

Effect of Bioaccumulation of Heavy Metals in *Oreochromis niloticus* Tissues of an Urban Lake in Southern Brazil

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Abstract:

In this study, the effect of the bioaccumulation of metals on *Oreochromis niloticus* tissues was evaluated. Significant Co, Mn and Ni concentrations were found in the lake water near the fertilizer industries. In the sediment was identified the presence of Cd ($4.612 \pm 0.930 \text{ mg kg}^{-1}$), Ni ($46.847 \pm 3.801 \text{ mg kg}^{-1}$) and Zn ($865.534 \pm 89.437 \text{ mg kg}^{-1}$) in concentrations above the probable effect level (PEL). For Cu ($141.963 \pm 5.148 \text{ mg kg}^{-1}$) and Pb ($53.362 \pm 6.621 \text{ mg kg}^{-1}$) concentrations between the threshold effect (TEL) or probable effect (PEL) levels. Regarding *Oreochromis niloticus* tissues, the concentration of metals in the liver was higher than found in muscle. The biomarkers indicated that the hepatic cells of *Oreochromis niloticus* are under oxidative stress, explained by the higher levels of reduced glutathione (GSH), lipid peroxidation (LPO) and protein carbonylation (PCO) when compared to control fish tissues. The set of biomarkers presented in this study contribute to define the effects of the bioaccumulation of metals in *Oreochromis niloticus* tissues.

Keywords: metals; *Oreochromis niloticus*; oxidative stress; urban lake

1. Introduction

Industries and urban environments are sources of contamination of rivers and lakes by potentially toxic metals. In this sense is important to know the level of impact and the risk of exposure to both biota and human populations. The industrial activities are in general the primary anthropic origin of metals to aquatic environment. Additionally, metals can be deposited in the soil and be transported to lakes and rivers mainly by drainage [1]. Potentially toxic metals can contaminate the environment since the generation rate through artificial cycles is higher when compared to the natural cycles [2].

In superficial water, these metals might be associated to colloids, particulate material and in the dissolved fraction. Due to the deposition of organic matter which favors metal concentration, the sediments might accumulate great amounts of these metals [3].

Sediments contaminated through the high concentration of metals might influence the water biota depending on this concentration. The Geoaccumulation index (I_{geo}) shows the level of contamination through metals in sediment in relation to the region background value, indicating the influence of the metal concentration increase due to anthropic action [3–6]. The Environment Canadian Council [7] and the Florida

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Environmental Protection Department [8] established the relation between metal concentration associated with sediment and the potential biological effects, through three levels of contaminant concentration, namely, threshold effect (TEL), possible effect, and probable effect (PEL) levels. The definition for metal bioavailability is given as the maximum amount of contaminant which is available or dissolved in the gastrointestinal tract of an organism [9]. Besides their presence in the environment, metals are bioaccumulated, catalyzing and favoring the unbalance between reactive and antioxidant chemical species present in the cell medium [10].

The use of toxicological tools favors the chemical study, since the cell responses measured in the tissues of aquatic organisms might help the detection of alteration provoked by contaminants such as metals. The combination of some biomarkers such as lipid peroxidation (LPO), protein carbonylation and reduced glutathione (GSH) has been recently used to evaluate the risk of exposure to contaminants including metals [11–13].

The fishes is established as contamination indicators by the contact with water and sediment including its important role in the food chain [14,15] or as vehicle to human exposure. Several studies on the bioaccumulation of metals in fish were published and show the relation importance of contaminated sediment as source of them [16]. Studies also show that metal accumulation in fish from lakes are higher when compared to fish living in rivers. *Oreochromis niloticus* is a species widely distributed and an important source of proteins in world [17,18]. Moreover, due to its resistance to pollution and good response to chemicals, this fish species could also be considered a good candidate to use as a sentinel species in monitoring programs [19]. Studies focused on the effects of bioaccumulation of metals on *Oreochromis niloticus* tissues is scarce. Many studies about this species were directed to the evaluation of food parameters, parasitism and cytogenetic issues [20–25].

The present study uses the species *Oreochromis niloticus* as a multibiomarker to evaluate the impact of contamination by metals in an urban lake in order to evaluate the risk of exposure to biota and human populations.

