Ecotoxicology and Environmental Safety 153 (2018) 188–194

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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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ARTICLE INFO

Keywords:
Antioxidants
Bioaccumulation
Biotransformation
Metal
Metallothionein
Oxidative stress

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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https://doi.org/10.1016/j.ecoenv.2018.02.023
Received 26 July 2017; Received in revised form 2 February 2018; Accepted 5 February 2018

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

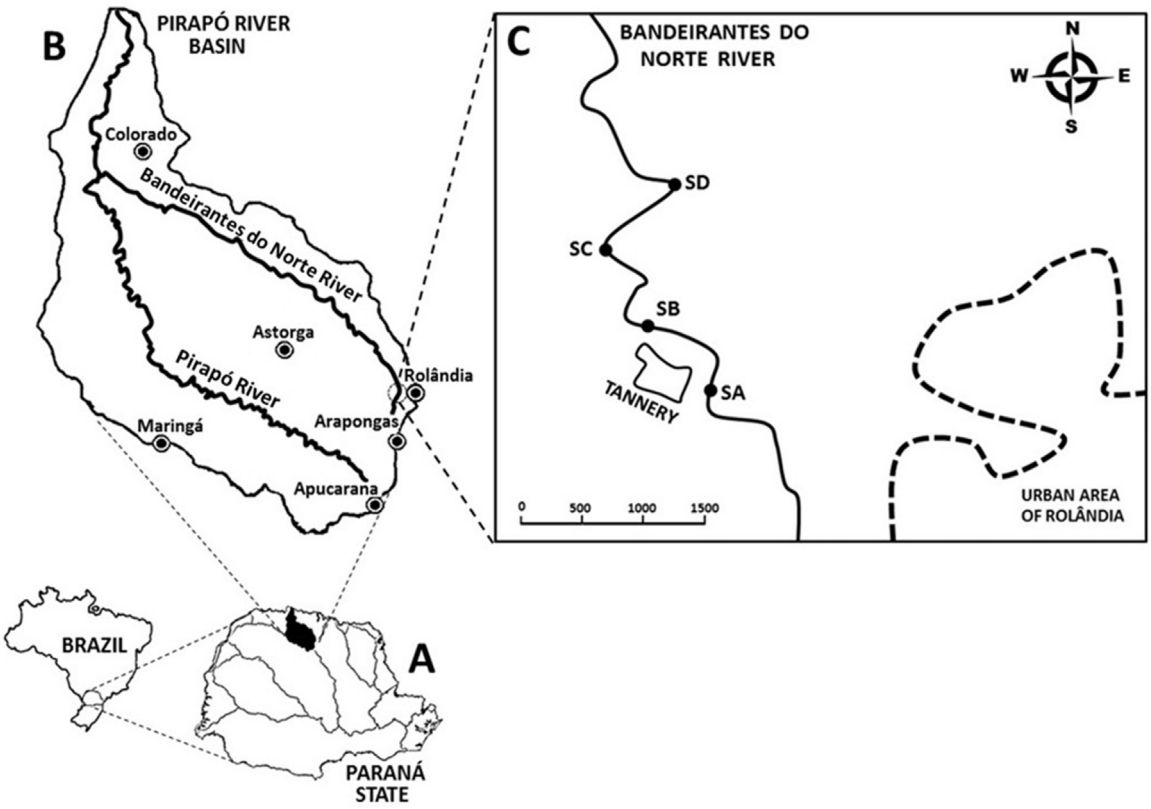

Fig. 1. Maps showing Paraná State, in Southern Brazil (A), Bandeirantes do Norte river, in the Pirapó River Basin (B) and a stretch of Bandeirantes do Norte river (C) indicating the four experimental sites along of the stream where fish were caged (Site A: SA, Site B: SB, Site C: SC and Site D: SD).