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Toxicity test and Cd, Cr, Pb and Zn bioccumulation in *Phalloceros caudimaculatus*

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ABSTRACT

The use of animals as bioindicators has been useful, especially in the evaluation of environmental impact of pollutant discharges in aquatic ecosystems. This study, aims to assess the fish *Phalloceros caudimaculatus* (known as Guaru) as a heavy metal sbioindicator. LC50 (96 h) acute toxicity tests to potassium dichromate salts, cadmium nitrate, lead II nitrate and zinc sulfate, and bioaccumulation of metals such as cadmium, chromium, lead and zinc were conducted. In the bioaccumulation test, for three months standard tanks were used, with different contamination levels above the levels set by local law, a pollution control tank was also set. The following data was obtained in acute toxicity tests LC50 (96 h): Potassium dichromate 164.58 ± 18.75 mg/L, Cadmium nitrate was 29.5 ± 1.21 mg/L, Lead nitrate was 15.5 ± 0.47 mg/L and zinc sulfate was 62.8 ± 2.81 mg/L. The bioconcentration factor (BCF) maintained the order Zn > Pb > Cd > Cr, where the resulting values were considered high for the four elements ranging from 92.4 (Cr) to 1793.1 (Zn). Based on the test results, the Guaru fish proved resistant to the presence of these metals and a showed a high bioaccumulation rate. Thus, it can be used as a bioindicator of heavy metals.

Introduction

The gradual increase of industrial manufacturing and urban sprawl has contributed to the increase in the amount of pollutants in the environment [1]. This increase in environmental pollution may cause a change in the balance of aquatic ecosystems and may influence significant changes in their biota [1,2].

The use of animals as bioindicators is extremely important, especially for the evaluation of environmental impacts resulting from human action such as sewage and waste dump into water bodies [3–5]. Bioindicators are species that are used as primary indicators of environmental contamination [5], which are defined by responses or reactions of any kind, from an organism to an environmental contaminant that can be measured in biological individual matrix, indicating a deviation of its homeostasis [4]. Organisms such as aquatic plants, algae, crustaceans, mollusks, fish, mammals, birds, can be considered bioindicators [5,6].

An ideal bioindicator should live in a healthy environment, as well as survive while resisting contaminants to which it will be exposed. It is also important to consider the abundance of this species in the environment, and it's ability to adapt to laboratory tests [5,6].

Fish suffer bioaccumulation of heavy metals through the absorption and retention of such materials along the food chain. This is facilitated in the aquatic environment by water filtration and retention of particles in suspension by filtrating organisms like the phytoplankton and shellfish. Fish accumulate metals by feeding and also through the gills [5,7,8].

The toxicity of heavy metals is related to their ability to interfere in enzymatic processes and their low mobility in the organism due to their small size and their unsaturated chemical bonds, favoring their accumulation and consequent alteration in the metabolism of organisms [2]. This Heavy metal toxicity in aquatic organisms and its balance depends on several limnological factors which determine its concentration in the environment and the availability of aquatic biota such as pH, alkalinity, hardness, organic matter, total solids and sedimentation [8,9]. Since these metals interact chemically with other elements, binding to the suspended particulate material, being able to precipitate next to the sediment or to be in the water column, being available to the biota [2]. The elevation of pH, for example, can increase adsorption of Zn to suspended particulate matter, decreasing its dissolved concentration [2].

Fish are also good bioindicators because they react with these mutagenic agents even at low concentrations, and physiological

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characteristics allow fish to survive in different habitats, and in adverse environmental conditions, which makes them appropriate indicators of the environment they live in, especially in regard to persistent and cumulative effects along the food chain [8,10].

Among the species of fish commonly used in aquatic toxicology studies are the *Poecilia reticulata* [11,12] and Zebrafish *Danio rerio* [12]. Among the Brazilian native species that have been used for this purpose are: *Piaractus mesopotamicus* and *Hyphessobrycon eques* [13] and *Hemigrammus marginatus* [12]. The *Phallocerus caudimaculatus* was used by Henares et al. (2011) [14] in the assessment of herbicide toxicity in aquatic weeds and proved efficient for toxicological tests.

