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Binde Doria, Halina; Voigt, Carmen Lúcia; de Campos, Sandro Xavier; Ferreira Randi, Marco Antonio

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Metal pollution assessment in a Brazilian hydroelectric reservoir: *Geophagus brasiliensis* as a suitable bioindicator organism

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Halina Binde Doria[1]*; Carmen Lúcia Voigt[2]; Sandro Xavier de Campos[2]; Marco Antonio Ferreira Randi[1]

[1]Universidade Federal do Paraná (UFPR), Curitiba, PR, Brasil
Departamento de Biologia Celular e Molecular
[2]Universidade Estadual de Ponta Grossa (UEPG), Ponta Grossa, PR, Brasil
Departamento de Química
*Corresponding author: e-mail: halinaplant@gmail.com,
clvoigt@uepg.br, campos@uepg.br, mafrandi@ufpr.br

ABSTRACT

Vossoroca is a reservoir in the Brazilian state of Paraná. Although it is located near big cities and can be used as a human water supply, it has remained unstudied. Concentrations of toxic metals and arsenic in sediments, water, liver, gills, and muscle of *Geophagus brasiliensis* from the reservoir were analyzed using atomic absorption spectrometry. Histological analyses were performed on the gills and the livers using scanning electron microscopy and light microscopy, respectively. The results showed that Vossoroca sediments were moderately polluted by copper, chromium, nickel and arsenic. Cadmium was above legal limits in the water. Histopathological assessment revealed epithelial alterations in the secondary lamella to be the most common abnormality observed in the gills and necrosis, melanomacrophage centers in the livers. In conclusion, although the reservoir is located in an Environmental Protection Area, it is negatively affected by human activity. Further, *Geophagus brasiliensis* was a suitable bioindicator for metal pollution studies.

Keywords: freshwater fish, reservoir, toxic metals.

Avaliação da poluição por metais em um reservatório hidroelétrico brasileiro: *Geophagus brasiliensis* como adequado organismo bioindicador

RESUMO

Vossoroca é um reservatório localizado no estado Paraná - Brasil. Embora esteja próximo de grandes cidades e possa ser usado para abastecimento de água para população humana, ele permaneceu sem ser estudado. As concentrações de metais tóxicos e arsênico nos sedimentos, água, fígado, brânquias e músculo de *Geophagus brasiliensis* do reservatório foram analisadas por espectrometria de absorção atômica. As análises histológicas foram realizadas em brânquia e fígado por microscopia eletrônica de varredura e microscopia de luz, respectivamente. Os resultados mostraram que os sedimentos de Vossoroca estão moderadamente poluídos pelo cobre, cromo, níquel e arsênico. O cádmio estava acima dos limites legais na água. A avaliação histopatológica revelou alterações epiteliais na lamela secundária como a anormalidade mais comum observada nas brânquias, e necrose e centros melanomacrófagos, no fígado. Em conclusão, O *Geophagus brasiliensis* é um bioindicador adequado para estudos de poluição de metais. Embora o reservatório esteja localizado em uma Área de Proteção Ambiental, ele é afetado negativamente pela atividade humana.

Palavras-chave: peixe dulcícola, metais tóxicos, reservatório.

1. INTRODUCTION

Due to the increase in environmental awareness seen in Brazil and around the world in recent decades, water consumption has become a major societal concern. Because of the desire to improve water quality both to protect aquatic life and to make water available for humans, knowledge of how reservoirs are impaired by human pollution is not only necessary, but vital. The study area of this work is known in Brazil as the Vossoroca Reservoir. It was created in 1949 in Paraná State, Brazil for hydroelectric purposes (IAP, 2009). However, the local human population continues to grow, and with it, the demand for potable water. Thus, the Sanitation Company of Paraná State (SANEPAR) has begun searching for alternative reservoirs for the public water supply (Brembatti, 2014). Because the Vossoroca Reservoir is 50 km away from the capital city, Curitiba, and is near to other cities in the greater Curitiba area, it can be easily converted into a public water supply reservoir.

Metals are considered potentially hazardous and toxic. They tend to accumulate rapidly in the environment and can reach toxic levels in short periods. Arsenic, cadmium, chromium, copper, lead, nickel and zinc are the most common toxic metals found in water and can trigger adverse biological effects in the biota through redox-cycling reactions and free radical generation or bonding to sulfhydryl and thiol groups of proteins (Beijer and Jernelov, 1986; Jaishankar et al., 2104; Valko et al., 2005). Due to human activity, metal bioavailability in the environment has increased to alarming levels (Castro-González and Méndez-Armenta, 2008).

