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FULL PAPER

Amazon Riparian People's Exposure to Legacy Organochlorine Pesticides and Methylmercury from Catfish (*Ageneiosus brevifilis*) Intake

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Abstract:

The worldwide massive use of organochlorine pesticides (OCPs) and mercury (Hg) is taken as an environmental threat due to their high toxicity, potential for long-range transport, bioaccumulation and persistence. Both, OCPs and Hg, were widely used in the Brazilian Amazon region and deserve more attention in terms of environmental and public health. Focusing on expanding the knowledge about impacted areas and its consequences for local people, this study measured concentrations of OCPs and Hg in catfish (*Ageneiosus brevifilis*) samples. Moreover, a non-target analysis of organohalogen contaminants was carried out by means of full-scan screening. DDT and methylmercury showed the highest concentration values, ranging from 101 to 2061 µg kg^{-1} w.w. and 629 to 2009 µg kg^{-1} w.w., respectively. Pentachloroanisole was the only non-target organohalogenated compound identified and it was present in all samples. Estimated daily intake values did not exceed the safe reference limits proposed by the World Health Organization for OCPs, with the exception in some cases of heptachlor. On the other hand, the safe reference value of methylmercury was surpassed in all the fish samples. Results dismiss a recent input of OCPs due to greater contribution of pesticide metabolites, however, they point out a health risk hazard to riparian people. The concentrations of methylmercury and total dichlorodiphenyltrichloroethane, were up to one order of importance higher than other contaminants, which endorses that this region is still a hotspot for these contaminants.

Keywords: estimated daily intake; organohalogen contaminants; persistent organic pollutants; risk assessment

1. Introduction

Contamination by anthropic activities has been taken as a worldwide concern issue, since the second half of last century, even in remote or pristine areas. However, there is still a lack of information about the real threats regarding high polluted areas or places which had a massive historical use of harmful substances, mainly if possible reactions in the environment and its metabolites are considered [1]. Nowadays, despite the restrictive measures adopted in recent years, organochlorine pesticides (OCPs) and mercury (Hg) continue to act as important threats to the environment because of their high potential for long-range transport, bioaccumulation, environmental persistence and high toxicity [2].

The usage of synthetic pesticides comes from the forties, with the discovery of legacy organochlorine pesticides and the beginning of their application [3]. They were widely produced and spread, until Rachel Carson (1962) warned about their environmental threats, publishing the 'Silent Spring' book [4]. Since then, each government started to implement restrictive measures concerning organic contaminants and in 2001, most of the countries decided to join the Stockholm Convention in a global treaty to protect human health and the environment from Persistent Organic Pollutants (POPs). The

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Stockholm Convention's restrictive measures entered into force in 2004, initially including twelve organohalogenated chemicals, and continues to list new substances once they are proven to behave like POPs [5].

The production of pesticides on an industrial scale made Brazil one of the main exporters of pesticides in South America [6]. The most used OCP in Brazil, perhaps in the world, was dichlorodiphenyltrichloroethane (DDT) [7]. Once in the environment, DDT can persist in its technical form over many years and can also breakdown into more toxic forms, or even into other still unstudied related compounds [1, 10]. Used in Brazil since 1945 for agricultural and sanitary (e.g. residual indoor spraying) purposes, DDT use was first forbidden in agriculture, together with other POPs, in 1985. However, its use in public health campaigns lasted until 1997. In 1998, the Brazilian Ministry of Health prohibited the use of DDT for any purpose in the country, yet, its total banishment only took place in 2009 [8]. Nonetheless, a recent study suggests an input of fresh DDT in the Brazilian Amazon [9].

Hg occurs naturally in the environment under several forms, but its massive use for different purposes has become an environmental concern due to an increase in its concentration at specific places [11]. Regarding Hg speciation, the most common organic form of Hg is methylmercury (MeHg), which is also one of the six most toxic substances in the world and, like POPs, MeHg increases in concentrations up trophic levels, reaching high concentrations in top-chain organisms [12]. During the last decades of the twentieth century, Hg contamination in the Brazilian Amazon region had been mostly associated with gold mining activities and its emissions have been estimated at 31 tons per year in Brazil [13]. However, more recent studies have also shown that the Amazon region soil is naturally abundant in Hg and its remobilization to water bodies leads to Hg enrichment in aquatic environments [14, 15].

