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Chromium accumulation and biomarker responses in the Neotropical fish *Prochilodus lineatus* caged in a river under the influence of tannery activities

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ABSTRACT

In order to assess the effects of tannery effluents (TE) in organism health, juveniles of *Prochilodus lineatus* were submitted to *in situ* tests at four different river locations: site A – upstream of the tannery; site B – next to the tannery; and sites C and D – downstream of the tannery. After 96 h exposure in the river, samples of fish tissue, river water and sediment were collected in order to quantify chromium (Cr) concentrations. Tissue samples were used to assess the activity of ethoxyresorufin-O-deethylase (EROD) and glutathione S-transferase (GST), the content of glutathione (GSH) and metallothionein (MT) and the occurrence of lipid peroxidation (LPO) and DNA damage. Higher Cr concentrations were detected in the water and sediments from site B and in the liver of fish confined at site B, compared to the other sites. Fish caged at site B demonstrated higher levels of liver MT and hepatic EROD activity in relation to fish caged at the other sites. Moreover, fish from site B presented increased liver and branchial GST activities, as well as more GSH in the liver, than fish from site A. There were no significant variations in the occurrence of LPO and DNA damage among fish caged at the different sites. Thus, TE increased Cr levels in the water, sediments, and fish livers and stimulated the synthesis of MT and GSH and the activities of EROD and GST. In conclusion, TE affect the quality of the river and promote changes in biochemical biomarkers and Cr accumulation in *P. lineatus*.

1. Introduction

Leather tanning is a common industry all over the world. Tanneries contribute to water deterioration by discharging wastewaters containing a wide range of contaminants (Shakir et al., 2012), of which a major concern is the presence of the metal chromium (Cr) (Tariq et al., 2006). In Brazil, tanneries represent the major industrial sources of anthropogenic chromium releases (Souza et al., 2016). Chromium toxicity in industrial effluents depends on its oxidation state. Oxidation of Cr(III) in tannery effluents to the more toxic Cr(VI) may occur depending on the characteristics of the receiving water body, such as pH, redox potential and sunlight (Rodrigues and Formoso, 2006; Markiewicz et al., 2015). Both Cr(III) and Cr(VI) can be biologically active, but they differ in their potential to cross biological membranes. Cr(III) does not cross cell membranes, however it can be environmentally converted into Cr(VI), an oxidant that enters cells through the sulfate anion transporter and becomes reduced to stable Cr (III), which adversely alters cell function. Intracellular Cr(VI) reduction generates reactive oxygen species (ROS), which are one probable cause of Cr toxicity (Reid, 2012).

Biomonitoring is the systematic use of live organisms to test for any environmental changes, particularly those caused by human actions (Buss et al., 2003). The use of animals in environmental monitoring allows for the detection of biomarkers that reflect sublethal effects of the contaminants in organisms (Zhou et al., 2008). Biomarkers reflect alterations at the cellular, biochemical, molecular, or physiological level, and they can be measured in cells, body fluids, tissues, and organs, as well as indicating the presence and/or effects of a xenobiotic (Lam, 2009).

In ecotoxicological studies, the simultaneous use of several types of biomarkers is highly recommended as single biomarkers cannot reflect the impairment of the health of an organism and/or the adaptation to impaired environmental conditions (Zorita et al., 2008). Enzymes that are involved in the detoxification of xenobiotics and their derivatives, such as ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST), the determination of oxidative stress, such as DNA damage and lipid peroxidation (LPO) and/or antioxidant responses, such as glutathione (GSH), are commonly employed as biomarkers in aquatic organisms (Monserrat et al., 2007; Lam, 2009). Metallothioneins (MT), a family of low molecular weight, cysteine-rich proteins, capable of

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binding metals, represent biomarkers normally associated with exposure to metals (Viarengo et al., 2007).

Fish are widely used as biological models to assess the quality of aquatic systems, and examinations of the bioaccumulation of contaminants and biomarkers in these animals are important tools for the biomonitoring of water pollution (Van der Oost et al., 2003; Zhou et al., 2008). *In situ* tests using caged fish are useful for assessing contaminant effects on the aquatic biota (Vieira et al., 2014). This active approach presents some advantages as it allows the exact knowledge of the exposure period and the standardization of organisms used in the tests, such as sex and size, making the comparison of results from different sites possible (Wepener, 2013; Vieira et al., 2017). The Neotropical fish *Prochilodus lineatus*, an ecologically and economically important species, has been successfully used in *in situ* tests (Camargo and Martinez, 2006; Cazenave et al., 2014; Vieira et al., 2016). This fish is a suitable biological model for environmental monitoring, as it is a sensitive bottom feeder fish, which is in contact to contaminants in the sediment, as well as dissolved in the water column (Simonato et al., 2016).

Therefore, the objectives of this study were to analyze biochemical and genotoxic biomarkers, as well as to determine Cr concentrations, in different tissues of *P. lineatus* submitted to *in situ* tests for 96 h in a river that receives tannery effluents. Complementary biomarkers, such as biotransformation enzymes, oxidative stress parameters, MTs and DNA damage were selected in order to understand fish responses to contamination as well as to evaluate which biomarkers could be used for effectively monitoring areas under the influence of tanneries.