When assessing aquatic environmental metal pollution, chemical analyses of metal concentrations cannot provide sufficient information because they cannot describe the true availability or even the adverse effects of metal mixtures in aquatic organisms (Maceda-Veiga et al., 2012). Therefore, fish are often used as bioindicators because they are considered sensitive; their life cycles are exclusively aquatic and bioaccumulation processes are easily detectable (Blanco et al., 2014; Lowe-McConnell, 1999; Mazzoni and Iglesias-Rios, 2002). *Geophagus brasiliensis*, a fish native to South America, has been found to be sensitive to environmental stressors and has been used as a bioindicator in several field studies (Benincá et al., 2012; Clemente et al., 2010; Osório et al., 2013; Paraguassú et al., 2005; Ruas et al., 2008; Wilhelm Filho et al., 2001), but its sensitivity to different metals has not yet been evaluated. In order to detect disturbances and evaluate both fish health and symptoms of environmental health, histopathological analyses serve as a biomarker that can show the cumulative effects of pollution on organisms chronically exposed (Au, 2004).

Though government monitoring programs for water quality control exist for several Brazilian reservoirs (IAP, 2009), there is little information available on the contaminants in the water, sediment, or fish in these water bodies. For this reason, the objectives of this study were to provide critical information on metal pollution levels in Vossoroca and to evaluate *Geophagus brasiliensis* as a bioindicator species in cases of metal pollution. To achieve this objective, current heavy metal contamination levels in the sediment and water of the reservoir were investigated, and a histopathological examination was performed on the liver and gills of a native fish species (*Geophagus brasiliensis*) that is chronically exposed to these waters. The metals considered in the analysis were copper (Cu), manganese (Mn), zinc (Zn), cobalt (Co), cadmium (Cd), chromium (Cr), silver (Ag), lead (Pb), nickel (Ni), aluminum (Al), and arsenic (As). The results of this study offer unprecedented data on the Vossoroca Reservoir, as well as critical information on the bioindicator species, *Geophagus brasiliensis*.

2. MATERIALS AND METHODS

2.1. Study area and bioindicator organism

The study area is known as the Vossoroca Reservoir. It is part of the upper portion of the São João Basin and is formed as the result of a dam on the São João River. The reservoir is in the city of Tijucas do Sul in Paraná State, Brazil and it is inside the Environmental Protection Area (EPA) of Guaratuba (IAP, 2009). Its geographical coordinates are 25º 52’ S/ 49º 00’ W (Figure 1). It covers an area of 5.1 km^2 and has an average depth of 8 m and maximum depth of 17 m (IAP, 2009). Additionally, a highway (BR-376) linking southwestern Brazil and southern Brazil crosses the reservoir, bringing with it intense traffic that includes many trucks. This activity is a potential source of pollutants and creates the risk of toxic substance spills. Because it is considered a small reservoir, an area next to the road that cuts through the reservoir and that is thought to be the main source of water contamination by toxic metals was chosen for the data collection and is identified as VOS in Figure 1.

