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ARTICLE
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Inorganic element assessments in water and a human population exposed to ore tailings

Avaliação de elementos inorgânicos em água e uma população humana exposta a rejeitos de minério

Francy Mendes Nogueira Cardoso[I,II]*

Caio Rodrigues Telles[I,II]

Wentony Mark Costa Milhomem[II]

Silas Vieira Trindade Junior[II]

Luany Janaira Gois Vidal[II]

Roberto Messias Bezerra[II]

Francinaldo Sarges Braga[II]

Huann Carllo Gentil Vasconcelos[II]

Amanda Furtado de Almeida[II]

Silvia Eline Alfaia Lima[II]

Madson Ralide Fonseca Gomes[I,II]

[I] Programa de Pós-graduação em Ciências da Saúde, Universidade Federal do Amapá (Unifap), Macapá, AP, Brasil

[II] Instituto Nacional de Ciência e Tecnologia Forense (INCT), Porto Alegre, RS, Brasil

* **E-mail:** francymnc35@gmail.com

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ABSTRACT

Introduction: Manganese exploration in Amapá and inadequate ore tailing storage in the Santana port area have generated environmental and social impacts for residents. **Objective:** Assess the presence of inorganic elements in Elesbão neighborhood inhabitants exposed to ore tailings. **Methods:** Metals were quantified in the water used by the population and in urine by Flame Atomic Absorption Spectrophotometry (F-AAS), and in hair Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Blood was obtained from 67 exposed and 99 unexposed women, totaling 65 urine and 18 hair samples. Hematological, biochemical and blood clotting analyses were performed in all participants, who also answered a socio-economic questionnaire. **Results:** Regarding age group, 60.0% of exposed residents ranged between 18 and 42 years old and lived in the area from 10 to 34 years. High concentrations of As, Cd, Cu, Fe, Mn and Pb were detected in the analyzed water. In urine, significance was observed for Mn and in hair, for Pb and Mn. Hb, Ht, VCM, HCM, monocytes, morphological changes in red cells and platelets were among the significant hematological parameters. Regarding the biochemical evaluations, alterations in the enzymes Alkaline Phosphatase, Bilirubins and Creatinine were observed. **Conclusions:** Environmental contamination by elements that can compromise the health of the exposed population was observed. However, more in-depth studies aimed at women's health are required, including hormones and genetic marker analyses, thus contributing to the action of competent authorities to improve the health of the exposed population.

KEYWORDS: Elements; Laboratory Evaluation; Amazon River; Elesbão; Amapá

RESUMO

Introdução: A exploração do manganês no Amapá e o armazenamento inadequado dos rejeitos de minério na área portuária de Santana geraram impactos ambientais e sociais aos moradores locais. **Objetivo:** Avaliar a presença de elementos inorgânicos nas moradoras do bairro Elesbão expostas aos rejeitos de minérios. **Método:** Por meio da Espectrofotometria de Absorção Atômica de Chama (F-AAS), quantificaram-se metais na água utilizada pela população e na matriz biológica urina, enquanto no cabelo utilizou-se Espectrometria de Massas com Plasma Indutivamente Acoplado (ICP-MS). Coletou-se o sangue de 67 mulheres expostas e de 99 não expostas, destas, coletou-se 65 amostras de urina e 18 de cabelo. Foram realizadas análises hematológica, bioquímica e de coagulação no sangue de todas as participantes que também responderam a um questionário socioeconômico. **Resultados:** Quanto à faixa etária, 60,0% das moradoras expostas estavam entre 18 e 42 anos e moravam na localidade entre 10 e 34 anos. Na água analisada, encontrou-se concentrações elevadas dos metais As, Cd, Cu, Fe, Mn e Pb. Na matriz urina, observou-se significância para o Mn e, no cabelo, para Pb e Mn. Dentre os parâmetros hematológicos significativos estavam Hb, Ht, VCM, HCM, monócitos, alterações morfológicas nas hemácias e plaquetas. Na avaliação bioquímica, observaram-se alterações nas enzimas fosfatase alcalina, bilirrubinas e creatinina. **Conclusões:** Há contaminação ambiental por elementos que podem comprometer a saúde da população exposta, quando em contato por tempo prolongado. Estudos mais aprofundados e direcionados à saúde da mulher precisam ser realizados, incluindo parâmetros como a análise de hormônios e marcadores genéticos, visando contribuir, assim, para uma ação das autoridades competentes em melhorar a saúde da população exposta.

PALAVRAS-CHAVE: Elementos Inorgânicos; Análise Laboratorial; Rio Amazonas; Elesbão; Amapá

INTRODUCTION

Many social, economic, and cultural factors existing in the regions of Brazil can influence the determination of vulnerabilities of the population, thus enhancing the effects of environmental pollution, which cause health risks[1].

In the Amazon region, productive enterprises were implemented through development plans and policies of the Federal Government, such as Indústria e Comércio de Minérios S/A (Icomi), which explored manganese ore in Serra do Navio, in the state of Amapá[2]. This ore was processed and shipped in the industrial and port area in the municipality of Santana, where the pelletizing plant operated. The tailings from this activity were improperly deposited at the site, remaining in the open until the present day. It thus represents a relevant risk of exposure to the population living in the vicinity of the company's area, including the Elesbão neighborhood[3,4].