Fishes are considered good monitors to evaluate environmental pollution, such as OCP and MeHg contamination. In addition, fishes are the main via to human exposure, especially those at high trophic level due to the enrichment of biomagnifying contaminants. Hence, carnivorous catfishes seem to be good species to assess environmental contamination and human exposure. Small catfish species, such as *Ageneiosus brevifilis,* have very low commercial value and are very commonly consumed by Amazon riparian dwellers [16]. Therefore, this study aimed to expand the knowledge on environmental contamination by massive used contaminants in Brazil and to indicate potential threats to Amazon riparian people regarding their main dietary protein source.

2. Materials and Methods

Barcelos is the biggest city of Amazonas state and the second biggest of Brazil in terms of area (122,476 km²). Located upside the Negro River (0° 58′ 30″ S, 62° 55′ 26″ W) (Figure 1), its weather is typically tropical. Barcelos population is estimated at 27,589 inhabitants, which means a population density of 0.23 inhabitant/km², extremely dependent on fishing for their economy and feeding habits [26].

Commonly known as *Mandubé* at the Amazon region, the *Ageneiosus brevifilis*, is a leathery fish well distributed in the Amazon basin freshwaters. When mature the species can reach up to 50 cm in total length and approximately 2.5 kg. *A. brevifilis* is described as a bottom feeding fish, which lives in contact with muddy water. Moreover, this fish species forages during nocturnal periods with a carnivorous diet. They feed mainly on small fishes and invertebrates, such as crustaceans and insects. The first sexual differentiation of *A. brevifilis* occurs close to 215 mm and the reproductive period goes from November to February [28]. Caught in abundance and sold cheaply, this species is a very important source of protein at most Amazon fish markets, mainly to the poorest riparian populations.

For this study, fish samples (n=12) were bought at the local fish market, in Barcelos city, Amazonas, Brazil (September, 2015). Samples were then kept frozen and transported to the Radioisotopes Laboratory Eduardo Penna Franca (LREPF) at the Federal University of Rio de Janeiro, Brazil. In order to obtain water content muscle fish samples were previously weighed and then freeze-dried by lyophilization before analytical steps for chemical determinations. OCP determinations were based on a miniaturized method optimized for exhaustive extraction and simultaneous purification of halogenated pollutants in fat containing matrices [29], with modifications. PCB 103 and 198 (100 µL) were added to ~0.5 g of catfish samples (*Ageneiosus brevifilis*) as internal standards. Extraction and purification steps were performed in a single column using matrix solid-phase dispersion with co-sorbents and modified silica gel layers, respectively. Extracts were dried and then reconstituted using 100 µL of injection standard tetrachloro-m-xylene (TCMX 100 ng g^{-1}) in isooctane.