The organism used as test in this study was the *Phalloceros caudimaculatus* fish (Fig. 1), known as Guaru, the Cyprinodontiformes order, Poeciliidae Family [15], found in Laguna dos Patos drainage basin, in the lower parts of the Uruguay river drainage basin, Tramandaí and Mampituba rivers drainage and also in coastal areas of Uruguay and Argentina [16].

According to Akanksha et al. (2010) [6], a suitable test organism should have characteristics: be ecologically representative group, viviparous with short reproductive cycle, easy availability, cosmopolitan and also a native species of Brazil [15].

Fig. 1. Specimens of *Phalloceros caudimaculatus* (A1) male; (A2) female; (Lucinda, 2008).

The objective of this study is to evaluate the possibility of using *Phalloceros caudimaculatus* as a bioindicator of heavy metals through toxicity tests and bioaccumulation of Cd, Cr, Pb and Zn.

Materials and methods

This work was carried upon approval of the ethics board for the use of vertebrate animals in laboratory of the Instituto Federal de Educação, Ciência e Tecnologia Sul-rio-grandense (IFSul). The experiments were conducted at the Laboratory of Environmental Contaminants (LACA), at the Grupo de Pesquisa em Contaminantes Ambientais, of the same institution.

The fish were collected from a random sampling from an urban streams in "Laranjal" in the Pelotas, Rio Grande do Sul, Brazil (Fig. 2).

Sampling was carried out with a satin net. Thereafter, the fish were brought to the laboratory and acclimatized in standard tanks with a volume of 30 L, kept for 30 days under constant aeration, and stabilized temperature of 20 ± 2 °C and pH within the neutrality range. Water quality parameters were monitored and management comprised of periodic waste siphoning with 20% volume water change. In the acclimatization period the fish were supplied with a daily diet of commercial feed Sera Vipan flakes™ and Alcon Basic™ with composition for omnivorous fish. During the acute toxicity tests the fish were not fed. The acclimation procedures and experimentation with the fish followed ABNT NBR 15088 [17] determinations.

Preceding the experimental phase, a fish biometry was performed and weight and length measured results showed an average of 0.14 ± 0.15 g (0.4 g Maximum and Minimum 0.03 g) and 1.9 ± 0.71 cm (3.0 cm Maximum and Minimum 1.1 cm), respectively. For the experiments, the fish were separated into batches and those with an average biometrics data were selected.

Acute toxicity test

Acute toxicity tests were performed in triplicate in static environment without water change for 96 h. For these tests, the fish were separated into batches of 8 individuals per treatment, given five treatments with each test substance at different concentrations, and a control treatment, the same experimental conditions,

