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Lead levels in teeth of inhabitants near a river contaminated with heavy metals: pilot study

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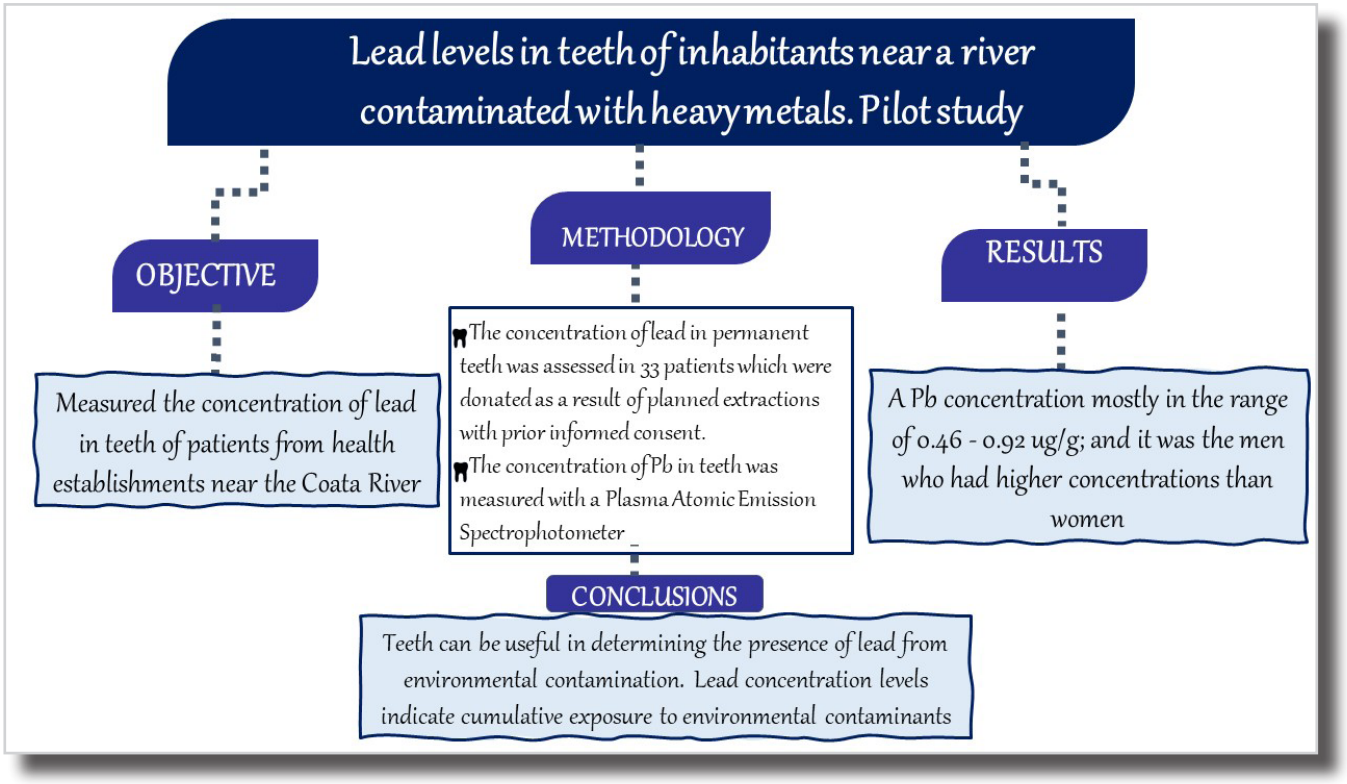
Graphical Abstract



Abstract

The objective of this study was measured the concentration of lead in teeth of patients from health establishments near the Coata River in Puno, Peru. This descriptive design study was approved by the Institutional Research Ethics Committee of the Universidad Nacional del Altiplano. The concentration of lead (Pb) in permanent teeth was assessed in 33 patients of both sexes which were donated as a result of planned extractions in the dental offices of the health establishments near the Coata River with prior informed consent. The concentration of Pb in teeth was measured with a Plasma Atomic Emission Spectrophotometer in the environmental monitoring and evaluation laboratory of the Faculty of Mining Engineering of the National University of the Altiplano Puno. The age range in Coata was 36 to 65 years and in Huata 35 to 63 years.The average age of the sample in the Coata district was 47.5 years and in Huata it was 47.6 years. The people living near the Coata River had a Pb concentration mostly in the range of 0.46 - 0.92 ug/g; and it was the men who had higher concentrations than women, without this difference being significant. It is concluded that teeth can be useful to determine Pb concentration due to chronic environmental exposure.

