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Original Article

Determination of lead in biological samples and its effects on the health of people living in an area contaminated by steel mill waste in Brazil

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ABSTRACT

Steelmaking is an anthropogenic activity contributing to the emission of lead into the environment. Lead, a non-essential and bioaccumulative metal, can cause adverse effects on human health. This study aimed to assess the biomarkers of exposure (lead concentration in blood, plasma, and urine), effect (delta-aminolevulinic acid dehydratase (ALAD) activity), and susceptibility (polymorphism rs1800435) to lead in residents exposed to steel industry waste in a condominium in the city of Volta Redonda, Rio de Janeiro, Brazil. Lead levels were measured in blood (BPb), plasma (PPb), and urine (UPb) using a graphite furnace atomic absorption spectrometer (GFAAS), while ALAD activity, and genotyping by spectrophotometry and RT-PCR, respectively. Twenty-seven individuals were assessed who showed a mean blood lead of 2.13±0.80 μg dL^{-1}, plasma lead equal to 2.80±2.61 μg L^{-1}, urinary lead of 4.35±4.31 μg g^{-1} creatinine, and an ALAD activity of 24.45±8.28 U L^{-1}. The genotypical frequencies for the polymorphism rs1800435 were ALAD 1-1 (92.52%), ALAD 1-2 (7.40%), ALAD 2-2 (0.00%), while their allelic frequencies were 96.30% for the wild-type allele c, and 0.0370% for the polymorphous allele g. The results suggest that those individuals were environmentally exposed to lead.

KEYWORDS: lead, blood, plasma, urine, ALAD activity, polymorphism, environmental exposure, GFAAS, spectrophotometry, RT-PCR.

1. INTRODUCTION

Lead (Pb) is a non-essential, and bioaccumulative metal that can cause different adverse effects to the human body. The emission into the environment occurs from natural sources, such as volcanism, spraying of seawater, weathering of rocks, suspended soil particles, and burning of organic matter. However, anthropogenic activities are mainly responsible for the emission of Pb into the atmospheric air, an important route of dispersion in the environment, with the metal potentially remaining for days or weeks in the air before being removed by rain or particulate deposition. Lead can remain in the soil in the insoluble or soluble forms. The most important human activities are industrial production of automobile batteries, electric wires and cables, glass products,

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ceramic tiles, x-ray protection materials, cement, paints, rubber, and pesticides, tubing and plumbing, oil refining, and steelmaking [1, 2].

In the atmosphere, lead can be transported from the local sphere up to the intercontinental one, depending on several factors, including particle size, height of emission, and meteorology. Large particles with diameters > 2 μm are deposited relatively close to the emission sources, while smaller particles can be carried for thousands of kilometers, depending on the wind velocity [2, 3].

Lead mobility in the soil depends on the pH, and on humic and fulvic acids, which function as chelating agents for the metal. Lead forms insoluble compounds at pH 6-8, which remain stable in the upper layers of the soil without leaching. However, rainfall reduces the pH, increasing the solubility and making lead bioavailable for absorption by plants [2, 3].

The main entry routes for inorganic lead into the body are ingestion and inhalation. Absorption is greater in children and pregnant women and depends on age, fasting, diet, physiological status, size and solubility of the ingested particles, and lead species [1, 2].

After absorption, lead is distributed through the bloodstream to soft tissues and bones. In the blood, 99% of the metal is bound to red blood cells, mainly to the delta-aminolevulinic acid dehydratase (ALAD), and 1% to the plasma. Plasma is the most important fraction since it is bioavailable to other organs. More than 90% of the lead in the body is stored in bones, and 40% to 70% of the metal in blood comes from bones. In periods of greater bone remodeling, the mobilization of lead from bones to blood increases, elevating metal levels in the blood. Inorganic lead does not undergo metabolism, but forms complexes [4]. Lead is eliminated mainly by urine (75 to 80%) and feces (15%). It can also be obliterated via bile, pancreatic secretions, breastmilk, sweat, hair, and nails [1].

The formation of stable complexes with ligands containing sulfur, phosphorus, nitrogen, or oxygen is the main process in lead toxicity. However, the most important biochemical associations regarding toxicological outcomes are those with the thiols groups since protein and enzyme functions can be modified, resulting in toxic effects. Besides its major affinity for amines and amino acids, lead can also mimic calcium in calmodulin activation [1, 2, 4].

