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*Original article*

# Nail Trace Element Profiling in Cancer Patients versus Healthy Controls: A Pilot Case-Control Study Using Energy-Dispersive X-ray Fluorescence Spectroscopy in Sebha City, Libya

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**ABSTRACT**
Trace element dysregulation is increasingly implicated in carcinogenesis, yet, to our knowledge, the nail metallome of Libyan cancer patients has not previously been characterized. Toenails provide a time-integrated, non-invasive biomarker of systemic metal exposure that is well suited to resource-constrained clinical settings. This pilot case–control study enrolled 16 histologically confirmed cancer patients and 7 healthy controls at Sebha Medical Oncology Centre, Libya. Concentrations of 12 elements (Cu, Zn, Se, Mn, Fe, Pb, Cd, As, Cr, Ni, Hg, Al) were measured in toenail specimens by energy-dispersive X-ray fluorescence (EDXRF) spectroscopy. Statistical comparisons used Mann–Whitney U and independent t-tests, with Bonferroni correction for multiple comparisons. Cancer patients showed significantly elevated copper (22.40 vs 12.86 µg/g; p = 0.0002) and reduced zinc (80.79 vs 127.26 µg/g; p = 0.0001) and selenium (0.55 vs 1.15 µg/g; p = 0.0001) relative to controls (all Bonferroni-corrected). Six of seven toxic metals (Pb, Cd, As, Cr, Ni, Hg) were significantly elevated after correction; exceedance of reference thresholds reached 100% for lead and 81.2% for arsenic. The Cu/Zn ratio was substantially higher in cancer patients (0.294 vs 0.101; p < 0.001; Cohen's d = 2.52), with 75% of cases exceeding the proposed threshold of 0.25. Robust inter-element antagonisms were identified between selenium and mercury (rho = −0.745) and between zinc and arsenic (rho = −0.674). This pilot study provides the first local evidence of nail trace element dyshomeostasis among Libyan cancer patients, characterized by concurrent essential element depletion and toxic metal accumulation. The Cu/Zn ratio emerges as a candidate composite biomarker. These hypothesis-generating findings support a larger, prospective, age- and sex-matched investigation.
**Keywords.** Nail Metallome, Trace Elements, Cancer, EDXRF, Copper–Zinc Ratio.

## Introduction

Cancer has become one of the defining public health challenges of the twenty-first century: GLOBOCAN 2020 estimates from the International Agency for Research on Cancer (IARC) recorded approximately 19.3 million new cases and 10.0 million deaths worldwide in 2020, with projections reaching 28.4 million new cases annually by 2040 [1]. In Libya, the burden is expanding, with roughly 7,750 new diagnoses and 5,290 cancer deaths recorded in 2020 [2]; breast cancer accounts for an estimated 14.3% of all Libyan malignancies, followed by colorectal (11.9%) and lung (11.4%) cancers, a distribution broadly confirmed by regional registry data from Tobruk, Benghazi, and Tripoli [3,4]. Among the potentially modifiable contributors to cancer risk, chronic trace metal dysregulation warrants particular attention: the term “metallome,” used here in an informal sense analogous to the genome or proteome, denotes the panel of metal ions measurable within a biological system [5].

Trace elements play dualistic roles in human physiology. Essential metals copper (Cu), zinc (Zn), selenium (Se), manganese (Mn), and iron (Fe) serve as cofactors for antioxidant enzymes, DNA repair machinery, and immune effectors [6]; their depletion can impair genomic integrity and facilitate malignant transformation. Toxic metals lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), nickel (Ni), mercury (Hg), and aluminium (Al) are classified by IARC as Group 1 or 2A human carcinogens acting through oxidative stress induction, epigenetic modification, and interference with essential metal metabolism [7,8]. Toenails offer a compelling biomonitoring matrix for this question: trace elements bind to keratin sulfhydryl groups during nail matrix synthesis, so toenail clippings accumulate a time-integrated record of systemic metal exposure spanning roughly the preceding four to twelve months [9,10].

Unlike blood or urine, which reflect only recent exposure, nails preserve this longer biological window without requiring refrigeration or rapid processing, a practical advantage in a resource-constrained clinical setting [9,10]. Standardized decontamination removes surface contaminants without altering endogenous concentrations [10], and nail concentrations of selenium, arsenic, cadmium, and mercury correlate meaningfully with dietary intake and environmental exposure in prior biomonitoring work [11]. Energy-dispersive X-ray fluorescence (EDXRF) spectroscopy allows simultaneous, non-destructive quantification of multiple elements in biological matrices, including nails, with minimal sample preparation [12,13]; its suitability for resource-constrained settings, where inductively coupled plasma-mass spectrometry may not be accessible, makes it an attractive option for Libyan clinical research, though its sensitivity is not uniform across all target elements — a limitation with direct implications for interpreting some of the findings reported below [14].

