

## Mercury Distribution in Organs of Two Species of Fish from Amazon Region

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**Abstract** Total mercury concentrations were determined in muscle, liver and kidney of *Cichlia ocellaris* and *Colossoma macropomum* sampled at Tapajos and Carnapijo Rivers in Amazon ecosystem during the flood period of 2009. In background area the highest levels of mercury were observed in liver of piscivorous ( $0.3 \pm 0.03$  ug/g dry wt) and non piscivorous fish ( $0.20 \pm 0.1$  ug/g dry wt), but in contaminated area the highest level of mercury in piscivorous fish was detected in liver ( $0.45 \pm 0.27$  ug/g dry wt) and in muscle ( $0.26 \pm 0.05$  ug/g dry wt) of non piscivorous fish. These results suggested that the presence of anthropogenic source plays a key role in the pattern of mercury distribution in fish tissues.

**Keywords** Mercury · Liver · Kidney · Distribution · Amazon

The use of mercury (Hg) in gold mining activities and the consequent contamination of ecosystem are well documented in Amazon basin, where its biogeochemical cycle, depends on a complexity of biotic and abiotic factors. In this context, fish play a key role in the distribution of Hg between the different biotic compartments as previously demonstrate in several studies in which elevated levels of Hg compounds were related in different species of fish

(Bastos et al. 2007; Bidone et al. 1997; Castilhos et al. 2001).

The exposure of fish to Hg can be by diet or water, via respiration at the gill barrier. After absorption, the pattern of distribution is closely related with the chemical form and to the uptake route. It is stored in fish protein matrices covalently bound to sulfhydryl groups, usually as methyl mercury (MeHg), which correspond approximately 75%–95% of all Hg accumulated in tissues. This binding results in a long half-life for elimination, ranged from 60 to 350 d in small (15 g) and large fish (100 g), respectively (Boudou and Ribeyre 2001; Hall et al. 1997; Mason et al. 2000).

The predictable associations between the fish species, trophic level, feeding habits, age/size and fish-Hg concentration may be easily disrupt in Amazon ecosystem, as an example, regular annual flooding can modify the aquatic environment and change the feeding habit of fish (Bastos et al. 2007). Also, a significant change in physical–chemical parameters of water, as pH, temperature and dissolved organic carbon may modify the degree of methylation/demethylation of Hg and consequently its accumulation in fish tissues. Finally, the human activities, especially deforestation, agriculture land use and gold mining activities can modulate the patterns of fish exposure to Hg (Barbosa et al. 2003; Hogstrand and Haux 1991; Maury-Brachet et al. 2006).

The presence of Hg in different fish species in contaminated and background areas from Amazon basin is generally report in skeletal muscle tissue, because the level of Hg in this biological media is closely related to human neuromotor disturbances and neuropathies as observed in the episodes of Minamata and Niigata in Japan (Harada 1995). However, few data demonstrated the pattern of distribution of Hg in liver and kidneys, which are considered the target organs of storage, elimination and

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detoxification of Hg in fish (Belger and Forsberg 2006; Bidone et al. 1997; Hogstrand and Haux 1991). Thus, in this study we determine the distribution of total mercury in muscle tissue, liver and kidneys of two species of fish from contaminated and background areas in Amazon basin. We expected test our hypothesis that the changes in the pattern of Hg distribution are mainly associated with the presence of anthropogenic source of metal.

## Materials and Methods

Two areas were selected for this study. The first site was localized around 10 km from Itaituba municipality (04° 16' 33" S and 55° 59' 02" O'), where the gold mining are distributed along the tributaries of Tapajos River. The background area was localized in Carnapijo river near Barcarena municipality, (01°30'21" S and 48°37'33"O) without gold mining activities and distant around 600 km from contaminated area (Fig. 1).