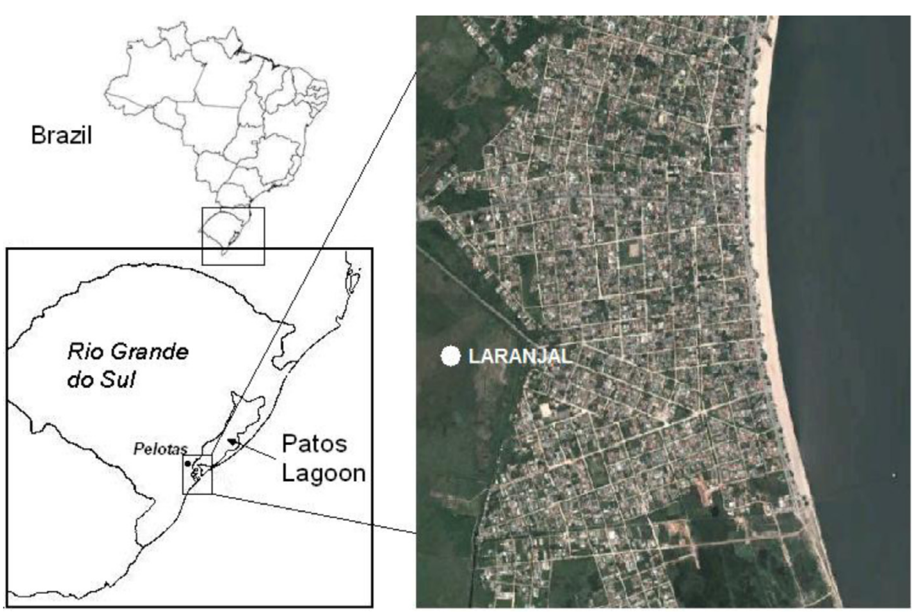

Fig. 2. Collection area location *Phallocerus caudimaculatus*, Google Earth.

with the exception of any test substances, which served to attest to the fish's health, 144 individual were used per test substance.

Tested substances were potassium dichromate, with concentrations of (50; 100; 150; 200 and 300 mg/L), cadmium nitrate (24; 27; 30; 33 and 36 mg/L), lead II nitrate (5, 10, 15, 20 and 25 mg/L) and zinc sulfate (30; 45; 60; 75 and 90 mg/L). The concentration range was defined from literature data such as used by Singh and Manjeet (2015) [18] and determined by preliminary tests. For the tests, the fish were placed in containers of 4 liters of water, with water quality parameters according to Georgetti (2010)[19], water hardness being of 40 mg/L $CaCO_3$, dissolved oxygen ranging from 7.0 to 7.5 mg/L O_2, pH between 6.90 and 7.10, and conductivity between 165.5 and 181.2 μS/cm, for 24 h. Under these conditions of pH and hardness there was no formation of precipitates in the solutions. After this period, the containers were contaminated and the fish remained under test for 96 h. Dead individuals were collected, quantified and parameters were measured and registered.

LC50 (96 h) data were statistically evaluated using "Behrens-Karber" method, though the application of Klassen's formula (1991) [20].

$$LC50 = LC100 - \frac{ab + \ldots + ab}{N}$$

LC50 and LC100 indicate the lethal dose for 50 % and 100% samples, respectively, wherein "a" is the difference between the two consecutive doses (mg/L), "b" is the average arithmetic mortality by two consecutive doses (mg/L) and "N" is the number of subjects in each group.

Bioaccumulation test

This test was performed in duplicate, using two 30 L capacity tanks, with 25 organisms per test tank, one of the tanks was labeled the control treatment to attest to the fish's health, and the other was contaminated with metals as cadmium, lead, chromium and zinc, as shown in Table 1. In both tanks, fish remained confined for 90 days.

Bioaccumulation tests were developed in low concentrations of the metals testes. The concentrations were defined above the quantification limits of the ICP OES method. The levels of the Pb, Cr and Cd metals were above the limits established by Resolution No. 357 – CONAMA, (March 17, 2005), by the Ministry of Environment [21], and measured by the proposed method varying according to metal.

Solutions and reagents

The materials used for handling and samples storage were subjected to a decontamination process using a HNO_3 solution at 10 % (v/v) for 24 h and then dried at 105 °C [22].

All reagents used were of analytical purity, being concentrated nitric acid 65 % (v/v) and perchloric acid 70% (v/v) used for the digestion of the samples. For acute toxicity and bioaccumulation tests, reagents used were: cadmium nitrate, lead nitrate II, potassium dichromate, zinc sulfate, all from Vetec. The ICP multi-element standard solution VIII – 100 mg/L (Merck) was used for the preparation of work solutions. In addition to this, dilutions in ultrapure water were applied.