Keywords: Teeth. Lead. Environmental Contamination.

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INTRODUCTION

Environmental contamination by heavy metals is a problem of great concern worldwide due to their entry into the body through routes such as ingestion, inhalation or dermal contact, which causes several adverse effects, such as neurotoxicity and cancer[1,2]. Although the main mechanisms of toxicity are known, there are still gaps in knowledge about the accumulation patterns and the most sensitive and specific biomarkers to detect their exposure and toxicity[3–5].

Lead (Pb) is a heavy metal with high toxicity and extensive historical use in industrial and domestic applications, which has made it a significant environmental pollutant. Even in small quantities, it can accumulate in the human body, especially in children, causing neurological and cognitive development problems[1,6,7]. The main sources of lead exposure vary between countries, including electronic waste, paints, ceramics, cosmetics and contaminated water[8]. In Peru, activities such as informal mining have caused serious problems of contamination of water bodies with heavy metals, including lead, affecting the health of riverside communities[9,10].

The Coata community in the Puno department of Peru has been facing significant contamination of its water sources for several years due to toxic waste from mining activities. These wastes include lead, mercury and other heavy metals, which pose serious risks to human health and the environment[11]. Cumulative exposure to lead can be assessed using bioindicators such as teeth, which offer advantages in being easily accessible and reflecting long-term chronic exposures, unlike other tissues such as blood, which only indicate recent exposure[12–15].

In this context, the present study aimed to assess lead exposure in inhabitants of the Coata community using teeth as biomarkers. The findings seek to provide useful information to characterize environmental contamination in the region and develop interventions aimed at protecting vulnerable populations during critical development windows.

METHODOLOGY

This was a non-experimental study with a descriptive scope. The sample consisted of 33 permanent teeth, obtained as a result of planned extractions in the dental offices of the Huata and Coata health centers, and donated with the informed consent of patients who had lived more than 5 years in areas near the Coata River. The sample size was based on some background from a literature review[16]. Likewise, because in the time it took us to collect, only 33 teeth met the inclusion criteria. Nowadays people prefer more conservative treatments.

The age range in Coata was 36 to 65 years and in Huata 35 to 63 years. The average age of the sample in the Coata district was 47.5 years and in Huata it was 47.6 years.

The study was approved by the Institutional Research Ethics Committee of the National University of the Altiplano with certificate No. 018-2023/CIEI UNA-Puno.

The evaluation of lead concentration in the teeth was carried out in the environmental monitoring and evaluation laboratory of the Faculty of Mining Engineering of the Universidad Nacional del Altiplano Puno (Peru).

Sample cleaning: The teeth after extraction were washed with distilled water and dried in an oven at low temperature.

Drying and pulverization: After being completely dried they were pulverized until a fine powder was obtained to facilitate greater homogeneity in the sample and better extraction of lead.

Acid digestion of the sample:

• **Reagents**: 5 ml of concentrated nitric acid (HNO_3) and 2 ml of perchloric acid ($HClO_4$) and a of 3:1 mixture of hydrochloric acid and nitric acid (aqua regia) were used. It is an acidic, corrosive and oxidative mixture.

• **Digestion process**: A specific amount of the tooth powder was weighed (usually between 0.1 and 0.5 grams) and then transferred to a suitable digestion vessel. An appropriate amount of nitric acid and, optionally, a small amount of perchloric acid was added. Nitric Acid digestion is suitable for Lead extraction. Nitrate provides a good matrix for spectrophotometric determinations. Perchloric Acid is added to achieve complete digestion. Both disintegrate the samples and preserve the metal for analysis.

The sample was heated at a controlled temperature (usually around 90-120°C) for 5 min until completely dissolved. The objective was to obtain a clear, particle-free solution.

• **Sample Dilution:** After digestion, the digested sample was cooled and diluted with deionized wa-

ter to a volume suitable for the analytical technique to be used; it was diluted to a final volume of 50 ml.

