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Accumulation of Chromium and Lead in Bryophytes and Pteridophytes in a Stream Affected by Tannery Wastewater

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Abstract The concentrations of Cr and Pb were determined in bryophytes and pteridophytes sampled in a stream near a tannery in Guarapuava, southern Brazil. The concentrations of Cr and Pb were measured by cathodic and anodic voltammetry, respectively. These plants were used to evaluate the spatial distribution of elements in the examined stream, and contained elevated levels of Cr (0.71–24.07 μg/g) and Pb (4.33–24.20 μg/g). Chromium levels in plants near the tannery greatly exceeded background levels, indicating a severe to extreme degree of contamination with this metal. Lead levels were elevated to a lesser degree, indicating slight to moderate contamination for most plants collected near the tannery.

Keywords Trace elements · Bioindicator · Plants · Water pollution

Introduction

Anthropogenic sources of heavy metals, mainly from industrial activities, have substantially increased their concentrations in aquatic ecosystems. The use of bioindicators is an efficient and cost-effective approach for monitoring water and sediment for contamination with metals (Seaward 1995). The bioindicators are organisms used to assist in detecting the quality of an environment. These organisms may respond to chemical stressors by physiological responses or by the accumulation of pollutants. Some bioindicator species may be considered to be accumulation indicators, which accumulate elements without visible symptoms of injury. Others may be response indicators, which present visible symptoms of damage from contact with small amounts of toxic substances (Esser 1986; Kaur et al. 2010).

Mosses are abundant in the environment. They usually lack a protective cuticle and thickened epidermal cell walls, making their tissues readily permeable to retain metallic ions from surface water, precipitation, and dry deposition of airborne particulates (Davis et al. 2001; Gerdol et al. 2000). Gonçalves et al. (1994) and Nimis et al. (2002) proved that the use of bryophytes (mosses, liverworts and hornworts) as accumulators has been one of the few effective techniques for detecting intermittent pollution (e.g., sporadic or seasonal), and in providing information on the bioavailable fraction of the metal. These bryophytes have a high ion exchange capacity with the environment, obtaining both nutrients and contaminants such as heavy metals and organic compounds, through the water, atmospheric deposition and soil (Otvos et al. 2003).

The present study deals with the use of mosses and ferns as bioindicators of heavy metals (Cr and Pb) in surface waters. The aim of our investigation was to elucidate the extent of pollution caused by tannery wastewater disposed of in an aquatic environment located in Guarapuava-PR, Brazil. The effluent, rich in salts of Cr, has been discharged into the river without prior treatment for more than 30 years. Previous studies have shown elevated levels of heavy metals (Cr and Pb) in sediments in the vicinity of the tannery (Pereira 2008; Quináia et al. 2009). The determination of Cr and Pb in plants was performed by mercury hanging drop electrode strip voltammetry.

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Materials and Methods

Plants were collected from a stream in the Rio das Pedras basin in Guarapuava City, Paraná State, Brazil at two sites: fish tank (−25.362942° latitude, −51.430795° longitude) and stream (−25.362806° latitude, −51.431535° longitude) near an effluent discharge for a tannery (Fig. 1). Plants were collected within 200 m of the confluence of the stream and effluent discharge at various points around the fish tank and stream. Eight plants were collected, including one from a clean environment (San Francisco Falls, located at −25.063492° latitude and −51.298331° longitude). The plants were collected and cleaned based on internationally approved techniques (U.S. EPA 1996). The plants, shown in Fig. 2, were named PA, PB, PC (for pteridophytes) and BD, BE, BF, BG, BH (for bryophytes). The bryophyte BH was collected at San Francisco Falls. San Francisco Falls is the largest waterfall in southern Brazil, and one of the largest in the country. Is is located 40 km upstream from the tannery, in the tri-border region between the towns of Guarapuava, Prudentópolis and Turvo, Paraná state, within the Environmental Conservation Area of Serra da Esperança.

Analytical grade reagents were used in the study. All solutions were prepared with ultra-pure water (Human UP 900®). The solutions of metal ions, Cr(VI) and Pb(II) were made by diluting a stock standard solution of 1,000 mg/L (J. T. Baker Instra Analysed, Mexico City, Mexico). Concentrated nitric acid and hydrogen peroxide, 30% v/v, were used for the wet digestion of samples. For the voltammetric determination of Cr, 0.05 mol/L diethyl triamine pentacetic acid (DTPA), 2.5 mol/L sodium nitrate, and 0.2 mol/L sodium acetate were added. For adjusting the pH of the reaction medium, 2.0 mol/L sodium hydroxide and pH 6.2 buffer (0.1 mol/L monobasic potassium phosphate and 0.1 mol/L sodium hydroxide) were used. An acetic acid/ammonium buffer at pH 4.6 was used for the quantification of Pb.

The plants were washed several times in running river water to reduce possible contamination. The samples were taken to the laboratory, and then washed three times with bi-distilled water. The plants (shoot and roots) were dried for 15 days in room temperature. The analytical procedure is summarized in Fig. 3. Potassium permanganate was used to promote the oxidation of Cr III in the samples after digestion.

