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Heavy Metal in Tissues of Three Fish Species from Different Trophic Levels in a Tropical Brazilian River

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Abstract The Paraíba do Sul river is located in one of the most developed part of Brazil and receives many organic and industrial effluents directly affecting the ichthyofauna. Concentration of four heavy metals (Cu, Cr, Zn and Pb) were determined in two tissues (muscle and gonads) of three abundant fish species from different trophic levels (*Oligosarcus hepsetus*—carnivore, *Geophagus brasiliensis*—omnivore and *Hypostomus luetkeni*—detritivore) between November 2002 and April 2003. The aim was to test the hypothesis that the trophic level and the proximity from impacted areas influence levels of contamination and to assess if these species are indicators of large-scale habitat quality. Levels of heavy metals were detected by Total Reflection X-ray Fluorescence with Synchrotron Radiation (SR-TXRF) at the Brazilian National Synchrotron Radiation Laboratory (LNLS). Generally, gonads showed higher metal concentration than muscles, except for Cr. All examined metals, but Cu, exceeded the maximum permitted concentration (mpc) by the Brazilian legislation for human consumption in at least one tissue. *O. hepsetus* (carnivore) showed the highest contamination levels, followed by *G. brasiliensis* (omnivore) and *H. luetkeni* (detritivore). The middle-upper segment, which encompasses large urban areas, showed the highest levels of metal contamination in most cases. *O. hepsetus* showed the highest levels of contamination in muscles for Pb in the middle-upper river segment (7.98±3.73; mpc=2.0 $\mu g\ g^{-1}$) and for Cr in the upper (5.53±0.05; mpc=0.10 $\mu g\ g^{-1}$) and middle-upper (4.20±0.85; mpc=0.10 $\mu g\ g^{-1}$) segments, which indicates that human population should avoid to consume these fishes species from these segments of the Paraíba do Sul river.

Keywords Fish · Metal pollution · Habitat quality · Paraíba do Sul river · Brazil

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1 Introduction

Heavy metal pollution in rivers has become a matter of great concern over the last few decades, not only because the threat to public water supplies, but also the hazards to human consumption of fishery resources. Heavy metals from natural and anthropogenic sources are continually released into rivers, and they are serious threats because of their toxicity, long persistence, bioaccumulation and bio-magnification in the food chain (Eisler 1988; Clark et al. 1997; Asuquo et al. 1999; Asuquo et al. 2004). Heavy metal contamination in water and its uptake by fishes is a direct consequence of urban and industrial pollution (Azcue et al. 1988; Campbell 1995; Chapman 1996).

The Paraíba do Sul river is an eighth-order river in southeast Brazil, draining one of the most industrial regions in the country. Its waters are used for human consume and c.a. 47,000 l/s are abstracted to supply Rio de Janeiro City only. On the other hand, one billion domestic effluent liters without any previous treatment per day are introduced into the river (Ceivap 2005). Recent surveys have indicated that the Paraíba do Sul is a suitable habitat for a variety species of fish which are target of regional exploitation for consumption and commercial purposes (Araújo et al. 2003; Pinto et al. 2006). However, information on contamination levels of fish from the Paraíba do Sul river is rather scarce (Azcue et al. 1988; Calza et al. 2004).

The degree of contamination depend on pollutant type, fish species, sampling location, trophic level and their mode of feeding (Asuquo et al. 2004). Monitoring heavy metal contamination in river systems by using fish tissues helps to assess the quality of aquatic ecosystems (Adams 2002). In this sense, fish samples are considered as one of the most indicative factors for estimation of trace metals pollution in freshwater systems (Cinier et al. 1999; Rashed 2001; Has-Schön et al. 2006). Heavy metal concentration in fish tissues reflects past or present exposure (Canli et al. 1998; Yilmaz 2003; Henry et al. 2004) and incorporation occurs mainly through the gills, skin or by food (Bordajandi et al. 2003).

Each fish species has a particular way to accumulate (and/or to eliminate) metal when exposed to such contaminants. Overall species in relatively low trophic levels are exposed to comparatively lower contamination, although plants can accumulate metals in high levels (Peakall and Burger 2003). On the other hand, fish in the upper food web position are prone to accumulate metals and cause human contamination, through food causing chronicle and acute diseases (Al-Yousuf et al. 2000; Has-Schön et al. 2006).

