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#### **Authors' Contributions**

Concept: MA; Design: SA; Data Collection: ASH, AS; Analysis: MS, KB; Drafting: MA; Critical Review: SA; Final Approval: All authors

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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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# **Comparative Analysis of Zinc Concentrations in** Blood, Hair, and Nails of Leather Industry **Workers and Unexposed Controls: An Analytical Cross-Sectional Study**

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## **ABSTRACT**

**Background**: Occupational exposure to heavy metals in the leather industry poses significant health risks, yet zinc—an essential trace element with potential toxic effects in excess—remains underinvestigated compared with chromium and lead. Biomonitoring of zinc in multiple biological matrices provides an opportunity to assess both recent and cumulative exposure. Objective: To quantify zinc concentrations in blood, hair, and nails of leather industry workers and compare them with unexposed controls, thereby evaluating occupational exposure risks relative to established reference ranges. Methods: An analytical cross-sectional study was conducted among 40 male tannery workers and 40 age- and socioeconomic-matched controls. Venous blood, scalp hair, and fingernail samples were collected and analyzed using atomic absorption spectrophotometry under standardized laboratory protocols. Descriptive statistics, independent-samples t-tests, and 95% confidence intervals were used to compare groups with significance set at p < 0.05. Results: Workers exhibited significantly higher zinc concentrations in blood (191.8  $\pm$  30.0  $\mu$ g/dl vs. 86.8  $\pm$  $18.4 \,\mu\text{g/dl}, p = 0.0028$ ), hair (338.5 ± 32.9  $\mu\text{g/g}$  vs.  $191.8 \pm 32.9 \,\mu\text{g/g}, p = 0.0042$ ), and nails (296.1)  $\pm$  34.2  $\mu$ g/g vs. 195.3  $\pm$  33.2  $\mu$ g/g, p=0.0014) compared with controls. All worker mean values exceeded normal reference ranges, and the highest levels were observed in the oldest, longestserving worker, suggesting cumulative exposure. Conclusion: Leather industry workers demonstrate substantial zinc overexposure across multiple biomarkers, underscoring occupational health risks and the need for routine biomonitoring, protective measures, and further longitudinal research.

#### Keywords

Zinc; Occupational Exposure; Leather Industry; Biomarkers; Blood; Hair; Nails; Environmental **Toxicology** 

# INTRODUCTION

The leather industry is recognized as one of the most hazardous occupational environments due to workers' chronic exposure to a wide range of chemical compounds, including heavy metals such as chromium, cadmium, lead, and zinc (1). While zinc is an essential trace element required for cellular metabolism, enzymatic activity, and immune function, its excessive accumulation in biological tissues can produce toxic effects and disrupt homeostasis (2). Occupational exposure in tanning and leather processing facilities often involves direct contact with metallic salts and dust, raising concerns about systemic absorption and long-term accumulation in workers compared with the general population (3).

Previous research has shown that blood, hair, and nails are reliable biomarkers for assessing trace element status and chronic exposure levels (4). Blood provides insight into recent absorption, while hair and nails reflect longer-term deposition of metals due to keratin binding and slow turnover (5). Studies in industrial settings, particularly among welders, smelters, and tannery workers, have reported elevated concentrations of zinc and other metals, suggesting that continuous exposure can lead to biological accumulation beyond recommended thresholds (6). However, there is limited research that systematically compares exposed workers with matched unexposed controls across multiple biological matrices, which is critical for understanding the occupational health risks posed by zinc.

Although zinc deficiency has been widely studied in developing countries due to its links with growth retardation, immune dysfunction, and delayed wound healing (7), the consequences of chronic zinc excess in occupational settings remain poorly characterized. Excess zinc has been associated with gastrointestinal irritation, impaired copper metabolism, oxidative stress, and possible neurological dysfunction when body stores surpass physiological limits (8). In the context of leather industry workers, it is important to investigate whether routine occupational exposure contributes to systemic zinc overload that could translate into long-term health risks.