Knowledge of the harmful effects of the interactions of inorganic elements with human health is essential, as they cause physical, biological, and socioeconomic impacts and generate damage to health, since they are elements widely found in soil, water, air, garbage, food, and sediment. Human intervention through industrial activities is primarily responsible for releasing metals into nature, contaminating water, plants, and animals, as metals have high levels of reactivity and bioaccumulation[5,6,7]. Each inorganic element has a characteristic and specificity for organs and tissues, but generally has a predilection for hepatic and renal tissues, where cellular detoxification processes occur, as well as for the reproductive system[8].

According to Brazilian epidemiological indicators, women get sick more often than men and are more predisposed to intoxication[9,10]. A study carried out in the state of Bahia showed cytogenetic alterations in women exposed to environmental pollution due to the presence of elements such as lead and cadmium[11]. In addition, studies have shown that differences in epigenetic mechanisms between the sexes influence detoxification and biotransformation of toxic substances[12].

In this context, the present work aims to evaluate the presence of inorganic elements in the environment and in the women of the Elesbão neighborhood. To this end, hematological and biochemical biomarkers were used in exposed individuals, as well as the evaluation of the presence of manganese (Mn), lead (Pb), and other toxic elements in the biological matrices urine and hair, which are important in monitoring the disease warning system, aiming to prevent health problems[13,14].

METHOD

Area and type of study

Conducted with volunteer women who have lived in the Elesbão neighborhood for at least 10 years, located near a mining company in the municipality of Santana, state of Amapá. Those in the group not exposed to metals lived in Macapá and all were between 18 and 65 years of age. According to the Brazilian Institute of Geography and Statistics (IBGE), in 2010, the population of Elesbão was 4,737 inhabitants, of which 2,332 were female. This is a quantitative cross-sectional study, allowing epidemiological and laboratory assessment of the residents of Elesbão exposed to ore tailings.

Sampling

After signing the Free and Informed Consent Term, with the norms of Resolution No. 196, of October 10, 1996, of the National Health Council (CNS), the study participants answered a socioeconomic questionnaire and collected blood, hair, and urine

A total of 283 blood samples were collected, 109 from exposed women and 174 from non-exposed women and, after passing the exclusion criteria, 67 samples belonged to exposed women and 99 samples from non-exposed women were eligible for the study. Women who smoke, women who had chemical procedures on their hair, workers in battery, foundry, metal polishing, and refining factories, welding workers, among others, did not participate in the research. This information was taken from the socioeconomic questionnaire that served as an exclusion criterion, since we sought to select residents who were not known to carry out activities related to handling metals.

The application of the questionnaires and the collections were carried out at the Basic Health Unit of the Elesbão neighborhood and at the Clinical Analysis Laboratory II of the Federal University of Amapá (Unifap), from November 2018 to June 2019. In all blood samples, laboratory tests were performed for hematological, biochemical, and coagulation analysis. Pb, Mn, and copper (Cu) elements were measured in 65 urine samples using Flame Atomic Absorption Spectrophotometry (FAAS) at the Laboratory of Atomic Absorption and Bioprospecting (LAAB) of Unifap and Pb, Mn, Cu, iron (Fe), cadmium (Cd), and arsenic (As) in 18 hair samples by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Laboratory of Atomic Spectrometry (LABSPECTRO) of the Department of Chemistry at the Pontifical Catholic University of Rio de Janeiro (PUC-RJ).

Water collection

Water samples were collected in the rainy season. One liter of water was collected at ten different points in the neighborhood, including streams, in appropriate, properly identified plastic containers. The samples were transported in thermal boxes with dry ice to the laboratory, where they remained under refrigeration (4ºC-8ºC) until analysis. Sample preparation followed the standards established by the American Public Health Association (APHA)[15].

After homogenization and filtration, the samples were placed in the FAAS spectrophotometer, model AA-6300 for determination of metals Cd, As, Cu, Fe, zinc (Zn), Pb and Mn in the Unifap's LAAB[16,17].

Calibration curves were determined for each analyzed metal from FAAS certified standard solutions provided by the National Institute of Standards and Technology of the United States of America (NIST-USA). Analyzes were performed in triplicate and the result expressed as mg.L^{-1}. The maximum permitted concentrations (CMP) of metals in fresh water are established in the Resolution of the National Environment Council (Conama) No. 357, of March 17, 2005[18].

Collection of biological material (blood, hair, and urine)

Blood was collected by means of vacuum venipuncture in tubes containing EDTA K3 anticoagulants for blood count tests, 3.2% sodium citrate for prothrombin time (PT) and activated partial thromboplastin time (APTT) and heparin, for the determination of heavy metals. For biochemical analyses, the tubes did not contain anticoagulant[6,19].

Blood analysis was performed at Unifap's Clinical Analysis Laboratory II. The blood count was performed on a Icounter 3D hematology analyzer and blood smears were prepared for differential and cellular morphological evaluation. Coagulation tests were performed using a Clo Timer coagulometer. Biochemical analyzes were performed on the LANDWIND LW C200i automatic analyzer with Labtest reagent kits.

Approximately 500 mg of hair was collected from the occipital region with the aid of titanium scissors, at a distance of up to 2 cm from the scalp. The samples were packed in hermetically sealed polyethylene envelopes with properly identified zippers, kept in a dry and clean place and transported to the LABSPECTRO of the Chemistry Department at PUC-RJ for analysis[20,21,22,23].