No published study, to our knowledge, has examined nail trace element profiles in Libyan cancer patients, and Libya's environmental context, including oil extraction and petrochemical processing, metalworking, phosphate mining, and years of civil disruption affecting environmental monitoring, may have shaped population-level toxic metal exposures in ways that


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no externally generated dataset can capture [15,16]. The copper-to-zinc ratio (Cu/Zn) has emerged as a promising composite biomarker in cancer biology: a large Swedish prospective cohort study found that an elevated serum Cu/Zn ratio was independently associated with poorer overall survival in breast cancer patients [17], yet this ratio has never been evaluated in a Libyan cohort, and combining EDXRF elemental profiling with conventional clinical biochemistry an approach that might surface associations between metal status and standard laboratory markers has not previously been applied in Libyan cancer research. This pilot study was accordingly designed with two pragmatic objectives: to generate the first local data on nail trace element profiles in Libyan cancer patients and healthy controls, measuring 12 elements simultaneously, and to estimate effect sizes and assess analytical feasibility to inform the design of a properly powered, age- and sex-matched investigation. Given the sample ultimately achieved 16 cases and 7 controls, substantially below the a priori target of 98 per group, all findings below are presented as hypothesis-generating rather than confirmatory, a framing maintained throughout.

## Methods

### *Study Design and Setting*

This was a pilot, cross-sectional case–control study conducted at Sebha Medical Oncology Centre, Sebha City, southwestern Libya. Cancer patients presenting to the oncology clinic were enrolled as cases and compared with healthy community volunteers (controls) with respect to nail trace element concentrations and a panel of clinical biochemistry parameters. An a priori sample size calculation using G*Power software (version 3.1; Heinrich-Heine-Universität Düsseldorf, Germany), targeting a medium effect size (Cohen's d = 0.40), alpha = 0.05, and 80% power, indicated 98 participants per group [18]. The final achieved sample of 23 participants (16 cases, 7 controls) fell substantially short of this target, reflecting limited patient flow through the single regional oncology service, difficulty recruiting healthy volunteers willing to provide nail specimens without direct clinical benefit, and broader disruption to research infrastructure during the study period. Consistent with established guidance on the conduct of pilot studies [19], this shortfall is treated as a defining design feature rather than an incidental limitation, and its implications are carried through explicitly in the Results and Discussion.

### *Participants*

Cases were patients aged 18 years or older with a histologically confirmed malignancy of any site, newly diagnosed or currently under active treatment, who were willing and able to provide written informed consent and sufficient toenail material. Controls were healthy community volunteers with no personal history of cancer, screened by self-reported history and basic clinical assessment. Exclusion criteria for both groups included prior chelation therapy for metal toxicity and a history of high-level occupational metal exposure, for example, smelting or battery manufacturing. A predefined frequency-matching protocol for age (within five years) and sex could not be implemented because of recruitment constraints. The resulting demographic imbalance, in that all seven controls were female with a mean age of 30.4 years, while cases were 81.2% female with a mean age of 57.7 years, represents a 27.3-year difference and a major unresolved confound, particularly for bioaccumulating toxic metals such as lead and cadmium, whose nail concentrations are strongly age-dependent [20].

### *Ethics*

This study was conducted in accordance with the Declaration of Helsinki (2013 revision) [21] and received prior approval from the Institutional Review Board of the Faculty of Medical Technology, Sabha University, and the Ethics Committee of Sebha Medical Oncology Centre. Written informed consent (2026/5/568) was obtained from all participants before enrolment. Participants were assigned unique study identification numbers; personal identifiers were stored separately on encrypted devices accessible only to the principal investigator.

### *Nail Specimen Collection and Preparation*

Toenail specimens were collected following a standardized protocol adapted from established epidemiological biomonitoring procedures [10,22]. Participants refrained from nail polish or cosmetic treatments for at least two weeks before collection. All available toenail clippings from both feet were collected using stainless steel clippers cleaned with acetone and deionized water between participants, targeting a minimum of 50 mg per specimen. Samples were decontaminated sequentially: a 10-minute soak in 1% Triton X-100 detergent (Sigma-Aldrich, St. Louis, MO, USA) with gentle agitation; three 5-minute rinses with deionized water; a 5-minute acetone wash; a final deionized water rinse; and drying at 60–70°C for 12–24 hours until constant weight. Dried specimens were pressed into acid-washed sample cups covered with 4-µm polypropylene film for analysis.

### *EDXRF Analysis*

Elemental analysis was performed using a silicon-drift-detector EDXRF spectrometer (Bruker S2 PUMA; Bruker AXS GmbH, Karlsruhe, Germany), fitted with a 50 W X-ray tube (operating range up to 50 kV, 2 mA) and a 10-position primary beam filter changer, configured for simultaneous quantification of 12 target elements. Calibration used National Institute of Environmental Studies (NIES) Certified Reference Materials No. 13 and No. 5 (Human Hair), with additional NIST standard


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reference materials for secondary verification; calibration curves required $r^2 > 0.99$ [13,23]. Method detection limits ranged from approximately 0.01 µg/g for Cd and Hg to 1–5 µg/g for Fe and Zn. A specific analytical caveat applies to selenium: its low fluorescence yield and spectral overlap with matrix elements mean that EDXRF detection limits sit close to the sub-1.5 µg/g concentrations measured in both groups, so selenium findings are presented as indicative rather than as precise quantitative estimates, and confirmation by an orthogonal method such as ICP-MS is recommended for future work [23,24]. Quality control included CRM recovery checks (target 85–115%; observed 97.3%), procedural blanks, random duplicate analyses (relative percent difference < 15%; observed 6.8%), and daily energy calibration. Concentrations are reported in µg/g dry nail tissue.

