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Tracing agrochemical footprints: heavy-metal contamination and esophageal squamous cell carcinoma Risk in Kashmir- the northernmost state of India

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Abstract

Background

Touching the border of the world-famous "*Esophageal Cancer Belt*" the Kashmir Valley has a high burden of esophageal squamous cell carcinoma (ESCC). The contribution of environmental exposures remains largely inconclusive. Horticulture and agriculture are considered the backbone of the Kashmir economy; however, such practices have raised concerns regarding the potential environmental toxic metal accumulation due to excessive use of pesticides, fungicides, insecticides and fertilizers. This study evaluated the distribution pattern of selected trace elements in the environment and staple food items across the Valley.

Methods

A total of 117 samples comprising soil (n = 30), drinking water (n = 30), collard greens (n = 30), and paddy/rice (n = 27) were collected across all 10 districts of the Kashmir Valley in this descriptive-analytical study. The samples were analysed for trace elements—zinc (Zn), selenium (Se), copper (Cu), lead (Pb), chromium (Cr), barium (Ba), cadmium (Cd), arsenic (As), and cobalt (Co)—using inductively coupled plasma mass spectrometry. Median and percentile measures were used for estimating elemental contamination. Differences across sample types and districts were assessed using Kruskal–Wallis tests and Benjamini–Hochberg false-discovery-rate correction. Spearman correlation and principal component analysis were used to assess inter-element associations and multivariate patterns.

Results

Trace-element concentrations varied significantly across the samples for all 9 elements (FDR-adjusted $P < 0.001$). Soil demonstrated high median concentrations of Zn (244.0 ppm), Cu (110.75ppm), Pb (108.4ppm), Cr (114.5ppm), Ba (396.0 ppm), Cd (12.25ppm), and As (133.0ppm). All soil samples exceeded the applied reference values for As and Cd, while 83.3%, 70.0%, and 70.0% exceeded those for Cr, Cu, and Pb, respectively. In collard greens, Pb and Cr exceeded the applied reference concentrations in all 30 samples, while As and Co exceeded them in 33.3% and 36.7%, respectively. In drinking water, Cu exceeded the applied reference concentration in 28/30 (93.3%) samples. We observed significant district-level heterogeneity for selected elements, with district Kupwara presenting substantially elevated median concentrations for multiple trace elements. Several elements presented with strong but positive correlations in collard greens and paddy samples.

Conclusions

The study demonstrates significant and spatially heterogeneous trace-element contamination across collected samples from high ESCC risk in the Kashmir Valley. Our study identifies potential environmental and dietary exposure pathways; however, the study findings are mainly hypothesis-generating and should not be considered as a causal relationship between heavy-metal exposure and ESCC until further

investigation integrating human biomonitoring, exposure assessment, agricultural practices, and district-level ESCC incidence.

Introduction

Rapid industrialisation, demand for high-quality agricultural production over recent decades have posed a significant threat to the quality of soil, water, plant vegetation, and air in terms of various hazardous chemical exposures [1, 2]. These exposures have emerged as a major global public health challenge due to their positive association with human diseases, including malignancies. Among various chemical pollutants, pesticides and fertilisers are the major contributors [3–5]. To improve the quality and manage higher production of crops, Food and Agriculture Organisation Corporate Statistical Database (FAOSTAT) reported that since 1990, worldwide agricultural pesticide use has doubled to 3.73 million tonnes [6]. This substantial increase in pesticide use has resulted in the persistence, bioaccumulation, and toxicity of these compounds and hence raised serious concerns about their impact on human health, confirmed by published studies across the globe [7–9]. Despite these observations, identifying parent pesticide compounds in environmental conditions remains challenging because many pesticides undergo rapid degradation through photolysis, hydrolysis, microbial activity, and enzymatic biotransformation [10]. This volatile nature of the majority of the pesticides makes them difficult to measure; however, pesticide residues often persist longer in the environment and may represent the principal toxic agents contributing to mutagenesis and chronic toxicity, as confirmed in published reports [11, 12].

Conventional agrochemicals frequently contain metals such as copper (Cu), cadmium (Cd), chromium (Cr), manganese (Mn), arsenic (As), lead (Pb), nickel (Ni), and zinc (Zn) etc [7]. Certain fungicides, including Bordeaux mixture, directly incorporate copper sulphate as an active ingredient [7]. At the same time, fertilisers used to enrich soil for better crop production further contribute to the accumulation of various types of HMs in agricultural and horticultural fields, as evidenced by published literature [1, 13]. The long-term persistence of these HMs in agricultural ecosystems may activate oxidative stress, genomic instability, and cellular damage, thereby increasing carcinogenic risk [14]. Contamination of soil, water, vegetables, fruits, etc by HMs due to agricultural practices has been associated with environmental toxicants at levels capable of causing various health issues, including malignancies, even at trace concentrations [15, 16]. Metals such as Cd, Pb, As, Cr, Mn, Se, and Zn have been implicated in the development of multiple cancers, including breast, lung, gastric, pancreatic, colorectal, and EC. Therefore, quantification of HM concentrations in the environment may provide an important indicator for evaluating environmental carcinogenic exposure in agriculturally intensive regions.

Kashmir Valley, owing to its favourable agro-climatic conditions, is among the most agricultural dependant region for its economy in terms of apple and rice production. The increasing dependency on agrochemicals in the region has raised concerns regarding their potential role in the rising burden of malignancies, particularly gastrointestinal malignancies (GI malignancies) [4, 17, 18]. Zargar et al., from a hospital-based study, reported that a total of 24,636 cancer patients were registered at the Regional Cancer Centre, SKIMS Srinagar, between 2018 and 2023, among which EC, predominantly Esophageal squamous cell carcinoma (ESCC), constituted 19.2% of the total cancer cases [19]. Interestingly, the southern districts of Kashmir,

which incidentally represent the major agricultural/horticultural belt of the region, have shown disproportionately higher incidences of upper gastrointestinal cancers, particularly ESCC [20, 21]. This interesting observation of intra-district variation in the incidence of ESCC risk, and the unavailability of any established risk factor as a causal agent for ESCC in Kashmir [22–25], highlights the need to investigate alternative risk determinants. Therefore, the potential link between agricultural contamination in the form of HM exposure remains an important yet unexplored area of research in this high ESCC risk region.

Materials and methods:

With the objectives as to find out the possible link between the high incidence of ESCC rates with biogeochemistry of agricultural soil, drinking water, and edible staple food sources, this descriptive-analytical study was conducted in the Kashmir Valley. The samples (soil, drinking water, and the edible portions of commonly consumed staple foods, specifically Rice (*Oryza sativa*) and Collard Greens (*Brassica oleracea var. viridis*), were collected from different locations across all 10 districts of the Kashmir Valley.

