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Effects of a Dam Reservoir on the Distribution of Heavy Metals in Two Chilean Native Freshwater Fish Species

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Abstract In order to determine the effect of a dam on metal concentrations in riverine fish species, we studied fish inhabiting the influent (Cachapoal River) and effluent (Rapel River) of the Rapel Reservoir in central Chile. Heavy metals were quantified in gills, liver and muscle of the catfish *Trichomycterus areolatus* and the silverside *Basilichthys microlepidotus*. Also, the bioaccumulation index (BAI) was estimated by considering heavy metal concentrations obtained from water and sediment. Results showed the presence of Al, Cu, Fe, Mn, Pb and Zn in the fish organs. The analysis showed high metal concentrations in catfish inhabiting the influent compared to those collected in the effluent. These results indicate a possible filter effect of the dam for most of the metals identified in the fish organs, because metal concentrations decreased in the effluent. Finally, catfish exhibited a larger BAI for most metals analyzed.

Keywords Heavy metals · Dams · Fish · Bioaccumulation index · Fish organs · Catfish (*Trichomycterus areolatus*) · Silverside (*Basilichthys microlepidotus*)

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Pollution has played one of the most important roles in water quality degradation, increasing mortality, decreasing fertility and changing the behavior of biota (Weis et al. 2001). Heavy metals are important pollutants in fluvial environments. Heavy metals can accumulate in the water, sediments and fauna of fluvial environments, and are subsequently transferred to humans through the food chain. Thus heavy metal pollution has become an important worldwide concern, not only because of the threat to biota, but also due to the health risks associated with fish consumption.

Muscle has been the main fish organ studied in order to survey human consumption (Fariba et al. 2009), however few studies have focused on the accumulation levels of heavy metals in other organs (Visnjic-Jeftic et al. 2010). Although diversity in source pathways (e.g. water or sediment) and living habits (e.g. pelagic or benthic) often affect metal uptake and regulation rates among fish species, few attempts have been made to probe the relationship between metals in fish and their surrounding environment, and to apply this correlation to verify the routes of metal transmission. Considering that water movement can release metals towards the water column, the presence of barriers to water flow leads to the accumulation of particulate matter to which trace elements are bound (Dauta et al. 1999). Thus when reservoirs release large amounts of water, the heavy metals become waterborne and are transported downstream (Lapaquellerie et al. 1995), becoming an additional source of heavy metal contamination. Also, sediment-associated metals pose a direct risk to detritus and may potentially be deposited in benthic fishes, which may also represent a long-term source of contamination since metals in sediment can be released into the water (Luoma 1983; Copaja et al. 2014).