Sample digestion methods

The fish samples were prepared from whole bodies, after stunning by benzocaine and following the death protocol approved by the Animal Ethics Committee of the institution. The fish were washed with distilled water, dried in an oven at 60 °C for 24 h, disintegrated (mortar and pestle) and separated in triplicate. For digestion, the fish samples were subjected to the method used by Dural et. al. (2007) [23], in which 0.5 g of sample were weighed, digested with a mixture of concentrated HNO_3 and $HClO_4$ (2: 1 v/v), heated at 60 °C for 72 h and the volume adjusted to 25 ml. Fish

Table 1
Physicochemical parameters of water: dissolved oxygen, pH and conductivity in different treatments (concentrations) of the test substances potassium dichromate, cadmium nitrate, lead nitrate II and zinc sulfate, in the acute toxicity test, for 96 h duration.

Cadmium nitrate	DissolvedOxygen (mg/L)				pH				Conductivity (μS/cm)			
Concentrations (mg/L)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
24	7.12	6.92	6.51	6.22	6.87	6.92	6.75	6.74	225.4	227.4	229.5	231.5
27	7.55	7.16	6.55	5.84	6.92	6.96	6.86	6.80	229.3	231.8	233.2	235.5
30	7.68	6.85	6.31	5.92	6.92	6.97	6.90	6.82	232.6	236.8	238.7	240.2
33	7.53	6.73	6.42	5.78	6.95	6.94	6.78	6.88	237.2	239.9	241.6	243.3
36	7.34	6.78	6.31	6.12	6.94	6.95	6.79	6.85	242.5	244.1	246.4	249.5
Potassium dichromate	DissolvedOxygen (mg/L)				pH				Conductivity (μS/cm)			
Concentrations (mg/L)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
50	7.23	6.98	6.55	6.12	7.09	6.96	6.74	7.01	221.3	224.5	226.5	227.1
100	7.53	6.97	6.45	5.55	7.00	6.99	6.72	7.00	250.2	253.9	255.3	256.1
150	7.26	7.12	6.48	5.54	6.85	6.94	6.78	6.94	286.9	291.3	295.8	297.5
200	7.62	6.78	6.53	5.58	6.69	6.97	6.84	6.51	315.8	319.3	322.3	324.4
300	7.64	6.87	6.34	5.57	6.84	6.98	6.87	6.64	413.3	418.6	418.6	421.3
Lead nitrate II	DissolvedOxygen (mg/L)				pH				Conductivity (μS/cm)			
Concentrations (mg/L)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
5	7.82	7.63	6.45	5.65	7.11	6.55	6.50	6.45	210.2	212.5	214.2	216.4
10	7.55	7.36	6.63	5.62	7.00	6.44	6.45	6.39	226.7	228.4	230.4	232.5
15	7.72	7.55	6.76	5.73	6.92	6.51	6.43	6.38	232.7	234.8	236.5	238.9
20	7.51	7.44	6.86	5.41	6.85	6.51	6.49	6.41	243.8	245.5	247.9	249.2
25	7.44	7.31	6.72	5.54	6.77	6.48	6.46	6.42	251.4	253.9	255.1	257.6
Zinc sulfate	DissolvedOxygen (mg/L)				pH				Conductivity (μS/cm)			
Concentrations (mg/L)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
30	7.95	7.52	6.55	5.85	6.59	6.57	6.61	6.62	207.2	209.4	211.3	213.4
45	7.86	7.44	6.42	5.96	6.55	6.56	6.60	6.63	214.1	216.3	218.3	220.3
60	7.59	7.65	6.76	5.63	6.55	6.52	6.54	6.66	223.2	224.5	226.5	228.6
75	7.68	7.58	6.75	5.58	6.58	6.49	6.63	6.67	234.3	235.2	237.4	238.4
90	7.75	7.35	6.34	5.44	6.68	6.48	6.62	6.65	243.2	245.4	247.1	248.5

samples digestions were followed by analysis of blank samples, a standard sample consisting of fish protein impregnated with trace metals, certified reference DORM-3® provided by the National Research Council of Canada.

Determination of metals

The determination of metals in bioaccumulation tests both in water and in extracts obtained from the chemical digestion of the test organisms was performed through ICP-OES equipment model "Optima 3300" by "PerkinElmer" using argon plasma at 15 L/min flow rate and 1300 W power, radio frequency.

