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Hg Biomagnification in the Ichthyofauna of the Tapajós River Region, Amazonia, Brazil

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Methylmercury (MeHg) is a well known human neurotoxin (Clarkson, 1994). The general population is primarily exposed to MeHg through fish consumption. In July 1969, United States Food and Drug Administration (FDA) set an Action Level of 0.5 $\mu g.g^{-1}$ -1 $\mu g.g^{-1}$ for concentration of Hg in fish.

Aquatic organisms accumulate Hg from food, water and sediment. It has been demonstrated that from 75% to 95% of all Hg accumulated in tissues of fish is usually MeHg, with inorganic Hg as the source (Huckabee, 1979). The direct bioaccumulation, or bioconcentration factor (BCF) of Hg is defined as the ratio of Hg concentration in fish tissue to the Hg concentration in water. The indirect bioaccumulation or biomagnification is the accumulation of a chemical in a given species according to its trophic levels in the food chain (Bruggeman, 1982). MeHg attains its highest concentration in the tissues of fish at the top of aquatic food chain (WHO,1989). It is generally agreed that Hg concentrations in carnivorous fish are higher than in non-carnivorous species (Watras & Huckabee, 1994). Carnivorous species in a food chain are placed at a higher trophic level than non-carnivorous species.

The aim of this article is the assessment of the indirect bioaccumulation process or biomagnification of Hg in the Amazonian ichthyofauna.

MATERIALS AND METHODS

Fish samples were collected from gold-mining contaminated area and the background area in the Tapajós River Region. The contaminated area is located in the Tapajós river between the cities of Jacareacanga and Itaituba (04°15'23"S-55°54'33"W), containing gold mining along the tributaries of the river. sites Akagi, et al. (1994) and Bidone, et al. (1997) have shown this area to be strongly contaminated by Hg from gold mining. The background site is located in a fluvial lacustrine system near Santarém city (02°25'11"S-54°42'16"W), 250 Km downstream the contaminated site. This site is not contaminated, but has the same basic characteristics as the contaminated site. We sampled and analyzed 541 specimens from 22 fish species: 238 specimens of 15 fish species from the contaminated site, and 303 specimens of 16 fish species from the control site. Each fish was classified, weighed, and its length was measured at the time of collection. They were placed in polyethylene bags and frozen. Hg was analyzed

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in the fish muscle through Atomic Absorption Spectrophotometer (A- G*N*ARIAN MODEL) using a Vapor Generation Accessory-VGA (CVAAS). The samples were digested in (1:1) sulfuric-nitric acid solution in the presence of vanadium pentoxido 0.1%; the oxidation completed by adding potassium permanganate 6% until the fixation of the violet color. Immediately before the determination, the excess of permanganate was reduced with hydroxylamine 50% (Campos, 1990). Reference standard IAEA-fish muscle tissue with a certified Hg concentration of 0.74±0.13 $\mu g.g^{-1}$ were also analyzed, giving a value of 0.73±0.08 $\mu g.g^{-1}$ (n=4).

Statistical approach (Student's T-test and non parametric analysis of variance followed by pos-hoc Kruskal-Wallis and Mann-Whitney U-Wilcoxon Rank Sum W Test were used when appropriate and $p<0.0001$ and $p<0.0005$ were accepted as confidence limit) was used in order to test the hypothesis about differences in Hg mean concentration in tissue related with food habit of fish. Also, FEF (FEF= $Hg_{contaminated\ site}$ -$Hg_{background}$ / $Hg_{background}$) calculation was performed in order to evaluate the increase of Hg concentration in fish tissue from contaminated area.

