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Methylmercury in Fish as a Tool for Understanding the Amazon Mercury Contamination

Helena do Amaral Kehrig* and Olaf Malm
Laboratório de Radioisótopos, IBCCF, CCS, UFRJ, Rio de Janeiro—RJ, CEP 21949-900, Brazil

In order to evaluate aquatic environmental mercury (Hg) and methylmercury (MeHg) contamination, a wide variety of fish species were sampled from some tributaries of the Brazilian Amazon river system, the Balbina reservoir and the Pantanal watersheds. These water bodies present different mercury inputs and biogeochemical characteristics. Amazon fish, which are the main pathway of MeHg to the local population, are the most important protein source for them, and fishing is a significant economic activity throughout these regions. MeHg in fish samples (164) were analysed with an efficient extraction technique and measured by GC–ECD. Analytical quality was checked through intercomparisons between two laboratories with local samples and a certified standard from IAEA. MeHg concentrations of carnivorous, omnivorous, detritivorous and herbivorous species ranged from 0.1 to 1.25 mg kg^{-1} wet wt and the mean percentages of MeHg to total mercury were usually higher than 80%. Carnivorous (piscivorous) fish, which represented 74% of all samples, effectively showed higher MeHg concentrations as well as a higher MeHg/total mercury ratio in muscle tissue than fish from lower trophic levels. In general, MeHg concentrations in carnivorous fish were higher in places close to goldmining activities, the Madeira river and the Tapajós river near Itaituba city. The MeHg/total mercury ratios in fish were higher in non-impacted areas and with smaller amounts of suspended particulate materials (Negro river, Balbina reservoir and Pantanal watershed). No MeHg seasonal variability was observed in *Serrasalmus rhombeus* (carnivorous fish) from the Madeira river basin during the year. High variability in MeHg levels was observed in muscle of the same carnivorous species fish with similar weights sampled at the same place and by similar nets.

Keywords: methylmercury; mercury; fish; goldmining; Brazilian Amazon; river basin; reservoir

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INTRODUCTION

In the Brazilian Amazon region, informal gold production, which uses large amounts of metallic mercury [Hg(0)] to amalgamate the fine gold particles, is one important source of environmental mercury pollution; it is the major health risk for occupationally exposed persons and is a potential risk for the general population.

Amazon fish are the most important protein source for the local riverine populations and fishing is a significant economic activity throughout the region. The local riverine populations, which have a high daily ingestion of fish, are exposed to methylmercury (MeHg) through consumption of contaminated fish from goldmining activities in rivers.[1]

Methylmercury is formed from inorganic mercury by methylation, preferably under anaerobic conditions,[2] and is then bioaccumulated in biota and biomagnified up through the aquatic food chain.[3] In particular, factors such as high bacterial activity under slightly acid conditions in the presence of high concentrations of dissolved organic carbon are very important for methylation.[4] Furthermore, temporary or permanent flooding of vegetation and soil may also increase mercury mobilization and consequently the concentrations in fish.[5] The Amazon aquatic ecosystems present

* Correspondence to: Helena do Amaral Kehrig, Laboratório de Radioisótopos, IBCCF, CCS, UFRJ, Rio de Janeiro—RJ, CEP 21949-900, Brazil. E-mail: kehrig@biof.ufrj.br

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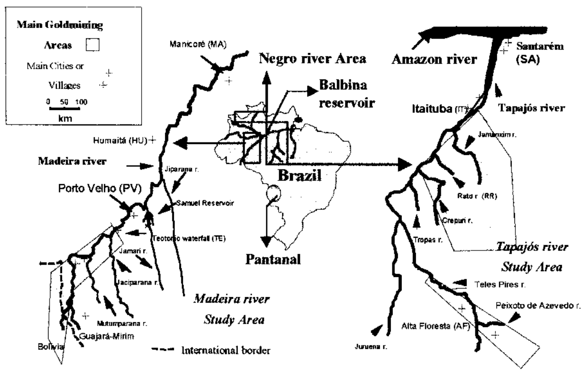

Figure 1 Study area.

these special conditions, which favour the mercury methylation rates.

