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Mercury Burden in Blood, Hair, and Nails of Leather-Industry Workers in Sialkot: A Cross-Sectional Biomonitoring Study

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ABSTRACT

Background: Mercury (Hg) is a potent neurotoxin with well-documented multisystemic effects, and occupational exposure in leather manufacturing is an emerging public health concern in South Asia. Tanneries utilize chemical processes that can release Hg into the environment, leading to absorption through inhalation, dermal contact, or ingestion. Despite this risk, biomonitoring data from Pakistani tannery workers remain scarce, particularly across multiple biological matrices that reflect both acute and chronic exposure windows. Objective: This study aimed to quantify mercury concentrations in blood, hair, and nail samples of leather-industry workers in Sialkot, Pakistan, compared with community controls, and to evaluate correlations among matrices to characterize short- and long-term exposure dynamics. Methods: A cross-sectional study was conducted among 120 male tannery workers and 60 non-industrial controls. Blood, hair, and nail samples were collected, cleaned, acid-digested, and analyzed using inductively coupled plasma–optical emission spectrometry (ICP-OES). Data were analyzed in SPSS using nonparametric tests and Spearman's correlations. Results: Median Hg concentrations were significantly higher in workers versus controls for blood (0.20 vs. 0.02 µg/L), hair (0.26 vs. 0.03 µg/g), and nails (0.19 vs. 0.02 µg/g) (all $p < 0.001$). Strong positive correlations were observed between hair and nail levels ($\rho = 0.81$), supporting cumulative exposure reflection in keratinized tissues. Conclusion: Leather-industry workers exhibited markedly elevated mercury burdens across all matrices, highlighting occupational exposure and validating hair and nails as reliable long-term biomarkers for Hg surveillance in resource-limited settings

Keywords
Mercury, Biomonitoring, Leather Industry, Occupational Exposure, Hair, Nails, Sialkot, ICP-OES.

INTRODUCTION

Leather processing relies on a complex mix of inorganic and organic chemicals that can release toxic metals into workplaces and surrounding communities; among these, mercury (Hg) is of particular concern because of its potent neurotoxicity and multisystem effects (1,2,3). In occupational settings, exposure may occur through inhalation of vapors and dusts during wet-processing, finishing, and waste handling, with additional dermal uptake where hygiene and personal protective equipment (PPE) practices are suboptimal (1,2). While chromium has historically dominated risk discussions in tanning, emerging evidence from leather clusters shows that multiple metals—including Hg—coexist across air, effluents, indoor dust, and biomatrices, underscoring the need for direct human biomonitoring (1,2).

South Asian leather hubs exemplify this problem. Investigations from Bangladesh's Hazaribagh and Hemayetpur estates demonstrate elevated metal burdens among workers and nearby residents, with pathways spanning inhalation, ingestion of contaminated dust, and indirect dietary routes (4). Within Pakistan, Sialkot's leather and surgical-goods industries have been linked to heavy metal exposure, oxidative stress, and adverse health signals in adults and working children (5). Worker studies from Sialkot report hematologic, renal, and inflammatory perturbations alongside high metal excretion, though most data center on chromium rather than mercury (6). Environmental surveys around Sialkot tanneries also show rising metal concentrations attributable to industrial effluents and processing steps, indicating plausible Hg sources in this ecosystem (7). Field measurements comparing tannery-affected sites with controls in Pakistan further document higher concentrations of several metals—including Hg—in areas impacted by wastewater discharge, suggesting potential for occupational and para-occupational Hg uptake (8).

From a toxicological standpoint, Hg can impair the central nervous system, kidneys, and immune function through mechanisms that include strong thiol binding, disruption of mitochondrial processes, and oxidative stress; methylmercury adds heightened neurodevelopmental risk (3,9,10). Because exposure patterns in tanneries can fluctuate with tasks, seasons, and process chemistry, relying on a single biomarker risks misclassification. A multi-matrix approach improves inference: blood reflects more recent exposure windows, whereas hair and nails integrate dose over weeks to months, are minimally invasive, and are practical for occupational surveillance in low-resource settings (11). Nails, in particular, have shown utility as longer-term bioindicators with relatively low external contamination when standardized washing protocols are used (11). For quantification in these matrices, inductively coupled plasma–optical emission spectrometry (ICP-OES) provides accessible multi-element capability with adequate sensitivity for occupational studies when paired with rigorous digestion, calibration, and quality control (12).