RESULTS AND DISCUSSION

In previous studies we showed that Hg concentrations in carnivorous fish were almost one order of magnitude higher than Hg in noncarnivorous fish from both areas (Student's t-test: $p<0.001$) the contaminated area and the background area. Likewise, in the contaminated site, carnivorous, noncarnivorous fish and whole fish had Hg concentrations about 1.8, 1.6 and 1.5 times higher, respectively, than those from background area, and these differences between means were statistically significant (Student's t-test; $p<0.001$) (Castilhos, et al., 1998). The original "fish enrichment factor" proposed for Hg, calculated as FEF= ($Hg_{contaminated\ site}$ -$Hg_{background}$) /$Hg_{background}$, showed that the contaminated site is richer *vis-à-vis* the background for total, noncarnivorous and carnivorous fish by 0.5, 0.6 to 0.8 (or plus 50%, 60% and 80%), respectively. In order to assess the Hg biomagnification processes in the ichthyofauna of the studied areas, the data were worked out whereby distinct food habits and their correspondent Hg concentrations were taken into account and analyzed. Trying to establish trophic sequences in the ichthyofauna from Amazon Region and their Hg accumulation, the present study aims to supplement the initial analysis that was done just between Hg levels in carnivorous and noncarnivorous species (Bidone, et al., 1997; Castilhos, et al., 1998)

The carnivorous fish species were classified as **(i)** omnivorous-predatory *(Plagioscium* sp. and *Serrassalmus* sp.) and **(ii)** ichthyophagous or tertiary consumers *(Pellona* sp., *Hydrolycus scomberoides, Brachyplatystoma flavicans, Brachyplatystoma filamentosum, Crenicichla* sp., *Pimelodus blochii, Brachyplatystoma vaillantii, Raphiodon* sp., *Pseudoplatystoma fasciaturn, Hoplias* sp., and *Cichla* sp.). The noncarnivorous fish species were classified as **(i)** detritivorous *(Geophagus surinamensis, Prochilodus nigricans, Semaprochilodus brama);* **(ii)** herbivorous *(Laemolyta* sp. and *Myleus* sp.); **(iii)** omnivorous *(Astronotus ocellatus, Brycon* sp., *Colossoma macropomum);* and **(iv)** planktivorous *(Hypophtalmus marginatus).*

The carnivorous ichthyophagous fish species are pelagics and they are active in water column. Adults feed mainly on other fish. Predatory-omnivorous species

feed on freshwater shrimps and fish. The herbivorous fish feed on fruits, roots, seeds and other parts of the plant material. For these species, the flooded forest is an important food source because during this season, many vegetable materials from terrestrial origin become available for them. The planktivorous fish feed exclusively on plankton. They live in the flooded areas and areas with stagnated or slow running water, such as marginal lakes and mouth of rivers. The omnivorous fish form the most numerous group. They can feed from either animal food or vegetable sources. Several species belong to this feeding habit and because of their high capacity for feeding different kinds of food, they do not show specific morphological structures. The detritivorous fish have as basic food source algae, bacteria and other microorganisms living in slime or mud and detritus.

Comparative analysis between mean Hg in fish tissues from each food habit was made for both background and contaminated areas and the results are shown in Table 1.

Student's T-test showed that mean Hg concentrations in both carnivorous and noncarnivorous fish as well as in their food was higher in fish from contaminated area than those in the control. Tendency was observed in positive values for FEF. Comparison of the Hg tissue levels between carnivorous ichthyophagous and omnivorous predatory showed no significant differences in the background and contaminated areas. Hg levels in background area were found to be herbivorous = detritivorous < omnivorous < planktivorous. For contaminated area sequence was herbivorous < detritivorous = omnivorous. Aula et al. (1994) reported that potentially contaminated area Hg levels were higher in planktivorous than in detritivorous and omnivorous fish. Taking into account the results obtained by these authors, in the contaminated area of this study, the sequence could be as herbivorous < detritivorous = onmivorous <planktivorous.

Table 1. Hg concentration (mean ± SD) in muscular tissues of fish ($\mu g.Kg^{-1}$) from the background [I] and the contaminated area [2]. "Fish enrichment factor", FEF= $(Hg_{\text{contaminated area}} - Hg_{\text{background area}}) / Hg_{\text{background area}}$.

