

Determination and Evaluation of the Metals and Metalloids in the *Chapeu-de-couro* (*Echinodorus macrophyllus* (Kunth) Micheli)

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Abstract The *Chapeu-de-couro* (*Echinodorus macrophyllus* (Kunth) Micheli) is a native plant from Brazil, which has been mainly used in medicinal application being a potent antirheumatic and diuretic, in the production of soft drinks, and also in the ornamentation of aquariums. In this paper, the metals and metalloids for the leaves of *chapeu-de-couro* collected in the Paraguaçu River from the city Cachoeira, Bahia State, Brazil, was determined and evaluated using multivariate analysis. The samples were digested using nitric acid and hydrogen peroxide and were analyzed using inductively coupled plasma mass spectrometry. The accuracy of the method was confirmed by analysis of a certified reference material of apple leaves, furnished by National Institute of Standard and Technology. The study involved 15 samples of the Paraguaçu River. The results expressed as milligrams of element per kilogram of sample demonstrated that the concentration ranges varied: 1.39–5.27 for chromium, 44.85–165.39 for manganese, 0.55–0.84 for arsenic, 0.01–3.94 for antimony, and 0.18–0.31 for lead. The principal component analysis and hierarchical cluster analysis evidenced that the concentrations of the metals and metalloids varied according with the variations in the water of the Paraguaçu.

Keywords Metals and metalloids · *Chapeu-de-couro* · *Echinodorus* · ICP-MS · PCA · HCA

Introduction

The determination of the metals toxic, metalloids, and mineral composition of fruits, leaves, and roots that are used as food for humans is a worldwide concern. These studies may reveal the nutritional potential of new plants and the toxicity of a given species, this way contributing to the formulation of food composition tables, which are used by nutritionists and doctors. Several studies have been performed with this objective [1–5].

The *chapeu-de-couro* (*Echinodorus macrophyllus* (Kunth) Micheli), also known by the names of “cha-da-campanha,” “erva-do-brejo,” and “erva-do-pantano,” is an herb of the Alismataceae family, which has been mainly used in the medicinal application being a potent antirheumatic and diuretic, in the production of soft drinks, and also in the ornamentation of aquariums. For this reason, some papers have been performed in order to evidence the composition of this plant [6]. Tanus-Rangel et al. evaluated the effects of leaf ethanolic extract from *E. macrophyllus* in acute and subchronic models of inflammation [7]. Lopes et al. evaluated the toxicological effects by in vitro and in vivo assays of an aqueous extract prepared from *E. macrophyllus* leaves [8]. Kobayashi et al. evaluated the potency of four new diterpenoids, isolated from the leaves of the plant *E. macrophyllus* [9]. Vidal et al. evaluated the genotoxicity and mutagenicity of an aqueous extract of *E. macrophyllus* leaves [10].

Multivariate analysis techniques—principal component analysis (PCA) and hierarchical cluster analysis (HCA)—have

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Table 1 Merit figures for validation of the analytical method used for quantification

Element	Achieved value (mg kg ⁻¹)	Certified value (mg kg ⁻¹)	LD ^a (mg kg ⁻¹)	LQ ^a (mg kg ⁻¹)	RSD (%)
Arsenic	0.118±0.028	0.112±0.004	0.016	0.055	4.52
Chromium	2.01±0.30	1.99±0.06	0.033	0.109	1.60
Manganese	247±3	246±8	0.948	3.161	2.43
Antimony	0.061±0.008	0.063±0.006	0.003	0.010	3.14
Lead	–	–	0.053	0.178	3.41

^aDetection limit ($3s/b$), quantification limit ($10s/b$); s (standard deviation of the blank) b (slope analytical curve)

been often employed for evaluation and characterization of analytical results [11–14]. PCA determines the diagonalization of the covariance or correlation matrix transforming the original chemical measurements into linear combinations of these measurements, which are the principal components (PCs) [15]. This corresponds to rotation of the coordinate space axes so that the explained variance of each PC is maximized. This technique allows for data reduction from higher to lower dimensional spaces to simplify their graphical representation. By contrast, HCA determines the similarities between samples by examining the interpoint distances representing all possible sample pairs in higher dimensional space. These techniques complement each other and have been widely used in solving classification problems [16–20].

Then, this paper had as objective the determination of the arsenic, chromium, cadmium, manganese, lead, and antimony in the leaves of this plant. The results were evaluated using the multivariate analysis techniques PCA and HCA.