Although fish gonads are hardly consumed by human populations, this tissue is very important for transferring contamination to the next fish generations, resulting in diseases or other illnesses. Muscle analyses are used to investigate direct transference of heavy metals to human population via fish consumption. The aim of the present study is to evaluate the level of contamination of four heavy metals (copper, chrome, zinc and lead) in muscle and gonads of three fish species in different trophic levels. The tested hypothesis is that fish in upper food web position are prone to accumulate more metals and that the proximity of urban-industrial effluents loads is more likely to have contaminated fish. Three abundant and widely distributed fish species in Paraíba do Sul river were studied: *Oligosarcus hepsetus*, a carnivorous Characiformes which use the water column; *Geophagus brasiliensis*, an omnivorous Cichlid species which feed near to the bottom, and *Hypostomus luetkeni*, a bottom dwelling detritivorous Siluriformes closely related to substrate. These species appear to have great economic and ecological importance and their populations are currently in decline because of environmental degradation due to human activities. Moreover, the use of these species aims to assess if they could be used as environmental indicators of aquatic ecosystems quality.

2 Study Area

The Paraíba do Sul watershed is located between latitudes 20°26′ and 23°38′ S and longitudes 41°00′ and 46°30′ W (Fig. 1). It is 1,100 km long with a large catchment area of about 55,500 km^2 in the most developed part of Brazil. The river receives large organic and industrial effluents loads without previous treatment (Molisani et al. 1999) containing heavy metals such as Zn, Pb, Cu e Cr (Azcue et al. 1988; Carvalho and Torres 2002). The watershed is divided in four environmental units according to Ab'Saber and Bernardes (1958) based on geomorphic and climate characteristics: (1) Upper segment: from the headwaters at 1,800 m above sea level in the Atlantic mountains crossing the upper slopes of the Sea Montains, along a 280 km long extent with hills and slopes predominating in the landscape; (2) Middle-Upper segment: a sinuous 300 km long, along plateaus encompassing large industrialized cities; (3) Middle-Lower segment: a 430 km long extent, encompassing different landscapes, from waterfalls to flooding areas; (4) Lower segment: is a 90 km long sedimentation area, dominated by floodplains up to the estuary at Atlantic Ocean.

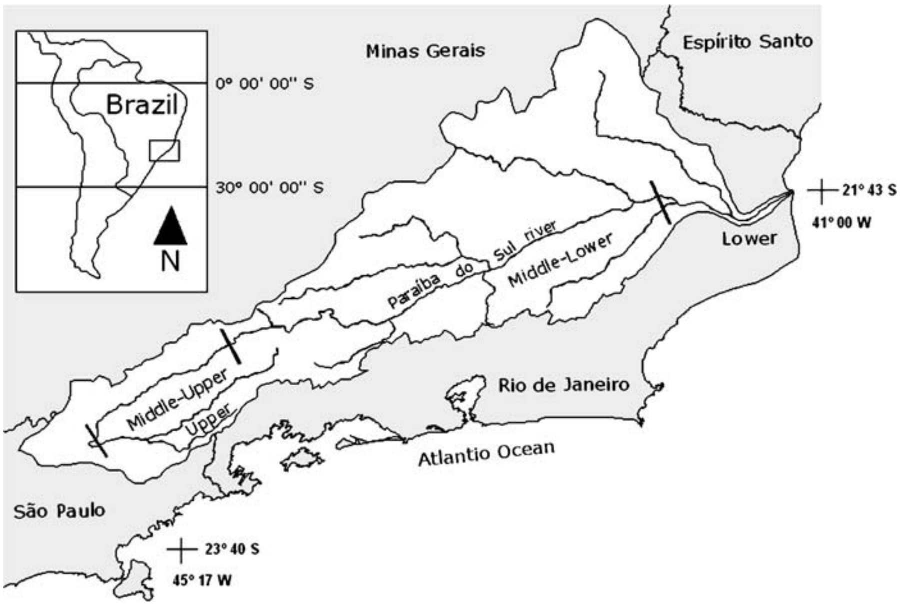

Fig. 1 Study area, Paraíba do Sul river, Brazil, showing the four river segments: upper, middle-upper, middle-lower and lower

Differentiated land uses and human interferences occur along the four rivers segments. The upper segment is the least impacted receiving urban effluent from small town and the run-off waters from subsistence agricultural activities only. The middle-upper and middle-lower segments are the most altered by human activities, consequently the most polluted due to large urban and industrial plants. The lower segment is dominated by agro-industries activities associated to sugar cane monoculture.

