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The concentrations of heavy metals and the incidence of micronucleated erythrocytes and liver EROD activity in two edible fish from the Paraíba do Sul river basin in Brazil

Concentração de metais pesados e incidência de eritrócitos micronucleados e atividade de EROD hepática em duas espécies de peixes comestíveis na bacia do Rio Paraíba do Sul, Brasil

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ABSTRACT

The Paraíba do Sul river (PSR) basin crosses one of the most populated and industrialized regions of Brazil and receives effluent from the metallurgic, paper mill and agricultural industries. PSR water has multiple uses, including being a supply of potable water and riverine populations which consume some species of its fish. In the present study, we investigated the responses of two biomarkers of aquatic pollution, EROD activity in the liver and micronuclei frequency in peripheral blood, as well as the concentrations of seven heavy metals in the muscle tissue of two species of fish native to the PSR: *Geophagus brasiliensis* and *Hypostomus* sp. Sampling was performed in both the wet and dry seasons of 2003, with a total of 339 fish collected from 16 sites along the PSR basin. Fish collected at Levy Gasparian, Barra Mansa and Três Rios both had higher EROD activity and micronuclei frequency. The results also suggested a moderate induction of EROD activity in fish collected from S.L. Paraitinga and Sapucaia. Micronuclei frequency was elevated in fish from S.J. Campos, Além Paraíba, Campos and S.J. Barra. In all cases, heavy metal concentrations did not exceed the maximum considered safe for fish meat consumption.

KEYWORDS: EROD; Condition Factor; *Geophagus*; Micronuclei; Biomonitoring

RESUMO

A bacia do rio Paraíba do Sul (PSR) atravessa uma das regiões mais povoadas e industrializadas do Brasil, recebendo efluentes de indústrias metalúrgicas, de papel e celulose e agrícolas. Os usos das águas do PSR são múltiplos, incluem o fornecimento de água potável, e as populações ribeirinhas consomem algumas de suas espécies de peixes. No presente trabalho foram investigadas as respostas de dois biomarcadores à poluição, a atividade hepática de EROD e a freqüência de micronúcleo em sangue periférico, e medidos os níveis de sete metais pesados no músculo de duas espécies de peixes nativas do PSR, *Geophagus brasiliensis* and *Hypostomus* sp.. As amostragens foram realizadas nos períodos de seca e de cheias de 2003. Ao todo foram coletados 339 peixes em 16 regiões ao longo da bacia do PSR. Os peixes coletados em Levy Gasparian, Barra Mansa e Três Rios apresentaram alta atividade de EROD e frequência de micronúcleo. Também foi detectada uma moderada indução de EROD nos peixes coletados em S.L. Paraitinga e Sapucaia. A frequência de micronúcleo foi elevada nos peixes coletados em S.J. Campos, Além Paraíba, Campos e S.J. Barra. Em todos os casos, as concentrações de metais pesados não excederam os valores máximos permitidos para carne de peixes.

PALAVRAS-CHAVE: EROD; Fator de Condição; *Geophagus*; Micronúcleo; Biomonitoramento

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INTRODUCTION

The Paraíba do Sul river (PSR) drainage basin extends over 55,400 km^2 crossing three of the most industrialized states of Brazil: São Paulo, Rio de Janeiro and Minas Gerais. The PSR can be divided into three stretches according to the effects of the prevailing economical activities on drainage. The upper stretch, closest to the source of the PSR, is characterized by small cities and localized agricultural activities. In the middle stretch the PSR receives untreated sewage from larger cities and discharge from various industries, including chemical, metallurgic and paper mill plants. The mouth of the Paraibuna river is one of the largest PSR tributaries, which has a record of contamination with polychlorinated biphenyls (PCB), [1] and is located in the middle stretch. The lower stretch is influenced by intensive agricultural practice (sugarcane plantations) and has increased salinity due to the surrounding river mouth delta. The water from the PSR is used for agricultural irrigation, by industry and for domestic purposes (including as a supply for drinking water). Fishing also occurs along the basin. Owing to the pollution of the PSR, consumption of its fish might pose health risks to the associated riverine population.