Table 2 Determination of chemical elements in *chapeu-de-couro* samples (milligrams of analyte per kilogram)

Samples	Cr	Mn	As	Sb	Pb
P1	5.37	44.85	0.84	0.06	0.29
P2	3.65	48.19	0.55	0.09	0.31
P3	3.68	45.39	0.55	0.06	0.21
P4	2.40	127.12	0.55	0.03	0.18
P5	2.49	123.63	0.55	0.02	0.18
P6	2.55	126.42	0.58	0.03	0.18
P7	2.35	87.29	0.55	0.03	0.26
P8	2.65	91.06	0.55	0.03	0.18
P9	2.60	84.57	0.55	3.94	0.23
P10	1.58	107.87	0.55	0.02	0.28
P11	1.39	103.33	0.55	0.01	0.30
P12	1.78	127.61	0.55	0.10	0.22
P13	2.15	165.39	0.55	0.05	0.18
P14	1.83	137.56	0.55	0.03	0.18
P15	1.81	138.63	0.55	0.03	0.20

Experimental

Instrumentation

A model Thermo X Series II inductively coupled plasma mass spectrometer (Germany) with axial viewing and a charge coupled device detector was used for multi-element determination. A concentric nebulizer was used. The metal determinations were carried out under manufacturer-recommended conditions for power (1.3 kW), plasma gas flow (16.0 L min⁻¹), auxiliary gas flow (0.7 L min⁻¹), and nebulizer gas flow (0.87 L min⁻¹). The isotopes monitored were ⁵²Cr, ⁵⁵Mn, ⁷⁵As, ¹²¹Sb, and ²⁰⁸Pb.

Chemicals and Reagents

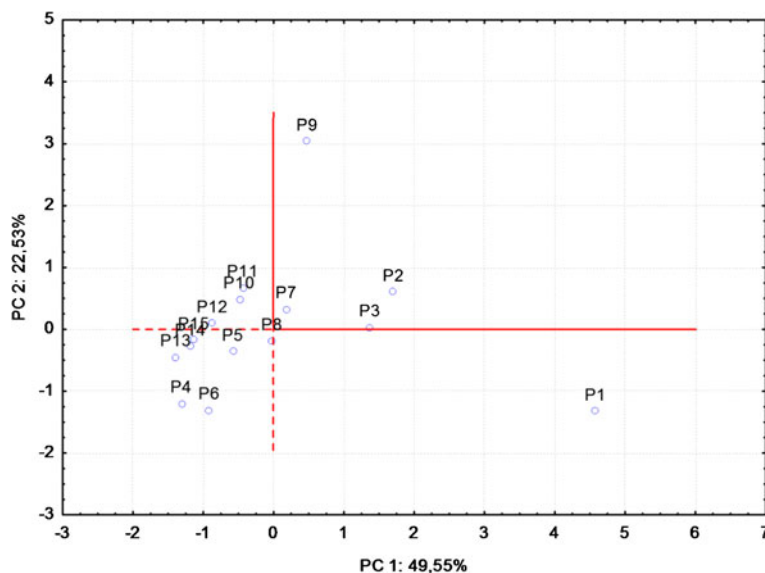
All chemical reagents used in the experiment were of analytical grade obtained from Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) from a Milli-Q system (Millipore, MA, USA) was used to prepare all solutions. Glass apparatus used were maintained in nitric acid solution (10 %, v/v) during 12 h for decontamination prior to use.

Stock standard solutions for all elements with a concentration of 1,000 mg L⁻¹ were used for prepared working standard solutions by diluting appropriate dilution of these solutions with 1 % (v/v) nitric acid. Nitric acids and hydrogen peroxide were used for sample digestion.

Table 3 Loadings of the variables for the first three principle components

Variable	PC1	PC2	PC3
Cr	0.9078	-0.2380	-0.2497
Mn	-0.8715	-0.2231	-0.0789
As	0.7856	-0.3887	-0.1639
Sb	0.0936	0.7938	-0.5832
Pb	0.5174	0.4885	0.6596
Total variance (%)	49.55	22.53	17.41
Cumulative variance (%)	49.55	72.08	89.49

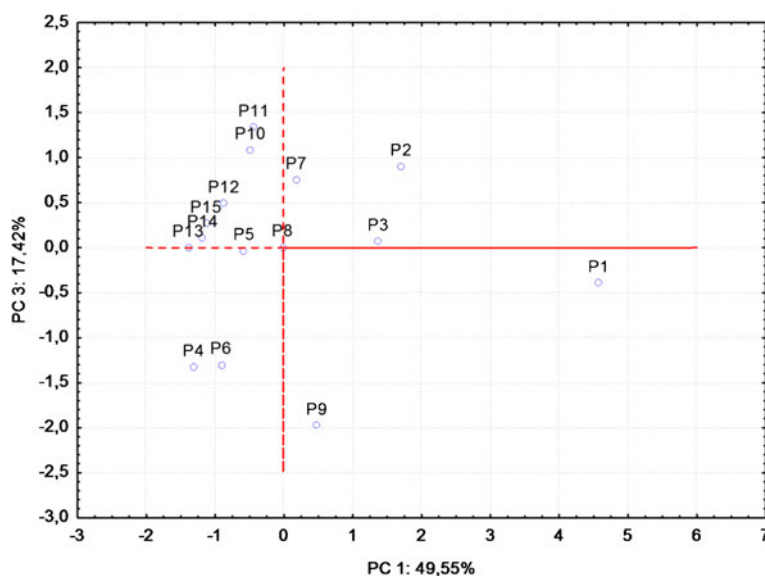
Fig. 1 Plot of PC1 versus PC2 for the 15 *E. macrophyllus* samples



