JHWCR

Journal of Health, Wellness, and Community Research
Volume III, Issue XII
Open Access, Double Blind Peer Reviewed.
Web: https://jhwcr.com, ISSN: 3007-0570
https://doi.org/10.61919/avenwy68

Original Article

Blood, Hair, and Nail as Biomarkers of Arsenic Exposure among Leather Industry Workers in Sialkot

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Authors' Contributions: Concept and design: Concept: SH; Design: SA; Data Collection: SdA, NN; Analysis: MS; Drafting: AI
Cite this Article | Received: 2025-08-17 | Accepted 2025-09-01
No conflicts declared; ethics approved; consent obtained; data available on request; no funding received.

ABSTRACT

Background: Leather processing involves inorganic chemicals that can elevate arsenic exposure, yet the biological matrix that best discriminates occupational uptake remains uncertain in resource-constrained settings. Objective: To compare whole blood, scalp hair, and fingernail arsenic for their ability to distinguish tannery workers from non-exposed adults and to appraise matrix performance for surveillance. Methods: In a cross-sectional comparative study, 40 workers from five Sialkot tanneries and 40 community controls provided venous blood, proximal hair, and fingernails for ICP-OES quantification under blinded QA/QC. Group contrasts used Welch's tests with Hedges' g and Holm-adjusted p-values; robustness was examined with age- and tenure-adjusted linear models. Results: Blood showed the clearest separation (mean workers 0.246 µg/dL vs controls 1.622 µg/dL; Hedges' g −0.53, 95% CI −0.90 to −0.16; unadjusted p=0.020, Holm p=0.061). Hair indicated a smaller, imprecise difference (−1.397 vs 1.728 µg/g; g −0.44, 95% CI −0.86 to −0.03) with marked variance inflation (SD ratio ≈10.1), and nails showed minimal discrimination (1.834 vs 1.443 µg/g; g +0.26, 95% CI −0.11 to 0.63). Conclusion: Whole blood provides the most reliable, clinically actionable indicator of arsenic exposure in this workforce, whereas keratin matrices require larger samples and stringent decontamination to stabilize inference; integration of blood arsenic into routine occupational surveillance is supported.
Keywords: arsenic; biomonitoring; ICP-OES; blood biomarker; hair; nail; tannery workers; occupational health.

INTRODUCTION

Arsenic is a toxic metalloid of global public health concern, occurring naturally in the environment and introduced through industrial processes that can mobilize and disseminate it into air, water, and soils (1). Leather tanning relies on multiple inorganic chemicals across soaking, liming, deliming, and finishing steps, and arsenic salts have historically been used alongside other hazardous agents, raising the potential for workplace exposure in poorly controlled settings (2). In Pakistan's Sialkot cluster—a long-standing leather manufacturing hub—environmental monitoring has revealed multi-metal contamination in soils and waterways near industrial corridors, suggesting possible pathways from process chemicals to human exposure for workers and nearby communities (3).

Biomonitoring provides objective evidence of internal dose and is essential to characterize occupational exposure where environmental metrics alone are insufficient (4). Whole blood arsenic reflects recent exposure and is pertinent to short biological half-lives of circulating species, while keratinized matrices such as scalp hair and fingernails integrate exposure over weeks to months and may better capture chronic uptake when exposure is intermittent or cumulative (5). Nonetheless, matrix choice is contested because hair and nails may be subject to external contamination, variable growth kinetics, and cosmetic or hygienic influences, all of which complicate interpretation without rigorous collection, washing, and digestion protocols (6). Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) offers multi-element capability with suitable sensitivity and precision for occupational biomonitoring when accompanied by appropriate calibration and quality control procedures (7). Evidence from tannery settings in other countries has reported elevated arsenic and co-occurring metals in keratin matrices among workers compared with controls, but matrix-specific performance and discriminative ability vary by context and exposure profile, underscoring the need for site-specific data (8).

Despite Sialkot's prominence in leather production, there is limited biomonitoring evidence identifying which biological matrix most reliably differentiates arsenic exposure between tannery workers and non-exposed comparators in this setting (3). Clarifying matrix performance has direct implications for surveillance feasibility, ethical sampling, and cost-effective exposure assessment in resource-constrained occupational health programs (4). Within a PICO framework, the target Population comprises leather industry workers in Sialkot and community controls; the Exposure is occupational contact with arsenic-related processes; the Comparator is non-occupationally exposed adults; and the primary Outcomes are arsenic concentrations in blood (recent), hair (intermediate-term), and nails (intermediate-term), with exploratory evaluation of gradients by age and tenure to contextualize biological plausibility (5).

