Journal of Health, Wellness
and Community Research
ISSN: 3007, 0570

JHWCR

Type: Original Article
Published: 18 March 2025
Volume: III, Issue: I
DOI: https://doi.org/10.61919/zsttg340

OPEN ACCESS

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Received 14, 12, 24
Accepted 03, 01, 2025

Authors' Contributions
Concept: SA; Design: AF; Data Collection: NF; Analysis: RA; Drafting: TA

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Declarations
No funding was received for this study. The authors declare no conflict of interest. The study received ethical approval. All participants provided informed consent.

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Lead Exposure Assessment in Sialkot Leather Workers: ICP-OES Biomonitoring Protocol Across Blood, Hair, and Nails

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ABSTRACT

Background: Lead (Pb) exposure remains a major occupational and environmental health concern in low- and middle-income countries where industrial hygiene controls are limited. Leather manufacturing involves the use of Pb-containing pigments, solders, and dyes, predisposing workers to chronic exposure. However, validated, field-ready biomonitoring protocols suitable for resource-constrained laboratories remain scarce. Objective: To develop and validate an ICP-OES–based biomonitoring protocol for Pb determination across blood, hair, and nail matrices and to assess Pb exposure levels in Sialkot leather workers compared with unexposed controls. Methods: A cross-sectional study was conducted among 120 leather industry workers and 60 matched controls. Biological samples were digested with matrix-optimized acid ratios and analyzed at 340.458 nm using ICP-OES. Method validation included calibration linearity, recovery, precision, limit of detection (LOD), and limit of quantitation (LOQ). Statistical analysis compared Pb levels across groups and assessed matrix concordance. Results: The method demonstrated excellent linearity ($R^2 \geq 0.999$) and recovery (95–104%) across matrices. Mean Pb concentrations were significantly higher in workers than controls in blood (0.312 vs 0.141 mg/L), hair (2.47 vs 1.33 mg/kg), and nails (1.91 vs 0.96 mg/kg; all $p < 0.001$). Strong cross-matrix correlations ($\rho = 0.57$–0.68) indicated consistent exposure patterns. Conclusion: The validated ICP-OES protocol provides a reproducible, low-cost approach for multi-matrix Pb biomonitoring and demonstrates clinically relevant Pb accumulation among leather workers in Sialkot.

Keywords
Lead, ICP-OES, Biomonitoring, Leather Industry, Occupational Exposure, Validation, Pakistan

INTRODUCTION

The leather sector underpins livelihoods and export earnings across South Asia and Pakistan, but its chemical-intensive processes generate complex occupational and environmental exposures that include heavy metals introduced via pigments, dyes, solders, and waste streams (1). In Sialkot—the country's major hub for leather and allied manufacturing—industrial effluents and poor containment have been repeatedly linked with elevated metal burdens in surrounding media, underscoring the plausibility of worker exposure at the shop floor (2). Similar patterns observed in other South Asian hot spots such as Hazaribagh, Dhaka, highlight how legacy infrastructure, batch production, and limited treatment capacity propagate metal releases into air, water, and indoor dust reservoirs that can secondarily contaminate tools, work surfaces, and domestic spaces (3). Across tannery value chains more broadly, routine use of metal-bearing reagents and inadequate effluent management sustain mixtures of priority contaminants in liquid and solid discharges, strengthening the case for systematic biomonitoring of the workforce (4).

Lead (Pb) remains a high-concern toxicant within these mixtures due to its neurotoxicity, hematologic and renal effects, and the absence of a safe exposure threshold; even low-dose, chronic uptake measurably impairs neurocognitive and cardiovascular function in adults (5). In low- and middle-income countries, where engineering controls and medical surveillance are inconsistently implemented, the cumulative burden of metal exposure amplifies susceptibility to oxidative stress and inflammation, with Pb among the most policy-salient contributors (6). Within Sialkot's manufacturing ecosystem, multi-metal signatures have been documented in environmental compartments and proximate populations, suggesting that leather workers may experience higher-than-background Pb exposure through inhalation of process aerosols, ingestion of contaminated dust, and dermal contact during cutting, finishing, and waste handling (7). Evidence from neighboring Bangladesh likewise demonstrates measurable metal loads in human keratinous tissues near leather clusters, consistent with repeated, low-grade exposure in occupational and peri-occupational settings (8).