3 Materials and Methods

Fishes were collected between November 2002 and April 2003, using gill and casting nets. Three fish species in different trophic levels were examined: *G. brasiliensis*—omnivore; *O. hepsetus*—generalist carnivore (Araújo et al. 2005) and *H. luetkeni*—detritivore (Delariva and Agostinho 2001).

Two tissues were examined for heavy metal contamination: (1) Muscle—the final metal contamination site and the main link to human contamination by fish consumption; 2) Gonads—to test the eventual transference to next generations through reproductive process (Lima-Júnior et al. 2002).

Samples were analyzed by X-rays total reflection fluorescence by Síncrotron radiation, at the Brazilian National Synchrotron Radiation Laboratory (LNLS). This method detects the multielementar chemical composition (Z>14), at trace and ultra-trace levels (Pérez et al. 1999). Composed samples were prepared from tissues of three to five individuals from size ranging between 170 and 240 mm and that each sample was analyzed in triplicates.; we have used adults species in a narrow size range and balanced sex ratio (similar number of females and males). The experimental design was to examine fish contamination from four metals (Cr, Cu, Zn and Pb) × three fish species (*G. brasiliensis*, *O. hepsetus* and *H. luetkeni*) × two tissues (muscle and gonad) × four river reaches (upper, middle-upper, middle-lower and lower).

All data was log-transformed to meet the parametric assumptions prior to statistical analysis. One-way analysis of variances (ANOVA) and Tukey HSD multiple comparison test were carried out to compare means values of each metal concentration among the river reaches per species and per tissues at the level of 0.05. We also use one-way ANOVA on $\log_{10}$-transformed data to compare means of metal concentration between species and between tissues in each river reaches.

Samples aperture were performed by liofilization and homogenization at the Nuclear Instrumentation Laboratory, UFRJ. Digestion of 100 mg of the sample were performed by adding 1 ml HNO3 65% in Teflon pumps at 120°C, during 4 h. Dilution was performed at room temperature (25°C), to 4 ml using deionized water (Milli-Q). An aliquot of 50 µl of selenium solution was added to 450 µl this solution,

Table 1 *F*-values and significant differences from ANOVA and Tukey test on comparisons of averages metal concentration among the river reaches per species and tissues

Metal	Species	Tissue	*n*	*F*	Significant differences (Tukey test)
Cr	*O. hepsetus*	Muscle	13	3,78ns	–
		Gonad	14	4,60*	UP > ML
	G. brasiliensis	Muscle	15	5,18*	UP, MU, ML > LO
		Gonad	16	5,31*	MU > ML
	H. luetkeni	Muscle	6	0,45ns	–
		Gonad	6	1,75ns	–
Cu	*O. hepsetus*	Muscle	12	9,14**	MU > ML, LO
		Gonad	12	5,16ns	MU >ML
	G. brasiliensis	Muscle	15	17,82**	LO>UP, MU, ML
		Gonad	15	3,01ns	–
	H. luetkeni	Muscle	7	2,05ns	–
		Gonad	6	613,5**	MU >UP, ML, LO
Zn	*O. hepsetus*	Muscle	12	7,17*	MU > ML
		Gonad	11	1,57ns	–
	G. brasiliensis	Muscle	15	0,69ns	–
		Gonad	13	4,14*	LO > ML
	H. luetkeni	Muscle	7	0,44ns	–
		Gonad	6	1,19ns	–
Pb	*O. hepsetus*	Muscle	8	2,79ns	–
		Gonad	13	2,83ns	–
	G. brasiliensis	Muscle	13	7,90**	MU > UP, LO
		Gonad	13	0,40ns	–
	H. luetkeni	Muscle	5	18,6ns	–
		Gonad	5	0,79ns	–

n=Number of composed samples, with each sample being comprised by 3 to 5 individuals.

River reaches: *UP* upper reaches; *MU* middle-upper; *ML* middle-lower; *LO* lower reaches.

