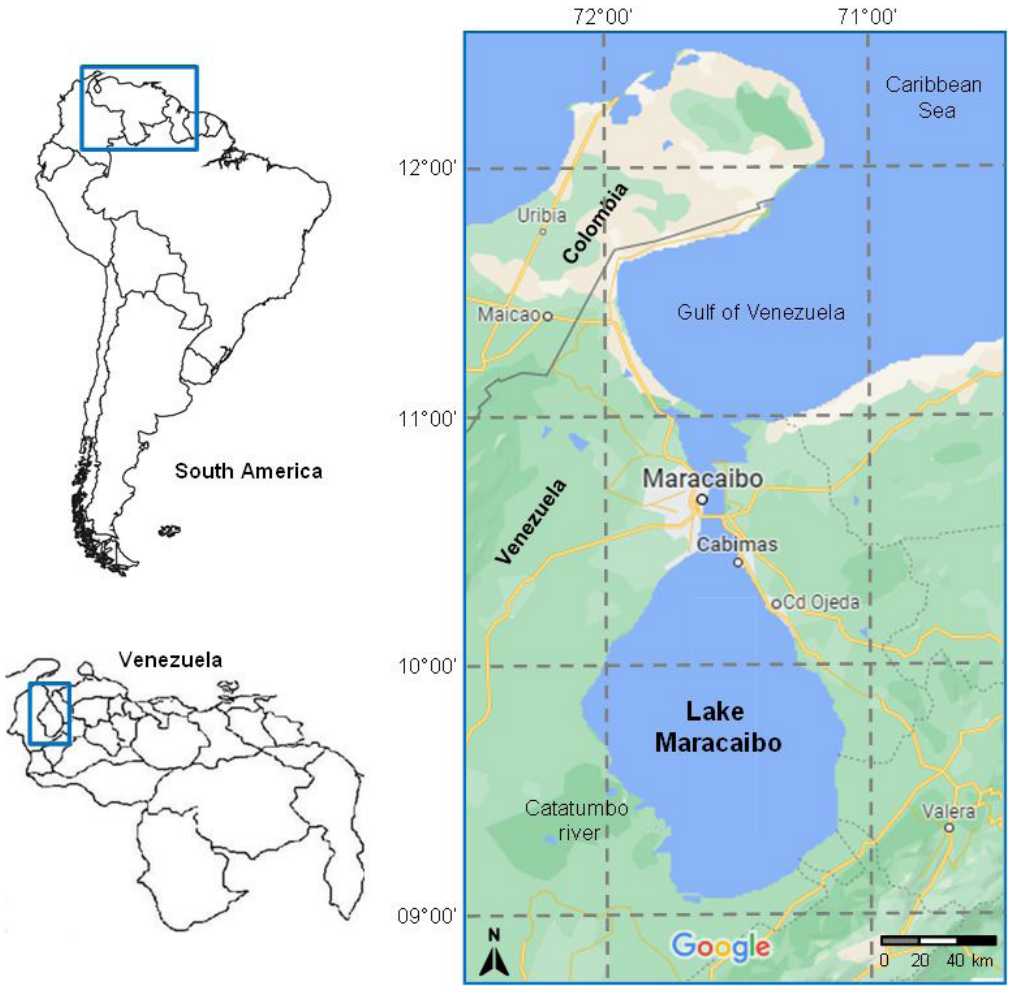

Figure 1. Map of the study area: Lake Maracaibo (Venezuela).

In surface water the following parameters were analyzed *in situ* (arithmetic mean ± standard deviation; n= 6): temperature (30.6 ± 1.1 ºC), pH (7.69 ± 0.63), redox potential (−80.84 ± 7.74 mV), salinity (1,701.3 ± 331.2 SPU), electric conductivity (3,471.7 ± 676.8 $\mu S.cm^{-1}$), and dissolved oxygen (3.94 ± 0.15 $mg.L^{-1}$), using a multiparameter probe (Thermo Scientific, model 5 Orion Star, USA). The total contents of chromium (Cr), cadmium (Cd) and vanadium (V) were determined by inductively coupled plasma spectrometry (Hewlett Packard 4500 apparatus, USA) on acidified and digested samples according to Colina (2001), using a certified reference material (NIST 1640a trace elements in natural water) as quality control. These concentrations were (arithmetic mean ± standard deviation; n= 3): 9.67 ± 1.30, 4.12 ± 1.56 and 15.13 ± 0.85 $\mu g.L^{-1}$, respectively.

2.3. Isolation and culture of ciliated protozoa

Small aliquots of water samples were observed under optical microscope (5X) to select the most abundant free-living ciliated protozoa using a capillary tube. The organisms were grown at an average temperature of 30.5 ± 1.5 °C in glass jars with pre-filtered lake water (0.22 μm) and oats to form oat liquor, and thereby promoting bacterial growth (Fried et al., 2002). The most abundant genera in the water samples were *Urodema* sp. (82.3%), *Euplotes* sp. (12.4%) and *Loxodes* sp. (5.3%), so they were used in the toxicity experiments. Microbial cultures were renewed every 48 h, centrifuged and observed under optical microscope to select organisms of the same species, using a capillary tube. These organisms were planted in fresh culture medium (per liter distilled water: 0.125 g K_2HPO_4, 0.025 g NH_4Cl, 0.4 g NaCl, 0.2 g $MgCl_2.6H_2O$, 0.15 g KCl and 0.25 g $CaCl_2.2H_2O$), until a monospecific cultivation of ciliates (Narayanan et al., 2007).

2.4. Taxonomic identification of ciliated protozoa

Cultures were, initially, stained with lugol to detail the internal and external structures of the specimens, such as: location and number of cirrus in different regions, morphology of the individual, location of cytostome contractile vacuole and then stained with methyl green and safranin, to observe the morphology and location of the nuclei (Lynn, 2008; Foissner, 1999; Foissner & Berger, 1996). Taxonomic identifications were made under the light microscope through specific taxonomic keys. The dichotomous keys allowed to verify the following distinctive structural characteristics: i) *Uronema* sp.: oval to ovoid morphology, elongated, slightly flattened, narrow preoral groove leads from the frontal plate to mouth at middle, inconspicuous peristome, ciliated right border, cytostome on the ventral side near the left border in the anterior half, confused cytopharynx, central spherical macronucleus and terminal contractile vacuole; ii) *Euplotes* sp.: inflexible ovoid body with flattened ventral surface and convex dorsal surface, longitudinally wrinkled, wide and triangular peristome, absence of frontal cirrus, frontal part of the adoral area in a flat groove, nine or more ventral frontal cirri (five anal and four scattered caudal), band-shaped macronucleus, a micronucleus, and posterior contractile vacuole; and iii) *Loxodes* sp.: larger, anterior part with beak-shaped curved end, mouth at posterior end of a concave indentation near the front of the cell, surface covered with a membrane bound with granules containing a yellow-brown pigment, a large part of the cell interior with mineral inclusions located in specific positions on the dorsal border and absence of contractile vacuole (Paiva et al., 2013; Lynn, 2008).

2.5. Acute toxicity bioassays with potentially toxic elements

Bioassays were performed using the following analytical grade salts (MERCK; purity >98%): $CrCl_3$, $K_2Cr_2O_7$, $CdCl_2$ and NH_4VO_3. Stock solutions were prepared with lake´s water pre-filtered in Millipore filters (0.22 μm) at concentrations high enough to prevent weighing errors and salinity changes (Shi et al., 2016). Experimental concentrations were chosen on the basis of preliminary trials (no deaths at low concentrations) and considering the reference levels of Bracho et al. (2016), Ávila et al. (2010), and Madoni & Romeo (2006). The concentration ranges of PTE solutions were: 2.5-700 $mgCr(III).L^{-1}$, 50-1,000 $mgCr(VI).L^{-1}$, 0.15-5.0 $mgCd(II).L^{-1}$ and 0.3-12.5 $mgV(V).L^{-1}$. Control tests were conducted with protozoa and pre-filtered lake´s water without addition of elements.