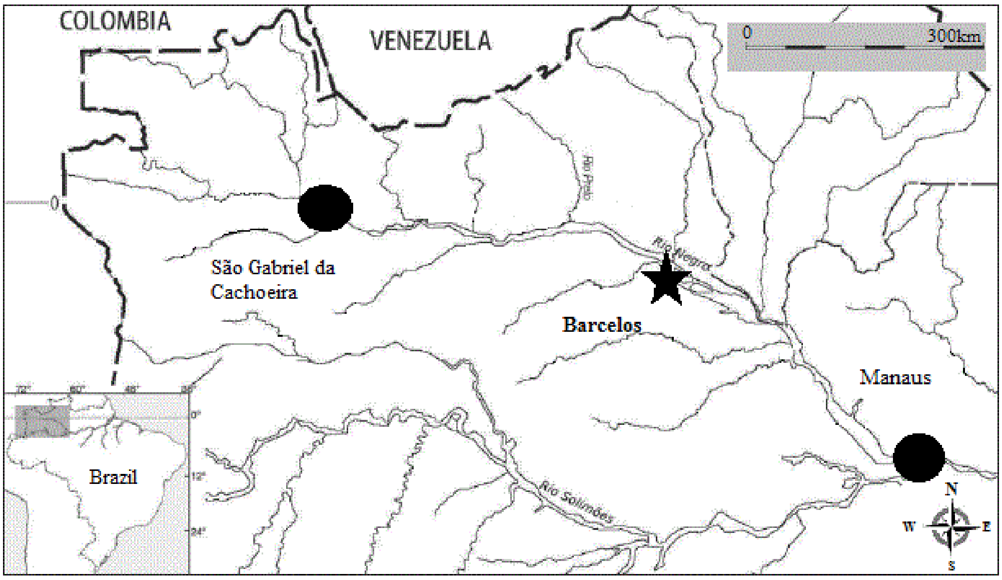

Figure 1. Negro River basin in the Brazilian Amazon region. Sampling point (black star) highlighted between the two main cities of Amazonas state, São Gabriel da Cachoeira and Manaus. Adapted from Emperaire & Eloy [27].

Organochlorine pollutants were determined using a 7890N gas chromatograph equipped with a DB5 fused silica capillary column (60 m x 0.25 mm x 0.25 µm film thickness) and coupled with a 5975C quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA). The equipment operated in the selected ion monitoring mode (SIM) for target compounds – 21 OCP related chemicals, from the standard solution Pesticide Mix 1 (AccuStandard, New Haven, USA): hexachlorobenzene (HCB); hexaclorocyclohexane (*α*, *β*, *δ* and *γ*-HCH); *Cis* and *Trans*-Chlordane; oxychlordane; dichlorodiphenyltrichloroethane (*o,p'* and *p,p'*-DDD, DDE and DDT); *α* and *β*-endosulfan; heptachlor; *cis* and *trans*-heptachlor epoxide; methoxychlor and mirex. Additionally, full-scan mode screening was performed to investigate non-target organohalogen contaminants. Negative chemical ionization source (NCI) was used in both cases. Sample injection (2 µL) was made at 260 ºC in pulsed hot splitless mode (4.0 min pulse and splitless time). Methane was used as carrier and reaction gas in a constant flow rate of 1.5 mL/min. The oven temperature programme was: 120 ºC for 4.2 min, 30 ºC/min to 200 ºC, 5 ºC/min to 275 ºC, 40 ºC/min to 300 ºC, held 10 min, and finally ramped at 10 ºC/min to 310 ºC and held 2 min. The temperatures of the transfer line, source and quadrupole were set at 300 ºC, 150 ºC and 150 ºC, respectively.

Total Mercury (THg) determinations were carried out following the method described by Bastos *et al.*, 1998 [30]. Briefly, the method consists in acid digestion (H_2SO_4 + HNO_3 - 1:1) followed by potassium permanganate oxidation ($KMnO_4$ 5%) of ~0.4 g (freeze-dried sample). Atomic absorption spectrophotometry coupled with a cold vapor generator, was used to measure THg concentrations (FIMS 400 Perkin-Elmer). Methylmercury concentrations were measured from ~0.03 g (freeze-dried sample) by KOH methanol (25%) digestion followed by ethylating with sodium tetraethyl borate as a derivatizing agent ($NaBEt_4$), according to Bloom, 1992 [31] and EPA, 2001 [32]. Gas chromatography coupled with atomic fluorescence spectrometry was used to measure MeHg concentrations (MERX™ Automated

Methylmercury Analytical System, Brooks Rand).

For quality assurance and quality control (QA/QC), analytical blanks (n=3) were included in each batch to perform blank correction from their average. For OCPs, the performance of PCB 103 and 198 as internal standards were satisfactory with mean values of 93% and a standard deviation of 12%. Mercury and methylmercury-based methods were evaluated, using a certified reference material from the National Research Council (Dorm 3, fish protein). Satisfactory recovery performances were obtained with 98 and 112% for THg and MeHg, respectively. The method limit of quantification (LOQ) was calculated by the average of the blanks plus 3 times its standard deviation.