Significance level: *$p<0.05$; **$p<0.01$.

used as intern pattern. After homogenization, 10 µl of the mixture was deposited in a lucite reflector disk. The reflectors were exposed to ultraviolet light up to complete drops dry. Each sample, with its respective reflector, was analyzed in triplicates. The obtained results were compared with the maximum permitted concentration by the Brazilian legislation for human consumption, that is, 0.1 $\mu g\ g^{-1}$ for Cr, 30 $\mu g\ g^{-1}$ for Cu, 100 $\mu g\ g^{-1}$ for Zn, and 2 $\mu g\ g^{-1}$ for Pb (ANVISA 2003).

4 Results

The results of ANOVA for comparisons of metal concentration means values among river reaches per tissue and per fish species are shown in Table 1. Comparisons between tissues and between species were included directly in the text. Size of *G. brasiliensis* ranged from 140 to 220 mm Total Length (TL), *O. hepsetus* ranged from 140 to 230 mm TL, and *H. luetkeni* ranged from 170 to 240 mm TL.

4.1 Chrome (Cr)

The maximum permitted concentration for Chrome (0.1 $\mu g\ g^{-1}$) was exceeded in all samples (Fig. 2). Overall, Cr concentrations did not vary significantly between tissues ($p>0.05$), except in *O. hepsetus* that showed higher concentration in muscles compared with gonads ($F=6.9$; $p=0.02$). Concentrations of Cr in the muscle tissue of *O. hepsetus* showed the higher values compared with the other two species ($F=14.02$; $p=0.03$), with the highest values being recorded in the upper (5.53±0.05 $\mu g\ g^{-1}$) and the middle-upper (4.20±0.85 $\mu g\ g^{-1}$) rivers segments. Likewise, *G. brasiliensis* showed the highest Cr concentration values in the upper (2.83±0.49 $\mu g\ g^{-1}$)

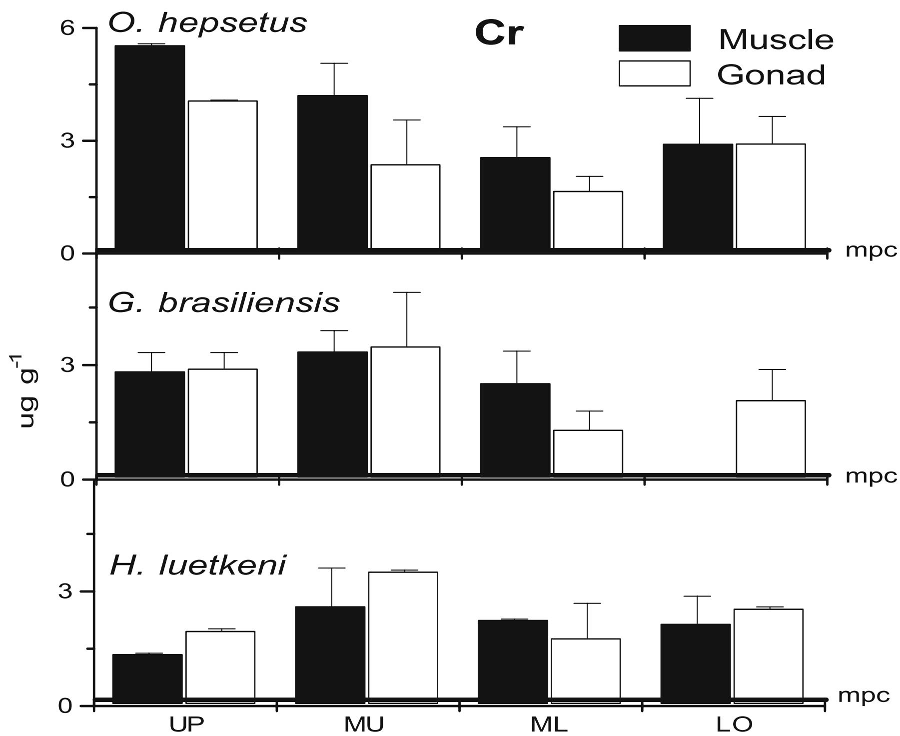

Fig. 2 Means of concentration of chrome in fish tissues from the Paraíba do Sul river. mpc, maximum permitted concentration. River segments: *UP*=upper; *MU*=middle-upper; *ML*=middle lower; *LO*=lower. The *vertical bars* denote ±1 standard deviation

and the middle-upper (3.35±0.55 μg g^{-1}) segments, but no Cr was detected in fish muscles from the lower segment. *G. brasiliensis* also had higher levels of Cr in gonads in middle-upper segment than in middle-lower segment (Table 1). Overall, the highest Cr concentration was found for the carnivorous *O. hepsetus*, followed by the omnivorous *G. brasiliensis* and the detritivorous *H. luetkeni*.