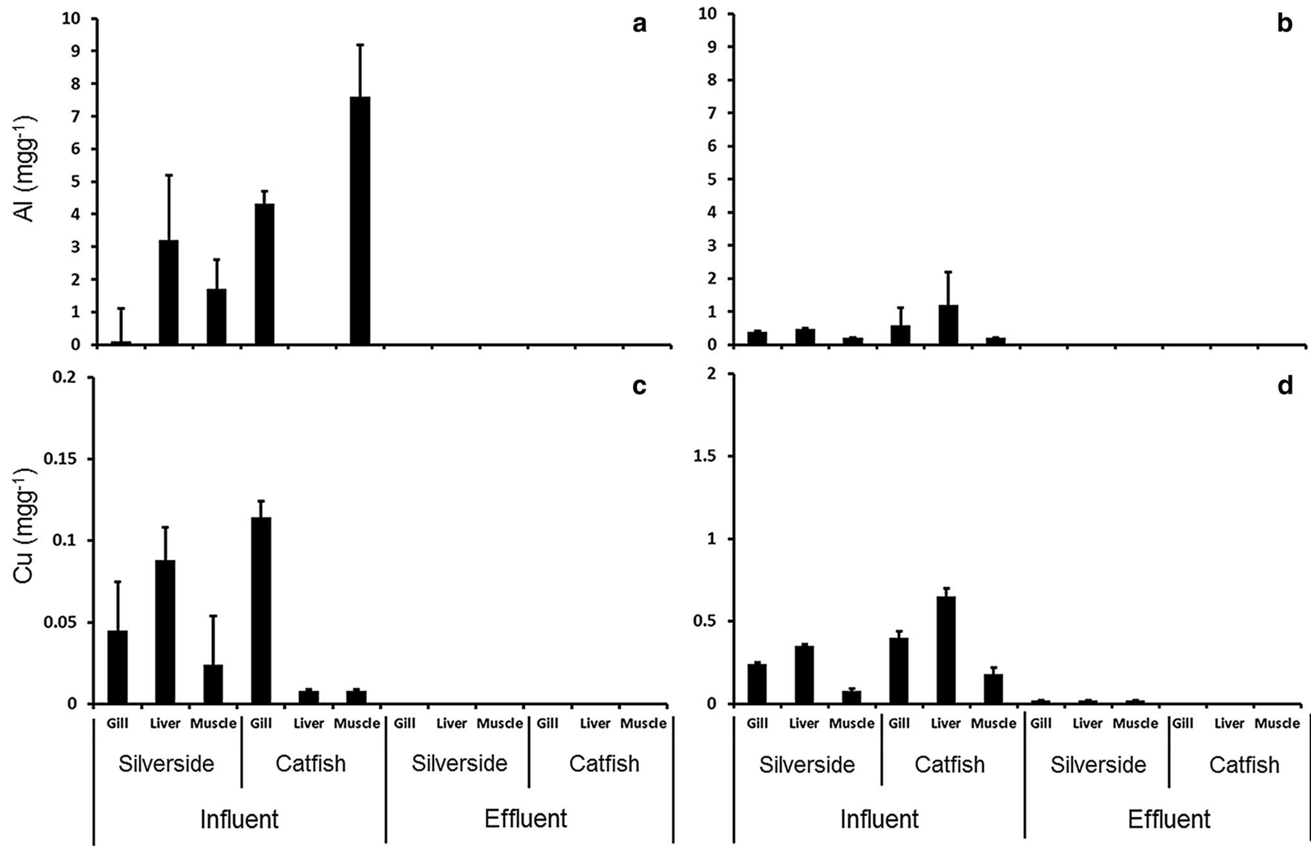

Fig. 1 Content of Al and Cu in the catfish, *T. areolatus* and the silverside, *B. microlepidotus* obtained from the influent and the effluent of Rapel reservoir, Cachapoal River. **a** and **c** high flow, **b** and **d** low flow season

The main goal of our study was to measure the concentrations of heavy metals in gills, liver and muscle of the benthic native catfish *Trichomycterus areolatus* and the pelagic native silverside *Basilichthys microlepidotus* on both sides of the reservoir; both are endemic and threatened species (Quezada-Romegialli et al. 2010; Veliz et al. 2012). Finally, the BAI was used to relate metals in fish to those in the water and sediments on both the inflow and outflow of the Rapel Reservoir.

Materials and Methods

The study sites were the Cachapoal River (influent) and the Rapel River (effluent) of the Rapel reservoir (33°53′S; 35°01′W). Three sites within each river were sampled during the winter of 2010 (high water flow) and the summer of 2011 (low water flow). Catfish sampled were as follows: during high flow season, four specimens were sampled from the influent. During the low flow season, eleven and fifteen individuals were sampled from the influent and the effluent, respectively. In the case of the silverside, four and twelve individuals were sampled from the influent during the high and low flow season, respectively. Fish were captured using an electrofishing device, euthanized with 100 mg L^{-1} tricaine methanosulfonate and stored at −20°C. Gills, liver and a piece of muscle were dried in an oven at 40°C to constant mass. These dried organs (0.25 g) were digested in a high resolution microwave (Marsx Press) (EPA Method 3015) with 10 mL 65 % Suprapur HNO_3 (Merck); digested samples were diluted 10 times with Milli-Q Millipore deionized water and stored in polyethylene plastic containers for analysis. The heavy metals analyzed in fish, water and label fraction of sediment, were Al, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn. Standard solutions for heavy metals were prepared with Titrisol 1000 mg L^{-1} (Merck) and determined using an atomic absorption spectrophotometer (AAS, Shimadzu 6800). The quality was controlled with certified standard references for water (ERM-CA615) and sediment (BCR-320R). Reference material (DOLT-4) was used to validate results.