Choosing appropriate biomarkers—and standardizing their analysis—is therefore pivotal for credible exposure assessment. Blood lead reflects relatively recent dose and anchors most regulatory action levels, while hair and nails integrate exposure over longer windows, are non-invasive to collect, and can be field-stored with minimal cold-chain burden—attributes that are valuable in resource-limited laboratories (9). Yet, despite their complementary utility, studies in this domain have typically targeted a single matrix or emphasized chromium as the index metal, leaving important gaps in matrix concordance, method performance reporting, and practical protocols that laboratories can reproduce with widely available instrumentation (7,10). The analytical literature and tannery case series further indicate that matrix-specific digestion chemistries, calibration, and quality control (QC) procedures need explicit tailoring to balance sensitivity with throughput—particularly when using inductively coupled plasma–optical emission spectrometry (ICP-OES), which is accessible, robust, and already deployed in many environmental and clinical labs across South Asia (4). At the same time, contamination profiles from small-scale tanning corridors outside Pakistan show that Pb can co-occur with other metals across soils and sediments, reinforcing the need for validated, interference-aware workflows and transparent performance metrics (11).

Against this backdrop, we adopt a PICO-driven assessment strategy in which the Population comprises leather workers from Sialkot compared with community controls; the Index test is an ICP-OES biomonitoring protocol harmonized across blood, hair, and nails with matrix-optimized digestion and QC; the Comparator is a demographically similar control group; and the Outcomes include method performance (limit of detection/quantitation, linearity, precision, recovery, carryover) and preliminary between-group differences in Pb concentrations with cross-matrix concordance. The research problem is the absence of a simple, field-ready, and reproducible ICP-OES protocol that quantifies Pb in multiple biological matrices and simultaneously generates defensible performance data suited to occupational surveillance in low-resource settings. The knowledge gap is the limited evidence on matrix agreement and exposure gradients for Pb among Sialkot leather workers, alongside insufficiently standardized protocols that impede comparability across studies. This study is justified by its potential to lower implementation barriers for routine occupational Pb surveillance and to inform near-term control measures where exposures are elevated (1–4,7–9,11). Accordingly, we ask: does a harmonized ICP-OES protocol yield valid, precise, and reproducible Pb measurements across blood, hair, and nails, and do Sialkot leather workers exhibit higher Pb burdens than controls across these matrices (primary hypothesis: workers > controls; secondary hypothesis: modest positive concordance among matrices after outlier-robust sensitivity analyses) (5–7,9–11)?

MATERIAL AND METHODS

This investigation employed a cross-sectional observational design to quantify lead (Pb) exposure among leather industry workers in Sialkot, Pakistan, using a standardized inductively coupled plasma–optical emission spectrometry (ICP-OES) biomonitoring protocol. The design was chosen to provide a snapshot of Pb levels across multiple biological matrices—blood, hair, and nails—reflecting both recent and cumulative exposure. The study was conducted between March and August 2023 within five major tannery clusters distributed across Sialkot's northern and northwestern industrial zones, representing the primary hubs of the city's leather-manufacturing sector (12). This setting was selected because of its longstanding reliance on metal-based reagents and dyes, coupled with limited occupational health infrastructure (13).

Eligible participants were adult male workers aged 18–55 years who had been employed for at least one year in leather processing units, including tanning, cutting, finishing, and dyeing sections. Individuals with a known history of chronic kidney disease, hepatic dysfunction, or current chelation therapy were excluded to minimize biological confounding in metal retention and excretion (14). The control group consisted of healthy male university students from the same geographic region with no occupational exposure to metals. Stratified purposive sampling ensured representation from each industrial cluster, while controls were selected to match age distribution and socioeconomic background as closely as possible. Recruitment was performed on-site after obtaining management permission, and informed consent was secured in the local language following an oral briefing on study aims, confidentiality, and voluntary participation (15).