2. Results and Discussion

2.1. Concentration of metals in the water

The results of analyses of metals in the water are summarized in Figure 1. According to the results among the investigated metals only Co ($0.143 \pm 0.018 \text{ mg L}^{-1}$), Ni ($0.098 \pm 0.006 \text{ mg L}^{-1}$) and Mn ($0.674 \pm 0.0150 \text{ mg L}^{-1}$) were quantified above the technical quantification limit, and dissolved metals Co ($0.133 \pm 0.002 \text{ mg L}^{-1}$), Mn ($0.628 \pm 0.007 \text{ mg L}^{-1}$) and Ni ($0.078 \pm 0.003 \text{ mg L}^{-1}$). The values found for Ni, Mn and Co are above two times the limit (0.025 , 0.100 and 0.05 mg L^{-1} , respectively) established for water class II in resolution CONAMA 430/2011, which establishes water quality standards where fishing occurs or cultivation of organisms for consumption, recreation of primary contact such as swimming, water skiing and diving [26]. These results may be explained by the proximity of the studied site with a fertilizer industry where the concentrations of Co and Mn are closer to the total metals.

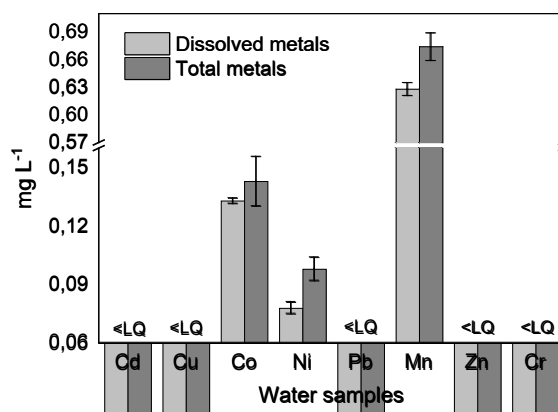


Figure 1. Metal concentration mean in the water of the lake under study.

Ni at trace level is considered a nutrient for the fish. However, in high concentrations it might affect the physiology of organisms due to the oxidative stress induction. According to Palermo et al. (2015) [27], *P. lineatus* exposed to Ni for 96 h increased the levels of lipid peroxidation in liver cells and DNA damages in blood cells.

Mn is an essential nutrient in water, however, the absorption of this metal at high levels is associated to adverse effects, since it acts as an inducer of oxidative stress, as shown by Amaeze et al. (2015) [28] in *Clarias gariepinus* naturally exposed in Nigeria. The same authors described

high levels of lipid peroxidation and other findings that suggested oxidative stress in the liver comparatively with individuals from a reference site.

The *Oreochromis niloticus* presents similar ecological characteristics described to *P. lineatus* and *Clarias gariepinus* as feed behavior (plankton, fingerlings, debris and decomposing organic matter) [29]. These aspects contribute to the metal concentration in the organism, leading cellular oxidative stress, which allows the comparison among the species regarding to the effects due to the metals exposure.

2.2. Metal concentration in the sediment

Table 1 shows the results of metal analyses identified in the sediment of lake. According to the Environment Canadian Council [7] and the Florida Environmental Protection Department [8], Cd, Ni and Zn are the metals values informing sediment quality for the protection of water life proposed by the metals that present concentrations above the probable effect level (PEL) are Cd, Ni and Zn. For Cu and Pb, the concentrations found were between the threshold level (TEL) and the probable effect level (PEL), indicating again the risk of exposure to biota. Co and Mn do not have reference values related to deleterious effects on biota.

Table 1. Mean of metal concentration in the sediment from the studied lake. (Mean \pm SD, n=3).