The Bioaccumulation Index (BAI) (see Zhu et al. 2004) is expressed in terms of the ratio between the amount of a pollutant in a living organism and the concentration in the habitat. This index is obtained by the following equation:

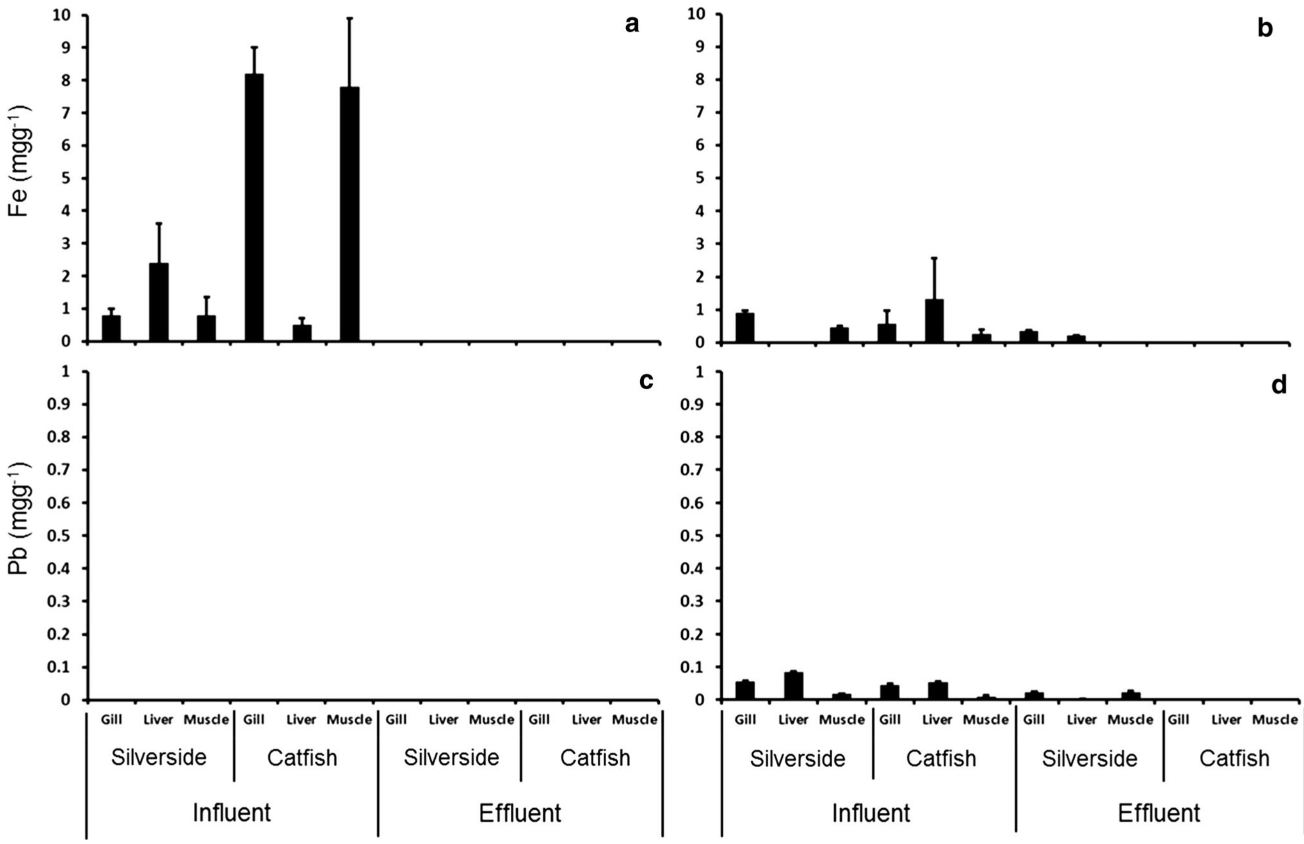

Fig. 2 Content of Fe and Pb in the catfish, *T. areolatus* and the silverside, *B. microlepidotus* obtained from the influent and the effluent of Rapel reservoir. **a** and **c** high flow, **b** and **d** low flow season

Metal in fish (mg kg^{-1})/Metal in sediment + water (mg kg^{-1}). To estimate the BAI, the concentration of metals in surface water, interstitial water and the sediment labile fraction was quantified in the same sites (six samples per area and time period). The metal concentrations from the interstitial water (IW) and the surface water (SW) were quantified following the protocols described by Golterman et al. (1978) and measured with an atomic absorption spectrophotometer (Shimadzu). For the analysis of the pelagic silverside, the BAI was estimated using metal concentrations obtained from IW and SW. For the benthic catfish, the BAI was estimated using LFS and IW. See "Appendix" for information about metal concentration in surface water, interstitial water and labile fraction of sediments used for this BAI calculation.

Results and Discussion