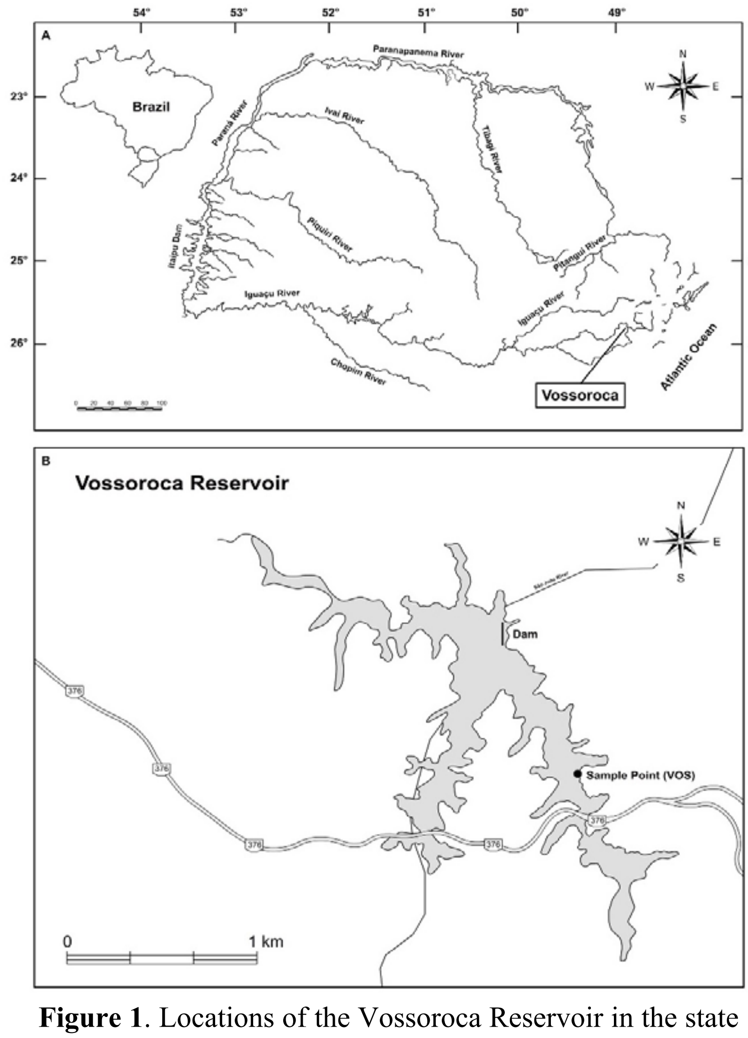

Figure 1. Locations of the Vossoroca Reservoir in the state of Paraná, Brazil; VOS - the sample site of the study.

The fish used as a bioindicator organism in this study was *Geophagus brasiliensis* (Perciformes, Cichlidae). It is one of the most abundant species in lentic aquatic ecosystems. It has omnivore benthopelagic habits, which recruits both bioavailable metals into the water column and in the sediment (Hauser-Davis et al., 2010) and has the key characteristics of an ideal bioindicator (Li et al., 2010; Mazzoni and Iglesias-Rios, 2002; Paraguassú et al., 2005).

2.2. Experimental design

Twenty-eight *G. brasiliensis* specimens were captured with cast nets and were anesthetized with MS-222 (1 g L^{-1}) and euthanized by spinal cord section. Average weight and average total length of the fish were found to be 23 g and 11.40 cm, respectively. For the histopathological analysis, the second right gill arch and a liver fragment were sampled. Muscle, liver and gill samples were stored at -80ºC. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Water and sediment samples were collected in triplicate according to the United States Environmental Protection Agency (USEPA, 2001) and were kept frozen at -20ºC.

2.3. Chemical analysis

Acid digestion for water, sediments and tissues samples were performed according to USEPA (1992; 1996). Arsenic levels were determined using an atomic absorption spectrometer (Varian®, AA 240Z) with electro thermal atomization in a graphite oven (model GTA 120) equipped with a transverse Zeeman corrector for background correction. A hollow cathode lamp was used for the procedure. In addition, argon was used as inert carrying gas with a 0.3 L min^{-1} flow. Pyrolytically-coated graphite tubes were used in all of the procedures.

The concentrations of the remaining metals were determined using a flame atomic absorption spectrometer (Varian®, AA 240FS) equipped with multi-element hollow cathode lamps, and with a deuterium lamp as a background corrector. In the case of Al, the flame used was a nitrous oxide acetylene flame with a flow of 10.24 L min^{-1} for the Al flame and of 6.95 L min^{-1} for the air oxidant acetylene flame. When the other metals were analyzed, the flame used was an air oxidant acetylene flame with a flow of 13.5 L min^{-1} for the air and of 2.00 L min^{-1} for the acetylene. Standard solutions were prepared with ultrapure water (reverse osmosis and a Gehaka® water filter), and the stock solutions were prepared with 1000 mg L^{-1} Qhemis High Purity® metals. The analytical curve was derived from these standards.

The bioconcentration factor (BCF) is the result of absorption, distribution and elimination of substances after an organism's exposure to contaminated water (Van Leeuwen and Vermeire, 2007). In the current study, this ratio was calculated using the metal found in the fish tissue and was expressed as µg g^{-1} dry weight, divided by the metal found in the water, expressed as µg mL^{-1}. The bioaccumulation factor (BAF) is used to determine the tendency of a given compound or element to accumulate in an organism as a result of its exposure to food or sediment (Van Leeuwen and Vermeire, 2007). This ratio was calculated using the concentration of metals found in the fish tissue divided by the concentration of metals found in the sediment, both of which were expressed as µg g^{-1} (Lau et al., 1998).