Study area:

Kashmir Valley mainly depends on agricultural and horticultural activities for its economy, making it one of the main areas of rice and apple production in India. The Kashmir Valley is situated between the Pir Panjal and the western end of the Great Himalayan range and comprises the extreme western part of the Himalayas (32.44°N and 74.54°E). The valley is approximately 120 km long, with an altitude ranging from 1,524 to 2,286 meters above mean sea level. The economy of the region predominantly suits agriculture practices, with rice and collard greens as main staple foods. This study is a joint effort conducted by the Department of Clinical Biochemistry at the Sher-i-Kashmir Institute of Medical Sciences (SKIMS) in collaboration with the Department of Allied Health Sciences at Lovely Professional University. It is part of a pilot study involving all 10 districts of Kashmir: Anantnag, Bandipora, Budgam, Baramulla, Ganderbal, Kulgam, Kupwara, Pulwama, Srinagar, and Shopian.

Reagents, Standards, and Labware

To eliminate cross-contamination, all polyethylene bottles, volumetric flasks, digestion vessels, and other labware were soaked in 10% (v/v) HNO_3 for at least 24 h, rinsed three times with ultra-pure deionised water, and dried in a class-100 clean environment before use for sampling [26]. Powder-free gloves were worn during all handling steps. Multi-element calibration standards were prepared by serial dilution of certified 1000 mg L^{-1} single- and multi-element stock solutions (traceable to NIST) in 2% (v/v) HNO_3. The target analytes were the ten trace elements zinc (Zn), selenium (Se), copper (Cu), lead (Pb), nickel (Ni), chromium (Cr), barium (Ba), cadmium (Cd), arsenic (As), and cobalt (Co).

Sample collection:

Samples were collected from three different locations of each district of Kashmir valley. These locations are mostly located in cultivation areas, which are free from the industrialization or factories. Geographic coordinate data of the selected locations were recorded, which includes latitude and longitude based on the WGS84 coordinate system. Seasonal samples of soil, water and foliage were collected and then pooled into a composite sample before analysis using ICP-MS. Combining triplicate samples across seasons into a single composite sample for trace element analysis is a recognized technique to manage local variability, reduce analytical costs, and provide a representative overview of long-term contamination or accumulation, rather than focusing on short-term or highly localized spikes [27].

Sample collection was done in different seasons, with the first and second sampling done in March-June and July- August 2025, followed by the third batch in September-November 2025 Autumn season. In the Autumn season, in addition to soil and water samples, rice/paddy samples and collard greens were collected from selected sites. In the Spring and summer seasons, the soil, water and collard greens were sampled. **Supplementary Table 1** shows the details of the soil, water, and foliage samples collected from the study site.

Soil sample Collection:

The sample was collected from the sample location using a clean shovel. At each point, the soil samples were collected from topsoil (0–15 cm depth) around the sample area, to produce a single composite sample. The sampling depth of 0–15 cm was chosen based on the most active zone of maximum root concentration and the most sensitive zone of erosion and atmospheric deposition [28, 29]. The samples were thoroughly mixed and transferred into clean and labelled polythene bags for onward analysis. The samples were first air-dried, then placed in an electric oven at a temperature of approximately 65°C for 3 hours. The samples were mixed and gently homogenized and sieved through a 2-mm mesh sieve. The resulting fine powder was kept at room temperature for the further digestion process [30, 31].

Water sample collection:

The water samples intended for heavy metals analysis were collected in pre-cleaned and sterilised polyethene bottles that had been rinsed and dried. The Samples were acidified to a pH of ≤ 2 with ultra-purified HNO3 ("trace metal' grade), to preserve trace metals in solution, suppress microbial activity, and prevent crystallization of trace elements before further analysis. These samples were then sealed, labelled, and stored in a dark refrigerator at 4ºC until analysis [32].

Foliage sample collection

Similar to soil and water samples, vegetables (collard greens) were sampled at selected sites. In the Autumn season Rice/paddy samples and Collard greens were sampled. In the Spring and summer seasons, only Collard greens were sampled as rice is a kharif crop and can be sampled only once in a year. The Fresh samples collected were first washed thoroughly under running tap water to dislodge adhering soil particles and dust and then rinsed three times with ultra-pure deionised water to remove residual tap-water ions and

surface contaminants. Later, the samples were air-dried first and then oven-dried at 60°C for 48 hours. These dried samples were homogenised and powdered uniformly with a grinder into a fine powder and stored in clean and labelled polyethene containers.

Digestion of samples

Microwave-assisted closed-vessel digestion

Complete mineralisation of the solid and liquid matrices was achieved using a closed-vessel microwave digestion system fitted with polytetrafluoroethylene (PTFE/TFM) pressure vessels and temperature- and pressure-feedback control. The closed-vessel approach was selected because it operates at elevated temperature and pressure in a sealed environment, ensuring complete decomposition of the matrix while preventing the loss of volatile analytes like As, Se, Cd, and Hg group elements and removing airborne contamination [33]. The digestion procedures were adapted from established microwave-assisted acid-digestion methods [30, 31]. Reagent blanks were processed through the same digestion programme for each batch.

Vegetable and water samples

For the vegetable matrix, an accurately weighed portion (≈ 0.25–0.50 g dry mass) of the homogenised powder was transferred into a clean digestion vessel and pre-digested with a mixture of 8 mL concentrated HNO_3 and 2 mL H_2O_2 (30%) ("trace metal' grade). For water samples, an aliquot (typically 10–25 mL) of the acidified, filtered sample was digested with 2–5 mL concentrated HNO_3 and 1–2 mL H_2O_2 to oxidise residual dissolved organic matter. The vessels were sealed and subjected to a controlled microwave programme in which the temperature was ramped to ≈ 180–200°C over approximately 15 min and held at that temperature for a further 15 min, followed by a cooling/ventilation step.

Soil samples

For total trace-element determination in soil, an accurately weighed portion (≈ 0.20–0.50 g) of the finely ground soil was digested with a mixture of concentrated HNO_3, HF, and HCl (a typical ratio being 6 mL HNO_3 : 3 mL HF: 2 mL HCl), the HCL being required to break down the silicate lattice and release lattice-bound elements. After the microwave programme (ramp to ≈ 190–200°C and hold), and once the vessels had cooled, excess/residual HF was complexed by adding a saturated boric acid (H_3BO_3) solution and reheating briefly; this step neutralises free fluoride, redissolves precipitated fluorides, and protects the quartz components and nickel cones of the ICP-MS from HF-induced damage. The digestion method followed in this study was based on the previously published reports [30, 31].