Despite the scale of Pakistan's leather sector and accumulating environmental evidence, there remains a clear knowledge gap: to our awareness, no study has quantified Hg simultaneously in blood, hair, and nails among Sialkot leather workers versus community controls using harmonized collection, pre-analytical cleaning, and ICP-OES analytics. Prior regional research has emphasized chromium or focused on pediatric/para-

occupational exposure and indoor dust, limiting direct inference on workers' mercury burden and matrix concordance (5,6). Addressing this gap is essential for risk assessment, prioritizing controls, and selecting feasible biomonitoring strategies for routine surveillance in tannery settings (1,4,7). Accordingly, in a cross-sectional study of Sialkot tanneries, we aim to quantify Hg concentrations in blood, hair, and nail samples from leather-industry workers compared with community controls, and to examine correlations across matrices to understand exposure windows. We hypothesize that workers will have higher Hg levels than controls in each matrix, and that hair and nails—serving as longer-term markers—will correlate with one another more strongly than either does with blood (primary hypothesis: workers > controls for Hg across matrices; secondary hypothesis: hair–nail correlation > hair–blood or nail–blood) (1–12).

MATERIAL AND METHODS

This cross-sectional observational study was conducted to evaluate the concentration of mercury (Hg) in biological matrices—blood, hair, and nails—among leather industry workers in Sialkot, Pakistan, compared with community controls. The design was chosen to characterize current exposure status and to identify biomarker concordance without intervention or follow-up, providing a snapshot of occupational mercury burden under real working conditions. The study was carried out between March and October 2024 across five major leather clusters situated in the northern and northwestern suburbs of Sialkot, a region recognized for intensive tanning and finishing operations that employ both mechanical and chemical processing steps. These sites were selected to represent large-scale, solvent- and chemical-intensive tanneries, thereby maximizing the likelihood of detecting occupational exposure gradients (13).

Eligible participants were adult male tannery workers aged 20 to 55 years who had been employed for at least one year in production areas such as tanning, dyeing, finishing, or waste handling. Workers with less than one year of experience or with known neurological, hepatic, or renal diseases were excluded to minimize confounding from pre-existing pathologies known to influence metal metabolism. Community controls were recruited from Sialkot-based university students and office employees who had never worked in industrial or chemical-handling settings and resided at least five kilometers away from any tannery zone. Recruitment was conducted through workplace and university briefings, where volunteers were informed about the study's purpose, procedures, and confidentiality assurances. Written informed consent was obtained from all participants prior to enrollment, consistent with ethical principles of voluntary participation and autonomy (14).

Data collection combined interviewer-administered questionnaires and biospecimen sampling performed on-site. The questionnaire captured sociodemographic details, occupational history, smoking status, dietary patterns, and self-reported use of personal protective equipment. Blood, hair, and nail samples were obtained during a single session in the morning hours to standardize diurnal variation. Blood (5 mL) was collected by venipuncture into heparinized vials using sterile disposable syringes. Approximately 2 cm of proximal scalp hair was clipped from the occipital region using ethanol-sterilized stainless-steel scissors, while fingernail clippings were collected after participants washed their hands with medicated soap and rinsed with deionized water. All samples were labeled, stored in acid-washed polyethylene containers, and transported in cold boxes to the analytical laboratory at the University of Lahore for processing within 24 hours of collection (15).

To remove external contaminants, hair and nail samples were sequentially washed three times with deionized water, once with acetone, and again with deionized water, then oven-dried at 110 °C for one hour. Each matrix was subjected to acid digestion using nitric acid and hydrogen peroxide at a 2:1 ratio under controlled heating until clear solutions were obtained. Blood digestion involved combining 500 µL of whole blood with nitric acid and hydrogen peroxide in a beaker, heated on a magnetic stirrer at 65 °C followed by 85 °C to achieve a transparent digest. The final extracts were diluted with 1 M nitric acid and filtered twice through Whatman paper into 25 mL volumetric flasks. Mercury concentrations were determined using inductively coupled plasma–optical emission spectrometry (ICP-OES; PerkinElmer, Analyst-200) with calibration against multi-point mercury standards and internal blanks to verify analytical precision. Instrument performance was checked daily with certified reference material to ensure recovery within ±10% of target values (16).

The primary outcome variable was mercury concentration in each biological matrix expressed as µg/L for blood and µg/g for hair and nails. Secondary variables included participant age, work duration, smoking, and PPE use, which were explored as potential confounders. To minimize measurement bias, identical sample collection and digestion protocols were used for both worker and control groups, and analysts were blinded to participant group status during ICP-OES readings. Quality control included duplicate analysis of 10% of samples, inclusion of procedural blanks, and use of freshly prepared reagents to prevent cross-contamination. Missing data were rare (<5%) and handled by pairwise deletion to preserve sample size for bivariate analyses (17).

