ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research

Vol. 10, Issue, 02, pp.44409-44414, February, 2021

https://doi.org/10.37118/ijdr.20996.02.2021

RESEARCH ARTICLE OPEN ACCESS

FACTOR ANALYSIS APPLIED TO METAL CONTENT IN HAIR: A STUDY ON THE CORRELATION BETWEEN NEIGHBORHOODS AND ASSOCIATED RISK - ALTAMIRA, PARÁ, BRAZIL

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ARTICLE INFO

Article History:

Received 20th December, 2020
Received in revised form
10th December, 2020
Accepted 21st January, 2021
Published online 24th February, 2021

Key Words:

Toxic risk, Hair, Principal Component Analysis.

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ABSTRACT

Few studies on capillary metals are conducted in the Amazon region despite the diversity of environmental problems that directly reflect on human health, as is the case of the population of Altamira. The hair matrix was selected because it is an indicator of past exposure, which enablesto correlate the concentration of trace elements in neighborhood residents in the metropolitan region to identify grouping by chemical similarities, using factor analysis as a statistical technique. The city was chosen due to its regional importance and the intense transformation process undergone over the years, among them the implantation of the Belo Monte Hydroelectric Plant, gold mining, and agriculture. These events brought development linked to social, environmental, and public health problems for the municipality. The elements were determined by Inductively coupled plasma atomic emission spectrometry (ICP-AOS). Among the elements surveyed, Mn, Ba, Sr, Ca, Mg, and Al stood out as the most important and responsible for the grouping of neighborhoods, with an explanation percentage of 76.08%. The elements Mn, Ba, Sr, Ca, and Mg are responsible for grouping the neighborhoods Esplanada do Xingu, Açaizal, Aparecida, Centro, Jardim Independente I and II, Sudam I and II, Jardim Oriente, and Jardim dos Estados with 58.96% of varianceexplained, and Al for the neighborhoods São Sebastião and Catedral with 17.12% of variance explained. These results indicated that the first group has a risk associated with the metals Mn and Ba, whereas the second with the metal Al, all with relevant toxic potential. Signs of health problems may be being demonstrated, as these results refer to extemporaneous exposure.

Citation: Pedro Moreira Sousa Junior, Simone Fátima Pinheiro Pereira, Ronaldo Magno Rocha, Orivan Maria Marques Teixeira Mateus Higo Daves Alves, Luciana Pinheiro Santos, Auriane Consolação da Silva Gonçalves, Daniel Pinheiro Nogueira and Kelson do Carmo Freitas Faial, 2021."Factor analysis applied to metal content in hair: a study on the correlation between neighborhoods and associated risk - Altamira, Pará, Brazil", *International Journal of Development Research*, 11, (02), 44409-44414

INTRODUCTION

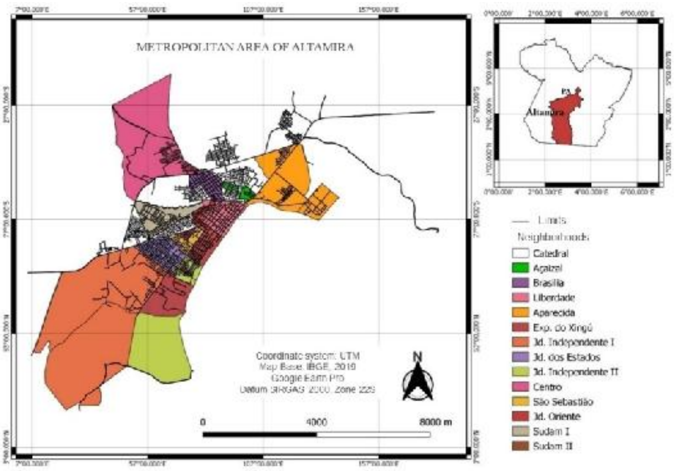
Currently, the biological monitoring of chemical elements has become an important tool in the scientific environment, as it allows investigative studies of harmful substances and their correlation with epidemics, pandemics, or even occupational exposures, especially in areas of high demographic density. The danger of trace elements is associated with their physical-chemical properties and routes of exposure.