2. Material and methods

2.1. Locations of the in situ tests

The Bandeirantes do Norte (BN) River flows along 149 km from its headwaters to its mouth in Pirapó River, in the north of the Paraná state in southern Brazil (Fig. 1; Martinez et al., 2011). A tannery adjacent to this river contributes to its contamination due to the disposal of liquid effluents. In addition, the BN River receives domestic and industrial effluents from its source, which is located in an industrialized urban area, as well as agricultural effluents, as this river flows through regions of intense agricultural activity.

In situ tests were performed at four sites along the BN River (Fig. 1) in an attempt to test for the existence of a contamination gradient caused by the tannery effluent. Site A (SA; 23°18'23.94"S/51°25'5.38"W): 1000 m upstream of the site of confluence of the tannery effluents with the BN River, represents a location that is not affected by the tannery effluents. Site B (SB; 23°18'9.34"S/51°25'27.75"W): only 50 m downstream of the site of discharge of the tannery effluents. Site C (SC; 23°17'51.31"S/51°25'35.67"W) and site D (SD; 23°17'32.11"S/51°25'20.44"W): located 800 m and 1750 m, respectively, downstream of the tannery effluents.

2.2. Experimental design

For the *in situ* tests, juveniles of *P. lineatus* (Valenciennes, 1836) with a total length of 12.30 ± 0.89 cm and weight of 22.53 ± 4.55 g (mean ± SD, n = 160) were obtained from the fish farming facility of the Londrina State University, Paraná, Brazil. *In situ* tests were performed in the winter, between July and August, which is the dry season in the region.

The animals were transported to the field in plastic bags containing water and oxygen and transferred to cages that had previously been placed under water, where they remained for 96 h. At each experimental location, 32 fish were confined, divided into two cages (two replicates per site). The cages were made of iron and had a size of 50 × 60 × 40 cm (volume of 120 L), and they were covered with a 5-mm mesh net that allowed water circulation and access to the sediment for the fish to feed. A group of fish (n = 20) was sampled before caging

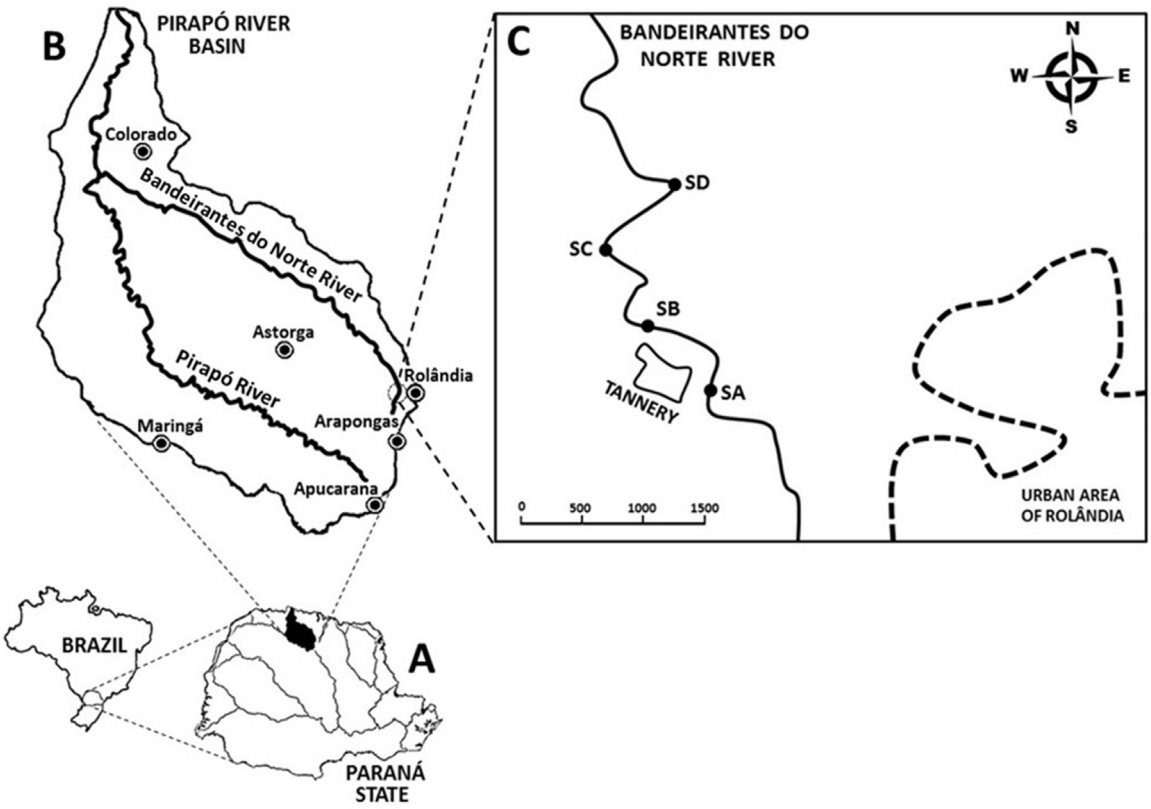

Fig. 1. Maps showing Paraná State, in Southern Brazil (A), Bandeirantes do Norte river, in the Pirapó River Basin (B) and a stretch of Bandeirantes do Norte river (C) indicating the four experimental sites along of the stream where fish were caged (Site A: SA, Site B: SB, Site C: SC and Site D: SD).