The present study was undertaken in order to evaluate the impact of contamination of the PSR basin by CYP1A inducers (Ethoxyresorufin-O-deethylase (EROD) activity) and genotoxic agents (micronuclei frequency in peripheral blood) on two edible fish. Concentrations of heavy metals in muscle tissue in conjunction with genotoxicity and *Ah*R-ligand (CYP1A, EROD) biomarkers not only indicate the extent of any contamination of edible fish meat by a diversity of industrial contaminants, which may pose risks to human health (such as polycyclic aromatic hydrocarbons (PAHs), polyhalogenated dibenzo-*p*-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs) and some highly toxic metals (Hg, Cd, Pb and others)), but also reveals the bioavailability of these pollutants to vertebrate species living in the PSR basin.

MATERIAL AND METHODS

Fish capture and handling

A total of 202 *Geophagus brasiliensis* and 137 *Hypostomus* sp., adults of both genders were collected from 16 sites along the PSR and its major tributaries during the wet (January-April) and dry (September-November) seasons of 2003. Table 1 shows the names of the closest cities and the river, and geographical coordinates of each sampling site. After capture, fish were immediately measured, weighed and decapitated.

Measurement of heavy metal concentrations

Fish muscle was dissected, as recommended by FAO [2]. For quantification of lead (Pb), copper (Cu), chromium (Cr), magnesium (Mg) and nickel (Ni), muscle samples were dried at 80°C for 24 hours and subsequently incubated at 450°C for at least 48 hours, or until calcination was complete. Samples were treated with nitric:chloridric acid solution (3:1), followed by concentrated chloridric acid and re-suspended in 10 mL 0.1N chloridric acid. For analysis of mercury (Hg) concentrations, muscle was chemically digested (hydrogen peroxide, sulfuric:nitric acid solution, 1:1, potassium permanganate and hydroxylamine chlorhydrate). Metal concentrations were determined using atomic absorption spectroscopy, with appropriate standard curves and reference samples [1].

Table 1. Location of Paraiba do Sul river (PSR) sample sites and the number of fish captured at each site in the wet and dry seasons.

#	City	River	GPS co-ordinates		Fish (N)	
			Latitude (South)	Longitude (West)	*Geophagus brasiliensis* (wet/dry)	*Hypostomus spp.* (wet/dry)
1	São Luiz do Paraitinga	Paraitinga	23° 21.249’	45° 12.183’	-/8	-/10
2	Paraibuna	PSR	23° 21.899’	45° 39.833’	10/10	5/2
3	São José dos Campos	PSR	23° 09.915’	45° 54.350’	4/3	10/4
4	Barra Mansa	PSR	22° 29.039’	44° 14.373’	11/6	-/9
5	Volta Redonda	PSR	22° 29.165’	44° 05.005’	10/10	3/7
6	Rio das Flores	Preto	22° 03.454’	43° 26.421’	2/10	8/8
7	Três Rios	PSR	22° 06.577’	43° 08.046’	-/2	-/2
8	Levi Gasparian	Paraibuna	22° 1’	43° 12’	3/21	17/10
9	Além Paraíba	PSR	22° 29.144’	44° 14.638’	4/-	2/-
10	Petrópolis	Piabanha	22° 17.439’	43° 07.584’	12/-	7/-
11	Sapucaia	PSR	22° 01.923’	42° 59.898’	-/11	-/9
12	Santo Antônio de Pádua	Pomba	21° 32.219’	42° 09.420’	2/5	5/-
13	São Fidelis	PSR	21° 38.744’	41° 44.343’	11/2	-/-
14	Italva	Muriaé	21° 24.708’	41° 41.598’	10/10	-/-
15	Campos	PSR	21° 43.470’	41° 20.953’	4/4	7/2
16	São João da Barra	PSR	21° 43.015’	41° 10.068’	3/-	3/-

(-) no fish caught.

Assessment of the frequency of micronuclei

Peripheral blood was collected and smeared on glass slides for micronuclei determination. Slides were fixed in absolute ethanol and stained with Giemsa. For each fish sample, a total of 1,000 erythrocytes were analyzed for nuclei abnormalities, according to the classification of Da Silva & Fontanetti [3].