• **Lead analysis:**

It was performed with an Agilent Microwave Plasma Atomic Emission Spectrometers 4210 MP: This method is more sensitive and accurate. The sample is introduced in a Nitrogen plasma, where the lead present in the sample emits light of a specific wavelength. The amount of light emmited is proportional to the concentration of lead. The wavelength for Pb analysis was in a sensitivity range of 405.781 nm

Statistical analysis

IBM SPSS version 27 and Microsoft Excel software were used for data analysis. A 95% confidence level was applied using the Chi-square test because we have independent nominal data. This test offers us a general test of the existence of differences between the categories that group the data of the dependent variable[17]. The variables evaluated were qualitative, such as: sex and origin (the place where the patient comes from). In addition, descriptive statistics were used to calculate percentages through frequency distribution, as well as measures of central tendency such as the mean. Dispersion measures such as range, standard deviation and variance were also calculated.

RESULTS

The majority of the teeth of the people evaluated were from Coata (51.5%). In relation to sex, more women were evaluated (54.5%). And most of the people (51.5%) had a higher Pb contamination of 0.46 - 0.92 (ug/g). The sociodemographic data of the sample studied are described in Table 1.

Table 1 - Sociodemographic characteristics of the sample according to origin and sex of the patients of the health facilities near the Coata and Huata rivers in the province of Puno, Peru 2024.

Variables		N	%
Origin	Coata	17	51.5
	Huata	16	48.5
Gender	Male	15	45.5
	Female	18	54.5
Lead	0.03 - 045	16	48.5
	0.46 - 0.92	17	51.5
Total		**33**	**100**

Source: Data collection form generated by the authors.

The results of Table 2 for the concentration of Pb [ug/g] in the health center establishment near the Coata River with a sample of 17 patients has a Range: 0.69 [µg/g], which indicates a moderate variation in the levels of Pb. The values of Minimum: 0.18 [ug/g] and Maximum: 0.87 [ug/g]. On the other hand, the Mean: 0.4076 [ug/g] micrograms of Pb, which indicates the average concentration of Pb in the sample, which means that, on average, each gram of the sample contains 0.4076 micrograms of Pb, this is the average of Pb in the tooth samples analyzed. Regarding the Standard Deviation (0.19392) : It indicates a moderate dispersion around the mean. And the Variance (0.038) indicates that the low variance suggests that the Pb levels in the samples are quite uniform, which could indicate a common source of exposure or a homogeneous environment. Related to the standard deviation, it indicates the extent of data dispersion.

Whereas, the concentration of Pb [ug/g] in Huata health center with a sample of 16 patients, which has a Range: 0.89 µg/g, higher than the first group, suggesting greater variability in lead levels. Likewise, the Minimum: 0.03 [ug/g], indicating low exposure in some samples. And the Maximum: 0.92 [ug/g], this being the highest value observed in the samples. The Mean: 0.5269 [ug/g], a higher mean than the previous group, which could reflect a higher average exposure. Regarding the Standard Deviation (0.22443): Higher than that of group 1, indicating

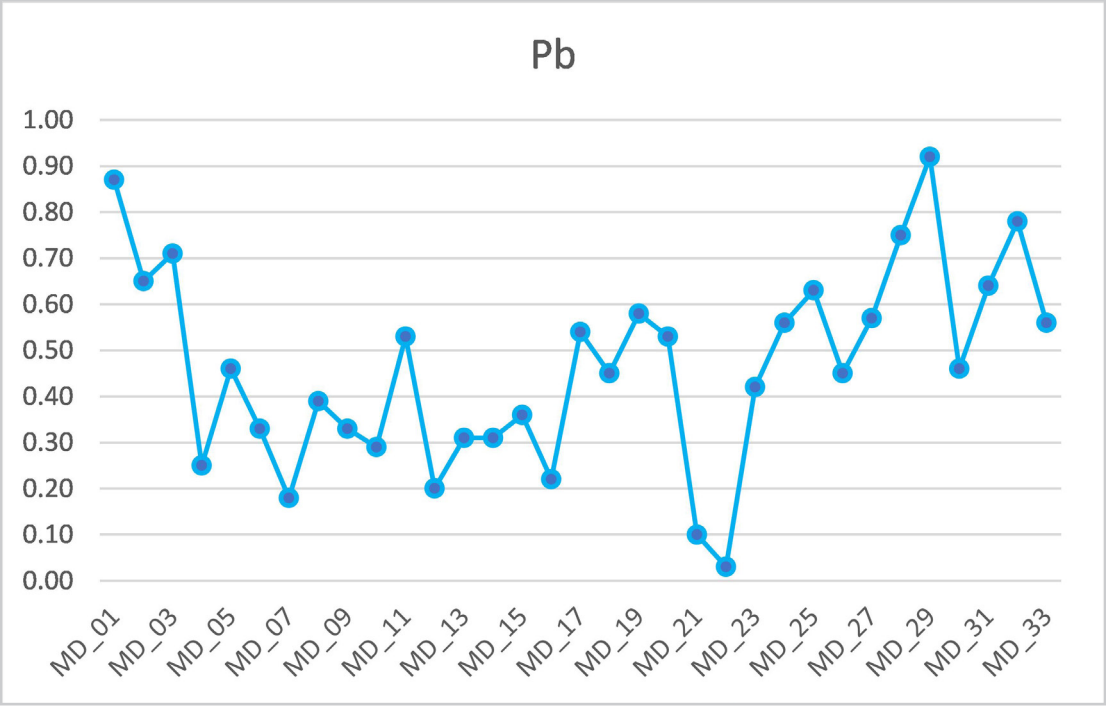
a greater dispersion of values in this group. Finally, the Variance (0.050): Higher variance means that lead levels are more dispersed compared to the first group. However, it is still low, indicating that, in general, Pb levels are relatively consistent among patients, despite the relatively wide range.