Sample Collection and Storage

The samples were collected in the Paraguacu River, waterfall in city, from Bahia, Brazil, between December of 2011 and February of 2012. A total of 15 samples were analyzed. In the laboratory, the samples were oven-dried (100 °C) and were lyophilized to ensure a greater homogenization. In the points of collection of samples of the plant in the river, water samples were collected to assess changes in concentrations according to the points in the rivers. The water samples passed through the measurement of pH, for this a pH meter Digimed DM20 (São Paulo, Brazil) was used. After the pH measurement, the samples were filtered to remove impurities and immediately acidified, with the addition of 0.2 mL of bidistilled HNO₃ to 10 mL of sample.

Fig. 2 Plot of PC1 versus PC3 for the 15 *E. macrophyllus* samples



Sample Digestion

About 0.1 g of each sample was placed into glass vessels. Two milliliters of concentrated nitric acid and 2.0 mL of 30 % (v/v) hydrogen peroxide were added to each vial. The samples were digested for about 4 h at 160 °C. The digested samples were later transferred to 15 mL centrifuge tubes and topped off with ultrapure water up to the 12 mL mark.

Accuracy of the Analytical Method Used for Quantification

The accuracy of the method used for determination of the elements was confirmed by analysis of a certified reference material furnished by National Institute of Standard and Technology (NIST; Gaithersburg, MD, USA), Tomato Leaves

NIST 1573a. Digestion of this material was performed with the same decomposition procedure used for the *chapeu-de-couro* samples. The results were in agreement with the certified values as Table 1. For lead, the accuracy was evaluated only by spike test, due to the fact that there were no any certified materials available to the metal. The recuperations values for the spike test for lead varied between 91.0 and 96.9 %.

Results and Discussion

Determination of Chemical Elements in *Chapeu-de-couro* Samples

The determination of the elements arsenic, cadmium, chromium, manganese, lead, and antimony were performed in the 15 samples. The results expressed as average of triplicates (milligrams of element per kilogram of sample) are shown in Table 2.

Data Evaluation Employing Principal Component Analysis

The results of the determination of the elements in the 15 samples analyzed were evaluated using PCA. A data matrix was constructed using the elements as columns and the *E. macrophyllus* samples as rows (Table 2). The evaluation was performed on auto-scaled data because of different orders of magnitude in element concentrations. The loadings of the original variables on the first three principal components and the variances explained by each component are given in Table 3.

The first two principal components were chosen for modeling the data because they describe about 72 % of the total variance. Chromium, arsenic, and manganese are the dominant variables for the first principal component (PC1)

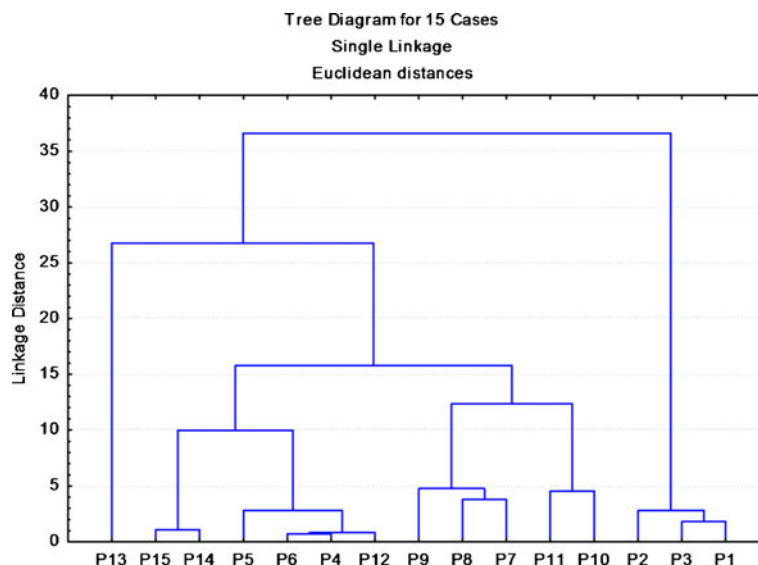
Table 4 Concentrations of chemical elements in the *chapeu-de-couro* samples (milligrams of element per kilogram of sample) and river water (micrograms per liter)

Element	River water Average concentration ($\mu\text{g L}^{-1}$)	<i>Echinodorus macrophyllus</i>	
		Average	Concentration range
Arsenic	0.65	0.34	0.55–0.84
Chromium	1.27	2.55	1.39–5.27
Manganese	10.00	103.93	44.85–165.39
Antimony	0.04	0.30	0.01–3.94
Lead	0.30	0.20	0.18–0.31

and represent 49.55 % of the total variance. These three elements contribute to the major variability presented in the samples, and arsenic and chromium are positively correlated. The second principal component (PC2) accounts for 22.53 % of the total variance and antimony as the dominant variable. The score plot of the first two components is shown in Fig. 1.