(basal group) to determine biomarker baseline levels for this species.

At each *in situ* experimental location, measures of temperature, pH, turbidity, and water conductivity were collected with a multiparameter meter (Horiba U-52) on the first day of cage exposure as well as on the day of their removal from the water. On those same days, samples of the superficial sediment and water were collected for Cr quantification. Half of the water samples were filtered (0.45-μm pore size) and subsequently acidified (1% HNO_3) for the quantification of the dissolved Cr concentration; the remaining samples were acidified without filtering for the quantification of total Cr concentration.

After 96 h, the *P. lineatus* fish were sampled in the field immediately after their removal from the cages. The fish were anesthetized with benzocaine (0.1 g L^{-1}) and blood samples were collected from the caudal vein. The animals were then killed by medullar section, and the gills, liver, kidney, dorsal axial muscle, and brain were collected. The samples were stored in ultrafreezer (−70 °C) until the quantification of the biomarkers and Cr accumulation. The Ethics Committee for the Use of Animals of Londrina State University approved this experimental design and the sampling procedures (Process No. 19623.2012.17).

2.3. Cr determination in water, sediment and tissue samples

In order to determine Cr concentration in sediment samples, the sediment was previously dried (60 °C) and then subjected to horizontal shaking, for 2 h, with hydrochloric acid (HCl) 0.1 M. After this period, the suspension was filtered (8 μm pore size) and the filtrate was used for Cr determination (Mozeto, 2004).

Cr concentrations were measured in the liver, muscle and kidney of the fish. Dried tissues were submitted to acidic digestion in suprapure nitric acid 5 N at 60 °C for 48 h, according to Alves and Wood (2006). Water samples (filtered and unfiltered), sediment and tissues digests were analyzed for metals through electrothermal atomic absorption spectrophotometry, using an atomic absorption spectrophotometer equipped with a graphite furnace atomizer (AAnalyst 700, Perkin Elmer, USA) against reference standard solutions (Specsol, Brazil).

2.4. Biochemical assays for EROD, GST, GSH and LPO

For the determination of EROD and GST activities, GSH concentration and the occurrence of LPO, liver and gills samples were homogenized (1:10 w/v for liver and 1:5 w/v for gills) in K phosphate buffer (0.1 M, pH 7.0), centrifuged (16,000 g, 20 min, 4 °C) and the supernatant was used for biochemical analyses.

2.4.1. Ethoxyresorufin-O-deethylase (EROD)

Liver EROD activity was estimated by the rate of conversion of 7-ethoxyresorufin to resorufin, according to the protocol of Eggens and Galgani (1992), with modifications. The reaction was initiated by adding the sample to the reactive mixture (0.1 M K phosphate buffer, pH 7.6, 2 mM NADPH and 0.1 mM 7-ethoxyresorufin). The progressive increase in fluorescence resulting from the formation of resorufin was measured at 1-min intervals for 10 min (ex/em: 530/590 nm). EROD activity was expressed in nmol resorufin min^{-1} mg $protein^{-1}$, based on a resorufin standard curve.

2.4.2. Glutathione S-transferase (GST)

The GST activity was determined both in the liver and gills, according to Keen et al. (1976) by monitoring the complexation of reduced glutathione (GSH) with the substrate 1-chloro-2,4-dinitrobenzene (CDNB), at 340 nm. GST activity was expressed in nmol CDNB min^{-1} mg $protein^{-1}$.

2.4.3. Glutathione (GSH)

The concentration of GSH was determined both in the liver and gills, according to Beutler et al. (1963), by the reaction of GSH with the color reagent 5,5-dithiobis-2-nitrobenzoic acid (DTNB), forming a thiolate anion (TNB), measured at 412 nm. The GSH concentration was expressed in μg GSH mg $protein^{-1}$, based on a standard curve for GSH.

2.4.4. Lipid peroxidation (LPO)

LPO was measured in both the liver and gills, through the quantification of malondialdehyde (MDA) according to the TBARS (thiobarbituric acid reactive substances) assay, following Camejo et al. (1998). Butylated hydroxytoluene (BHT 1 M), phosphate buffered saline (2 mM KCl; 1.4 mM NaH_2PO_4; 357 mM NaCl; 10 mM Na_2HPO_4; pH 7.4), trichloroacetic acid (TCA 50%) and thiobarbituric acid (TBA 1.3%), dissolved in 0.3% NaOH, were added to the supernatant. The mixture was then incubated for 1 h, at 60 °C, and a fluorescence reading was made (ex/em: 535/590 nm). LPO was expressed in equivalents of MDA as nmol MDA mg $protein^{-1}$, based on a malondialdehyde (MDA) standard curve.