Acute toxicity bioassays were conducted in Sedgewick-Rafter plates, in triplicate, according to APHA (2017). Each chamber had 1 mL of test solution and of 18 to 22 organisms. The exposure time to the PTE solutions was 1-h, during which counts and microscopic observations were performed every 5 min (Figure 2). After this time, the number of ciliated protozoa dead (non-moving) was determined (Girling et al., 2000).

2.6. Statistical analysis

For descriptive statistical calculations (arithmetic mean, range, and standard deviation), the program Microsoft Excel version 10 was used. LC_{10}, LC_{50} and LC_{90} calculations (including confidence limits 95%) for PTE were performed with the program PriProbit version 1.63. Two-way analysis of variance (ANOVA) with Tukey test *a posteriori* was used to determine statistical significance of the differences between LC_{50} values considering the element and the protozoa (genera), whereas the level of significance was accepted at $p<0.05$. These analyses were performed using the program SPSS version 20.0.

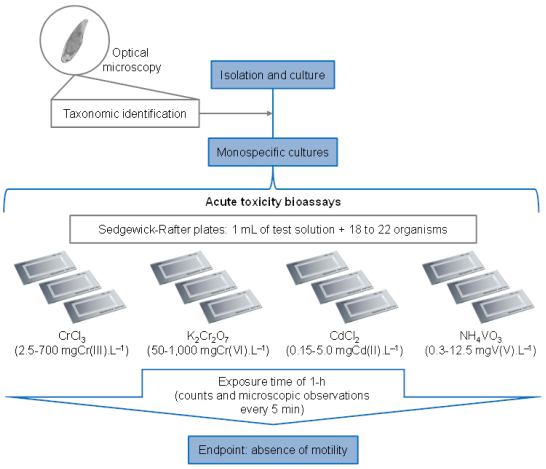

Figure 2. Scheme of the laboratory procedure to evaluate the acute ecotoxicological effects of potentially toxic elements on ciliated protozoa from Lake Maracaibo.

3. Results

Figures 3, 4 and 5 show the mortality percentages for the three genera of ciliated protozoa isolated from water samples of the Lake Maracaibo, according to the PTE concentrations in the acute toxicity bioassays observed every 5 min during 1-h. These percentages were less than 1% in all controls. The shortest time of exposure to PTE concentrations in which a mortality of 100% of exposed organisms was observed, was: *Uronema* sp. 5 min at 700 mgCr(III).L^{-1}, 25 min at 1,000 mgCr(VI).L^{-1}, 20 min at 5 mgCd(II).L^{-1} and 10 min at 12.5 mgV(V).L^{-1} (Figure 3); *Euplotes* sp. 5 min at 700 mgCr(III).L^{-1}, 30 min at

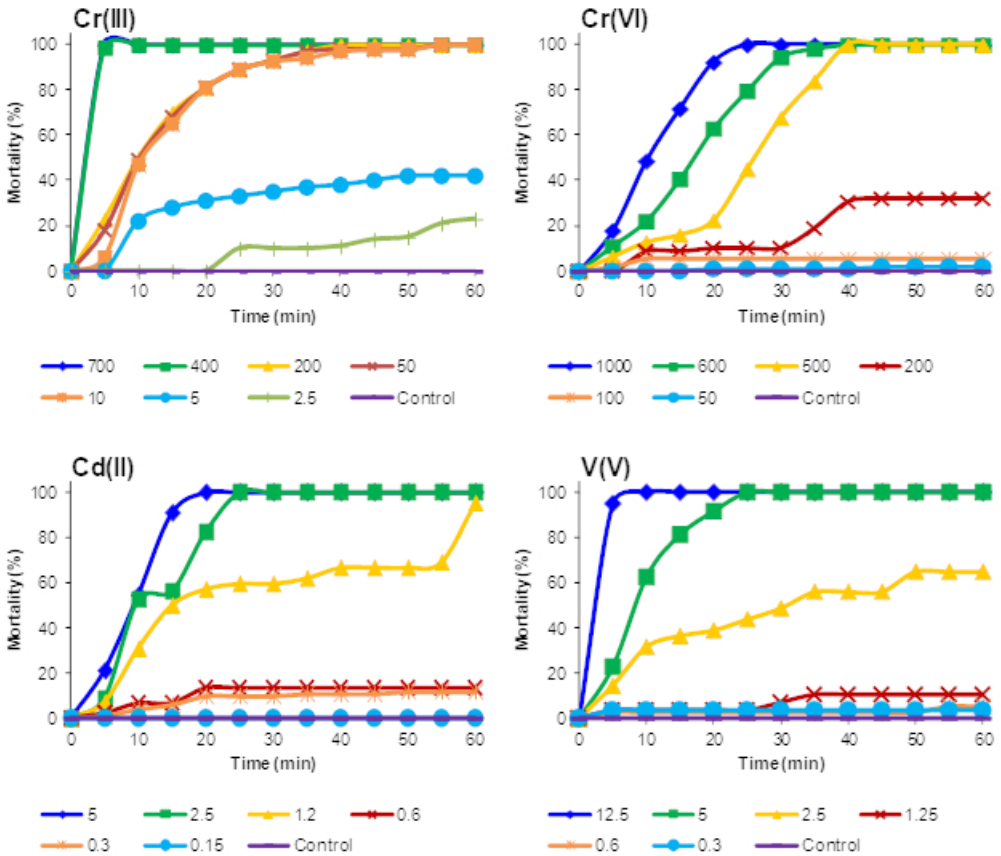

Figure 3. Mortality of *Uronema* sp. in acute toxicity bioassays with potentially toxic elements (mg.L^{-1}).

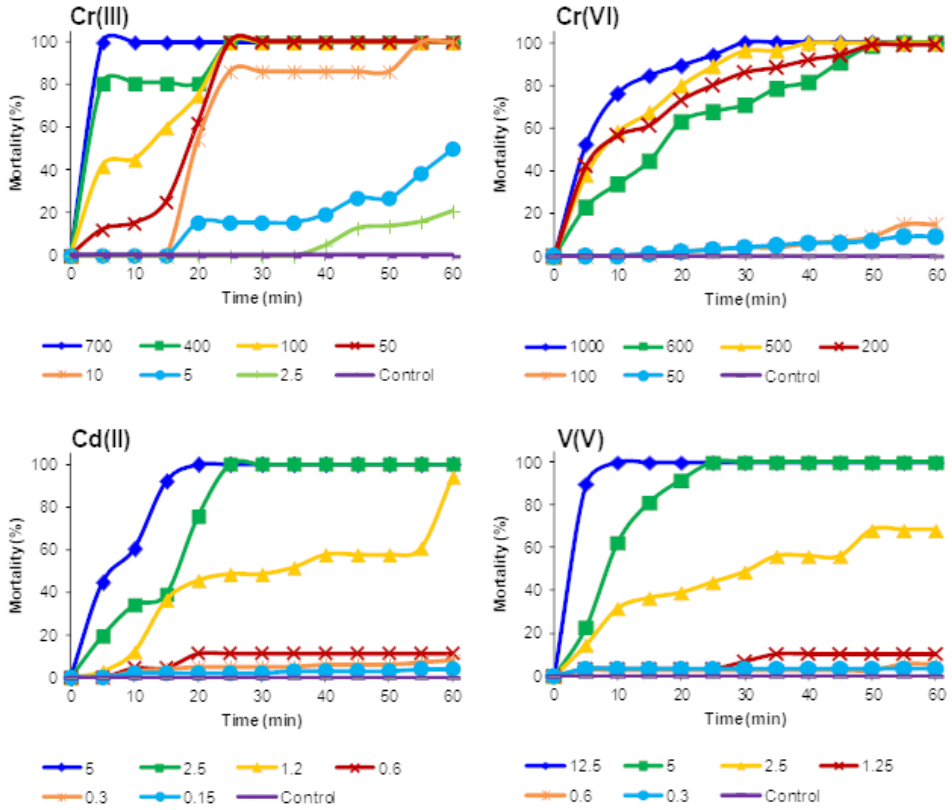

Figure 4. Mortality of *Euplotes* sp. in acute toxicity bioassays with potentially toxic elements (mg.L^{-1}).