This combination linked to the health condition and inherent vulnerability are the main factors that cause generalized problems to the population (Ali *et al*., 2019; Gil and Hernández, 2015). Toxic metals and metalloids are contaminants of great importance because they are widely distributed in air, water, soil and, other environmental compartments resulting from anthropogenic or geological emissions (Gupta, 2019). In this perspective, analyzes in hair samples stand out as the most used due to simplicity, little invasiveness, high sample mineralization, and irreversible incorporation of trace elements in this matrix (Chojnacka *et al*., 2006; Kosanovic and Jokanovic, 2011; Skalny *et al*., 2015). The city of Altamira, 720 km from Belém, is the most important city located in the Xingu region. Over the last 30 years, Altamira has been the scene of an intense migratory flow, with the propelling agents of mining, agriculture, and more recently the Belo Monte Hydroelectric Plant (Miranda Neto and Herrera, 2016). All this transforming process in the region brings advances, but also socio-environmental problems that mainly affect the biodiversity, food, and life quality (Gauthier and Moran, 2018). Given the above, studies on the influence of trace metals on the population living in the metropolitan region of Altamira become relevant. The use of the Factor Analysis emerges as an important tool in the selection of neighborhood groups and in the indication of which metals are important in the explanation of that behavior.

MATERIALS AND METHODS

Characteristics of the study area: Altamira stands out as one of the most important cities in Pará, according to IBGE data from 2014, the municipality of Altamira had an estimated population of 106,768 inhabitants, whereas in the 2010 Census the population was 99,075 inhabitants, and in 2000 its population was 77,439 inhabitants. This growth was driven by two factors: the first refers to the gold extraction and trading activities that started in the 1980s (Macedo, 2016), and the migration process due to the Belo Monte Hydroelectric Plant (Miranda Neto and Herrera, 2016). The population weight is relevant regarding the role played by Altamira, because according to the main theories of urban location, activities related to the supply of goods and services tend to be concentrated in urban centers, causing serious problems of urban and social infrastructure (Franco *et al*., 2018).

Sampling: The sampling was simple and random, contemplating a universe of 70 individuals of different ages, in a sample space of 14 neighborhoods (São Sebastião, Açaizal, Aparecida, Brasília, Catedral, Centro, Esplanada do Xingu, Jardim Independente I and II, Jardim dos Estados, Jardim do Oriente, Liberdade, and Sudam I and II), which were chosen considering the population density parameter (IBGE, 2014). In this study, all the ethical procedures foreseen in the Declaration of Helsinki and the Brazilian legislation were carried out, including the Free and Informed Consent Term (TCLE) according to the protocol of the internal ethics committee of the Federal University of Pará. All residences visited were georeferenced and the extraction of the hair sample occurred in only one volunteer member per household to which the TCLE was issued. The sample space where the study was developed is illustrated in Figure 1.



Source: Prepared by the authors.

Figure 1. Map of the metropolitan area of Altamira highlighting the neighborhoods sampled in the survey

Collection: The samples were collected on the nape of the neck (Katz and Chatt, 1988; Robbins and Robbins, 2012). This collection site is already standardized because it is less susceptible to external contamination, in addition to being the area that is more likely to have hair, even in bald individuals. Pozebon *et al*. (1999) and Wang *et al*. (2019) recommend that the sample be collected respecting the range from 0.5 cm to 1.0 cm in length, avoiding aesthetic damage. To determine the amount of metals Manganese (Mn), Barium (Ba), Strontium (Sr), Iron (Fe), Calcium (Ca), Magnesium (Mg), Sodium (Na), Aluminum (Al), Zinc (Zn), and Potassium (K), it is suggested between 1 to 2 g of hair extracted with the aid of gloves and titanium or stainless steel scissors (Semenova *et al*., 2019). The hair sample must be identified and conditioned in a plastic bag or polyethylene bottle and sent to the laboratory where the analyzes will be performed. Hair with different (natural) pigmentations from the same individual must be collected separately, since the absorption of the elements may vary according to their pigmentation (Pozebon *et al*., 1999; Marcineck-Jacel *et al*., 2017; Li *et al*., 2020).

Sample pre-treatment: All samples were treated analytically in the Analytical and Environmental Chemistry Laboratory (LAQUANAM) at the Federal University of Pará, Brazil. The pre-treatment consisted of washing the samples with non-ionic detergent and then submitting to the procedure deionized water/acetone/deionized water for 10 minutes in each step, for complete removal of external contamination, as described by Drobyshev *et al*. (2017) and Ali *et al*. (2019). In the next stage, the samples were conditioned in an oven at 50ºC for 2 hours and then crushed with a stainless steel instrument until fragments were smaller than 1cm (Sela *et al*., 2007).

