Amazonia

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Article

Levels of As, Cd, Pb and Hg Found in the Hair from People Living in Altamira, Pará, Brazil: Environmental Implications in the Belo Monte Area

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Amostras de cabelo foram utilizadas como bioindicador para avaliar os níveis de As, Cd, Pb e Hg na população residente na área "Volta Grande" do rio Xingu, no Norte do Brasil. As concentrações de As e Cd estão de acordo com os valores encontrados na literatura, no entanto, foram encontrados valores anômalos de Pb e Hg , o que pode sugerir uma variação inter-regional. Os elevados valores encontrados de Pb e Hg, em cabelos de moradores da Ilha do Canteiro, podem ser atribuídos à exposição ambiental das famílias, por estarem em uma área ambiental impactada pela mineração de ouro. A aplicação dos métodos de estatística multivariada mostrou que a concentração dos metais, estudados neste trabalho, pode ser classificatória entre pessoas de localidades diferentes do município de Altamira. Os resultados neste trabalho refletem o impacto ambiental destes elementos sobre a saúde dos habitantes desta área de garimpagem de ouro.

Human hair was used as a bioindicator to evaluate the levels of As, Cd, Pb and Hg in the resident population of the "Volta Grande" area of the Xingu river, in the North of Brazil. The concentrations of As and Cd are in agreement with the values found in the literature, however, the Pb and Hg concentration is very different, which can suggest an inter-regional variation. Moreover, the high values found for these elements in the residents of the Canteiro Island can be attributed to environmental exposure of this population in an area with environmental impact from activities of gold mining. The application of these methods showed that the concentrations of the metals studied in this work can correctly classify two different localities in the Altamira district. The results reflect the impact of these elements on the health of the inhabitants of this auriferous area.

Keywords: arsenic, cadmium, lead, mercury, hair, Altamira, environmental, chemometric methods, multivariate statistics

Introduction

The Amazon region possesses one of the biggest hydrographic basins of our planet. The construction of a hydroelectric dam in Monte Belo, Pará state, will help to resolve some of Brazil´s energy demand. However, its construction points to conflicts with respect to the social-economics of the environment and of the population living in Altamira and in other localities near the "Volta grande" area of the Xingu river, in the North of Brazil.

The exploitation of hydroeletric energy implies a significant environmental impact that can not be neglected. The formation of the accumulation basin behind the dam will provoke flooding of the forests, the disappearance of important fauna from the submersed area as well as land used for agriculture, and villages and towns with traditional and cultural knowledge. The dam will also provoke displacement of the population, changes in the quality of the water, increases in production of gaseous carbon compounds (CO_2, CH_4) and, nitrogen compounds (NO_3^-, O_2^-), phosphorous (PO_4^{3-}) and other disruptions.[1,2] All these environmental factors modify the biodisponibility of the metals in the aquatic environment and modifies the relationship between the man and metals in these localities through a process called biomagnification.[1–4]

The Brazilian Amazon presents a great area of mineral wealth but a high level of Hg contamination from the mines that are quite widespread. Studies of the presence of this dangerous element are quite common. However, very little is known about contaminations by As, Cd and Pb. For a long time the presence of Hg in the Amazon was associated with its use by prospectors action, but, recently, these high

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values have also been associated to its natural occurrence in the soil of this area.[5–9] In a general way, the Amazon area is characterized by its enormous amount of water that shelters a great number of fish species, which are considered to be the main source of animal protein in the feeding of the indigenous and riverine populations.[10,11] WHO[12] considers only 10 μg g^{-1} of Hg tobe the tolerable level.[13] Higher concentrations were found by Leino and Lodenius,[14] who encountered amounts of 240 μg g^{-1} in people around of the reservoir of Tucuruí. In addition, the municipal district of Altamira placed at the Xingu river's margins, also presents "ribeirinhos", a kind of population that also has fish as its main food. However, there is no information in the literature above the concentration of Hg or other heavy metals in the peoples of the Altamire region of Pará State.

Diverse studies had been carried through by our group using multivariate analysis models as support to understand problems of diverse natures and origins.[15–18] Principal component analysis (PCA), hierarchical cluster analysis (HCA) and discriminant analysis (DA) methods were used in the statistical treatment of the data to build a model to make possible the classification of these residents according to the amounts of As, Cd, Pb and Hg in their hair. This type of procedure has been quite successful in similar studies[19–22] with trace levels of chemical elements present in hair samples, and for distinction among persons diagnosed with cancer.[23]

This paper reports part of the results of a cooperative research project between UFPA and Eletronorte, developed in the municipal district of Altamira, this being an area characterized by intense activity of clandestine prospectors who present risks from As, Cd, Pb and Hg during the extraction of gold. The concentrations of these elements in hair samples of inhabitants of the Altamira and of Canteiro island in Brazil are presented. The Hg was determined by atomic absorption spectrometry (CVAAS) and other elements were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). In addition, principal component analysis (PCA), hierarchical cluster analysis (HCA) and discriminant analysis (DA) were employed to investigate the possible relationships between the concentrations of As, Cd, Pb and Hg and these two localities.