The urine samples were collected by the research participants in proper bottles, properly identified, stored, and transported. They remained frozen at -80ºC until analysis, performed at LAAB/Unifap[6].

Preparation of samples for analysis

Urine samples were diluted 1 + 7 in 0.2% nitric acid (v/v), according to the methodology used by Marinha[24]. After homogenization, measurements were performed in triplicate for each metal in FAAS model AA-6300 equipment.

Each hair sample (about 200 mg to 250 mg) was washed, according to a methodology adapted from the International Atomic Energy Agency (IAEA), in which ultrapure water and acetone were used. First, it was washed once with acetone, three times with ultrapure water, and once more with acetone. The samples remained submerged for 10 min in an ultrasonic bath in each solvent. After washing, the samples were dried in an oven at 50ºC for 12 h.

The samples were weighed after drying and, according to the remaining mass, they were separated into groups and the amounts of 65% nitric acid and hydrogen peroxide that would be used in the digestion step were determined.

After the addition of nitric acid, the samples were dried on hot plates at 80ºC for 12 h. Then, hydrogen peroxide was added, and a new drying step was performed for 20 min at 80°C. After reaching room temperature, the samples were diluted with ultrapure water in the same proportion used for the peroxide. To finally be analyzed in the LABSPECTRO's ICP-MS equipment of the Chemistry Department of PUC-RJ.

Ethical considerations

The study was approved by the Ethics and Research Committee of Unifap, through opinion No. 2853167 and No. CAE 94256218.0.0000.0003, on July 18, 2018.

Data processing and analysis

Mean and standard deviation were performed using Microsoft Excel 2010. Using the BioEstat 5.0 program, the Kolmogorov-Smirnov normality test was performed, then, for the analysis of parametric data, the t-student (t) test was performed and for non-parametric tests, the Mann-Whitney (U) test. The significance level considered was p = 0.05.

RESULTS AND DISCUSSION

Socioeconomic questionnaire

In this study, it was observed that 40.9% of the women lived in Elesbão between 22 and 34 years old. Among the socioeconomic characteristics, it is highlighted that 60.0% of the women belonged to the age group between 18 and 42 years, in both groups. Regarding education, about 40.0% of the exposed women had completed high school, followed by 28.0% with incomplete elementary education, while 30.3% of the non-exposed women had incomplete higher education, followed by 26.2% with complete higher education. Of those who worked, most had a monthly income of up to half a minimum wage in the exposed group, of up to one minimum wage in the unexposed group, and most had no monthly income.

Studies have shown that the level of education and income are factors that directly impact people's perception of health, negatively affecting the health status of individuals who live in regions with a lot of inequality, reducing the chance of being healthy by around 4% when compared to those who live in less unequal regions[25]. Individuals with higher levels of education and income have more access to health goods and services, which provides them with a better quality of life[26].

The most used drinking water in both groups was the well, followed by the supply network, and only four exposed women used river water as the only source of consumption.

Among the symptoms reported by exposed women are: anemia, muscle pain, emotional instability, and sleep and gastrointestinal (GI) tract disorders. Regarding fertility, 25.4% of the women reported having a decrease in libido and 20.0% had already had an abortion. Among couples, 16.0% were considered sterile. As for the incidence of cancer in family members, 48.0% of the women responded positively, and 30.0% were from the cervix.

Heavy metals (As, Cd, Pb) cause effects on human infertility and congenital malformations, interfering with the embryo's neurological development, abortions, premature births, and hormonal changes[27].

Water analysis

Changes were observed at several collection points (Table 1). The metals As, Cu, Fe, Mn, Cd, and Pb exhibited concentrations above the maximum values allowed by current legislation. A worrying fact, as they are potentially toxic, risking the health of the population that uses the water.

Studies have shown that there is contamination by heavy metals in the stream that runs through the Elesbão neighborhood and flows into the Amazon River, in which high concentrations of As and Fe[2] were found, corroborating the results of this study.

According to other water analysis studies carried out in the Elesbão neighborhood, during the Amazon summer period, high levels of Mn and Fe[13,14] were demonstrated. The Amazonian summer is a period of drought in which there is little or no rain, while the Amazonian winter is a rainy period, in which the waters of the rivers rise, allowing the release of concentrations of metals that were possibly found in the sediment. In the present study, carried out during the Amazonian winter, we found concentrations of different metals.

In a study by Facundes[4], a high content of As was reported in two wells located at the bottom of the tailings dam. However, the results of samples taken from other water collection points for consumption revealed that As levels were higher in the Amazon River than in the wells opened by private individuals in the vicinity of the Icomi industrial area. These As values varied greatly throughout the year, being higher in the rainy seasons, indicating that the Amazon River was an important carrier of As[4].

Analysis of metals in biological matrices (urine and hair)

In the average concentrations found in this research (Table 2), there was a significant difference between the groups for urinary Mn ($p < 0.0001$) and for PB and hair Mn ($p < 0.05$).

As for the mean concentration of urinary Pb, it was observed that the mean value found in the group of exposed women is within the reference value and in the group of non-exposed women, this metal was not detected.