	Total metals (mg kg ⁻¹)	Bioavailable metals (mg kg ⁻¹)	TEL* (mg kg ⁻¹)	PEL** (mg kg ⁻¹)	Bioavailability (%)	Igeo
Cd	4.612 \pm 0.930	< LQ	0.6	3.5	-	3.9
Co	24.891 \pm 2.578	13.681 \pm 0.251	-	-	55	3.0
Cr	< LQ	< LQ	37.3	90	-	-
Cu	141.963 \pm 5.148	40.578 \pm 0.290	35.7	197	28	5.0
Mn	839.431 \pm 89.437	484.916 \pm 81.115	-	-	58	10.0
Ni	46.847 \pm 3.801	12.927 \pm 0.193	18	35,9	27	5.0
Pb	53.362 \pm 6.621	31.852 \pm 0.293	35	91.3	59	3.6
Zn	865.534 \pm 89.437	473.175 \pm 38.216	123	315	55	5.2

* (Threshold effect level); ** (probable effect level)

The aspects utilized to evaluate the quality of sediments are based on the concentration of total metals. According to the results Co, Mn, Pb and Zn are the most bioavailable of the metals identified in the sediment exceeding in 50% the relation with the total metals.

When comparing the bioavailability results with the ecological risk defined by CCME and FDEP, among the metals with concentration above the PEL value, Cd is the only metal that does not present bioavailable concentration. However, Ni is found in the least bioavailable form suggesting that this metal including Mn do not present risk to *Oreochromis niloticus*, differently that observed to Zn, this a high bioavailable metal indicating risk of exposure. The Cu and Pb concentrations are between the PEL and TEL levels. The Cu presented low bioavailability, while Pb is among the most bioavailable, indicating a high risk of exposure to the biota. The bioaccumulation of Pb in sediment and biota can be explained by the proximity of the lake with highways and railways. In general the urban activities are related with the release of toxic metals presented in lubricants,

fuels, metal displacement few industries along the roads, contributing significantly to contamination of aquatic environments [30, 31]. According to Rzetala (2014) [32] the enrichment of sediments with metals in 20 water bodies located at south of Poland, is related directly with anthropic contamination due to the urban activities.

According to the Geoaccumulation index classification Table 1, Mn and Zn were classified in Class 6, showing extreme contamination to the sediment, while Cd, Co, Cu, Ni and Pb were classified between Classes 4 and 5. Such information reveals the influence of urban anthropic contamination as the levels of metals from a less impacted reservoir were significantly lowers [33]. According to the same authors the soil was classified as non-contaminated to moderately contaminated by Mn and Zn (Classes 1 and 2), while Pb, Cu and Cd presented moderate contamination (class 3).

2.3. Concentrations of metals to *Oreochromis niloticus*

The metal concentrations in *Oreochromis niloticus* from lake studied are presented in the Table 2. The bioaccumulation of metals in the eleven animals of the control group showed levels below the limit of quantification for Cd, Co, Ni, Pb,

Mn, Cr and Cu in muscle and liver. For Zn, mean values of $73.390 \pm 11.101 \text{ mg kg}^{-1}$ were observed in the liver and values below the limit of quantification in the muscle.

Table 2. Mean concentration of metals in muscle and liver of *Oreochromis niloticus*. (Mean \pm SD, n=3).