Table 2 - Descriptive statistics of lead concentration in teeth of patients in health facilities near the Coata and Huata rivers in the province of Puno, Peru 2024.

	N	Range	Minimum	Maximun	Mean	Standart deviation	Variance
Lead Concentration (Pb) [ug/g] Coata	17	.69	.18	.87	.4076	.19392	.038
Lead Concentration (Pb) [ug/g] Huata	16	.89	.03	.92	.5269	.22443	.050

Source: Data collection form generated by the authors.

The results presented in Figure 1 for Pb concentration in patients, expressed in µg/g (micrograms per gram), show significant variations among the different individuals (identified as MD_01 to MD_33). The following results show the highest concentrations in patients with the highest lead concentrations are MD_29 (0.92 µg/g), MD_01 (0.87 µg/g), MD_32 (0.78), MD_28 (0.75 µg/g), MD_03 (0.71 µg/g) and MD_02 (0.65 µg/g). These patients could have a higher Pb exposure. While moderate concentrations have a significant group of patients presenting Pb concentrations between 0.4 and 0.6 µg/g, which could be considered a moderate level of exposure. Likewise, with low concentrations, some patients, such as MD_22 (0.03 µg/g) and MD_21 (with a concentration below 0.10 µg/g), have very low levels, suggesting minimal exposure to Pb.



Source: Data collection form generated by the authors

Figure 1 - Lead concentrations [ug/g] in teeth as a bioindicator of environmental exposure to lead in patients in health facilities near the Coata River in the province of Puno, Peru 2024.

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The inhabitants of Coata mostly had lower Pb concentrations of 0.03 - 0.45 ug/g (64%); while 35.3% of the people had higher Pb concentrations of 0.46 - 0.92 ug/g.

In Huata, the villagers mostly had higher Pb concentrations of 0.46 - 0.92 ug/g (68.8%); and only 31% had lower Pb concentrations of 0.03 - 0.45 ug/g. This means that Huata villagers may be more exposed to Pb contamination. However, when relating lead concentrations in teeth to origin, no statistically significant differences were found (p = 0.055).

In relation to sex, 40% of the male sex had low concentrations of Pb, while 60% had high concentrations. The female sex had 55.6% low Pb concentrations and 44.4% high concentrations. This indicates that women have a more balanced distribution between low and high lead levels. Regarding the value of p = 0.373 suggests that there is no statistically significant difference between males and females in terms of Pb levels in teeth. Therefore, sex is not a determining factor in Pb exposure (Table 3).

Table 3 - Analysis with the chi-square test in relation to sex and origin in teeth as a bioindicator of environmental exposure in patients of health facilities near the Coata and Huata rivers in the province of Puno, Peru 2024.

Variables		Lead (ug/g)		P
		0.03 - 045, N (%)	0.46 - 0.92, N (%)	
Origin	Coata	11 (64.7)	6 (35.3)	0.055
	Huata	5 (31.2)	11 (68.8)	
Gender	Male	6 (40)	9 (60)	0.373
	Female	10 (55.6)	8 (44.4)	
Total		**16 (48.5)**	**17 (51.5)**	

Chi-square test.
Source: Data collection form generated by the authors.