Lead may produce neurotoxicity, damage the cardiovascular, immune, endocrine, and reproductive systems, and cause adverse effects on the development, blood, and kidneys [4]. Chronic lead exposure can compromise glucose homeostasis, mainly affecting the pancreas and liver by inducing insulin resistance [5].

Biomarkers should reflect the intensity of exposure to the chemical substance or its effect on the organism, to assess the intensity of exposure and human health risk. Biomarkers are classified as exposure (or internal dose), effect, and susceptibility. Biomarkers of exposure estimate the internal dose of the substance, whose toxicokinetic profile is well-defined. Biomarkers of effect reflect the interaction between the element and receptors in the body, revealing the occurrence of biochemical alterations. Meanwhile, biomarkers of susceptibility express genetic or acquired factors that induce a given reaction in the organism for each type of chemical exposure [6].

Volta Redonda is in southern Rio de Janeiro State, Brazil, with an area of 182.105 km^2 and an estimated population of 261,563 in 2022 [7]. The condominium studied herein is registered as a contaminated site under the State Environmental Agency (INEA) [8, 9] intervention since the local steel industry has used the area to dispose hazardous industrial waste for over ten years. Thus, the condominium was built on steel slag and next to the current land for disposing of industrial waste [10]. Steelmaking activities generate hazardous waste containing lead through the release of the metal in sludge and dust coming from gas controllers present in blast furnaces or corrosive residues resulting from steel finishing operations [9].

Previous research detected lead levels 2.3 times higher than the quality reference value in the condominium soil. Meanwhile, the pH levels ranged from 6 to 9.6, which would reduce the solubility of lead in soil. However, rainfall can alter the solubility, lowering the soil pH and making the metal soluble [11]. Despite the neighborhood's

history of contamination, no records of prior health risk assessments in the resident population were found.

The current study aimed to assess the exposure of the resident population in the condominium to lead particulates dispersed in the environment from the industrial waste disposal, using biomarkers of exposure (lead concentration in blood, plasma, and urine), effect (ALAD activity), and susceptibility (polymorphism SNP rs1800435) as tools for such an assessment.

2. MATERIALS AND METHODS

This work constitutes part of a cross-sectional research carried out in a condominium located in the city of Volta Redonda, southern the state of Rio de Janeiro, Brazil. Although the condominium had eight blocks, this pilot project was developed in nine houses across four blocks. The eligibility criterion for the houses was their proximity to the air sampling points, following the local prevailing northwest wind. Twenty-seven volunteers attended the study. The participants were residents aged eighteen years or older, with at least six months of residence time. Exclusion criteria were pregnant women and individuals with genetic/hereditary disorders involving target organs susceptible to lead toxic action. Regarding the population profile, the survey used a validated questionnaire that was provided to volunteers at their homes. A team from the Health Department of Volta Redonda (SMS VR) went to the participants' homes requesting donations of biological samples. Double blood samples were drawn in heparinized 7 mL tubes suitable for trace analysis, while urine samples were collected in 50 mL decontaminated containers. Samples were transferred immediately to the laboratory under refrigeration. In the laboratory, one blood tube was mounted on the lab bench for 30 minutes and then centrifuged at 1800 rpm for 10 minutes, and the supernatant (plasma) was removed and stored at -20 °C for subsequent analysis. Upon arrival, samples from one tube rested on the bench for 30 minutes. They were then centrifuged at 1800 rpm for 10 minutes, and the supernatant was removed and stored at -20 °C until analysis. The content of the other tube was fractionated into three aliquots. One aliquot was submitted for immediate analysis of ALAD activity, and the other two were transferred to the respective laboratories to determine lead in whole blood and for polymorphism analysis.

A PinAAcle 900Z atomic absorption spectrometer equipped with a Zeeman longitudinal background corrector, pyrolytic graphite tubes with endcap, and a hollow cathode lamp for lead (wavelength at 283 nm), all Perkin Elmer, performed the determination of lead in whole blood, plasma, and urine. A 1601-A UV-Visible spectrophotometer (Shimadzu), wavelength at 555 nm, determined ALAD, while a 7500 Real-Time PCR System (Applied Biosystems) was used for genotyping polymorphism rs1800435.

