Research Article

Association of Drinking Water Heavy Metal Exposure with Salivary Inflammatory Biomarkers and Oral Health Status among Residents of Tirunelveli and Kanyakumari Districts, Tamil Nadu

Dr Ankit Vasudeva[1], Dr Shobhit Tayal[2], Dr. Ashok Kumar[3], Dr. Shivani Bansal[4]

[1]Department of Physiology, Rajas Dental College and Hospital, Kavalkinaru Junction, Tirunelveli, Tamil Nadu, 627105, India. Email: dr.arathi@rajasdentalcollege.edu.in
[2]Undergraduate student, Rajas Dental College and Hospital, Kavalkinaru Junction, Tirunelveli, Tamil Nadu, 627105, India. Email: sanaafsanaa@gmail.com
[3]Undergraduate student, Rajas Dental College and Hospital, Kavalkinaru Junction, Tirunelveli, Tamil Nadu, 627105, India.
[4]Undergraduate student, Rajas Dental College and Hospital, Kavalkinaru Junction, Tirunelveli, Tamil Nadu, 627105, India.
[5]Undergraduate student, Rajas Dental College and Hospital, Kavalkinaru Junction, Tirunelveli, Tamil Nadu, 627105, India.
[6].Department of Orthodontics and Dentofacial Orthopaedics, Rajas Dental College and Hospital, Kavalkinaru Junction, Tirunelveli, Tamil Nadu, 627105, India. Email id: dr.shahinajueith@rajasdentalcollege.edu.in

Abstract

Heavy metal contamination of drinking water is a growing public health concern. Saliva may serve as a non-invasive biomarker of exposure, but regional evidence remains limited. This study assessed the association between drinking-water heavy metal exposure, salivary inflammatory biomarkers, and oral health status among residents of Tirunelveli and Kanyakumari districts, Tamil Nadu. A community-based comparative study assessed adults exposed to elevated-lead drinking water and controls. Drinking water and saliva were analysed for lead and iron by UV–visible spectrophotometry, while salivary total protein and nitric oxide were assessed as inflammatory biomarkers and oral health was evaluated using Plaque and Gingival Indices. Lead was detected in drinking water and corresponding saliva samples, with water lead exceeding the WHO/BIS guideline by approximately 381-fold. Salivary lead was significantly lower than paired water lead ($p = 0.021$), while salivary protein correlated significantly with nitrate ($\rho = 0.638$, $p = 0.019$). Higher-exposure localities showed elevated salivary biomarkers and poorer oral-health measures ($r = 0.76–0.89$). These findings suggest a potential association between drinking-water lead exposure, salivary biomarkers, and oral health, supporting further evaluation of saliva as a non-invasive exposure-monitoring tool.

Keywords: Heavy metals; Drinking water; Salivary biomarkers; Oral health; Lead exposure

1. Introduction

Heavy metal contamination of drinking water is an increasing public health concern worldwide [1]. Chronic exposure to heavy metals may affect multiple biological systems and contribute to adverse systemic and oral health outcomes [2]. As the oral cavity is continuously exposed to ingested water, it may represent an early site of environmental metal exposure. Saliva, as an easily accessible and non-invasive biofluid, may therefore provide a practical matrix for assessing metal exposure and associated inflammatory changes [2,3].

Groundwater quality in the Tirunelveli and Kanyakumari districts of Tamil Nadu may be influenced by geological and anthropogenic factors, including quarrying, potentially increasing the risk of heavy-metal contamination [4].. However, regional evidence linking drinking-water metal exposure with salivary biomarkers and oral health remains limited. Concurrent assessment of environmental exposure, salivary responses, and oral health may therefore provide a broader understanding of the potential health implications of heavy-metal contamination in these communities.