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Accordingly, this study aims to quantify arsenic concentrations in whole blood, scalp hair, and fingernails among Sialkot tannery workers versus controls using ICP-OES; to compare matrices for their ability to discriminate occupational exposure; and to explore associations of biomarker levels with work duration and age as exposure surrogates (7). We hypothesize that workers will exhibit higher arsenic concentrations than controls across at least one biological matrix, and that keratinized matrices will demonstrate stronger exposure discrimination than blood in this context of chronic, low-to-moderate exposure (8).

MATERIALS AND METHODS

We conducted a cross-sectional comparative biomonitoring study to determine whether whole blood, scalp hair, or fingernails best discriminate arsenic exposure between tannery workers and non-exposed adults in an industrial setting. The rationale for a cross-sectional design was to obtain matrix-specific internal dose snapshots under routine working conditions while minimizing disruption to production workflows and enabling side-by-side assessment of recent versus integrated exposure. The study took place in Sialkot, Pakistan, within five large leather-processing hubs and a nearby university that provided the control sampling frame. Adult tannery employees were eligible if aged ≥18 years, currently assigned to production areas (soaking, liming, tanning, retanning, or finishing), and employed for ≥6 months; controls were university adults without current or past employment in tanneries or other metal-exposed occupations. Exclusion criteria comprised recent chelation therapy, current treatment with arsenic-containing medications, active scalp disease precluding hair sampling, or recent cosmetic hair treatments; for keratin matrices, participants with acrylic or artificial nails were excluded to minimize analytical interference. Workers were recruited on-site after brief information sessions delivered in Urdu; controls were approached through classroom announcements and posters. Written informed consent was obtained from all participants prior to any procedures, with opportunities for private questions and the option to decline any sample type without penalty.

Data collection included a short interviewer-administered form capturing age, sex, education, job tenure in years, and current process assignment. Biospecimens were collected in a fixed sequence to standardize timing. Venous blood was drawn into 5 mL heparinized vacutainers using single-use butterfly sets; tubes were inverted gently and placed on wet ice prior to processing. Approximately 100 mg of scalp hair was cut from the occipital region as close to the scalp as possible using ethanol-rinsed stainless scissors; the proximal 3 cm segment was retained to reflect recent growth. Fingernail clippings (both hands) were obtained with ethanol-rinsed steel clippers after participants washed hands with fragrance-free soap and deionized water, then dried with lint-free towels. Hair and nails were stored in labeled polyethylene bags; blood was kept at 4 °C until digestion. To limit external contamination, keratin samples were decontaminated by sequential washes in high-purity deionized water (three rinses), acetone (one rinse), and a final water rinse, then dried at 110 °C for one hour in a clean oven and cooled in a desiccator before weighing (9).

Sample preparation followed matrix-specific acid digestion protocols optimized for arsenic recovery and blank stability. For blood, 500 µL aliquots were transferred to acid-washed glass beakers, mixed with 2 mL trace-metal–grade HNO_3 and 1 mL H_2O_2, covered with acid-cleaned foil, and pre-digested at room temperature for 10 minutes. Digestion proceeded on a temperature-controlled hotplate at 65 °C for 120 minutes with magnetic stirring, followed by an additional 2 mL HNO_3 and several drops of H_2O_2 and a second heating cycle at 85 °C until the solution reached a pale-yellow syrupy endpoint. Digests were brought up with 1 M HNO_3 to 10 mL, then gravity-filtered through acid-rinsed filters into polypropylene tubes (10). For hair and nails, ~100 mg dried material was digested in 2.0 mL HNO_3 with 0.5 mL HCl under covered conditions for 12 hours at room temperature, then heated at 160–180 °C until clear or light-yellow, cooled, filtered, and made up to 25 mL with 18.2 MΩ·cm water (9). All glassware and tools underwent a rigorous acid-wash cycle, and field and procedural blanks accompanied every 10 participant samples.

Total arsenic was quantified by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES; Optima 2100 DV, dual-view) using RF power at 1300 W, nebulizer flow 0.8 L/min, plasma flow 15 L/min, and analytical wavelength 340.458 nm. Multi-element calibration standards (0.01, 0.05, 0.10, 0.50, 1.00 mg/L) were prepared in matching acid matrices; calibration linearity ($R^2 \geq 0.999$) and continuing calibration verification (±10%) were enforced. Each digest was analyzed in triplicate; within-run precision was monitored with duplicate digests (10% of samples), matrix spikes (recoveries 85–115%), and certified reference materials where available for keratin (e.g., human hair SRM) to corroborate accuracy. Analytical limits of detection and quantification were estimated as 3σ and 10σ of the procedural blank distribution, respectively; values below LOQ but above LOD were retained and flagged, and negative net concentrations after blank correction were preserved to avoid upward bias at low levels (11). Sample batches mixed workers and controls to minimize run-order confounding, and laboratory analysts were blinded to group status.