Sampling showed that both fish do not inhabit both sides of the Rapel reservoir. While catfish were found in the influent and the effluent, silversides were only found in the influent. Silversides were described 15 years ago in the effluent area (Vila et al. 2000); however no specimen of this species was found in six additional field trips.

Seven out of the ten metals were detected in the fish, namely Al, Cu, Fe, Mo, Mn, Pb and Zn.

Aluminum This metal was detected in all organs of both species from the influent in the high flow period. In catfish, the highest concentration was detected in muscle (7.39 mg g^{-1}), while at low flow the highest concentration was found in liver (1.86 mg g^{-1}). In silverside, the highest concentration was found in the liver in the influent during the high flow period (3.18 mg g^{-1}) (Fig. 1a, b).

Copper This metal was detected during the high flow period in both species. In catfish, the highest concentration was detected in gills (0.11 mg g^{-1}) in samples obtained from the influent of the Rapel Reservoir. In low flow, catfish liver tissue showed higher concentrations in the influent (0.66 mg g^{-1}). Copper concentration was greater in the liver of silverside, in high flow than low flow (0.10 and 0.35 mg g^{-1}, respectively) (Fig. 1c, d).

Iron This metal was detected in both species. In catfish, the highest concentrations were measured in gills (8.26 mg g^{-1}) and muscle (7.92 mg g^{-1}) in the influent during the high flow period. In low flow, the highest