The results obtained in this work will be used as the base for future studies after the start up of the hydroelectric plant of Belo Monte.

Experimental

Reagents

All chemicals were of analytical reagent grade from Merck (Darmstadt, Germany), unless otherwise indicated. Distilled, doubly deionized, high purity water provided by an Elga Purelab Ultra purification system with a maximum specific conductivity of 0.052 μS cm^{-1}, was used to prepare all solutions. Stock solutions Titrisol (Merck, Germany) of 1000 μg g^{-1} of As, Hg, Cd and Pb were used to prepare the standard solutions.

Study area

The study was performed in Altamira city (02° 34' 45" S and 51º 57' 15" W Gr) and on Canteiro island (03° 24' 36.8" S and 51º 56' 10" W Gr), Brazil (a map is shown in the supplementary information, Figure S1). Altamira is also known as the "capital of Transamazônica". With an area around 161,445.93 km^2. The population of the municipal district is 85,901 inhabitants.[24] On Canteiro island, the inhabitants consume mainly fish as their source of protein and use water from the Xingu river for drinking and domestic purposes. A person who lives on the island survives mainly by extraction of rubber latex (*Hevea brasiliensis*) and by collecting Brasil nuts (*Bertholletia excelsa*), complementing these activities with manioc cultivation, hunting and fishing. [25]

The illusion of possible wealth provoked an invasion of people from others parts of Brazil into the district of Altamira. With this process, the area now has an ecological imbalance accelerated as a result of the activities of mineral extraction. In the area next to the Xingu river, there is a great diversity of natural resources, including fauna, flora and an immense mineralogical potential. However, human activities, for example, in the mining of gold, have caused irreparable damage to the principal natural resource of the studied area.

Sample collection and study population

The hair samples of 81 persons were collected in Altamira (29 males and 52 females) with ages ranging from 3 to 87. On Canteiro island 9 individuals (4 males and 4 females) were submitted to collection, with an age range of 8 to 52. The hair samples were collected from volunteers by a barber according to IAEA recommendations.[26] Only those who had lived for at least 2 years at the same address were included in the study. For the children, all the parents permitted the study and completed a questionnaire. The questionnaire was used as a tool to investigate the important factors in their lives that might influence the levels of elements found in each individual. These items reported such informations: socio-economic factors; nationality; nutrition; exposure to tobacco smoke within their environment; as well as housing conditions; hair color and the type of hair products used.

Sample treatment and data analysis

About 600 mg of hair sample were collected from each person. These were washed with acetone and water for 10 min in three consecutive steps. Then, approximately 150 mg of washed and dried sample were accurately weighed and transferred to a closed microwave digestion vessel. Into the vessel were added slowly 1.5 mL of concentrated HNO_3 and 0.25 mL of 30% (m/v) H_2O_2. The mixture was allowed to react until vigorous reactions ceased. The vessels were closed and heated using the following MW program: 4 min at 200 W; 3 min at 0 W and 4 min at 250 W. After the end of the program the digested samples were transferred to a 50 mL volumetric flask and diluted with water. A Provecto DGT 100 microwave oven and Hostaflon bombs (Provecto, Brazil) were used for the development of the microwave digestion procedures.

Analytical method

The element Hg was analyzed by CVAAS. For mercury analysis, a Varian Spectra 55 flame atomic absorption spectrophotometer with an Hg hollow cathode lamp (Varian, Australia) operated at 4 mA along with a deuterium background correction lamp, equipped with a Varian VGA 77 continuous vapor generation accessory, was used. The sample was continuously mixed with a reducing reagent (25% (m/v) $SnCl_2$ in 20% (m/v) HCl), according to the methodology described by Rahman *et al*.[27]

For analysis of As, Cd and Pb a Varian Vista Pro ICP-OES was used. A Y internal standard was added to each sample solution, according to the methodology of D'Ilio *et al*.[28] Argon with a minimum purity of 99.999% (White Martins, Brazil) was used to sustain the plasma. Integrated absorbance was used for measurement.

The calibration data obtained for each element are summarized in Table 1. The accuracy of the methodology developed was appraised through the analysis of NCS Certified Reference Material DC73347 (GBW07601) from the Chinese National Analysis Center and of CRM 397 (Trace Elements in human hair). Samples of the reference material were prepared three times according to the methodologies used for the collected samples. These results are also summarized in Table 1 where it can be seen that the elemental concentrations found for these reference samples are in agreement with the certified or indicated concentrations of the reference materials.

The instrumental detection and quantification limits were obtained from the values of 20 blank samples as recommended by Skoog *et al*.[29] These values are also reported in Table 1.

Statistics treatment

Data analysis

All data are presented as the mean ± standard deviation. Student's two-sample *t*-test was employed for statistical analysis of the data. The significance level was set at $P < 0.05$. Data analyses were performed with the computer statistical analysis system Statistic 6.0, StatSoft In.