From May to August/2009 which correspond to the period of peak of flooding in the Tapajos and Carnapijo rivers system were sampled specimens of *Cichlia ocellaris* and *Colossoma macropomum* by local professional fishermen using nets, fishhooks and harpoons. The first is the piscivorous specie most consumed in Amazon region; it is located at the top of food chain and may be considered a good indicator of Hg accumulation in the Amazon ecosystem especially because of its territorial behavior and time-integration capacity. *Colossoma macropomum* feeds

abundantly on fruits and seeds, which represent its energy source during the flooding period, while during the dry period it feed on protein rich sources such a zooplankton.

The species were identified (Elliot et al. 2007), weighed on a digital electric balance and standard lengths recorded using a ruler attached to a measuring board with a precision of +1 mm. For each species, an initial selection was carried out in order to select homogeneous groups with maximum similarity in standard length and weight. The sex was determined through macroscopic examination of the gonads. All specimens analyzed were adult females. Fish were immediately dissected and samples were collected from different organs: dorsal skeletal muscle (samples between 2 and 5 g, wet wt.) under the dorsal fin; liver (whole) and kidneys (whole). These samples were kept in plastic bags and maintained frozen until analysis for Total Hg at the Tropical Medicine Center at Para Federal University.

Approximately 20 mg from each sample were cut in small pieces and washed in ultrasonic bath for 5 min as follows: ultrapure water (<18 mΩ), acetone and ultrapure water (<18 mΩ). They were then oven-dried at 40°C for 24 h. The dried samples were weighed in sample boats and placed in a dessicator. The total Hg content was determined using previously described technique (Pinheiro et al. 2006). Briefly, the boats containing the samples are introduced in the equipment and heated in oven at 800°C. The Hg vapor formed is collected in two successive gold traps and determination is performed by atomic absorption spectrophotometry (Nippon Instruments Corporation). The results were expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  on a dry-weight basis. A blank sample without fish tissue was analyzed after the samples of each specimen.

The detection limit was considered as three times the value of standard deviation of the blank at least 8 blanks and was equal to  $3\text{ ng}\cdot\text{g}^{-1}$ . The accuracy and precision of the procedure used were tested by submitting to the same treatment replicates of a dog fish muscle (DORM—2) certified reference material (National Research Council, Canada); measured values were always within  $\pm 10\%$  of the reference value ( $4.64\text{ }\mu\text{g}\cdot\text{g}^{-1}$ ). Repeatability was estimated by the coefficient of variation was always lower than 15%.

A *t* test was conducted in order to test for differences in Hg concentration between tissues independently. ANOVA, and subsequently Tukey multiple comparison tests (*post hoc*), were used to test the differences between Hg ratios between tissues. The ratios between Hg concentrations in liver or kidney and in the skeletal muscle, considered as the reference tissue for biomagnification effects, were calculated. All tests were conducted at a significance level of 5%. Statistical analyses were performed using STATISTICA 8.0 (StatSoft 2008).

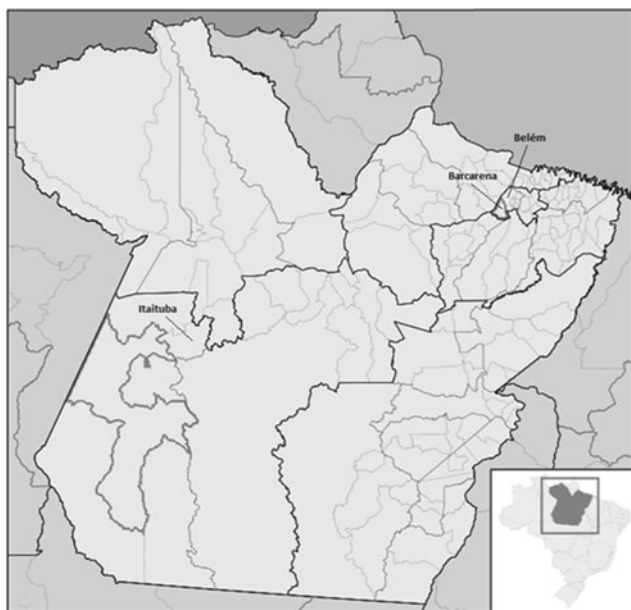


Fig. 1 Localization of studies areas

## Results and Discussion

A total of 48 specimens of *C. ocellaris* weighting approximately 735 g and 23.1 cm ( $\pm 10.1$ ) total length average and 50 specimens of *C. macroporum* weighting approximately 614 g and total length of 20.3 cm ( $\pm 9.1$ ) were sampled in each place.