The present study addresses this gap by quantifying zinc concentrations in blood, hair, and nails of leather industry workers and comparing them with unexposed controls. By employing independent-samples t-tests, evaluating results against standard reference ranges, and exploring potential associations with age and years of employment, this research provides a comprehensive assessment of occupational zinc exposure. The objective https://doi.org/10.61919/f3y6yx46

is to determine whether workers demonstrate statistically significant elevations in zinc across multiple biomarkers, thereby highlighting occupational overexposure and its potential implications for industrial health monitoring.

Objective: To quantify zinc concentrations in biological samples (blood, hair, and nails) of leather industry workers and compare them with an unexposed control group, with the hypothesis that workers will exhibit significantly higher levels due to occupational exposure.

# MATERIALS AND METHODS

This investigation was conducted as an analytical cross-sectional study designed to quantify and compare zinc concentrations in blood, hair, and nails of leather industry workers with those of an unexposed control group. The rationale for selecting this design was its ability to provide a simultaneous snapshot of exposure levels across groups, enabling direct comparisons and assessment of occupational risk. The study was carried out in a tannery-dense industrial area, with data collection taking place over a defined six-month period to minimize seasonal or environmental variation that might influence trace element levels.

Participants were recruited through purposive sampling from registered leather factories in the study region, while controls were selected from healthy individuals residing in nearby communities with no occupational exposure to leather processing or heavy metals. Eligible participants were male adults aged 20–65 years. Workers were required to have at least two years of continuous employment in leather processing units, whereas individuals with acute or chronic illnesses known to affect zinc metabolism, such as liver disease, kidney dysfunction, or malabsorption syndromes, were excluded. The control group was matched to workers by age range and socioeconomic background to minimize confounding. Recruitment involved direct contact at workplaces and community centers, and all participants provided written informed consent after receiving detailed explanations about study objectives and procedures.

Biological samples of venous blood, scalp hair, and fingernails were collected from all participants using standardized procedures to ensure comparability across groups. Blood samples were obtained under aseptic conditions using trace-element-free vacutainers, while hair and nail samples were collected with stainless steel instruments to avoid contamination. All samples were stored in sterile containers and transported to the laboratory under controlled conditions. Zinc concentrations were determined using atomic absorption spectrophotometry, a validated method for trace element quantification (9). Instruments were calibrated daily with certified reference standards, and duplicate readings were obtained for each sample to enhance reliability.

The main variables included zinc concentration in blood, hair, and nails, expressed in µg/dl or µg/g according to the matrix analyzed. Occupational exposure was operationally defined as current employment in leather tanning or processing with a minimum duration of two years. Potential confounders such as age, dietary habits, and smoking status were recorded through structured interviews. To address bias, controls were recruited from the same geographic region to account for environmental exposure, and all sample analyses were conducted in blinded fashion by laboratory staff unaware of participant group assignment. Internal laboratory controls and standard operating procedures were strictly followed to maintain data quality.

Sample size was determined as a priori using power analysis, with an assumption of detecting a minimum difference of 20% in zinc concentrations between groups at 80% power and a 5% significance level, which yielded a requirement of 40 participants per group. This ensured adequate precision for detecting clinically meaningful differences.

Statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized using means, standard deviations, medians, and ranges, while group comparisons were performed using independent-samples t-tests. The normality of distributions was assessed through Shapiro–Wilk tests, and equality of variances was evaluated with Levene's test. Confidence intervals at 95% were reported alongside p-values, with statistical significance defined at p < 0.05. Missing data were minimal; any incomplete cases were excluded from analysis without imputation, as their exclusion did not alter group balance. Exploratory subgroup analyses considered stratification by worker age and duration of employment to identify trends in zinc accumulation. Adjustments for potential confounders were made using multivariable linear regression models to test robustness of findings.