Instrumental analytical procedures: In this stage, the samples were microwave digested in 6 ml polytetrafluoroethylene (PTFE) containers with mixtures of 2 ml of concentrated HNO_3 and 0.25 ml of concentrated H_2O_2 (Pereira *et al*., 2009; Tipple *et al*., 2013; Hu *et al*., 2019). The equipment used in the digestion procedure is the PROVECTO ANALÍTICA brand and model DGT 100 Plus. The recommended program was followed (Tipple *et al*., 2013), which is heating at 200ºC at a rate of 13.3 C/min and maintained for 15 min. After the cooling step, the sample was reduced to 2 mL, transferred to a 10 mL volumetric flask, calibrated with pure water, and transferred to a 15 mL Falcon tube, for later analysis of the metals. The technique used was Inductively coupled plasma optical emission (ICP OES) due to its high sensitivity, low detection and quantification limits, and, mainly, to enable multi-element analysis (Skoog *et al*., 2008). The equipment used for quantifying the elements is from the Varian brand (PRO view model). The calibration and analysis methodology followed the manufacturer's instructions (Charles and Fredeen, 1997; Pereira *et al*, 2007). The initial specifications of the equipment can be observed in Table 01. The study of accuracy and precision was performed with analysis of the standard hair reference material (GBW07601 of the China National Analysis Center) (Hair *et al*., 2009). In general, the quantification of all trace elements (Mn, Ba, Sr, Mn, Fe, Ca, Mg, Na, Al, and Zn) performed in this study presented a recovery rate (95.3 - 101.0%) in the reference standard. The recovery after digested solution showed an interval between 96 and 100.0% with the acceptable precision level in a range of ± 5 in weight%. The calibration curves were linear within the range (r >0.99) demonstrating the precision and consistency of technical analysis for determining the analytes. The descriptive data for this stage were analyzed using the software SPSS® version 21.

Results Analysis and Interpretation: The analytical results were treated statistically with the help of SPSS® software version 21 for Windows (Statistical Package for the Social Sciences, Inc.). In the preliminary study, the methods of descriptive analysis were used, evaluating the mean and standard deviation, in addition to the bivariate correlation, seeking the strongest interactions in the data set. Both methods corroborate practices developed by Ballesteros *et al*. (2017) and suggested by Hair *et al*. (2009). Regarding the Factor Analysis, the Kaiser-Meyer-Olkin (KMO) tests were performed to assess the proportion of variance that the parameters present between themselves, assuming reasonable KMO values between 0.6 and 0.7, average values between 0.7 and 0.8, good values between 0.8 and 0.9, and very good values greater than 0.9 (Hair *et al*., 2009; Dini *et al*., 2014). Figueiredo Filho and Silva (2010) suggests the need to use

Bartlett's test of sphericity to verify the possibility of the matrix is identity and the condition for non-occurrence, and Bartlett's significance value is less than 0.05. Although there is no consensus on patterns of variance and commonality, authors report that to ensure more robust results in Principal Component Analysis (PCA), the cumulative explanation variance must present values above 60% and communalities greater than 0.6 to certify that the variable is representative in the data matrix (Hair *et al*., 2009; Dini *et al*., 2014; Li *et al*., 2020; Kumar *et al*., 2019).

RESULTS AND DISCUSSION

Univariate statistics: Univariate statistical tests are important because they allow an overview of the individual behavior of the variables. The descriptive analysis explains the average levels and their variability and thus identifies whether there is a homogeneous or heterogeneous sampling behavior.