2.4. Histopathology

Liver samples were preserved in FAA fixative (ethanol 80%, formaldehyde 40%, and glacial acetic acid 5%) for 16 hours. After dehydration with a graded ethanol series, the samples were purified in xylene and embedded in Paraplast-Plus resin (Sigma). Sections were cut to 3–5 µm in length, prepared in a Leica RM2145 microtome, and stained with hematoxilin and eosin/floxin. The second right gill arch was fixed in a Karnovsky solution (glutaraldehyde 2.5%; paraformaldehyde 4% and cacodylate buffer 0.1 M) at 4ºC. The tissue was dehydrated with an ethanol series before critical point drying was applied using liquid CO2. The dried tissue was metalized with gold and examined under a JEOL JSM-6360LV scanning electron microscope. Histopathological assessment in liver and gills were performed by examination of lesions described by Bernet et al. (1999) and were expressed in relative frequencies.

3. RESULTS AND DISCUSSION

3.1. Vossoroca sediments are moderately polluted by metals and arsenic

Concentrations of the metals and of arsenic measured in the sediments are provided in Table 1. Aluminum exhibited the highest concentration, followed by Mn> Zn> Cr> Cu> Ni > Co> Pb> Ag> As> Cd. Bioavailability of the metals revealed the following decreasing order of metal concentrations: Al> Mn> Cr> Co ~ Cu> Pb> Zn> Ni> Ag> Cd> As.

Four of the eleven metals analyzed herein - Cr, Cu, Ni and Cd - and As are present in Vossoroca Reservoir above national or international limits (CONAMA, 2012; CEQG 2014; MacDonald et al., 2000; Thomas, 1987). As metal distribution in sediments provides a record of the spatial and temporal pollution history of an ecosystem (Alloway, 2013), this signifies that, although the reservoir is inside the Guaratuba EPA, it has been suffering from the consequences of human activity for a considerable amount of time.

The Al element was the most abundant metal in sediments, but was not found in either the water samples or any fish tissues. This is likely due to the physicochemical parameters of the water such as the near-neutral pH (Camargo et al., 2009). Metals in sediments are being continually made bioavailable to and are in constant exchange with the water column; thus, concentration levels in the two compartments vary significantly (Camargo et al., 2009; Ferreira et al., 2010).

Table 1. Analysis of metal and arsenic concentrations in sediments, water, and fish tissues.

	Sediments		Water	Fish Tissues		
	Bioavailable	Total	Total	Liver	Gills	Muscle
Cu	1.68±0.08	28.85±0.61	0.0034±0.0002	28.44±1.05	3.65±0.61	14.09±0.53
Mn	21.03±0.46	267.08±0.52	0.0093±0.0002	18.61±0.86	34.22±2.46	13.18±1.07
Zn	1.06±0.07	51.89±1.11	0.0035±0.0002	45.63±0.59	24.90±1.45	16.82±0.78
Co	1.69±0.11	19.77±0.84	0.0020±0.0001	*	*	*
Cd	0.025±0.01	1.91±0.05	0.0014±0.0001	*	*	*
Cr	2.71±0.04	34.04±0.55	0.0162±0.0004	*	*	*
Ag	0.52±0.09	4.30±0.42	0.0057±0.0001	14.32±0.72	17.48±1.36	4.51±0.41
Pb	1.52±0.08	19.44±1.27	*	11.42±0.42	4.63±0.48	1.65±0.12
Ni	0.69±0.02	23.20±0.75	0.0058±0.0004	9.93±0.33	6.61±0.34	2.78±0.32
Al	68427.35±104	713.95±63.90	*	*	*	*
As	0.14±0.002	3.91±0.05	*	1.19±0.02	0.55±0.03	*

Note: Sediment and fish tissue results are expressed as mg/kg dry weight; water results are expressed as mg/L. All values are expressed as means ± standard error of the mean.

* indicates a result below method's detection limit.

3.2. *G. brasiliensis* may be able to decrease the residual time of Cd in tissues

Water from Vossoroca Reservoir had an average pH of 6.7. Concentrations of metals and arsenic in water samples and metal accumulation in *Geophagus brasiliensis* tissue samples are presented in Table 1. In water, Cr had the highest concentration, followed by

Mn> Ni> Ag> Zn> Cu> Co> Cd. Cd and Cr were neither bioaccumulated nor bioconcentrated in any of the tissues extracted from *G. brasiliensis*.