The determination of Pb in plants was performed by anodic stripping voltammetry with a hanging mercury drop electrode. In this method, Pb dissolved in solution is first deposited on the mercury electrode with a constant cathodic potential. In the determination stage, differential pulse voltammetry is applied in the anodic direction. An acetic acid/ammonia buffer (3 mL) was added to the polarographic cell with the digested sample.

Fig. 1 Map showing the study area: stream, fish tank, tannery (Image: Google Earth) and San Francisco Falls (Photo by Sueli Pércio Quináia)

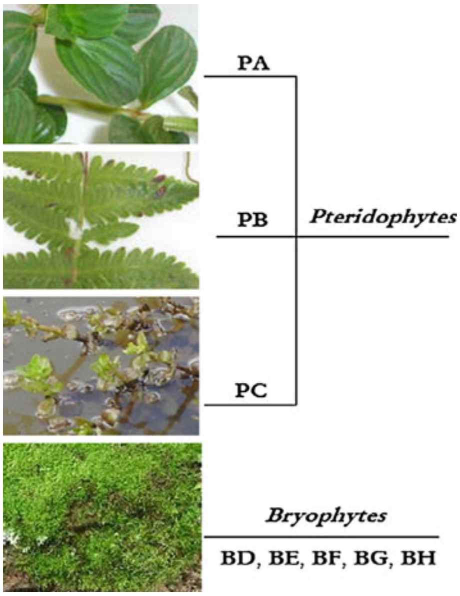

Fig. 2 Examples of pteridophytes (**a**) and bryophytes (**b**) from the fish tank and stream (Photos by Caroline M. M. Repula)

The quantification of Cr was carried out by adsorptive cathodic voltammetry. In this method, the Cr in solution is complexed by DTPA. In the accumulation step, the Cr-DTPA complex is adsorbed on the mercury drop. In the stage of determination, differential pulse voltammetry in the cathodic direction is applied for the stripping and determination of analyte. The determination of Cr(III) by this method is difficult because only a small fraction of Cr(III) provides current response and this response declines steadily over time (Li and Xue 2001). To circumvent this problem, after digestion of the sample, all the Cr(III) is oxidized to Cr(VI), as indicated in Fig. 3.

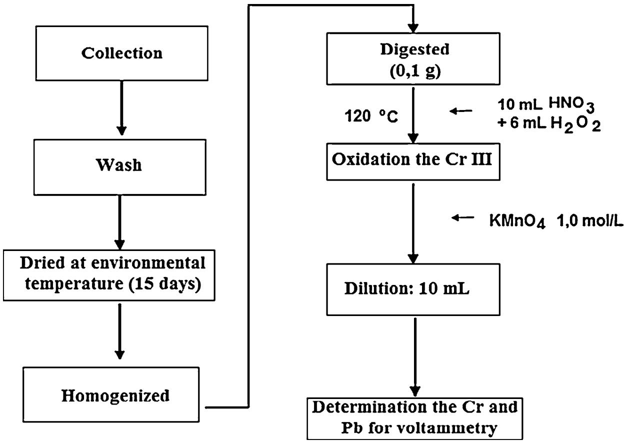

Fig. 3 Analytical procedure for the preparation of plant samples

Table 1 Parameters used for the voltammetric determination of Pb and Cr

Parameters	Pb	Cr
Working electrode	HMDE	HMDE
Purge time	300 (s)	300 (s)
Deposition time	60 (s)	60 (s)
Amplitude	−50 (mV)	−50 (mV)
Initial potential	−0.8 (V)	−1.0 (V)
Final potential	1.0 (V)	−1.45 (V)
Increment potential time	0.2 (s)	0.3 (s)
Increment potential	0.006 (V)	0.009 (V)
Scan rate	30 (mV/s)	33.3 (mV/s)
Equilibrium time	10 (s)	10 (s)
Deposition potential	0.8 (V)	−1

Table 2 Heavy metal concentrations in plants (n = 3)

	Pb (μg/g, Dry Wt.)	Cr (μg/g, Dry Wt.)
Pteridophyte (PA)	4.81 ± 0.10	0.71 ± 0.01
Pteridophyte (PB)	24.20 ± 0.40	3.06 ± 0.27
Pteridophyte (PC)	8.94 ± 0.30	1.43 ± 0.26
Bryophyte (BD)	8.72 ± 0.77	1.74 ± 0.23
Bryophyte (BE)	4.33 ± 0.89	24.07 ± 0.25
Bryophyte (BF)	6.93 ± 0.14	1.97 ± 0.03
Bryophyte (BG)	6.44 ± 0.11	22.29 ± 2.23
Bryophyte (BH)[a]	2.04 ± 0.09	<LD

LD = 0.03 μg/g

[a] Plants were collected from the non-polluted San Francisco Falls reference site