Feeding Habit	Hg mean concentration ($\mu g.Kg^{-1}$) [1]	[2]	Student's T-test	FEF
Carnivorous	228±171 (159)	420±230 (98)	p<0.0001	0.8
Ichthyophagous	236 ± 189 (115)	448 ± 195 (59)	p<0.0001	0.9
Omnivorous	206 ± 104 (44)	381 ±281 (39)	n.s.	0.8
Student's T-test	n.s.	n.s.	n.s.	
Noncarnívorous	39±47 (144)	62± 53 (140)	p<0.0001	0.6
Detritivorous	26± 20 (57)	90± 67 (43)	p<0.0001	2.5
Herbivorous	30± 40 (58)	49± 41 (89)	p<0.05	0.6
Omnivorous	36 ± 13 (17)	72 ± 30 (8)	p<0.01	1.0
Planktivorous	149 ± 60 (12)			

(n) is the number of samples

In both background and contaminated areas, highest Hg concentrations were found in tissues of carnivorous fish. Hg concentration below these were found in planktivorous fish. Hg concentration in the planktivorous fish were lower than that found in carnivors but higher than the noncarnivors. The planktivorous fish fed almost exclusively on phytoplankton and zooplankton. Both dissolved, inorganic

Hg and MeHg, accumulate in phytoplankton by passive diffusion across the membrane (Mason et al., 1995). However, in contrast to MeHg, inorganic Hg is not biomagnified as the trophic transfer from phytoplankton to zooplankton. Mason et al (1995) suggested that there are differences in partitioning within phytoplankton cells between inorganic Hg- which is principally membrane bound- and MeHg- which accumulates in the cytoplasm. In the model "eat- the -grapes- and- spit- the- skins" (Mason et al.,1995) of zooplankton feeding assumes that the zooplanktons digest the dissolved cytoplasmatic content but simply defecate the membrane material, would explain the efficient assimilation of MeHg and the poor assimilation of inorganic Hg. Perhaps the highest levels of Hg in planktivorous fish in the noncarnivorous group can be related with dissolved Hg, which is the most bioavailable to phytoplankton accumulation.

Hg concentration in Amazonian river water is linked to particulate suspended material and is estimated to be about 10^{-1} mg.Kg^{-1} (Aula et al., 1994;Roulet et al., 1998). Mean concentration of dissolved inorganic Hg has been estimate to be $\leq 10^{-2}$ mg.Kg^{-1} (Solomons and Förstner, 1984). The organic matter (O.M.) is a well known carrier of Hg in natural and polluted water and this complex is suggested to reduce the metal bioavailability. A positive and statistically significant relationship is reported between Hg concentration in Amazonian river water and organic matter present in suspended or nonfiltrable materials (Aula et al., 1994). As the size of plankton range from 1µm to 100µm, they may be included in the suspended materials and may compose part of the O.M. total content in the suspended materials. The other part of the suspended O.M. is of plant origin and comes from soils or forest litter erosion. This portion presents mean Hg around 10^{-2} mg.kg^{-1}. Nonorganic matter in suspended materials are clay minerals originating from rocks and soil through weathering process. They have Hg baseline mean concentration around 10^{-2} mg.kg^{-1} or less.

Hg concentration associated with O.M. is estimated to be in the magnitude of 10^{-1}mg.kg^{-1} If it is assumed that (i) the total O.M is 10% of the suspended material and Hg concentration in this suspended material is around 10^{-1}mg.kg^{-1}, then Hg concentration of nonorganic component, which is the remainder 90% of suspended material would be 10^{-2} mg.kg^{-1}. These assumptions and other alternatives are shown in Table 2.

Table 2. Estimates of Hg concentration in organic matter (O.M.), nonorganic (N.O.M.) components of suspended solids (S.S.), plankton fraction of O.M. and non-plankton fraction of O.M. from Amazonian River water.