2.5. Metallothionein-like proteins (MT-like)

The content of MT-like proteins was determined only in the liver following Viarengo et al. (1997) with modifications. Liver samples were homogenized (1:5 w/v) in buffer (0.5 M sucrose, 26 mM Tris, 0.5 mM phenylmethylsulfonyl fluoride, 1.3 mM β-mercaptoethanol) and centrifuged (18,650 g, 45 min, 4 °C). The supernatant was subjected to ethanol/acid chloroform fractionation to obtain a partially purified metalloprotein fraction. In this fraction sulfhydryl groups (–SH) were quantified at 412 nm, using Ellman's reagent (2 M NaCl, 0.43 mM DTNB buffered with 0.2 M Na-phosphate, pH 8). Reduced glutathione (GSH) was used as standard and the MT-like content was expressed in nmol de GSH mg $protein^{-1}$.

2.6. Total proteins

The results of biochemical biomarkers were expressed per mg protein, determined by the method of Bradford (1976), based on the reaction of proteins with Coomassie Brilliant Blue G-250 dye. The calibration curve was prepared with bovine serum albumin (BSA) and absorbance was determined at 595 nm.

2.7. DNA damage – Comet assay

The alkaline comet assay (pH > 13) with erythrocytes was performed according to Singh et al. (1988), with some modifications described by Ramsdorf et al. (2009). Only blood samples with cell viability above 80%, determined by the Trypan blue exclusion method, were used in the comet assay. After sampling, an aliquot of blood mixed with fetal bovine serum (1:100 - v/v) was added to the low melting point agarose. This mixture was placed on a glass slide previously covered with standard agarose (1.5%), covered with a coverslip, and stored in the refrigerator (4–8 °C) for 30 min. Next, the coverslips were removed and the slides were subjected to: a) lysis: 1 h at 4–8 °C in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 10% DMSO, 1 mL Triton X-100, pH 10.0); b) DNA denaturation: 30 min in the dark in an electrophoresis buffer (0.3 N NaOH, 1 mM EDTA, pH > 13); c) electrophoresis: 20 min, 300 mA, 25 V; and d) neutralization: three rinses for 5 min each with buffer (0.4 M Tris, pH 7.5). The slides were then fixed with absolute ethanol for 10 min and placed in the refrigerator until analysis.

The slides, stained with GelRed (Uniscience ®) were analyzed under a Leica microscope (DM 2500) adapted for fluorescence/epi-fluorescence at 1000× magnification. All slides were blind-reviewed. The extent of DNA damage was visually determined in 100 randomly selected and non-overlapping nucleoids per fish. DNA damage was classified into four classes – 0: no visible damage; 1: a short tail smaller than the diameter of the nucleoid; 2: a tail length 1–2 times the diameter of the nucleoid; 3: a tail length > 2 times the diameter of the nucleoid. The score of DNA damage was obtained by multiplying the

Table 1
Water physical and chemical parameters in the first and last day of caging and metals (Cr, Cd, Cu and Pb) concentrations (mean ± SE, n = 4) in the water (total and dissolved) and in the sediment collected from sites where *in situ* tests were performed. Different letters indicate significant differences among sites (P < 0.05).

Sites	Site A	Site B	Site C	Site D
Temperature (°C)	17.8–16.7	17.1–18.4	17.9–17.5	17.8–16.4
pH	7.04–7.15	7.35–7.57	7.14–7.05	6.90–7.18
Conductivity ($\mu S\ cm^{-1}$)	167–208	414–560	398–406	263–612
Turbidity (NTU)	23.6–30.0	42.2–47.3	30.7–25.8	24.1–28.7
Water Total Cr ($\mu g.\ L^{-1}$)	2.97 ± 0.21 ^{a}	17.08 ± 2.41 ^{b}	6.57 ± 0.84 ^{c}	6.21 ± 0.53 ^{c}
Water Dissolved Cr ($\mu g.\ L^{-1}$)	2.52 ± 0.28 ^{a}	7.73 ± 1.13 ^{b}	3.48 ± 0.08 ^{a}	3.86 ± 0.19 ^{a}
Sediment Cr ($\mu g.g^{-1}$)	5.75 ± 0.46 ^{a}	47.49 ± 3.34 ^{b}	29.84 ± 0.58 ^{c}	5.10 ± 0.33 ^{a}
Water Total Cd ($\mu g.\ L^{-1}$)	0.08 ± 0.05 ^{a}	0.12 ± 0.02 ^{a}	0.27 ± 0.08 ^{a}	0.29 ± 0.12 ^{a}
Water Dissolved Cd ($\mu g.\ L^{-1}$)	0.04 ± 0.02 ^{a}	0.05 ± 0.01 ^{a}	0.16 ± 0.05 ^{b}	0.27 ± 0.03 ^{c}
Water Total Cu ($\mu g.\ L^{-1}$)	1.20 ± 0.81 ^{a}	1.15 ± 0.30 ^{a}	1.23 ± 0.47 ^{a}	0.74 ± 0.27 ^{a}
Water Dissolved Cu ($\mu g.\ L^{-1}$)	0.08 ± 0.05 ^{a}	0.69 ± 0.44 ^{a}	0.62 ± 0.36 ^{a}	0.39 ± 0.26 ^{a}
Water Total Pb ($\mu g.\ L^{-1}$)	0.62 ± 0.36 ^{a}	0.75 ± 0.46 ^{a}	1.57 ± 0.60 ^{a}	1.75 ± 0.64 ^{a}
Water Dissolved Pb ($\mu g.\ L^{-1}$)	0.50 ± 0.18 ^{a}	0.15 ± 0.12 ^{a}	1.26 ± 0.73 ^{a}	1.72 ± 0.71 ^{a}

number of cells in each class by the damage class, and ranged from 0 (all undamaged) to 300 (all maximally damaged).