4.2 Copper (Cu)

The Cu concentration did not reach the maximum permitted concentration in any examined fish species (Fig. 3). Gonads showed comparatively higher values than muscles for all fish species (*O. hepsetus*—F=24.3, p=0.001; *G. brasiliensis*—F=25.1, p=0.001; *H. luetkeni*—F=356.2, p=0.000) with the highest concentrations been recorded for *O. hepsetus* in the middle-upper segment (16.8±8.02 μg g^{-1}). Muscle tissues showed the highest Cu concentration for *G. brasiliensis* (5.7±0.93) in the lower segment, followed by *O. hepsetus* (3.70±0.83 μg g^{-1}) in the middle-upper segment. The highest Cu concentration was recorded in gonads of the carnivorous *O. hepsetus* in middle-upper segment compared with the other two species.

4.3 Zinc (Zn)

The maximum permitted concentration for Zn was not reached in muscle for all three species (Fig. 4). On the other hand, this limit was reached by all the three species for gonads. Significant differences between tissues were shown for all three species (*O. hepsetus*—F=20.3, p=0.001; *G. brasiliensis*—F=95.8, p=0.000; *H. luetkeni*—F=164.0, p=0.000), with *O. hepsetus* (carnivore) showing the highest Zn concentration in gonads (412.59±35.32 μg g^{-1}) for the middle-upper segment. *G. brasiliensis* and *H. luetkeni* showed similar Zn concentrations in gonads, with the highest values in the lower segment (380.25±1.19; 241.23±39.36 μg g^{-1}) respectively, followed by the middle-upper segment (261.52±74.51; 235.68±0.36). Overall, the highest Zn concentration in both was found for the carnivorous *O. hepsetus* when compared with the other two species (F=6.3, p=0.03).

4.4 Lead (Pb)

The maximum Pb permitted concentration (2 μg g^{-1}) was reached in gonads for all three species (Fig. 5). The concentrations in muscles exceeded the maximum permitted limit for all three species in the

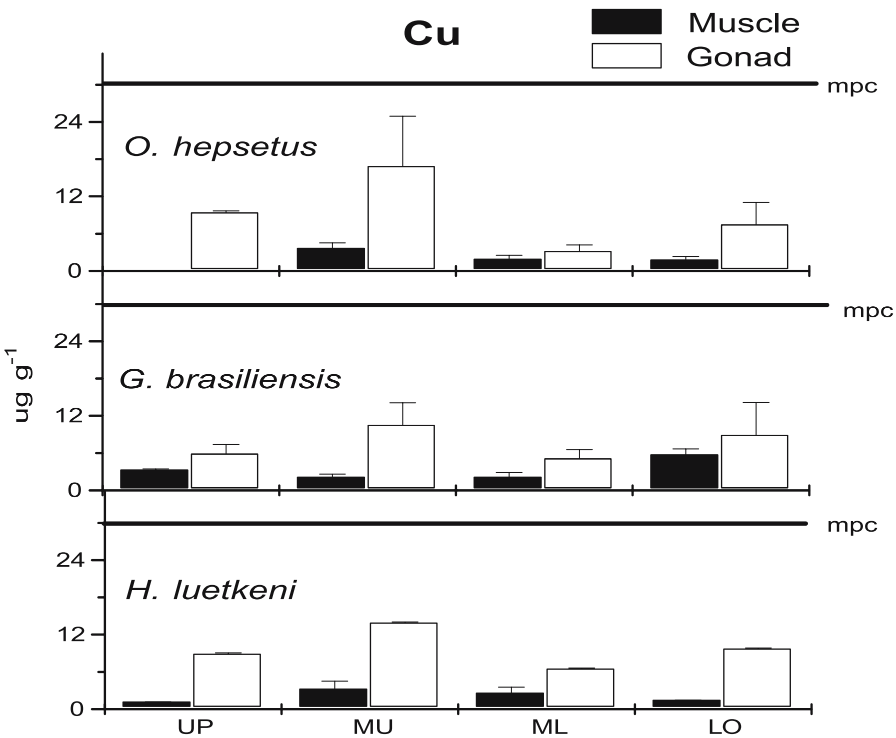

Fig. 3 Means of concentration of copper in fish tissues from the Paraíba do Sul river. mpc, maximum permitted concentration. River segments: *UP*=upper; *MU*=middle-upper; *ML*=middle lower; *LO*=lower. The *vertical bars* denote ±1 standard deviation