The present study aimed to assess the association between heavy-metal exposure through drinking water, salivary inflammatory biomarkers, and oral health status among residents of the Tirunelveli and Kanyakumari districts of Tamil Nadu. Specifically, the study aimed to (i) detect and quantify selected heavy metals in drinking water and saliva, (ii) evaluate their association with salivary inflammatory biomarkers between exposed and control groups, and (iii) assess differences in oral health status in relation to heavy-metal exposure.

Methodology

A community-based comparative cross-sectional study was conducted in the Tirunelveli and Kanyakumari districts of Tamil Nadu, India, following approval from the Institutional Ethics Committee of Rajas Dental College & Hospital.

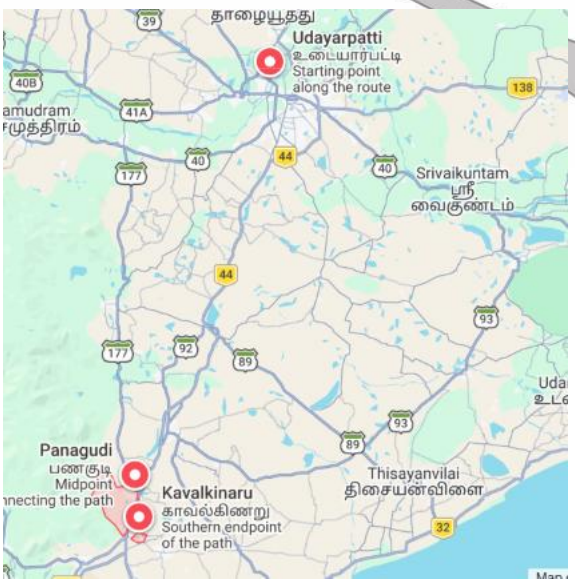

Fig. 1. Sampling sites: Kavalkinaru around 8.276°N, 77.581°E, Panagudi around 8.323°N, 77.579°E, and Udayarpatti around 8.741°N, 77.711°E.

Thirty eligible adults were recruited after written informed consent. Household drinking-water samples were initially screened for selected heavy metals, including lead and iron. Lead was identified as the predominant metal and, based on the applicable permissible limit, participants were classified into elevated-lead (n=15) and control (n=15) groups. Water [5] and unstimulated whole-saliva [6] samples were collected under standardized conditions. Lead [7], and iron [8], concentrations were determined by UV–visible spectrophotometry while salivary total protein and nitric oxide were measured using the Bradford [8] and Griess [9] methods, respectively. Oral health was assessed using the Silness–Loe Plaque and Gingival Indices, with DMFT and CPI recorded in selected localities [10]. Data were analysed using appropriate parametric or non-parametric tests based on distribution, with Pearson/Spearman correlation used to assess associations among exposure, salivary biomarkers, and oral health parameters. Analyses were performed using Python (SciPy/NumPy), with $p<0.05$ considered statistically significant.

Results And Discussion

Quantification was performed using linear calibration curves based on absorbance at 510 nm, with six replicates per standard. Both analytes showed good linearity over 0.5–3.0 mg/L, with R^2 values of 0.9918 for lead and 0.9969 for iron (Figures 2A and 2B).