2.8. Statistical analysis

After checking for normality and homoscedasticity, the results of each parameter were compared between sites (Site A x Site B x Site C x Site D) by parametric (ANOVA) or non-parametric analysis of variance (Kruskal-Wallis), and differences between treatment groups were identified by the Student-Newman-Keuls (SNK) or Dunn's test, respectively. Values of P < 0.05 were considered significant.

3. Results

Temperature and pH did not demonstrate important variations between the tested sites (Table 1). However, conductivity was higher at the sites downstream of the tannery (site B, site C, and site D) and turbidity was higher in site B than in the other locations (Table 1). Site B also presented significantly higher concentrations of both total Cr (P < 0.001) and dissolved Cr (P < 0.001) in the water, as well as Cr in the sediment (P = 0.004), in comparison to all other sites. Sites C and D showed higher total Cr concentrations in water compared to site A. In the sediment, site C also demostrated higher chromium concentrations in relation to sites A and D (Table 1).

Fish caged at site B presented significantly higher Cr concentration in the liver (P < 0.001) than those caged at the other sites. Cr concentrations in the kidney (P = 0.604) and muscle (P = 0.44) did not

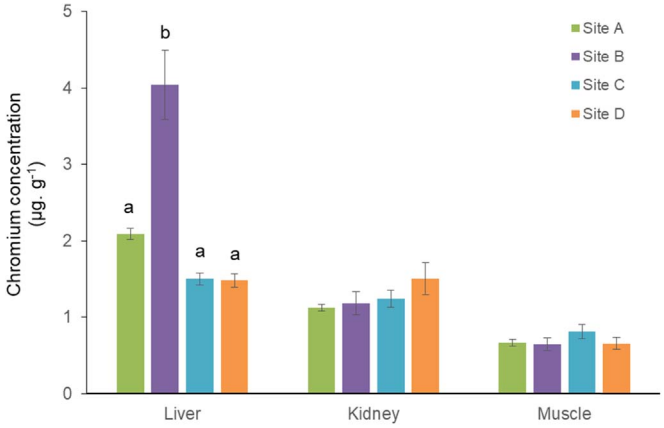

Fig. 2. Concentrations of chromium (mean ± SE, n = 6–8) in the liver, kidney and muscle of *P. lineatus* caged at different sites (Site A, Site B, Site C and Site D) in the BN river. Different letters indicate significant differences between fish caged at different sites (P < 0.05).

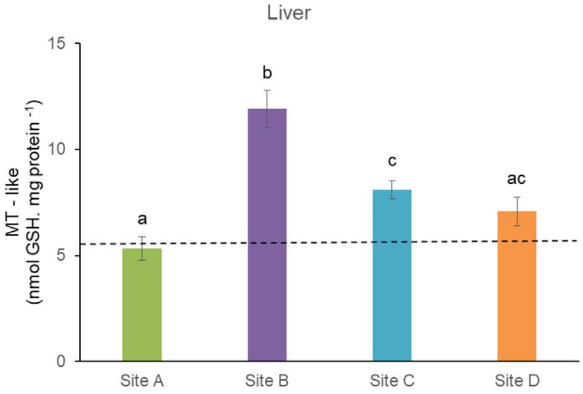

Fig. 3. Concentrations of MT-like proteins (mean ± SE, n = 6–8) in the liver of *P. lineatus* caged at different sites (Site A, Site B, Site C and Site D) in the BN river. Different letters indicate significant differences between fish caged at different sites (P < 0.05). The dashed line represents the mean baseline value of the biomarker measured in a group of fish sampled before caging (5.56 ± 0.45, n = 7).

vary significantly between fish caged at different sites (Fig. 2). The hepatic content of MT-like proteins (Fig. 3) was significantly higher in fish caged at site B, followed by fish from site C, in relation to fish from site A (P < 0.001). Fish caged at site D showed liver MT-like proteins levels statistically similar to those from the fish at site A and site C.

The hepatic activity of EROD (Fig. 4) was significantly higher in fish caged at site B than in fish at site A and site D (P = 0.005), while fish caged at sites A, C and D demostrated statistically similar liver EROD activity. The GST activity was significantly higher in the liver of fish caged at site B and site D (P < 0.001) than in fish at the other locations (Fig. 5A). In the gills, GST activity was significantly higher (P < 0.001) only in fish caged at site B in comparison to fish caged at all the other sites (Fig. 5B). The GSH concentration was higher (P < 0.001) in the liver of fish caged at sites B and D than in the other groups (Fig. 6A). In contrast, gill GSH concentrations were not statistically different (P = 0.131) between fish caged at different sites (Fig. 6B).

Lipid peroxidation (Fig. 7A and B) was not statistically different between fish caged at different sites, both in the liver (P = 0.434) and in the gills (P = 0.282). In addition, there were no significant differences in the scores of DNA damage (Fig. 7C) in the erythrocytes of *P. lineatus* caged at the different sites in the BN River (P = 0.087).