The Hg concentrations were significantly higher in muscle and liver of *C. ocellaris* in both areas (Tables 1, 2). This feature was previously documented in this region, and results of Hg bioaccumulation through the trophic levels and the carnivorous fish are in the top of food chain (Barbosa et al. 2003; Castilhos et al. 2001; Dorea et al. 2006). Previous studies in fish from Amazon basin showed that MeHg corresponds to more than 90% of total Hg concentration and the principal accumulators are the carnivorous species (Barbosa et al. 2003; Bidone et al. 1997; Dorea et al. 2006). Mercury contamination of fish followed the trend piscivores > herbivores > omnivores > detritivores (Kasper et al. 2009; Mason et al. 2000; Maury-Brachet et al. 2006).

The Hg concentrations in muscle, liver and kidney were significantly higher near gold mining area in *C. ocellaris*, but in *C. macroporum* a significant difference was showed only in muscle tissues (Table 2). Subsequently, these levels reduces gradually downstream as show at Barcarena, where Hg concentrations in fish tissues are similar to others background areas (Belger and Forsberg 2006; Dorea et al. 2006).

The species sampled are non-migratory fish and the Hg concentrations tend to reflect the characteristics of the local

aquatic environment (Castilhos et al. 2001) In fact, the low mobility of Hg in Amazon basin was previously described, and relatively high values of Hg in fish sampled near gold mining areas were related, whereas far downstream the levels were lower even in piscivorous fish. Hence, the impact of Hg released by gold mining is localized and the disruption of ecosystems impacts fish habitat and community structure and can change the fish Hg concentrations (Bastos et al. 2007; Belger and Forsberg 2006; Castilhos et al. 2001; Dorea et al. 2006).

The specimens were sampled during the flood period of Tapajos and Carnapijo Rivers, when the water level may dilute the Hg, reflecting on Hg concentrations in muscle samples in both fish species were lower than the safety limit for human ingestion (WHO 1989). Indeed, the flooding system of Amazon basin can be more relevant than size or others biological variables as demonstrate in some fish species, in which the levels of Hg were five to eight times higher during descending and low waters. Also, explain the wide intra- and inter-species variations in the concentrations of Hg in fish even from the same source (Barbosa et al. 2003; Bastos et al. 2007; Belger and Forsberg 2006; Castilhos et al. 2001; Dorea et al. 2006).

The absorbed Hg is transported via blood to all tissues and distributed in the different internal compartments. In skeletal muscle, it is bound to cysteine rich proteins and when compared to liver or kidney usually has the lowest essential and nonessential metal concentration, (Bloom 1992; Boudou and Ribeyre 2001; Mason et al. 2000). Only *C. macroporum* from contaminated area presents Hg muscle concentrations higher than liver and kidney. These organs are actively involved in heavy-metal metabolism and acts as an active site of storage and pathological effects induced by Hg (Hogstrand and Haux 1991; Kasper et al. 2009; Mason et al. 2000). The ratios between [Hg] Liver/Kidney, [Hg] Liver/Muscle and [Hg] Kidney/Muscle in *C. ocellaris* sampled at contaminated area were  $3.48 \pm 3.1$ ;  $1.27 \pm 0.02$  and  $0.46 \pm 0.10$ , respectively. These ratios were significantly higher than background area, which were  $1.17 \pm 0.07$ ;  $1.27 \pm 0.1$  and  $1.06 \pm 0.18$ , respectively. Thus, in contaminated area these ratios following this trend: [Hg] Liver/Kidney > [Hg] Liver/Muscle > [Hg] Kidney/Muscle, but in background area the trend change to: [Hg] Liver/Muscle > [Hg] Liver/Kidney > [Hg] Kidney/Muscle.