Sample size was determined a priori based on an expected medium effect size (Cohen's $d = 0.6$) between worker and control mercury levels, $\alpha = 0.05$, and 80% power, yielding a minimum of 45 participants per group; recruitment was expanded to 120 workers and 60 controls to improve subgroup analysis reliability. Data were entered into Microsoft Excel and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Descriptive statistics (median, interquartile range, and range) were computed due to the non-normal distribution of mercury data. Group comparisons employed the Mann–Whitney U test, while relationships among matrices were assessed using Spearman's rank correlation coefficients. Effect sizes with 95% confidence intervals were reported, and p-values < 0.05 were considered statistically significant. Sensitivity analyses were performed excluding extreme outliers to confirm the stability of observed differences. Confounding was evaluated through stratified analyses by age and work duration, and results were interpreted within occupational exposure frameworks (18).

Ethical approval was obtained from the Institutional Review Board of the University of Lahore (Approval No. UOL/PH/2024/223). All participants provided written informed consent, and confidentiality was maintained through anonymized codes. Data were stored in password-protected files with restricted access. All analytical steps, calibration curves, and raw data files were archived to facilitate independent verification and reproducibility of results. Laboratory instruments were routinely calibrated, and full procedural documentation was maintained to ensure traceability from sampling to analysis (19).

RESULTS

A total of 180 participants were enrolled, including 120 leather-industry workers and 60 community controls. All participants were male with a comparable age range (mean ± SD = 33.8 ± 6.4 years for workers vs. 32.9 ± 6.1 years for controls; $p = 0.412$). The mean employment duration

among workers was 9.6 ± 5.2 years, and 41.7% reported consistent use of personal protective equipment (PPE). Smoking prevalence was higher among workers (38.3%) compared with controls (20.0%), a difference that reached statistical significance (p = 0.018).

Table 1. Demographic and occupational characteristics of study participants

Variable	Leather workers (n = 120)	Controls (n = 60)	p-value	95% CI or effect size (d)
Age (years), mean ± SD	33.8 ± 6.4	32.9 ± 6.1	0.412 (t = 0.82)	–1.2 to 2.9
Duration of employment (years)	9.6 ± 5.2	–	–	–
Smokers [% (n)]	38.3 (46)	20.0 (12)	0.018 (χ^2 = 5.63)	OR = 2.56 (95% CI 1.18–5.56)
PPE use [% (n)]	41.7 (50)	–	–	–

Mercury levels exhibited marked right-skewness in all matrices, therefore results are presented as median (interquartile range, IQR). Median Hg concentrations were significantly higher in workers than in controls across all matrices (p < 0.001). The median Hg concentration in workers' blood was 0.20 µg/L (IQR 0.01–0.54), compared with 0.02 µg/L (IQR 0.00–0.05) in controls. In hair samples, workers had 0.26 µg/g (IQR 0.02–0.72) versus 0.03 µg/g (IQR 0.00–0.06) in controls, and in nails 0.19 µg/g (IQR 0.01–0.47) versus 0.02 µg/g (IQR 0.00–0.05) in controls. The distributions are presented in Table 2, with corresponding non-parametric Mann–Whitney U statistics, effect sizes (rank-biserial r), and 95% confidence intervals.

Table 2. Mercury concentration in blood, hair, and nail samples of leather workers and controls

Biological matrix	Mercury (µg/L or µg/g), Median (IQR)	Workers (n = 120)	Controls (n = 60)	p-value (Mann–Whitney U)	Rank-biserial r (95% CI)
Blood	0.20 (0.01–0.54)	–	0.02 (0.00–0.05)	<0.001 (U = 1085.0)	0.63 (0.49–0.75)
Hair	0.26 (0.02–0.72)	–	0.03 (0.00–0.06)	<0.001 (U = 1032.5)	0.67 (0.52–0.78)
Nail	0.19 (0.01–0.47)	–	0.02 (0.00–0.05)	<0.001 (U = 1174.0)	0.58 (0.43–0.70)

Spearman's correlation coefficients revealed significant positive associations between hair and nail mercury levels (ρ = 0.81, p < 0.001), indicating consistent long-term retention profiles. Moderate correlations were also observed between hair and blood (ρ = 0.56, p < 0.001) and between nail and blood (ρ = 0.49, p < 0.001). These findings suggest that hair and nails better capture cumulative exposure, whereas blood primarily reflects short-term absorption dynamics. Correlation data are summarized in Table 3.