The local populations are also exposed to high mercury (Hg) levels from certain fish species collected in new reservoirs.[5,6] New regulated reservoirs with a high dissolved organic matter content favour increased methylation of mercury, which may lead to increased bioaccumulation of methylmercury in fish.[7,8]

Fish, which is the critical pathway of MeHg to the population, must be monitored.[3] The enormous diversity in fish species in the Amazon basin makes food chains extremely rich but more complex and therefore the monitoring program is also complex.[9] There are around 200 different fish species of commercial importance in each river basin.[10]

In order to evaluate aquatic environmental mercury and MeHg contamination, a wide variety of fish species, which are those most frequently consumed by the local population, were sampled. They came from several different tributaries of the Amazon River system (Madeira river, Negro river and Tapajós river), the Balbina reservoir and from the Pantanal watersheds (Bento Gomes river and Fazenda Ipiranga lake) (Fig. 1). These water bodies present different mercury inputs as well as particular biogeochemical characteristics. Even inside each basin, peculiarities of each ecosystem influence mercury incorporation through the biota and consequently along the food chain.

MATERIALS AND METHODS

During the last 10 years the UFRJ laboratory (Rio de Janeiro, Brazil) have been working in several different areas in the Brazilian Amazon and have collected a large number of fish samples. From this fish sample collection we chose more representative groups of individuals based on previous total mercury data, the biogeochemical characteristics of the areas and the possible influence of goldmining activity. Some observations on seasonal variations were made.

A total of 164 fish samples were selected from a collection sampled in different periods (1991 to 1996) with distinct feeding habits, mainly carnivorous (piscivorous) and omnivorous species. Table 1 shows the number of individuals selected from each fish species according to their feeding habits.

Analytical methodology

Total mercury and methylmercury analyses were performed for fish samples at the Laboratório de Radioisótopos (UFRJ). For total mercury, muscle tissue of fish samples were acid-digested and subjected to atomic absorption spectrometry with an AA 1475 Varian instrument, equipped with a cold vapour generator accessory (Varian VGA-76), with sodium borohydride as a reducing agent.[11] For methylmercury, we used an analytical procedure developed at the National Institute for Minamata Disease (NIMD) laboratory and adapted at the UFRJ laboratory. Methylmercury muscle tissue of fish was analysed the combined method of dithizone–benzene extraction and analysis by ECD–GC.[6,12] This method is based on the fact that methylmercury dithizonate in the final solution in benzene is converted into its chloride form as soon as it is subjected to conventional ECD–GC. Therefore, a special column with about 0.2 g of NaCl crystals added on the top of column (Hg-20[A]; G1 Science Ltd) was used throughout methylmercury measurement in this study.[12]

Methylmercury analysis in fish

Muscle tissue (0.5 g) was digested with 10 ml of 1 N alcoholic potassium hydroxide solution in a 50-ml screw-capped centrifuge tube at 100 °C in a water bath for 45 min. The digested sample was slightly acidified with 10 ml of 1 N HCl. After washing with 5 ml of n-hexane, the methylmercury was extracted with 10 ml of 0.05% dithizone in benzene or toluene purified with an equal volume of 0.1 N NaOH just before use. The organic layer was then washed twice with 5 ml of 1 N NaOH to remove the excess dithizone. An aliquot (5 ml) of the organic layer was back-extracted with 2 ml of 0.01% Na_2S in 0.1 N NaOH/ethanol (1:1). The excess sulphite ions from the methylmercury

Table 1 Total number of individuals of fish species according to feeding habits, sampling and date from the several different study areas

Fish species according to feeding habits	Sampling areas and dates						
				Tapajós river (1996)		Pantanal (1995)	
	Madeira river[a] (1994)	Negro river[b] (1991)	Balbina reservoir[b] (1996)	Santarém[b]	Itaituba[a]	Bento Gomes river[b]	FazendaI-piranga lake[b]
Carnivorous							
Tucunaré (*Ciclha* spp.)	8	5	17	5	5		
Aruanã (*Osteoglossum ferreir*)		6					
Peixe Cachorro (*Hydrolycus* sp.)	6	6	1				
Piranha (*Serrasalmus rhombeus*)	10	8	4			6	6
Mapará (*Hypophtalmus edentatus*)	4						
Bagre (*Ageneiosus brevifilis*)						6	
Traíra (*Hoplias malabaricus*)			1			6	
Pescada (*Plagioscion* sp)				6	5		
Omnivorous							
Pacu (*Mylossoma duriventre*)	3						
Piau (*Leporinus trifasciatus*)	3						
Acará (*Geophagus surinamensis*)			10				
Sardinha (*Trportheus elongatus*)	1						
Detritivorous							
Branquinha (*Potamorhina latior*)	6						
Branquinha (*Curimata vittata*)		4					
Cará (*Geophagus* sp.)		5					
Aracu (*Laemolyta taeniata*)		4					
Others	3						
Herbivorous							
Aracu (*Leporinus* sp.)				4			
Total	**44**	**38**	**33**	**15**	**10**	**18**	**6**