Pre-processing of the data

Before applying the multivariate methods to the 90 samples under study, each calculated property (variable or descriptor) was autoscaled. In the autoscaling method, each variable is scaled to a mean of zero and a variance of unity. This method is very important because each variable is weighted equally and this provides a measure of the ability of a variable to discriminate classes of samples. With this method, we can compare all variables at the same level although each presents different units.[30,31]

Multivariate methods

Principal component analysis (PCA)

PCA is a multivariate statistical technique and the central idea of PCA is to reduce the dimensionality of a data set that presents a large number of interrelated variables, while retaining as much as possible of the variation present in the data set. This is achieved

Table 1. Recuperation, detection and quantification limits for As, Cd and Pb determinations in the certified reference material DC73347 (GBW07601) by ICP-OES and for Hg in CRM by CVAAS

Elements	Detection limit[a] 3SD* / (μg g^{-1})	Quantification limit[a] 10SD / (μg g^{-1})	Certified Value / (μg g^{-1})	Observed Value / (μg g^{-1})	Recuperation / (%)
As	0.0019	0.0063	0.28 ± 0.04	0.25 ± 0.09	92.36
Cd	0.0660	0.2190	0.11 ± 0.02	0.10 ± 0.05	91.30
Pb	0.0097	0.0032	8.80 ± 0.90	8.20 ± 0.20	93.18
Hg	0.0050	0.0170	12.30 ± 0.50	14.01 ± 0.09	113.90

*SD Standard deviation; [a]Detection and quantification limits based on 20 runs of the blank.

by transforming the original data set to a new set of variables, called the principal components (PC), which are not correlated between themselves, and are ordered so that the first PC retain most of the variation present in all of the original variables.[32] Three general aspects can be attained when PCA analysis is employed: (*i*) the original set of variables can be reduced to a smaller set that accounts for most of the variance in the data set; (*ii*) PCA can search the data for qualitative and quantitative distinctions in situations where the number of data available is quite large; (*iii*) PCA can be used to test hypotheses about the qualitative and quantitative distinctions in the data set.[15-18,33,34]

Hierarchical cluster analysis (HCA)

The HCA technique examines the distances between the samples in a data set and represents this information as a two-dimensional plot called a dendrogram. The HCA method is an excellent tool for preliminary data analysis. It is useful for examining data sets for expected or unexpected clusters, including the presence of outliers. It is informative to examine the dendrogram in conjunction with PCA as they give similar information in different forms. In HCA, each point forms a single cluster initially and then the similarity matrix is analyzed. The most similar points are grouped forming one cluster and the process is repeated until all the points belong to a unique group.[33,35]

Discriminant analysis (DA)

The main objective of DA is to determine discriminant functions using the measured variables that separate the groupings as distinctly as possible. The DA is a classification procedure that maximizes the variance between categories and minimizes the variance within categories. This method renders a number of orthogonal discriminant functions equal to the number of categories minus one.[35]

Results and Discussion

A recent study related to this region, *Programa Levantamentos Geológicos Básicos do Brasil*,[36] supplies more complete information of the geological and geochemistry of this region. The morphologies, genetic types and the association of 39 different mineral concentrations, besides the distribution of the elements gold and tin, show significant and divergent distributions in the area. Other elements are distributed in a sporadic way, of which Pb, As and Cd can be mentioned.

The toxic elements and the environmentally exposed groups (in Altamira city and on Canteiro island)

The statistic summary with the values of the means (both arithmetic and geometric), medians and standard deviations from the chemical analysis of the heavy elements As, Cd, Pb and Hg in the residents' hair from Altamira city and Canteiro island is presented in Table 2 and Figure S2 (supplementary information).

Several papers found in the literature have reported that chemical element concentrations in human hair depend on age and sex, geographic changes, nutritional level and environmental factors. With the exception of mercury, there is little information about the concentration levels of the different elements in this region. The results were compared with several studies realized around the world and summarized in Table 3. From the information obtained from the social-environmental questionnaire, applied to these two groups of residents, and also from the values of the averages of the concentrations of these elements in other control populations (Table 3), it is possible to verify the level of environmental exposure related to these people. The data distribution is very important and can be used to help in the interpretation of the studied persons. The present paper shows the all elements show a log-normal distribution. The same aspect was observed by

Table 2. Concentrations of As, Cd, Pb and Hg (μg g^{-1}) in human hair samples of the resident populations in Altamira city and on Canteiro Island, in the North of Brazil

Element	Altamira city							Canteiro island						
	N	AM*	GM*	Median*	SD*	CV / (%)	Range Min-Max	N	AM*	GM*	Median*	SD*	CV / (%)	Range Min-Max
As	67	0.434	0.373	0.344	0.287	66.12	0.140 - 1.507	8	0.279	0.263	0.254	0.112	40.14	0.173 - 0.523
Cd	75	0.259	0.187	0.191	0.250	96.53	0.040 - 1.695	9	0.230	0.202	0.193	0.130	56.52	0.085 - 0.465
Pb	76	6.550	4.606	4.765	6.151	93.91	0.822 - 33.761	9	23.233	20.835	19.888	11.154	48.01	8.059 - 45.350
Hg	80	2.556	0.907	0.945	1.259	49.26	0.114 - 4.832	9	12.787	9.177	15.309	6.867	53.70	0.541 - 20.316

GM Geometric mean; AM Arithmetic mean; SD Standard deviation; CV(%) Percent coefficient variation; *Concentration in μg g^{-1}.