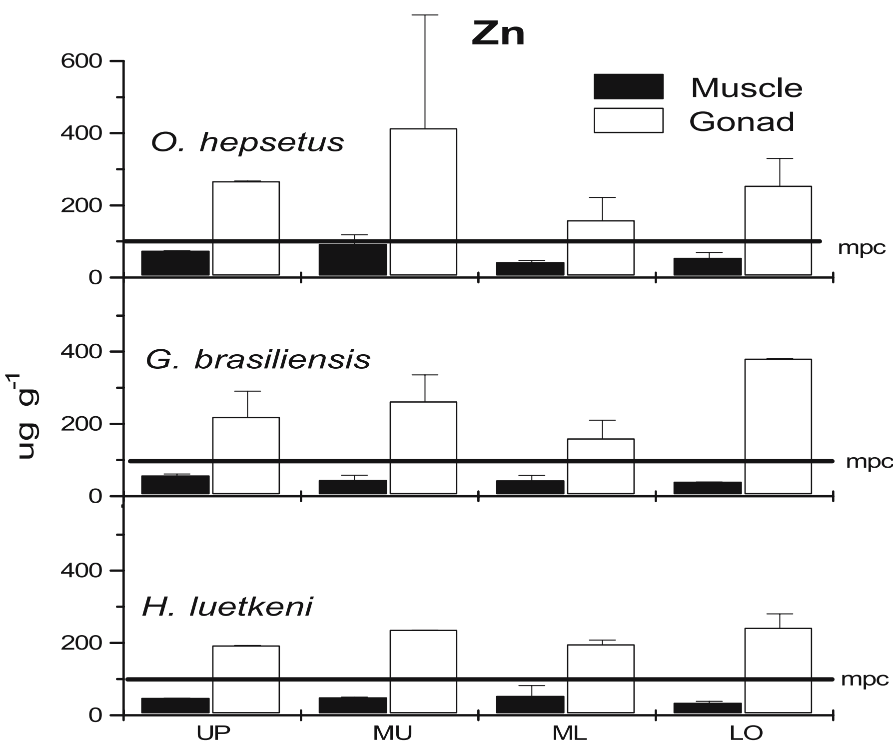

Fig. 4 Means of concentration of zinc in fish tissues from the Paraíba do Sul river. mpc, maximum permitted concentration. River segments: *UP*=upper; *MU*=middle-upper; *ML*=middle-lower; *LO*=lower. The *vertical bars* denote ±1 standard deviation