The procedure for the quantification of Cr was performed by adding 30 mL of buffer (pH 6.2) and 2.5 mL of electrolyte (DTPA 0.05 mol/L + Na_2NO_3 2.5 mol/L + CH_3COONa 0.2 mol/L). The pH was adjusted to approximately 6.2 with NaOH 2.0 mol/L. An aliquot of the digested sample (usually 0.1 mL) was added. The sequence of procedure must be strictly followed, because if the pH is adjusted after the introduction of the sample the relative standard deviation among triplicates increases. Nitrogen was used as purge gas to remove the oxygen present in the reaction medium. Table 1 contains the parameters for the determination of Pb and Cr by anodic and cathodic stripping voltammetry, respectively. For both determinations, a Metrohm 757 polarograph (Metrohm Ltd., Herisau, Switzerland) with a three-electrode system was used. The reference electrode used was Ag/AgCl with a solution of KCl 3.0 mol/L, the auxiliary electrode was a platinum wire, and the working electrode was a hanging drop of mercury. The detection limits for Pb and Cr were 0.01 and 0.03 μg/g, respectively.

Results and Discussion

The advantage of using bryophytes and pteridophytes as bioindicators is that they reflect the accumulation of

Table 3 Contamination factor for Pb and Cr in plants

	Sampling points	Pb	Cr
Pteridophyte (PA)	Stream (230 m from the tannery)	2.4	23.4
Pteridophyte (PB)	Stream (150 m from the tannery)	11.9	102.0
Pteridophyte (PC)	Fish tank	4.4	47.6
Bryophyte (BD)	Fish tank	4.3	58.0
Bryophyte (BE)	Fish tank	2.1	802.3
Bryophyte (BF)	Stream (115 m from the tannery)	3.4	65.7
Bryophyte (BG)	Stream (300 m from the tannery)	3.2	743.0

Contamination factor (CF) categories

CF > 1 No contamination CF > 3.5–8 Moderate contamination

CF > 1–2 Suspected contamination CF > 8–27 Severe contamination

CF > 2–3.5 Slight contamination CF > 27 Extreme contamination

Table 4 Comparison of present results with information cited in the literature

	Pb (μg/g)	Cr (μg/g)	References
Bryophytes and Pteridophytes	2.04–24.20	<LD–24.07	This work
Bryophytes	20.00–220	4.00–250	Ghatge et al. (2011)
Bryophytes	4.19–14.7	1.47–1.83	Harmens et al. (2010)
Bryophytes	1.06–26.09	0.50–585.63	Nimis et al. (2002)
Pteridophyte	6.30–2029.00	nd	Liu et al. (2008)

LD detection limit, *nd* not determined

environmental pollutants over time, due to the affinity of their chelating agents for metal ions (Palmieri et al. 2005; Davis et al. 2001; Gerdol et al. 2000). Pereira (2008) determined the levels of Cr in suspended particulate matter, water and sediments collected in various parts of the stream. The results revealed high concentrations of Cr in suspended particulate matter (1,580–2,877 μg/g) and sediments (873–1,691 μg/g) far above those specified by the Canadian Council of Ministers of the Environment (CCME 1999) in their sediment quality guidelines (90 μg/g). Quináia et al. (2009) analyzed sediment samples from the same stream, and found that Cr and Pb concentrations were elevated compared to levels normally found in uncontaminated sediments. Concentrations of Cr and Pb ranged from 10 to 4,400 μg/g and 7 to 320 μg/g, respectively.

Cr and Pb concentrations in plant tissue varied with species and collection site (Table 2). The bryophyte (BH) collected in an area free from contamination (San Francisco Falls) presented the lowest levels of Cr and Pb. The pteridophyte (PB) showed the highest concentration of Pb, while plants BE and BG showed higher levels of Cr.

To estimate the environmental impact, we calculated the contamination factor (CF) as a relationship between the levels of certain metals in plants and the background concentration found in plants collected at the reference site, San Francisco Falls (Table 3). Fernandez and Carballeira (2001) proposed a scale of contamination based on the values of CF. The scale consists of six categories, ranging from the absence of contamination (CF < 1) to extreme contamination (CF > 27) (Fernandez and Carballeira 2001).

Serious contamination by Cr was observed in plants collected around the stream due to the discharge of tannery wastewater. The concentrations of Pb in plant shoots ranged from 4.81 to 24.20 μg/g, which were 2.40–11.00 times higher than in plants collected from the non-contaminated site. The concentration of Cr ranged from 0.71 to 24.07 μg/g, with an average value of 7.89 μg/g. Most samples showed a CF greater than 27 for Cr. For Pb, four samples showed a CF between 2.0 and 3.5, indicating slight contamination. Two samples showed moderate contamination and one showed severe contamination. The slightly elevated levels of Pb in the environment may be due to the urban setting of the stream, and emissions from road traffic. Table 4 presents comparative information from some studies on the use of biological indicators for determination of Cr and Pb in contaminated environments.

The plant species studied are good biomonitors for the accumulation of Pb and Cr. Comparing the samples of plants from the reference site with the sites near the tannery effluent discharge, it was concluded that the biomonitors contained Cr and Pb concentrations well above the natural baseline. This confirms the results from earlier studies by

Pereira (2008) and Quináia et al. (2009) showing heavy metal pollution in this stream.

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