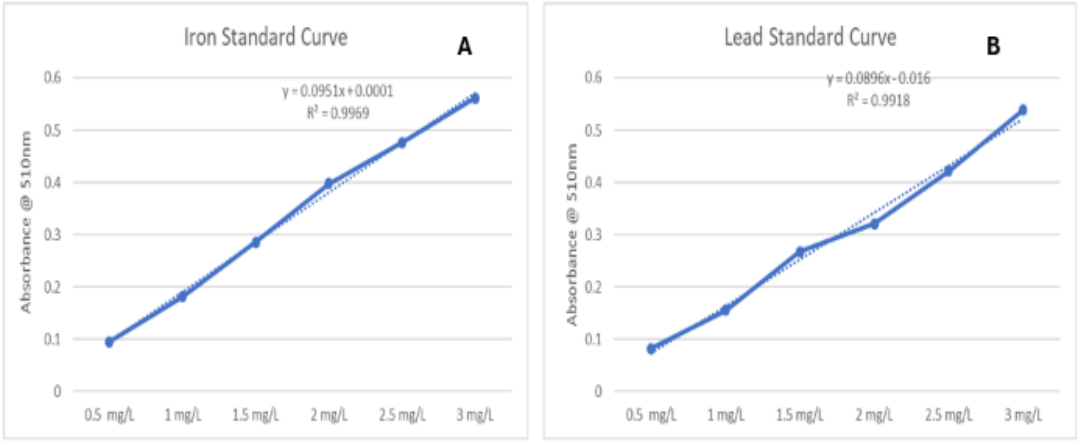

Figure 2. Calibration curves determined by UV–visible spectrophotometry at 510 nm. (A) Iron (n = 6 replicates; y = 0.0951x + 0.0001; R^2 = 0.9969). (B) Lead (n = 6 replicates; y = 0.0896x – 0.016; R^2 = 0.9918).

The concentrations of the analysed salivary and drinking-water analytes showed varying degrees of variability. Lead and iron were detected in 13 of 30 drinking-water samples and in the corresponding saliva samples. Salivary total protein was 75.66 ± 53.10 µg/mL and followed a normal distribution (p = 0.250). Salivary iron (0.255 ± 0.238 mg/L), lead (3.189 ± 0.698 mg/L), and nitrate (18.16 ± 5.68 µM) showed non-normal distributions (p < 0.01). Drinking-water iron (0.353 ± 0.237 mg/L) and lead (3.812 ± 0.143 mg/L) were normally distributed (p = 0.120 and 0.994, respectively) (Table .1). Salivary iron showed the highest relative variability (CV = 93.6%), whereas drinking-water lead showed the lowest (CV = 3.8%).

Table 1. Descriptive statistics and normality assessment of drinking-water and salivary analytes.

Analyte (unit)	Mean ± SD	CV %	Median (IQR)	Range	Shapiro–Wilk
Salivary total protein (µg/mL)	75.66± 53.10	70.2	66.05(35.33105.48)	13.82 – 197.64	p = 0.250 (normal)
Salivary iron (mg/L)	0.255± 0.238	93.6	0.136 (0.094–0.293)	0.051 – 0.851	p = 0.006 (non-normal)
Drinking-water iron (mg/L)	0.353± 0.237	67.2	0.356 (0.167–0.430)	0.094 – 0.893	p = 0.120 (normal)
Salivary lead (mg/L)	3.189± 0.698	21.9	3.114 (2.768–3.370)	2.455 – 5.212	p = 0.005 (non-normal)
Drinking-water lead (mg/L)	3.812± 0.143	3.8	3.817 (3.728–3.895)	3.571 – 4.051	p = 0.994 (normal)
Salivary nitrate (µM)	18.16 ± 5.68	31.3	17.70 (12.39–23.85)	11.80 – 24.04	p = 0.002 (non-normal)

Table 2, lead concentrations exceeded the WHO/BIS guideline value in all 13 drinking-water samples, with a mean concentration of 3.81 ± 0.14 mg/L (range: 3.57–4.05 mg/L). Iron concentrations ranged from 0.094 to 0.893 mg/L, with a mean of 0.35 ± 0.24 mg/L. Seven samples exceeded the BIS acceptable limit of 0.30 mg/L; however, none exceeded the permissible limit of 1.0 mg/L. Lead levels were significantly higher than the recommended guideline value, whereas iron concentrations remained below the BIS permissible limit (Fig.3)

Table 2. Heavy metal concentrations in drinking water samples and comparison with WHO/BIS guideline limits.