Cd concentration was higher than the legal limits (CONAMA 357, 2005), but its presence was not detected in any of the three tissues analyzed. It is known that not all metals taken up by organisms are bioaccumulated; fish can, to a certain degree, regulate metal concentration in tissues (Maceda-Veiga et al., 2012; Pourang et al., 2005), but Cd is one of the most toxic metals, as it is nonessential, and can quickly bioaccumulate in fish (Liu et al., 2011). Even though metallothioneins tend to detoxify nonessential elements (Hansen et al., 2007), their excretion is a slow process (Besirovic et al., 2010). Accordingly, a previous study by Calza et al. (2004) also demonstrated the absence of Cd accumulation in *G. brasiliensis* tissues. Therefore, the results suggest that *G. brasiliensis* may not bioaccumulate cadmium, thus raising the possibility that it is not a good bioindicator organism for the study of this specific metal.

Similar results were found for Cr, which was the most concentrated metal in the water and which was found above USEPA limits in the sediment; however, it was below detection limits in the fish samples. Nevertheless, unlike Cd, Cr is an essential element and is required for the metabolism of sugar and fat (Anderson, 1997; Lall, 2002). Still, both situations provide evidence that measuring metal concentrations either in water or sediment does not provide information on the risk posed by metal bioaccumulation to the biota.

3.3. Behavioral and dietary habits of *G. brasiliensis* greatly influence the bioaccumulation of metals and metalloids

Bioaccumulation and bioconcentration factors are shown in Table 2. The liver was found to have greater affinity for metal and arsenic bioconcentration when compared with muscle and gill tissues. A higher BCF and BAF of Mn and Ag was found in the gills. Muscle tissue did not exhibit elevated bioaccumulation of metals when compared with the other tissues analyzed. All metal concentrations in the muscle tissues were below the limits established by Brazilian law (ANVISA, 1965; 1998). However, Pb concentration in the muscle tissues was found to be above the limit established by the European Commission, - 0.5 mg kg^{-1} dry weight (EC, 2006).

Table 2. Bioconcentration factors (BCF) and bioaccumulation factors (BAF) in *G. brasiliensis* tissues.

	BCF			BAF		
	Liver	Gills	Muscle	Liver	Gills	Muscle
Cu	8364.70	1073.53	4144.12	0.985	0.126	0.488
Mn	2001.07	3679.57	1417.20	0.069	0.128	0.049
Zn	13037.14	7114.28	4805.71	0.879	0.479	0.324
Co	0	0	0	0	0	0
Cd	0	0	0	0	0	0
Cr	0	0	0	0	0	0
Ag	2512.28	3066.66	791.23	3.330	4.065	1.049
Pb	-	-	-	0.587	0.238	0.085
Ni	1712.07	1139.65	479.31	0.428	0.285	0.119
Al	-	-	-	0	0	0
As	-	-	-	0.304	0.141	0

Note: - indicates error when divided by zero.

Pb and As were not detected in the surface waters, but Pb was found to have bioaccumulated in all three of the tissues analyzed and As was found in the gills and liver of *Geophagus brasiliensis*. These results are likely a consequence of the fish's behavior. The species is largely dependent on sediments; its feeding habits are based on sediment foraging (Lowe-McConnell, 1999; Mazzoni and Iglesias-Rios, 2002; Moraes et al., 2004) and it has an omnivorous diet that ranges from algae and seeds to small animals such as crustaceans, mollusks, and aquatic insects (Gomiero and Braga, 2008; Moraes et al., 2004). It also builds nests on the slope of the reservoir to protect its offspring (Suzuki and Agostinho, 1997). It is important to emphasize that sediment-associated metals pose a direct risk to detrital and deposit-feeding benthic organisms and may represent long-term sources of contamination in higher trophic levels (Mendil and Uluozlu, 2007). Finally, this result highlights the fact that metals and arsenic can accumulate in fish both through water and through the direct contact of fish with sediments, and emphasizes the difficulty of comparing metal accumulation between the same tissues extracted from different species due to the ecological particularities of each species (Demirak, et al., 2006; Mendil and Uluozlu, 2007).

3.4. Essential metals involved in detoxification processes were the most bioaccumulated metals

It is likely that the high degree of bioaccumulation of Zn, Mn and Cu are linked to their intrinsic metabolic roles (Maceda-Veiga et al., 2012). These metals are involved in the functions of several enzymes and are considered essential metals. They are mainly involved the regulation of key enzymatic detoxification processes such as superoxide dismutase and metallothioneins (Agtas et al., 2007).