Regarding chronic exposure to some harmful substances, the World Health Organization (WHO) proposes an Acceptable Daily Intake (ADI) as the maximum quantity of a toxic substance that offers no risk, even if ingested daily, during an individual's entire life. The ADI value is not definitive and is expressed in μg kg^{-1} day^{-1} (body weight) [33]. Brazilian data for fish consumption is estimated in 10.6 kg per inhabitant per year, mean value of 29 g per inhabitant per day [25]. Nonetheless, previous studies have reported that riparian people eat much more fish than the national average and they suggest a mean value of 200 g per inhabitant per day [24-36]. Thus, Estimated Daily Intakes (EDI) were measured according to local fish consumption, for riparian people, and adult mean body weight of 70 kg, according to Equation 1. Eq. (1) represents the EDI as the contaminant concentration in microgram per kilo per day (μg kg^{-1} day^{-1}), which is equal to the contaminant concentration, in microgram per gram in wet weight (μg g^{-1} w.w.), per sample, multiplied by the local fish intake per body weight (g/inhabitant/day/70 kg). EDI values were then compared to each ADI [37].

$$\mathrm{EDI}\left(\frac{\mu\mathrm{g\,kg^{-1}}}{\mathrm{day}}\right) = Conc.\left(\frac{\mu g}{g\,(w.w.)}\right) * \frac{g/inhabitant/day}{70\ \mathrm{kg}}$$

Eq. (1)

The Graphpad Prism 5.0 Statistical Software System was used for statistical analyses and graphic composition. Shapiro–Wilk test was used in order to test data normality. Kruskal–Wallis and Dunn multiple comparison tests were applied for comparing different contaminants concentration of non-parametric data. The adopted significance level was 5% for all tests.

3. Results and Discussion

For this study, Hg and legacy OCPs were frequently detected in all fish samples at some level. Results were grouped by the sum of pesticide related substances. Only MeHg concentrations were discussed in this study, because of its predominance in muscle tissues of the analyzed samples and its toxicological relevance. The mean percentage of MeHg regarding the THg, for all samples, was of 98 ± 6%. Concentration details of OCPs, MeHg and THg are described in Table 1. The sum of pesticides are presented as: HCHs = $\alpha + \beta + \delta + \gamma$–HCH; chlordanes = *Cis* and *Trans*-chlordane + oxychlordane; Dichlorodiphenyltrichloroethane and its metabolites (DDX) = *o,p'* and *p,p'*-DDD + DDE + DDT. Concentrations were expressed in μg kg^{-1} of wet weight, in order to assess contamination levels for fresh fishes, as they are consumed.

For non-target screening analysis, there was no detectable concentration of any compound related to organohalogen contaminants, besides pentachloroanisole (PCA). PCA is a common metabolite of pentachlorophenol (PCP) in fish. Although it is not well documented in Brazil, PCP is commonly used in wood protection, as part of technical formulations for fungicide, algaecide and insecticide [17]. Therefore, PCP might have been used in this region.

The greatest mean values were measured for MeHg (1100 μg kg^{-1}) and ∑-DDX (600 μg kg^{-1}), followed by methoxychlor (240 μg kg^{-1}), ∑-chlordane (72 μg kg^{-1}), heptachlor (70 μg kg^{-1}), ∑-HCH (54 μg kg^{-1}) and HCB (48 μg kg^{-1}). There was no correlation between catfish length and any measured contaminant. Although MeHg and DDT come from completely different sources and were used independently from each other, they presented the greatest values, at the same significance level ($p < 0.05$), in contrast with other OCPs (Figure 2). These results could be explained by the massive input of DDT in the Amazon region, mainly related to fighting vectors of tropical diseases [18]. Even though we cannot dismiss Hg contamination related to outlawed mining activities, that may continue in the surrounding area, some authors have shown that the Amazon region is naturally rich with Hg [14, 15, 19]. Sometimes THg concentrations in fish muscle tissue can be higher than those reported even for areas influenced by gold mining activities [20, 21]. Meanwhile, other OCP concentrations could be more related to diffused sources, such as agrochemical inputs, lower usage in local sanitary campaigns or even long-range atmospheric transport [22].