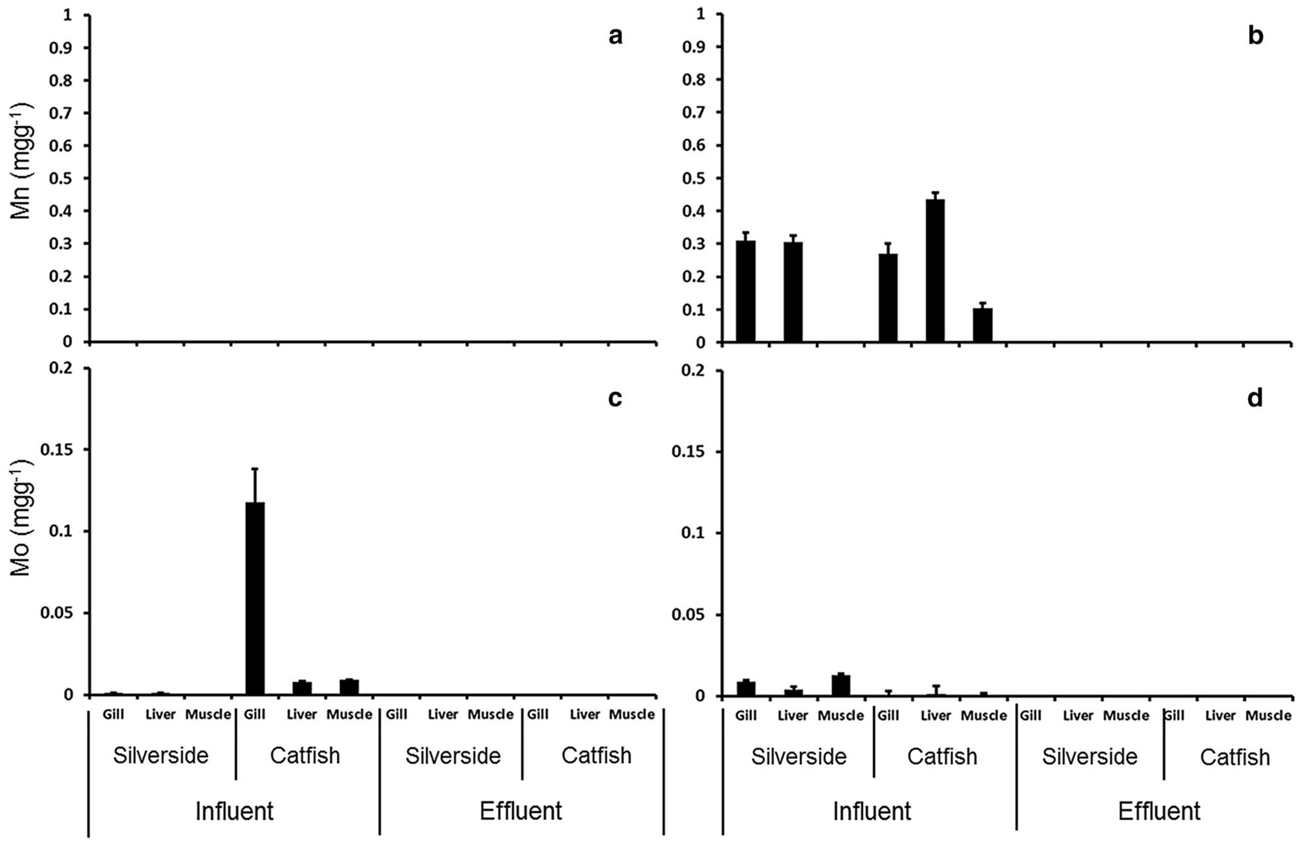

Fig. 3 Content of Mn and Mo in the catfish, *T. areolatus* and the silverside, *B. microlepidotus* obtained from the influent and the effluent of Rapel reservoir. **a** and **c** high flow, **b** and **d** low flow season