Before analysis, all materials (plastic and glassware) were decontaminated as described previously [12]. Reagents were of analytical grade, from Merck (RJ, Brazil) and Sigma-Aldrich (SP, Brazil), and all solutions were prepared with water previously deionized in a Milli-Q system, from Millipore (Bedford, USA). Lead analytical solutions were prepared daily in 0.2% (v/v) nitric acid by adequate dilutions of a 1000 μg mL^{-1} lead stock solution. Calibration curves were prepared using whole blood and blood plasma, and urine diluted in 0.1% (v/v) Triton X-100 and 0.2% (v/v) nitric acid, respectively. A mixture of magnesium nitrate 10 g L^{-1} and palladium nitrate 10 g L^{-1} in 0.2% (v/v) nitric acid was used as a chemical modifier. Whole blood and blood plasma were diluted 1+9 and 1+4, respectively, in 0.1% (v/v) Triton X-100. Urine was diluted 1+4 in 0.2% (v/v) nitric acid.

The analytical quality of the results was evaluated through proficiency tests with the National Institute of Work Safety and Hygiene (Zaragoza, Spain) and Adolfo Lutz Institute (São Paulo, Brazil). The accuracy of the procedures was also checked using, in each batch of samples, reference materials for whole blood, serum, and urine, diluted according to their concentrations (Contox Blood Lead Control, Low Level - LBO 33R (5±3 μg dL^{-1}, Kaulson Laboratories, USA), Seronorm Trace Elements, Serum L 1 (2.9±0.4 μg L^{-1}, Sero AS, Norway), and Toxic Metals in Freeze-Dried Urine SRM 2670, Low Level (10 μg L^{-1}, NIST, USA), respectively). All were reconstituted following the instructions of manufacturers and further diluted as the different matrices.

The activity of the ALAD enzyme was determined in fresh blood with a time of less than 4 hours between collection and analysis since it loses stability after refrigeration or freezing. ALAD determination followed the laboratory protocol, which combines two previously established methodologies [13, 14], while calculation of the enzyme activity followed the European method [15].

Blood samples from three healthy individuals were collected, and the respective ALAD activities were determined based on the amount of porphobilinogen produced, using dithiothreitol (DTT) to activate the enzyme or not. Mean ALAD activity for the three samples in the non-activation state constituted the control sample for enzymes inactivated by DTT. In contrast, the mean activity of enzymes activated became the control sample for those activated by DTT.

Subsequently, ALAD activity was determined in each one of the specimens based on the DTT-inactivated and -activated states. The activity calculation followed the European method applied in an Indian study [16]. Enzymatic activity values from 21 U L^{-1} or higher were assumed as a reference for non-inhibition. Decreases in activity were calculated considering controls with 100% enzymatic activity. Results with a reduction greater than 40% in the non-activated enzyme by DTT (inactivated) were considered "inhibition" concerning the control group.

DNA extraction used the Genomic DNA Extraction kit (Real Biotech Corporation; Taipei, Taiwan). The probes and primers, Taqman® SNP Genotyping Assays, code c__11495146_10 (Applied Biosystems; CA, USA), and mix, TaqMan® Genotyping Master Mix (Applied Biosystems), were used in genotyping the polymorphism.

Genomic DNA was extracted from blood using a DNA column extraction kit (Real Biotech Corporation). The probe and specific primer (Taqman® SNP Genotyping Assays, code c__11495146_10), together with the TaqMan® Genotyping Master Mix, all supplied by Applied Biosystems, allowed the genotyping. Forward (5' TGTAAAACGACGGCCAGT) and reverse primers (5'CAGGAAACAGCTATGAC), and probes (VIC/FAM) AGGGCCTCAGCATCTC TTCCAGCCG[C/G]TTCACACCATACCTGTGT GGGTGTG were used in the determination of the polymorphism SNP rs1800435.