Pb is a toxic metal that accumulates in the body and can cause damage to the central nervous system, altering neurocognitive and neurophysiological functions in both children and adults. Studies have shown that there is no safe level of Pb presence

Table 1. Results of the mean and standard deviation of the concentrations of metals in $mg.L^{-1}$ analyzed in the ten collection points of the Elesbão neighborhood, in the rainy season of the region.

Collection Points	Cu ($mg.L^{-1}$)	Mn ($mg.L^{-1}$)	Fe ($mg.L^{-1}$)	Cd ($mg.L^{-1}$)	Pb ($mg.L^{-1}$)	As ($mg.L^{-1}$)	Zn ($mg.L^{-1}$)
	(MD ± SD)	(MD ± SD)	(MD ± SD)	(MD ± SD)	(MD ± SD)	(MD ± SD)	(MD ± SD)
RV Conama*	0.009	0.1	0.3	0.001	0.01	0.01	0.18
Point 1	0.033 ± 0.018	0.046 ± 0.005	0.905 ± 0.003	0.002 ± 0.001	0.058 ± 0.006	1.4 ± 0.1	0.053 ± 0.001
Point 2	0.048 ± 0.017	0.071 ± 0.001	0.295 ± 0.004	ND	ND	2.6 ± 1.2	0.032 ± 0.002
Point 3	0.014 ± 0.014	0.061 ± 0.003	0.916 ± 0.029	ND	ND	3.8 ±0.9	0.029 ± 0.002
Point 4	0.022 ± 0.012	0.110 ± 0.010	0.910 ± 0.011	ND	ND	5.4 ± 0.9	0.035 ± 0.003
Point 5	0.017 ± 0.011	0.041 ± 0.003	0.489 ± 0.001	ND	ND	7.5 ± 0.7	0.007 ± 0.003
Point 6	0.025 ± 0.010	0.049 ± 0.002	0.821 ± 0.052	ND	ND	10.9 ± 0.9	0.01 ± 0.002
Point 7	0.012 ± 0.01	0.014 ± 0.003	0.034 ± 0.005	ND	ND	10.3 ± 1.0	0.026 ± 0.001
Point 8	0.019 ± 0.009	0.027 ± 0.007	0.069 ± 0.006	ND	ND	11.4 ± 1.1	0.018 ± 0.004
Point 9	0.025 ± 0.009	0.344 ± 0.016	1.666 ± 0.104	ND	ND	13.9 ± 0.9	0.018 ± 0.005
Point 10	0.024 ± 0.009	0.055 ± 0.001	0.846 ± 0.055	ND	ND	16.9 ± 1.2	0.008 ± 0.002

Source: Elaborated by the authors, 2021.
* Reference values in $mg.L^{-1}$ according to Conama Resolution No. 357, of March 17, 2005[18].
ND: not detected; MD ± SD: mean and standard deviation.

Table 2. Mean concentrations of Pb, Cu, Mn, Cd, As and Fe in urine and hair in the groups composed of exposed (n = 29) and non-exposed (n = 36) women. Mean values (MD), standard deviation (DP), statistical test, and p value.

Metals	RV	Group		Test result	p-value
		Exposed (MD ± SD)	Non-exposed (MD ± SD)		
Urine					
Pb	up to 50.00 μg.g^{-1}	16.90 ± 54.30	0.00	U = 473	0.0800
Cu	up to 60.00 μg.L^{-1}	45.40 ± 84.20	34.9 ± 49.70	U = 512.5	0.9000
Mn	up to 10.00 μg.L^{-1}	29.00 ± 33.40	117.7 ± 46.70	t = -8.4	< 0.0001
Hair					
Pb	up to 9.30 mg.L^{-1}	2.40 ± 2.70	0.3 ± 0.30	U = 13.5	0.0200
Cd	up to 0.30 mg.L^{-1}	0.07 ± 0.05	0.01 ± 001	U = 19.5	0.0800
Cu	10.00 to 32.00 mg.L^{-1}	11.90 ± 4.30	8.4 ± 2.00	U = 18.0	0.0600
Mn	0.15 to 1.20 mg.L^{-1}	4.70 ± 3.00	0.6 ± 0.70	U = 7.0	0.004
As	up to 0.15 mg.L^{-1}	0.03 ± 0.01	0.02 ± 0.01	t = 1.3	0.1000
Fe	7.00 to 18.00 mg.L^{-1}	34.90 ± 17.40	23.1 ± 28.70	t = 0.9	0.3000

Source: Elaborated by the authors, 2021.
RV: reference value; MD ± SD: mean and standard deviation; U: Mann-Whitney statistical test; t: t-student statistical test.
urine RV - RV for Pb according to Regulatory Norm n° 7[59] and Cu and Mn RV according to Burtis and Burns[28].
hair RV - RV according to data obtained from LABSPECTRO of the Chemistry Department at PUC-RJ.

without harm to the body, making any source of exposure to this metal a concern for public health[29].

The organism exposed to Pb for a long period results in the inhibition of hemoglobin synthesis, in addition to reducing the circulation time of red blood cells, which causes mild to moderate anemia in adults and severe anemia in children[30] Anemia was one of the pathologies reported by the women, with about 70.0% being in the exposed group and 35.0% in the unexposed group, but the presence of the metal in the urine of the latter was not detected. Despite being detected only at one point of water collection, it is a relevant data that deserves further investigation, emphasizing that it should not be found in the organism, as found in the present study.