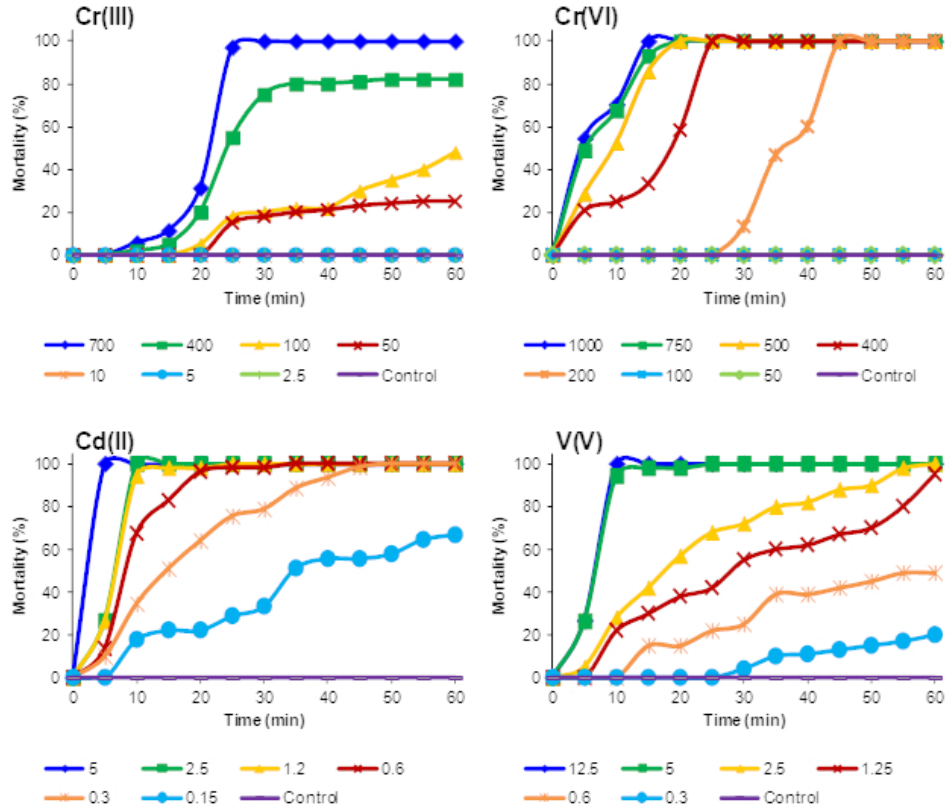

Figure 5. Mortality of *Loxodes* sp. in acute toxicity bioassays with potentially toxic elements (mg.L^{-1}).

1,000 mgCr(VI).L^{-1}, 20 min at 5 mgCd(II).L^{-1} and 10 min at 12.5 mgV(V).L^{-1} (Figure 4); and *Loxodes* sp. 30 min at 700 mgCr(III).L^{-1}, 15 min at 1,000 mgCr(VI).L^{-1}, 5 min at 5 mgCd(II).L^{-1} and 10 min at 12.5 mgV(V).L^{-1} (Figure 5).

The 1-h LC_{50} values for ciliated protozoa from Lake Maracaibo ranged between 0.14 mg.L^{-1} (Cd(II), *Loxodes* sp.) and 213.59 mg.L^{-1} (Cr(VI), *Uronema* sp.) (Table 1). The LC_{10} and LC_{90} values have been included to relate them to the concentrations that could cause minor effects on the population and those that would have a greater effect on it, respectively.

The two-way ANOVA showed highly significant differences (p<0.001) of the LC_{50} values with respect to the genera of ciliated protozoa (p<0.001, F value = 98.00), to the PTE (p<0.001, F value = 1271.70) and for the interaction element*protozoan (p<0.001, F value = 119.86). The Tukey test revealed three independent groups (p<0.05) with the three genera of ciliated protozoa (Table 2). Thus, the toxic effect of the elements on ciliated protozoa was Cd(II) = V(V) > Cr(III) > Cr(VI) (Table 1). *Loxodes* sp. was the protozoan most sensitive to Cd(II) and V(V), while *Uronema* sp. was the most tolerant to Cr(VI), however, *Euplotes* sp. showed the highest sensitivity to the set of PTEs tested (1-h mean LC_{50} 30.77 mg.L^{-1}) (Figure 6).

4. Discussion

In this work, the adverse toxic effects of the PTEs tested on four genera of protozoa isolated from surface water samples from Lake Maracaibo were confirmed, allowing the estimation of the LC_{50} for an exposure time of 1-h and with the following pattern: Cd(II) = V(V) > Cr(III) > Cr(VI).

4.1. Toxicity of potentially toxic elements on ciliated protozoa

In aquatic organisms, in general, Cr(III) is less toxic than Cr(VI), mainly due to: weak permeability in the cell membrane, use as a trace

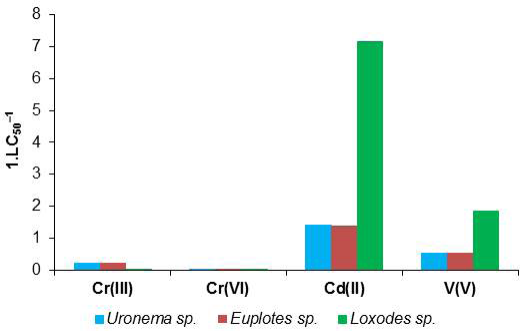

Figure 6. General ecotoxicity levels (1.LC_{50}^{-1}) of potentially toxic elements to ciliated protozoa of Lake Maracaibo.

Table 1. Mean lethal concentration (LC) and confidence limits 95% (in parentheses) of potentially toxic elements (mg.L^{-1}) to ciliated protozoa of Lake Maracaibo (exposure time 1-h, n= 3).

Protozoa	LC	Cr(III)	Cr(VI)	Cd(II)	V(V)
Uronema sp.	LC_{10}	2.17 (1.03-2.94)	118.38 (37.16-174.21)	0.39 (0.12-0.56)	0.87 (0.09-1.48)
	LC_{50}	4.39 (3.35-5.72)	213.59 (133.98-362.14)	0.71 (0.47-1.21)	1.92 0.96-4.48
	LC_{90}	8.88 (6.57-18.40)	385.36 (256.64-1,408.92)	1.32 (0.88-5.48)	4.24 (2.42-57.88)
Euplotes sp.	LC_{10}	2.19 (1.39-2.77)	68.35 (18.70-96.28)	0.36 (0.01-0.62)	0.86 (0.08-1.47)
	LC_{50}	4.23 (3.50-5.08)	116.25 (77.08-204.05)	0.72 (0.31-2.53)	1.88 (0.93-4.46)
	LC_{90}	8.18 (6.50-12.59)	197.72 (136.34-1,007.72)	1.47 (0.83-136.02)	4.1 (2.35-61.39)
Loxodes sp.	LC_{10}	29.83 (22.54-37.30)	145.76 (119.84-179.71)	0.12 (0.11-0.13)	0.26 (0.21-0.30)
	LC_{50}	111.24 (95.36-129.43)	154.08 (126.46-190.33)	0.14 (0.13-0.15)	0.54 (0.49-0.59)
	LC_{90}	414.83 (335.81-539.62)	162.88 (133.43-201.59)	0.17 (0.16-0.18)	1.13 (0.99-1.35)

Table 2. Multiple comparisons of the mean 1-h LC_{50} values of ciliated protozoa from Lake Maracaibo for the potentially toxic elements tested by means of the Tukey test.