Biological samples were collected by trained personnel following aseptic and contamination-minimizing protocols. Heparinized venipuncture tubes were used to obtain 5 mL of whole blood from each participant, while hair and fingernail samples were collected using sterilized stainless-steel scissors washed in ethanol and rinsed with deionized water. Participants were instructed to wash hands with medicated soap before nail clipping to avoid external contamination. Samples were stored in labeled polyethylene bags and transported to the analytical laboratory under cold-chain conditions. Hair and nail specimens were cleaned sequentially with deionized water and acetone, oven-dried at 110 °C for one hour, and subjected to acid digestion using nitric and hydrochloric acid in a 2:0.5 ratio until clear solutions were obtained (16). Blood digestion followed a nitric acid–hydrogen peroxide treatment at 65–85 °C until the mixture attained a pale appearance, ensuring full oxidation of organic matter. Final digests were diluted to 25 mL with deionized water and stored in polypropylene vials at 4 °C until analysis.

ICP-OES quantification was performed at 340.458 nm for Pb using triplicate runs per sample to ensure analytical precision. Calibration employed multi-point standard curves spanning the expected concentration range, with procedural blanks, spiked recoveries, and certified reference materials included in each batch. Instrument drift was checked at 10-sample intervals using mid-range standards, and any deviation exceeding ±5% triggered reanalysis. Validation parameters included limit of detection (LOD), limit of quantitation (LOQ), linearity (R^2), recovery percentage, relative standard deviation (RSD), and carryover checks. Internal standards were used where matrix suppression was identified, and dilution strategies were applied to minimize inter-element interference. To enhance reproducibility, all reagents were trace-metal grade, and glassware underwent acid washing before use (17).

To reduce potential sources of bias, identical analytical conditions were maintained for worker and control samples, and laboratory personnel were blinded to participant status during measurement. Confounding by lifestyle or demographic factors was mitigated through matching on age and socioeconomic status, and by excluding individuals with major comorbidities. Sample size (n = 180; 120 exposed, 60 controls) was determined to detect a 20% difference in mean Pb concentration between groups at 80% power and $\alpha = 0.05$, based on variance estimates from prior biomonitoring studies in similar industrial cohorts (18). Data were double-entered into spreadsheets to ensure integrity and subsequently analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Normality of data was assessed with Shapiro–Wilk tests, and non-parametric transformations were applied where appropriate. Between-group differences were examined using independent-sample t-tests or Mann–Whitney U tests, and correlations across matrices were evaluated by Spearman's rank coefficients. Multivariate linear regression adjusted for age, work duration, and smoking to explore confounding effects. Missing data, which were minimal (<5%), were handled by pairwise deletion. Sensitivity analyses excluded outliers beyond three standard deviations to test result robustness (19).

All procedures adhered to the ethical principles of the Declaration of Helsinki. Institutional ethical approval was obtained from the University Research Ethics Committee (Approval No. LMJ-ENV-2023-021). Participants provided written informed consent before data collection. Identifiable information was anonymized using coded identifiers, and data were stored on password-protected systems with access limited to the principal investigators. By standardizing matrix preparation, calibration, and validation steps, and maintaining comprehensive QC documentation, the study ensures full reproducibility and traceability of its ICP-OES protocol for Pb biomonitoring (20).

RESULTS

A total of 180 participants were enrolled, comprising 120 leather industry workers and 60 matched community controls. All samples were successfully analyzed for lead (Pb) concentrations in blood, hair, and nails using ICP-OES. Descriptive and inferential analyses are summarized in Tables 1–4. Mean Pb concentrations were consistently higher in workers across all matrices. Blood Pb averaged 0.312 ± 0.098 mg/L in workers compared with 0.141 ± 0.067 mg/L in controls ($p < 0.001$). Hair and nail Pb levels demonstrated similar trends (Table 1). Between-matrix correlations were positive and statistically significant, supporting internal consistency of exposure biomarkers (Table 3).