G. brasiliensis organs expressed affinity for metals at different levels. The liver had the highest metal bioaccumulation of the three tissues analyzed, followed by the gills, which is consistent with other studies on the differences in metal accumulation in fish tissues (Jezierska and Witeska, 2006; Maceda-Veiga et al., 2012; Papagiannis et al., 2004; Pyle et al., 2005; Rashed, 2001; Wei et al., 2014). It also reinforces existing evidence that, because the liver reflects metal bioavailability in both the water and sediments, it is an important target organ for detecting bioaccumulation processes (Jezierska and Witeska, 2006; Karadede et al., 2004).

3.5. Exposure to toxic metals induced histopathological damages in liver and gills

Liver histopathology revealed few abnormalities with low relative frequencies (Table 3 and Figure 2 A-D). Necrosis, the most frequent alteration observed in liver, manifests as irreversible tissue lesions that are closely linked to chronic exposure to pollutants, including metals such as Pb, Cu, Mn and Ni (Bernet et al., 1999; Costa et al., 2009; Mela et al., 2013; Rabitto et al., 2005; Van Dyk et al., 2009; Benincá et al., 2010). Cell death associated with this type of injury not only induces an inflammatory response, but also decreases the number of functional cells in the tissue, a process that has deleterious consequences for organ function (Akaishi et al., 2004). Moreover, because MMCs play a role in cleaning and particle elimination and appear to be sites of inflammation (Balamurugan et al., 2012), a high incidence of MMCs, as was observed in this study, may be a sensitive indicator of impaired fish health and stressful environmental conditions (Agius and Roberts, 2003; Balamurugan et al., 2012; Camargo and Martinez, 2007; Hinton et al., 2008).

Table 3. Frequency of morphological changes observed in liver and gills of *Geophagus brasiliensis.*

Relative Frequencies (%)			
Liver		Gills	
Necrosis	70	Epithelial Alteration	100
MMCs	60	Desquamation	50
Cholestasis	30	Necrosis	25
Infiltration	20	Lamellar Fusion	25
Hemorrhage	0	Neoplasia	12.5
Steatosis	0	Hyperplasia	12.5
Tissue Differentiation	0	Aneurysm	0
Granulomatosis	0	Parasites	0
Necrosis	70	Epithelial Alteration	100
MMCs	60	Desquamation	50
Cholestasis	30	Necrosis	25