samples. The high amplitude in the sample results is due to environmental factors such as exposure to contaminated environments (place of residence or work), eating habits with food intake from the bioaccumulation chain, to biologicals conditions where the differentiated physiology between male and female stands out concerning the absorption of chemical elements (Miekeley *et al*., 1998; Abdulrahman *et al*., 2012; Gibb *et al*. 2016, Ali *et al*., 2019). Another important piece of information deals with the groupings discretized according to the variables. The contents of Ba, Fe, Ca, and Al are responsible for grouping most of the neighborhoods (S. Sebastião, Esp. Dos Xingu, Liberdade, Açaizal, Aparecida, Catedral, Independent Jd. II, and Jd. Oriente); Sr e Mg integrates the neighborhoods São Sebastião, Esp. do Xingu, and Liberdade; Zn and the neighborhoods S. Sebastião, Esp. Dos Xingu, Liberdade, Aparecida, Catedral, Jd. Independent I and II, Jd. Oriente, and Centro; and the metals Na and K did not show any influence on the neighborhood grouping. The parameters Mn, Ba, Ca, and Mg showed the highest bivariate correlations (sig. <0.05) (Table 3).

Table 1. ICP-OES parameters for hair elements analyzes

Parameters	*Value*
Radiofrequency power (KW)	1.4
Nebulizer pressure (KPa)	180
Plasma argon flow-rate (L/min)	15
Auxiliary argon flow-rate (L/min)	0.75
Sample introduction (s)	40
Washing time (s)	30
Stabilization time (s)	15
Read time (s)	1
Peristaltic pump speed (rpm)	15

Table 2. Descriptive statistics of metal contents (µg/g) discretized by neighborhood

Neighborhood		Ba	Sr	Fe	Ca	Mg	Na	Al	Zn	K
S. Sebastião	Mean	14.02	4.95	26.27	676.43	78.91	164.89	45.28	429.73	26.27
	S. D.	*19.17*	*2.68*	*15.22*	*270.79*	*42.75*	*87.29*	*28.41*	*595.92*	*15.22*
Esp. do Xingu	Mean	4.82	3.11	19.67	567.63	69.65	113.68	16.10	136.16	19.67
	S. D.	*4.02*	*1.35*	*7.74*	*193.14*	*51.44*	*88.79*	*6.22*	*44.41*	*7.74*
Liberdade	Mean	8.20	4.04	32.96	565.54	75.78	162.28	20.13	112.87	64.45
	S. D.	*4.35*	*2.80*	*18.52*	*350.61*	*64.34*	*108.51*	*11.79*	*31.64*	*36.96*
Aparecida	Mean	6.59	2.37	29.76	598.19	45.10	117.66	23.20	125.16	76.96
	S. D.	*8.67*	*1.84*	*22.16*	*312.37*	*42.23*	*47.45*	*8.89*	*33.42*	*72.23*
Catedral	Mean	22.12	7.33	27.52	908.96	146.57	171.01	29.90	134.69	96.01
	S. D.	*15.44*	*4.65*	*2.94*	*543.42*	*87.06*	*81.84*	*20.51*	*31.01*	*27.71*
Jd. Independente I e II	Mean	3.56	1.60	35.96	529.10	33.25	167.22	38.68	106.40	79.09
	S. D.	*1.33*	*0.77*	*27.42*	*264.54*	*14.59*	*76.38*	*26.99*	*43.36*	*19.97*
Sudam I e II	Mean	7.15	1.85	29.98	288.92	27.69	95.52	14.85	77.98	42.44
	S. D.	*0.61*	*0.22*	*10.62*	*38.25*	*10.18*	*19.45*	*7.04*	*43.72*	*2.23*
Jd. Oriente	Mean	7.87	2.96	18.91	689.14	104.24	277.87	39.64	192.53	120.97
	S. D.	*4.12*	*2.62*	*11.30*	*571.37*	*84.07*	*80.35*	*62.56*	*162.23*	*62.96*
Jd. dos Estados	Mean	5.74	2.08	21.79	211.82	13.05	60.73	37.87	107.01	53.80
	S. D.	*8.60*	*2.39*	*23.60*	*270.29*	*22.37*	*52.35*	*16.17*	*29.01*	*25.38*
Centro	Mean	10.63	2.96	3.19	71.74	3.80	30.30	31.11	140.80	65.40
	S. D.	*17.16*	*4.74*	*1.94*	*54.36*	*7.16*	*17.85*	*10.96*	*25.69*	*23.20*
Mean value	----	*9.07*	*3.32*	*24.60*	*510.74*	*59.80*	*136.11*	*29.67*	*156.33*	*64.50*
Mean standard deviation	----	*8.34*	*2.40*	*14.14*	*286.91*	*42.61*	*66.02*	*19.95*	*104.04*	*29.36*
Rodushkin and Mikael (2000)	*Mean*	*1.04*	*2.84*	*13.95*	*1501.50*	*74.75*	*343.50*	*14.15*	*133.00*	*213.70*
Goullé et al. (2005)	*Mean*	*0.81*	*2.40*	---	---	---	---	*2.78*	*169.00*	---
Batista et al. (2009)	*Mean*	*1.06*	---	---	---	---	---	*3.36*	*142.00*	---
Ballesteros et al. (2017)	*Mean*	*1.00*	*3.46*	*19.75*	---	---	---	*3.41*	*148.95*	---
Mean value	----	*0.97*	*2.90*	*16.85*	*1501.50*	*74.75*	*343.50*	*5.92*	*148.23*	*213.70*