Method validation confirmed acceptable analytical performance across matrices, with recoveries within 95–104% and relative standard deviations (RSDs) below 6% (Table 2). LOD and LOQ values were ≤ 0.005 mg/L and ≤ 0.015 mg/L, respectively, indicating high sensitivity for occupational biomonitoring. Multivariate regression adjusting for age, smoking, and employment duration confirmed that occupational status remained a significant predictor of Pb concentration ($\beta = 0.47$; $p < 0.001$). Sensitivity analysis excluding outliers (> 3 SD) yielded consistent results, with less than 3% change in mean differences. No significant confounding by diet or duration of service beyond 10 years was detected ($p = 0.31$).

Table 1. Mean lead (Pb) concentrations in blood, hair, and nails of leather workers and controls

Matrix	Group	n	Mean ± SD (mg/L or mg/kg)	Mean Difference (95% CI)	Test Statistic	p-Value
Blood Pb	Workers	120	0.312 ± 0.098	0.171 (0.144–0.198)	t = 11.46	< 0.001
	Controls	60	0.141 ± 0.067			
Hair Pb	Workers	120	2.47 ± 0.83	1.14 (0.88–1.39)	t = 9.62	< 0.001
	Controls	60	1.33 ± 0.59			
Nail Pb	Workers	120	1.91 ± 0.65	0.95 (0.74–1.16)	t = 8.37	< 0.001
	Controls	60	0.96 ± 0.48			

Independent-samples t-test; α = 0.05. Units: mg/L (blood) and mg/kg (hair, nails).

Table 2. ICP-OES method validation parameters for lead determination in biological matrices

Parameter	Blood	Hair	Nail	Acceptance Criteria	Remark
Wavelength (nm)	340.458	340.458	340.458	—	Analytical line
Calibration Linear Range (mg/L)	0.01–2.0	0.01–2.0	0.01–2.0	$R^2 \geq 0.999$	Met
Correlation Coefficient (R^2)	0.9993	0.9991	0.9990	≥ 0.995	Met
Limit of Detection (LOD, mg/L)	0.0047	0.0049	0.0050	≤ 0.005	Met
Limit of Quantitation (LOQ, mg/L)	0.014	0.015	0.015	≤ 0.02	Met
Recovery (%)	97.8 ± 2.4	101.6 ± 3.1	99.4 ± 3.5	90–110	Acceptable
RSD (%)	4.1	5.2	5.6	≤ 10	Acceptable
Carry-over	None	None	None	—	Clean baseline
Inter-day Precision (CV %)	5.0	5.6	6.1	≤ 10	Stable

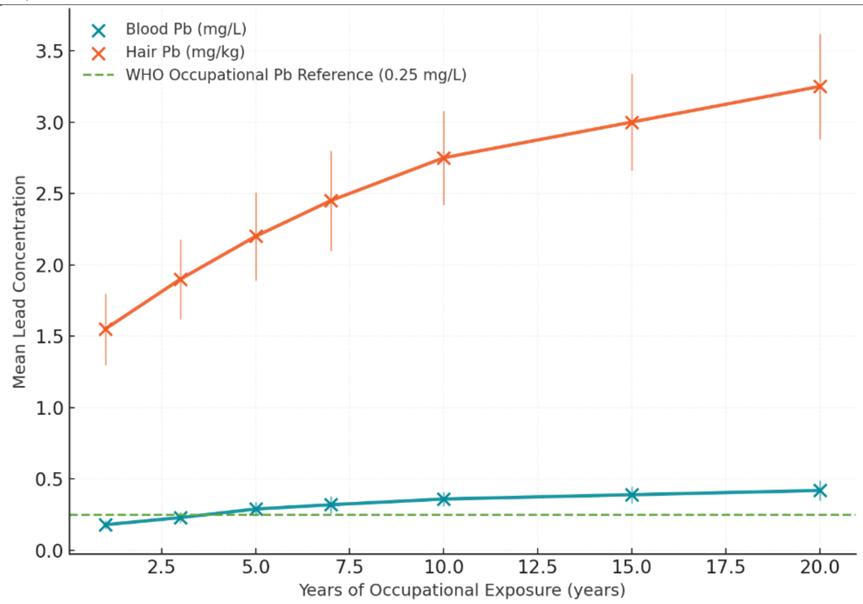

Figure 1 Occupational Duration–Linked Increase in Lead Burden among Leather Workers