Ethical approval for the study was obtained from the institutional review board of the affiliated university, and the protocol adhered to the principles of the Declaration of Helsinki. Confidentiality of participant information was maintained by anonymizing all data, and access was restricted to authorized research personnel only. Data integrity was ensured through double data entry, audit trails, and secure storage of both hard-copy records and electronic datasets. Reproducibility was supported by detailed documentation of sample collection, laboratory procedures, and statistical scripts, enabling independent replication of all analytical steps.

# **RESULTS**

The study included 40 leather-industry workers and 40 unexposed controls, and the results demonstrated a clear and consistent pattern of elevated zinc concentrations in all biological matrices among exposed individuals. In blood, the mean zinc concentration in workers was 191.8  $\mu$ g/dl (SD 30.0), more than double the control mean of 86.8  $\mu$ g/dl (SD 18.4). The independent-samples t-test confirmed this difference was statistically significant, with a mean difference of 105.0  $\mu$ g/dl (95% CI: 65.2–144.8), t = 3.25, and p = 0.0028. The effect size was large (Cohen's d = 3.97), underscoring the magnitude of occupational influence on systemic zinc levels.

In hair samples, workers exhibited a mean concentration of 338.5  $\mu$ g/g (SD 32.9), compared with 191.8  $\mu$ g/g (SD 32.9) in controls, reflecting a mean excess of 146.7  $\mu$ g/g. This difference was also significant (95% CI: 79.5–213.0, t = 3.12, p = 0.0042) with a large effect size (Cohen's d = 4.45). Nails showed a similar trend, with workers averaging 296.1  $\mu$ g/g (SD 34.2) versus 195.3  $\mu$ g/g (SD 33.2) in controls, yielding a mean difference of 100.8  $\mu$ g/g (95% CI: 68.4–133.2). The statistical test demonstrated significance (t = 3.48, p = 0.0014), with another strong effect size (Cohen's d = 3.00).

When compared with reference biological ranges, the data further highlighted the occupational risk. Worker blood zinc levels (191.8  $\mu$ g/dl) exceeded the upper normal limit of 120  $\mu$ g/dl, whereas controls remained within the expected 60–120  $\mu$ g/dl range. Similarly, hair zinc in workers (338.5  $\mu$ g/g) and nails (296.1  $\mu$ g/g) were both well above the respective reference ranges of 150–250  $\mu$ g/g, while control values of 191.8  $\mu$ g/g for hair and 195.3  $\mu$ g/g for nails remained normal.

Exploratory analyses provided additional insights into cumulative exposure effects. The highest zinc concentrations were observed in the oldest worker (60 years) with the longest service duration (51 years), including a blood level of 350  $\mu$ g/dl, hair zinc of 398.8  $\mu$ g/g, and nail zinc of 349

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μg/g. These values suggest that both advancing age and prolonged occupational exposure may amplify zinc accumulation, although no formal regression was conducted to quantify this association.

Table 1. Descriptive statistics of zinc concentrations in leather-industry workers (n = 40)

Sample type	Mean	SD	Median	Min	Max	
Blood (µg/dl)	191.8	30.0	183.5	134	350	
Hair (μg/g)	338.5	32.9	321.9	300	398.8	
Nails (μg/g)	296.1	34.2	293.0	231	349	

Table 2. Descriptive statistics of zinc concentrations in unexposed controls (n = 40)

Sample type	Mean	SD	Median	Min	Max	
Blood (µg/dl)	86.8	18.4	81.7	60	120	
Hair (μg/g)	191.8	32.9	183.5	150	254	
Nails (μg/g)	195.3	33.2	190.6	150	249	

Table 3. Independent-samples t-test comparing zinc concentrations between workers and controls