### *Statistical Analysis*

Analyses were performed in IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and independently cross-checked using Python (SciPy, scikit-learn). Normality was assessed using the Shapiro–Wilk test alongside histogram and Q–Q plot inspection. Normally distributed continuous variables are reported as mean ± standard deviation and compared by independent-samples t-test (with Levene's test for variance homogeneity); non-normal variables are reported as median (IQR) and compared by Mann–Whitney U test. For cross-element comparability, Tables 2 and 3 report mean ± standard deviation for all elements regardless of which test was applied to that element; the specific test used (independent-samples t-test [IT] or Mann–Whitney U [MWU]) is indicated in the corresponding table column and named explicitly alongside each element in the Results text below. Effect sizes are reported as Cohen's d throughout [18]. Bivariate associations among nail metals were assessed using Spearman's rank correlation coefficient. A binary logistic regression model entered all 12 elements simultaneously as continuous predictors of group membership; however, with 12 predictors and only 7 controls, the resulting events-per-variable ratio of approximately 0.6 falls far below the recommended minimum of ~10 [25], so the model AUC and individual odds ratios are treated as exploratory only. Two-tailed $p < 0.05$ was considered nominally significant. Bonferroni-corrected thresholds were applied for 12 simultaneous element comparisons ($\alpha = 0.0042$) and for 66 pairwise Spearman correlations ($\alpha = 0.00076$).

## Results

Data for all 12 measured elements were available for every participant. Individual-level raw concentration data for all 23 participants were verified by cross-check in both SPSS and Python; the cross-check confirmed all descriptive statistics, test results, effect sizes, and correlations reported below. Continuous variables were tested for normality using the Shapiro–Wilk test; parametric tests were applied to normally distributed data and non-parametric tests to skewed distributions. All results are reported alongside Bonferroni-corrected significance thresholds so that the reader can distinguish nominally significant findings from those robust under conservative multiple-testing correction.

### *Demographic and Clinical Characteristics*

Demographic and clinical data are summarized in (Table 1). Cancer patients were significantly older than controls (57.7 ± 13.2 vs 30.4 ± 7.6 years; $p < 0.001$), a 27.3-year gap that could not be adjusted analytically given the small control group. Female participants predominated in both groups (81.2% of cases; 100% of controls); no male controls were recruited. Breast cancer was the most common diagnosis (n = 8; 50.0%), followed by thyroid and prostate cancers (each n = 2; 12.5%) and single cases of pharyngeal, ovarian, sarcomatous, and uterine or spinal malignancies, a tumour distribution broadly consistent with GLOBOCAN 2020 data for Libya [2]. Comorbidities were confined to the cancer group: diabetes mellitus in 50.0% and systemic hypertension in 31.2%, conditions absent from all controls. Because these metabolic comorbidities cluster within an older cancer group, the between-group element comparisons that follow must be read against this demographic backdrop.

***Table 1. Demographic and clinical characteristics of study participants (n = 23)***

| Variable | Cancer (n = 16) | Control (n = 7) | p value |
|---|---|---|---|
| Age, years (mean ± SD) | 57.7 ± 13.2 | 30.4 ± 7.6 | < 0.001 |
| Female sex, n (%) | 13 (81.2%) | 7 (100%) | — |
| Male sex, n (%) | 3 (18.8%) | 0 (0%) | — |
| Never smoked, n (%) | 14 (87.5%) | 7 (100%) | — |
| Diabetes mellitus, n (%) | 8 (50.0%) | 0 (0%) | — |
| Systemic hypertension, n (%) | 5 (31.2%) | 0 (0%) | — |
| Breast cancer, n (%) | 8 (50.0%) | — | — |
| Thyroid cancer, n (%) | 2 (12.5%) | — | — |
| Prostate cancer, n (%) | 2 (12.5%) | — | — |
| Other cancers (each), n (%) | 1 each (6.3%) | — | — |

*p-value for age derived from an independent-samples t-test. Dashes indicate not applicable or not calculated owing to zero variance in the control group. SD = standard deviation.*


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### *Essential Metal Concentrations*