Liver		Cd	Co	Ni	Pb	Zn	Mn	Cu
Mean		5.783	16.79	10.691	25.796	91.017	20.246	1855.389
SD	1	0.146	0.918	0.167	4.276	1.674	0.222	111.386
Mean		5.33	18.497	11.286	28.688	82.223	7.197	545.54
SD	2	0.018	0.368	1.807	0.004	1.016	0.677	135.591
Mean		5.584	16.426	10.482	22.167	90.506	22.461	669.16
SD	3	0.588	0.78	0.767	3.774	4.838	1.162	0.813
Mean		4.79	16.106	9.581	31.366	119.303	31.132	330.638
SD	4	0.014	0.692	2.447	8.629	10.915	2.069	50.349
Mean		5.32	19.41	12.349	24.989	88.527	9.102	1590.361
SD	5	0.108	0.234	0.07	1.856	3.935	0.37	119.837
Mean		3.899	12.547	9.952	21.019	84.598	4.012	1550.259
SD	6	0.284	0.119	0.795	1.416	2.293	0.213	59.592
Mean		3.521	16.88	7.352	16.534	94.524	13.405	1055.645
SD	7	0.221	0.027	0.587	0.118	14.817	3.415	35.564
Mean		3.38	18.923	10.331	18.959	83.042	5.831	1089.641
SD	8	0.043	0.334	0.463	0.708	2.889	0.379	180.826
Mean		3.725	17.5	9.509	22.204	84.735	14.403	768.162
SD	9	0.936	1.57	1.843	4.381	1.715	0.829	179.848
Mean		5.916	17.543	12.136	31.855	121.357	10.36	986.576
SD	10	0.166	0.295	0.274	1.737	0.565	1.554	4.09
Mean		4.234	12.598	11.201	30.032	119.382	5.182	608.849
SD	11	1.218	2.377	0.189	0.165	6.942	1.194	33.305
Muscle		Cd	Co	Ni	Pb	Zn	Mn	Cu
Mean		< LQ	8.005	5.238	26.152	53.129	< LQ	< LQ
SD	1	< LQ	0.061	0.244	0.345	22.783	< LQ	< LQ
Mean		< LQ	8.251	6.526	26.699	29.896	< LQ	< LQ
SD	2	< LQ	0.54	0.61	1.956	1.07	< LQ	< LQ
Mean		< LQ	8.683	5.105	25.937	33.127	< LQ	< LQ
SD	3	< LQ	1.46	0.088	2.923	1.183	< LQ	< LQ
Mean		< LQ	11.713	5.328	28.816	45.869	< LQ	< LQ
SD	4	< LQ	1.104	0.141	1.518	7.447	< LQ	< LQ
Mean		< LQ	13.405	5.945	30.533	33.404	< LQ	< LQ
SD	5	< LQ	0.401	1.162	0.101	5.567	< LQ	< LQ
Mean		< LQ	12.685	6.278	28.878	36.051	< LQ	< LQ
SD	6	< LQ	0.422	0.253	1.136	8.444	< LQ	< LQ
Mean		< LQ	11.551	6.084	31.283	33.977	< LQ	< LQ
SD	7	< LQ	0.379	2.077	5.184	6.355	< LQ	< LQ
Mean		< LQ	10.927	4.729	28.74	27.46	< LQ	< LQ
SD	8	< LQ	0.087	0.987	2.276	6.034	< LQ	< LQ
Mean		< LQ	11.46	5.488	30.765	20.669	< LQ	< LQ
SD	9	< LQ	0.291	0.367	0.998	1.264	< LQ	< LQ
Mean		< LQ	10.829	5.213	33.634	27.316	< LQ	< LQ
SD	10	< LQ	0.507	0.018	3.66	7.039	< LQ	< LQ
Mean		< LQ	10.492	5.752	32.817	51.89	< LQ	< LQ
SD	11	< LQ	0.255	1.292	2.529	16.332	< LQ	< LQ

Results presented in mg kg^{-1} (dry weight).

Table 2 shows that the Cd, Cu, Ni, Zn and Mn metals are bioaccumulated preferentially in the liver of *Oreochromis niloticus*. However, Pb and Co presented similar findings in muscle and liver. When comparing the concentrations found in the species of the group control and the sampling group, the influence of the urban environment in

the water medium can be noticed. Similar studies [20, 21, 23] found the highest concentrations of the metals under study in the sampling group when compared to the control group. These results indicate that the consumption of this fish might result in health risk due to the exposure to these metals. Pb was the metal that presented the

highest bioavailability for *Oreochromis niloticus*. According to US.EPA, (2000) [34] the value of $0.0108 \text{ mg kg}^{-1}\text{day}^{-1}$ was obtained for the average daily ingestion of Pb considering an adult person weighting 70 kg. From the health adverse effect index resulting from metal ingestion from the relation between the daily ingestion average and the reference dose FAO/WHO [35], the value obtained was 3.050, concluding that the consumption of *Oreochromis niloticus* from the studied lake may present risk of health adverse effects.

Pb might be absorbed by the intestine and stored in bones. The bone is continuously formed and reabsorbed, allowing the Pb^{2+} to circulate in the blood reaching critical targets in nervous tissues and blood forming tissues as liver. Pb in the blood, even if at trace level, is related to growth and hearing impairment, as well problems of mental development [36].

The metal Zn is more related with sediment source than water column, but despite of that the results showed that this metal presents a different way to bioavailability in *Oreochromis niloticus*. Since Zn is among the most bioavailable metals, this might indicate that the presence of Zn in the sediment influences the absorption of this metal by the *Oreochromis niloticus* through food chain.