S. D. – Standard Deviation

The bivariate correlation seeks to identify the direct linear relationships between the variables and the possible existing associations. Evaluating the results of Table 2, the variables Mn (5.74 ± 5.06), Ba (9.07 ± 8.34), Sr (3.32 ± 2.40), Fe (24, 60 ± 14.14), Ca (510.74 ± 286.91), Mg (59.80 ± 42.61), Al (29.67 ± 19.95), and Zn (156.33 ± 104.04) stood out for presenting levels above those found by previous studies conducted over approximately 17 years (Rodushkin and Axelsson, 2000; Goullé *et al*., 2005; Batista *et al*., 2009; Ballesteros *et al*., 2017). The considerations regarding this information refer to the high sample variability, indicating that there is no pattern in the content of the elements selected in the hair

This characteristic remains constant in both tests, directing the understanding that they are responsible for most of the explanatory content of the matrix, serving as a statistical method complementary to other techniques such as linear regression or factor analysis (Filippini *et al*., 2018; Ainley *et al*., 2020). Rodushkin and Axelsson (2000) report that the first explanatory factor for the interrelation of chemical elements is due to their chemical properties, which highlights the alkaline nature for example, which at that moment, can explain part of the correlation and why they are positive. As it indicates past contamination, other authors suggest that these chemical groups are due to the individual's social condition, whereas

Table 3. Result of Pearson's bivariate correlation between the variables tested

Correlation		Mn	Ba	Sr	Ca	Mg	Al	Fe	Na	Zn	K
Significance of Pearson (<0.05)	Mn										
	Ba	0.001									
	Sr	0.001	0.001								
	Ca	0.001	0.001	0.001							
	Mg	0.001	0.001	0.001	0.001						
	Al	0.410	0.058	0.107	0.007	0.269					
	Fe	0.014	0.052	0.177	0.001	0.038	0.018				
	Na	0.234	0.322	0.447	0.003	0.001	0.369	0.187			
	Zn	0.189	0.278	0.063	0.009	0.070	0.007	0.422	0.254		
	K	0.486	0.241	0.177	0.030	0.005	0.066	0.417	0.001	0.380	

Table 4. Statistical result of matrix adjustment

KMO and Bartlett tests (Mn, Ba, Sr, Fe, Ca, Mg, Al e Zn)		**Values**
Kaiser-Meyer-Olkin measure of sampling adequacy.		0.601
Bartlett's test of Sphericity	Approx. Chi-Square	222.594
	Significance	0.050
Commonalities	Fe	0.268
	Zn	0.469
Teste de KMO e Bartlett (Mn, Ba, Sr, Ca, Mg e Al)		**Valores**
Kaiser-Meyer-Olkin measure of sampling adequacy.		0.714
Bartlett's test of Sphericity	Approx. Chi-Square	189.705
	Significance	0.001
Commonalities	Mn	0.710
	Ba	0.708
	Sr	0.816
	Ca	0.712
	Mg	0.746
	Al	0.963

Table 5. Results of the variance matrix and component weights

Components	Initial eigenvalues			Components Matrix and Weights		
	Total	% of variance	% cumulative	Variables	PC 1	PC 2
1	3.538	58.965	58.965	Mn	0.768	-0.291
2	1.027	17.122	76.087	Ba	0.809	-0.010
3	0.749	12.480	88.567	Sr	0.900	-0.082
4	0.433	7.222	95.789	Ca	0.823	0.187
5	0.154	2.567	98.356	Mg	0.853	-0.135
6	0.099	1.644	100.000	Al	0.284	0.939

is highlighted factors that influence the levels in hair samples, such as the type of work, place and time of residence, and the type of food consumed (Zheng *et al*., 2010; Mehra and Thakur, 2016).