[a] Near goldmining area.
[b] Non-impacted area.

solution was eliminated with continued bubbling (50 ml$\,$min^{-1}) for a further 5 min with nitrogen gas and some drops of 1 M HCl. To the sample solution, 2 ml of Walpole's buffer (pH 3.0) was added. The methylmercury from this inorganic layer was re-extracted with 1 ml of 0.05% purified dithizone–benzene. The organic layer was then washed twice with 2 ml of 1 M NaOH to remove the excess dithizone and subsequently with 5 ml of distilled water and acidified with a few drops of 1 N HCl followed by ECD–GC measurement.

The quality of the analytical methylmercury

Table 2 Mean methylmercury concentrations[a] and ratios to total mercury in fish muscle tissue from the different study areas[b]

Sampling area	[MeHg] (mg kg^{-1} wet wt) C	O	D	H	All fish[c]	[MeHg] (%)[c]
Madeira river basin[d]	0.57 ± 0.24	0.15 ± 0.11	0.18 ± 0.14		0.41 ± 0.23 (0.01–1.18)	91 (64–100)
Negro river basin[e]	0.61 ± 0.28		0.09 ± 0.03		0.43 ± 0.26 (0.03–1.39)	90 (63–100)
Balbina reservoir[e]	0.32 ± 0.25	0.06 ± 0.02			0.24 ± 0.18 (0.03–0.92)	96 (82–100)
Tapajós river basin (Santarém)[e]	0.14 ± 0.08			0.05 ± 0.02	0.12 ± 0.08 (0.03–0.36)	88 (65–99)
Tapajós river basin (Itaituba)[d]	0.81 ± 0.35				0.81 ± 0.35 (0.34–1.25)	84 (74–96)
Pantanal (Bento Gomes river)[e]	0.12 ± 0.05				0.12 ± 0.05 (0.03–0.21)	97 (89–100)
Pantanal (Cuiabá river)[e]	0.07 ± 0.03				0.07 ± 0.03 (0.04–0.11)	96 (82–100)

[a] Mean values ± SD.
[b] C, carnivorous; O, omnivorous; D, detritivorous; H, herbivorous.
[c] Values in paranthesis are the range, (min.–max.).
[d] Near goldmining area.
[e] Non-impacted area.

and total mercury was certified through intercomparison exercises between the two laboratories (NIMD and UFRJ) using fish samples[6,13,14] and a certified standard sample from the International Atomic Energy Agency (IAEA). The NIMD has checked its methodology against other laboratories and methodologies.[15]

The variability of MeHg and total mercury concentrations in fish species samples from different study areas was investigated using the coefficient of variation (CV). Differences between those variables were compared and expressed as percentages (%).

Statistical analysis

After verification of the normal distribution of each data set, an analysis of variance and 'Tuckey's' multiple comparison were used to compare the methylmercury results in muscle tissue of fish from all the different study areas. Mean comparisons were carried out using the Student's *t*-test.[16]

RESULTS AND DISCUSSION

Comparison between the methylmercury results of the analysis performed at the two laboratories for 32 fish samples collected at the Balbina reservoir showed good agreement with a highly significant correlation ($r^2 = 0.99$); a paired *t*-test showed that they are similar ($0.50 < P_{t32} < 0.25$).

Our methylmercury results of analysis ($N = 31$), 3.59 ± 0.36 mg kg^{-1}, of a tuna-fish certified reference sample from the IAEA, which has a certified MeHg value of 3.65 ± 0.35 mg kg^{-1}, demonstrated the high precision and accuracy of the analytical method.

A total of 164 fish samples from the Amazon and Pantanal watershed were analysed for methylmercury in this study; 121 (74%) of them are carnivorous species, mainly *Ciclha* sp. (tucunaré), *Plagioscion* sp. (pescada comum), *Hypophtalmus edentaus* (mapará), and *Serrasalmus rhombeus* (piranha) (Table 1). Tucunare and pescada comum are the most important commercially used species, and much less is piranha consumed.[17]

Carnivorous species, which are on the top end of the aquatic food chain, are a good indicator of mercury in fish.[18] Of these, 45 (37%) had a mercury concentration above the maximum limit of 0.5 mg kg^{-1} wet wt. established for food by Brazilian legislation.[19] The mean percentages of MeHg relative to total mercury were more than 80%, indicating that organic mercury was the predominant form of mercury in the fish muscle tissue (Table 2).