Carneiro *et al.*[37] in Brazil. Box plots (Figure 2S in supplementary information) show that the average values are quite homogenous in the studied populations. In particular, the boxes include concentration values between the 25th and 75th percentiles of distribution, while inside the box represents the average values. According to the student *t*-test (see Figure 2S), it was verified that, for independent samples, the element distribution between the sites did not show significant differences for As and Cd. In particular, the shape of distributions and the absence of a unique mode for part of them suggest the presence of distinct subgroups in the populations of Altamira city and Canteiro island. A criterion treatment, based on residence site led to more regular distributions for Pb and Hg and pointed to significant differences in some element concentrations among the groups. Otherwise, the test showed significant differences for Pb ($p < 0.05$) and Hg ($p < 0.05$) in relation to the two places studied. Our investigation found higher Pb and Hg concentrations in residents of Canteiro island in relation to Altamira city (see Figure 2S). These elements are chemically related in hair and depend on environmental exposure.

Regarding concentration of As and Cd, it was verified that the values of the averages among the two places are very close amongst themselves and in relation to other control populations. By contrast, the elements Pb and Hg presented concentrations, on average, very different for the two locations, which suggests an inter-regional variation. In addition, the high values found in the residents of Canteiro island can be attributed the environmental exposure in an area of environmental impact from gold mining activity.

Arsenic

WHO[12] indicates a maximum value of 1.00 μg g^{-1} of arsenic in human hair. Our study found only four people that presented results above this limit. This result showed that 94% of the people presented arsenic concentrations smaller than 1 μg g^{-1}. Furthermore, 76% of persons tested had levels smaller than 0.500 μg g^{-1}.

Comparing the means of other populations from around of the world, the values found in this study are higher than values from North America and Europe, and also in areas of Canada (0.016 μg g^{-1}) and Poland (0.020 μg g^{-1}) with little industrial activity.[36] Also, the values from this study were four times higher than those found by Caroli *et al.*[39] in Italy and by Oluwole *et al.*[40] in Nigeria, 0.090 μg g^{-1}. The values found in this work were closer to the mean found (0.280 μg g^{-1}) in Malaysia,[40] and in Japan.[41] With respect to arsenic, special mention should be made of a study of the population of Rio de Janeiro (Brazil), where for a group of 1434 people, a mean of 0.070 μg g^{-1} of arsenic was found.[37]

Table 3. Comparison of the concentrations of some toxic elements (μg g^{-1}) in the hair of populations from various parts of the world

Country	As	Cd	Pb	Hg	Reference
South America					
Brazil	0.434	0.259	6.550	2.556	Our study (Altamira)
	0.279	0.230	23.233	12.787	Our study (Canteiro island)
	0.020	0.170	N.A.*	1.160	Vasconcellos *et al.*[42]
	0.070	0.170	6.410	1.440	Carneiro *et al.*[37]
Colombia	N.A.	N.A.	N.A.	1,330	Olivero *et al.*[50]
North America					
Canada	0.010	0.500	5.380	0.930	Takagi *et al.*[38]
USA	0.010	<0.100	2.430	0.490	Dipietro *et al.*[48]
Europe					
Poland	0.020	0.310	2.520	0.280	Takagi *et al.*[38]
Italy	0.090	0.210	8.100	N.A.	Caroli *et al.*[39]
	N.A.	N.A.	0.680	2.600	D'Ilio *et al.*[28]
Portugal	0.040	0.890	N.A.	N.A.	Pereira *et al.*[46]
Africa					
Nigeria	0.090	N.A.	N.A.	0.300	Oluwole *et al.*[40]
Asia					
India	0.610	0.320	13.200	1.300	Takagi *et al.*[38]
	2.290	0.130	4.650	0.880	Samanta *et al.*[21]
Singapure	N.A.	0.170	6.740	5.920	Foo e Tan[45]
Japan	0.050	0.280	3.620	2.200	Takagi and *et al.*[38]
	0.230	N.A.	4.800	3.180	Sera *et al.*[41]
Indonesia	N.A.	0.320	15.100	5.590	Foo e Tan[43]
	N.A.	0.600	15.700	N.A.	Ponzeta *et al.*[47]
Malaysia	0.280	N.A.	N.A.	2.970	Oluwole *et al.*[40]

* N.A. Not available.

In Altamira (Table 2), about 93% of donors showed arsenic levels lower than 0.900 μg g^{-1}. Another study, in an indigenous reservation named Parque do Xingu, a mean arsenic content of 0.020 μg g^{-1} varying form 0.0067 μg g^{-1} to 0.2120 μg g^{-1}, was found.[42] Our work has verified that 88% of the people showed arsenic contents higher than 0.212 μg g^{-1}.