Lakes present different characteristics from rivers, due to their closed and still system that contribute to increase the concentration of metals in the sediment and consequently its bioavailability [27, 28]. The trend of metal concentration in the muscle was $\text{Zn} > \text{Pb} > \text{Co} > \text{Ni}$ and Cd, Cr, Cu and Mn were below the limit quantification. The concentration of metals in the liver followed the trend $\text{Zn} > \text{Pb} > \text{Co} > \text{Mn} > \text{Ni} > \text{Cd}$ where Cr and Cu were below the limit quantification. The concentrations of metals in the liver were higher than those found in the muscles. The liver is a target organ for xenobiotic and present an important role in the metabolism of organic pollutants [33]. The accumulation of metals in the liver might trigger cell reactions which are responsible to damage in macromolecules and lead to a failure of cellular physiology.

2.4. Oxidative stress in the liver of *Oreochromis niloticus* exposed to toxic

metals

The biochemical effects in liver of *Oreochromis niloticus* are demonstrated in the Figure 2. The levels of GSH is higher in liver of individuals from the studied site if compared with the control group. This finding may explain the activation of antioxidant mechanisms where GSH is an acceptor of reactive species, decreasing the oxidant state of the cell [37–39]. Also the increase levels of GSH may suggest the decrease of P450 activation proteins, by inhibition of the system through direct interaction of metals with enzymes of indirect effect due to a pro oxidant state in the cell. As shown in Figure 2, the *Oreochromis niloticus* presented higher lipid peroxidation and protein carbonylation levels when compared to the control group. These findings are strong indicators of oxidative stress in the hepatocytes. The lipid peroxidation and protein carbonylation are common effects induced by the presence of oxygen reactive species when the antioxidant mechanisms is not efficient, also as an effect of the exposure to toxic metals [38].

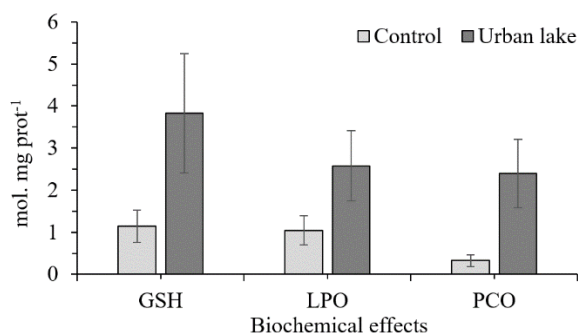


Figure 2. Concentration of reduced glutathione (GSH) mol. mg prot⁻¹; Lipid peroxidation in proteins (LPO) mol [10⁻⁴]. mg prot⁻¹; protein carbonylation (PCO) mol [10⁻²]. mg prot⁻¹.

The current results are in accordance with Palermo et al. (2015) [27], that described experimental studies with *Prochilodus lineatus* exposed to Ni (0.025; 0.250 and 2.500 mg L⁻¹) and described the occurrence of lipid peroxidation in liver after 96 h of exposure. These data suggest that the concentration as found for Ni ($10.59 \pm 0.52 \text{ mg L}^{-1}$) in liver of *Oreochromis niloticus* may explain the increase of LPO and GSH levels. On this way the bioavailability of toxic metals and the generation of reactive species indicate that the origin of effects is in general the oxidative stress in cells from liver or even a failure of antioxidant

mechanism. This hypothesis may be applied also to the bioavailability of other toxic metals as Pb. Currently, Souid et al. (2015) [40] described the oxidative stress in *Sparus aurata* experimentally exposed to Pb (0.75 mg L^{-1}) for 2 h, 4 h and 24 h. The authors showed the increase in the GSH levels after 2 h of exposure and the 35% increase in lipid peroxidation after 24 h. Although these data are not from individuals exposed in field as showed in the presented study, they are important to confirm the relationship between these metals exposure and oxidative stress induction.

The current study add new data related with the bioavailability of pollutants in the studied lake, confirming the studies of Bussolaro et al., (2012) [41] and Miranda et al., (2008) [42]. Additionally, provides evidence of the influence of toxic metals as potent agent to disturb cell physiology even under chronic and field exposure. Probably the bioaccumulation of these toxic metals are the origin of the related damages. Finally, the

monitoring studies must be stimulated in the studied lake in order to understand the geochemical behavior of metals and organic pollutants and establish safety levels permitting fishing activities for example. In this sense, these data and other as mentioned above are the base of a better risk management for the lake.