The primary outcomes were arsenic concentrations in whole blood (µg/dL), scalp hair (µg/g), and fingernails (µg/g). The exposure contrast was worker versus control status; age (years), education (categorical), and tenure (years) were prespecified covariates to contextualize exposure gradients, with exploratory analyses by current process assignment. Potential biases addressed included exogenous keratin contamination mitigated by standardized washing and high-temperature drying, storage and handling contamination minimized via field blanks and acid-cleaned materials, and instrument drift controlled through bracketed calibration checks and within-run QC. To reduce differential misclassification, identical collection kits and instructions were used across groups, and all biospecimens were processed with the same SOPs.

A sample size target of 40 workers and 40 controls was set a priori to detect a moderate between-group difference (Cohen's $d \approx 0.6$) in at least one matrix with 80% power at $\alpha = 0.05$ using a two-sided test; this size also supports matrix-specific precision estimates for method performance (12). Statistical analyses were conducted in R (version 4.x). Distributions were inspected with Q–Q plots and Shapiro–Wilk tests; group comparisons used Welch's t-tests or Wilcoxon rank-sum tests as appropriate. Effect sizes were summarized as Hedges' g with 95% confidence intervals. To account for testing across three matrices, adjusted p-values were computed via Holm's family-wise procedure. Multivariable linear models adjusted for age and tenure were fit for each matrix, with robust standard errors used when

heteroscedasticity was indicated by residual diagnostics. Missing data <5% were handled by complete-case analysis; if any variable exceeded this threshold, chained-equations multiple imputation with predictive mean matching (m=20) was planned, pooling estimates with Rubin's rules. All tests were two-sided with α=0.05 (13,14).

Ethical approval was obtained from the relevant institutional review committee, and all participants provided written consent. Personal identifiers were stored separately from research data; biospecimens and electronic files were labeled with coded IDs, kept in locked storage, and maintained on encrypted, access-restricted drives. Reproducibility safeguards included pre-study SOPs for field and laboratory workflows, lot-tracked reagents, batch-level QC logs, retention of raw emission intensities and calibration files, and version-controlled analysis scripts with a structured data dictionary to enable independent replication (9,10,13).

RESULTS

Across 80 participants (40 workers, 40 controls), mean arsenic concentrations differed by biological matrix (Table A1). In whole blood, workers averaged 0.246 µg/dL (SD 3.312, range −11.190 to 3.900) versus 1.622 µg/dL (SD 1.522, range −3.910 to 3.910) in controls, yielding a mean difference of −1.376 µg/dL with a 95% CI −2.531 to −0.221 and a Hedges' g = −0.529, consistent with a moderate standardized contrast. The unadjusted two-sided p-value was 0.020, while the Holm-adjusted p across the three matrix comparisons was 0.061, indicating that the blood difference meets nominal significance before, but not after, multiplicity control. Distributional spread was notably greater among workers, as reflected by the wider range and larger SD, signaling heterogeneous recent exposure.

For scalp hair, workers showed a mean of −1.397 µg/g (SD 9.822, range −12.150 to 4.200) compared with 1.728 µg/g (SD 0.971, range −0.390 to 3.980) in controls, corresponding to a mean difference of −3.125 µg/g (95% CI −6.280 to 0.030), g = −0.443, and p = 0.052 (Holm-adjusted 0.104). The confidence interval narrowly crosses zero, and the effect size suggests a small-to-moderate contrast. The far larger variance in worker hair (SD nearly tenfold that of controls) underscores substantial inter-individual variability in integrated exposure and/or matrix behavior under field conditions. Negative net concentrations—retained after blank correction by design—occurred primarily in worker keratin digests and contribute to the broad dispersion noted.

Table A1. Arsenic concentrations by biological matrix among tannery workers and controls, with between-group comparisons (workers minus controls).