Our results show that there is no statistical difference ($p > 0.05$) in arsenic content between men and women. In comparison with the results of Samanta *et al.*,[21] their means were 6 times higher than our work. There, 63% of the total population of West Bengal, in India, live in arsenic contaminated areas. The Amazonian area also shows arsenic contaminated areas. Contamination by arsenic and manganese in Santana city, Amapá state, is well known. Studies to understand the pathways of arsenic that justifies higher arsenic contents in population of a non-industrial area are needed.

Cadmium

For cadmium, 93% of the studied population showed contents above the detection limit. The values proposed by Pozebon *et al.*[43] and Iyengar *et al.*[44] for a normal range were 0.04 to 5.30 μg g^{-1} and 0.24 to 2.70 μg g^{-1}, respectively. These results are quite similar to those found in our work (range from 0.04 to 1.70 μg g^{-1}).

The mean value of 0.259 μg g^{-1} found in Altamira residents is below the results of other works in the literature. Even so, the results are very close to those from Altamira and Canteiro island (see Figure 1S). In Altamira city 96.5% of the population had cadmium contents lower than 1 μg g^{-1}, 50% lower than 0.391 μg g^{-1} and 25% lower than 0.173 μg g^{-1}.

In relation to the works of Takagi *et al.*[38] in India, Canada and Poland, of Foo and Tan[45] in Indonesia; and of Pereira *et al.*[46] in Portugal, our mean values of cadmium content were lower. Ponzetta *et al.*,[47] in Indonesia, related the cadmium content found in human hair to the vegetarian habits of some people, so this type of food can be assumed as a possible source for cadmium content in inhabitants of these regions.

The study done by Carneiro *et al.*[37] for cadmium, obtained a mean content of 0.17 μg g^{-1} with minimum and maximum values of 0.0436 and 1.22 μg g^{-1}, respectively, so, it is in agreement with the results obtained in our work. Therefore, our study has shown that 1.4% (n = 2) of the people studied showed cadmium content higher than 1.22 μg g^{-1}. Thus median obtained for this element is 2.3 times smaller than the average value.

Lead

The lead contents found in local inhabitants were higher than the detection limit (0.0097 μg g^{-1}, see Table 1) in 94% of the total population studied. In this population 72% of the results were lower than 10 μg g^{-1} and 25% were lower than 4 μg g^{-1}. In Altamira city the mean value found was 6.55 μg g^{-1} with minimum and maximum values of 0.82 and 33.8 μg g^{-1}, respectively, while on Canteiro island the mean was 23.3 μg g^{-1} with a minimum of 8.06 μg g^{-1} and a maximum of 45.3 μg g^{-1}. Pozebon *et al.*[43] found a range of 0.04 to 5.30 μg g^{-1} and they considered this as normal values for human hair. On Canteiro island, the values found were 8 times higher than this range.

The average in Altamira (6.55 μg g^{-1}) is very close to that found by Carneiro *et al.*,[37] which was an index of the lead content in the hair of the population of an urban area. These values are close to those found in Singapore by Foo and Tan[45] and by Taksagi *et al.*[38] in Canada, but are above those found by Taksagi *et al.*[38] in Japan and in Poland, and also by Dipietro *et al.*[48] in the United States.

Folio *et al.*[49] showed differences between the quantities of this element in the hair of people from urban areas and from rural areas. These authors obtained a range of 1.81 to 91.42 μg g^{-1} for urban areas and 0.5 to 35.6 μg g^{-1} for rural areas. Comparison of these concentration ranges in the hair of the residents of Altamira indicates classification as a rural area. The work of D'Ilio *et al.*[28] found lead levels for an area exposed to lead contamination, of around 3.3 ± 6.94 μg g^{-1}. This value represents an amount inferior to that found for the residents of the islands and of Altamira city. For instance, the lead level on Canteiro island was 7 times higher than that found by D'Ilio *et al.*[28]

In this study, the range of concentrations for this element among the residents of Altamira are from 0.11 to 4.83 μg g^{-1}. This is in agreement with the values proposed by Pozebon *et al.*,[43] who registered 0.300 to 12.2 μg g^{-1}. But, among the residents of the island the values were higher, with a maximum value of 20.32 μg g^{-1} (see Table 2 and Figure 2S).

The mean value found at Canteiro island (12.8 μg g^{-1}) was approximately five times higher than in Altamira, and it is very much higher than those found in the literature. However, the coefficient of variation is high when compared with values from the literature, such as in the work of Elivero *et al.*[50] However, in the study of Foo and Tan,[45] carried out in Singapure and in Indonesia, mean values as high as our values were encountered.

In the study of D'Ilio *et al.*,[28] the level of Hg for an auriferous area was around 2.3 ± 1.2 μg g^{-1}. The mercury concentration found on Canteiro island was 5.5 times that found by D'Ilio *et al.*[28] although their value was similar to that for the residents of Altamira. Another study, by Olivero *et al.*,[50] in an auriferous area of Colombia, presented results similar to this work. The average mercury content found in the hair samples of 157 miners was of 2.83 μg g^{-1} with a maximum of 28.3 μg g^{-1}, while, in the control area an average of 1.33 μg g^{-1} was verified with a maximum content of 3.00 μg g^{-1}, close to values found in our work.