3. Material and Methods

3.1. Study area

The studied site is located at Ponta Grossa city (Paraná State) in Southern of Brazil (Figure 3). This is an artificial lake, belonging to an entertainment and leisure club, built in the mid-1970s and embedded in a region constantly impacted by agricultural activities, fertilizer industries, soybean processing, highway and railway [42]. Fishing and other entertainment activities are frequent in the lake.

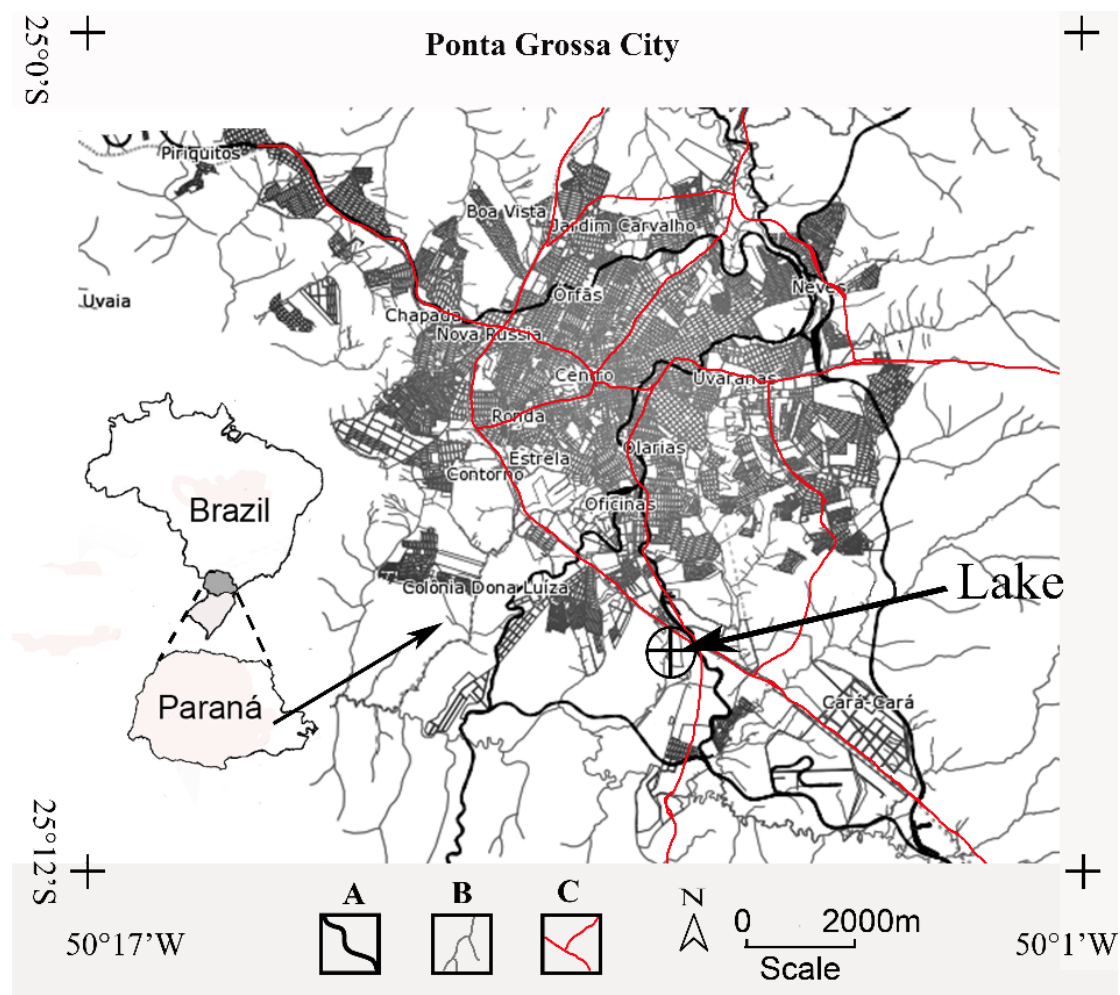


Figure 3. Localization of the studied area, Ponta Grossa Lake-South of Brazil. A: Railways; B: Hydrology; C: Highways.