Metal	Limit (mg/L)	Mean ± SD	Range	Samples > limit
Lead (Pb)	0.01 (WHO/BIS)	3.81 ± 0.14	3.57–4.05	13/13 (≈381×)
Iron (Fe)	0.30 (BIS acceptable)	0.35 ± 0.24	0.094–0.893	7/13
Iron (Fe)	1.00 (BIS permissible)	0.35 ± 0.24	0.094–0.893	0/13

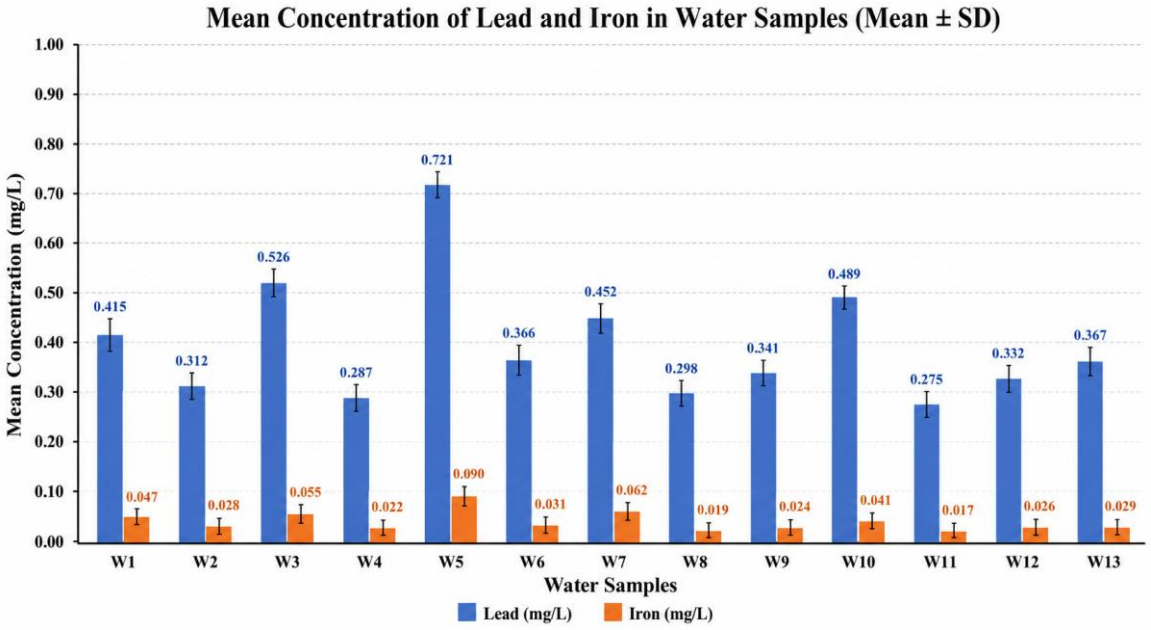

Figure 3. Mean (± SD) concentrations of lead and iron in drinking water samples (W1–W13).

Among the 13 samples, mean water concentrations of iron and lead were 0.353 and 3.812 mg/L, respectively, compared with 0.255 and 3.189 mg/L in saliva. Iron concentrations did not differ significantly between water and saliva (p = 0.364), whereas lead concentrations showed a significant difference (p = 0.021). Spearman correlation revealed a significant negative association between water and saliva lead concentrations (ρ = −0.608, p = 0.028), while correlations for iron were not significant (Table 3). The corresponding saliva concentrations are shown in Figure 4.

Table 3. Water–saliva paired comparison and correlation, by metal.

Metal	Water mean	Saliva mean	Mean diff.	Paired test (statistic, p)	Pearson r (p)	Spearman ρ (p)
Iron (mg/L)	0.353	0.255	+0.099	Paired t = 0.94, p = 0.364 (NS)	−0.260 (0.392)	−0.322 (0.284)
Lead (mg/L)	3.812	3.189	+0.622	Wilcoxon W = 13, p = 0.021*	−0.363 (0.223)	−0.608 (0.028)*