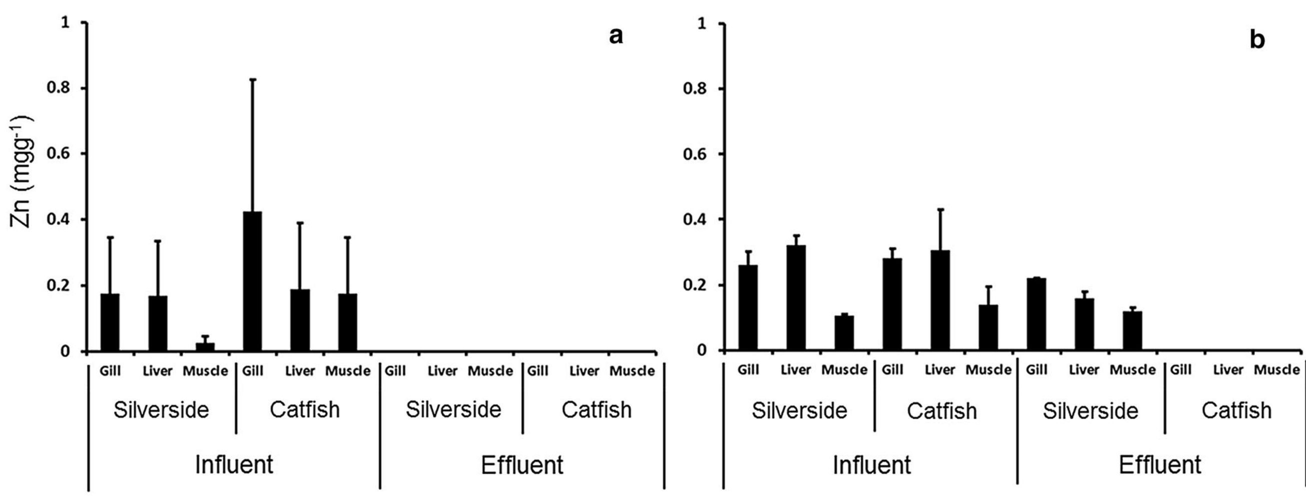

Fig. 4 Content of Zn in the catfish, *T. areolatus* and the silverside, *B. microlepidotus* obtained from the influent and the effluents of Rapel reservoir, Cachapoal River. **a** high flow, **b** low flow season

concentration was measured in liver (1.33 mg g^{-1}). For silverside, the highest value was found in liver (3.43 mg g^{-1}) and gills (0.94 mg g^{-1}) during high respectively (Fig. 2a, b).

Lead For both species, this metal was under the detection limit in the influent and effluent during the high flow period. In the low flow period, in the influent catfish exhibited this metal in gill (0.046 mg g^{-1}), and liver

Table 1 BAI values in high flow and low flow seasons for the catfish and the silverside

			Al	Cu	Fe	Zn
High flow						
Catfish (Fish/IW + LFS)	Influent	Gill	8.33	0.06	5.65	18.78
		Liver	*	0.004	0.43	8.02
		Muscle	14.14	0.004	5.42	6.01
Silverside (Fish/SW + LFS)	Effluent	Gill	0.96	0.03	0.51	5.97
		Liver	6.08	0.05	2.35	5.66
		Muscle	2.92	0.01	0.52	2.18

			Cu	Fe	Zn
Low flow					
Catfish (Fish/IW + LFS)	Influent	Gill	5.57	4.89	6.56
		Liver	9.09	11.23	7.27
		Muscle	2.39	2.23	3.21
	Effluent	Gill	0.02	*	3.32
		Liver	0.03	*	2.25
		Muscle	*	*	1.77
Silverside (Fish/SW + FLS)	Influent	Gill	0.65	8.03	6.23
		Liver	0.97	*	7.56
		Muscle	0.24	3.02	2.55

IW interstitial water, *LFS* labile fraction of sediments, *SW* surface water (μg g^{-1})

* No sample for this measure

(0.054 mg g^{-1}). In silverside, the highest concentration was found in gills (0.086 mg g^{-1}) during in the influent (Fig. 2c, d).

Manganese This metal was under the limit of detection for both species, in the influent during the high flow period. During low flow, the highest concentration was detected in liver (0.43 mg g^{-1}) of catfish and in gills of the silverside (0.31 mg g^{-1}) (Fig. 3a, b).

Molybdenum This metal was detected in all organs of the catfish in the effluent during high flow; the highest concentration was found in gills (0.11 mg g^{-1}), while the highest concentration during low flow was found in gills in the effluent (0.002 mg g^{-1}). This metal was detected in all organs of silverside during low flow, with the highest concentration in muscle (0.013 mg g^{-1}) (Fig. 3c, d).