According to Ramos[6], when evaluating environmental exposure to Mn in a resident population close to a shipyard in Angra dos Reis, RJ, the mean concentrations of this metal in urine did not have significant differences with results within the reference range. These results do not corroborate the present study.

However, in another study that evaluated the level of exposure to metals in shipbuilding workers, it was observed that about 50% of urine samples contained Mn above the reference value, indicating exposure[31].

Oral contamination by excess Mn causes damage to the body. Foods rich in Mn can be plant-based, such as rice, wheat, cereals, as well as seafood and water. Conditions found not only in the municipality of Santana, but also in Macapá, bathed by the Amazon River. The sediment analyzed in the rainy and dry seasons on the edge of Macapá contained heavy metals in various concentrations, such as As, Zn, Ni, and Mn[32].

The average concentrations of Mn in the hair of adults living in an area located near an iron-manganese alloy industry, in Bahia, were about eight times higher than the recommended value. It was shown that the measurement of Mn in hair is an important environmental biomarker[33], corroborating the present study, in which the average concentration of Mn in the hair of the residents of Elesbão is about four times above the recommended value.

Other studies found high levels of Mn in the hair of children exposed to minerals in a community in Bahia, with 15.20 μg Mn/g hair, while the unexposed population had 1.37 μg Mn/g hair[33,34]. Evidencing that the greater the exposure, the greater the accumulation of the element in the organism.

The analysis of Pb in the hair of the residents of Elesbão showed significance between the groups studied, with the exposed group showing results about six times higher than the unexposed group, although both are within the reference range. A worrying factor, as a high level of Pb was found at a point of collection of river water, characterizing environmental exposure.

After the rupture of the ore dam in Mariana, there was an increase in the concentrations of metals such as Pb and Mn in the hair samples of the local population when compared to previous periods. In addition, the consumption of water and food from the contaminated river can influence these indices[35].

The analysis of hair from residents of the communities on the islands of Volta Grande do Rio Xingu and Belo Monte and Altamira in Pará highlighted Pb and mercury in high average concentrations. Although it is a mining area, we emphasize the importance of contamination of the river water used by the population, which had the highest average concentration of Pb with about four times the average value of other types of water[36].

Thus, we emphasize that the Elesbão neighborhood is also susceptible to contamination, as shown by the result in the hair samples of this population.

Hematological evaluation

A significant difference ($p < 0.05$) was found between the groups studied in the parameters: hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV) and mean corpuscular hemoglobin (MCM), with individuals in the exposed group showing slightly lower averages than those in the unexposed group. Regarding monocytes ($p = 0.0494$), both groups had mean values slightly lower than the reference values; and in the prothrombin time (PT), in the international normalization ratio (INR) and in the activated partial thromboplastin time (APTT), the mean value is within the recommended values. In addition to the mean concentration of mean platelet volume (MPV), which is slightly above the reference value (Table 3).

Hematological alterations are evident in intoxication by metals such as Pb, As, Fe, and Cd. In chronic Pb intoxication, we find normocytic normochromic or microcytic and hypochromic anemia with decreased heme synthesis and increased hemolysis. Anisocytosis and poikilocytosis, nucleated red blood cells, and polychromasia also occur[37]. These changes described above corroborate the results found in this work.

A study that looked at 192 patients with a history of Pb poisoning over 50 years, comparing them with a control group, showed that exposed individuals were seven times more likely to develop hypertension and lower hematocrit than control individuals[38].

Correlating a study carried out in the exposed population of Elesbão, a significant difference was found in the hematocrit

Table 3. Analysis of hematological parameters (blood count and coagulogram) in exposed (n = 67) and non-exposed (n = 99) groups, showing mean values (MD), standard deviation (SD), statistical test, and p value.

Hematological parameters	RV*	Group		Test result	p-value
		Exposed	Non-exposed		
		MD ± SD	MD ± SD		
Erythrocytes (x 10^{12}/L)	3.8 to 4.8	4.7 ± 0.3	4.8 ± 0.3	t = -0.4	0.6860
Hemoglobin (g/dL)	12.0 to 15.0	12.5 ± 1.1	13.0 ± 0.9	U = 2,623.0	0.0220
Hematocrit (%)	36.0 to 46.0	39.2 ± 2.9	40.4 ± 2.3	U = 2,494.0	0.006
MCV (fL)	83.0 to 101.0	82.1 ± 4.9	84.1 ± 4.3	t = -2.7	0.0070
MCM (pg)	27.0 to 32.0	26.4 ± 0.8	27.0 ± 1.5	t = -2.7	0.0070
MCHC (g/dL)	31.5 to 34.5	31.9 ± 0.5	31.8 ± 2.9	U = 2,986.5	0.2770
RDW CV (%)	11.0 to 14.0	10.8 ± 0.8	11.0 ± 3.0	U = 2,986.5	0.9750
Leukocytes (/mm^3)	3,500.0 to 10,000.0	5,482.0 ± 1,177.0	5,715.0 ± 1,341.0	t = -1.1	0.2570
Neutrophils (/mm^3)	1,700.0 to 8,000.0	3,105.0 ± 921.0	3,394.0 ± 1,079.0	t = -1.6	0.0950
Lymphocytes (/mm^3)	900.0 to 2,900.0	1,841.0 ± 464.0	1,890.0 ± 489.0	t = -0.6	0.5180
Monocytes (/mm^3)	300.0 to 900.0	241.0 ± 219.0	264.0 ± 108.0	U = 2,719.0	0.0400
Eosinophils (/mm^3)	50.0 to 500.0	213.0 ± 222.0	174.0 ± 121.0	U = 3,266.0	0.8000
Basophiles (/mm^3)	Up to 200.0	0.7 ± 6.3	0.5 ± 5.5	t = 0.2	0.8000
Platelets (/mm^3)	140,000.0 to 450,000.0	248,700.0 ± 58,900.0	250,800.0 ± 49,200.0	t = -0.2	0.8000
MPV (µm^3)	7.4 to 10.4	10.5 ± 0.6	10.4 ± 0.9	U = 2,991.5	0.2000
PDW (%)	15.0 to 17.0	11.2 ± 1.3	11.1 ± 1.7	t = 0.4	0.6000
P-LCR (%)	15.8	25.7 ± 5.4	24.8 ± 7.0	U = 3,046.0	0.3000
PT (seg)	10.0 to 14.0	10.3 ± 3.9	10.6 ± 1.8	U = 2,287.0	0.0007
TAP (%)	70.0 to 100.0	92.5 ± 13.0	89.5 ± 14.2	t = 1.3	0.1000
INR	0.8 to 1.0	0.9 ± 0.1	1.0 ± 0.2	U = 2,300.0	0.0008
APTT (seg)	24.0 to 40.0	33.8 ± 8.2	36.9 ± 8.6	U = 2,606.5	0.0100