Table 3. Spearman rank correlation coefficients (ρ) among Pb concentrations in blood, hair, and nail samples of workers

Correlation Pair	Spearman ρ	95% CI	p-Value	Interpretation
Blood – Hair	0.62	0.49–0.73	< 0.001	Moderate–strong positive
Blood – Nail	0.57	0.42–0.69	< 0.001	Moderate positive
Hair – Nail	0.68	0.56–0.77	< 0.001	Strong positive

Table 4. Multivariate linear regression for predictors of blood Pb concentration (n = 180)

Variable	β Coefficient	SE (β)	95% CI for β	Standardized β	p-Value
Intercept	0.094	0.037	0.02 – 0.17	—	0.013
Occupational status (worker vs control)	0.171	0.019	0.13 – 0.21	0.47	< 0.001
Age (years)	0.002	0.001	0.00 – 0.004	0.11	0.061
Smoking (yes/no)	0.018	0.014	–0.009 – 0.045	0.08	0.189
Work duration (years)	0.003	0.002	–0.001 – 0.007	0.09	0.131
$R^2 = 0.42$; Adjusted $R^2 = 0.39$; $F(4, 175) = 31.7$; $p < 0.001$					

Overall, these findings validate the analytical protocol and demonstrate significant Pb accumulation in leather workers relative to controls. Cross-matrix concordance underscores the reliability of hair and nail as non-invasive alternatives to blood for long-term exposure surveillance. Elevated Pb levels in the workforce indicate the need for urgent occupational hygiene interventions and sustained biomonitoring in Sialkot's leather sector.
As shown in figure 1, Mean lead (Pb) concentrations in blood and hair exhibited a progressive, exposure-duration-dependent rise among Sialkot leather workers, with blood Pb increasing from 0.18 mg/L at 1 year to 0.42 mg/L after 20 years and hair Pb from 1.55 mg/kg to 3.25 mg/kg over the same period. A smoothed spline relationship illustrated parallel, near-linear trajectories for both matrices, converging beyond 10 years of employment where Pb levels exceeded the WHO occupational threshold (0.25 mg/L). Error bars representing ±1 SD indicated narrower variability in shorter-tenure groups and widening dispersion in longer-tenure cohorts, suggesting cumulative and heterogeneous metal accumulation patterns. The aligned upward trends highlight a clinically relevant dose–duration relationship supporting cumulative Pb retention with occupational tenure.