According to IPCS (International Programme on Chemical Safety),[51] methylmercury is listed as one of the six most of the dangerous chemical substances in the environment. The Amazonian population is exposed mainly to methylmercury through fish consumption, and the toxicological risks are increased in the Amazonian riverine populations, because fish is their main food. Comparing these mercury concentrations of our work with the values found in the works of de Folio *et al.*,[49] these workers found differences between the contents of this element in the hair of people from urban areas (0.01-0.04 μg g^{-1}) and rural areas (0.01-2.92 μg g^{-1}).

The hair of the residents of Altamira was above these values, in agreement with its classification as a rural area.

It was observed that the level of this element in the residents of Canteiro island was higher than the another locations, being higher than that obtained by Carneiro *et al*.[39] The mercury level reported by Vasconcellos *et al*.,[52] showed a highest value of 15.75 μg g^{-1}, in agreement to that found among the residents Canteiro island, and a lowest 1.16 μg g^{-1}, close of the general value for the area (among 0.541 to 20.316 μg g^{-1}). Thus, 65.2% of the hair donors presented mercury contents higher than 1.16 μg g^{-1}. As a result the median is about 2.3 times less than mean. A correlation analysis was applied to verify the level of the relationship of mercury with the other elements (As, Pb, Cd) and the time of residence classified accordingly to the place of current residence. The relationships between the mercury, arsenic, lead and cadmium in all groups, except the correlations already discussed, presented weak correlations.

In the work of Leino and Lodenius,[14] carried at several places in the region of the Tucuruí reservoir an average of this element in the hair of 12 Indians of 8.5 μg g^{-1}, varying among 3.3 to 12 μg g^{-1}, was found. The results of our work present background levels for this area as those from Tucuruí are much higher than those found in Altamira.

Statistics Analysis

Correlations

The interaction between elements in hair was evaluated through of the correlation matrix (correlation coefficients are shown in the supplementary information, Table 1S). A higher correlation between the variables As and Pb, As and Hg, Pb and Hg, Cd and As was observed for samples of Canteiro island (0.286, 0.107, –0.758 and 0.692, respectively), while for Altamira city the highest correlation was found for Cd and As. For the element Hg in hair in people from Cantero island, a significant negative correlation was verified with age ($r = -0.742$, $p < 0.05$). This same correlation was found by Asano *et al*.[53] for children from 1 up to 5 years of age. Also in this group a significant positive correlation with age was observed for Pb ($r = +0.751$, $p < 0.05$), As ($r = +0.505$, $p < 0.05$) and Cd ($r = +0.404$, $p < 0.05$) in hair. Asano *et al*.[53] suggest close correlations in this value for a group of children with one five years for Cd. Moderate but significant positive correlations between As-Cd in the hair of people from Canteiro island ($r = +0.692$, $p < 0.05$) and Altamira city ($r = +0.461$, $p < 0.05$) are also seen.

Multivariate methods

Twenty-four hair samples of the ninety samples studied in this word present experimental values below the detection limit. As a consequence, these samples (23 from Altamira city and one from Canteiro island) were not included in the multivariate analysis. Therefore, the PCA, HCA and DA presented in this study were constructed with only 66 samples (these samples are tested in the Supplementary Information, Table S2).

Principal component analysis

In the discrimination between hair samples of inhabitants of Altamira city (58 samples) and Canteiro island (8 samples) four variables were used (arsenic, lead, mercury and cadmium concentrations in the hair samples). The 66 samples were divided into two groups: group A (hair samples from Altamira city) and B (hair samples from Canteiro island). After several analyses, the best separation was obtained by using all variables: The values of the scores obtained for the four elements (arsenic, lead, mercury and cadmium) are presented in the supplementary information. The PCA results showed that the first component (PC1) is responsible for 37.8% of the variance of the data. Considering the first (PC1) and second (PC2) components, the accumulated variance increases to 70.7%. Figure 1 presents the score plots. This projection has 71% of the variance of the original data set and provides a reasonably accurate representation of the higher order space. From Figure 1 we can observe that the hair samples, with exception of sample B9 (See Table S2), are separated into two groups: group A (hair samples of Altamira) and B (hair samples of Canteiro). Also from Figure 1, we can see that PC2 alone is responsible for the separation between the two groups.

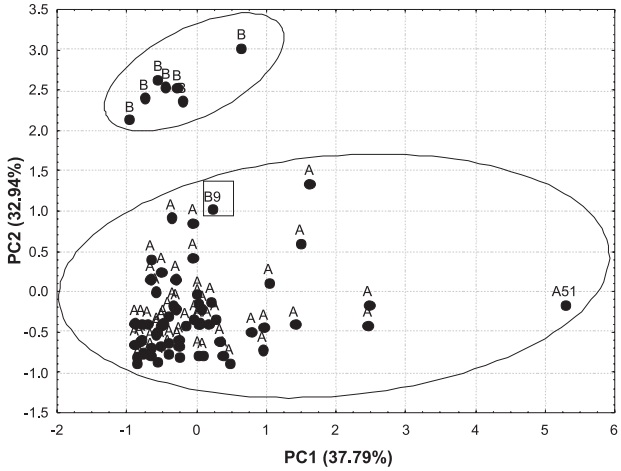

Figure 1. Two-dimensional scores plot of the hair samples from the resident populations of Altamira city and Canteiro island.