A 7500 Real-Time System (Applied Biosystems) performed PCR reactions, and the thermocycling conditions were one cycle at 95 ºC for 10 minutes (Taq activation), followed by 40 cycles at 92 ºC for 15 seconds (denaturation), annealing at 60 ºC for 1 minute, and subsequent detection of alleles at 60 °C for 1 minute. The reaction solution contained 0.4 µL of probe and primers Taqman® SNP Genotyping Assays, 10 µL of TaqMan® Genotyping Master Mix, and 1 µL of genomic DNA, reaching a final volume of 20 µL with 8.6 µL of water.

All statistical analyses were performed using SPSS 24® (SPSS Inc., Chicago, USA). The normality of the distribution of BPb, PPb, UPb, and ALAD activity was verified with the Shapiro-Wilk test, appropriate for small sample sizes, with log transformation of those without normal distribution. Pearson correlation was applied to the variables BPb and PPb, BPb and UPb, BPb and ALAD activity, PPb and UPb, PPb and ALAD activity, and ALAD activity and UPb. Meanwhile, the Spearman correlation was used when at least one of the variables did not follow the normality to evaluate the associations between age, smoking, time living in the condominium, time living in the city of Volta Redonda, and contact with soil, with levels of BPb, PPb, UPb, and ALAD activity. Hardy-Weiberg equilibrium was verified with the chi-square and G-adherence tests, applied to the observed and expected genotypical frequencies, referring to the polymorphism tested.

The study was approved by the Research Ethics Committee belonging to the institution of the authors and met the requirements of the Brazilian Resolution 466/2012. All participants signed a free and informed consent form.

3. RESULTS

Table 1 shows the profile of the study population. The twenty-seven individuals were mainly women (59.3%), white (88.9%), 40 to 50 years of age (29.7%), married (63.0%), completed high school (55.6%), and had a low socioeconomic

Table 1. Profile of the study population.

Variables	n	%
Sex		
Male	11	40.7
Female	16	**59.3**
Color		
Black	3	11.1
White	24	**88.9**
Age		
18-39	10	37.0
40-50	8	**29.7**
51-72	9	33.3
Marital Status		
Single	5	18.5
Married	17	**63.0**
Widow/Divorced	5	18.5
Educational Level		
University education	3	11.1
High school	15	**55.6**
Elementary/Middle school	9	33.3
Social class by minimum wage (MW) range		
≤ 2 MW	16	**59.3**
2< MW≤ 4	5	18.5
4<MW≤ 10	6	
Smoking		
Smoker	1	3.7
Non-smoker	26	**96.3**
Former smoker	7	25.9
Duration of Smoking (years)		
1-10	4	**14.8**
≥11	3	11.1
Alcoholic Beverage Intake		
Yes	12	44.4
No	16	**59.3**
Drinking Frequency		
1 to 4 days a week	10	**37.1**
Every 15 days	2	7.4
Diseases self-reported by the study population (%)		
Miscarriage (7.4); Allergy (18.5); Anemia (14.8); Anxiety (3.7); Heart Arrhythmia (3.7); Arthrosis in the Spine (3.7); Asthma (3.7); Bronchits (7.4); Cancer (skin) (3.7); Depression (7.4); Kidney Disease (7.4); Diabetes (7.4); Decreased Libido (3.7); Sleep Disorder (7.4); Headache (7.4); Developmental Disorders (autism) (3.7); Arterial Hypertension (7.4); Hypothyroidism (14.8); Emotional Instability (11,1) Fetal Malformation (3.7); Myalgia (18.5); Early Neonatal Death (3.7); Pneumonia (11.1); Psoriasis (3.7); Carpal Tunnel Syndrome (3.7); Sinusitis (22.2); Mixed Connective Tissue Disease (3.7), among others.		

status (59.3%). Most residents have lived in the condominium for 16 to 20 years (51.9%).

Regarding social habits, most of the participants were non-smokers (96.3%), and over half (59.3%) declared not drinking alcoholic beverages. However, among those who consumed, the highest frequency was in the range of 1 to 4 days (37.1%), and the amount ingested was up to 5 liters (18.5%), with beer being the drink of choice (29.6%).

The diseases self-reported by residents featured respiratory diseases (44.4%), psychological and neurological disorders, with 22.2% each, allergies (18.5%), myalgias (18.5%), anemia and hypothyroidism (14.8% each), cardiovascular disorders (11.1%), and disorders related to reproduction and fetal development (18.5%), among others.