Multivariate Statistics: The Factor Analysis (FA) technique was adopted to provide data reduction and the indication of variables with important scores (components) in the sample set, based on previous research carried out on the application of the (Saleem *et al*., 2019; Njuguna *et al*., 2020). This approach allows a deeper and more comprehensive analysis of which variables are relevant in the neighborhood grouping. As suggested (Hair *et al*., 2009; Dini *et al*., 2014; Li *et al*., 2020; Kumar *et al*., 2019), the present study evaluated that the worked matrix, considering the initial selection of the variables Mn, Ba, Sr, Fe, Ca, Mg, Al, and Zn did not present results of the KMO test (0.60), Bartlett's sphericity (0.05), and accumulated variance for two components with (63.44%) satisfactory. As for commonality, weights were identified below 0.6 for Fe (0.268) and Zn (0.469), disagreeing with the established limit (Hair *et al*., 2009; Figueiredo Filho and Silva, 2010). Excluding the aforementioned metals, the sample set of variables that initially consisted of 9 was reduced to 6 (Mn, Ba, Sr, Ca, Mg, and Al) with results of 0.71 for the KMO test, 0.001 for Bartlett's sphericity, and 76.08% for accumulated variance in two components, making it an adjusted matrix with commonality above 0.7 as shown in Table 4. Table 5 shows the information on accumulated variance and the component matrix with their respective weights. The first Principal Component (PC1) provided by the Factor Analysis presented a percentage of 58.96% of variance contained, whereas the second Principal Component (PC 2) contains 17.22%, totaling 76.08% of explanation on the studied group and 23.91% of unexplained interaction. Another data extracted is related to the elements Mn, Ba, Sr, Ca, and Mg that lead to the explanation of the neighborhood grouping in the city of Altamira in PC 1, whereas Al is the main metal in the explanation of the group of associated neighborhoods in PC 2. Regarding the first component, Hu *et al*. (2019) report that elements such as Ca and Mg are considered biogenic or endogenous in the hair and for this reason, their concentrations are more susceptible to physiological processes such as ingestion of supplements, for example, than due to environmental contamination. Regarding Mn, Ba, and Sr, their most common contamination pathways are exogenous. Previous studies report that these elements have a relatively high susceptibility to contamination from the environmental condition (Gellein *et al*. 2008; Hu *et al*., 2019). Although the absorption pathways are different, Ballesteros *et al*. (2017) suggest that the aforementioned elements tend to mutually associate in the organism due to chemical and biochemical factors, possibly influenced by age or sex, as reported in Madrid, Spain, and Italy (Senofonte *et al*., 2000). Figures 2 and 3 show the result of the groupings referring to the selected variables and the influence on the formation of clusters, respectively. In the first figure, there is the separation into two distinct groups, one formed by the elements Mn, Ba, Ca, and Sr referring to PC 1, which presents the highest explanatory percentage, and the other with only Al referring to PC 2. In Principal Component 1, the association between the following neighborhoods stands out: Esplanada do Xingu, Açaizal, Aparecida, Centro, Jardim Independente I and II, Sudam I and II, Jardim Oriente, and Jardim dos Estados.