Methylmercury concentrations (Table 2) showed decreasing values along the food chain from carnivorous to omnivorous, detritivorous and herbivorous species. This observation indicates that biomagnification is probably occurring in these food chains. These species were collected in all sampling areas and presented a methylmercury range from 0.1 to 1.25 mg kg^{-1} wet wt. These data can be considered consistent with data from the Madeira river basin and the Tapajós river basin,[20–22] the Balbina reservoir,[6] the Negro river basin[23] and the Pantanal watershed.[24]

We observed a high variability in methylmercury levels in muscle tissue of the same fish species with similar weights sampled at the same place and time, such as tucunare and piranha from Balbina, tucunare and pescada comum from the Tapajós river basin near Itaituba city, and piranha from the Madeira river. This observation can be considered consistent with preliminary results from Ref. 25.

Tucunare and piranha sampled at the Balbina reservoir presented a methylmercury range from 0.06 to 0.73 mg kg^{-1} wet wt. for the former and 0.05 to 0.92 mg kg^{-1} wet wt. for the latter. Piranha collected at the Madeira river presented a range from 0.2 to 1.1 mg kg^{-1} wet wt. This large interval is probably due to the abundance and assortment of food offers, which vary during the year. 'Piranha' has a quite specific meaning: a carnivorous species, feeding on small pieces bitten from prey, but also on some fruits and seeds, mainly endosperm material.[26]

The variation in mercury and methylmercury levels in fish of each trophic level is a result of a varied diet of the fish through the dry and rainy seasons. The contents of protein and fat on fish vary significantly throughout the dry and rainy seasons.[14]

Nevertheless, no methylmercury seasonal variability was observed in the muscle tissue of *Serrasalmus rhombeus* (Piranha) collected during the dry and rainy seasons at the Madeira river basin. A comparison of means using Student's *t*-test showed that mean MeHg for on dry and rainy seasons (0.69 ± 0.32 and 0.56 ± 0.27 mg kg^{-1} wet wt) presented no significant difference ($t = 0.764$; $P > 0.23$).

In general, concentrations of MeHg in carnivorous fish were higher in places close to goldmining activity (Table 2), such as in the Madeira river basin (0.57 ± 0.24 mg kg^{-1} wet wt) and the Tapajós river basin near Itaituba city (0.81 ± 0.35 mg kg^{-1} wet wt).

Ratios of MeHg to total mercury in carnivorous fish were higher in non-impacted areas, such as the Negro river basin (97%), the Balbina reservoir (97%), the Bento Gomes river (97%) and the Fazenda Ipiranga lake (96%). These areas usually presented low amounts of suspended particulate materials.[24,27]

These high mean concentrations and low percentages of MeHg in fish from the Madeira and the middle of the Tapajós (Itaituba) river basins (91 and 84%) may be due, in part, to the influence of goldmining activities near their systems. Furthermore, the Madeira river basin is considered the most mineralized river from the Brazilian Amazon and presents high amounts of suspended particulate materials.[11]

Two carnivorous fish collected along the Tapajós river basin (*Ciclha* sp. and *Plagioscion* sp.) presented a distinct variation in mercury and MeHg concentrations along the sampling areas, showing a decreasing pattern from the middle of the river to further downstream. The mean methylmercury for *Ciclha* sp. and *Plagioscion* sp. was 0.99 ± 0.33 and 0.63 ± 0.31 mg kg^{-1} wet wt at Itaituba and 0.18 ± 0.11 and 0.11 ± 0.03 mg kg^{-1} wet wt at Santarém. This observation may reflect the possible influence of the higher mercury discharges in the middle reaches of the Tapajós river.[18] The Tapajós river basin, which has been prospected during the last 30 years, is considered a major gold-producing area in Brazil.