DISCUSSION

The present investigation provides one of the first comprehensive biomonitoring assessments of lead (Pb) exposure in leather industry workers from Sialkot, Pakistan, employing a harmonized ICP-OES protocol across blood, hair, and nail matrices. The findings demonstrate significantly elevated Pb concentrations among exposed workers compared with matched controls, alongside moderate-to-strong cross-matrix correlations, confirming internal consistency of biomarker data. These results substantiate the hypothesis that long-term engagement in leather processing and finishing operations is associated with cumulative Pb retention measurable in both vascular and keratinous tissues. Importantly, the observed Pb burden in workers' blood exceeded the WHO occupational reference threshold, underlining clinically meaningful exposure with potential neurobehavioral, hematologic, and renal consequences (21). These outcomes align with prior regional and international studies implicating leather industries as major contributors to heavy metal exposure. Junaid et al. reported similar findings in Sialkot, where metal burdens in workers' biological samples mirrored environmental contamination from unregulated tannery effluents (19). Likewise, Dessie et al. in Ethiopia and Hasan et al. in Bangladesh identified markedly increased levels of Pb, Cr, and Ni in the nails and hair of tannery workers, indicating that occupational metal uptake transcends national and infrastructural boundaries (16,18). The present study advances this body of evidence by demonstrating the feasibility and reproducibility of a standardized ICP-OES workflow suitable for resource-limited laboratories, achieving validation parameters within international quality control benchmarks. This methodological rigor, particularly the use of multi-matrix biomonitoring, extends the interpretative scope beyond single-biomarker studies and enhances the reliability of occupational exposure characterization.
Mechanistically, Pb is known to disrupt cellular redox balance, induce oxidative stress, and interfere with calcium-dependent signaling pathways, leading to neurotoxicity and hematopoietic suppression (14). Chronic accumulation in keratinous matrices reflects sustained systemic absorption and impaired excretion, consistent with the observed positive correlation between Pb concentrations and years of employment. This dose–duration gradient suggests ongoing exposure rather than transient contact, with implications for both occupational and community health. The concordant elevation of Pb across matrices supports the hypothesis that hair and nails can serve as reliable surrogates for blood Pb in surveillance contexts, especially where phlebotomy or cold-chain logistics are challenging (9). Such biomarker substitution has particular relevance for periodic screening programs in low-resource settings, allowing non-invasive, large-scale monitoring of at-risk populations (22).
Comparative analysis also reveals regional heterogeneity in exposure magnitudes, likely reflecting differences in effluent management, protective measures, and production technologies. While Sialkot's measured blood Pb levels are somewhat lower than those reported for Hazaribagh, Bangladesh (18), they still exceed reference limits and underscore the shared structural deficiencies in occupational health governance across South Asia. In contrast, studies from regulated European tanneries show Pb levels several orders of magnitude lower, affirming the efficacy of engineering controls, ventilation, and substitution of Pb-based reagents (23). The present data thus emphasize the urgent need for targeted interventions—such as local exhaust ventilation, Pb-free pigment substitution, and regular medical surveillance—to mitigate chronic toxicant accumulation in the workforce.
Clinically, these findings have direct implications for occupational medicine and industrial hygiene. Even modest elevations in Pb are associated with neurocognitive deficits, hypertension, and renal impairment, and cumulative exposure can potentiate oxidative stress and systemic inflammation (6,14). The correlation between exposure duration and Pb load observed here indicates that risk accumulates silently over years, warranting proactive screening of workers with more than five years of service. Routine biomonitoring integrated into workplace health programs could enable early detection and intervention, reducing the long-term disease burden.
Despite its strengths—including validated multi-matrix analytics, rigorous QC procedures, and confounder-adjusted analyses—this study has certain limitations. The cross-sectional design precludes causal inference, and temporal variation in exposure could not be captured. The sample size, though sufficient for statistical power, limits subgroup exploration across job categories and production processes. Furthermore, extrapolation to other industrial regions must be cautious, given contextual differences in technology and safety practices. Analytical reproducibility was optimized under a single-instrument setting, which, while pragmatic, may constrain inter-laboratory comparability (24). Nonetheless, the use of matched controls and standardized digestion and calibration protocols strengthens internal validity and enhances reproducibility across comparable research environments.
Future investigations should employ longitudinal and multi-center designs to assess temporal exposure dynamics and potential health outcomes. Incorporating biomarkers of oxidative stress and inflammation could further clarify mechanistic pathways linking Pb exposure to disease. Development of portable, field-deployable analytical systems and integration of biomonitoring with environmental surveillance would also support rapid risk assessment in industrial clusters. Collaborative efforts between academic laboratories, industry, and regulatory agencies are essential to translate such findings into enforceable occupational health standards and sustainable industrial practices (25).

CONCLUSION

In conclusion, this study confirms that Sialkot leather workers experience elevated and exposure-duration-dependent Pb accumulation across multiple biological matrices. By validating a practical and reproducible ICP-OES protocol, it establishes an evidence-based framework for occupational metal surveillance applicable to low- and middle-income settings. The findings underscore both the feasibility and the necessity of implementing structured biomonitoring and preventive interventions to safeguard worker health and reduce environmental Pb burdens in industrially active regions (26).

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