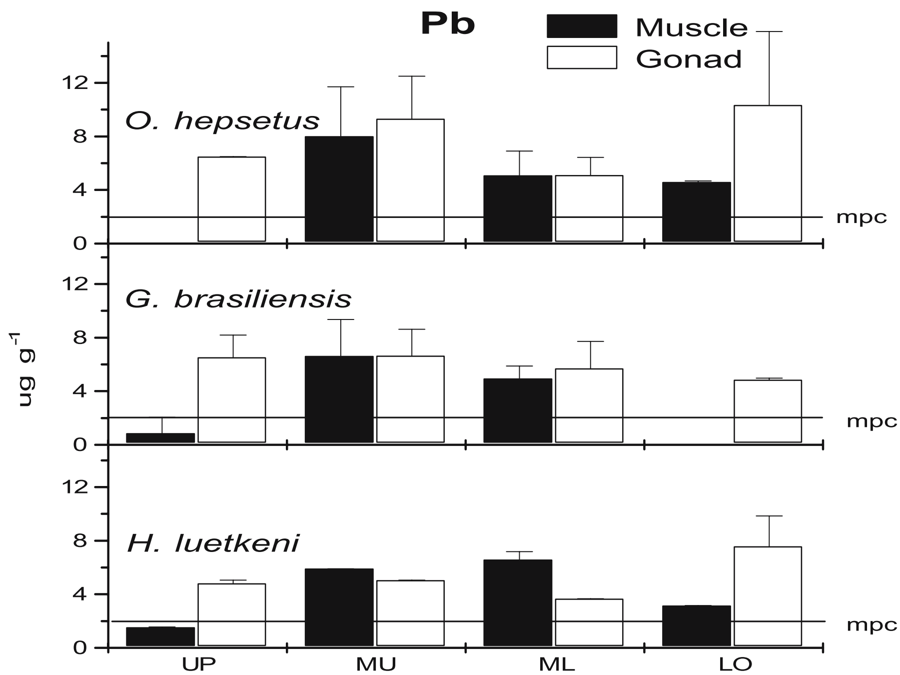

Fig. 5 Means of concentration of lead in fish tissues from the Paraíba do Sul river. mpc, maximum permitted concentration. River segments: *UP*=upper; *MU*=middle-upper; *ML*=middle-lower; *LO*=lower. The *vertical bars* denote ±1 standard deviation

middle-upper and middle-lower river segments. This limit was not reached only in the upper segment (all three species) and in the lower segment for *G. brasiliensis*. *G. brasiliensis* had higher levels of Pb in muscle in middle-upper segment than in upper and lower segments (Table 1). Gonads showed significantly higher values than muscles for *G. brasiliensis* (F=11.6, p=0.003) only. *O. hepsetus* showed the highest Pb concentration in both gonads (9.30±3.18 µg g^{-1}) and muscles (7.98±3.73 µg g^{-1}) in the middle-upper segment, and in gonads (10.33±5.50 µg g^{-1}) for the lower segment. An increased Pb concentration was shown for gonads according to trophic level: *H. luetkeni* showed slightly lower Pb in muscles tissues than *G. brasiliensis,* while the carnivorous *O. hepsetus* showed the highest concentrations.

5 Discussion

The hypothesis of higher metal concentration in the highest trophic levels was met for the examined fish species in the Paraíba do Sul river since *O. hepsetus* (carnivore) showed the highest Cr, Pb and Zn concentration than *G. brasiliensis* (omnivore) and *H. luetkeni* (detritivore). On the other hand, Cu concentrations changed according to fish tissues and river segments for all fish species but the recorded values were well below the maximum permitted concentration by the Brazilian legislation for human consumption (ANVISA 2003). According to Phillips (1990) carnivorous species are prone to have higher metal concentrations than herbivorous, omnivorous or planktivorous species, but this general pattern can change. Other factors like pollution exposure, diet and behavior can influence metals accumulation (Florence 1982; Cinier et al. 1999; Henry et al. 2004). It is generally accepted that heavy metal uptake occurs mainly from water, food and sediment. Dallinger and Kautzky (1985) indicated that the uptake of copper and zinc from food could be the main route of metal uptake by rainbow trout *Salmo gairdneri*, although high levels of elimination through faeces occur. Different concentration of heavy metals in different fish species might be a result of different ecological needs, metabolism and feeding patterns (Allen-Gil and Martynov 1995). Moreover, species in the lower trophic levels in very impacted sites can present higher metal contamination, with the local playing a more important role than the trophic web fish position. Camusso et al. (1995) found that two different stations of the river Po showed different metal concentration, indicating that stations contaminated heavily caused the fish to accumulate heavy metals in greater level.

O. hepsetus showed the highest Cu concentration in gonads for the middle-upper segment although such values were below the maximum permitted concentration. It is believed that Cu is not harmful for the fish species in Paraíba do Sul river since it was never recorded in levels above the maximum permitted concentration. According to Bordajandi et al. (2003), the diet has a remarkable role in the bioconcentration process for some metals, mainly for the Cu and Zn. According to Asuquo et al. (2004) fish that ingest pollutants directly or indirectly form water as detritus (food) and sediment trends to show acute and chronic diseases. In the present study, *H. luetkeni* was the species to present such feeding behavior but showed generally the lowest level of metal concentration. In fact there is little information on the mechanism of pollutant absorption and elimination that could clarify this process that is peculiar for each species.