**** Significant at p < 0.05; NS = not significant.***

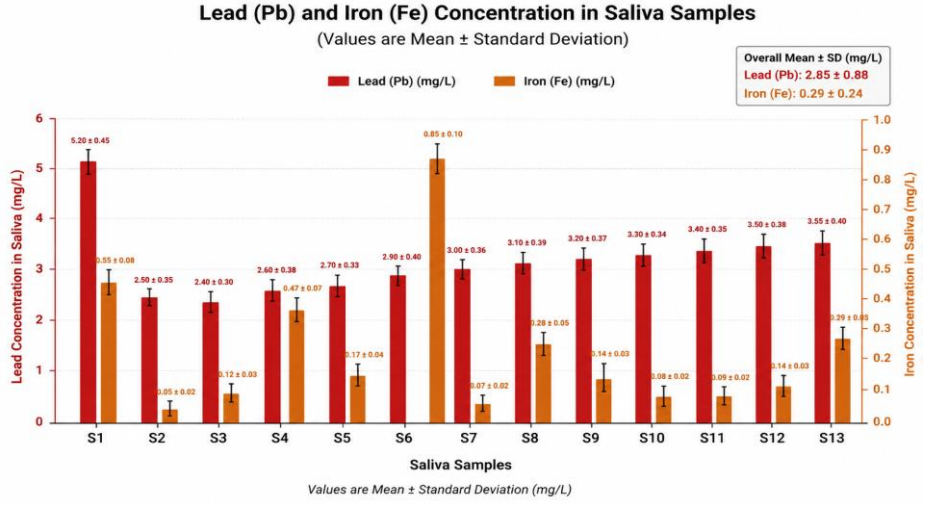

Figure 4. Mean (± SD) lead and iron concentrations in the 13 corresponding saliva samples (S1–S13). Lead is plotted against the left-hand axis and iron against the right-hand axis. Error bars represent standard deviation.

Salivary protein showed a significant positive correlation with nitrate (Spearman ρ = 0.638, p < 0.05). Protein showed negligible correlation with iron (ρ = −0.039) and lead (ρ = 0.066). Iron showed weak positive correlations with lead (ρ = 0.228) and a weak negative correlation with nitrate (ρ = −0.180). Lead also showed a weak positive correlation with nitrate (ρ = 0.396); however, none of these associations reached statistical significance (Table 4). The corresponding variation in salivary nitric oxide and total protein concentrations across the 13 samples is presented in Figure 6.

Table 4. Salivary inter-analyte Spearman correlation matrix (ρ; * p < 0.05).

	Protein	Iron	Lead	Nitrate
Protein	1.000	−0.039	+0.066	+0.638*
Iron	−0.039	1.000	+0.228	−0.180
Lead	+0.066	+0.228	1.000	+0.396
Nitrate	+0.638*	−0.180	+0.396	1.000