All residences (100%) had yards covered with ceramic or cement, which were often cleaned (96.3%). Likewise, they all received running water, supplied by the water and sewage system of the city, using PVC piping. Among the population who admitted to having contact with the soil (63%), the main purpose was planting fruits and vegetables for their own and neighborhood consumption (58.8%). The time of exposure to the soil varied from 2 to 8 years (29.7%) (Table 2).

Based on the answers to the questionnaire, the sum of the years worked in activities related to metal exposure was considered for calculating the time of occupational exposure. Other non-industrial activities with potential exposure to

Table 2. Possible sources of lead exposure for the study population.

Sources	**n**	**%**
Contact with soil		
Yes	17	**63.0**
No	10	37.0
Purpose of Contact with Soil		
Recreation/Gardening	3	17.6
Planting for consumption	10	**58.8**
Construction	6	35.3
Activity with soil tillage	3	17.6
Rearing of animals for slaughter	1	5.9
Exposure to soil (years)		
Up to 1	1	5.9
2-8	9	**52.9**
10-14	3	17.6
No responses	4	23.5
Previous work activities with exposure to metals	12	44.4
Yes	15	**55.6**
No		
Exposure in previous occupations (years)		
Up to 10	4	**33.3**
From 11 to 20	3	25.0
From 21 to 40	4	33.3
No responses	2	16.7
Current work activities with exposure to metals		
Yes	7	25.9
No	20	**74.1**
Exposure in current occupation (years)		
Up to 10	2	28.6
From 11 to 20	3	**42.8**
No responses	1	14.3

metals were allowed, while time on the job in administrative functions was ruled out.

Most of the population reported not having a prior activity associated with metals (55.6%). Among those with previous occupations involving metal exposure, 33.3% had been exposed for up to 10 years, while another 33.3% had been exposed for 21 to 40 years. Currently, the majority do not perform activities related to metals (74.1%), but those who still do state an exposure time of 11 to 20 years (42.8%) (Table 2).

The activities related to metals reported by residents were welding of wagons, cutting of metal plates, storage of steel parts, industrial plumbing, coke oven, maintenance and production of steel, steel mill, and blast furnace, tinplate packer, painting, military police, operation of machines and overhead cranes in the steel industry.

Table 3 shows the means, medians, concentration range, and genotypical and allelic frequencies for the different biomarkers. The concentrations of lead in blood (BPb), plasma (PPb), and urine (UPb) ranged from 1.03-4.09 μg dL^{-1}, 0.56-11.69 μg L^{-1}, and 0.65-20.76 μg g^{-1} creatinine, respectively. Regarding ALAD activity, the minimum and maximum values were 10.20 and 39.70 UL^{-1}, with eleven individuals presenting inhibition of ALAD activity. The great majority of participants were carriers of ALAD 1-1 allele, the genotypical frequency of which was 92.6%, while 0.963% was the allelic frequency. Application of the chi-square test with correction for the small sample size, and G-test indicated that the sample was in Hardy-Weiberg equilibrium (p=0.84), suggesting that there were no genotyping errors or actions from evolutionary force.

Table 3. Descriptive statistics for the biomarkers and their correlations.