EROD assay

Ethoxyresorufin-O-deethylase (EROD) activity was measured in hepatic S9 fractions. Immediately after fishing, fish livers were removed, weighed, wrapped in aluminum foil, and frozen in liquid nitrogen until further use. Liver S9 fractions were prepared by tissue homogenization and centrifugation at 9,000 g for 30 minutes [4]. The supernatant was used for total protein and EROD determination. Total protein concentrations were quantified using the Bradford assay [5] and EROD activity was determined spectrofluorometrically [6].

RESULTS

Heavy metal concentrations

In any muscle sample obtained from either species of fish, the concentrations of mercury and lead did not exceed the maximum allowable level established by the Brazilian Sanitary Agency (ANVISA) regulations. The concentrations of the other four metals analyzed in the present study were also within the range of values generally found in the literature for non-contaminated fish meat [7, 8, 9] (Table 2, shows some of the data collected).

Frequency of erythrocytes with micronuclei

The frequency of micronucleated erythrocytes found in the peripheral blood of both the species of fish examined in this study (0-0.67‰ in *G. brasiliensis* and 0-2‰ in *Hypostomus* sp.) was within the range of values generally reported for other species of fish caught in areas that are unpolluted. The highest incidence of micronucleated erythrocytes was recorded in fish captured in São José dos Campos, Barra Mansa, Três Rios, Campos and São João da Barra for *G. Brasiliensis*, and in São José dos Campos, Levy Gasparian, Além Paraíba and São João da Barra for *Hypostomus* sp.(Table 3).

EROD activity

G. brasiliensis and *Hypostomus* sp. exhibited a marked difference in their liver EROD activity. While for G. brasiliensis EROD activity ranged from 16 to 260 pmol resorufin.min^{-1}.miligram of protein^{-1} (Figure 1), in most *Hypostomus* sp. samples EROD activity was undetectable (data not shown). As far as *G. brasiliensis* samples were concerned, sampling sites were ranked according to a four-category classification scheme, based on the average level of EROD activity. Categories of this ranking scheme were defined, taking into account the statistical distribution of the individual values within the whole sample. Locations with a fish liver mean EROD activity value lower than that of the lowest quartile were placed into category 1. Locations where the mean EROD activity values were in between the lower quartile and the median were allocated to category 2, while category 3 comprised locations where the mean EROD activity values of fish were in between the median and the upper quartile, and locations for which mean liver EROD activity values were higher than that of the upper quartile were allocated to category 4 (Table 4). In both seasons, *G. brasiliensis* collected at Italva were consistently classified into category 1 (lowest activity), while samples from Levy Gasparian were classified into category 4 (highest activity). Fish from Barra Mansa collected during the wet season were classified as category 2, while those caught during the dry season were classified as category 4. Fish from São Luiz do Paraitinga, Três Rios and Sapucaia collected in the dry season were classified as category 4 (the category with the highest activity). During the wet season fish were not sampled at these locations.

Table 2. The concentration of heavy metals in the muscle tissue of *Geophagus brasiliensis* ("acará") and *Hypostomus* spp. ("cascudo") caught during the dry season.

	Heavy metal (µg/g of wet weight)						
	Cr	Mn	Pb	Zn	Cu	Hg	Ni
G. brasiliensis							
2	0.11 ± 0.11 (5)	ND (5)	-	3.00 ± 0.44 (4)	0.11 ± 0.07 (5)	0.12 ± 0.04 (5)	0.16 ± 0.14 (5)
5	0.20 ± 0.08 (5)	ND (5)	0.18 ± 0.20 (5)	4.03 ± 0.85 (5)	0.21 ± 0.21 (5)	-	0.04 ± 0.05 (5)
6	0.18 ± 0.09 (5)	ND (3)	0.08 ± 0.08 (5)	1.96 ± 1.70 (3)	0.25 ± 0.20 (5)	0.11 ± 0.02 (5)	0.03 ± 0.03 (5)
8	0.13 ± 0.13 (5)	ND (5)	0.18 ± 0.15 (5)	3.36 ± 0.32 (5)	0.58 ± 0.90 (5)	0.10 ± 0.01 (5)	0.22 ± 0.21 (5)
10	0.16 ± 0.04 (4)	0.35 ± 0.04 (2)	0.19 ± 0.22 (4)	3.43 ± 0.67 (4)	0.30 ± 0.14 (4)	-	ND (3)
12	0.15 ± 0.08 (3)	0.48 ± 0.10 (4)	0.53 ± 0.27 (5)	2.80 ± 0.34 (4)	0.28 ± 0.07 (4)	-	0.02 ± 0.04 (5)
Hypostomus spp.							
13	0.96 ± 1.98 (5)	0.18 ± 0.06 (5)	0.25 ± 0.24 (5)	2.74 ± 0.50 (5)	0.14 ± 0.13 (5)	-	0.03 ± 0.04 (5)