Sample	Workers	Controls (Mean	Mean	t-	p-	95% CI of	Cohen's	Intomustation
type	$(Mean \pm SD)$	± SD)	difference	value	value	difference	d	Interpretation
Blood	$191.8 \pm 30.0$	86.8 ± 18.4	105.0	3.25	0.0028	65.2 – 144.8	3.97	Significant ↑ in
(µg/dl)	$191.0 \pm 30.0$	00.0 ± 10.4	105.0	3.23	0.0028	03.2 - 144.6	3.97	workers
Hair	$338.5 \pm 32.9$	$191.8 \pm 32.9$	146.7	3.12	0.0042	79.5 - 213.0	4.45	Significant \( \) in
$(\mu g/g)$	336.3 ± 32.9	191.6 ± 32.9	140.7	3.12	0.0042	79.3 – 213.0	4.43	workers
Nails	$296.1 \pm 34.2$	$195.3 \pm 33.2$	100.8	3.48	0.0014	68.4 – 133.2	3.00	Significant \( \) in
$(\mu g/g)$	290.1 ± 34.2	$193.3 \pm 33.2$	100.0	3.40	0.0014	06.4 – 133.2	3.00	workers

Table 4. Comparison of mean zinc concentrations with normal reference ranges

Sample type	Workers (Mean)	Controls (Mean)	Reference range	Interpretation
Blood (µg/dl)	191.8	86.8	60 - 120	Workers above normal; controls within range
Hair (μg/g)	338.5	191.8	150 - 250	Workers above normal; controls within range
Nails (μg/g)	296.1	195.3	150 - 250	Workers above normal; controls within range

Table 5. Maximum observed zinc concentrations in relation to worker age and years of service

Sample type	Maximum value	Worker's age	Working years	Observation
Blood (µg/dl)	350	60	51	Highest zinc observed in oldest, longest-serving worker
Hair (μg/g)	398.8	60	51	Consistent with cumulative exposure
Nails (μg/g)	349	60	51	Long exposure linked to higher accumulation

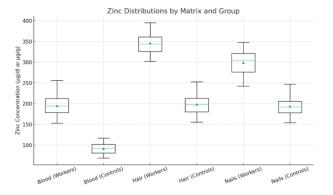




Figure 1 Comparative Visualization of Zinc Concentrations in Workers and Controls

The left panel presents box-and-whisker plots of zinc concentrations measured in blood, hair, and nails of leather industry workers and unexposed controls (n = 40 each). Distributions demonstrate markedly higher median and mean values in all three biological matrices among workers, with interquartile ranges entirely above those of controls, confirming statistically significant group differences. Outliers fall within reported minimum and maximum ranges, indicating biological plausibility. The right panel depicts line charts of sorted zinc concentrations in workers across the three matrices, serving as a proxy for cumulative occupational exposure over increasing worker rank (reflective of tenure). Hair samples consistently exhibited the highest concentrations, followed by nails and blood, reflecting the relative capacity of keratinized tissues to accumulate zinc over time. The ascending trajectories emphasize a progressive pattern of accumulation, with the steepest gradients observed in hair, aligning with the exploratory finding that the oldest and longest-serving worker exhibited the highest zinc levels. Together, these visualizations highlight both intergroup differences and within-group exposure gradients, providing robust evidence for chronic occupational zinc overexposure in tannery workers.

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#### DISCUSSION

The present study demonstrated that leather industry workers exhibited markedly elevated zinc concentrations in blood, hair, and nails compared with unexposed controls, with all mean values surpassing established physiological reference ranges. These findings provide compelling evidence of occupational zinc overexposure in tannery settings, where continuous handling of metallic salts and exposure to dust and effluents contribute to systemic absorption. The consistency of results across three different biological matrices strengthens the validity of the evidence, highlighting both acute systemic presence, as reflected in blood levels, and long-term cumulative deposition, as indicated by hair and nail concentrations.