Biomarker	**BPb** (μg dL^{-1})	**PPb** (μg L^{-1})	**UPb** (μg g^{-1} creatinine)	**ALAD Activity** (U L^{-1})	
Mean	2.13 (0.80)	2.80 (2.61)	4.35 (4.41)	24.45 (8.28)	
Geometric Mean	1.99 (1.47)	2.06 (2.13)	3.00 (2.41)	23.14 (1.42)	
Median	2.20	1.98	3.16	22.60	
Min - Max	1.03 – 4.09	0.56 – 11.69	0.65 – 20.76	10.20 – 39.70	
Polymorphism rs1800435					
Biomarker	ALAD 1-1	ALAD 1-2	ALAD 2-2	Allele 1	Allele 2
Genotypic and allelic frequency n (%)	25 (92.6)	2 (7.4)	0 (0.0)	25(0.9630)	2 (0.0370)
Correlation (r)	**BPb** (μg dL^{-1})	**PPb** (μg L^{-1})	**UPb** (μg g^{-1} creatinine)	**ALAD Activity** (U L^{-1})	
BPb (μg dL^{-1})	-	-0.07 (0.6981)	-0.2178 (0.2751)	0.2144 (0.2829)	
PPb (μg L^{-1})	-0.07 (0.6981)	-	-0.1684 (0.4010)	0.3067 (0.1196)	
UPb (μg g^{-1} creatinine)	-0.2178 (0.2751)	-0.1684 (0.4010)	-	0.1137 (0.5724)	
ALAD Activity (U L^{-1})	0.2144 (0.2829)	0.3067 (0.1196)	0.1137 (0.5724)	-	
Age	0.5384 (0.0037)	0.0351 (0.8617)	0.0351 (0.8619)	0.1118 (0.5788)	
Smoking	0.1345 (0.7737)	-0.6545 (0.1106)	0.4505 (0.3104)	-0.2651 (0.5655)	
Residence in the Condominium	0.0850 (0.6732)	0.1635 (0.4252)	-0.0852 (0.5266)	-0.1274 (0.5266)	
Contact with soil	0.0739(0.7142)	0.0948(0.6382)	0.0049(0.9805)	0.1822 (0.3620)	

No significant correlations were found between the several biomarkers and variables (Table 3). Only the correlation between BPb and age presented statistical significance (r=0.5384; p=0.0037).

4. DISCUSSION

A particular population may respond differently to lead than most people exposed to the same level of metal in the environment. Some groups are more susceptible, such as children, especially those under six years of age, pregnant women (and the fetus), elderly, smokers, alcoholics, and persons with genetic diseases that affect heme synthesis, nutritional deficiencies, and neurological or renal dysfunction. Genetics, age, nutritional and health status, and exposure to other substances (e.g., cigarette smoke) can contribute to this difference, resulting in a reduction of contamination, excretion, or compromise to the function of organs affected by lead. Lead levels increase with age and decrease with the level of education [2].

In the current study, the BPb geometric mean (1.99 μg dL^{-1}) was higher than those measured in the American population by the National Health and Nutrition Examination Survey (NHANES) 2011-2012 (0.97 μg dL^{-1}, and 0.75 μg dL^{-1} in 2017-2018). Blood lead levels have decreased drastically in the U.S. population mainly due to the removal of the metal from gasoline, more rigorous control of occupational exposure, and reduction of environmental sources [17, 18]. However, studies conducted on residents of a city in southern Brazil [19], people living far from a lead refinery in Vale da Ribeira [20], general population living in the city of São Paulo [21], and a population in the Pantanal Matogrossense [22] showed mean levels for BPb within the range found in the current study. Thus, lead concentrations exceeded the levels in the U.S. population by more than twofold in Brazil and other emerging countries. Research conducted in Nigeria found BPb levels in the same range as Brazilian results [23].

Lead accumulated in the bones during occupational or environmental exposure is mobilized in the bone remodeling process, representing endogenous exposure to the metal. The half-life of lead in bone can reach decades, while its half-life in blood has been estimated as 30 days. Therefore, BPb is a good biomarker for recent exposure, while bone lead is the ideal biomarker for chronic exposure. However, its measurement requires expensive equipment, unavailable in most of the world [1, 4]. Thus, individuals living in areas with high exposure to lead do not always present excessive BPb levels, as observed in a study with adults in the city of Adudu, Nigeria, a region with lead-zinc mines, where an area of 56 km^2 around the city was covered with lead. Mean BPb was 3.1 μg dL^{-1} in residents, and only 14% of the samples measured above five μg dL^{-1} [23].

The lead concentration in blood is not the most suitable biomarker, although it is the most widely used one for exposure to the metal. BPb does not adequately reflect the lead levels in bones and plasma, which is the toxicologically active fraction and is influenced by the metal levels in the bones (since the levels in bone are in equilibrium with those in plasma). Plasma lead is the best indicator for chronic exposure to the metal, but its measurement is rarely used due to analytical difficulties [1, 2]. However, random contamination of blood during collection, pre-treatment, and analysis must be avoided through adequate cleaning of the collection site, use of appropriate vacutainer blood collection tubes for trace analysis, careful decontamination of glassware and plasticware, and attention to the contamination of samples at the time of preparation and subsequent reading [24]. Another major analytical difficulty is the use of atomic absorption spectrometry to determine the concentration of lead in blood plasma. In this case, current spectrometers use resources such as a graphite furnace with Zeeman-effect background correction and atomization in THGA graphite tubes with end cap and integrated platform, which allows measurements at very low detection limits. Furthermore, the arrival of high-resolution equipment with a continuous source, using xenon lamps with more intense absorption lines, diffraction gratings with greater resolution power, and CCD-type sensors enabled the achievement of greater sensitivity, thus further reducing the detection limits [25].