Source: Elaborated by the authors, 2021.
*Reference values according to the Brazilian Society of Clinical Analysis; package inserts for the reagents used; Melo and Silveira[60] and Henry[40].
MCV: Mean cell volume; MCM: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: MCV variation coefficient; MPV: mean platelet volume; PDW: platelet distribution width; P-LCR: percentage of large platelets; PT: prothrombin time; TAP: prothrombin activity; INR: international normalization ratio; APTT: activated partial thromboplastin time; MD: mean values; SD: standard deviation; U: Mann-Whitney statistical test; t: t-student statistical test.

parameter[13,14]. However, in the present study, the other hematimetric parameters that had significance were not evidenced in the aforementioned study[14], which may be related to the sample n, which in the present study is greater.

Monocytes are myeloid cells that derive from the same progenitor cell as neutrophils. After formation in the bone marrow, they are released into the bloodstream and migrate to the tissues, forming the reticuloendothelial system, which is important in combating microorganisms[39,40].

The study by Lima showed significance between the groups only in terms of lymphocytes, with a slightly lower mean in the exposed group[13,14]. Comparing with the present study, significance was observed in the concentration of monocytes, although the average concentrations of neutrophils, lymphocytes, and monocytes are within the reference values and lower in the exposed group, indicating a slight alteration in the defense cells of the organism of the population studied.

According to a study that evaluated chronic exposure to Pb, no significant difference was found in the levels of lymphocytes and neutrophils between the groups studied, corroborating the present study[41].

The platelet count of this study did not show significance as in another study involving the same population[13,14]. This disagreement was attributed to the sample number of each study, since in this study 67 individuals were analyzed in the exposed group and 99 in the non-exposed group, all female, while in the above study about 50 people participated, of both sexes in the exposed group and 50 in the control group.

However, the present study and those by Lima[13,14] are in agreement regarding changes in platelet morphology, in which macroplatelets and giant platelets were frequently found.

The literature does not detail the correlation between coagulation tests and the presence of metals in the body. However, once the clotting factors are produced in the liver, considering that some metals accumulate in this organ and depending on the concentration of these elements or their simple presence, they can compromise its functioning causing hematological alterations altering the laboratory tests.

Examples are: As which, after being absorbed, accumulates primarily in the liver, interfering with enzymatic activities; Pb that causes alterations in the hematological system; Cu that has the liver responsible for its homeostasis and, when in excess, overloads the hepatocytes degenerating the organ; and Fe which, when in excess, is stored as ferritin or hemosiderin, mainly in the liver and spleen[7,27,28,42,43,44,45].

Laboratory tests to assess clotting factors are important parameters for monitoring blood clotting. Among these, the PT assesses the extrinsic and common pathways of coagulation. It is a test requested for preoperative examination, in the investigation of coagulopathy or to monitor treatment with oral anticoagulants[43,46].

The INR aims to reduce the variation in the PT result between different clinical laboratories and is widely used to control oral anticoagulation, preventing thromboembolic phenomena and becoming an effective resource to reduce these complications[40,43,46,47,48,49].

The APTT assesses the functioning of the intrinsic and common coagulation pathway, detecting deficiencies in factors VIII, IX, XI, and XII, prekallikrein and high molecular weight kininogen, among others.

Therefore, it is important that further studies are carried out to assess whether the hematological changes in coagulation are due to the presence of metal in the body.

Morphological changes

Blood smears from participants in both groups were analyzed and the frequency of occurrence of the main morphological changes was observed. In the exposed group, the presence of macroplatelets stands out in 38.8% of the samples, followed by anisocytosis (28.3%), poikilocytosis (28.3%), and hypochromia (20.8%), in addition to 8.0% of giant platelets, while the unexposed group had 2.0% macroplatelets, 17.0% anisocytosis, 5.0% poikilocytosis, 9.0% hypochromia, and absence of giant platelets.