Protozoa		Difference of means (I-J)	Typical error	p	Confidence interval 95%	
					Lower limit	Upper limit
Euplotes sp.	*Loxodes* sp.	–35.8008*	2.60980	0.001	–42.3183	–29.2834
	Uronema sp.	–24.2242*	2.60980	0.001	–30.7416	–17.7067
Loxodes sp.	*Euplotes* sp.	35.8008*	2.60980	0.001	29.2834	42.3183
	Uronema sp.	11.5767*	2.60980	0.001	5.0592	18.0941
Uronema sp.	*Euplotes* sp.	24.2242*	2.60980	0.001	17.7067	30.7416
	Loxodes sp.	–11.5767*	2.60980	0.001	–18.0941	–5.0592

*: the difference in means is significant at the level 0.05.

element in metabolic processes, greater stability and low oxidizing power, between other factors; while Cr(VI) is more active in penetration through passages for isoelectric and isostructural anions (Hong et al., 2020; Jaishankar et al., 2014; WHO, 2009; CCME, 1999). Inside the cell, reactions between Cr(VI) and biological reducers such as thiols and ascorbate, result in the production of reactive oxygen species such as superoxide ion, hydrogen peroxide and hydroxyl radical, which in ultimately leads to oxidative stress in the cell that damages DNA and proteins (Jaishankar et al., 2014; Rico et al., 2009). However, in the isolated protozoa from Lake Maracaibo, the trivalent form of chromium showed a higher degree of toxicity in laboratory tests carried out (Figures 1, 2 and 3), possibly due to: solubility and mobility of chemical species (WHO, 2009; Panda & Choudhury, 2005), water hardness (USEPA, 1985; Pickering & Henderson, 1966), water pH (Aslam & Yousafzai, 2017), age of organisms (Guertin, 2005), use of non-specific salts (Vignati et al., 2010), direct effects on DNA (Fang et al., 2014; Plaper et al., 2002), genetic variations (Martín-González et al., 2006), among others.

Vignatiet al. (2010) reported acute toxicities of 0.65-0.81 mgCr(III).L^{-1} and 1.58-2.24 mgCr(VI).L^{-1} for the microalgae *Pseudokirchneriella subcapitata*, emphasizing that Cr(III) can be more toxic to certain aquatic microorganisms, even at concentrations below current environmental quality criteria and within the concentration range typically observed in aquatic systems impacted by industrial discharges. Also, it has eventually been observed that fish may be more sensitive to Cr(III) than to Cr(VI) (Aslam & Yousafzai, 2017; WHO, 2009), being able to generate tissue damage, including hyperplasia, toothpick formation in the gill lamellae and necrosis, as well as impaired ability to osmoregulate and breathe (Moore, 1991).

Several authors have shown the toxic effect of Cr(III) on various aquatic organisms, for example: the microcrustacean *Daphnia magna* with an acute toxicity of 22 mg.L^{-1} (Kuhn et al., 1989), the protozoan *Tetrahymena pyriformis* with 50-62 mg.L^{-1} (Sauvant et al., 1995), the microalgae *Chlorella kessleri* with 0.08-0.12 mg.L^{-1} (Vignati et al., 2010), the fish *Cyprinus carpio* with 97.7 mg.L^{-1} (Wong et al., 1982), and the amphipod *Crangonyx pseudogracilis* with 291 mg.L^{-1} (Martin & Holdich, 1986). Similarly, in heterotrophic bacteria from Lake Maracaibo, a greater ecotoxic effect of Cr(III) was found compared to Cr(VI) in the sampling site of the present study (Lake Park Vereda), possibly due to spatial variability in terms of PTE sources' contribution in the basin and its effect on local microbial populations (Castro & Marín, 2018).

Regarding cadmium, it has been reported that protozoa can bioaccumulate between 0.05 and 332.75 mgCd.kg^{-1} dry weight (Fernandez-Leborans & Olalla Herrero, 2000), with percentages between 13.5 and 86.4% of the Cd available in their environment (Martín-González et al., 2006). In aquatic environments, Cd(II) shows relative mobility, which depends on pH, alkalinity, temperature, presence of organic molecules and water hardness. Thus, marine organisms are more resistant to Cd poisoning than those living in a freshwater environment (Borsari, 2014; CCME, 2014). Cd indirectly generates reactive oxygen species and ultimately increases oxidation of proteins, lipids, and DNA in cells, leading to DNA damage and tumor growth (Liu et al., 2009; Wang et al., 2004). It can also block the calcium absorption in water (CCME, 2014), also exhibiting embryotoxic, teratogenic and mutagenic properties (Sanders, 1986).

In various investigations the sensitivity of protozoa and other aquatic organisms to Cd(II) has been described, for example: *Colpoda steinii* (protozoa) of 0.5-5.0 mg.L^{-1} (Díaz et al., 2006), *Tetrahymena owiformis* (protozoa) of 3-10 mg.L^{-1} (Sauvant et al., 1995), *D. magna* (microcrustacean) of 0.004 mg.L^{-1} (Okamoto et al., 2015), *Corbicula fluminalis* (bivalve) of 0.52-0.56 mg.L^{-1} (Abdel-Gawad, 2006) and *Channa punctatus* (fish) of 5.2-8.4 mg.L^{-1} (Gupta & Rajbanshi, 1988). In contrast, it has been reported that the tolerance of protozoa to Cd(II) may be due to the following mechanisms: i) formation of granules resistant to higher concentrations of the element (Nilsson, 1981), which can contain several elements and function as structures involved in detoxification or regulation of metal ions (Dunlop & Chapman, 1981); ii) formation of a metallothionein related to stress regulation by PTE (Martín-González et al., 2006), where Cd induces a Cd-metallothionein tetramer similar in amino acid composition to metallothioneins from certain invertebrates and vertebrates (Piccinni et al., 1987). For Lake Maracaibo, Cd(II) ecotoxicological analysis on heterotrophic bacteria of 75->5,000 mg.L^{-1} (Castro & Marín, 2018), *P. solida* (bivalve) of 128.25 mg.L^{-1} (Rojas, 2012) and bacterial strains associated with *Lemna*

sp. with a minimum inhibitory concentration >2,000 mg.L^{-1} (Díaz-Borrego et al., 2007) have been reported, showing the ecotoxic effect of this element on organisms of different trophic levels.

On the other hand, the ecotoxic effects of vanadium come mainly from the similar structure of vanadate (VO_4^{3-}, oxidation state +5) and o-phosphate and, therefore, from interference with the function of biomolecules containing P, turning out to be the most toxic, soluble and stable form of vanadium in the environment (Gustafsson, 2019; Mannazzu, 2001). Furthermore, vanadium appears to interfere with different enzymes such as ATPases, protein kinases, ribonucleases, and phosphatases (Mukherjee et al., 2013). Some examples of the acute toxicity of pentavalent vanadium on aquatic organisms are: *Pseudomonas putida* (bacteria) 180-200 mg.L^{-1}, *Trachelophyllum* sp. (protozoa) of 120-130 mg.L^{-1} (Kamika & Momba, 2014), *D. magna* (microcrustacean) of 1.2 mg.L^{-1} (Okamoto et al., 2015) and *Salmo gairdneri* (fish) of 2.0-13.2 mg.L^{-1} (Stendahl & Sprague, 1982). In the case of Lake Maracaibo, an ecotoxicity of V(V) for *P. solida* of 401.96 mg.L^{-1} has been documented; this bivalve has been proposed as a bioindicator for PTE in this ecosystem, due to its ability to incorporate, tolerate and bioaccumulate elements in higher concentrations and proportional to exposure levels (Rojas, 2012).