4. Discussion

The input of liquid effluents from the tannery can be corroborated

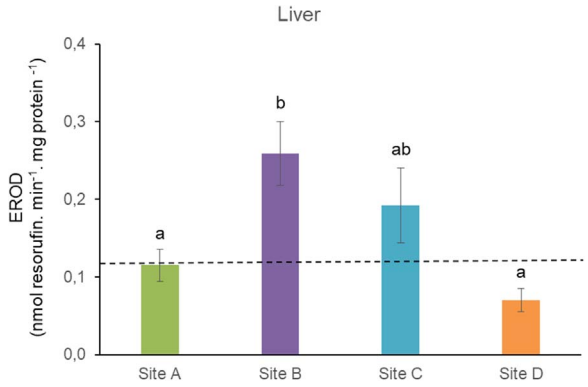

Fig. 4. Activity of EROD (mean ± SE, n = 6–8) in the liver of *P. lineatus* caged at different sites (Site A, Site B, Site C and Site D) in the BN river. Different letters indicate significant differences between fish caged at different sites ($P < 0.05$). The dashed line represents the mean baseline value of the biomarker measured in a group of fish sampled before caging (0.16 ± 0.01, n = 6).

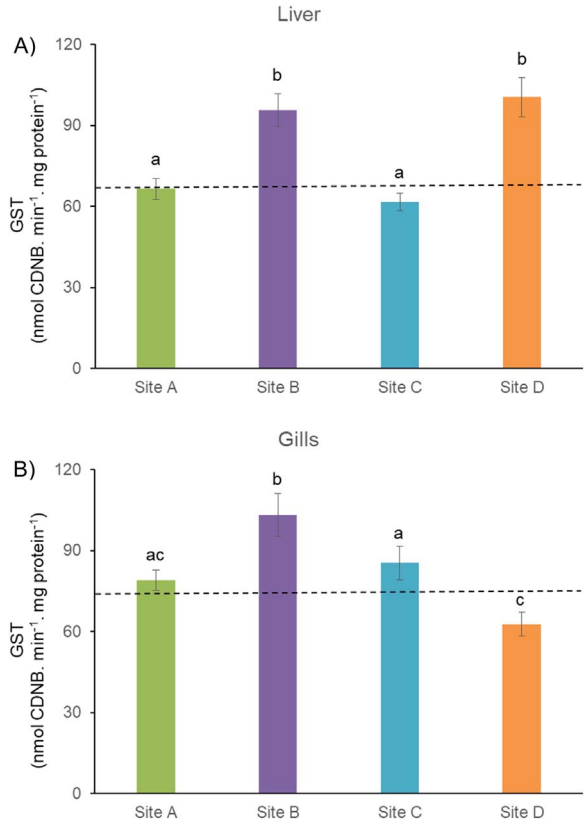

Fig. 5. Activity of GST (mean ± SE, n = 6–8) in the liver (A) and gills (B) of *P. lineatus* caged at different sites (Site A, Site B, Site C and Site D) in the BN river. Different letters indicate significant differences between fish caged at different sites ($P < 0.05$). The dashed line represents the mean baseline value of the biomarker measured in a group of fish sampled before caging (Liver: 65.63 ± 7.26, n = 6; Gills: 73.06 ± 6.39, n = 5).

by the increased conductivity between site A and site B, which are located before and immediately after the tannery, respectively, indicating higher concentrations of dissolved ions in the water at the downstream location. The increase in turbidity downstream of the tannery, to the extent that the water at this location was opaque, suggests the presence of phenolics compounds, sulfates, phosphates and metals, which are commonly released from tannery industries (Chandra et al., 2009). The pH values, which affect the bioavailability of several metals and their toxicity (Camargo et al., 2009; Velma et al., 2009), were not significantly different between the various sites studied and varied between 6 and 8. According to Velma et al. (2009), the highest toxicity of