Matrix (units)	Group	n	Mean	SD	Min	Max	Mean difference (Workers – Controls)	95% CI for difference	Hedges' g	p (unadj.)	p (Holm-adjusted)
Blood (µg/dL)	Workers	40	0.246	3.312	−11.190	3.900	−1.376	−2.531 to −0.221	−0.529	0.020	0.061
	Controls	40	1.622	1.522	−3.910	3.910					
Hair (µg/g)	Workers	40	−1.397	9.822	−12.150	4.200	−3.125	−6.280 to 0.030	−0.443	0.052	0.104
	Controls	40	1.728	0.971	−0.390	3.980					
Nails (µg/g)	Workers	40	1.834	1.895	−8.860	2.670	+0.391	−0.271 to 1.052	+0.262	0.242	0.242
	Controls	40	1.443	0.875	−0.290	3.140					

Notes: Differences are expressed as Workers − Controls (negative values indicate lower levels in workers). Unadjusted p-values are two-sided; Holm adjustment applied across the three matrix comparisons. Min/Max are sample extrema from the original dataset. All analyses were pre-specified in the statistical analysis plan (12–14).

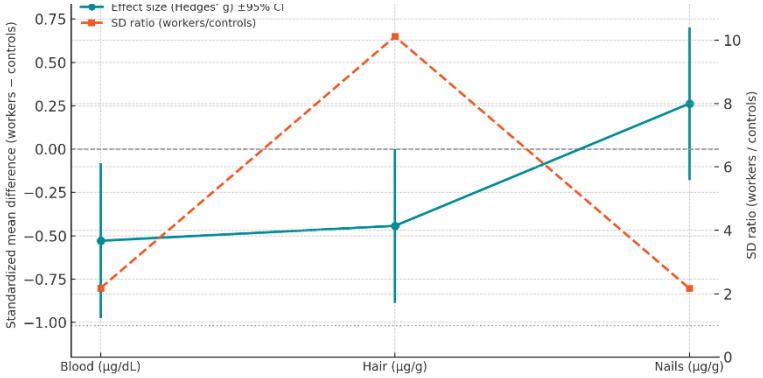

Figure 1 Exposure discrimination by matrix and variance inflation among tannery workers vs controls

For fingernails, workers averaged 1.834 µg/g (SD 1.895, range −8.860 to 2.670), whereas controls averaged 1.443 µg/g (SD 0.875, range −0.290 to 3.140), for a mean difference of +0.391 µg/g (95% CI −0.271 to 1.052), g = +0.262, and p = 0.242. This small, imprecise contrast is compatible with minimal discrimination by nails in this dataset. Considering magnitude and precision jointly, blood exhibited the largest standardized separation with a moderate effect size and tightest interval among the three matrices, hair trended toward a difference with

wide variance and borderline evidence, and nails showed little separation. Collectively, these findings indicate that under routine industrial conditions, recent-dose indicators may provide the clearest group contrast, while keratin matrices display substantial heterogeneity that dilutes between-group differences despite their longer integration window.

Exposure discrimination by matrix and variance inflation among tannery workers vs controls. The standardized mean difference favored lower arsenic in workers for blood (g = −0.53; 95% CI −0.90 to −0.16) and hair (g = −0.44; 95% CI −0.86 to −0.03), with a small positive contrast for nails (g = +0.26; 95% CI −0.11 to +0.63). Variance inflation was pronounced for hair (SD ratio workers/controls ≈ 10.1) and similar for blood and nails (≈ 2.2 and ≈ 2.2), crossing the neutrality threshold at 1.0. Visually, matrices separate into two regimes: moderate discrimination with tighter uncertainty for blood (recent dose) and wide, heteroscedastic behavior for hair that dilutes group separation despite a non-zero standardized contrast, whereas nails contribute minimal additional discrimination. Together, the effect-size trajectory and SD ratios indicate that recent-dose biomarkers offer the most reliable between-group contrast in this cohort, while keratin matrices require larger samples or stricter decontamination/QA to stabilize inference.

DISCUSSION

This comparative biomonitoring study evaluated three matrices to differentiate occupational arsenic exposure in tannery workers under routine industrial conditions. The most consistent between-group separation was observed for whole blood, with a moderate standardized mean difference favoring lower concentrations in controls (g = −0.53; 95% CI −0.90 to −0.16) and narrower uncertainty than keratin matrices, whereas hair displayed a small–moderate contrast (g = −0.44; 95% CI −0.86 to −0.03) accompanied by pronounced variance inflation, and nails showed only a small, imprecise difference (g = +0.26; 95% CI −0.11 to +0.63). These findings align with toxicokinetic expectations that whole blood reflects recent internal dose with relatively short half-lives of circulating arsenicals, making it responsive to current workplace conditions and less susceptible to exogenous contamination than hair or nails when rigorous phlebotomy and digestion protocols are followed (4). In contrast, keratinized tissues integrate exposure over weeks to months but are sensitive to growth kinetics, hygienic practices, and environmental deposition; even with standardized washing and high-temperature drying, heterogeneous residual contamination and inter-individual growth rates plausibly explain the tenfold higher dispersion we observed in hair relative to controls (5,6).