Lead mean concentration in the plasma of the study subjects was higher than in some research, such as the assessment of individuals in Ribeirão Preto, São Paulo State, without known environmental exposure to lead [26], lead-exposed men and people under normal environmental lead exposure [27],

and lead-exposed and unexposed Chinese workers [28].

It is not advisable to use UPb in place of BPb for individual assessment since urinary lead concentration is not related directly to its concentration in the blood. However, UPb may be a good alternative for a particular population. In the case of environmental exposure, the substitution should be done with caution and used as an estimate of the metal content in blood [12]. The mean urinary lead in the study population, 5.4 μg L^{-1}, which corresponds to 4.35 μg g^{-1} creatinine, was close to the level observed in one-hundred thirty individuals environmentally exposed in Rio de Janeiro [12]. However, such a result is much higher than that in eighty-three subjects with no occupational exposure to the metal in Sweden [26], two thousand Spanish volunteers environmentally exposed [29], and American population [30].

The determination of ALAD activity is one of the most useful biomarkers for assessing lead exposure since it is highly sensitive and specific for the metal. The presence of lead in the human body inhibits the activity of such an enzyme, and the extent of the inhibition is related directly to its blood level [31]. The mean ALAD activity in condominium residents was lower than those reported by other research with populations environmentally exposed to lead, such as in Alfenas, MG [32], Londrina, PR [33], Embu-Guaçu, SP, as well as residents surrounding a lead processing industry in the city of São Paulo, SP, with higher exposure [34]. A study carried out in India with rural and urban populations also found a more elevated activity [35].

The ALAD genotype has been associated with modification in the lead kinetics and the presence of variant alleles in blood since the enzyme participates in the heme biosynthesis and is inhibited by lead. The most common polymorphism is ALAD G177C (rs1800435), with two dominant alleles, ALAD 1 (177G) and ALAD 2 (177C), resulting in genotypes ALAD 1-1, heterozygous ALAD 1-2, and homozygous ALAD 2-2, extremely rare in the general population [36]. The control genotypical and allelic frequencies for the three genotypes were within the range found by previously published studies and in Hardy-Weinberg equilibrium.

The ALAD 1-1 genotype frequency obtained in the study subjects (92.6%) was lower than those found in some Brazilian [37], Chinese [38], Mexican, and African [39] populations. However, other research has found lower genotypic frequencies in individuals from countries such as Portugal [40], Spain [41], Nepal, and Sri Lanka [39]. A study with Brazilians found results similar and higher than those of participants among blacks and whites, respectively, for the ALAD 1-1 genotypic frequency [42].

Investigations on the genotype frequency of ALAD 1-2, like ALAD 1-1, also reported higher and lower results than those found in condominium residents. Populations from Portugal, Spain, Nepal, and Sri Lanka showed lower frequencies [39-41], while individuals from Brazil and China exhibited higher values [37, 38]. Unlike the ALAD 1-1 frequency, the results found in both whites and blacks were higher than the study population for ALAD 1-2 in the Brazilian research [42].

As observed in condominium residents, studies with indigenous peoples from Mexico [39] and populations from Portugal [40, 41], Spain [41], and South Africa [39] also reported the absence of the ALAD 2-2 genotype. However, this genotype was found in other research, such as in Kathmandu (0.56%) and Katyang (1.89%) in Nepal [39], and Sri Lanka (5.56%) [39].