The loading value for each variable indicates its contribution to a PC. From Equations (1) and (2) we can observe that the metals As and Cd have high PC1 loadings, while the elements Pb and Hg have high loadings in PC2.

This result is in according with Figure 1, which shows that the concentrations of Pb and Hg metals are higher on Canteiro island than in Altamira city.

$$PC1 = 0.843As + 0.073Pb – 0.0125Hg + 0.853Cd \quad (1)$$

$$PC2 = –0.137As + 0.825Pb – 0.815Hg + 0.083Cd \quad (2)$$

From equation 2 and Figure 1 we can see that the hair samples from Canteiro island present the most positive values for PC2, while the hair samples from Altamira present a progressive decrease in the PC2 values. Figure 2 displays the PCA scores plot for these first two principal components (PC1 and PC2). PC1 indicated that Pb and Hg form a cluster in right side, while As and Cd form a cluster in left side. These results are in agreement with the correlation results, and this figure shows how to identify the variables responsible for the separation of the studying groups.

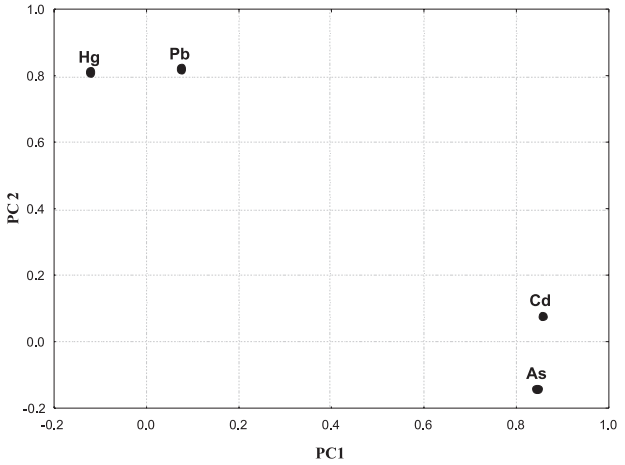

Figure 2. Principal component analysis for the four elements in hair samples from the resident populations of Altamira city and Canteiro island.

The median values among population from Canteiro island are, in fact, much higher than those observed for Altamira city for elements Pb and Hg. This aspect is also found when the association between As and Cd, with another for Hg and Pb at the two sites are compared for the same elements groups. Figure 2 indicates that Pb and Hg forms one cluster and As and Cd form another.The more important variables for this separation are Pb and Hg, clear here and also in the Box plots (Figure 2S). That is to say, the samples from Canteiro island have larger concentrations of this metal than do the samples from Altamira. This concentrations of Pb and Hg in hair are considerably influenced by the environmental exposures in group B (hair samples of Canteiro). Comparison of our data with other studies suggests that the levels of these two elements in hair among individuals vary due to the environment of two studied areas. The distribution of the elements Pb and Hg is similar to the disposition of group B, in Figure 1. This indicates that the concentrations of these elements in hair has directly influenced the separation between groups A and B.

Samanta *et al.*[21] have shown that, due to environmental exposure, each element significantly influences the structure of hair. Finally, it can be concluded that the concentration of the four elements in hair form a strong cluster indicating that there is environmental exposure to these elements.

Hierarchical cluster analysis (HCA)

The results obtained with the HCA analysis were similar to those obtained with PCA and are shown in the dendrogram (Figure 3). The dendrogram can be used to provide information on chemical behavior and to verify the results obtained by PCA. In the dendrogram of Figure 3, the vertical lines represent the hair samples and the horizontal lines represent the similarities between individuals in terms of Euclidean distances which originate from the cluster analysis between pairs of samples, between a sample and a group of samples and between groups of samples. From Figure 3 we can note that the Euclidean distances observed between the two groups is 4.23 (the maxim distance is 6.71). In Figure 3 the groups A and B correspond to the same groups A and B in Figure 1 (PCA analysis). Both PCA and HCA methods, with the exception of sample A51, classified the 66 hair samples studied into two groups exactly in the same manner. Based on the classification obtained with PCA and HCA we can say that As, Pb, Hg and Cd are responsible for the separation between resident people in Altamira city and Canteito island.

Figure 3 shows that sample A51 cannot be classified among either groups A or group B, but this sample was still considered significant for this model. Its absence in a new model generates results that cause a 66% reduction in the accumulated variance of the new principal components.