Zinc This metal was found in both species for both periods analyzed. During the high flow period, catfish showed the highest concentration in gills (0.45 mg g^{-1}) in the influent, followed by liver (0.19 mg g^{-1}) and muscle (0.14 mg g^{-1}). At low flow, the highest concentration measured in the influent was found in liver tissue (0.31 mg g^{-1}), while concentrations in the effluent decreased; the highest values were found in gills (0.22 mg g^{-1}). During the high flow period, the silverside presented the highest concentration in gills (0.14 mg g^{-1}), while in the effluent during the low flow the highest concentration was found in liver (0.32 mg g^{-1}) (Fig. 4a, b).

As mentioned in the descriptive analysis performed per element, the results showed two main features: (a) the heavy metals showed high values in the influent compared to those observed in the effluent and (b) higher concentrations of these elements were detected in the high flow season. The first result suggests that the Rapel Reservoir produces a filtering effect by reducing metal concentrations downstream from the dam. To our knowledge, no other study has shown this kind of pattern.

The second result suggests that riverine water interacts more with the sediment during this season in the influent, leading to an increase in heavy metals in the water, thus facilitating uptake by fish. While metal levels are currently measured in muscle of different fish due to their importance in human health, liver and gills are also analyzed because these organs are vulnerable to bioaccumulation of metals (Marcovecchio et al. 1991). These organs are used as indicators of chronic exposure to heavy metals because of its importance in metal metabolism. In this context, gills are often considered to constitute a good indicator of the concentration of metals and their concentrations could be proportional to those present in the habitat (Dural et al. 2007). Kargin (1996) reported the influence of factors on the accumulation of heavy metals in different organs of aquatic species such as seasonality and the physical–chemical characteristics of the water. The results of these studies showed that the accumulation of heavy metals is higher in liver and gills, while it is lower in muscle (Yilmaz 2003; Ebrahimpour et al. 2011). Further, the liver has been deemed to be the best environmental indicator of water pollution and chronic exposure to heavy metals because of its ability to store and bioaccumulate metals due to the large amount of metallothioneins produced by fish liver tissue (Dural et al. 2007; Agah et al. 2009; Massaoudi et al. 2009).

In our study, liver tissue showed the highest metal concentration when compared with muscle and gills. Such differences in the values obtained were also observed by Karadede and Ünlü (2000), who reported highest metal concentrations in liver and gills compared to the muscle in the mugil fish (*Liza abu*) and the catfish *Silurus triostegus*. Visnjic-Jeftic et al. (2010) also found that, with the exception of Al, liver tissue had significantly higher concentrations of metals and trace elements than those in muscle. The difference in accumulation in these two tissue can be explained by the activity of metallothioneins, which are proteins observed in the liver but not in muscle and which have the ability to bind to certain heavy metals, thus allowing the tissue to bioaccumulate. These results are in accordance with the analysis reported by Poleksic et al. (2010), who found 40 and 35 times more Cu and Fe in liver tissue than in muscle and gills from the sterlet fish *Acipenser ruthenus*.

Bioaccumulation Index Analysis (*BAI*) Concentrations of metals obtained from interstitial water and sediment are shown in Table 1. Of the seven metals considered in this work only was possible to calculated BAI with four of them (Al, Cu, Fe and Zn) in high flow season and three of them (Cu, Fe and Zn) in low flow season. In the case of the catfish in the high flow season, Al showed high BAI values for muscle (14.14), followed by gill (8.33); both indicated possible bioaccumulation in these organs. For the liver this relationship could not be established. There was evidence of Fe bioaccumulation in gills (5.65) and muscle (5.42); and Zn showed the highest BAI values compared to other metals. In the silverside Al showed BAI values of 6.08 in liver and 2.92 in muscle. Cu showed values of BAI <1 in all organs and Fe BAI values >1 were observed only in the liver (2.35). In the low flow season, the BAI was estimated for three metals: Cu, Fe, and Zn. For silverside, BAI values were >1 in the influent for the three organs and metals. The highest BAI values were found in fish sampled in the influent; Zn in gills (6.56) and liver (11.23) and Fe in muscle (3.21). BAI values for Cu were < 1 and values for Zn were >1 in all organs, thus showing a potential for bioaccumulation. In the case of silverside, Fe showed levels of bioaccumulation in gills (8.03) and muscle (3.02), while Zn exhibited bioaccumulation in gills, liver and muscle (6.23, 7.56 and 2.55, respectively).