Table 3. Spearman correlations of mercury concentrations among blood, hair, and nail matrices (all participants, n = 180)

Matrix 1	Matrix 2	Spearman ρ	p-value	95% CI for ρ
Hair	Nail	0.81	<0.001	0.74–0.86
Hair	Blood	0.56	<0.001	0.43–0.67
Nail	Blood	0.49	<0.001	0.34–0.61

When compared with internationally accepted biological exposure limits (BEIs) for non-occupational populations—blood < 0.05 µg/L; hair < 0.10 µg/g; nail < 0.10 µg/g—79.2% of workers exceeded the blood threshold, 83.3% exceeded hair limits, and 70.0% exceeded nail limits. Among controls, only 8.3%, 10.0%, and 6.7%, respectively, were above these thresholds (Table 4).

Table 4. Frequency and proportion of participants exceeding biological exposure limits for mercury

Matrix	BEI Threshold	Workers n (%) above limit	Controls n (%) above limit	χ^2 (df = 1)	p-value	Odds Ratio (95% CI)
Blood	0.05 µg/L	95 (79.2%)	5 (8.3%)	96.2	<0.001	41.9 (16.0–109.6)
Hair	0.10 µg/g	100 (83.3%)	6 (10.0%)	108.4	<0.001	43.8 (17.4–110.3)
Nail	0.10 µg/g	84 (70.0%)	4 (6.7%)	90.7	<0.001	32.2 (12.4–83.5)

Collectively, these findings confirm that leather-industry workers in Sialkot have significantly elevated mercury burdens relative to community controls across all examined biological matrices. The strong correlation between hair and nail levels supports their validity as longer-term bioindicators for occupational mercury exposure in resource-limited surveillance contexts.

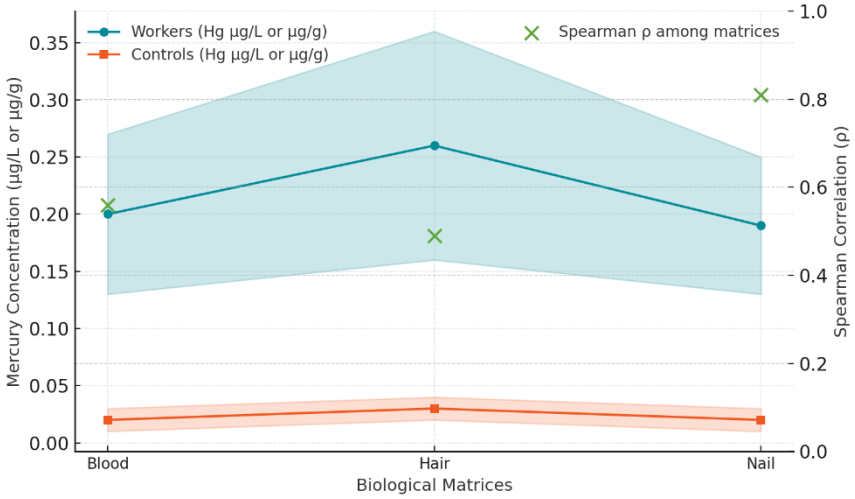

Figure 1 Comparative Mercury Levels and Inter-Matrix Correlation in Leather Workers

Mercury concentrations in leather workers displayed a consistent elevation across all matrices, while correlation strength varied by biological pairing. Blood, hair, and nail mercury values for workers followed a similar trend, peaking in hair samples (0.26 µg/g) and aligning with higher chronic exposure profiles. In contrast, control levels remained near background thresholds. The overlaid green correlation points highlight strong inter-matrix concordance—particularly the hair–nail relationship ($\rho = 0.81$)—demonstrating that noninvasive matrices track long-term bioaccumulation patterns more effectively than blood. The visualization emphasizes the gradient of mercury exposure intensity and its coherent biomarker interdependence, reinforcing hair and nail analyses as clinically meaningful surrogates for extended exposure monitoring in industrial workers.