The LC_{50} values observed for the ciliated protozoa of Lake Maracaibo, in general, are relatively high and comparable to those of other water bodies (Maurya & Pandey, 2020; Kamika & Momba, 2014; Madoni & Romeo, 2006; Madoni et al., 1996). Madoni & Romeo (2006) reported toxic effects of 0.30, 0.89, 0.59 and 0.07 mgCd(II).L^{-1}, and of 110, 108, 0.1 and 0.1 mgCr(VI).L^{-1} for the ciliated protozoa *Dexiotricha granulosa*, *Colpidium colpoda*, *Euplotes aediculatus* and *Halteria grandinella*, respectively, showing great differences in tolerance, as observed in other species of ciliates. These variations make it difficult to define an absolute scale of PTE toxicity for aquatic organisms, but they establish a greater sensitivity of ciliated microorganisms compared to invertebrate metazoans. Kamika & Momba (2013), for their part, highlighted the synergistic effect of the combination of V(V) and Ni(II), increasing toxicity and impairing the microbial survival and its ability to eliminate PTE, due to presence resistant genes and microbial diversity in the environment.

Tolerance and adaptability processes are related to prolonged exposure to PTEs (Poirier et al., 2013; Gadd, 2010; Martín-González et al., 2006), which could be occurring in the Lake Maracaibo basin, as a result of constant discharge of large volumes of untreated industrial wastewaters (e.g. petrochemicals, gas processing, oil production and transportation, tanneries, metal-mechanics, among others), especially in the Strait of Maracaibo area, as well as the activity of approximately 10,000 oil drilling platforms located in the lake central area (CGR, 2010; Rodríguez, 2000; Parra-Pardi, 1979). In this sense, Martín-González et al. (2006) pointed to bioaccumulation as an important resistance mechanism for these xenobiotic substances in the environment, possibly being mediated by the presence of metallothioneins. Bioaccumulation in biota negatively affects the health of exposed organisms and their consumers. PTEs are transferred from the abiotic environment to living organisms (bioconcentration) and accumulate in biota, causing contamination of food chains with these elements. PTEs can enrich successive trophic levels of food webs, leading to their biomagnification. In this way, the bioconcentration, bioaccumulation and biomagnification of PTE in food chains have important implications for wildlife and human health (Ali & Khan, 2018).

Another mechanism that promotes tolerance to the PTE presence in aquatic environments is microbial reduction. When an element is microbially reduced to a lower redox state, its mobility and toxicity can be greatly decreased (Gadd, 2010). In this way, the bacteria-protozoan association could allow the survival of non-tolerant communities by reducing toxic forms of elements to more innocuous ones. In this regard, Kamika & Momba (2014) revealed that the V reduction capacity, adopted by *Pseudomonas putida*, can be an effective strategy to eliminate V from polluted environments.

The higher relative sensitivity of *Euplotes* sp. to the PTE tested (Table 1, Figure 4) makes it look like a possible bioindicator for PTE contamination in the Lake Maracaibo. The high sensitivity of ciliated protozoa to toxic compounds, coupled with their structural simplicity (unicellular models), ease of culture, physiological changes, behavioral activity, responses of cell organelles and short generational period (Maurya & Pandey, 2020; Gomiero et al., 2013; Mortimer et al., 2010; Qing-Hua et al., 2008; Martín-González et al., 2006) makes them good candidates for use as bioindicators, biosensors, and models for ecotoxicity studies.

4.2. Toxic effect of potentially toxic elements at different exposure times on ciliated protozoa

Comparing the toxic effects of chemicals on ciliated protozoa is a complicated matter, due to the differences between experimental conditions used in studies (Amaro et al., 2011; Rico et al., 2009; Madoni & Romeo, 2006; Madoni et al., 1996; Sauvant et al., 1995; Simanov, 1987). While most research is conducted using a 24-hour exposure time; shorter periods offer the opportunity to better assess the existence of oxidative stress (Rico et al., 2009; Nalecz-Jawecki et al., 1993), demonstrate the early effects of toxicity on natural populations (Sauvant et al., 1995), act promptly (early warning systems) in contamination events by PTEs (Isibor et al., 2020), as well as development of sensitive and rapid biomonitoring methods for PTE detections in the environment (Amaro et al., 2011; Blanck & Wängberg, 1988). In this sense, Mortimer et al. (2010) reported that the toxic effects of copper compounds on the ciliated protozoan *Tetrahymena thermophila* did not depend on the exposure time (4 and 24 h), while the toxicity of zinc compounds was approximately 1.5 times lower after 24-h of exposure than after 4-h, probably due to adaptation. These findings support the need to further study the influence of exposure time in PTE toxicity tests on ciliated protozoa, in order to establish the most suitable periods of experimentation and achieve similar conditions to carry out the laboratory tests. Also, Nalecz-Jawecki et al. (1993) suggest that the tests with short exposure periods (1-h) allow to observe the adverse effects in a more rapid and sensitive way before the death of organism occurs, also pointing out that the effective concentration values (1-h EC_{50}) of the protozoan *Spirostomum ambiguum* for Hg(II), Ag(I), Cu(II) and Cd(II) were almost the same as the 24 and 48-h LC_{50}.

The acute toxicity data (1-h LC_{50}) for Cr(III), Cr(VI), Cd(II) and V(V) of the ciliated protozoa of Lake Maracaibo are comparable to those reported for other water bodies at different exposure times (Table 3). *Euplotes* sp. and *Loxodes* sp. from Lake Maracaibo are the organisms most sensitive to Cr(III) and V(V), while *Euplotes aediculatus* (Garda Lake, Italy) (Madoni & Romeo, 2006) and *Bresslauides* sp. (canal of water, Thailand) (Pudpong & Chantangsi,

Table 3. Comparison of LC_{50} values at different exposure times of potentially toxic elements for ciliated protozoa.

Protozoa	Source	Element (mg.L^{-1})				Time (h)	Reference
		Cr(III)	Cr(VI)	Cd(II)	V(V)		
Uronema sp.	Lake Maracaibo (Venezuela)	4.39	213.59	0.71	1.92	1	This study
Euplotes sp.	Lake Maracaibo (Venezuela)	4.23	116.25	0.72	1.88	1	This study
Loxodes sp.	Lake Maracaibo (Venezuela)	111.24	154.08	0.14	0.54	1	This study
Spirostomum ambiguum	Municipal waterworks (Poland)			0.5-40.0		1	Nalecz-Jawecki et al. (1993)
Tetrahymena sp.	Tinto River (Spain)			0.52		1	Rico et al. (2009)
Tetrahymena Thermophile	University of Georgia (USA)			0.56-5.62		2	Amaro et al. (2011)
Dexiostoma campylum	Oder river (Czech Republic)			1.61		2	Simanov (1987)
Tetrahymena pyriformis	France	62		10		3	Sauvant et al. (1995)
Tetrahymena pyriformis	France	54		3		6	Sauvant et al. (1995)
Tetrahymena pyriformis	France	50		3		9	Sauvant et al. (1995)
Euplotes patella	Activated sludge (Italy)		9.47			24	Madoni et al. (1994)
Peranema sp.	Wastewater (South Africa)				160-200	24	Kamika & Momba (2014)
Trachelophyllum sp.	Wastewater (South Africa)				120-130	24	Kamika & Momba (2014)
Bresslauides sp.	Canal of water in Chulalongkorn (Thailand)			0.09		24	Pudpong & Chantangsi (2015)
Euplotes sp.	Activated sludge (Italy)		38.6	2.66		24	Madoni et al. (1996)
Opercularia coarctata	Activated sludge (Italy)		211	3.75		24	Madoni et al. (1996)
Euplotes aediculatus	Garda Lake (Italy)		0.1	0.59		24	Madoni & Romeo (2006)
Colpidium colpoda	Garda Lake (Italy)		108	0.89		24	Madoni & Romeo (2006)

2015), are the most sensitive to Cr(VI) and Cd(II), respectively. The highest tolerance levels are observed in *Loxodes* sp. (Lake Maracaibo) for Cr(III), *Uronema* sp. (Lake Maracaibo) for Cr(VI), *Spirostomum ambiguum* (municipal waterworks, Poland) (Nalecz-Jawecki et al., 1993) for Cd(II) and *Peranema* sp. (wastewater, South Africa) (Kamika & Momba, 2014) for V(V). In general, these data suggest large differences in the levels of sensitivity and tolerance of ciliated protozoa to PTEs, regardless of the exposure time in laboratory tests.