Post-digestion handling

After digestion, the vessels were allowed to cool to room temperature before being carefully vented in a fume hood to release accumulated gases. The clear, cooled digests were quantitatively transferred into acid-cleaned volumetric flasks. Each digest was then made up to the final volume with ultra-pure (Milli-Q®) water. Where necessary, digests were further diluted with 2% (v/v) HNO_3 to bring analyte concentrations

within the linear calibration range and to reduce the total dissolved solids loading and were filtered (0.45 µm) if any residual particulate remained before instrumental analysis.

Quality assurance and quality control

Assessment of contamination and reliability of data was done as a part of quality control measures. Blank and quality control standard samples were examined after every ten samples to ensure that the obtained results are within the range. The Certified Reference Materials (CRM) for every metal obtained from the International Atomic Energy Agency (IAEA) were used to check for accuracy and precision of the analysis of each metal in the water, soil and vegetable samples. The ICP-MS calibration standard solutions were prepared for elements via dilution of 1 mg L − 1 standards for ICP-MS or 1000 mg L − 1 standards for ICP (Sigma Aldrich, Trace CERT).

Analysis:

The concentrations of the selected 10 target trace elements in the final digests were determined using a PerkinElmer ICP-MS, following established ICP-MS methods for trace-element determination [34]. Before measurement, the instrument was optimised (torch alignment, lens voltages, nebuliser gas flow, and mass calibration) using a daily performance/tuning solution to maximise sensitivity and minimise oxide (CeO^+/Ce^+ < 2–3%) and doubly charged ion ratios. The instrument was calibrated using external multi-element calibration standards bracketing the expected sample concentrations, and calibration curves showed correlation coefficients (r) ≥ 0.999 for all analytes. To overcome spectral overlaps from argon-, chloride-, and matrix-based polyatomic species, the instrument was operated in Kinetic Energy Discrimination (KED) mode using helium as the collision gas. In KED mode, the larger collisional cross-section of polyatomic interferences relative to the analyte ions of the same nominal mass causes the interferences to lose more kinetic energy during collisions with helium; a downstream energy barrier then preferentially rejects the lower-energy polyatomic ions while transmitting the monatomic analyte ions [35]. This effectively suppressed interferences such as $^{40}Ar^{35}Cl$ on ^{75}As, $^{40}Ar^{40}Ar$ on ^{80}Se, $^{40}Ar^{12}C$ on ^{52}Cr, and $^{40}Ar^{23}Na$ / $^{43}Ca^{16}O$ on ^{63}Cu, enabling accurate quantification even in high-matrix soil digests. Representative isotopes monitored were ^{66}Zn, ^{78}Se, ^{63}Cu, ^{208}Pb, ^{60}Ni, ^{52}Cr, ^{137}Ba, ^{111}Cd, ^{75}As, and ^{59}Co.

Data Analysis

All statistical analyses were performed using Stata/SE version 17.0 (StataCorp LLC, College Station, TX, USA). After checking the completeness of the dataset, the concentrations of all 09 elements—zinc (Zn), selenium (Se), copper (Cu), lead (Pb), chromium (Cr), barium (Ba), cadmium (Cd), arsenic (As), and cobalt (Co)—were analysed across 04 sample types from all ten districts of Kashmir Valley. Shapiro–Wilk test was used to assess distributional assumptions. The elemental concentrations were summarised primarily by median and appropriate percentile measures, since the data were presented predominantly in continuous form. We used the Kruskal–Wallis rank-sum test to check the differences in elemental concentrations; however, the pairwise differences were evaluated using Dunn's post-hoc test were appropriate. All tests were two-sided, and an adjusted $P < 0.05$ was considered statistically significant. For multiple comparisons, the Benjamini–Hochberg false discovery rate (FDR) procedure was used to adjust *P* values.

The district-level differences in the elemental concentrations were calculated as district-specific median concentrations for each element across all 10 districts using heatmaps and other graphical presentations.

Inter-elemental associations were checked using Spearman's rank correlation coefficient (ρ), considering the non-normal distribution of our data. Similarly, the correlation analyses were performed separately for each sample type to look for any sample-specific patterns of elemental co-variation using heatmaps. In our analysis, we also employed principal component analysis (PCA) to characterise multivariate patterns and identify groups of elements contributing to common dimensions of variation.

So, all the obtained results were presented using median concentrations, district-level summaries, exceedance proportions, correlation coefficients, and publication-quality boxplots, heatmaps, and PCA plots. Given the observational nature of the study, statistical associations were interpreted as evidence of matrix-specific and geographic variation rather than proof of causality.

Results

Environmental samples comprising 30 collard-green, 30 soil, 30 water samples, and 27 Paddy samples from all 10 districts of the Kashmir Valley were selected for quantification for possible heavy metal contamination. As per sample matrix, we observed marked differences in elemental concentrations in collected sample types, with soil and plant-associated samples generally presenting with substantially higher concentrations than water samples.

Differences in heavy-metal concentrations among sample matrices

We observed substantial differences among the four collected sample matrices and found that the median concentrations in soil were higher as compared to water for the majority of the samples. Median soil concentrations were 244.0 ppm for Zn, 110.75 ppm for Cu, 108.4 ppm for Pb, 114.5 ppm for Cr, 396 ppm for Ba, 12.25 ppm for Cd and 133 ppm for As, as compared to the presence of these element concentrations in drinking water samples **(Table 1 & 2)**.

Kruskal–Wallis analysis revealed that elemental distribution patterns were strongly influenced by the environmental matrix and showed significant differences across the four sample matrices for all nine trace elements examined. We observed that Zn ($H = 98.91$, FDR-adjusted $P < 0.001$), Se ($H = 106.87$, FDR-adjusted $P < 0.001$), Cu ($H = 96.74$, FDR-adjusted $P < 0.001$), Pb ($H = 97.40$, FDR-adjusted $P < 0.001$), Cr ($H = 85.38$, FDR-adjusted $P < 0.001$), Ba ($H = 106.96$, FDR-adjusted $P < 0.001$), Cd ($H = 107.35$, FDR-adjusted $P < 0.001$), As ($H = 103.91$, FDR-adjusted $P < 0.001$), and Co ($H = 97.13$, FDR-adjusted $P < 0.001$) all exhibited highly significant variation **(Table 4)**.

Contamination of collard greens

Ccompared with the reference concentrations, collard-green samples showed the presence of contamination by several trace elements. Specifically, Cr and Pb exceeded their respective reference

concentrations in all 30 collard-green samples. 33.3% of the samples showed As concentrations exceeding the reference values, while the percentage of samples with higher levels further increased to 36.7% for Co. Cu exceeded the reference concentration in 10.0% of samples, whereas Zn and Cd exceeded their respective reference standards in one sample each (3.3%).

In collard greens, the median concentrations were 23.94 ppm for Cr, 19.0 ppm for Zn, 18.50 ppm for Pb, 12.34 ppm for Ba, 11.43 ppm for Cu, 4.41 ppm for Co, 1.19 ppm for Se, 1.02 ppm for As and 0.086 ppm for Cd. However, the substantial increase in contamination was particularly observed for Pb and Cr, with median concentration-to-reference ratios of approximately 61.7-fold and 23.9-fold, respectively **(Table 1 & 2)**.