Pollutants rarely distribute uniformly with the body tissues of fish, but are accumulated by particular target organs. Overall the highest concentration was shown for gonads than muscles (Pb, Cu and Zn) although Cr has been higher in muscles, which is hardly expected. Gonads, similarly to liver, kidney and gills, are target organs for metals accumulation since they are metabolically actives accumulating metals sometimes in high levels (Yilmaz 2003). According to Allen-Gil and Martynov (1995) the low concentrations of Cu and Zn in the muscle of fish species may reflect the low levels of binding proteins in the muscle. Yilmaz (2003) also found that skin and gonads showed higher metal concentrations than muscle for *Mugil cephalus* and *Trachurus medítterraneus*. Cinier et al. (1999) found that Cadmium accumulation in carps kidney was fourfold higher than in liver and 50-fold higher than in muscle, and that toxic concentration increased as the concentration of pollutant in water increased; conversely, they found that the loss of accumulated cadmium was rapid and immediate in muscle and no loss of cadmium was observed in kidney and liver. Therefore, is it reasonable to suppose that fish species in Paraiba do Sul river have undergone exposure from pollutants, mainly Cr and Pb, and to a lesser extent, Zn that remain in gonads as result of past exposure.

Fishes normally endure to high pollutant exposure as they can develop pollutant-sequestering detoxifying systems which make the metal relatively inert and not toxic to the organism. Kito et al. (1986) showed that carp exposed to cadmium synthesize detoxifying proteins such as metallothioneins in kidney and liver. A similar process could be developed by the studied fish in Paraíba do Sul, for Cr and Pb, since their levels of concentration were remarkable high in the study case. The synthesis of such metal-binding proteins inhibits the toxic effects of the pollutant and could explain the high metals accumulation level in gonads.

The highest Zn values found in gonads for all examined species may be also associated to reproductive processes. Papagiannis et al. (2004) also found higher Zn levels in gonads than in muscle of four species in Lake Pamvotis. Gonads are likely to have high Zn concentration due to their participation in cellular division and growth processes (Lacerda et al. 1989). Studies focusing the underlying mechanisms on metal transference via reproduction processes are still lacking.

The middle-upper river segment showed consistently the highest metal concentration compared with the other segments. The middle-upper segment is the most impacted river extent due to high industrial and urban concentration, where large untreated organic and industrial effluents are discharge into the river without any previous treatment. It is expected that metal concentration in the tissues of fish samples from this river segment would be the highest. In fact, the metal concentration in middle-upper segment was usually higher when compared with other segments.

The upper and middle-upper segments showed the highest Cr concentrations in tissues of *O. hepsetus* and *G. brasiliensis*. It was not expected to find high Cr concentrations in the upper segment that is considered the less impacted river extent. An explanation for such findings may be due to air deposition, since only small towns are located in this river segment. The metal contamination, mainly Cr and Pb, in the middle-lower and the lower segment was also recorded for most cases. It could be associated to effluent discharges from a major industrial steel and siderurgic plant in middle-lower segment, and to agro-industrial activities, mainly sugar cane monoculture and associated industries in lower segment. The maximum permitted Cr and Pb concentrations for fish tissues was exceeded in most cases according to Brazilian legislation, indicating the low water quality of Paraíba do Sul river. Whatever the source of metals, the results of the present study showed that concentrations of these two metals were very high at nearly all stations, being even higher than the acceptable values for human consumption designated by various health organizations (Merian 1991). The major findings of this study are that heavy Cr and Pb concentrations in muscles tissues of *O. hepsetus*, *G. brasiliensis* and *H. luetkeni* and that human population should avoid to consume these fishes species from the Paraíba do Sul river.

6 Conclusions

1. All examined metals, but Cu, exceeded the maximum permitted concentration in muscles and/or gonads for the three examined fish species (*O. hepsetus*, *G. brasiliensis* and *H. luetkeni*).
2. The carnivorous species (*O. hepsetus*) showed the highest metal concentrations in most cases, confirming the hypothesis that carnivorous species are prone to incorporate more heavy metals than fish in other trophic levels.
3. The middle-upper segment was consistently the most polluted, suggesting that the proximity of discharges of effluents loads mainly form urban and industrial areas were associated to this pattern.
4. Muscles of all fish were contaminated by Cr and Pb, which seem to be the most concern on metal fish pollution in the Paraíba do Sul river.

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