3.2. Sampling procedures

Water was collected in sampling polyethylene bags and 1 mL 65% nitric acid was added during collection to preserve the sample for metals determination. The samples were stored at 4 °C up to preparation and determination [43,44]. An Eckman-Birge collector was used for sampling the sediments. The material was placed in polypropylene container previously sterilized and stored at -20 °C for later lyophilization until analyses [43,44].

Eleven individuals of *Oreochromis niloticus* were collected, comprising 5 female and 6 male specimens, weighing 600 g on average and transported to Laboratory where the specimens were sacrificed using the anesthetics MS-222 at 0.2% followed by spinal puncture and later excision of liver and muscle for chemical and biological analyses [45]. The tissue samples were preserved at liquid nitrogen, transported to cell toxicology laboratory and stored at -86 °C until analysis. For chemical and biological analyzes, an equivalent number of individuals (control) were obtained from the Institute of Environmental Aquaculture Research (IEAR) of the University of the West of Paraná (UNIOESTE), grown in a controlled laboratory.

3.3. Metal determination

Water samples were submitted to the acid digestion process using the method 3005A [46]. The sediment and *Oreochromis niloticus* samples were submitted to the acid digestion process and the standard method 3050B [47] to determine total metals. For determination of bioavailable metals in sediment, 0.5±0.001 g of sample was acidified with 12.5 mL of 0.1 mol L⁻¹ HCl, followed by stirring at 200 rpm for 2 h. The obtained material was filtered and completed in 50 mL with ultrapure water [48].

To certify the accuracy of the extraction and analyze methods a reference material certified by the European Reference Materials ERM-CE278 (mussel tissue) from the (Institute for Reference Materials and Measurements – IRMM) was used for Cd, Cr, Cu, Mn, Pb and Zn. Additionally, the MESS-2 - marine sediment from the National Research Council of Canada (NRCC) was used.

Metal determination was carried out using a

flame atomic absorption spectrometer (FAAS) (Varian®, AA 240FS), with automatic dilution system, equipped with deuterium lamp as background corrector and multi element hollow cathode lamps. Air-acetylene oxidant flame was employed.

The analytical curve was obtained through the standard solutions, prepared with ultrapure water (water ultra-purification system and reverse osmosis) and High Purity® standards of the following metals: Copper (Cu), Manganese (Mn), Zinc (Zn), Cobalt (Co), Cadmium (Cd), Chrome (Cr), Lead (Pb) and Nickel (Ni) in the 1,0 mg L⁻¹ concentration. The analytical curve was derived from these standards to the points 0.25; 0.5; 0.75 and 1.0 mg L⁻¹. The quantification limits and accuracy are shown in Table 3.

3.4. Biochemical biomarker procedures

The lipid peroxidation (LPO) and protein carbonylation (PCO) was measured with 0.3 g of liver homogenized in buffer Tris-HCl 20 mmol L⁻¹, EDTA 1.0 mmol L⁻¹, pH 7.6, PMSF 1.0 mmol L⁻¹. For the determination, a microplate spectrophotometer, Infinite® 200 PRO – Tecan, was used to read the absorbance at 595 nm [49–52]. Concentration of reduced glutathione (GSH) levels were determined by the addition of 48% trichloroacetic acid in 200 µL of sample for protein precipitation. Subsequently, 50 µL of the supernatant was centrifuged and pipetted into microplate. Then 230 µL was added with 0.4 mol L⁻¹ tris-base buffer, pH 8.9 and 20 µL of 5,5'-dithio-bis-2-nitrobenzoic acid [53]. GSH levels were measured at 412 nm.