When analysis of collard-green samples was done based on district-level analysis demonstrated significant heterogeneity for Se, Cr, Ba and As after false-discovery-rate correction (FDR-adjusted P=0.0218, 0.0337, 0.0218 and 0.0218, respectively). However, there was an insignificant difference in the distribution of trace elements like Zn, Cu, Pb, Cd or Co among samples collected from districts **(Table 3)**.

Soil contamination

In comparison to the reference values for trace elements in soil, we observed substantially higher levels for several elements. The median concentrations were 244.0 ppm for Zn, 110.75 ppm for Cu, 108.4 ppm for Pb, 114.5 ppm for Cr, 396 ppm for Ba, 12.25 ppm for Cd, 133 ppm for As and 29 ppm for Co. we observed that As and Cd surpassed their respective reference concentrations in all 30 samples. In the case of Cr 25 (83.3%) samples presented exceeded values as compared to its reference concentration, while Cu and Pb showed exceeded values compared to their respective reference concentrations in 21 (70.0%) samples each. Zn concentration in soil samples surpassed the normal reference ranges in 2 (6.7%) samples. However, none of the soil samples exceeded the applied Co or Se reference concentrations **(Table 1, 2)**.

However, we did not find any significant district-wise distribution differences in these elements after FDR correction. This hints at relatively widespread contamination across the sampled districts rather than being restricted to a single geographic location.

Water contamination

From drinking water sources, we observed that the median Cu concentration in water was 0.281 ppm, and 28 of 30 samples (93.3%) exceeded the reference concentration of 0.05 ppm. 05 (16.7%) samples exceeded the applied Pb reference concentration. We could not find any samples exceeding the reference concentrations for Zn, Se, Ba, Cd, Cr or As. However, the results demonstrated significant variation in Cu concentration across districts (Kruskal–Wallis P=0.0326), although this finding should be interpreted cautiously because of the relatively small number of samples per district **(Table 1, 2, 3 and 4)**.

Spatial heterogeneity across districts

When stratifying sample analysis by location (district-wise), we observed spatial heterogeneity in median values of heavy metal contamination. It was observed that Kupwara district showed the highest concentrations of most of the heavy metals, with median concentrations of 36.782 ppm for Zn, 46.939 ppm for Cu, 49.305 ppm for Pb, 10.414 ppm for Cr, 14.274 ppm for Ba and 13.454 ppm for Co. IN Kupwara, we also observed the highest median Se concentration (0.542 ppm). Anantnag, Srinagar, Ganderbal and showed the highest median concentrations of Zn (37.92 ppm), Ba (20.30 ppm), As (1.86 ppm) and As (1.67 ppm), respectively **(Table 3)**.

The district-level variance in heavy metal contamination was also reflected in the collected sample types. The highest mean concentration-to-reference ratio was observed in collard-green samples collected from district Srinagar, followed by Kulgam and Kupwara. In the soil samples, we observed a comparatively higher heavy metal contamination ratio across all districts than in drinking water samples. However, because this ratio is a descriptive comparison with selected reference concentrations rather than a validated human-health risk index, we used it only to characterise relative contamination burden.

Correlation analysis

When analysis was done based on the correlations between two sample matrices, Spearman correlation revealed that there is a strong positive association among heavy metals. This positive correlation was particularly visible within collard-green and paddy samples. In collard greens, the positive correlations were observed between Cu and Co (ρ=0.885), Zn and Co (ρ=0.856), Zn and Cu (ρ=0.868), and Se and As (ρ=0.798), while as in the paddy samples Zn and Cu (ρ=0.803), Cu and Cr (ρ=0.803), Cu and Ba (ρ=0.817), and Pb and Cr (ρ=0.769), presented with the strong positive correlations. Among the various studied heavy metals, the correlational analysis from soil samples demonstrated a more heterogeneous structure, including negative associations between Zn and Cd (ρ=−0.738), Cd and As (ρ=−0.666), Ba and Cd (ρ=−0.558), and Pb and Cd (ρ=−0.528). Since, there was very little heterogeneity of these metals in drinking water samples, we could not get any conclusive information regarding the possible correlations and concentrations were clustered close to the analytical lower range **(Table 5)**.

Overall environmental contamination profile

Collectively, the study findings revealed that there is a substantial and spatially heterogeneous trace element contamination across the collected samples from all 10 districts of Kashmir Valley. Among the findings, the most prominent results were the widespread elevation of Pb and Cr in collard greens. Similarly, we observed As and Cd contamination of soil, and the high frequency of Cu exceedance in water. Interestingly, we observed Kupwara as having the median level of the majority of the studied trace elements on the higher side, while as other districts presented with element-specific elevations. Thus, these findings highlighted the quantifiable environmental contamination gradient across the Kashmir Valley and provide a basis for subsequent investigation of potential exposure pathways, including transfer of contaminants from water to soil and soil to agricultural products. However, these findings need to be interpreted as exposure indicators rather than evidence of causality for ESCC in Kashmir. Linking environmental contamination to

cancer risk requires integration with individual exposure data, dietary patterns, occupational/agricultural practices and district-level cancer epidemiology.

PCA and possible contamination sources

For checking the possible multielement contamination gradient, we employed the PCA. Our analysis revealed that PC1 presented with positive loadings for elements like Zn, Cu, Pb, Ba, Cd, As and Co and accounted for 72.2% of total variance. In case of PC2 which accounted for 14.4% with dominance shown by elements like Se and Cr (**Table 6, a & b**).

Graphical representation of study findings

The overall distribution, spatial pattern and correlational analysis of trace-element contamination are further illustrated in **Figures 1–6. Figure 1** summarises the differences in median trace-element concentrations across the collected sample types, presenting the marked difference in elemental contamination. Figure 2 highlights spatial variation in median concentrations across districts, with significant district-specific differences in some elements. **Figure 3** highlights the frequency and sample-specific extent of elevated trace elements and the proportion of samples exceeding the reference concentrations. We have provided the multivariate representation of the standardised trace-element concentrations across the four sample matrices in **Figure 4**, while **Figure 5** illustrates the distribution and concentration ranges of the elements which have an established role in carcinogenicity. These visual presentations strengthen the results discussed above and offer an integrated representation of the environmental contamination profile observed across the Kashmir Valley.