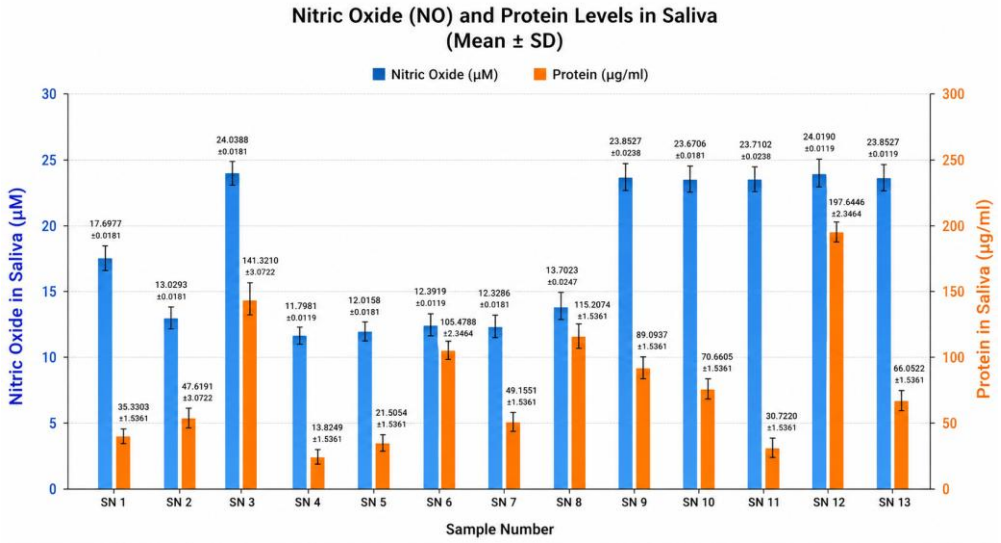

Figure 5. Mean (± SD) salivary nitric oxide and total protein concentrations across the 13 saliva samples (SN1–SN13). Nitric oxide is plotted against the left-hand axis and total protein against the right-hand axis.

Oral health assessment of the 13 corresponding participants showed a mean Plaque Index of 1.11, with 7 participants classified as having good oral hygiene, 5 as fair, and 1 as poor. The mean Gingival Index was 0.79, with mild gingivitis observed in 9 participants and moderate gingivitis in 4. Clinical examination of the exposed group revealed generalized gingival recession and greater plaque accumulation compared with the control group, in which no visible gingival recession or plaque accumulation was observed.

Table 5. Oral health indices among the 13 exposed participants with detectable water/salivary lead.2.2 Preparation of leaf extract

Parameter	Mean / Distribution	Range
Plaque Index	1.11; Good n=7, Fair n=5, Poor n=1	0.4 – 2.3
Gingival Index	0.79; Mild n=9, Moderate n=4	0.3 – 1.7

The measured water, salivary, and oral health parameters varied across the three study locations. Kavalkinaru showed the highest mean values, followed by Panagudi, while Udayarpatti showed the lowest values. Water iron and lead

concentrations were 0.47 ± 0.22 and 3.96 ± 0.54 mg/L at Kavalkinaru, compared with 0.10 ± 0.03 and 0.88 ± 0.20 mg/L at Udayarpatti. Salivary nitric oxide, total protein, iron, and lead concentrations showed a similar pattern. Gingival Index, Plaque Index, DMFT, and CPI scores were also highest at Kavalkinaru and lowest at Udayarpatti.

Table 6. Comparison of water heavy metal levels, salivary biomarkers, and oral health outcomes across three localities representing a gradient of exposure (mean ± SD).

Parameter	Kavalkinaru (Highest)	Panagudi (High)	Udayarpatti (Lowest)
Water iron (mg/L)	0.47 ± 0.22	0.40 ± 0.18	0.10 ± 0.03
Water lead (mg/L)	3.96 ± 0.54	3.22 ± 0.41	0.88 ± 0.20
Salivary nitric oxide (µM)	23.30 ± 1.82	19.80 ± 1.66	8.98 ± 0.75
Salivary total protein (µg/mL)	205.75 ± 18.05	167.04 ± 16.50	61.93 ± 5.88
Salivary iron (µg/L)	0.93 ± 0.10	0.65 ± 0.07	0.09 ± 0.02
Salivary lead (µg/L)	5.45 ± 0.34	4.42 ± 0.28	1.05 ± 0.07
Gingival Index	2.21 ± 0.25	1.63 ± 0.19	0.68 ± 0.12
Plaque Index	2.18 ± 0.24	1.59 ± 0.20	0.62 ± 0.10
DMFT Index	4.75 ± 0.55	3.18 ± 0.40	1.15 ± 0.20
CPI score	2.06 ± 0.23	1.41 ± 0.18	0.55 ± 0.09

The present study identified marked lead contamination in the drinking-water sources, with concentrations approximately 381-fold higher than the WHO guideline value of 0.01 mg/L. This is of considerable public-health concern because lead exposure can adversely affect the neurological, cardiovascular, renal, haematological, reproductive, and developmental systems, and WHO recommends keeping lead concentrations in drinking water as low as reasonably practicable [11]