Discriminant analysis (DA)

The main objective of DA is to determine discriminant functions using the measured variables that separate the groups as distinctly as possible. In this work we considered two groups: group A (hair samples of Altamira) and B (hair samples of Canteiro). The discriminant functions obtained in this work are given in equations 3 and 4:

$$Group\ A = –0.12As – 13.94Pb – 21.05Hg – 6.32Cd \quad (3)$$

$$Group\ B = –2.33As + 1.56Pb + 2.95Hg – 6.54Cd \quad (4)$$

Through the discriminate functions and the values of each variable for the elements studied, we can obtain the

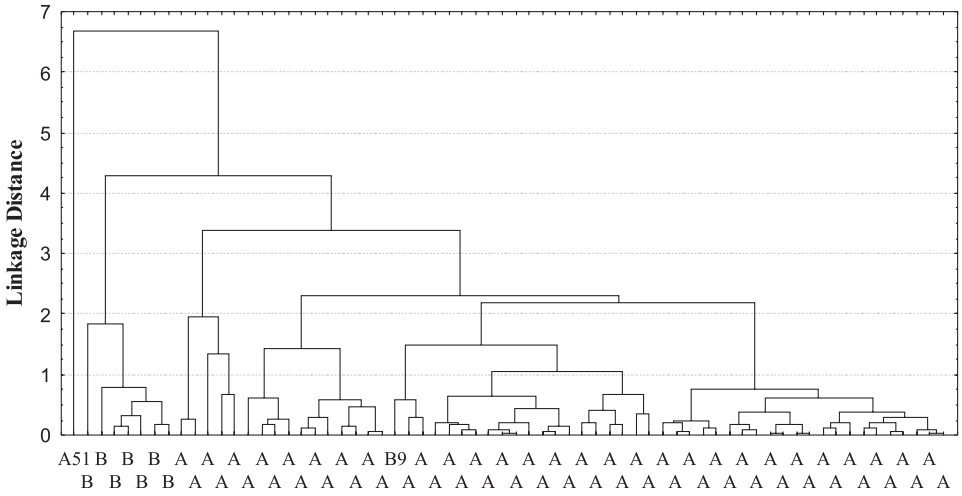

Figure 3. HCA dendrogram for the discrimination between resident populations of Altamira city (A) and Canteiro island (B).

classification matrix by using all samples in the analysis (see Table 4). The classification error rate was 1.51%, resulting in a satisfactory separation of the two groups. From Figure 4 we can also notice that the groups A and B correspond to the same groups A and B in Figures 1 and 3 (PCA and HCA).

Table 4. Summary of classification used in the DA, with variables As, Cd, Pb and Hg in hair from the resident populations in Altamira city and Canteiro island, p = 0.01

Individuals	Previous classification		Total	Classification / (%)
	Altamira city (p = 0.878)	Canteiro island (p = 0.121)		
Altamira city	58	1	59	100
Canteiro island	0	7	7	87.5
Total			66	98.49

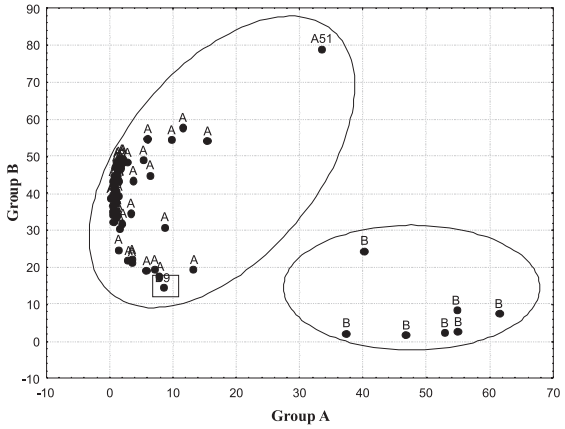

Figure 4. Two-dimensional scores plot of the group from Altamira city (A) and Canteiro island (C), obtained by DA.

The results are agree with those obtained by PC for the classification groups A and B (see Table 4) from which hair samples of the populations from Altamira city and Canteiro island are more distant for group A than for group B in the two-dimensional plot formed by the two first components (group A). Results from discriminat analysis classified sample B9 in group A (Figure 4). This shows the significance of this model for sample classification, confirming the PCA analysis, so this sample cannot be called an outlier in this sampling.

Conclusions

The values of the concentrations of As, Cd, Pb and Hg obtained in this study can be considered as the background levels for the elements in this area. This assumption is supported by the concentrations of some elements that are higher in the hair from Canteiro island than from Altamira city, mainly Pb and Hg.

The average values of the concentrations of As and Cd between the two places are quite close, and also close to those of other control populations. In contrast, the elements Pb and Hg in presented values of concentrations, on average, very different from each other, which suggests an inter-regional variation. The high values in these elements found in the residents of Canteiro island can be attributed to environmental exposure from the mining activity for gold in this areas.

Finally, PCA, HCA and DA were used in the statistical treatment of the data to build a model to classify the resident persons by the amounts of As, Cd, Pb and Hg in their hair. The PCA results showed that the variables As, Cd, Pb and Hg are responsible for the correct classification into two groups: group A (hair samples of Altamira) and B (hair samples of Canteiro). The HCA and DA results were similar to those obtained with PCA, *i.e.* both methods classified the samples in exactly same way.

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Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br, as PDF file.

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