Discussion

The present study demonstrates the burden and spatial heavy-metal contamination across collected environmental samples of the Kashmir Valley. The findings are potentially relevant for understanding environmental exposure pathways in a population with a historically high burden of gastrointestinal malignancies, with no conclusive aetiology for its high incidence. However, interpret the present environmental exposure data cautiously and do not interpret them as evidence that heavy-metal contamination causes ESCC. Rather, the findings provide an environmental exposure hypothesis that requires confirmation through individual-level exposure assessment and epidemiological investigation. Previous Kashmir-specific literature regarding the unusually high incidence of ESCC (evidenced by population-based cancer registry [36]) is inconclusive. Despite multi-etiological factors involving dietary, lifestyle and environmental factors being associated with this high incidence [22–25, 37, 38], none of the factors have been identified as a causal agent to date.

In our study, we observed a significant difference in concentrations of all 09 analysed trace elements from the collected samples. After correction for false discovery rate for Zn, Se, Cu, Pb, Cr, Ba, Cd, As and Co, the analysis/distribution remained significant, indicating that the elemental profile was strongly dependent on environmental partitioning. This difference in distribution of heavy metals in different sample types seems

biologically plausible because metal mobility, retention and bioavailability are influenced by soil characteristics, pH, organic matter, redox conditions, mineral composition and plant uptake mechanisms [39–41]. However, this difference is particularly important from a human-exposure perspective. Soil contamination may be associated with and may represent an environmental reservoir. But the contaminated crops and drinking water could directly put humans at risk of exposure, and research studies have shown toxic metal exposure due to diet [39]. This type of exposure becomes more significant in areas where agricultural and horticultural production is higher, and people mainly depend on their self-production of crops and vegetables (like Kashmir). Therefore, the continuous assessment of soil, water and edible plant material in the present study provides significant information regarding the potential exposure pathways compared with analysis of heavy metal exposure to humans by studying one sample type at a time.

Soil samples demonstrated that all 30 soil samples exceeded the reference concentrations applied in this study for As and Cd, while 83.3%, 70.0% and 70.0% exceeded the respective reference values for Cr, Cu and Pb respectively. Although these findings indicate substantial contamination relative to the selected benchmarks, soil concentrations should not be equated directly with human-health risk because reference concentrations for soil are not equivalent to tolerable daily intake or food-safety limits. The current study findings are validated by a recent large-scale investigation from Kashmir [40]. The authors of the study analysed 200 agricultural and grazing soils from two districts of Kashmir valley (Bandipora–Ganderbal region) and observed elevated concentrations of several potentially hazardous elements, including As and Cr. They reported that areas with more agricultural, habitation and hydroelectric project areas were more contaminated than forest and grazing areas and suggested that pesticides, fertilizers, construction activities and vehicular emissions may contribute to contamination. Importantly, their study results further hint that both anthropogenic and geogenic processes may contribute to the observed elemental distribution [40]. Thus. The agreement between our study and the regional study strengthens the evidence that potentially toxic-element contamination of Kashmir soils warrants systematic environmental surveillance. Therefore, the soil contamination may become a significant human health burden only when the contaminants are transferred from soil into edible crops or drinking water sources. Their accumulation in crop-producing soils can represent a long-term environmental reservoir, especially where repeated application of agrochemicals for quality agricultural production [40–42].

Our results regarding the presence of heavy metals in collard greens are of great significance, as it provides a direct dietary exposure pathway. Pb and Cr exceeded the acceptable reference guideline concentrations in all 30 collard-green samples. Similarly, the presence of As and Co in 33.3% and 36.7% of samples, respectively, exceeded the corresponding reference concentrations. Thus, the consistent occurrence of elevated Pb and Cr as well as the moderate presence of As and Co in our samples warrants further investigations regarding their source (soils and irrigation water). The exceedingly high concentration of such metals in soil results in greater uptake by plants, aided by soil physicochemical properties and plant-specific physiological characteristics [42]. Recent studies in the form of systematic reviews have highlighted that the simultaneous presence of elevated metals in soil and edible vegetables results in potential soil-to-crop transfer [39]. From the present findings, the presence of As in particular deserves more attention, as inorganic arsenic is a well-established human carcinogen, and chronic exposure through food and drinking water has been associated with several adverse health outcomes [43, 44]. More importantly, in the current

study, As exceeded the applied reference concentration in one-third of collard-green samples and in all soil samples. As per the updated 2024 EFSA assessment, inorganic As is a genotoxic carcinogen [45]. These findings are particularly relevant to populations in which environmental and dietary exposure may occur simultaneously, considering the dependence of populations on agriculture and horticultural practices. The limiting factor in the current study is that we measured total As rather than As species. Therefore, the detected As concentrations cannot be assumed to represent inorganic As specifically. Similarly, total Cr measurements do not distinguish between Cr(III) and the more toxic and carcinogenic Cr(VI) species. This distinction is essential because IARC classifies Cr(VI) compounds as carcinogenic to humans, whereas the carcinogenicity of metallic chromium and Cr(III) compounds cannot be inferred from the Cr(VI) classification [46]. So, we recommend further evaluation to stratify the carcinogenic species of these metals.

Although drinking water samples contained heavy metal concentrations within the acceptable limits, Cu represented a notable exception. In 93.3% (28 out of 30) of the drinking water samples, the Cu concentrations exceeded the reference concentration. These reports suggest that drinking water may constitute an important exposure pathway and warrant the importance of drinking and irrigation water in environmental surveillance. The source of the elevated Cu concentrations cannot be established from the present dataset; however, the plausible contributors include agricultural runoff, corrosion of water-distribution infrastructure, domestic plumbing and other anthropogenic inputs. The lack of substantial exceedance for most other elements suggests that the water contamination profile differs from that of soil and vegetables.

District-level heterogeneity in water Cu concentrations was statistically significant at the nominal level. Larger longitudinal studies incorporating seasonal sampling and characterisation of water sources are required to determine whether the observed Cu pattern is persistent.

As per the objectives, one of the main findings of the present study was the considerable district-wise heterogeneity in heavy metal concentrations. Kupwara demonstrated the most pronounced multielement median profile, with relatively high median concentrations of Zn, Cu, Pb, Cr, Ba and Co. Anantnag showed a comparatively high median Zn concentration; Srinagar demonstrated a distinctive elevation in Ba, while Ganderbal and Shopian showed relatively high median As concentrations. The identification of district Kupwara as having an elevated multielement profile is potentially important for hypothesis generation. The increased concentrations of other elements elevated in different sample sources could reflect shared environmental determinants, including agricultural practices, soil characteristics, irrigation practices, or other anthropogenic activities. The study findings and recent study findings [40] warrant further investigation from all sample sources. The district-wise figures observed in the present study should be viewed as spatial exposure signatures, rather than definitive evidence of pesticide or fertilizer contamination in the respective districts.