S.S.	Hg water (mg. L^{-1})	Participation in SS (%)	Hg (mg.kg^{-1})
	50	100	10^{-1}
$\sum$ N.O.M. in S.S.	45	90	10^{-2}
$\sum$ O.M. in S.S.	5	10	10^{1} *
Non-Plankton in O.M			10^{-2}
Plankton in $\sum$ O.M.	5	100	10^{1}
	0.5	10	10^{2} **
	0.05	1	10^{3}

* Hg in S.S= (Hg N.O.M. * 0.9) + (Hg $\sum$0.M.* 0,1) / 10

** Hg $\sum$0.M. = (Hg nonplankton * 0,9 + Hg plankton * 0,1) / 10

Bidone et al. (1993) estimated primary production of algae in pristine amazonian rivers as 10^{-1} mg.l^{-1} suspended material level fewer than 20mg.l^{-1} and the O.M in this suspended material as far as 30%. The actual value for algae production could be several times higher than this estimated value. During rainy season rivers overflow forming 'igapós", i.e., riparian zones, flooded savannas and extensive floodplains. These areas filter material derived from uplands and regulate inputs to the river channel. The plankton usually represents only a percentage of the total O.M. component of river suspended material. Despite their low numbers, plankton must be able to bioconcentrate Hg in large quantities. For instance, if plankton represents 1% of the total O.M. which has Hg concentration estimated as 10^{-1} mg.l^{-1}, the estimated Hg for plankton could be 10^{3} mg.l^{-1}.

Lowest Hg tissue levels were found in herbivorous and detritivorous fish. Herbivorous fish generally have low Hg concentration. Stable isotope data indicate that detritivorous Characiformes species (such as *Prochilodus nigricans* and *Semaprochilodus brama)* must receive a large fraction of their carbon from phytoplankton and very littler from other plant groups (Araujo-Lima et al, 1986). However, the authors suggested that, as organic matter encountered in stomach analysis was largely unrecognizable, it was not clear whether these fish were receiving their energy, directly from phytoplankton by consuming their sedimented remains or indirectly by consuming the remains of organisms at higher trophic level (Araujo-Lima et al, 1986). The resulting ^{13}C merely indicate that carbon in these fish was derived from a food chain with phytoplankton as its initial stage.

The estimated mean Hg concentrations in fluvial sediments from noncontaminated areas in Amazon Region are 0.05mg.kg^{-1} or less (Malm, 1991). These estimated values are close to 0.03mg.kg^{-1} which is the estimated value for global background (Solomons and Förstner, 1984). From contaminated areas of Amazon Region (usually areas sampled are inside "garimpos" area) the Hg levels in fluvial sediment have been found one to two orders of magnitude higher than uncontaminated areas. This Hg contamination in sediments, which is the main compartment for detritivorous food source, agree with the significant difference ($p<0.0001$) found between detritivorous from background and contaminated area, with FEF resulting 2.5, the higher FEF calculated in Table 1. Considering these ideas the relation of Hg tissue levels between herbivorous and detritivorous from contaminated area are very well explained. This relation has been found in other contaminated areas in Amazon Region (Malm, 1991; Lacerda et al., 1994). Therefore, Hg levels in fluvial sediments in the background area should have explained the depressed Hg concentration in detritivorous fish close to herbivorous fish, which allowing igualitary relation between Hg levels in herbivorous and detritivorous fish.

The sediment contamination might not affect the Hg concentration in herbivorous fish, because the difference observed between their Hg levels from background and from contaminated areas showed the lowest calculated FEF (FEF=0,6) as presented in Table 1, whereas the Student's T-test resulted in significant difference. On the other hand the herbivorous fish get used to feed from floating plants or seeds and fruits from alloctone vegetable sources, usually feed themselves in "igapós" areas. These plants do not absorve large quantities of Hg and have no tendency for accumulation of Hg very much. Aula et al. (1994) have

demonstrated that Hg levels in sedimentable solids, organic matter of sediments and soil are at least one order the magnitude higher than in floating plants in the reservoir. So, herbivorous fish usually show the Hg levels similar in both contaminated and background areas.