Finally, the need for further research on the influence of exposure time in both acute and chronic toxicity tests with ciliated protozoa is pointed out, considering that this variable is critical in the response of these organisms in the presence of chemical contaminants such as PTE. This response is also dependent on the natural condition to which the organism is accustomed in its ecosystem.

5. Conclusions

The PTE Cr(III), Cr(VI), Cd(II) and V(V) revealed a toxic effect on ciliated protozoa isolated from Lake Maracaibo. The performance of *Euplotes* sp. makes it a sensitive and convenient biomonitor for PTE contamination in this lake.

The short exposure time (1-h) and the simplicity of the laboratory procedure used in this work allowed obtaining LC_{50} levels comparable to traditional studies with 24-h experiments. These minor periods facilitate the application of toxicity essays in the development of rapid and sensitive biomonitoring programs for the detection of PTEs in the aquatic environment, as well as in emergency and early warning situations that require immediate responses.

The results obtained reveal the importance of continuing to develop studies aimed at understanding and explaining the ecological risk related to the presence of PTE, particularly in tropical environments where native species are threatened by the constant dumping of untreated liquid wastes, limiting their survival and altering the functionality of food webs, as well as the integrity of ecosystems as a whole.

Acknowledgements

The authors thank Dr. Cristiano V. M. Araújo for the critical review of the draft of this work.

References

Abdel-Gawad, S.S., 2006. Toxicity and bioaccumulation of cadmium in the freshwater bivalve *Corbicula fluminalis* Müller, 1774. Egypt. J. Aquat. Biol. Fish. 10(4), 33-43. http://dx.doi.org/10.21608/ejabf.2006.1899.

Alayo, M., & Iannacone, J., 2002. Ensayos ecotoxicológicos con petróleo crudo, diésel 2 y diésel 6 con dos subespecies de *Brachionus plicatilis* Müller 1786 (Rotifera: Monogononta). Gayana, 66(1), 45-58. http://dx.doi.org/10.4067/S0717-65382002000100007.

Ali, H., & Khan, E., 2018. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs - concepts and implications for wildlife and human health. Hum. Ecol. Risk Assess. 2018, 1353-1376.

Amaro, F., Turkewitz, A.P., Martín-González, A., & Gutiérrez, J.C., 2011. Whole-cell biosensors for detection of heavy metal ions in environmental samples based on metallothionein promoters from *Tetrahymena thermophila.* Microb. Biotechnol. 4(4), 513-522. PMid:21366892. http://dx.doi.org/10.1111/j.1751-7915.2011.00252.x.

American Public Health Association – APHA, 2017. Standard methods for the examination of water and wastewater (23th ed.). Washington: APHA.

Aslam, S., & Yousafzai, A.M., 2017. Chromium toxicity in fish: a review article. J. Entomol. Zool. Stud. 5(3), 1483-1488.

Ávila, H., Gutiérrez, E., Ledo, H., Araujo, M., & Sánquiz, M., 2010. Heavy metals distribution in superficial sediments of Maracaibo Lake (Venezuela). Rev. Tec. Fac. Ing. Univ. Zulia, 33(2), 122-129.

Blanck, H., & Wängberg, S., 1988. Validity of an ecotoxicological test system: short-term and long-term effects of arsenate on marine periphyton communities in laboratory systems. Can. J. Fish. Aquat. Sci. 45(10), 1807-1815. http://dx.doi.org/10.1139/f88-212.

Borsari, M., 2014. Cadmium: inorganic chemistry. In: Scott, R.A., ed. Encyclopedia of inorganic and bioinorganic chemistry. New York: John Wiley & Sons. p. 56-87.

Bracho, G., Cuador-Gil, J.Q., & Rodríguez-Fernández, R.M., 2016. Calidad del agua y sedimento en el Lago de Maracaibo, estado Zulia. Miner. Geol. 32(1), 1-14.

Cáceres, A., 2012. La Creole Petroleum Corporation en Venezuela: la gran fusión petrolera de los años cuarenta. Debates IESA, 2012(17), 58-61.

Canadian Council of Ministers of the Environment – CCME, 1999. Canadian water quality guidelines for the protection of aquatic life: chromium-hexavalent chromium and trivalent chromium. In: Canadian environmental quality guidelines. Winnipeg: CCME.

Canadian Council of Ministers of the Environment – CCME, 2014. Canadian water quality guidelines for

the protection of aquatic life: cadmium. In: Canadian environmental quality guidelines. Winnipeg: CCME.

Castro, F., & Marín, J., 2018. Comparación de la ecotoxicidad por metales pesados sobre bacterias heterótrofas de dos sitios contrastados del Lago de Maracaibo (Venezuela). Rev. Fac. Cienc. Básicas 14, 1-10.

Colina de Vargas, M., & Romero, R., 1992. Mercury determination by cold vapour atomic absorption spectrometry in several biological indicators from Lake Maracaibo, Venezuela. Analyst, 117(3), 645-647. PMid:1580413. http://dx.doi.org/10.1039/an9921700645.

Colina, M., 2001. Determination of nutrients and heavy metal species in samples from Lake Maracaibo [Doctoral dissertation]. Sheffield: Sheffield Hallam University.

Contraloría General de la República – CGR, 2010. Actuación coordinada en el sistema nacional de control fiscal para evaluar los problemas ambientales y el deterioro de las relaciones ecológicas en la cuenca del río más importante de cada entidad federal [online]. Caracas. Retrieved in 2020, May 12, from: http://www.cgr.gob.ve

Corona, J., 2013. Contaminación antropogénica en el Lago de Maracaibo, Venezuela. Biocenosis 27(1-2), 85-93.

Díaz, S., Martín-González, A., & Gutiérrez, J.C., 2006. Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. Environ. Int. 32(6), 711-717. PMid:16650895. http://dx.doi.org/10.1016/j.envint.2006.03.004.

Díaz-Borrego, L., Dupontt, J., Espina, K., Rincón, N., García, M., & Atencio, L., 2007. Utilización de sustratos orgánicos y resistencia a metales pesados por bacterias asociadas a *Lemna* spp. Bol. Cent. Invest Biol. 41(1), 27-43.

Dunlop, S., & Chapman, G., 1981. Detoxification of zinc and cadmium by the freshwater protozoan *Tetrahymena pyriformis.* Environ. Res. 24(2), 264-274. http://dx.doi.org/10.1016/0013-9351(81)90156-0.

El-Serehy, H., Al-Rasheid, K., & Shafik, H., 2012. Microbial loop populations: their abundances and trophodynamics in the Gulf of Aqaba, Red Sea. Turk. J. Fish. Aquat. Sci. 12, 565-573.

Fang, Z., Zhao, M., Zhen, H., Chen, L., Shi, P., & Huang, Z., 2014. Genotoxicity of tri- and hexavalent chromium compounds *in vivo* and their modes of action on DNA damage *in vitro.* PLoS One 9(8), e103194. PMid:25111056. http://dx.doi.org/10.1371/journal.pone.0103194.

Faure, V., Pinazo, C., Torréton, J., & Jacquet, S., 2010. Modelling the spatial and temporal variability of the SW lagoon of New Caledonia I: a new biogeochemical model based on microbial loop recycling. Mar. Pollut. Bull. 61(7-12), 465-479. PMid:20667554. http://dx.doi.org/10.1016/j.marpolbul.2010.06.041.

Fernandez-Leborans, G., & Olalla Herrero, Y., 2000. Toxicity and bioaccumulation of lead and cadmium in marine protozoan communities. Ecotoxicol. Environ. Saf. 47(3), 266-276. PMid:11139180. http://dx.doi.org/10.1006/eesa.2000.1944.