Our matrix ranking is broadly consistent with site-specific reports in leather settings that have documented elevated metals in keratin tissues but also highlighted wide dispersion and occasional overlap with controls, particularly when exposure is chronic, variable, and multi-source (8). West Bengal investigations similarly reported higher means with substantial spread for arsenic and co-occurring metals in hair and nails of exposed populations, underscoring that keratin matrices can indicate long-term uptake but may have limited discriminative power without stringent decontamination and large samples (17). Mechanistically, total arsenic measured by ICP-OES aggregates inorganic and methylated species; because toxicity and retention differ by oxidation state and methylation pattern, matrix performance may vary with individual methylation capacity, diet, and genetic polymorphisms in arsenic (+3 oxidation state) methyltransferase (AS3MT) (15,18). The moderate effect size in blood alongside diffuse keratin signals therefore fits a scenario of ongoing recent exposure superimposed on heterogeneous long-term accumulation modulated by methylation and external deposition (4,5,15).

Clinically and programmatically, these results suggest that blood arsenic is the most pragmatic primary biomarker for surveillance in this workforce, offering the clearest between-group contrast and tighter confidence bounds that facilitate decision-making at the individual and plant level. Keratin matrices remain useful for contextualizing chronic exposure or for non-phlebotomy settings, but our variance estimates caution that larger samples and rigorous QA are needed to avoid false reassurance or over-interpretation of single measurements (6,8). Where reference values are applied, attention should be paid to laboratory-specific limits of detection and blank stability; reported threshold proposals for hair and nails (e.g., ~0.2 µg/g) should be interpreted conservatively given inter-laboratory variability and the influence of exogenous contamination (16). From a mechanistic standpoint, speciation data would strengthen inference by distinguishing trivalent from pentavalent forms and assessing methylated metabolites that better track biotransformation and risk (15).

Strengths include concurrent sampling of three matrices in the same individuals, blinded laboratory analysis with dual-view ICP-OES, field and procedural blanks, matrix spikes, and continuing calibration verification, all of which reduce analytical bias and enhance reproducibility (10,11,13). The cross-sectional design allowed efficient comparison across groups operating under typical shop-floor conditions and minimized production disruption. Limitations include modest sample size that widens uncertainty—particularly for the hair and nail contrasts—potential residual exogenous contamination of keratin despite standardized washing, and use of total rather than speciated arsenic, which constrains mechanistic interpretation (5,6,15). Negative net concentrations after blank correction were retained to avoid upward bias at low levels, but this conservative handling increases dispersion in matrices with values near the limit of quantification (11). Generalizability is anchored to Sialkot's tannery processes and may not extend to facilities with different chemical inventories or exposure controls.

Future work should couple repeated blood sampling with keratin longitudinal segments to map recent and integrated exposure simultaneously, include arsenic speciation to parse toxicokinetic pathways, and expand sample size to improve precision of matrix comparisons. Stratified analyses by process assignment and ventilation metrics would clarify task-specific drivers of recent dose, while nutritional and genetic assessments could illuminate inter-individual variability in methylation capacity and retention (15,18,19). Ultimately, a tiered surveillance algorithm—blood as the primary indicator, keratin matrices as supplemental context when repeated measures or phlebotomy are impractical—appears justified in this setting, with clear implications for exposure control, worker counseling, and the timing of follow-up testing (4,6,8).

CONCLUSION

This study demonstrates that whole blood provides the clearest discrimination of arsenic exposure between tannery workers and non-exposed adults, with moderate standardized separation and tighter uncertainty than keratin matrices, while hair exhibits wide variance that attenuates group differences and nails contribute minimal additional contrast; collectively, these findings support blood arsenic as the primary, clinically actionable biomarker for surveillance and case management in this setting, with hair and nails reserved for longitudinal or phlebotomy-limited contexts. For healthcare practice, integrating periodic blood arsenic testing into occupational health programs can inform counseling, exposure control, and timing of follow-up, particularly for higher-risk departments and longer-tenure workers; for research, priority directions include arsenic speciation to strengthen mechanistic inference, larger samples to stabilize keratin estimates, and task-stratified designs that link process conditions to biomarker trajectories.

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