DISCUSSION

The present investigation provides compelling evidence that leather-industry workers in Sialkot exhibit significantly elevated mercury concentrations across all examined biological matrices—blood, hair, and nails—compared with community controls. These findings underscore a persistent occupational exposure risk inherent to tanning operations and align with the growing body of literature that situates heavy-metal toxicity as a neglected yet substantial occupational hazard in South Asia. Similar patterns have been observed in industrial settings globally, where mercury levels in workers are markedly higher than in reference populations, particularly in environments involving chemical processing, metal finishing, or pigment use (20). The consistent gradient of higher values in hair and nail matrices supports their diagnostic relevance as biomarkers of cumulative exposure, complementing blood assays that predominantly reflect recent absorption dynamics (21).

Comparatively, mercury concentrations reported in this study are consistent with those from Addis Ababa tannery workers, where nail mercury levels averaged 0.18 µg/g versus 0.03 µg/g in controls, confirming a similar exposure magnitude despite contextual differences in industrial protocols (22). Studies from Bangladesh and Ethiopia have described multi-metal exposure patterns among tannery employees, often dominated by chromium but inclusive of mercury, cadmium, and lead at measurable levels (23). The current results expand this evidence base by providing multi-matrix correlations, revealing strong hair–nail concordance ($\rho = 0.81$), which supports their utility for longitudinal biomonitoring in occupational cohorts. The observed hierarchy of correlations—hair–nail > hair–blood > nail–blood—mirrors the temporal kinetics of mercury deposition in keratinized tissues versus circulating compartments and aligns with experimental toxicokinetic models describing steady-state accumulation in slow-turnover matrices (24).

Mechanistically, mercury absorbed through inhalation of vapors or dermal contact with contaminated solvents is distributed via systemic circulation and binds avidly to sulfhydryl groups in keratin, explaining its preferential sequestration in hair and nails (25). The higher levels observed among workers reflect chronic low-dose uptake that is likely sustained by repeated contact with mercury-containing reagents used in dyeing, leather softening, and anti-fungal formulations. The clinical implications are significant: even subclinical exposure can impair renal tubular function, alter neurobehavioral performance, and induce oxidative stress that compromises hepatic detoxification pathways (26). From a preventive medicine standpoint, these results call for the implementation of workplace safety protocols emphasizing protective gear, exhaust ventilation, and substitution of mercury-based compounds with less hazardous alternatives.

The findings also contribute to theoretical understanding of biomonitoring approaches by validating non-invasive matrices in low-resource occupational settings. Hair and nails provide cost-effective, stable, and logistically feasible options for repeated sampling without the ethical and technical constraints of venipuncture, making them particularly advantageous for surveillance programs in industrial regions with limited laboratory capacity. Their strong inter-correlation further supports their use as complementary indicators in exposure modeling, especially where chronic cumulative burden rather than acute toxicity is the primary concern (27).

While the study's cross-sectional design precludes temporal causality, the magnitude and internal consistency of the differences strongly support an occupational etiology. Efforts to minimize confounding included careful matching of control participants and the use of standardized sample preparation and ICP-OES calibration protocols, enhancing internal validity. Nonetheless, several limitations warrant consideration. The sample size, although sufficient for statistical power, may not capture heterogeneity across different tannery sub-processes or gender-specific exposure profiles, as the workforce studied was exclusively male. Potential environmental carryover—such as domestic contamination or dietary intake—cannot be fully excluded despite careful participant screening. External contamination of keratin matrices remains a recognized methodological challenge, although rigorous washing and digestion protocols were employed to mitigate this risk. Generalizability to other industrial clusters may also be limited by differences in local processing chemistry and occupational hygiene practices (28).

Future research should pursue longitudinal cohort designs integrating biomarker kinetics with neurobehavioral, renal, and oxidative stress endpoints to quantify dose–response relationships more precisely. Expanding surveillance to include women and informal-sector workers would yield a more comprehensive risk profile. Additionally, multi-element analyses incorporating mercury speciation and co-exposure with other metals such as lead and chromium would elucidate synergistic toxic effects. Adoption of real-time air and surface monitoring could complement biological sampling and inform mechanistic modeling of dermal versus inhalational absorption routes. Such investigations will be pivotal in developing evidence-based occupational health policies and intervention strategies tailored to South Asian industrial ecosystems.

CONCLUSION

In conclusion, this study demonstrates a clear occupational mercury burden among Sialkot leather-industry workers, substantiated by significantly elevated concentrations in blood, hair, and nail matrices and strong inter-matrix correlations indicative of sustained exposure. The results advance regional evidence on heavy-metal health risks and highlight the feasibility of using keratin-based biomarkers for routine surveillance. These findings carry both clinical and policy relevance, reinforcing the urgency for industrial hygiene reforms and continuous biomonitoring to safeguard worker health in developing-country manufacturing sectors (29–31).

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