OCP profiles were quite similar for most of the samples (Figure 3). Regarding DDX specific contributions, it is important to highlight that *p,p'*-isomers showed the highest values compared to *o,p'* and, considering both isomers, DDE showed the greatest mean values (266 μg kg^{-1} w.w.) followed by DDT (180 μg kg^{-1} w.w.) and DDD (154 μg kg^{-1} w.w.). The ratio between the metabolite *p,p'*-DDE and all *p,p'*-DDX (DDE + DDD + DDT) was used in previous studies to evaluate possible fresh input of technical DDT in the environment [23, 24]. The closer to 1, the later the input should be. This is the case for this study which shows a value of 0.8 for the ratio, suggesting a greater contribution of the metabolite negating an assumption of recent inputs of technical DDT.

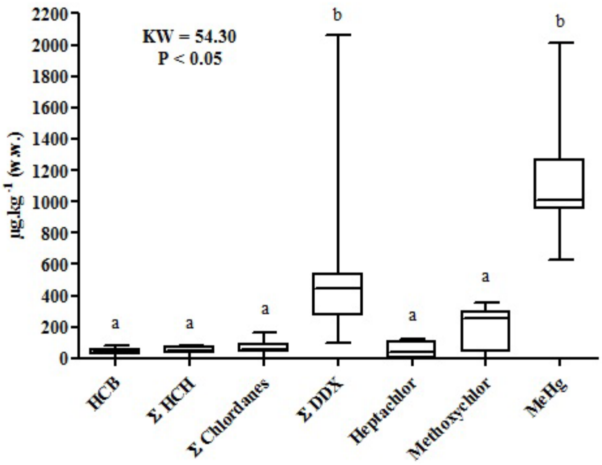

Figure 2. Boxplot representing: (-) Median, ([]) 25% - 75%, (I) minimum and maximum of each contaminant concentration in the catfish samples. Contaminants that differ from each other at the significance level of 0.05 are presented with a different letter.

Table 1. Contaminant concentrations, average ± standard deviation (SD) and limit of quantification (LOQ) in μg kg^{-1}, wet weight, in catfish (*Ageneiosus brevifilis* = *Ab*) muscle tissue samples from Barcelos, Amazonas, Brazil.

Sample	Length	HCB	∑-HCH	∑-Chlordane	∑-DDX	Heptachlor	Methoxychlor	MeHg	THg
***Ab* 1**	35 cm	< LOQ	< LOQ	< LOQ	100	< LOQ	81	990	840
***Ab* 2**	37 cm	44	84	89	510	74	350	1000	760
***Ab* 3**	39 cm	58	48	130	390	100	300	2000	1800
***Ab* 4**	38 cm	57	77	170	2100	110	280	1100	1100
***Ab* 5**	35 cm	43	53	48	200	72	230	1200	1100
***Ab* 6**	41 cm	27	37	64	530	5	32	1600	1300
***Ab* 7**	38 cm	56	56	71	540	15	300	630	630
***Ab* 8**	35 cm	< LOQ	46	10	230	< LOQ	< LOQ	990	1000
***Ab* 9**	37 cm	26	16	42	480	16	< LOQ	760	780
***Ab* 10**	41 cm	83	28	44	420	120	280	950	900
***Ab* 11**	33 cm	33	81	48	380	< LOQ	170	1300	1100
***Ab* 12**	30 cm	51	70	84	1400	130	350	950	760
Average±SD	**37±3 cm**	**48±16**	**54±21**	**72±41**	**600±560**	**70±47**	**240±100**	**1100±380**	**1000±300**
LOQ		**0.2**	**0.1**	**0.4**	**0.6**	**0.5**	**1.5**	**0.3**	**3.2**

∑-HCH = *α* + *β* + *δ* + *γ* –HCH; ∑-chlordane = *Cis* and *Trans*-chlordane + oxychlordane; Dichlorodiphenyltrichloroethane and its metabolites (∑-DDX) = *o,p'* and *p,p'*-DDD + DDE + DDT; methylmercury = MeHg and total mercury = THg.