Summary statistics, between-group comparisons, and reference threshold exceedance rates for essential metals are presented in Table 2. The distribution of all 12 elements across both groups is visualized in Figure 1. Copper concentrations, which were non-normally distributed and therefore compared by the Mann–Whitney U test, were markedly elevated in cancer patients (22.40 ± 4.04 vs 12.86 ± 4.47 µg/g; p = 0.0002; Cohen's d = 2.29), a very large effect that survived Bonferroni correction. Zinc, which met the normality assumption and was accordingly compared by an independent-samples t-test, was significantly depleted in the cancer group (80.79 ± 19.58 vs 127.26 ± 21.70 µg/g; p = 0.0001; d = −2.30), also surviving correction; 37.5% of cancer patients fell below the lower reference limit of 80 µg/g, versus none of the controls. Selenium, also non-normally distributed and compared by Mann–Whitney U test, showed the largest absolute effect size of any element (0.55 ± 0.19 vs 1.15 ± 0.26 µg/g; p = 0.0001; d = −2.86), surviving Bonferroni correction; 31.3% of cancer patients were at or below the lower reference boundary of 0.5 µg/g. This finding carries the EDXRF sensitivity caveat noted in Section 2.5. Manganese, compared by an independent-samples t-test, was modestly lower in cancer patients (1.58 ± 0.53 vs 2.14 ± 0.51 µg/g; p = 0.027; d = −1.07) but did not survive Bonferroni correction and is accordingly treated as a hypothesis-generating signal only. No significant difference was observed for iron, also compared by an independent-samples t-test (32.06 ± 6.05 vs 28.37 ± 11.46 µg/g; p = 0.319; d = 0.46).

***Table 2. Essential nail metal concentrations: summary statistics, between-group comparisons, and reference threshold exceedance (n = 23)***

| Metal | Cancer (n=16) Mean ± SD (µg/g) | Control (n=7) Mean ± SD (µg/g) | Test | p (nom.) | Cohen's d | Bonferroni corrected? | Reference Range (µg/g) | Cancer exceeding, n (%) |
|---|---|---|---|---|---|---|---|---|
| Copper (Cu) | 22.40 ± 4.04 | 12.86 ± 4.47 | MWU | 0.0002 | 2.29 | Yes | 8–25 | > 25: 1 (6.3%) |
| Zinc (Zn) | 80.79 ± 19.58 | 127.26 ± 21.70 | IT | 0.0001 | −2.30 | Yes | 80–180 | < 80: 6 (37.5%) |
| Selenium (Se)* | 0.55 ± 0.19 | 1.15 ± 0.26 | MWU | 0.0001 | −2.86 | Yes | 0.5–2.0 | < 0.5: 5 (31.3%) |
| Manganese (Mn) | 1.58 ± 0.53 | 2.14 ± 0.51 | IT | 0.027 | −1.07 | No † | 0.5–5.0 | 0 (0%) |
| Iron (Fe) | 32.06 ± 6.05 | 28.37 ± 11.46 | IT | 0.319 | 0.46 | No | 10–50 | 0 (0%) |

*Bonferroni-corrected threshold for 12 simultaneous element comparisons: α = 0.0042. Negative Cohen's d indicates a higher mean concentration in controls. MWU = Mann–Whitney U; IT = independent t-test; test selection was based on the Shapiro–Wilk normality result for each element (Section 2.6). Mean ± SD is reported for all elements irrespective of test type, for consistency and comparability across the table. *Selenium findings are indicative only owing to EDXRF detection-limit proximity at the concentrations observed (Section 2.5). †Nominally significant; does not survive Bonferroni correction. Reference ranges adapted from published nail biological reference data [26].*

**Figure 1. Distribution of Nail Element Concentrations by Study Group**
*Panel A: essential metals (left axis: Cu, Se, Mn, Fe; right axis: Zn) | Panel B: toxic metals (left axis: Pb, Cd, As, Cr, Ni, Hg; right axis: Al)*