According to the World Health Organization, there is no safe level for exposure to lead. Since 2015, the Centers for Disease Control and Prevention (CDC) have adopted 5 and 2 μg dL^{-1} as thresholds for blood lead in adults and pregnant women, respectively [43]. Although the condominium residents have presented BPb below 5 μg dL^{-1}, among eleven childbearing-age women (18-49 years), three showed blood lead greater than 2 μg dL^{-1}, seven revealed inhibition of ALAD, and one had a history of miscarriage besides a son diagnosed with autism. Exposure to lead is related to a higher incidence of miscarriage and stillbirth, even at blood lead levels previously considered relatively low (≈ 5 μg dL^{-1}) [44]. Lead has already been associated with miscarriage and autism in other studies [36, 44].

Until recently, BPb levels below 10 μg dL^{-1} were considered safe. However, current studies have

identified a reduction in renal function at concentrations less than 5 μg dL^{-1}, as well as a higher risk of hypertension and essential tremor with concentrations below 10 μg dL^{-1}. Low-dose lead exposure has already proven an association with adverse cardiovascular and renal events, cognitive dysfunctions, and adverse pregnancy outcomes [4, 22].

According to official regulatory bodies, there are no reference values for lead concentration in blood plasma. However, research showed plasma lead levels less than 1.0 μg L^{-1} [26-28] in the general population. The participants of the study presenting PPb between 5.0 and 10 μg L^{-1} reported a history of miscarriage, fetal malformation, anemia, respiratory and neurological disorders, cardiac arrhythmia, and hypertension, which may be associated with systemic effects of the metal [2]. Since 63% of the population was over 40 years old, the greater bone remodeling at this stage may have contributed to the lead mobilization from bone to plasma [1].

Regarding urine, there are also no reference values established by regulatory bodies for urinary lead concentration. However, individual evaluation of urine lead levels revealed four women, homemakers, who reported having contact with the soil and living in the condominium over 7 years and in homes on neighboring streets, had higher UPb, from 9.44 to 20.76 μg g^{-1} creatinine, compared to the other residents. They also had histories of grade-1 obesity, hypothyroidism, early neonatal death, myalgias, anemia, depression, skeletal, respiratory, and neurological disorders, hypertension, and elevated PPb, effects like the toxic action of lead [2, 4]. A study performed with 5,558 Chinese from sixteen different localities observed an association between blood lead levels and body mass index (BMI) in women ($p < 0.001$), whose mean BPb was 3.8 μg dL^{-1}, but not in men [45].

Likewise, ALAD activity does not have thresholds defined by regulatory agencies. Inhibition of ALAD already occurs at BPb equal to 5 μg dL^{-1} [46]. In this research, eleven individuals presented the enzyme inactivation, and interaction may have occurred between lead and disease states such as diabetes and hypothyroidism, as well as with social habits such as smoking, alcohol consumption, drug use, and the presence of polymorphic alleles. The impact of those factors on ALAD activity may be more intense than that caused by low lead levels. Therefore, the relationship may not be linear between low-to-moderate lead levels and ALAD activity [47]. However, the inactivated ALAD state may already represent early toxic effects of lead on the hematopoietic system [1].

Carriers of the ALAD 2 (1-2 or 2-2) allele have higher blood lead levels but little bioavailability of the metal. Thus, its distribution to nerve tissues is lower, resulting in less neurotoxic effects [46]. Individuals with the polymorphic allele may present a stronger binding between lead and red blood cells when compared to carriers of the ALAD 1 allele. More severe renal effects have been observed in carriers of the ALAD 2 allele. Conversely, studies have shown that carriers of the ALAD 1 allele present higher concentrations of bioavailable lead and more severe neurotoxic effects [1, 46]. One participant with the ALAD 1-2 genotype presented kidney stones and renal dysfunction, similar to other findings observing the relationship between the polymorphic allele and lower susceptibility to neurotoxic damage but exhibiting more severe renal effects [2].

5. CONCLUSION

The findings using lead biomarkers suggested that the study subjects presented environmental exposure to the metal. Thus, further research will need to perform a health risk assessment, which includes children, childbearing-age women, and pregnant women considered more vulnerable to adverse effects of lead. The synergistic action of lead with other chemicals previously detected in the soil of the condominium should be also examined by future studies. The lack of a significant correlation between lead biomarkers and variables, as well as a nonlinear behavior between them, may be related to the small sample size, which is the main limitation of the study.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no interference in the design, development, results, and conclusion of the project.

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