Platelets are cytoplasmic fragments of megakaryocytes, anucleated, discoid, produced by the bone marrow, responsible for primary homeostasis. Macroplatelets are 4 to 7 µm in size and giant platelets are usually 10 to 20 µm (Figure 1A). The formation of macroplatelets and giant platelets is related to accelerated platelet turnover, as demonstrated by the increase in MPV[46,50,51,52].

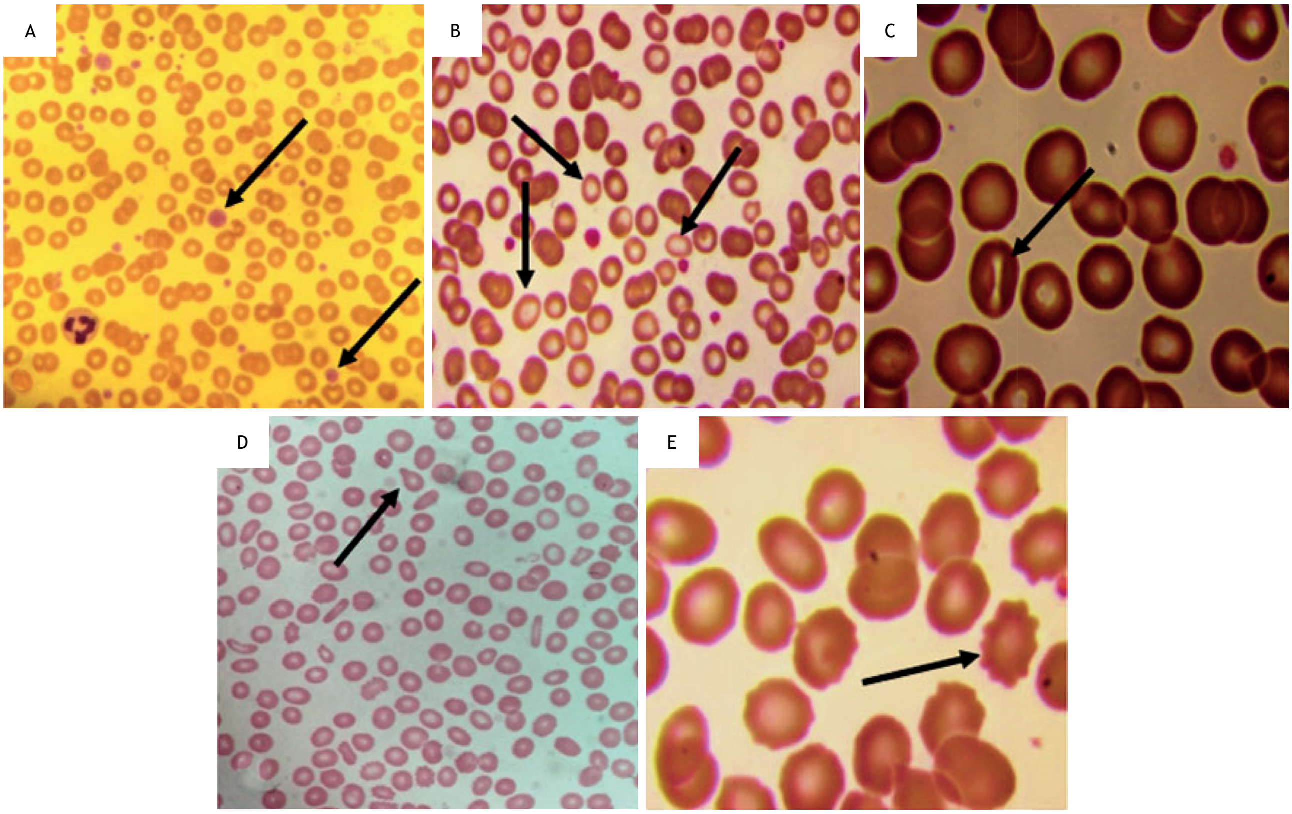
The study carried out with the female and male population of Elesbão, showed a high quantity of macroplatelets, as well as the presence of poikilocytosis, especially dacriocytes and echinocytes in the exposed group[13,14].

In the exposed group of the present study, poikilocytosis was frequently observed with the presence of stomatocytes, dacrocytes, and echinocytes, as well as the presence of hypochromia (Figure 1B). Stomatocytes, erythrocytes with a central halo called a stoma and similar to fish mouth (Figure 1C), occasionally occur in normal blood distensions and in hereditary stomatocytosis, liver diseases, alcoholism, and also due to exposure of erythrocytes to cationic substances and drugs[43,53].

Dacrocytes (Figure 1D) are present in pathologies such as myelofibrosis, acquired hemolytic anemia, megaloblastic anemia, and hypersplenism. Echinocytes, red blood cells with alterations in their discoid shape, presenting themselves covered by 10 to 30 spicules with a relatively regular shape and distribution (Figure 1E), may be present in renal and hepatic disease, in addition to being an artifact of the preparation of the extension (alkaline pH of the glass) or excess of EDTA[43,53].

Biochemical tests (markers of liver and kidney function)

Based on the results of liver and kidney markers of the groups studied, we found a significant difference in the parameters:



Source: Elaborated by the authors, 2021.

Figure 1. (A) Macroplates and Giant Platelets, (B) Hypochromia and Anisocytosis, (C) Stomatocytes, (D) Dacrocytes, (E), Echinocytes.