Taking into account these arguments, one could suggest a general trophic sequence for biomagnification of Hg in the ichthyofauna from the studied region as: herbivorous = or <detritivorous< omnivorous< planktivorous< carnivorous omnivorous= carnivorous ichthyophagous. Table 3 shows a synthesis of the sequence proposed for biomagnification.

Table 3. Synthesis of the main elements affecting the generic trophic sequence proposed for the ichthyofauna for study areas.

Trophic sequence	**Herbivorous < or = detritivorous < omnnivorous < planktivorous < carnivorous**				
Food habit	herbivorous	detritivorous	omnivorous	planktivorous	carnivorous
Food	vegetables	detritus	diverse	plankton	fish
Hg in food	10^{-3} (*)	---------->		10^{-3} (**)	10^{-3} - 1
MeHg in food	n.d. (***)	---------->		15% (****)	75-90%(#)
BCF (1)	10^4			10^5	10^5
BCF (2)	10^4			10^5	10^5

(*) Aula et al., 1994; (**) Estimation in this study; (***) Lodenius, 1994; (****) Porcela, 1994, Akagi, 1994 (#).

Concentrations of Hg in principal items of fish food are shown in magnitude order of $mg.Kg^{-1}$ (dry weight). The estimated Hg concentration in water (10^{-3} $\mu g.l^{-1}$) and the Hg concentration in fish presented in Table 1 were used to calculate the fish BCF for both background (1) and (2) contaminated areas.

Elimination of MeHg by fish is very slow relative to the rates of uptake and the accumulation. Positive correlation between concentrations in muscle and both size, length and/or age for a given specie have been well documented (WHO,1989). However, the present results showed no correlation between fish Hg concentration and weight or length for any group. Maybe it is because we are dealing with many species within each group. The results showed that carnivorous ichthyophagous from background area are higher ($t=7.86$; $p<0.001$) and heavier ($t=3.01$, $p<0.05$) than those from contaminated area. The carnivorous omnivorous from background area are heavier than those from contaminated area ($t=2,32$; $p<0.05$) also, but showed no different size. So, the actual FEF could be higher than FEF calculated in both carnivorous ichthyophagous and carnivorous omnivorous. Except noncarnivorous onmivorous, the other noncarnivorous showed no differences in length or weight between background and contaminated areas. The omnivorous from contaminated area are bigger and more contaminated than those from background area. Their FEF could be lower than FEF calculated and shown in Table 1. These factors certainly do not interfere with the proposed Hg biomagnification sequence in the food chain structure for the study area.

We selected 188 specimens from four species belonging to three different food habits (carnivorous - *Brachyplatystoma vaillantii* and *Cichla* sp - , detritivorous - *Semaprochilodus brama* - and herbivorous - *Myleus* sp). They were estimated to be one year old or in the first maturation, inferred by their length (Ruffino & Isaac, 1995). Their Hg concentration (which are not presented in this paper) were

compared by using these equations: Hg carnivorous - Hg detritivorous/ Hg detritivorous; Hg detritivorous - Hg herbivorous/Hg herbivorous; Hg carnivorous - Hg herbivorous/Hg herbivorous. The “fish biomagnification (enrichment) factors” for Hg, proposed here, showed that the carnivorous fish were enriched *vis-a-vis* the detritivorous and herbivorous fish from 2 to 10 (or plus 200% and 1000%) and detritivorous fish were enriched vis-a vis herbivorous fish close to 200%, for both areas. These results agree with previous ones.

These results must be seen with caution because several other parameters may influence the Hg bioconcentration and biomagnification. Among them, one could mention the Amazonian seasons, which show a pronounced waterlevel fluctuation. Drastic environmental and related fish food supply changes result from the great monomodal floodpattern.

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