The catfish showed higher levels of bioaccumulation (based on BAI) than silverside. This could be explained by the nature the behavior displayed by the fish, that is, considering that silverside are pelagic and catfish are benthic. In this sense, catfish interact more with sediments, which would explain the higher levels of metals observed in their tissue.

It is important to highlight the influences of the reservoir on the distribution of metals in the rivers separated by a dam. Such influences include the “filtering effect”, which refers to a decrease in metal concentration downstream of the dam, and the “accumulative effect” where metal concentrations increase downstream (Klaver et al. 2007; De et al. 2010). Because metal concentrations decreased in the effluent, our analysis suggests that there is a filter effect for all metals identified in different organs in catfish *T. areolatus*.

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Appendix

See Table 2.

Table 2 Heavy metals concentration in surface water, interstitial water (mg L^{-1}) and sediment labile fraction (mg g^{-1})

Concentration (mgL^{-1})		Al	Cu	Fe	Mo	Mn	Pb	Zn
High flow								
Superficial water	Influent	4.04 ± 0.01	0.63 ± 0.03	4.46 ± 0.02	0.19 ± 0.01	4.77 ± 0.01	<LD	0.16 ± 0.003
	Effluent	1.52 ± 0.02	0.21 ± 0.01	0.93 ± 0.01	0.02 ± 0.005	4.20 ± 0.01	<LD	0.09 ± 0.004
Interstitial water	Influent	0.44 ± 0.01	7.17 ± 0.06	1.74 ± 0.04	0.02 ± 0.005	4.67 ± 0.02	0.05 ± 0.01	0.09 ± 0.005
	Effluent	0.35 ± 0.01	0.20 ± 0.01	0.84 ± 0.02	0.02 ± 0.005	0.44 ± 0.01	0.11 ± 0.006	0.15 ± 0.02
Low flow								
Superficial water	Influent	<LD	1.35 ± 0.02	0.42 ± 0.01	<LD	<LD	0.41 ± 0.005	0.45 ± 0.01
	Effluent	<LD	<LD	0.93 ± 0.004	<LD	<LD	0.33 ± 0.004	<LD

Table 2 continued

Concentration (mgL^{-1})		Al	Cu	Fe	Mo	Mn	Pb	Zn
Interstitial water	Influent	<LD	0.31 ± 0.005	1.90 ± 0.02	<LD	3.73 ± 0.02	<LD	0.28 ± 0.005
	Effluent	<LD	23.20 ± 0.04	2.24 ± 0.04	0.72 ± 0.01	7.70 ± 0.05	<LD	0.56 ± 0.02
Concentration (mgg^{-1})		**Al**	**Cu**	**Fe**	**Mo**	**Mn**	**Pb**	**Zn**
High flow								
Sediment-label fraction	Influent	0.52 ± 0.005	1.89 ± 0.03	1.45 ± 0.02	<LD	4.07 ± 0.03	<LD	0.02 ± 0.001
	Effluent	0.16 ± 0.002	0.29 ± 0.01	<LD	<LD	4.27 ± 0.04	<LD	0.02 ± 0.001
Low flow								
Sediment-label fraction	Influent	<LD	0.36 ± 0.005	0.12 ± 0.001	<LD	1.23 ± 0.004	<LD	0.04 ± 0.001
	Effluent	<LD	0.16 ± 0.002	<LD	<LD	1.46 ± 0.006	<LD	0.67 ± 0.01

± values represents the standard deviation

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