Foissner, W., & Berger, H., 1996. A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshw. Biol. 35(2), 375-482. http://dx.doi.org/10.1111/j.1365-2427.1996.tb01775.x.

Foissner, W., 1999. Protist diversity: estimates of the near-imponderable. Protist 150(4), 363-368. PMid:10714770. http://dx.doi.org/10.1016/S1434-4610(99)70037-4.

Fried, J., Ludwig, W., Psenner, R., & Schleifer, K.H., 2002. Improvement of ciliate identification: a new protocol for fluorescence *in situ* hybridization (FISH) in combination with silver stain techniques. Syst. Appl. Microbiol. 25(4), 555-571. PMid:12583717. http://dx.doi.org/10.1078/07232020260517706.

Gadd, G.M., 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156(Pt 3), 609-643. PMid:20019082. http://dx.doi.org/10.1099/mic.0.037143-0.

Girling, A.E., Pascoe, D., Janssen, C.R., Peither, A., Wenzel, A., Schäfer, H., Neumeier, B., Mitchell, G.C., Taylor, E.J., Maund, S.J., Lay, J.P., Jüttner, I., Crossland, N.O., Stephenson, R.R., & Persoone, G., 2000. Development of methods for evaluating toxicity to freshwater ecosystems. Ecotoxicol. Environ. Saf. 45(2), 148-176. PMid:10648133. http://dx.doi.org/10.1006/eesa.1999.1847.

Gomiero, A., Dagnino, A., Nasci, C., & Viarengo, A., 2013. The use of protozoa in ecotoxicology: application of multiple endpoint tests of the ciliate *E. crassus* for the evaluation of sediment quality in coastal marine ecosystems. Sci. Total Environ. 442, 534-544. PMid:23202299. http://dx.doi.org/10.1016/j.scitotenv.2012.10.023.

Gomiero, A., Sforzini, S., Dagnino, A., Nasci, C., & Viarengo, A., 2012. The use of multiple endpoints to assess cellular responses to environmental contaminants in the interstitial marine ciliate *Euplotes crassus.* Aquat. Toxicol. 114-115, 206-216. PMid:22459342. http://dx.doi.org/10.1016/j.aquatox.2012.02.030.

Guertin, J., 2005. Toxicity and health effects of chromium (all oxidation states). In: Guertin, J., Avakiam, C.P. & Jacobs, J.A., eds. Chromium (VI) handbook. Boca Raton: CRC Press, 216-234.

Gupta, A.K., & Rajbanshi, V.K., 1988. Acute toxicity of cadmium to *Channa punctatus* (BLOCH). Acta

Hydrochim. Hydrobiol. 16(5), 525-535. http://dx.doi.org/10.1002/aheh.19880160513.

Gustafsson, J.P., 2019. Vanadium geochemistry in the biogeosphere -speciation, solid-solution interactions, and ecotoxicity. J. Appl. Geochem. 102, 1-25. http://dx.doi.org/10.1016/j.apgeochem.2018.12.027.

Hong, Y.J., Liao, W., Yan, Z.F., Bai, Y.C., Feng, C.L., Xu, Z.X., & Xu, D.Y., 2020. Progress in the research of the toxicity effect mechanisms of heavy metals on freshwater organisms and their water quality criteria in China. J. Chem. 2020, 1-12. http://dx.doi.org/10.1155/2020/9010348.

Isibor, P.O., Imoobe, T.O., Dedeke, G.A., Adagunodo, T.A., & Taiwo, O.S., 2020. Health risk indices and zooplankton-based assessment of a tropical rainforest river contaminated with iron, lead, cadmium, and chromium. Sci. Rep. 10(1), 16896. PMid:33037243. http://dx.doi.org/10.1038/s41598-020-72526-1.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B., & Beeregowda, K., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxicol. 7(2), 60-72. PMid:26109881. http://dx.doi.org/10.2478/intox-2014-0009.

Kamika, I., & Momba, M.N.B., 2013. Synergistic effects of vanadium and nickel on heavy metal-tolerant microbial species in wastewater systems. Desalin. Water Treat. 51(40-42), 7431-7446. http://dx.doi.org/10.1080/19443994.2013.777680.

Kamika, I., & Momba, M.N.B., 2014. Effect of vanadium toxicity at its different oxidation states on selected bacterial and protozoan isolates in wastewater systems. Environ. Technol. 35(13-16), 2075-2085. PMid:24956802. http://dx.doi.org/10.1080/09593330.2014.893023.

Kuhn, R., Pattard, M., Pernak, K., & Winter, A., 1989. Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. Water Res. 23(4), 501-510. http://dx.doi.org/10.1016/0043-1354(89)90142-5.

Liu, J., Qu, W., & Kadiiska, M.B., 2009. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol. Appl. Pharmacol. 238(3), 209-214. PMid:19236887. http://dx.doi.org/10.1016/j.taap.2009.01.029.

Lynn, D., 2008. The ciliated protozoa (3rd ed.). Bern: Springer Science and Business Media B.V.

Madoni, P., & Romeo, M.G., 2006. Acute toxicity of heavy metals towards freshwater ciliated protists. Environ. Pollut. 141(1), 1-7. PMid:16198032. http://dx.doi.org/10.1016/j.envpol.2005.08.025.

Madoni, P., Davoli, D., & Gorbi, G., 1994. Acute toxicity of lead, chromium and other heavy metals to ciliates from activated sludge plants. Bull. Environ. Contam. Toxicol. 53(3), 420-425. PMid:7919720. http://dx.doi.org/10.1007/BF00197235.

Madoni, P., Davoli, D., Gorbi, G., & Vescovi, L., 1996. Toxic effect of heavy metals on the activated Sludge protozoan community. Water Res. 30(1), 135-141. http://dx.doi.org/10.1016/0043-1354(95)00124-4.

Mannazzu, I., 2001. Vanadium detoxification and resistance in yeast: a mini-review. Ann. Microbiol. 51(1), 1-9.

Marín-Leal, J.C., Carrasquero-Ferrer, S.C., Pire-Sierra, M.C., & Behling de Calmón, E.H., 2017. Dynamic of priority pollutants and wastewater adequacy in the Lake Maracaibo basin (Venezuela). In: Araújo, C.V.M. & Shinn, C., eds. Ecotoxicology in Latin America. New York: Nova Science Publishers, Chapter 29, 457-479.

Martin, T.R., & Holdich, D.M., 1986. The acute lethal toxicity of heavy metals to peracarid crustaceans (with particular reference to fresh-water asellids and gammarids). Water Res. 20(9), 1137-1147. http://dx.doi.org/10.1016/0043-1354(86)90060-6.

Martín-González, A., Díaz, S., Borniquel, S., Gallego, A., & Gutiérrez, J.C., 2006. Cytotoxicity and bioaccumulation of heavy metals by ciliated protozoa isolated from urban wastewater treatment plants. Res. Microbiol. 157(2), 108-118. PMid:16129584. http://dx.doi.org/10.1016/j.resmic.2005.06.005.

Maurya, R., & Pandey, A.K., 2020. Importance of protozoa *Tetrahymena* in toxicological studies: a review. Sci. Total Environ. 741, 140058. PMid:32599397. http://dx.doi.org/10.1016/j.scitotenv.2020.140058.

Moore, J.W., 1991. Chromium. In: Moore, J.W., ed. Inorganic contaminants of surface water: research and monitoring priorities. New York: Springer-Verlag New York, 83-97. http://dx.doi.org/10.1007/978-1-4612-3004-5_9.

Mortimer, M., Kasemets, K., & Kahru, A., 2010. Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophila*. Toxicology 269(2-3), 182-189. PMid:19622384. http://dx.doi.org/10.1016/j.tox.2009.07.007.