The high mean MeHg concentration and percentage of MeHg in carnivorous fish sampled at the Negro river (0.61 ± 0.28 mg kg^{-1} wet wt and 97%), which have small local goldmining operations, may reflect a natural background of mercury present in the Negro amplified by its unique biogeochemical characteristics.[23] The Negro river has a high density of podsols in its drainage basin, which results in high dissolved organic carbon (DOC) concentrations and a high capacity for complexation and transport of mercury. It is also extremely low in pH and conductivity, which favours the methylation of mercury.[23]

Mercury and methylmercury levels in fish samples, which were collected from one goldmining area in the vicinity of the Manu National Park (Peru–Amazon) borders, were high and ranged from 0.051 to 1.54 mg kg^{-1} wet wt. The ratio of MeHg to total mercury in fish muscle was 61–97%.[28]

Freshwater fish from areas considered to be unpolluted (without any anthropogenic mercury point source input) usually have levels lower than 0.20 μg Hg g^{-1} wet wt.[29] The Balbina reservoir,

Table 3 Variability of methylmercury and total mercury in muscle tissues of carnivorous fish species

Area	Fish species	Environmental CV[a], MeHg (%)	Difference (%)	Environmental CV[a], Total Hg (%)
Balbina	*S. rhombeus*	67	0	67
	Ciclha sp.	63	+5	60
Madeira	*S. rhombeus*	62	+24	50
	Ciclha sp.	45	0	45
	Hydrolycus sp.	27	+35	20
Negro	*S. rhombeus*	53	−2	54
	Ciclha sp.	16	−33	24
	Hydrolycus sp.	53	−2	54
Pantanal	*H. malabaricus*	23	−8	25
	A. brevifilis	20	0	20
	S. rhombeus (Bento Gomes river)	63	0	63
	S. rhombeus (Fazenda Ipiranga lake)	34	+9	31
Tapajós	*Ciclha* sp. (Itaituba)	33	0	33
	Plagioscion sp. (Itaituba)	48	−4	50
	Ciclha sp. (Santarem)	23	−8	24
	Plagioscion sp. (Santarém)	23	−8	24

[a] CV, standard deviation/mean concentration.

which has no known goldmining activity in its watershed, presented a mean methylmercury concentration ($N = 32$) of 0.24 ± 0.18 mg kg^{-1} wet wt in all fish species (Table 2). Of these, 13 fish samples analysed (40%) presented MeHg concentrations higher than 0.20 mg kg^{-1} wet wt. Furthermore, carnivorous species of fish, which represented 69% of the fish sampled, showed a mean methylmercury concentration of 0.32 ± 0.25 mg kg^{-1} wet wt. Tucunare (*Ciclha* sp.) seemed to be good indicator species for mercury pollution in the Balbina reservoir. This carnivorous species presented a mean methylmercury concentration of 0.24 ± 0.16 mg kg^{-1} wet wt ($N = 17$).

One of the parameters which may affect the mercury concentration in Balbina fish is the age of the reservoir, which was filled in 1989. Fish of newly impounded reservoirs in the USA, Canada and Finland, with a high organic matter content in the water, presented an elevated mercury concentration.[5,7,8] In new reservoirs, the mercury load introduced originated from inundated soil and vegetation. Moreover, addition of organic material in water, associated with enhanced bacterial activity, is known to increase methylation of mercury, which then may favour bioaccumulation of methylmercury in fish.[7,8,30] The Atmospheric deposition is expected to be small and, together with soil leaching, is the main mercury source to the Balbina watershed.[6]

In a new hydroelectric reservoir, at Tucuruí on the Brazilian Amazon, (situated about 110 km to the north-east of the Serra Pelada goldmining area), all carnivorous fish presented high mercury concentrations and the mean mercury content was 1.30 mg kg^{-1} wet wt ($N = 121$).[14] Tucunare (*Ciclha temensis*) and pescada (*Plagioscion squamosissimus*) seemed to be good indicator species for mercury pollution in the Tucuruí reservoir.[14]

The carnivorous fish samples from the Bento Gomes river and the Fazenda Ipiranga lake at the Pantanal watersheds, which presented very low MeHg concentrations, can be considered as natural background ($\leq$0.20 mg kg^{-1} wet wt[29]). At the Pantanal watershed, high biological production might allow a large dilution capacity for any mercury present in the drainage. Also, mercury exported associated with suspended particles is efficiently trapped by the various flood plains, swamps and lakes present in the drainage system.[24]

Occurrence of mercury in fish in areas far from goldmining centres in the Amazon may be associated with deforestation due to burning of cleared vegetation and releases of mercury contained in plant tissues.[31] The levels of mercury in aquatic systems are also influenced by terrestrial systems. Leaching of minerals from the soil as well as soil erosion may constitute important sources of mercury for Amazon ecosystems.[9] Otherwise, fish migration acts as a dispersal agents for pollutants along the river basins and could explain the presence of high mercury levels in fish in rivers never exposed to goldmining.