The analytical curves were performed with solutions diluted at concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 mg/L (with standard ICP for multi-element 100 mg/L (Merck), obtaining linear and angular correlation coefficients. The standard of 1.0 mg/L was analyzed 11 times to evaluate accuracy. To assess the recovery method, a standard certified DORM-3® (National Research Council of Canada) sample reference was analyzed.

The blank reading was performed 10 times for the measurement of the limit of quantitation (LOQ) and the limit of detection (LOD) of the instrument, which were in accordance with the IUPAC (1997) [24] recommendation.

Results and discussion

This is the first work that attests to the *Phallocerus caudimaculatus* as an important bio-indicator species of environmental contamination by metals Cd, Cr, Pb and Zn, and this can be seen through the results achieved.

The physicochemical parameters data of the water quality were monitored for 96 h during the tests of acute toxicity and are presented in Table 1. Dissolved oxygen ranged between 5.41 and 7.86 mg/L and the pH varied between 6.38 and 7.11 for the contaminated tanks. For the control tank, oxygen ranged between 5.4 and 7.9 mg/L and the pH varied between 6.4 and 7.5.

In Table 1, you will find that there was a reduction in the concentration of dissolved oxygen in all treatments for all analytes, as well as an increase in conductivity, for all treatments, ranging from 220.4 to 260.1 µS/cm. The increase in conductivity can be the result of an increase of dissolved ions and possibly other substances released by the fish [9]. As this was a static test, i.e. without water renewal, the small variation of these parameters may be due to fish's breathing and excretion, the toxicity may be directly linked to the concentration of the substance of exposure and, in this case, not necessarily, to the physicochemical parameters, unlike what happens in the aquatic environment [8,9].

As for the acute toxicity test, *P. caudimaculatus* has shown resistance to the contaminants tested, with greater sensitivity to lead II nitrate, cadmium nitrate and zinc sulfate, respectively, and higher potassium dichromate resistance, in the following order of metals sensitivity: Pb > Cd > Zn > Cr.

The LC50 results obtained (Table 2) enable the practical identification of contamination intensity in the environment where fish lives given that, if submitted to a level of contamination higher than the concentration obtained by LC50, fish will no longer live at the site. Biomonitoring of this species can contribute to environmental impacts mitigation actions in these environments.

The *P. caudimaculatus* was more sensitive to lead II nitrate than other salt tested in this study. According to Mary et al. (2014) [25] the lead II nitrate is toxic to fish, since it affects the gills, liver and muscle due to complete melting of gill lamellae and edema in the filamentary epithelium. According to Table 2, the value obtained in LC50 (96 h) of the present study was 15.5 ± 0.47 mg/L for $Pb(NO_3)_2$ and 9.70 ± 0.29 mg/L for Pb (table 3).

However, if compared with other freshwater species, the *P. Caudimaculatus* proved more resistant, for example: to the ornamental goldfish which is larger in size than the *P. Caudimaculatus* [26] obtained LC50 (96 h) 5.02 ± 0.54 mg/L for $Pb(NO_3)_2$ to *C. auratus*. For smaller fish such as *Rasbora sumatrana* and *Poecilia reticulate* that were exposed to various metals, both species were more sensitive to copper, cadmium and zinc, respectively, than lead [27].

This demonstrates that different species have different sensitivity to Pb, as observed by Mohanambal and Puvaneswari (2013) [28] also highlighting the influence of factors such as fish's age and water hardness in lead toxicity to fish. Thus, the development of future work on lead II nitrate toxicity tests with *P. caudimaculatus* in different concentrations, and in treatments with different water hardness, pHs and dissolved organic treatments, envisioning greater precision when monitoring the natural environment is of utmost importance, once, this study demonstrated the high toxic potential of this substance.

The LC50 obtained with cadmium nitrate for the *P. caudimaculatus* in our study was 29.47 mg/L, similar to that obtained by

Table 2
Maximum allowable values for Cadmium, Chromium, Lead and Zinc, established by Ordinance No. 1469, 12/29/2000, the Ministry of Health (Brazil, 2000); the concentrations (mg/L) for contaminated and uncontaminated tanks, and their limit of detection (LOD) and quantitation (LOQ).