The correlation analysis revealed strong positive associations among several elements, particularly in collard greens and paddy samples. Such interactions indicate that exposure to multiple elements may occur simultaneously rather than as isolated single-metal exposures. The positive correlations may also result from the common source of these trace elements. In the present study, overall correlations across all

matrices are likely to be strongly influenced by the substantial differences in concentration ranges between soil, plant and water samples. The heterogeneous distribution of elements across districts and across sample types further hints at multiple sources of possible heavy metal toxicity. These observations are in agreement with the regional evidence from Kashmir showing that different trace elements may have distinct geochemical and anthropogenic signatures [40].

PCA and possible contamination sources

Although, the PCA alone cannot identify the underlying source, but the strong multielement contamination clustering (PC1) in our study indicates some common environmental determinants. This sought of interaction is particularly important and possible due to combined analysis of multiple type of samples (soil, water, collard greens and rice)-together. Thus, hints at the matrix effects in contributing to strong PC1. Such correlational analysis can be helpful in identifying the potential group sources (group of elements) towards disease susceptibility. In agreement with our findings, published studies have reported similar findings [46]. In the context of high agrarian populations, the potential sources include pesticides, insecticides and fertilisers. In one of the recent regional studies, heavy metal contamination from fertilizers, pesticides, building construction and vehicular emissions as potential contributors [46].

Heavy metals and potential relevance to ESCC in study population

The epidemiology of ESCC in Kashmir is very complex, with several factors associated with it. Previously published reports identified multiple potential contributors, including dietary practices, nutritional factors, lifestyle characteristics and environmental exposures [22–24, 37, 38, 47]. However, no single factor could explain the increased incidence. More importantly, the global evidence similarly supports a multifactorial model for ESCC involving tobacco, alcohol, nutritional factors, environmental exposures and other determinants [48]. In our population, the presence of a comparatively higher distribution of heavy metals suggests that the possible relevance to ESCC arises from the merging of the three most common observations, like: Kashmir being considered as one of the highest incidences of ESCC; the Kashmiri population has agriculture and horticulture as main sources of economy and, incidentally, is the pesticides/insecticide applicators for quality crop and fruit production and environmental contamination involving several potentially toxic elements; and some of these contaminants have been proved as established to have carcinogenic potential.

In the current study, As is particularly relevant because chronic exposure to inorganic arsenic is causally associated with cancer. The 2024 EFSA risk assessment concluded that chronic dietary exposure to inorganic As raises health concerns, while WHO identifies it as a major health burden that needs immediate steps to stop its exposure [43, 44, 49]. Similarly, Pb and Cd also have a role in carcinogenicity as well as established health hazards [39, 50, 51]. Similar to As, Cr(VI) compounds are classified as carcinogenic to humans, but the study analysis represents total Cr and does not establish the presence or concentration of Cr(VI) [46]. Thus, the high total Cr concentrations observed in collard greens and soil should be interpreted as evidence of chromium contamination rather than evidence of exposure to carcinogenic Cr(VI), though a

few studies have reported that the reduction of Cr(VI) in humans proceeds through Cr(V) and Cr(IV) intermediates, which are implicated in DNA damage and mutagenesis [52, 53].

Therefore, the association between ESCC and potential environmental heavy metal contamination needs proper investigation. The district-wise environmental measurements should be interpreted and linked with cancer-registry data, separate dietary and occupational exposure details. Similarly, using blood, urine, hair or other samples from suspected populations for biomonitoring could determine whether environmental contamination translates into increased health issues and possible toxicity/carcinogenicity. To substantially strengthen causal interpretation, we need specific mechanistic models to interpret this association.

Implications for environmental and public-health surveillance

Looking at the study findings, the implications seem long-lasting and hence need immediate steps. Identification of extensive contamination in vegetable, garden and paddy field soils as well as in staple food hints at strict environmental monitoring as part of broader food-safety and disease-prevention strategies. Since the economy of Kashmir is mainly dependent on agricultural practices, particular attention needs to be given to areas where people are directly involved with agricultural activity, with drinking water in close proximity. The study also highlights the possibility of targeted and uniform environmental surveillance. The areas with relatively high median concentrations for multiple elements (like district Kupwara) may be considered as hot spot areas for broadening sampling. Similarly, other districts with higher ESCC incidence and accompanied by high median concentrations of heavy metals may warrant focused investigation.

Despite these findings, the reference-limit exceedance analysis needs to be interpreted cautiously. The reference benchmark used for defining the exceedance in the study does not automatically translate into an individual-level health risk due to different heavy metals presented higher values in different samples from different study areas. For proper and specific measurement, an assessment would require exposure frequency, consumption quantity, body weight, bioavailability, cooking practices and contaminant speciation [41, 54].

Strengths and limitations

The major strengths of the study include real-time assessment of 09 elements across 04 sample types collected across all 10 districts of Kashmir Valley. Simultaneously, the district-level analysis and integration of descriptive, non-parametric, correlation and multivariate analyses further add significance to the obtained results. More importantly, the inclusion of drinking water, paddy, and edible plant material provides a more comprehensive exposure framework. The collection of samples at different seasons also adds significance to the study by remove bias of any temporal variability across districts.

Despite, the above-mentioned strengths, several limitations should nevertheless be acknowledged. First, the relatively small number of samples per district limits statistical power for detecting subtle spatial differences. The total elemental concentrations were measured without stratifying them into carcinogenic and potential toxic species like in the case As or Cr again limits the study conclusions. Finally, the study

results also lack direct measurements of pesticide/fertilizer composition, land-use history and individual human exposure.

Conclusion

In conclusion, the present study identifies a substantial and heterogeneous burden of potentially toxic elements present in drinking water, soil and edible plants across Kashmir Valley. The widespread exceedance of various heavy metals above the reference ranges in collard greens, soil, and drinking water is particularly noteworthy. These findings are consistent with the possibility of multiple environmental exposure pathways involving agricultural soils, food crops and drinking water. Despite the comparatively higher concentrations of heavy metals, the obtained results need to be considered hypothesis-generating rather than evidence of causality for ESCC. Therefore, a combination of environmental monitoring, human biomonitoring, exposure assessment and cancer epidemiology will be necessary to determine whether the observed contamination contributes meaningfully to the unusually high burden of ESCC in Kashmir. The present study nevertheless provides an important environmental baseline and identifies specific districts, contaminants and exposure pathways that warrant further investigation.

Declarations

Author Contribution

ANS and GAB: Conceptualization, methodology, investigation, sample collection and coordination, data curation, formal statistical analysis, visualization, interpretation of results, and and revision of the manuscript.. GAB developed the study protocol, interpretation of trace-element findings. ANS prepared the initial draft of the manuscript, tables and figures. SM and SK contributed to study supervision and critical review and editing of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for the work.

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Data Availability

NA

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Tables

Table 1. Median concentrations of trace elements by sample matrix.