At the Madeira river no significant difference was observed between mean percentages of MeHg relative to total mercury in carnivorous species (93%) and omnivorous species (92.5%) ($t_{21} = 2.173$ and $P > 0.05$). Omnivorous species had a mean MeHg percentage significantly higher than detritivorous species (76.7%, $t_{14} = 3.761$ and $P < 0.001$). Otherwise, in the Negro river, a significant difference was observed between mean percentages of MeHg of carnivorous species (98.6%) and detritivorous species (77.6%) ($t_{31} = 2.142$ and $P < 0.02$). No significant difference ($t_{29} = 0.1568$, $P > 0.50$) in the mean percentages of MeHg relative to total mercury was observed between carnivorous and omnivorous species from the Balbina reservoir (97.3% for carnivorous species, and 96.4% for omnivorous species).

We observed a tendency for increased MeHg concentration with fish weight in some carnivorous fish, such as *Cichla* sp. (tucunaré) and *Serrasalmus rhombeus* (piranha). The body weight of *Cichla* sp. (tucunaré) sampled from the Madeira ($N = 8$) and Tapajós (Itaituba) ($N = 5$) river basins correlated significantly with MeHg concentrations ($r = 0.75$ and 0.88; $P < 0.5$) and this relationship was linear. With the same fish species from the Negro river ($N = 5$) and from the Balbina reservoir ($N = 17$), fish body weight did not present good correlation with MeHg concentration ($r = 0.34$ and 0.39; $P < 0.5$).

Analysis of variance and Tukey's multiple comparison procedure showed that MeHg concentrations in the muscle of *Cichla* sp. (tucunaré) from the Madeira, Negro, Tapajós river basins and the Balbina reservoir presented a significant difference ($F = 12.583$; $P = 2 \times 10^{-6}$).

Tukey's multiple comparison procedure showed that MeHg concentrations of tucunaré from the Tapajós river basin (Itaituba) were significantly higher than those from other areas such as the Madeira, Negro and Tapajós (Santarém) river basins and the Balbina reservoir ($P < 0.05$). MeHg concentrations of tucunaré from the Madeira and Negro river basins were similar and significantly higher than from the Balbina reservoir and the Tapajós river basin near Santarém city. Tucunaré samples from the Balbina reservoir and the Tapajós river basin (Santarém) presented similar MeHg levels.

Analysis of variance and Tukey's multiple comparison procedure verified that MeHg concentrations in the muscle of *Serrasalmus rhombeus* (piranha) from the Madeira and Negro river basins, the Bento Gomes river and the Balbina reservoir presented a significant difference ($F = 11.7863$; $P = 6.1 \times 10^{-5}$).

Tukey's multiple comparison procedure showed that MeHg concentrations of piranha from the Bento Gomes river were significantly lower than these from other areas such as the Madeira and Negro river basins and the Balbina reservoir ($P < 0.05$). MeHg concentrations in piranha in the Madeira and Negro river basins and the Balbina reservoir were similar.

In general, the percentage differences between the environmental coefficients of variation of MeHg compared with total mercury for fish species samples presented positive and high variability on sampling sites close to goldmining activity, such as observed for *Serrasalmus rhombeus* (piranha) (+24%) and *Hydrolycus* sp (peixe cachorro) (+35%) from the Madeira river (Table 3). The Madeira river basin is a typical white-water area with a high suspended particles content and with high biological productivity, which may give rise to a large dilution capacity for mercury present in its drainage system and a consequent increase in the percentage of environmental variation coefficients relative to total mercury for the different fish species samples. The percentage difference between the environmental coefficients of variation of MeHg and total mercury for fish species samples was negative and high in non-impacted areas, such as the Negro river basin. *Ciclha* sp. (tucunaré) collected at the Negro river presented a negative difference of 33%. The Negro river basin is a typical black-water area with a high density of hydromorphic podsoils in its drainage basin, high organic matter content in the water and low pH.[23] This combination of environmental factors and low productivity may result on a decrease in the percentage of environmental variation coefficients of MeHg to total mercury in the different fish species samples.

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