Lead was also detected in the corresponding saliva samples. However, the significant difference between water and salivary lead concentrations ($p = 0.021$) and the inverse individual-level correlation ($\rho = -0.608$, $p = 0.028$) indicate that salivary lead may not directly reflect drinking-water exposure. Previous studies have similarly highlighted the limitations of saliva as a standalone biomarker of lead exposure and identified blood lead as the more established biomarker of internal exposure [12]

At the locality level, the positive associations between water-metal concentrations, salivary biomarkers, and oral-health measures ($r = 0.76–0.89$) indicate a consistent environmental exposure gradient. The higher salivary nitric oxide and total protein levels, together with greater plaque accumulation, gingival inflammation, caries experience, and periodontal scores in higher-exposure localities, may reflect alterations in the oral inflammatory environment. The significant correlation between salivary protein and nitrate ($\rho = 0.638$, $p = 0.019$) further supports their potential relationship with local oral physiological or inflammatory processes. Evidence from previous studies also suggests an association between lead exposure and dental caries [13] although the available evidence is not sufficient to establish causality

Overall, the findings provide preliminary evidence connecting drinking-water metal contamination with salivary biochemical alterations and oral-health indicators. Given the small sample size and cross-sectional design, these findings should be interpreted cautiously and confirmed through larger studies incorporating individual-level exposure assessment and established biomarkers such as blood lead.

Conclusion

The observed locality-level exposure gradient highlights a potential environmental–oral health relationship, while the individual-level findings demonstrate the complexity of using saliva to assess lead exposure. These findings support the

need for regular drinking-water surveillance, community awareness, and appropriate oral-health monitoring, with larger longitudinal studies required to clarify the biological and clinical significance of these associations.

Limitation of the study.

The study was limited by its small sample size, cross-sectional design, single-point sampling, and potential matrix interference associated with UV–Visible spectrophotometry.

Future Scope

This study highlights the potential of saliva as a simple biomarker for assessing metal exposure and supports future environmental monitoring.

Acknowledgments

The authors thank the study participants and Rajas Dental College & Hospital for their support and facilities.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Olowoyo JO, Olaiya OO, Oharisi OOL, Olusola JA, Tshoni UA, Oladeji OM. Heavy metals burden in drinking water: global patterns, sources, and public health implications. Water. 2026;18(8):886.
2. Bogdan-Andreescu CF, Cadar E, Bubulac L, Eremia IA, Tudor V, Albu CC, et al. Heavy metal-driven oral dysbiosis: salivary toxicometallomics at the host–microbiome interface across pathologies. Life. 2026;16(6):920.
3. Bogdan-Andreescu CF, Bubulac L, Albu CC, Slăvescu DA, Bănăţeanu AM, Botoacă O, et al. Toward smart salivary diagnostics: a comprehensive review of heavy metal biomarkers and digital risk modeling. Diagnostics. 2026;16(4):635.
4. Ikpi GE, Nganje TN, Edet A, Adamu CI, Eyong GA. Investigation of hydrochemical characteristic, water quality and associated health risks of metals and metalloids in water resources in the vicinity of Akamkpa quarry district, southeastern Nigeria. Geochem Trans. 2024;25(1):7.
5. Batley GE, Gardner D. Sampling and storage of natural waters for trace metal analysis. Water Res. 1977;11(9):745-756.
6. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci. 1993;694(1):72-77.
7. Mendham J, Denny RC, Thomas MJK. Vogel's textbook of quantitative chemical analysis. Vol. 562. London: Prentice Hall; 2000. p. 338.
8. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248-254.
9. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982;126(1):131-138.
10. Peter S. Essentials of preventive and community dentistry. Arya (Medi) Publishing House; 2009.
11. World Health Organization. Exposure to lead: a major public health concern. Preventing disease through healthy environments. Geneva: World Health Organization; 2025.
12. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect. 2005;113(12):1669-1674.
13. Lee G, Zhang Y, Yang Q, Angley M, Lu L, Kahe K. The association between lead exposure and dental caries: a systematic review. Caries Res. 2024;58(3):141-152.