Values are means ± standard deviation (sample size, N); ND: not detected (below the limit of quantification).

Table 3. Frequency of micronuclei frequency (‰) in the erythrocytes of G. brasiliensis (“acará”) and Hypostomus spp. (“cascudo”) collected at different sampling sites in the Paraíba do Sul river (PSR) basin during the wet and dry seasons

	Micronuclei frequency (‰)			
	Geophagus brasiliensis		Hypostomus spp.	
Location	Wet	Dry	Wet	Dry
1	-	0.13 ± 0.35	-	0.33 ± 0.71
2	0.10 ± 0.32	0.10 ± 0.32	0.0	0.50 ± 0.71
3	0.50 ± 1.0	0.0	-	1.50 ± 1.29
4	0.56 ± 1.33	0.17 ± 0.41	1.0	0.33 ± 0.52
5	0.33 ± 0.82	0.10 ± 0.32	-	0.0
6	0.0	0.20 ± 0.63	-	0.80 ± 0.84
7	-	0.50 ± 0.71	1.50 ± 0.71	0.0
8	0.0	0.22 ± 0.44	1.17 ± 0.98	2.0
9	0.0	-	-	-
10	0.30 ± 0.48	-	0.57 ± 0.79	-
11	-	0.18 ± 0.40	-	0.0
12	0.0	0.0	0.17 ± 1.41	-
13	-	0.0	-	-
14	0.40 ± 0.89	0.10 ± 0.32	-	-
15	0.50 ± 0.58	0.67 ± 1.15	0.17 ± 0.41	0.0
16	0.67 ± 0.58	-	1.00 ± 1.00	-

Values are means ± standard deviation of the frequencies of micronucleated erythrocytes (per 1000 erythrocytes) in fish peripheral blood.

DISCUSSION

The Data in this study were derived from samples of edible fish collected in the dry and wet seasons of 2003. Therefore, they are a snapshot of the contamination of PSR fish meat by a diverse range of industrial pollutants (genotoxic and AhR ligand agents, and toxic metals), which are relevant to human health at the present time. Although these two species and other fish can be used as “sentinel” species for monitoring PSR basin water pollution, it should be borne in mind that the biomarkers respond to the bioavailability of contaminants to target organisms. Persistent compounds with poor solubility in water are deposited at the bottom of the river over time. Contaminants remaining trapped in the sedimentary strata are not bioavailable to fish living in the water column and, thus, these pollutants are not detected by these “sentinel” species. However, if a river sediment is disturbed by natural phenomena or human activity, contaminants deposited on river beds may become re-suspended and ingested (with particulate matter) by fish. Aquatic pollution and contamination of the biota are dynamic phenomena and historical data records are needed to evaluate the impact of anthropological activities and the effectiveness of interventions to control pollution. Retrospective epidemiological studies on the impact of pollutants on human health, for instance, would require historical data records in order to estimate past exposure. Some current diseases (e.g. cancer) can be influenced by exposure to contaminants in the environment that took place years or even over a decade ago.