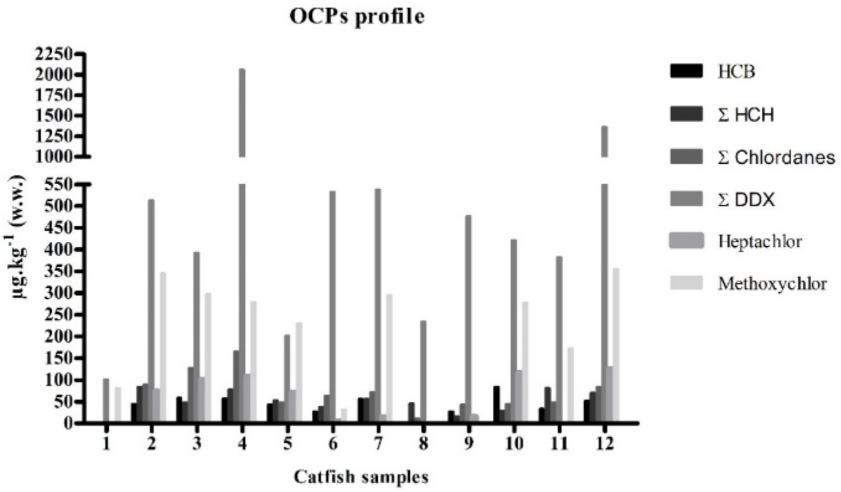

Figure 3. Organochlorine pesticide (OCPs) profile in catfish (*Ageneiosus brevifilis*) samples from Barcelos, Amazonas, Brazil.

In order to evaluate the human exposure to contaminants through catfish consumption, EDI values were compared to the ADI for each contaminant group, regarding the provisory values given by the WHO [25]. ADI provisory values for OCPs provided by the WHO in 2009 are 0.6 μg kg^{-1} day^{-1} for HCB, 5 μg kg^{-1} day^{-1} for ∑-HCH, 0.5 μg kg^{-1} day^{-1} for ∑-chlordane, 10 μg kg^{-1} day^{-1} for ∑-DDX, 0.1 μg kg^{-1} day^{-1} for heptachlor, 10 μg kg^{-1} day^{-1} for methoxychlor and 0.1 μg kg^{-1} day^{-1} for Hg. For most OCPs the EDI did not reach the respective ADI proposed value. Only the heptachlor EDI average (0.15 μg kg^{-1} day^{-1}) exceeded the ADI, with half of the samples (n=6) ranging from 0.2 to 0.36 μg kg^{-1} day^{-1}. Nevertheless, 100% of the catfish samples exceeded the reference acceptable value for mercury, ranging from 1.79 to 5.74 μg kg^{-1} day^{-1}, with an average value of 3.22 μg kg^{-1} day^{-1}, which means an EDI 30 times higher than the ADI.

These values were obtained using a single value, which should comprise a standard for the riparian adult fish consumption and body weight in the Amazon region. Therefore, this evaluation could underestimate the errors associated with the biometric factors of the whole population as well as their fish intake amount. Furthermore, children might represent a high-risk group, if they consume similar amounts of catfish as adults.

4. Conclusions

As depicted by our results, the higher concentrations of ∑-DDX and MeHg, up to one order of importance more than the other contaminants, reflect and endorse the massive use and persistence of DDT as well as the unsettling levels of MeHg, that should be carefully monitored, in the region. Even though the recent input of legacy pesticides could be dismissed, these chemicals can still be a threat for those who live in contaminated areas and have local fishes as their main protein source. More studies ought to be done in order to shelter local people by encouraging them to consume less contaminated species and avoid species that represent a health risk hazard, such as the studied catfish that exceed the ADI for heptachlor and methylmercury.

Regarding the non-target screening, although the presence of PCA could be traced in all samples using the full-scan mode, a more sensitive screening analysis based on SIM mode is needed to investigate the presence of less abundant contaminants. Additionally, quantitative measurement should be carried out to evaluate the risk associated with PCA and contamination.

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