Metal	Maximum Allowed Value (mg/L) (Ministry of Health)	Uncontaminated Aquarium	Contaminated Aquarium	Limit of Detection (LOD) (mg/L)	Limit of Quantification (LOQ) (mg/L)
Cadmium (Cd)	0.005	nd	0.028	0.007	0.025
Chromium (Cr)	0.05	nd	0.013	0.003	0.010
Lead (Pb)	0.01	nd	0.085	0.021	0.071
Zinc (Zn)	5	+	0.290	0.075	0.250

Subtitle: (+) = Detected; (nd) Not detected.

Table 3
LC50 Data *Phalloceros caudimaculatus* for substances (potassium dichromate, cadmium nitrate, lead nitrate II and zinc sulfate), and their respective metals, Cr, Cd, Pb and Zn, in an acute toxicity test (96 h).

Substance	LC50 (Mean ± SD) (mg/L)	Metal	LC50 (Mean ± SD) (mg/L)
Potassium dichromate ($K_2Cr_2O_7$)	162.5 ± 18.75	Chromium (Cr)	57.49 ± 6.63
Cadmium nitrate ($Cd(NO_3)_2$)	29.5 ± 1.21	Cadmium (Cd)	13.99 ± 0.57
Lead II nitrate ($Pb(NO_3)_2$)	15.5 ± 0.47	Lead (Pb)	9.70 ± 0.29
Zinc sulfate (Z_nSO_4)	62.8 ± 2.81	Zinc (Zn)	14.23 ± 0.63

Yilmaz et al. (2004) [11], of 30.4 mg/L for the *Poecilia* crosslinked, another similarly sized poecilid, which has been used as a bioindicator body for toxicity testing. This sensitivity similarity may be related to the similar size of the bodies. According to Yi and Zhang (2012) [29], the size of the bodies relates to the retention and accumulation of metals such as cadmium, zinc and lead, in most fish species. It can also be related to the similarities between poecilids in terms of ecological need and metabolic activity, since these characteristics can affect metal concentrations in fish [29].

Potassium dichromate was evaluated by Da Cruz et al. (2008) [13] in *Piaractus mesopotamicus*, *Hyphessobrycon eques* and *Phallocerus caudimaculatus*. When compared to other fish species, *P. caudimaculatus* showed greater resistance to potassium dichromate, with LC50 of 154.39 ± 6.72 mg/L, while *Piaractus mesopotamicus* and *Hyphessobrycon eques* obtained LC50 of 144,50 ± 19.67 mg/L and 130.79 ± 10.02 mg/L (salt), respectively. Our results of LC50 162.5 ± 18.75 mg/L for $K_2Cr_2O_7$ and 57.49 ± 6.63 mg/L for Cr obtained with potassium dichromate to *P. caudimaculatus* is similar to the result obtained by Da Cruz et al. (2008) [13] and reinforces the perception that *P. caudimaculatus* can be an effective bioindicator body of environmental contamination.

Compared to Bertoletti (2009) [12], who researched the toxicity of certain substances, including zinc sulfate in zebrafish *Daniorerio* (average weight 0.15 g and LC50 (96 h) 22.0 mg/L), *Poecilia reticulata* (average weight 0.20 g and LC50 (96 h) 28.0 mg/L) and *Hemigrammu smarginatus* (average weight 0.40 g and LC50 (96 h) 8.2 mg/L), the results found in this study was higher, showing that *P. caudimaculatus* with an average mass of 0.14 g and LC50 (96 h) 62.8 mg/L for Z_nSO_4 and 14.23 mg/L for Zn (table 3) has greater resistance to this contaminant. Even though Bertoletti (2009) [12] demonstrated greater sensitivity of some exotic species to the detriment of native species, one should reflect on the use of exotic species because, in addition to the damage involved with their introduction, they may not provide the necessary effectiveness since they are not natural to the environment in question.