Table 4. Biochemical parameters total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase (ALF), AST/TGO, ALT/TGP, GT Gamma, urea, and creatinine performed in the exposed and unexposed groups. Mean values (MD), standard deviation (DP), statistical test, and p value.

Biochemical tests	RV*	Group		Test result	p-value
		Exposed	Non-exposed		
		MD ± SD	MD ± SD		
Total bilirubin	Up to 1.2	0.3 ± 0.2	0.4 ± 0.2	t = -3.90	0.0001
Direct bilirubin	Up to 0.4	0.1 ± 0.1	0.1 ± 0.1	t= -3.70	0.0003
Indirect bilirubin	Up to 0.8	0.2 ± 0.1	0.3 ± 0.2	U= 2,511.50	0.0080
Alkaline phosphatase	27.0 to 100.0	73.3 ± 25.0	50.0 ± 24.6	t= 5.90	< 0,0001
AST/TGO (U/L)	Up to 42.0	22.8 ± 9.1	20.9 ± 8.4	U = 2,935.50	0.2000
ALT/TGP (U/L)	Up to 42.0	20.0 ± 10.2	19.5 ± 12.7	t = -1.80	0,0600
Gama GT(U/L)	5.0 to 58.0	36.7 ± 28.1	37.5 ± 29.6	t = -0.09	0,9000
Urea	10.0 to 45.0	30.3 ± 9.8	31.7 ± 8.2	t = -0.70	0.4000
Creatinine	0.4 to 1.3	0.5 ± 0.2	0.8 ± 0.2	t = -7.00	< 0,0001

Source: Elaborated by the authors, 2021.
*RV: Reference value according to the Labtest biochemistry reagent kits used in the dosages. AST/TGO: Aspartate aminotransferase or oxalacetic transaminase; ALT/TGP: Alanine aminotransferase; U: Mann-Whitney statistical test; t: t-student statistical test.

total bilirubin (p = 0.0001), direct bilirubin (p = 0.0003), indirect bilirubin (p = 0.0081), alkaline phosphatase (p = < 0.0001), and creatinine (p < 0.0001) (Table 4), with mean concentrations within the reference values.

Although the average concentrations of the studied groups are within the recommended reference intervals, it is observed that the alkaline phosphatase (ALF) of the exposed population is higher than that of the unexposed, with 12.0% of exposed women presenting FAL above the maximum allowed, while only 3.0% of non-exposed women had altered values.

Considering that exposure to metals can cause liver diseases, the biochemical data may be correlated with the metals detected in the drinking water studied. Mn, when in excess in the body, is found at higher levels in the liver, conjugated to bile salts. Excess Fe in the blood causes hemochromatosis, a disease that accumulates iron in the form of ferritin in the muscles, liver, pancreas, and joints, causing damage to them. As accumulates mainly in the liver, as well as Pb[41,42,54].

In this study, hepatic transaminases (AST, ALT) did not show a significant difference between the groups, not corroborating another study carried out in which a significance was observed for ALT in the female population studied[13,14]. However, in both, the results of the exposed population are higher than those of the control group, within the recommended reference value.

Chronic exposure to metals can influence these results, however, creatinine can be altered by several factors, such as physical exertion, action of some medications and malnutrition[55,56]. According to Ramos and Marini[57], the elevation of the serum creatinine level can be found in skeletal muscle necrosis or atrophy, congestive heart failure, shock, diarrhea, or gastrointestinal fistulas, uncontrolled diabetes, among others[57]. Factors such as ethnicity, race, age, and sex also influence the serum level of creatinine[58].

A study that compared groups of renal function markers in the Elesbão population did not find statistical significance. However, in their individual renal assessment, it was evidenced that seven individuals in the exposed group had alterations in creatinine and one in urea and in the non-exposed group there were no alterations[14]. When comparing the analysis of the groups with the present study, it can be observed that the results of urea corroborate the study mentioned above. However, those for creatinine showed statistical significance.

CONCLUSIONS

After analyzing samples of water from the Amazon River and streams, used by the residents of Elesbão, contamination by metals was detected. Prolonged exposure can compromise the health of the population and may be related to changes in hematological, morphological, biochemical, and coagulation tests.

When analyzing the biological matrices urine and hair, we found significantly high concentrations of Mn, the main ore of local extraction.

However, more in-depth studies aimed at women's health need to be carried out with the increase in the number of participants and the inclusion of other parameters such as the analysis of hormones and genetic markers.

The high presence of the As metal in the river water must be evaluated in the biological matrices as well as the inclusion of the population of children, as it is a group especially sensitive to the toxicity of metals.

Finally, the investigation of other sources of exposure to metals is extremely important so that preventive measures can be taken to eliminate or minimize the risks of adverse effects related to exposure and, thus, improve the quality of life of the population.

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Author's Contributions

Cardoso FMN, Gomes MRF - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Cardoso FMN, Telles CR, Milhomem WMC, Trindade Junior SV, Vidal LJG, Bezerra RM, Braga FS, Vasconcelos HCG, Almeida AF, Lima SEA - Acquisition, analysis, and data interpretation. All authors approved the final version of the work.

Conflict of Interests

The authors inform that there is no potential conflict of interest with peers and institutions, politicians, or financial in this study.