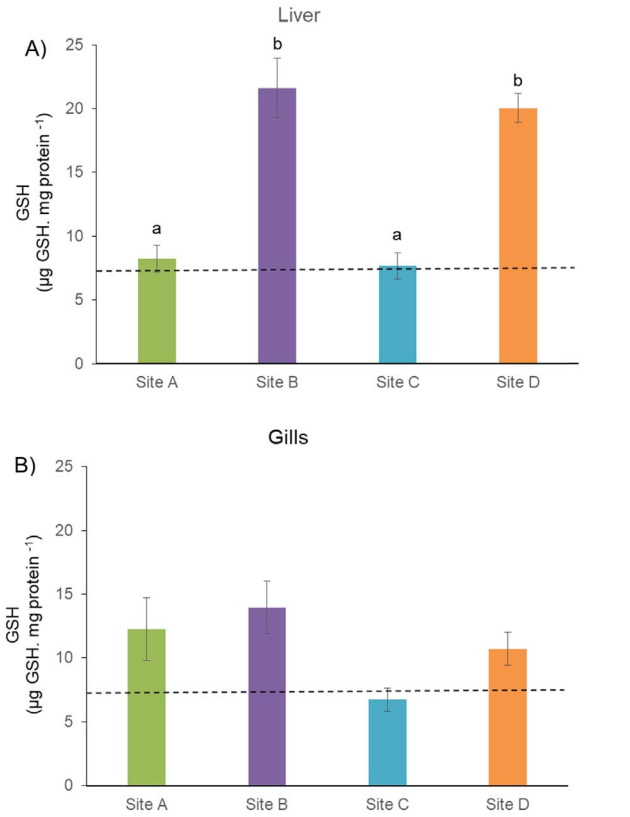

Fig. 6. Concentrations of GSH (mean ± SE, n = 6–8) in the liver (A) and gills (B) of *P. lineatus* caged at different sites (Site A, Site B, Site C and Site D) in the BN river. Different letters indicate significant differences between fish caged at different sites ($P < 0.05$). The dashed line represents the mean baseline value of the biomarker measured in a group of fish sampled before caging (Liver: 7.04 ± 0.60, n = 7; Gills: 7.16 ± 1.12, n = 5).

Cr on teleost fish occurs in waters with pH values between 6.4 and 7.8. Therefore, the pH levels that were measured in the BN River could contribute to the toxicity caused by chromium to fish.

The concentrations of total Cr found in the water downstream of the tannery (sites B, C and D) were higher than upstream of the tannery (site A). In the sediment, Cr was 8 times higher at SB and 5 times higher at site C in comparison to site A. These values of Cr concentrations suggest either recent water contamination from the residual waters discharged by the tannery or chronic contamination, with Cr remaining in the sediment and possibly being released into the water. Even so, these Cr concentrations in the water (Table 1) are below the values permitted by Brazilian legislation (CONAMA, 2005), which stipulate 50 $\mu g\,L^{-1}$ as the maximum permitted total Cr concentration in inland waters. In Brazilian streams located near tanning industries, Jordão et al. (1999) have already demonstrated that 32% of the water samples presented Cr concentrations above the maximum allowed by the Brazilian Guidelines and Matsumoto et al. (2003) determined Cr concentrations in the water of up to 380 $\mu g\,L^{-1}$. In the sediment of the BN River, Cr concentrations were also below the levels determined by Jordão et al. (1997) and Tagliari et al. (2004) in the sediment from Brazilian rivers affected by tannery industries. A possible explanation for these lower Cr concentrations in the water and sediment of the BN River could be the treatment of the effluents provided by the tannery located near this river. Nevertheless, the results of the present study showed that the Cr was not completely removed from the effluents.

The Cr concentrations found in the liver of fish caged at site B were higher than those of fish exposed at other locations and are directly related to the presence of this metal in the water and sediment at this location. Following metal exposure, the organs with higher metabolic activities, which are responsible for the detoxification and elimination

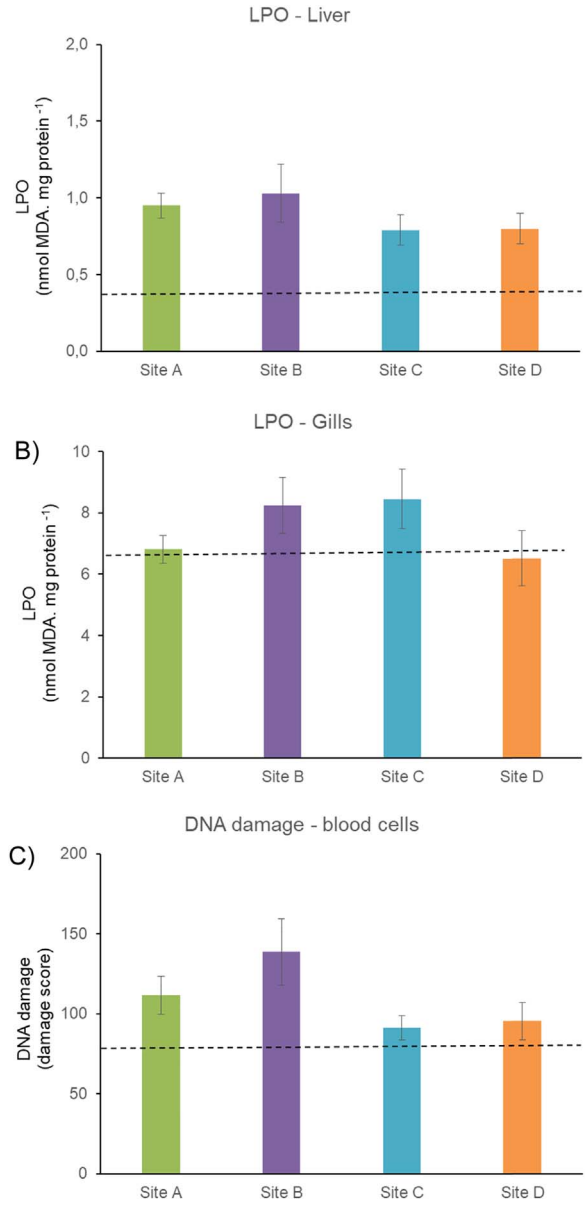

Fig. 7. Lipid peroxidation (LPO) in the liver (A) and gills (B) and score of DNA damage in erythrocytes (C) of *P. lineatus* caged at different sites (Site A, Site B, Site C and Site D) in the BN river. Values are presented as mean ± SE (n = 6–10). The dashed line represents the mean baseline value of the biomarker measured in a group of fish sampled before caging (LPO liver: 0.34 ± 0.05, n = 6; LPO gills: 6.17 ± 1.33, n = 5; DNA damage: 68.00 ± 8.42, n = 9).