Figure 1 shows no systematic separation between the varieties analyzed. Samples with high concentrations of manganese had low scores for PC1 because the loading for the element is negative. This way, samples P1, P2, and P3 had low concentrations of the manganese. Sample P1 had the highest concentration of arsenic and chromium, and P13 had the highest concentration of manganese. Samples with higher scores on PC1 had the smallest concentrations of chromium, arsenic, and lead. Samples with lower scores on PC2 (P1, P6, and P4) had lower concentrations of the antimony, considering that the element has negative loading on PC2 (Table 3). On the other hand, sample P9 had high score on PC2, therefore high

Fig. 3 Dendrogram for *E. macrophyllus* samples showing single linkage with Euclidean distances



concentration for the antimony. The third principal component (PC3) presented about 17 % of the total variance, where lead was the dominant variable. They were, however, inversely correlated because antimony had negative loadings and lead showed a positive loading. Figure 2 depicts the score graph for the first and third PCs. An evaluation of this figure reveals that some samples were separated. Samples P4, P6, and P9 had a higher negative score on PC3; however, antimony has the highest concentration. Sample P9 had a high concentration of antimony and the lowest concentration of lead.

Evaluation of Data Using Hierarchical Cluster Analysis

HCA was applied to the auto-scaled data using the single linkage method with Euclidean distances to calculate sample interpoint distances and similarities. The dendrogram obtained is shown in Fig. 3.

Some results found by PCA were also achieved using HCA. Samples P1, P2, and P3 were found to be very separated on the graph compared to other samples using the method of HCA. Using PCA, the sample was found to have the highest concentration of chromium. Sample P13 analyzed by HCA showed that the sample is a single group (Fig. 3). Samples P14, P15, P4, P6, P12, and P5 can also be observed in Fig. 3. By PCA, some of these samples had low scores for PC1 because they have high concentrations of chromium, arsenic, and lead, which are the dominant variables for PC1.

Determination of the Metals Toxics and Metalloids in the *Chapeu-de-couro*

The results obtained by PCA and HCA demonstrated that there was no systematic difference in metal and metalloids composition of the *chapeu-de-couro* samples collected in the Paraguacu River. The average concentrations of the elements were calculated using the data obtained for the 15 samples analyzed. The average concentrations and ranges of concentration were found to be the following, respectively: 0.34 and 35.80–140.27 mg kg⁻¹ for arsenic, 0.01 and 0.11–0.37 mg kg⁻¹ for cadmium, 2.55 and 1.39–5.27 mg kg⁻¹ for chromium, 103.93 and 44.85–165.39 mg kg⁻¹ for manganese, 0.30 and 7.67–51.40 mg kg⁻¹ for antimony, and 0.20 and 0.05–0.64 mg kg⁻¹ for lead. The concentrations found in the leaves of *E. macrophyllus* were inversely proportional to the concentrations in the river water, in other words, when concentrations of the elements were higher in the river water, lesser were in the leaves—the results for the elements are shown in Table 4. This direct relationship between the concentration of these elements in the river and leaves of *E. macrophyllus* could be related to the fact that heavy metal absorption by plant roots be inevitable because during the process of mineral nutrition, the facilitated entry of nutrients into the body of the plant by these same mechanisms allows the entry of toxic elements. These

toxic metals, as well as minerals, reach the leaves through the xylem [21]. In the aquatic plants, the leaves may represent another route of absorption, by establish direct contact with the absorption solution containing heavy metal [22]. Therefore, the origin of the plant used must be known because depending on the cultivated locality it can present values that are considered toxic for consumption established for the Brazilian legislation [23].

Conclusions

The method developed for the determination of metals and metalloids in *chapeu-de-couro* by ICP-MS was satisfactory for the quantification of arsenic, chromium, manganese, antimony, and lead.

Chromium, arsenic, and manganese are the elements that contribute to the major variability presented in the samples and all are positively correlated for chromium and arsenic. It means that the assimilation processes of these elements by this vegetable probably are similar.

The PCA and HCA techniques demonstrated that there is small difference in the composition for elements determined between the various points of the river.

The results evidenced that *chapeu-de-couro* can present toxicity if cultivated in contaminated locality because of metals and metalloids absorption during mineral nutrition.

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