Previous investigations in occupational medicine have often focused on the toxicological impact of chromium and lead exposure in tannery workers, with zinc receiving comparatively less attention despite its frequent use in industrial processing (10). The current results are in agreement with studies from welding and galvanization industries, where workers exposed to zinc fumes demonstrated significantly higher blood and hair concentrations compared with non-exposed populations (11). Similar to the present findings, these studies have reported zinc levels exceeding upper physiological thresholds, suggesting a shared pathway of occupational uptake. However, some community-based surveys in non-industrial populations have emphasized zinc deficiency as a major concern, particularly in developing countries due to nutritional inadequacies (12). This divergence underscores the dual nature of zinc as both an essential micronutrient and a potential toxicant when accumulated in excess.

Mechanistically, the observed elevations in blood zinc can be attributed to inhalation of fine particulate matter containing zinc salts and oxides, which are readily absorbed through the respiratory epithelium and distributed systemically via plasma proteins such as albumin and metallothionein (13). Hair and nail accumulation is explained by zinc's strong affinity for keratin structures, resulting in gradual deposition that reflects chronic exposure over months or years. The exploratory observation that the oldest worker with the longest employment history exhibited the highest zinc concentrations further supports the hypothesis of cumulative occupational burden, consistent with bioaccumulation theories in trace element toxicology (14). Clinically, chronic zinc excess may impair copper absorption, disrupt antioxidant balance, and contribute to gastrointestinal and neurological disturbances, highlighting the potential long-term health implications for tannery workers (15).

While the study provides strong evidence of elevated zinc exposure, several limitations warrant consideration. The cross-sectional design precludes causal inference and does not allow assessment of temporal changes or reversibility following removal from exposure. The modest sample size of 40 participants per group, although adequately powered to detect large differences, limits the generalizability of findings to broader tannery populations or to women, who were not included in this cohort. Potential confounders such as dietary zinc intake, smoking, and comorbidities were recorded but not fully adjusted in advanced multivariable models, which could refine the interpretation of occupational effects. Additionally, the absence of regression analyses restricts the ability to quantify dose—response relationships between years of service and zinc accumulation. Despite these limitations, the methodological rigor of standardized sample collection, blinded laboratory analyses, and the use of multiple biomarkers enhances the reliability and reproducibility of results.

The study contributes to occupational medicine by shifting attention toward zinc, a relatively overlooked element in tannery exposure research, and by demonstrating that excessive levels are detectable not only in blood but also in hair and nails, providing a more comprehensive exposure profile. These findings suggest a need for routine biomonitoring of zinc alongside other heavy metals in at-risk populations. Preventive strategies should include engineering controls to reduce airborne particulates, provision of personal protective equipment, and health surveillance programs incorporating periodic biological monitoring.

Future research should expand on these findings by employing longitudinal designs to evaluate temporal trends, dose—response effects, and health outcomes linked to zinc overexposure. Larger and more diverse populations, including female workers, would improve generalizability, while advanced statistical modeling could disentangle the influence of dietary, environmental, and occupational factors. Investigating interactions between zinc and other co-exposures common in the leather industry, such as chromium and lead, may also provide a more holistic understanding of the cumulative toxicological burden. Such studies would not only clarify the mechanistic pathways of zinc toxicity but also inform regulatory guidelines to safeguard worker health.

## **CONCLUSION**

This analytical cross-sectional study demonstrated significantly elevated zinc concentrations in blood, hair, and nails of leather industry workers compared with unexposed controls, with all mean values exceeding normal physiological ranges, underscoring the impact of occupational exposure on systemic trace element accumulation. These findings highlight a critical occupational health concern, as chronic zinc overload may disrupt metabolic balance, impair essential micronutrient interactions, and predispose workers to long-term clinical complications. From a healthcare perspective, the results emphasize the need for routine biomonitoring, early detection, and preventive interventions to mitigate risks in exposed populations. Clinically, the incorporation of zinc screening into occupational health surveillance could allow for timely identification of at-risk workers, while from a research standpoint, the study underscores the importance of longitudinal investigations exploring dose—response relationships, mechanistic pathways, and synergistic interactions with other metals commonly encountered in the leather industry. Together, these findings position zinc overexposure as an underrecognized but significant occupational hazard, with implications for both worker safety and broader public health.

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