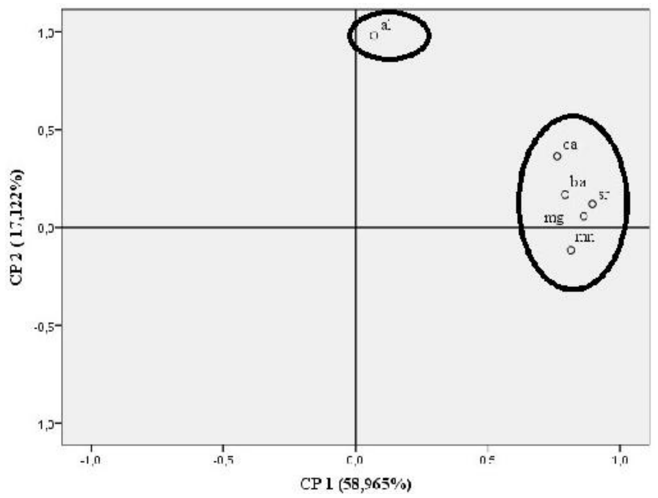


Figure 2. Illustration of the grouping according to the variables

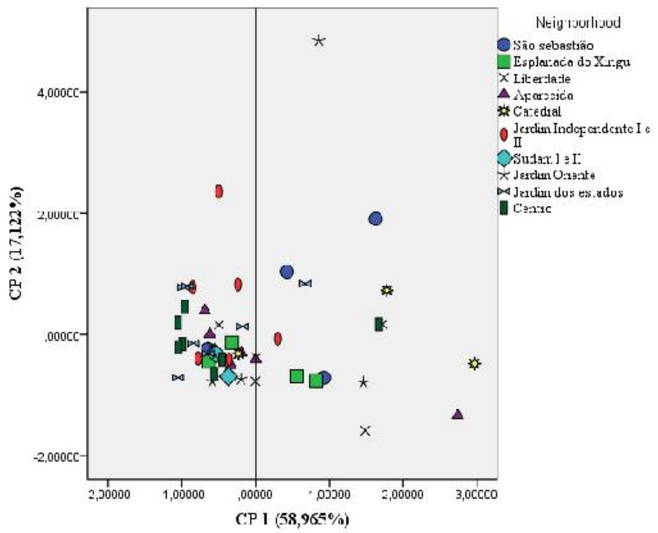


Figure 3. Illustration of the grouping according to the neighborhoods

Considerations about the behavior shown indicate that the presence of metals in resident individuals suggests special attention, above all, due to the presence of metals Mn and Ba, as according to Skalny *et al*. (2015) and Irizar *et al*. (2019), can be explained by its frequent occurrence as an impurity of barite, which can increase the concentration in drinking water samples (6.6 µg/g) as occurred in Bangladesh and Russia. As for Mn, Gil and Hernández (2015) and Pragst *et al.* (2017) mention in their study that several factors are linked to high levels of this metal, such as smoking, living in industrial districts, and situations related to the emission of pollutants by traffic. Ca, Mg, and Sr generally do not exhibit any essential chronic toxicity condition capable of bringing any type of risk to human health. Principal Component 2 shows the grouping of São Sebastião and Catedral neighborhoods. Regarding PC 2, this relationship explains the risk to which these individuals are susceptible since the aluminum present in the hair is the result of exogenous exposure linked to the consumption of water without proper treatment, use of Al-containing products, or even direct exposure from contaminated food or water (Irizar *et al*., 2019). Tinkov *et al*. (2019) reinforce the presence of metal in the body associated with poor eating habits, which can alter the lipid metabolism of adipose tissue, contributing to obesity and metabolic syndrome, as occurred in Hong Kong. Krewski *et al*. (2007) add that epidemiological studies have reported a positive association between aluminum levels in drinking water and the risk of dementia with cognitive impairment.

Scientific counterpart to society: In view of the goals of the project that originated the present study, the findings were offered to the municipal health department of Altamira, which was responsible for planning actions aimed at this problem. Furthermore, the results were forwarded to the voluntary participants along with health guidelines for contamination prevention.

CONCLUSIONS

Biomonitoring indicates that the inhabitants of the neighborhoods surveyed in Altamira need special attention regarding the concentration of the trace elements evaluated in hair samples. The elements Mn, Ba, Sr, Ca, Mg, and Al were the most relevant to explain the grouping between neighborhoods, with the aid of the univariate and multivariate statistics. Esplanada do Xingu, Açaizal, Aparecida, Centro, Independent Garden I and II, Sudam I and II, Jardim Oriente, and Jardim dos Estados were correlated due to similar values of Mn, Ba, Sr, Ca, and Mg, with emphasis on the first two elements that have toxic action and need constant monitoring. Concerning São Sebastião and Catedral, the element responsible for its correlation is Al, and for this reason, it is necessary to pay more attention to these residents due to the considerable level of toxicity that this element can cause to people. All the results served as a scientific support document for the health department of Altamira to promote actions to control and mitigate possible health problems.

ACKNOWLEDGMENTS

The authors would like to thank Eletronorte for the logistical support, ANEEL for the financing of this project, and CAPES and CNPq for undergraduate and graduate scholarships.

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