The ratios [Hg] Liver/Kidney, [Hg] Liver/Muscle and [Hg] Kidney/Muscle in *C. macroporum* sampled at contaminated area were  $1.4 \pm 0.8$ ;  $1.17 \pm 0.03$  and  $0.68 \pm 0.02$ , respectively. In background area these ratios were  $1.31 \pm 0.16$ ;  $1.09 \pm 0.15$  and  $1.2 \pm 0.13$ , respectively. In contaminated area the ratios following the same trend of *C. ocellaris*, but in background area change to [Hg] Liver/Kidney > [Hg] Kidney/Muscle > [Hg] Liver/Muscle.

**Table 1** Hg concentrations in different tissues of *C. ocellaris* (muscle, liver and kidney) of fish sampled at Itaituba and Barcarena (Mean  $\pm$  SD)

Municipality	N	Muscle Hg (ug/g dry wt)	Liver Hg (ug/g dry wt)	Kidney Hg (ug/g dry wt)
Itaituba	50	$0.36 \pm 0.11$	$0.45 \pm 0.27$	$0.15 \pm 0.03$
Barcarena	50	$0.25 \pm 0.07$	$0.3 \pm 0.03$	$0.25 \pm 0.02$

**Table 2** Hg concentrations in different tissues of *C. macroporum* (muscle, liver and kidney) of fish sampled at Itaituba and Barcarena (Mean  $\pm$  SD)

Municipality	n	Muscle Hg (ug/g dry wt)	Liver Hg (ug/g dry wt)	Kidney Hg (ug/g dry wt)
Itaituba	48	$0.26 \pm 0.05$	$0.22 \pm 0.13$	$0.18 \pm 0.04$
Barcarena	48	$0.18 \pm 0.1$	$0.20 \pm 0.1$	$0.15 \pm 0.01$

The liver concentrates Hg absorbed by the digestive tract and from enterohepatic circulation, and demonstrate a high bioaccumulation capacity, because the presence of metallothioneins which interact with the metal mainly in the organic form (Bloom 1992; Boudou and Ribeyre 2001; Hogstrand and Haux 1991; Mason et al. 2000). As expected, the concentration of Hg in liver was significantly higher than muscle and kidney in *C. ocellaris* sampled at both areas and in *C. macroporum* from background area.

The concentrations of Hg in kidney were significantly lower in *C. ocellaris* from contaminated area. On other hand, no differences were demonstrated in concentrations of Hg in kidney of *C. macroporum* between two areas. Kidney could accumulate Hg binds to metallothionein in renal tubular epithelium with subsequent tubular damage; however, this organ is preferentially associated with Hg excretion, since the results of this study suggested that liver and skeletal muscles are the most important organs of inorganic and organic Hg storage, respectively. (Bloom 1992; Boudou and Ribeyre 2001; Hogstrand and Haux 1991; Mason et al. 2000).

Significant difference was showed in Hg distribution in 12 fish species, sampled at Maroni River in French Guiana, where piscivorous presents the lowest value of the [Hg] organs/[Hg] muscle ratio and benthivorous, periphytophagous and herbivorous, with the highest ratio values. Our study show a similar trend in ratios of Hg concentrations in tissues in both specie from contaminated area; however, this pattern was different in both species from background area, indicating that the pattern of Hg distribution is dose dependent and closely associated to the presence of anthropogenic source of metal (Kasper et al. 2009; Maury-Brachet et al. 2006).

The results of this study confirm our hypothesis that the presence of anthropogenic source of Hg plays a key role in the pattern of Hg distribution in fish tissues. Further studies should be encouraged for a better understand of distribution and speciation of Hg on others tissues in fish species from Amazon ecosystem.

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