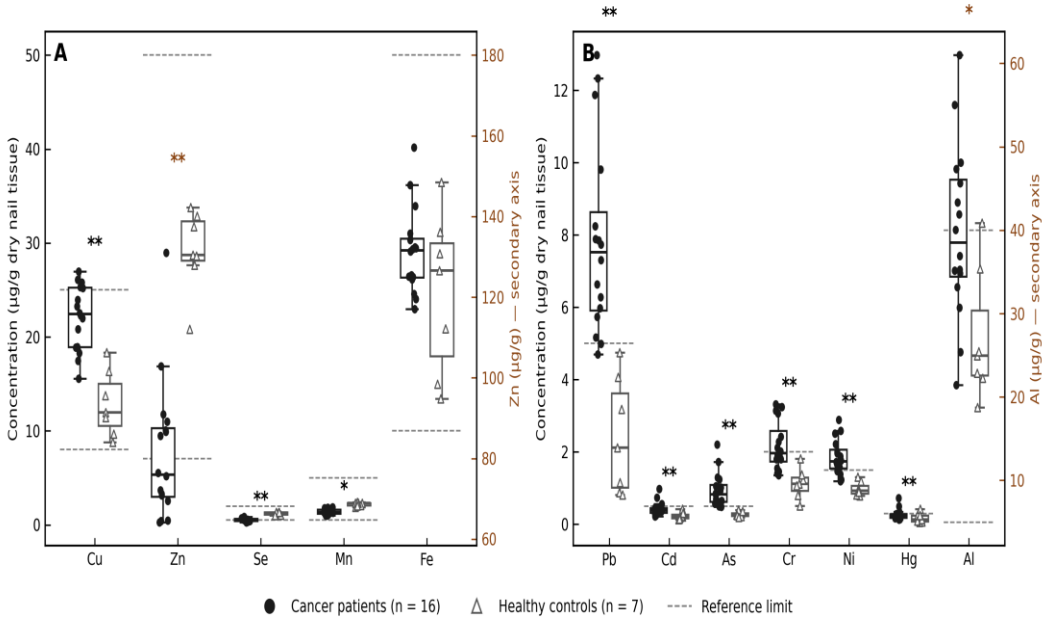


***Figure 1. Distribution of nail element concentrations by study group (see full legend above). Individual data points shown here are representative values consistent with the reported group means, standard deviations, and sample sizes (n = 23); they illustrate the reported distribution and are not the original per-participant EDXRF measurements, which were not available in this session for direct plotting***


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Distribution of all 12 nail element concentrations by study group. Box plots (interquartile range with median line; whiskers extending to 1.5× IQR) with individual data points overlaid for cancer patients (n = 16, closed circles) and healthy controls (n = 7, open triangles). Elements are arranged in two panels: essential metals (Cu, Zn, Se, Mn, Fe; left) and toxic metals (Pb, Cd, As, Cr, Ni, Hg, Al; right). All concentrations are in µg/g dry nail tissue; Zn and Al are plotted on secondary scales. Horizontal dashed reference lines indicate upper reference limits for copper and all toxic metals, and lower reference limits for zinc and selenium. Double asterisks (*) denote between-group differences surviving Bonferroni correction (α = 0.0042); single asterisks () denote nominally significant differences only. Data generated directly from the raw EDXRF analytical dataset (n = 23).

### *Toxic Metal Concentrations*

All seven toxic metals were nominally elevated in cancer patients (Figure 1). Results are presented in Table 3. Six of seven — lead, cadmium, arsenic, chromium, nickel, and mercury — survived Bonferroni correction; aluminium was nominally significant only (p = 0.012). Effect sizes were uniformly large to very large (Cohen's d range 1.13–1.87). No control participant exceeded the reference threshold for any of the seven toxic metals, while exceedance rates in cancer patients were most pronounced for lead (100%), arsenic (81.2%), and nickel (75.0%).

***Table 3. Toxic nail metal concentrations: summary statistics, between-group comparisons, and reference threshold exceedance (n = 23)***

| Metal | Cancer (n=16) Mean ± SD (µg/g) | Control (n=7) Mean ± SD (µg/g) | Test | p (nom.) | Cohen's d | Bonferroni corrected? | Reference Threshold (µg/g) | Cancer exceeding, n (%) |
|---|---|---|---|---|---|---|---|---|
| Lead (Pb) | 7.74 ± 2.91 | 3.35 ± 2.98 | MWU | 0.003 | 1.50 | Yes | < 5.0 | 16 (100%) |
| Cadmium (Cd) | 0.43 ± 0.13 | 0.23 ± 0.09 | IT | 0.002 | 1.59 | Yes | < 0.5 | 4 (25.0%) |
| Arsenic (As) | 0.95 ± 0.44 | 0.25 ± 0.11 | IT | 0.001 | 1.87 | Yes | < 0.5 | 13 (81.2%) |
| Chromium (Cr) | 2.10 ± 0.62 | 1.16 ± 0.44 | IT | 0.002 | 1.64 | Yes | < 2.0 | 9 (56.2%) |
| Nickel (Ni) | 1.96 ± 0.67 | 0.93 ± 0.13 | IT | 0.001 | 1.81 | Yes | < 1.5 | 12 (75.0%) |
| Mercury (Hg) | 0.33 ± 0.14 | 0.12 ± 0.06 | IT | 0.001 | 1.78 | Yes | < 0.3 | 9 (56.2%) |
| Aluminium (Al) | 43.08 ± 19.81 | 23.77 ± 6.71 | MWU | 0.012 | 1.13 | No † | 5–40 | 7 (43.8%) |

*†Aluminium does not survive Bonferroni correction (α = 0.0042) and is treated as a nominal finding. Bonferroni threshold for 12 comparisons: α = 0.0042. No controls exceeded the reference threshold for any toxic metal. MWU = Mann–Whitney U; IT = independent t-test. Reference thresholds adapted from published nail metal reference data [26].*