3.5. Risk evaluation through *Oreochromis niloticus* muscle ingestion

Aiming at calculating the level of exposure resulting from the consumption of fish contaminated with metals, the mean daily dose (MDD) equation was used (mean daily ingestion of a specific chemical product throughout life) according to US.EPA (2000) [34] :

$$\text{MDD (mg kg}^{-1} \text{ day}^{-1}) = (\text{C} \times \text{IR} \times \text{EF} \times \text{ED}) / (\text{BW} \times \text{AT})$$

Where, C is the metal concentration in the tissue (mg kg⁻¹), IR represents the mean ingestion band (0.0312 - 0.1424 kg day⁻¹ for ordinary

consumers and regular fish consumers, respectively). EF is the exposure frequency (365/days / year), ED is the exposure lifetime (70 years), BW is the consumer weight (70 kg) and AT is the average time (70 years' x 365 days/year). The risk evaluation was estimated through the calculation of the hazard index (HI) which indicates whether there are adverse effects of the metal in the food. This is expressed by the relation between MDD and the Oral Reference Dose (RfD) based on the highest level of metal ingestion for an adult consumer with 70 kg average body

weight:

$$HI = \text{DDM/RfD}$$

where, RfD suggested by the Food and Agricultural Organization and World Health Organization (FAO/WHO) for Pb is equal 0.00357 mg kg⁻¹ day⁻¹ [35]. If the HI is lower than 1.0, it indicates that the health adverse effects are not likely to occur due to tissue consumption. However, if the HI value is higher or equal 1.0, it is assumed that the consumption might result in health adverse effects.

Table 3. Quantification and accuracy of the metals under analysis. (Mean ± SD; n: 3).

		Certified Values (mg kg ⁻¹)	Values found (mg kg ⁻¹)	Recovery (%)	LQ
MESS-2	Cd	0.24±0.01	0.23±0.02	95.8	0.017
	Co	13.8±1.4	14.7±0.01	106	0.03
	Cr	106.0±8.0	97.7±3.5	92	0.033
	Cu	39.3±2.0	42.0±2.7	106	0.04
	Mn	365.0±21.0	338.2±3.2	92.6	0.014
	Ni	49.3±1.8	46.0±1.5	93.3	0.044
	Pb	21.9±1.2	20.0±1.0	91.3	0.24
	Zn	172.0±1.6	171.6±6.5	99.9	0.092
ERMCE278	Cd	0.348±0.007	0.3±0.01	92	-
	Cr	0.78±0.6	0.7±0.02	90	-
	Cu	9.45±0.13	10.0±0.15	106	-
	Mn	7.69±0.23	7.9±0.19	103	-
	Pb	2.00±0.04	1.8±0.05	91	-
	Zn	83.10±1.7	82±2.0	99	-

Results presented in dry weight; LQ: Limit Quantification

3.6. Geoaccumulation index

The geoaccumulation index was calculated using the following equation [54]:

$$I_{\text{geo}} = \log_2 (C_n/1.5B_n)$$

where, C_n is the metal concentration in the sediment and B_n is the metal background concentration [55]. The results for I_{geo} are classified as: Class 0 Uncontaminated ($I_{\text{geo}} \leq 0$). Class 1 uncontaminated to moderately contaminated ($0 < I_{\text{geo}} < 1$). Class 2 moderately contaminated ($1 < I_{\text{geo}} < 2$). Class 3 moderately to heavily contaminated ($2 < I_{\text{geo}} < 3$). Class 4 heavily contaminated ($3 < I_{\text{geo}} < 4$). Class 5 heavily to extremely contaminated ($4 < I_{\text{geo}} < 5$). Class 6 extremely contaminated ($I_{\text{geo}} > 5$) [54].

4. Conclusions

The metal level in lake water was higher than the maximum established in the legislation for

metals Ni, Mn, Co. Copper presented low bioavailability in the lake sediment, while Pb is among the most bioavailable, indicating a potential risk of exposure to living organisms. The values found for the geoaccumulation indices point to the high sediment contamination caused by the intense anthropic activity of the region. The metal concentrations in the *Oreochromis niloticus* tissues of the lake were higher than those found in the fish tissues of the control group. The data observed in the multibiomarker approach applied in this study revealed significant differences in the health of the *Oreochromis niloticus* individuals collected in the lake and the control group. The data suggest that the multibiomarker approach adequately distinguishes the effects caused by metals in *Oreochromis niloticus*. In summary, results obtained from this research can be useful in identifying the source of chemicals released in the environment and their toxic effects in aquatic ecosystems.

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