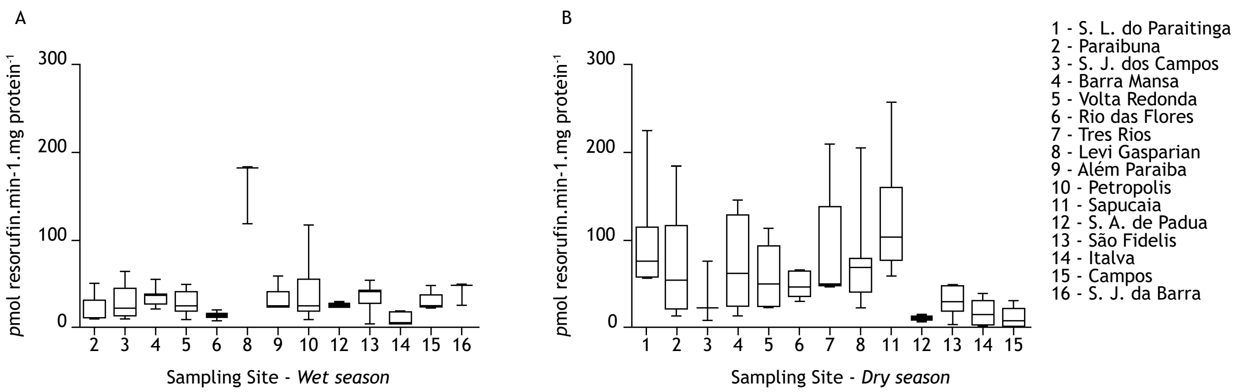

Figure. EROD activity (pmol resorufin.min^{-1}.milligram protein^{-1}) in liver S9 fractions of *Geophagus brasiliensis* (“acará”) collected at different sampling sites (x axis) during the wet (A) and dry (B) seasons.

Table 4. Classification of sampling locations according to average EROD activity (pmol resorufin.min^{-1}.mg of protein^{-1}) recorded in the *Geophagus brasiliensis* (“acará”) liver.

Categories	EROD (pmol resorufin. min^{-1}.mg protein^{-1})	Sampling Location	
		Wet season	Dry season
1	≤ 16	2, 6, 14	12, 14, 15
2	16 to 36	3, 4, 5, 10, 9, 12, 15	3, 13
3	36 to 61	13, 16	2, 5, 6, 7
4	≥ 61	8	1, 4, 7, 8, 11

Taken together, the concentrations of heavy metals in muscle tissue, liver EROD activities, and the frequency of erythrocytes with micronuclei all indicate that the contamination of edible fish from the PSR basin by metals, PAHs, PCDD/Fs PCBs and related compounds, was not a threat to human health at the time of sampling (2003). Some pollution hotspots, however, were detected. The highest values of micronuclei frequency and EROD activity were found in fish collected in the middle stretch of the river basin between the cities of São José dos Campos and Sapucaia. This region is the most industrialized part of the basin, where the Paraibuna river, one of the largest tributaries, flows into the PSR. The elevated biomarker values in fish from the middle stretch suggests that industrial discharges and contaminants carried in the Paraibuna river affects the fish community. A continuous biomonitoring programme should be implemented in this region. High micronuclei frequency was found in fish caught at Campos and São João da Barra, two adjacent cities close to the delta of the river mouth. This might have been due to the intensive use of pesticides at sugarcane plantations or to the physiological stress caused by the high salinity found around the river mouth. The presence of mutagenic compounds in the stretch of river traversing this region should be investigated further. Curiously, high EROD activity was detected in fish collected at São Luiz do Paraitinga. This small city is located in the upper part of the river, close to its origin. There is no obvious source of contamination here and so a more comprehensive and systematic investigation should be performed in order to identify the origins of any pollution that exists here. The lack of detectable EROD activity in *Hypostomus* spp. liver is an intriguing question, which has been investigated by our research group [10, 11].

CONCLUSIONS

Although the PSR basin is spread over a highly urbanized and industrialized area of south-eastern Brazil, this study found no indication that consumption of its most appreciated edible fish is likely to pose significant health risks. However, the data used for this investigation were obtained in 2003 and the aquatic pollution, and the extent to which contaminants are bioavailable to both species of fish may have changed since then.

As far as the biomarkers for pollution and metal contamination evaluated in the present study are concerned, the greatest impact was indicated on the middle stretch of the PSR basin, between the cities of São José dos Campos and Sapucaia, near the border of the states of São Paulo and Rio de Janeiro. We suggest, therefore, that a continuous biomonitoring program should be implemented, in order to protect areas, which have experienced little impact and to assess further the effects of industrial discharge on the middle stretch of the PSR basin.

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