DISCUSSION

Dental tissue has long been considered a reliable reservoir and have been used to assess lead exposures among populations[18]. Several studies have concluded that teeth are better indicators of long-term lead exposure than blood, hair, and nails[19,20]. It has been suggested that the presence of lead in human teeth is present in small amounts[21] however, the WHO states that there is no level of lead exposure that does not have harmful effects[22].

The results of this study showed that the evaluated teeth of the people living near the Coata River presented a lead concentration in a range of 0.03 - 0.92 (µg/g), similar to those shown in other studies such as in Mitrovica (22.3 µg/g), Klina (3.1 µg/g) and Graz (1.6 µg/g)[23,24].

Lead accumulated in teeth in the long term is related to the environment where people live such as industrial areas, smelters, refineries, flotation, battery factories, contamination of mining tailings among others[16]; and after chronic exposure, lead accumulates in the human body, especially in bones and teeth, with very serious effects on the nervous system, reproduction, fertility, as well as genotoxicity and carcinogenicity[25].

The Coata River is contaminated by toxic wastes and discharges from nearby mining operations; water contamination includes mercury, cyanide, arsenic, and lead[11].

According to the technical report (No. 172 - 2015 - ANA -AAA.TIT-SDGCRH) of the National Water Authority (ANA), lead, among others, exceeds environmental quality standards in the Coata River, posing a health risk to residents living along the river, as they are contaminated with mining wastewater and sewage. Likewise, the National Center for Occupational Health and Environmental Protection of the National Institute of Health has evidenced the exposure of people to toxic metals by performing blood and urine tests and, as a result of toxic metal concentrations exceeding the Maximum Permissible Limits in water wells for human consumption, a State of Emergency was declared for imminent danger, due to contamination of water for human consumption, in the districts of Coata, Huata, Capachica, Caracoto and Juliaca since 2019[26]. In March 2024, the Civil Court of Juliaca ratified the so-

called historic ruling, holding state institutions in Puno responsible for the unhealthy conditions in which the inhabitants living near the Coata River reside[27].

The results of this study in relation to the concentration of lead in teeth according to sex showed a higher concentration in the male group in a range of 0.46 – 0.92 µg/g; although this difference was not significant, similar to those reported in studies in inhabitants of Kuwait, Spain and Poland, whose mean lead concentration was higher in men with values of 6.8 ± 4.7 µg/g, 8.45 ± 0 .98 µg/g and 0.63 µg/g, respectively.

The results of the study show the usefulness of teeth as indicators of prolonged exposure to environmental substances[24,28,29].

The limitations of this study lie in the use of a reduced sample, and having included only permanent teeth, deciduous teeth may be more useful in determining environmental lead exposure and accumulated exposure of their mothers[24]; as well as there may be differences in lead concentration according to tooth morphology[29]. Although the concentration of lead in permanent teeth of people living near the Coata River was not very high, it is important to continue research in this area in order to mitigate the health risks of environmental contamination.

CONCLUSION

This research has shown that teeth are useful in determining the presence of lead non-invasively. Concentration levels indicate cumulative exposure to environmental contaminants. People living near the Coata River had a Pb concentration mostly in the range of 0.46 - 0.92 ug/g; and it was men who had higher concentrations than women without this difference being significant. Based on these conclusions, it is suggested to implement public health policies that monitor the residents affected by the contamination of mining relatives, and it is suggested to carry out studies that analyze the complications of their health in general.

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CREdiT author statement

Conceptualization: Padilla Cáceres, TC. Methodology: Padilla Cáceres, TC; Caballero Apaza, L. Validation: Padilla Cáceres, TC; Caballero Apaza, L; Casa Coila, MD; Mamani Cori, V. Statistical analysis: Casa Coila, MD; Arbildo Vega, HI. Formal analysis: Padilla Cáceres, TC, Arbildo Vega, HI. Investigation: Padilla Cáceres, TC; Caballero Apaza, L; Casa Coila, MD; Mamani Cori, V. Resources: Padilla Cáceres, TC; Caballero Apaza, L; Casa Coila, MD. Writing-original draft preparation: Padilla Cáceres, TC; Caballero Apaza, L; Casa Coila, MD. Writing-review and editing: Padilla Cáceres, TC; Caballero Apaza, L; Casa Coila, MD, Arbildo Vega, HI. Supervision: Padilla Cáceres, TC. Project administration: Padilla Cáceres, TC.

All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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