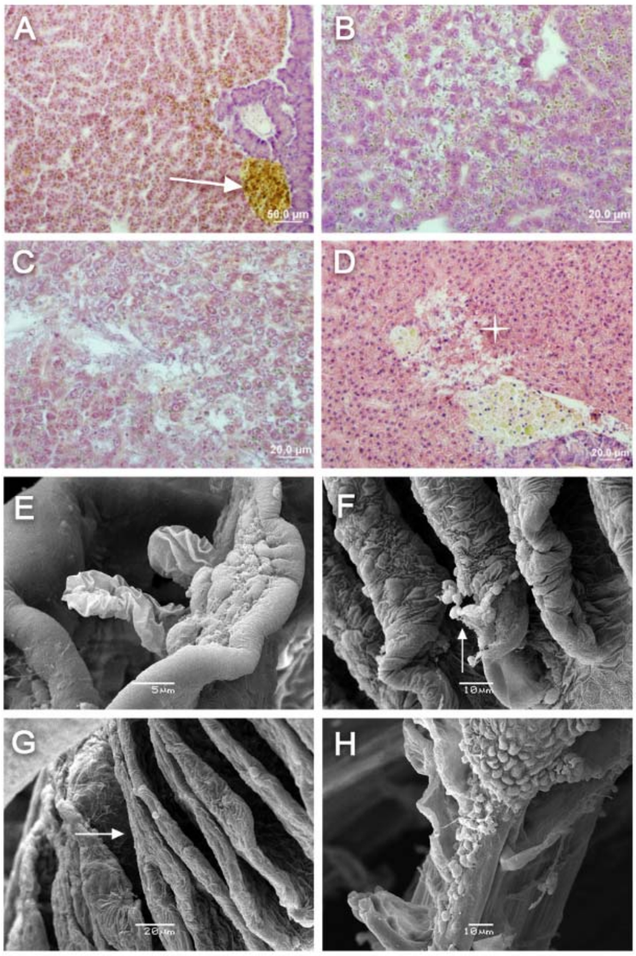

Figure 2. Histopathological alterations found in the liver of *Geophagus brasiliensis* (A-D); (A) Cholestasis and MMCs (arrow); (B) Cholestasis; (C) Onset of necrosis and cholestasis; (D) Necrotic area (star) and MMCs. Histopathological alterations found in the gills of *Geophagus brasiliensis* (E-H); (E) Cell projections in secondary lamella. (F) Epithelium desquamation in secondary lamella (arrow). (G) Lamellar fusion (arrow). (H) Necrotic area of primary lamella and loss of secondary lamellae.

Intrahepatic cholestasis was another frequent alteration observed in *G. brasiliensis* specimens from the Vossoroca Reservoir. The presence of intrahepatic cholestasis is not well understood in aquatic organisms due to the low frequency of its observation in ecotoxicological studies (Safahieh et al., 2011; Wolf and Wolfe, 2005). This situation can cause membrane damage due to detergent action of bile salts; it may lead to cell death either by apoptosis or by necrosis (Biagianti-Risbourg et al., 1998; Jaescheke et al., 2002). Some studies have associated exposure to metals with the onset of cholestasis in the livers of different fish species (Ayas et al., 2007; Jaescheke et al., 2002; Moreira et al., 2014; Safahieh et al., 2011). Therefore, it is possible that the relatively high frequency of necrosis is a result of both direct damage produced by chronic exposure to the metals and indirect damage to the membrane caused by bile salts, with subsequent cell death.

Several morphological alterations were observed in the gills of *Geophagus brasiliensis* (Figure 2 E-H) and are expressed in their relative frequencies in Table 3. The gills are a highly vascularized and the first tissue to contact bioavailable pollutants in water. For these reasons, they are vulnerable to histopathological alterations and may even reflect early deleterious effects of xenobiotics present at low concentrations (Pawert et al., 1998). In the current study, the three most frequent histopathological disturbances observed were epithelial changes in the secondary lamella, desquamation, and necrosis, alterations induced by high concentrations of metals in water (Pandey et al., 2008). These are regressive changes that culminate in the functional impairment of the organ (Bernet et al, 1999; Biagini et al., 2009), directly reflecting physiological difficulties in gas exchange between the gill epithelium and the aquatic environment.

Correspondingly, *G. brasiliensis* chronic exposed to environments where deleterious anthropogenic activities take place exhibited the same lesions observed in the present work (Benincá et al., 2010; Osório et al., 2013). These changes in liver and gills also show that the species chosen for the study is sensitive to low concentrations of metals and is therefore a suitable bioindicator for evaluating toxic metal pollution in lentic aquatic ecosystems in South America.

3.6. Chemical analysis and biological data corroborate Vossoroca Reservoir as impaired by anthropogenic activities

It is understood that the Vossoroca Reservoir is impaired by human activity. Therefore, more attention should be given to this ecosystem, and regular and long-term monitoring of the lake and its fish species is essential. The results of this study show that even within the legal limits, arsenic and some metals can still have negative effects on the aquatic life. This information can assist authorities in decision-making processes in their attempts to protect local wildlife and improve water quality; ideally, this information will ultimately make the water viable for human use.

This conclusion was possible only because of the combination of analyses: metal concentrations in the water, in the sediment and in different fish tissues, and data on the biological effects on fish.

4. CONCLUSIONS

This is the first study conducted in the Vossoroca Reservoir in Brazil, and although the reservoir is in an environmental protection area, concentrations of Cd in water, and levels of Cr, Cu, Ni, Cd and As in sediment and Pb in fish muscle were above the limits established by Brazilian law and some international parameters. It must therefore be noted that chronic exposure to the conditions found in the reservoir modifies the basic morphology of target organs, such as the liver and gills, as was seen in *G. brasiliensis*. Those alterations may impair the proper functioning of these tissues**.**

The current study also offers important information on the response to stressors that a suitable bioindicator and a native fish species, *Geophagus brasiliensis*, may exhibit as a result of chronic exposure to metal contaminants. Thus, only through a careful review of the current Brazilian legislature on legal thresholds for toxicants can the water in this reservoir become safe, both for the local biota and for human consumption. Legal limits that are more consistent with international standards and that are then enforced in the country will result in a larger variety of potable water sources.

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