Sample matrix	n	Zn (ppm)	Se (ppm)	Cu (ppm)	Pb (ppm)	Cr (ppm)	Ba (ppm)	Cd (ppm)	As (ppm)	Co (ppm)
Collard greens	30	18.98	1.19	11.43	18.50	23.94	12.34	0.09	1.02	4.41
Rice	27	20.35	0.59	17.04	18.27	32.36	3.54	0.20	1.77	6.04
Soil	30	244.00	0.08	110.75	108.40	114.50	396.00	12.25	133.00	29.00
Water	30	0.06	0.00	0.28	0.00	0.00	0.01	0.00	0.01	0.00

Table 2. Samples exceeding the applied reference limits.

Matrix	Element	N	Reference upper limit (ppm)	Exceeding n (%)
Collard greens	Zn	30	50.00	1 (3.3)
Collard greens	Cu	30	30.00	3 (10.0)
Collard greens	Pb	30	0.30	30 (100.0)
Collard greens	Cr	30	1.00	30 (100.0)
Collard greens	Cd	30	0.20	1 (3.3)
Collard greens	As	30	1.10	10 (33.3)
Collard greens	Co	30	5.00	11 (36.7)
Rice	Zn	27	50.00	2 (7.4)
Rice	Cu	27	30.00	9 (33.3)
Rice	Pb	27	0.20	27 (100.0)
Rice	Cd	27	0.40	1 (3.7)
Rice	As	27	0.20	27 (100.0)
Rice	Co	27	5.00	16 (59.3)
Soil	Zn	30	300.00	2 (6.7)
Soil	Se	30	5.00	0 (0.0)
Soil	Cu	30	100.00	21 (70.0)
Soil	Pb	30	100.00	21 (70.0)
Soil	Cr	30	100.00	25 (83.3)
Soil	Cd	30	3.00	30 (100.0)
Soil	As	30	20.00	30 (100.0)
Soil	Co	30	50.00	0 (0.0)
Water	Zn	30	5.00	0 (0.0)
Water	Se	30	0.01	0 (0.0)
Water	Cu	30	0.05	28 (93.3)
Water	Pb	30	0.01	5 (0.0)
Water	Cr	30	0.05	0 (0.0)
Water	Ba	30	0.70	0 (0.0)
Water	Cd	30	0.03	0 (0.0)
Water	As	30	0.10	0 (0.0)

Table 3. District-wise median trace-element concentrations.

District	Zn (ppm)	Se (ppm)	Cu (ppm)	Pb (ppm)	Cr (ppm)	Ba (ppm)	Cd (ppm)	As (ppm)	Co (ppm)
Anantnag	37.92	0.30	23.15	20.32	35.50	6.30	0.20	1.18	12.84
Bandipora	15.44	0.20	11.48	17.01	22.01	5.71	0.11	1.21	2.62
Baramulla	19.56	0.23	10.34	14.88	24.15	6.76	0.09	1.22	2.94
Budgam	18.50	0.35	8.36	17.41	20.84	7.43	0.12	1.22	8.56
Ganderbal	18.52	0.34	7.27	20.31	15.63	7.01	0.16	1.86	8.08
Kulgam	15.36	0.22	10.67	15.97	17.35	8.27	0.10	1.29	2.99
Kupwara	36.78	0.54	46.94	49.30	49.39	14.27	0.19	1.48	13.45
Pulwama	15.56	0.36	16.32	15.05	20.57	7.28	0.12	1.27	2.78
Srinagar	27.97	0.08	20.50	22.70	82.00	20.30	0.08	1.18	7.14
Shopian	22.52	0.26	15.91	10.48	17.47	5.07	0.11	1.67	5.37

Table 4. Kruskal–Wallis comparison across 4 sample types with FDR correction.

Element	Kruskal-Wallis H	*P* value	FDR-adjusted P
Zn	98.91	<0.0001	<0.0001
Se	106.86	<0.0001	<0.0001
Cu	96.74	<0.0001	<0.0001
Pb	97.40	<0.0001	<0.0001
Cr	85.38	<0.0001	<0.0001
Ba	106.96	<0.0001	<0.0001
Cd	107.35	<0.0001	<0.0001
As	103.91	<0.0001	<0.0001
Co	97.13	<0.0001	<0.0001

Table 5. Spearman correlations coefficient (≥ 0.70) within sample types.

Matrix	Element 1	Element 2	Spearman's correlation coefficient (ρ)
Collard greens	Zn	Cu	0.87
Collard greens	Zn	Cd	0.73
Collard greens	Zn	Co	0.86
Collard greens	Se	As	0.79
Collard greens	Cu	Cr	0.73
Collard greens	Cu	Co	0.88
Collard greens	Cd	Co	0.78
Rice	Zn	Cu	0.80
Rice	Zn	Pb	0.75
Rice	Zn	Co	0.70
Rice	Cu	Pb	0.76
Rice	Cu	Cr	0.80
Rice	Cu	Ba	0.82
Rice	Pb	Cr	0.77
Rice	Pb	Ba	0.70
Rice	Cr	Ba	0.80
Soil	Zn	Cd	-0.74

Table 6A. Principal component analysis (PCA) loadings for PC1 and PC2.

Element	PC1	PC2
Zn	0.38	-0.06
Se	-0.14	0.68
Cu	0.38	0.15
Pb	0.37	0.18
Cr	0.17	0.63
Ba	0.37	-0.14
Cd	0.33	-0.15
As	0.38	-0.14
Co	0.36	0.12

Table 6B. Principal component analysis (PCA) variance explained.

Component	Variance explained (%)	Cumulative variance (%)
PC1	72.19	72.19
PC2	14.38	86.57
PC3	6.63	93.21