During the acute toxicity tests, the fish exposed to the highest concentrations of metals, showed behavioral changes such as near the surface erratic swimming and high opercular beat. These behaviors suggest the occurrence of change in homeostasis, indicating that the test substance may be affecting their physiology through breathing damage [8,10].

The observed LC50 (table 3) are considered high, even for extremely polluted systems. However, the laboratory situation elucidates the tolerance of the species by such pollutants, making possible the presence or absence of the same in their habitat, the identification of environmental pollution by these metals. Moreover, knowing these sublethal levels for the species it is possible to verify if the pollution has exceeded or not such limits, allowing the use of this species as a bioindicator.

The results of metal concentration for the test organisms of the chronic toxicity tests, and the maximum levels of potability allowed by CONAMA – 357, the Ministry of the Environment, [21], are presented in Table 3.

Cadmium, chromium, lead and zinc levels in *P. caudimaculatus* as well as the bioconcentration factor (BCF), which is the ratio between the concentration of a substance from a body and the concentration of this substance in water [8] and values of detection (LOD) and limits of quantification (LOQ) are shown in Table 4. The recovery values obtained from the standard sample certified DORM 3® ranged from 87.5% for cadmium and 98.7% for lead, which is also shown in Table 4.

After three months of exposure, the bioconcentration occurred in the order Zn > Pb > Cd > Cr, and BCF values obtained were: 1793.1 for Zn; 1192.3 for Pb; 982.14 for Cd and 92.35 for Cr. The BCF numbers for these four elements were high, but the bioaccumulation of cadmium and lead should be highlighted due to its high relative concentration found in the fish.

Relating the sensitivity of *P. caudimaculatus* to the tested metals, as measured by the acute toxicity test (Pb > Cd > Zn > Cr) and bioconcentration (Zn > Pb > Cd > Cr), by the chronic toxicity test, lead and cadmium proved to be more toxic to the species. Zinc obtained higher bioconcentration and lower toxicity than these metals. On the other hand, the chromium, bioconcentrated less and was more tolerated by the species than the other metals tested. However, according to McGeer et al. (2003) [30], for a correct prediction of how hazardous are these metals, it should be based on the intrinsic characteristics of each metal, regardless of concentration of exposure. Since it cannot be assumed that the hazard will be reduced as the concentration increases.

Zinc and chromium are trace elements necessary for organisms and, in small amounts, are essential for realization of metabolic processes [8,31]. In excess, they become harmful and toxic to these organisms [8]. However, due to sequestration and retention for essential functions, McGeer et al. (2003) [30], suggest that some animals may control the bioaccumulation of some metals such as zinc and mentions that the adverse effects of zinc are independent of accumulation and may have an inverse relationship of accumulation in tissues when the concentration of exposure increases. Compared with data from McGeer et al. (2003) [30] for fish, with a mean BCF value of 2941 at a concentration of 0.110 mg/L. In this work, the concentration of exposure to zinc was higher, being 0.290 mg/L (table 2) and lower BCF, being 1793.1 (table 3), which corroborates this hypothesis.

Cadmium, has no defined essentiality, and lead does not act on the functioning of biological metabolism, both are considered non-essential and, therefore, highly harmful to organisms [11]. In this work, the exposure concentration of Cadmium was 0.028 mg/L and BCF 982.14, compared to the BCF of 2.623 obtained by McGeer et al. (2003) [30] at a lower exposure concentration of 0.003 mg/L. Despite the limited evidence of regulation of internal Cadmium concentrations, this author speculates that control over Cadmuim accumulation can be achieved, with accumulated Cadmium detoxification being a common process with Cadmium binding to low molecular weight molecules, such as metallothionein that occurs in many animals. Although some species have physiological mechanisms to mitigate the damages. This does not guarantee the protection of the species.