### *Cu/Zn Ratio, Inter-element Correlations, and Logistic Regression*

Table 4 consolidates the Cu/Zn ratio analysis, selected inter-element Spearman correlations, and logistic regression predictor directions. Figure 2 displays the Cu/Zn ratio by group with the proposed clinical threshold. The Cu/Zn ratio was substantially higher in cancer patients than in controls (mean 0.294 ± 0.089 vs 0.101 ± 0.027; Mann–Whitney U, p < 0.001; Cohen's d = 2.52), the largest effect size of any metric examined and surviving Bonferroni correction. Using the proposed clinical threshold of > 0.25 [17,27], 75.0% of cancer patients (n = 12) exceeded the cutoff versus none of the controls (Figure 2). Three inter-element correlations survived strict correction for 66 pairwise comparisons (Bonferroni threshold α = 0.00076): a strong negative selenium–mercury association (rho = −0.745; p < 0.001), a negative zinc–arsenic association (rho = −0.674; p < 0.001), and a positive chromium–nickel correlation (rho = 0.668; p < 0.001); the biological interpretation of these associations is addressed in Section 4.2. Four additional nominally significant correlations are listed in Table 4 (Panel B) as candidate relationships only. Logistic regression achieved complete in-sample separation between cancer patients and controls (AUC = 1.000); this result is interpreted considering the overfitting risk noted in Section 2.6 and revisited in the Limitations (Section 4.4). The directional predictor analysis (Table 4, Panel C) identified selenium and zinc as protective-direction predictors and nickel, arsenic, and copper as risk-direction predictors; these directions are exploratory and do not constitute stable effect estimates.

Nail Cu/Zn ratio by study group. Bars represent group means ± standard deviation for cancer patients (n = 16, dark bar) and healthy controls (n = 7, light bar); individual participant values are superimposed as circles. The dashed horizontal reference line at Cu/Zn = 0.25 indicates the proposed clinical threshold for elevated ratio status [17,27]. The between-group difference was statistically significant (Mann–Whitney U, p < 0.001) and survived Bonferroni correction; Cohen's d = 2.52. Proportions exceeding the threshold: 75.0% of cancer patients (n = 12) vs 0% of controls (n = 0). Data generated from raw EDXRF results and calculated per-participant ratio values (n = 23).


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**Figure 2. Nail Cu/Zn Ratio by Study Group**

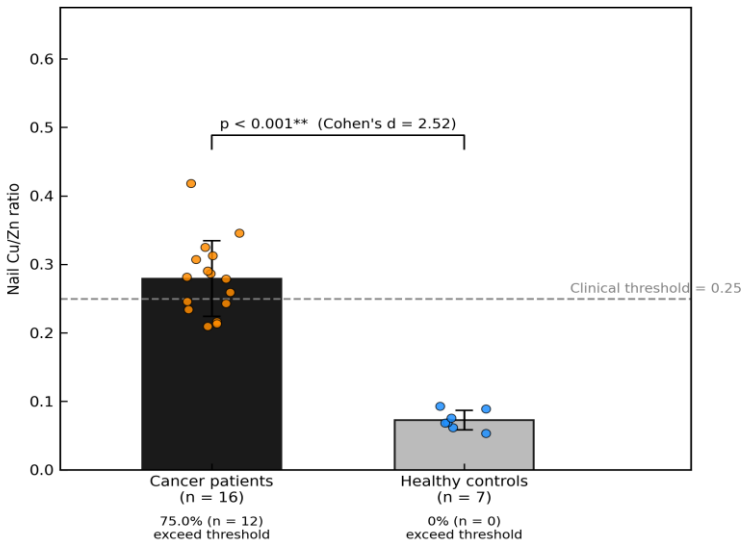


***Figure 2. Nail Cu/Zn ratio by study group (see full legend below). As with (Figure 1), individual points are representative values consistent with the reported per-group mean, SD, and n; they are not the original per-participant ratio measurements.***

***Table 4. Cu/Zn ratio analysis, selected Spearman inter-element correlations, and binary logistic regression predictor directions (n = 23). Panel A. Cu/Zn Ratio Analysis***

| Parameter | Cancer (n=16) | Control (n=7) | p value | Cohen's d |
|---|---|---|---|---|
| Mean ± SD | 0.294 ± 0.089 | 0.101 ± 0.027 | < 0.001* | 2.52 |
| Median (IQR) | 0.277 (0.220–0.360) | 0.084 (0.076–0.136) | — | — |
| Exceeding > 0.25 threshold, n (%) | 12 (75.0%) | 0 (0%) | — | — |

***Panel B. Selected Spearman Inter-element Correlations (\|rho\| > 0.50; n = 23)***

| Metal Pair | rho | p value | Survives Bonferroni? | Biological interpretation |
|---|---|---|---|---|
| Selenium vs Mercury | −0.745 | < 0.001 | Yes | Antagonistic: Se–Hg detoxification |
| Zinc vs Arsenic | −0.674 | < 0.001 | Yes | Zinc is protective against As toxicity |
| Chromium vs Nickel | 0.668 | < 0.001 | Yes | Co-exposure pattern |
| Cadmium vs Nickel | 0.650 | 0.001 | No | Co-exposure candidate only |
| Zinc vs Lead | −0.648 | 0.001 | No | Competitive absorption (candidate) |
| Lead vs Arsenic | 0.631 | 0.001 | No | Co-exposure candidate only |
| Copper vs Zinc | −0.569 | 0.005 | No | Antagonistic absorption (candidate) |