Mukherjee, B., Halder, S., Ghosh, M.K., & Manasadeepa, R., 2013. Vanadium ions and proteins, distribution, metabolism, and biological significance. In: Uversky, V.N., ed. Encyclopedia of metalloproteins. Bern: Springer Science, 2306-2316. http://dx.doi.org/10.1007/978-1-4614-1533-6_136.

Nalecz-Jawecki, G., Demkowicz-Dobrzainski, K., & Sawicki, J., 1993. Protozoan *Spirostomum ambiguum* as a highly sensitive bioindicator for rapid and easy determination of water quality. Sci. Total Environ. 134(Suppl.1993), 1227-1234. http://dx.doi.org/10.1016/S0048-9697(05)80128-7.

Narayanan, N., Priya, M., Haridas, A., & Manilal, V.B., 2007. Isolation and culturing of a most common anaerobic ciliate, *Metopus* sp. Anaerobe 13(1), 14-20. PMid:17223583. http://dx.doi.org/10.1016/j.anaerobe.2006.10.003.

Nilsson, J., 1981. Effects of copper on phagocytosis in *Tetrahymena*. Protoplasma 109(3-4), 359-370. http://dx.doi.org/10.1007/BF01287453.

Okamoto, A., Yamamuro, M., & Tatarazako, N., 2015. Acute toxicity of 50 metals to *Daphnia magna*. J. Appl. Toxicol. 35(7), 824-830. PMid:25382633. http://dx.doi.org/10.1002/jat.3078.

Paiva, T.S., Borges, B.N., & Silva-Neto, I.D., 2013. Phylogenetic study of class Armophorea (Alveolata, Ciliophora) based on 18S-rDNA data. Genet. Mol. Biol. 36(4), 571-585. PMid:24385862. http://dx.doi.org/10.1590/S1415-47572013000400017.

Panda, S.K., & Choudhury, S., 2005. Chromium stress in plants. Braz. J. Plant Physiol. 17(1), 95-102. http://dx.doi.org/10.1590/S1677-04202005000100008.

Parra-Pardi, G., 1979. Estudio integral sobre la contaminación del Lago de Maracaibo y sus afluentes, parte II: evaluación del proceso de eutroficación. Caracas: Ministerio del Ambiente y de los Recursos Naturales Renovables.

Piccinni, E., Irato, P., Coppellotti, O., & Guidolin, L., 1987. Biochemical and ultrastructural data on *Tetrahymena pyriformis* treated with copper and cadmium. J. Cell Sci. 88(Pt 3), 283-293. PMid:3129439. http://dx.doi.org/10.1242/jcs.88.3.283.

Pickering, Q.H., & Henderson, C., 1966. The acute toxicity of some heavy metals of different species of warmwater fishes. Air Water Pollut. 10(6), 453-463. PMid:5946974.

Plaper, A., Jenko-Brinovec, S., Premzl, A., Kos, J., & Raspor, P., 2002. Genotoxicity of trivalent chromium in bacterial cells. Possible effects on DNA topology. Chem. Res. Toxicol. 15(7), 943-949. PMid:12119005. http://dx.doi.org/10.1021/tx010096q.

Poirier, I., Hammann, P., Kuhn, L., & Bertrand, M., 2013. Strategies developed by the marine bacterium *Pseudomonas fluorescens* BA3SM1 to resist metals: a proteome analysis. Aquat. Toxicol. 128-129, 215-232. PMid:23314334. http://dx.doi.org/10.1016/j.aquatox.2012.12.006.

Premke, K., & Arndt, H., 2000. Predation on heterotrophic flagellates by protist: food selectivity determined using a live-staining technique. Arch. Hydrobiol. 150(1), 17-28. http://dx.doi.org/10.1127/archiv-hydrobiol/150/2000/17.

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(2), 117-125.

Qing-Hua, C., Run-Lin, X., Nora, F.Y.T., Siu G.C. & Paul, K.S.S., 2008. Use of ciliates (Protozoa: Ciliophora) as bioindicator to assess sediment quality of two constructed mangrove sewage treatment belts in Southern China. Mar. Pollut. Bull. 57(6-12), 689-694.

Rico, D., Martín-González, A., Díaz, S., De Lucas, P., & Gutiérrez, J.C., 2009. Heavy metals generate reactive oxygen species in terrestrial and aquatic ciliated protozoa. Comp. Biochem. Physiol. Part C Toxicol Pharmacol. 149(1), 90-96. PMid:18725323. http://dx.doi.org/10.1016/j.cbpc.2008.07.016.

Rodríguez, G., 2000. El sistema de Maracaibo, biología y ambiente. Caracas: Instituto Venezolano de Investigaciones Científicas.

Rojas, J., 2012. *Polymesoda solida* como bioindicador de metales pesados en el sistema del Lago de Maracaibo [Doctoral dissertation]. Maracaibo: Universidad del Zulia.

Rojas, J., Rincón, J., Marín, J., Ortega, P., Buonocore, R., Colina, M., & Montilla, J., 2015. Toxicidad y bioacumulación de cromo (Cr^{+6}) en la almeja *Polymesoda solida* del sistema estuarino Lago de Maracaibo. Bol. Cent. Inv. Biol. 49(1), 5-25.

Sanders, C.L., 1986. Toxicological aspect of energy production. New York: MacMillan Publishing Company.

Sauvant, M.P., Pepin, D., Groliere, C.A., & Bohatier, J., 1995. Effects of organic and inorganic substances on the cell proliferation of L-929 fibroblasts and *Tetrahymena pyriformis* GL protozoa used for toxicological bioassays. Bull. Environ. Contam. Toxicol. 55(2), 171-178. PMid:7579920. http://dx.doi.org/10.1007/BF00203006.

Shi, W., Zhao, X., Han, Y., Che, Z., Chai, X., & Liu, G., 2016. Ocean acidification increases cadmium accumulation in marine bivalves: a potential threat to seafood safety. Sci. Rep. 6(1), 20197. PMid:26795597. http://dx.doi.org/10.1038/srep20197.

Simanov, L., 1987. Toxic effect of copper and cadmium on algae and protozoa. Inst. Land Ecol. Czech. Acad. Sci. 28, 81-82.

Stendahl, D.H., & Sprague, J.B., 1982. Effects of water hardness and pH on vanadium lethality to rainbow trout. Water Res. 16(10), 1479-1488. http://dx.doi.org/10.1016/0043-1354(82)90246-9.

U. S. Environmental Protection Agency – USEPA, 1985. Ambient water quality criteria for chromium - 1984, EPA-440/5-84-031. Washington: USEPA.

Vignati, D.A.L., Dominik, J., Beye, M.L., Pettine, M., & Ferrari, B.J.D., 2010. Chromium (VI) is more toxic than chromium (III) to fresh water algae: a paradigm to revise? Ecotoxicol. Environ. Saf. 73(5), 743-749. PMid:20138363. http://dx.doi.org/10.1016/j.ecoenv.2010.01.011.

Vilas-Boas, J.A., Xavier, M., & Pedroso, R., 2020. Ciliates in ecotoxicological studies: a minireview. Acta Limnol. Bras. 32, e202. http://dx.doi.org/10.1590/s2179-975x6719.

Wang, Y., Fang, J., Leonard, S.S., & Krishna Rao, K.M., 2004. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic. Biol. Med. 36(11), 1434-1443. PMid:15135180. http://dx.doi.org/10.1016/j.freeradbiomed.2004.03.010.

Wong, M.H., Lau, W.M., Tong, T.Y., Liu, W.K., & Luk, K.C., 1982. Toxic effects of chromic sulphate on the common carp, *Cyprinus carpio.* Toxicol. Lett. 10(2-3), 225-232. PMid:7080089. http://dx.doi.org/10.1016/0378-4274(82)90079-0.

World Health Organization – WHO, 2009. Inorganic chromium (III) compounds, concise international chemical assessment document 76. Huntingdon: WHO.

Received: 13 December 2021
Accepted: 16 August 2022

Associate Editor: André Andrian Padial.