Fish affected by Lead are killed by axficia. According to the FAO (2017) [32], the maximum permissible concentration of lead in water is 0.004 to 0.008 mg/L for salmonids and 0.07 mg/L for cyprinids. According to McGeer et al. (2003) [30], the concentration of 0.015 mg/L obtained BCF of 410 and mentions that all groups

Table 4
Concentration of metals in the control fish, and the fish in the contaminated aquarium in (Mean ± SD) (mg/kg) with their bioconcentration factors, LOD and LOQ for each metal, in chronic toxicity test after three months of exposure.

Metal	Fish Control aquarium	Fish Contaminated aquarium	Bioconcentration factor (BCF)	LOD (mg/kg)	LOQ (mg/kg)	DORM 3® (mg/kg)	Rec (%)
Cd	2.3 ± 0.07	25.5 ± 2.12	982.14	0.094	0.312	0.29 ± 0.020	87.5
Cr	2.5 ± 0.49	7.8 ± 1.48	92.35	0.037	0.125	1.89 ± 0.17	89.4
Pb	nd	15.5 ± 3.54	1192.3	0.268	0.887	0.395 ± 0.050	98.7
Zn	300 ± 56.57	520 ± 70.71	1793.1	0.094	0.312	51.30 ± 3.1	97.3

Subtitle: (DORM 3®) – Amostra padrão de referência certificada pelo Conselho Nacional de Pesquisa do Canadá; Rec (%) – percentual de recuperação; (nd) – Notdetected.

evaluated showed an increase in body concentrations as exposure levels increased. In this work, the concentration used of 0.085 mg/L (table 2), considered high in comparison to FAO [32] data and *P. caudimaculatus* obtained BCF 1192.3, confirming this behavior mentioned by McGeer et al. (2003) [30].

The toxicity of lead to aquatic organisms depends on the solubility of the lead compounds and the calcium concentration. As a result, it is influenced by water quality, with increasing hardness, alkalinity and pH, increasing the precipitation of these compounds, reducing their toxicity in the water column (FAO, 2017) [32]. What did not happen in this work, because the pH was in neutrality. Furthermore, according to Singh and Manjeet, 2015 [18], the toxicity of Lead Nitrate is proportional to concentration and time of exposure, increasing mortality.

The monitoring of these metals in the environment is extremely important. The use of *P. caudimaculatus* as bioindicator of these contaminants has proven to be very efficient through the tests performed. Since these fish are related to all the lower chain in their habitat of origin, they may indicate responses of chronic, accumulative and persistent effects at chain level, in addition to direct effects at individual level, as observed by Lins et al. (2010) [5], which reinforces the importance of its use for such purpose.

The *P. caudimaculatus* lives in healthy natural environment, and survives in environments subject to contamination, such as urban streams, which implies resistance to contaminants, as demonstrated in this work. It is abundant, cosmopolitan and easily obtainable species. Also, it has a short breeding period, easily breed in captivity, and may easily adapt to the laboratory environment. All these characteristics according to Akanksha et al. (2010) [6], makes it an ideal bioindicator organism.

Many exotic species are efficiently used in toxicity tests [12]. However, the use of non-commercial and easy to breed in captivity native species as bioindicators is important to environmental protection, mostly because the introduction of non-native in the natural environment is unwanted, since they damage the local aquatic community due to multiplication and competition for resources. Therefore, it is important to know the biota of the site to be protected, not only in ecological terms, but in their susceptibility to contaminants.

Considering that the contamination of the aquatic environment by metals such as cadmium, lead, chromium and zinc affect both the aquatic biota, and those who depend on it along the food chain [8]. The attesting of *P. Caudimaculatus* as a bioindicator of heavy metals confers environmental protection against them. Whereas, it makes it possible indicate the contaminated sites to resolve the problem.

Conclusion

Based on the experiment conducted in this study, the lead nitrate II was the most toxic salt for the *Phalloceros caudimaculatus* species, followed by cadmium nitrate, zinc sulfate and potassium dichromate respectively. Despite the bioconcentration *P. caudimaculatus* is an efficient bio-indicator of Cd, Cr, Pb and Zn.

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