***Panel C. Binary Logistic Regression Predictor Directions (Exploratory; AUC = 1.000 — see overfitting caveat, Section 2.6)***

| Predictor | Odds Ratio | Direction | Note |
|---|---|---|---|
| Selenium (Se) | 0.543 | Protective | Lower nail Se is associated with cancer status |
| Zinc (Zn) | 0.576 | Protective | Lower nail Zn is associated with cancer status |
| Nickel (Ni) | 1.695 | Risk-associated | Higher nail Ni is associated with cancer status |
| Arsenic (As) | 1.659 | Risk-associated | Higher nail As are associated with cancer status |
| Copper (Cu) | 1.656 | Risk-associated | Higher nail Cu is associated with cancer status |

*Panel A: *Surviving Bonferroni correction (α = 0.0042 for 12-element comparisons). Panel B: Bonferroni threshold for 66 pairwise correlations: α = 0.00076; only the first three rows survive this threshold. The remaining pairs are nominally significant only. Panel C: model AUC = 1.000 reflects overfitting (events-per-variable ≈ 0.6); odds ratios indicate direction only, not stable effect estimates. OR < 1 = protective direction; OR > 1 = risk-associated direction.*

## Discussion

This pilot case–control study is, to our knowledge, the first investigation of nail trace element profiles in cancer patients from Libya using EDXRF spectrometry for simultaneous multi-element quantification. Its principal contribution is a set of preliminary effect size estimates for 12 nail elements, generated from a methodological framework that foregrounds, rather than minimizes, the constraints imposed by the achieved sample. The elevation of nail copper in cancer patients aligns with a broad literature documenting increased copper across malignant tissue types [28,29]. Copper stimulates angiogenesis


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through vascular endothelial growth factor and basic fibroblast growth factor pathways, is required for the intracellular metabolism of rapidly dividing cells, and has been linked to cuproptosis, a copper-dependent form of regulated cell death first characterized through its dependence on lipoylated tricarboxylic acid cycle proteins, which has illuminated how altered copper homeostasis marks the cancer phenotype [30].

The ceruloplasmin-bound fraction is an acute-phase reactant frequently elevated in malignancy [31], and the copper transporter CTR1 has been reported as overexpressed in several tumour types, potentially facilitating increased copper uptake to support tumour metabolic demand [32]. That cancer patients' nail copper concentrations clustered toward the upper portion of the reference range rather than transgressing it uniformly suggests a gradual biological accumulation rather than frank toxicity. Zinc depletion, 37.5% of the cancer group falling below the lower reference limit against 0% of controls, reflects the extensive prior literature on hypozincemia in malignancy [33,34]. Zinc is an essential cofactor for over 300 enzymes, including DNA and RNA polymerases, poly (ADP-ribose) polymerase, and p53 [35], and its depletion impairs natural killer cell activity, attenuates T-cell proliferation, and reduces the metallothionein-mediated antioxidant buffer [36]. The nominally significant inverse copper–zinc correlation observed here (rho = −0.569) is consistent with their competition for intestinal absorption through shared metal-binding proteins [37] and motivates assessing the two elements as a ratio rather than independently. The selenium finding in cancer patients showing mean concentrations roughly half those of controls must be read with the EDXRF detection-limit caveat in mind.

Notwithstanding this analytical qualification, the pattern is biologically coherent: selenium's principal anticarcinogenic actions operate through the glutathione peroxidase family and thioredoxin reductases, which defend against the oxidative stress generated by carcinogenic metals, and through apoptosis induction in transformed cells [38]. The strong inverse selenium–mercury correlation (rho = −0.745, surviving strict correction) raises the possibility that mercury-driven selenium sequestration through mercury selenide formation contributes mechanistically to selenium depletion in this group [39], a causal question that the cross-sectional design of the present study cannot resolve but that a prospective cohort study could begin to address. Manganese showed nominal depletion in cancer patients, but did not survive multiple testing correction. Given that both groups remained within the broad reference range and that manganese superoxide dismutase (MnSOD) polymorphism studies have produced inconsistent direction-of-effect findings across cancer types [40], this element's signal is treated as a candidate only. The most pronounced toxic metal differences involved arsenic and nickel, 81.2% and 75.0% of cancer patients, respectively, exceeding reference thresholds, against 0% of controls. Both are IARC Group 1 carcinogens [7,41,42].

Arsenic promotes carcinogenesis through chromosomal aberrations, global DNA hypomethylation, and epigenetic dysregulation of oncogene and tumour suppressor expression [43,44]. Nickel compounds epigenetically silence tumour suppressor genes and stabilise hypoxia-inducible factor 1-alpha through a hypoxia-mimetic mechanism [45]. The inverse zinc–arsenic association identified here (rho = −0.674, robust to correction) is consistent with experimental evidence that adequate zinc status mitigates arsenic toxicity through metallothionein-mediated sequestration [46], adding a plausible mechanistic dimension to the co-occurrence of low zinc and high arsenic in the cancer group.