of substances, such as the liver, tend to accumulate higher levels of metals, partially due to the presence of MTs (Klavins et al., 2009). In this context fish caged at site B and site C exhibited higher levels of liver MT-like proteins. The biological functions of MTs include the detoxification of both essential metals and non-essential metals, thus protecting the organism from the toxic effects of these compounds (Monserrat et al., 2007). They can also act as antioxidants by scavenging reactive oxygen species (ROS) and avoiding the possible occurrence of oxidative lesions (Falfushynska and Stolyar, 2009). According to Fleet et al. (1990), Cr (III) can induce the synthesis of MTs at a larger scale than Cr (VI). This would explain the induced synthesis of these proteins in fish caged at the sites downstream of the tannery (site B and site C), where Cr in sediment was higher, as most of the Cr temporarily retained in the sediment is probably in the trivalent state (Rodrigues and Formoso, 2006).

There were no significant increases in the Cr concentrations in the kidney and muscle of *P. lineatus*, which suggesting that these tissues require longer exposures to the metal before it starts to accumulate it.

In fish, phase I of the biotransformation pathway is mainly mediated by enzymes of the cytochrome P450 1A (CYP1A) subfamily, and EROD activity is one way of measuring CYP1A activity, while phase II comprises the xenobiotic conjugation reactions or its metabolites to GSH by the action of GST (Van der Oost et al., 2003). *In vitro* studies with liver microsomes of the fish *Liza saliens* showed that, regardless of their oxidation state, Cr and other metals inhibit the activity of NADPH-P-450 reductase, which is required for the activity of CYP1A (Bozcaarmutlu and Arinç, 2007). Therefore, Cr would be expected to decrease EROD activity, which is in contrast with the results reported in this study. Similarly, GST activity was higher in the liver and gills of fish caged at site B, indicating the activation of phase-II of biotransformation. Chromium has been shown to lead to a reduction in the activity of GST in the liver of *Carassius auratus* (Lushchak et al., 2009; Kubrak et al., 2010) and in the gills of *Danio rerio* (Domingues et al., 2010). Taken together, the results of EROD and GST indicate that the tannery effluents were capable of activating biotransformation processes both in the liver and gills of *P. lineatus* caged at site B and suggest that these responses were probably due to the presence of organic contaminants in the tannery effluents.

Metals and organic contaminants can stimulate ROS production in organisms by different ways and if not properly scavenged, ROS can cause oxidative stress and lead to the oxidative damage of cellular components (Livingstone, 2001). GSH is an efficient antioxidant and serves as a cofactor of many redox enzymes, particularly a large class of glutathione-dependent peroxidases and transferases (Halliwell and Gutteridge, 2005). In the present study the concentration of GSH was higher in the liver of *P. lineatus* caged at site B and site D than in fish caged at the other sites. This increase in GSH concentrations probably resulted from enhanced synthesis and not from reduced consumption, considering that GSH serve as a cofactor for GST, which exhibited increased activity in the liver of *P. lineatus* caged at site B and site D and in the gills of fish caged at site B. In addition, GSH can act directly as an antioxidant (Lushchak, 2011) thus protecting the cells from the noxious effects of the contaminants in the tannery effluents and directly neutralizing the pro-oxidative compounds. This is consistent with a Cr-mediated increase in GSH concentrations in the liver of *Carassius auratus* exposed to this metal (Lushchak et al., 2009; Kubrak et al., 2010).

When the antioxidant defense system is not sufficient or adequate to inactivate the ROS, the redox levels of the cells can be altered and imbalanced towards pro-oxidation, which is a condition known as oxidative stress and that can cause cell and tissue injuries, such as lipid peroxidation, protein damage, enzyme inactivation, and DNA breaks (Amado et al., 2009). Chromium may perturb free radical processes in animals, and causes oxidative stress *in vivo* in fish tissues (Lushchak et al., 2008). In this context, Tagliari et al. (2004) showed increased lipid peroxidation in the liver of the fish *Gymnogeophagus gymnogenys* collected from a river under the influence of tanneries. However, in the present study there was no evidence of oxidative damage in the fish at any site. This is possibly because the concentrations of the GSH and MT-like proteins were higher in fish caged at the sites downstream of the tannery, which directly or indirectly avoided oxidative damage.

In summary, this study showed that tannery effluents affect the quality of the river water, which resulted in Cr accumulation and altered biochemical biomarkers in *P. lineatus* caged in this river. Compared to the fish caged upstream of the tannery (SA), downstream fish exhibited higher Cr accumulation in the liver, increased EROD and GST liver activities, and higher concentrations of MT-like proteins and GSH. In the light of these results, the use of a set of biomarkers in *P. lineatus* might become a suitable tool for monitoring the quality of water bodies under influence of tannery activities so that precautionary measures can be implemented and damage reduced.

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