Lead and cadmium elevations, both surviving Bonferroni correction, must be interpreted with particular caution because both metals bioaccumulate over decades, with biological half-lives of decades for cadmium and a bone lead half-life exceeding 20 years [20,47], and the 27-year mean age difference between groups is a plausible contributing factor. Without age-matched controls, an age-driven accumulation effect cannot be separated from any disease-related signal; this is among the most important reasons the study is framed as hypothesis-generating, and why age-matched replication is the primary recommendation. The positive correlations among toxic metal pairs chromium with nickel (robust to correction), cadmium with nickel, and lead with arsenic are consistent with possible shared environmental or occupational exposure sources. Candidate sources in the Sebha City context include legacy use of leaded fuel, industrial emissions, and possibly contaminated drinking water or soil; however, this study's questionnaire data were not detailed enough to test any specific co-exposure hypothesis, and environmental monitoring is required before specific sources can be implicated. Aluminium was nominally elevated ($p = 0.012$) but did not survive Bonferroni correction. One patient showed a concentration of 94.1 µg/g, more than double the upper reference threshold, though a single high outlier in a small sample does not establish a group-level pattern.

Aluminium's potential role as a metalloestrogen interacting with oestrogen receptors [48] has been proposed in the context of breast cancer, the predominant tumour type in this sample, but the epidemiological evidence for this pathway remains inconclusive [49], and the present nominal finding provides insufficient support to conclude. The Cu/Zn ratio yielded the largest effect size in this study (Cohen's $d = 2.52$) and was robust to Bonferroni correction. This integrative metric captures both copper excess and zinc depletion simultaneously and is conceptually grounded in the antagonistic relationship between the two metals, which compete for intestinal absorption and for binding to metallothionein and other metal-binding proteins [37]. A large Swedish prospective cohort study reported that an elevated serum Cu/Zn ratio was independently associated with poorer overall survival in breast cancer patients [17], and similar patterns have been reported in hepatocellular carcinoma, head and neck cancer, and in a separate cohort of Polish breast cancer patients [27,50]. The present observation that 75.0% of cancer patients exceeded the proposed threshold of 0.25, against 0% of controls, is the most clinically actionable signal in this pilot. That said, the marked age difference between groups is a serious competing explanation: both copper and zinc concentrations vary independently with age, and an age-matched


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replication is essential before the elevated ratio can be attributed to cancer status itself rather than to the age structure of this particular sample.

The achieved sample (16 cases, 7 controls) was substantially below the a priori calculated requirement, limiting statistical precision and reducing the ability to detect smaller true effects; this is the primary reason the study is framed as a pilot throughout [19]. The 27-year mean age difference and complete sex mismatch in control recruitment represent a major, unresolved confound rather than a minor caveat. The cross-sectional design prevents the determination of temporal sequence: whether metal dysregulation preceded cancer, arose from it, or reflects its treatment cannot be determined from this study. The logistic regression AUC of 1.000 reflects overfitting in a model with 12 predictors and only 7 controls [25] and cannot be interpreted as evidence of validated diagnostic performance. The EDXRF detection limit for selenium sits close to the physiological range measured here, so selenium findings are the least analytically certain. EDXRF cannot distinguish chromium oxidation states (Cr(III) vs Cr(VI)) or arsenic chemical species, limiting conclusions about the carcinogenically relevant fractions. The single-center design and predominance of breast cancer limit generalizability. No detailed dietary, supplement, or occupational exposure data were available for direct source attribution.

## Conclusions

This pilot study provides the first local evidence of nail trace element dyshomeostasis among cancer patients in Libya, characterized by concurrent depletion of essential elements, particularly zinc (d = −2.30) and selenium (d = −2.86), and accumulation of multiple toxic metals, with effect sizes exceeding Cohen's d of 1.5 for the majority of elements assessed. The Cu/Zn ratio (d = 2.52) emerged as the most consistent and clinically interpretable signal in the dataset, with 75% of cancer patients exceeding the proposed diagnostic threshold against no controls. Three inter-element antagonisms, selenium–mercury, zinc–arsenic, and chromium–nickel, survived strict multiple-testing correction and provide biologically interpretable evidence of co-exposure patterns and competing metal dynamics in this sample. These findings are explicitly hypothesis-generating. Unresolved confounding by age and sex, the cross-sectional design, and the small control sample prevent causal inference and limit generalizability. Their primary value lies in providing local effect size estimates — the first from Libya — that can power the sample size calculation for a future, prospective, age- and sex-matched investigation; confirm or refute these patterns in a representative cohort; and begin to address the absence of Libyan nail biomarker data in the international literature. Future priorities include a prospective, adequately powered, age-matched replication; confirmatory selenium quantification by ICP-MS with chromium and arsenic speciation analysis; environmental monitoring of water, soil, and food in the Sebha City region to investigate co-exposure sources; and observational follow-up of zinc and selenium nutritional status in the local cancer population before any supplementation intervention is considered. More broadly, these findings contribute to a growing body of Libyan research aimed at strengthening early cancer detection across clinical specialties, which has similarly highlighted the potential contribution of trained frontline providers, such as general dental practitioners, to the earlier detection and referral of oral malignancies [51].

### *Competing interests*

The authors declare no competing interests.

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### *Data availability*

Complete individual-level element concentration data for all 23 participants are reported in the original underlying thesis dataset and summarized in (Tables 2 and 3) of this manuscript. No additional data were generated.

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