Figures

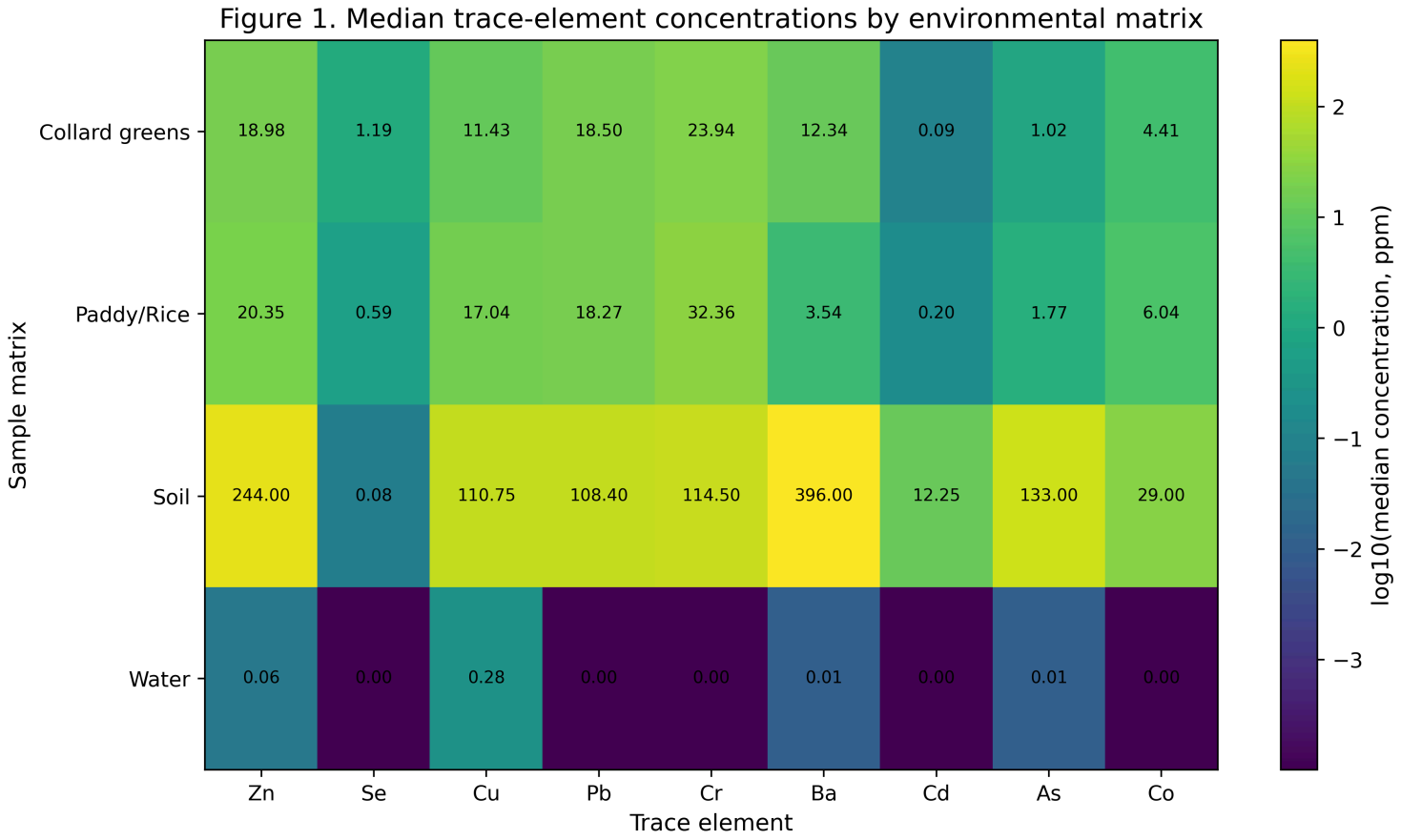


Figure 1

Median trace-element concentrations across the four sample types.

Note: The heatmap uses a log10 transformation for visual comparability because concentrations differ substantially between matrices

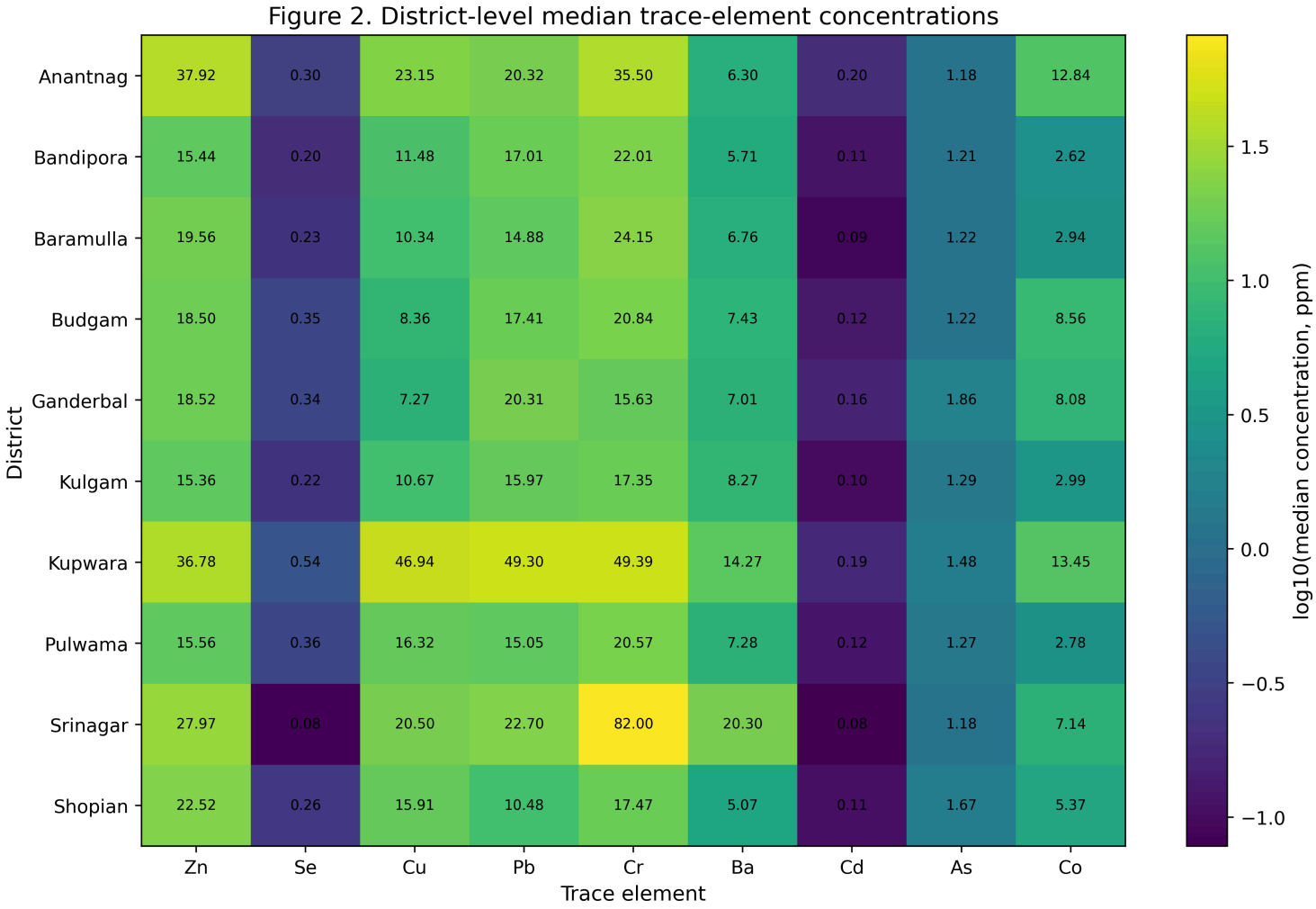


Figure 2

District-level median concentrations across all available samples. Values

are shown numerically within the cells; log10 scaling is used for visual comparability.

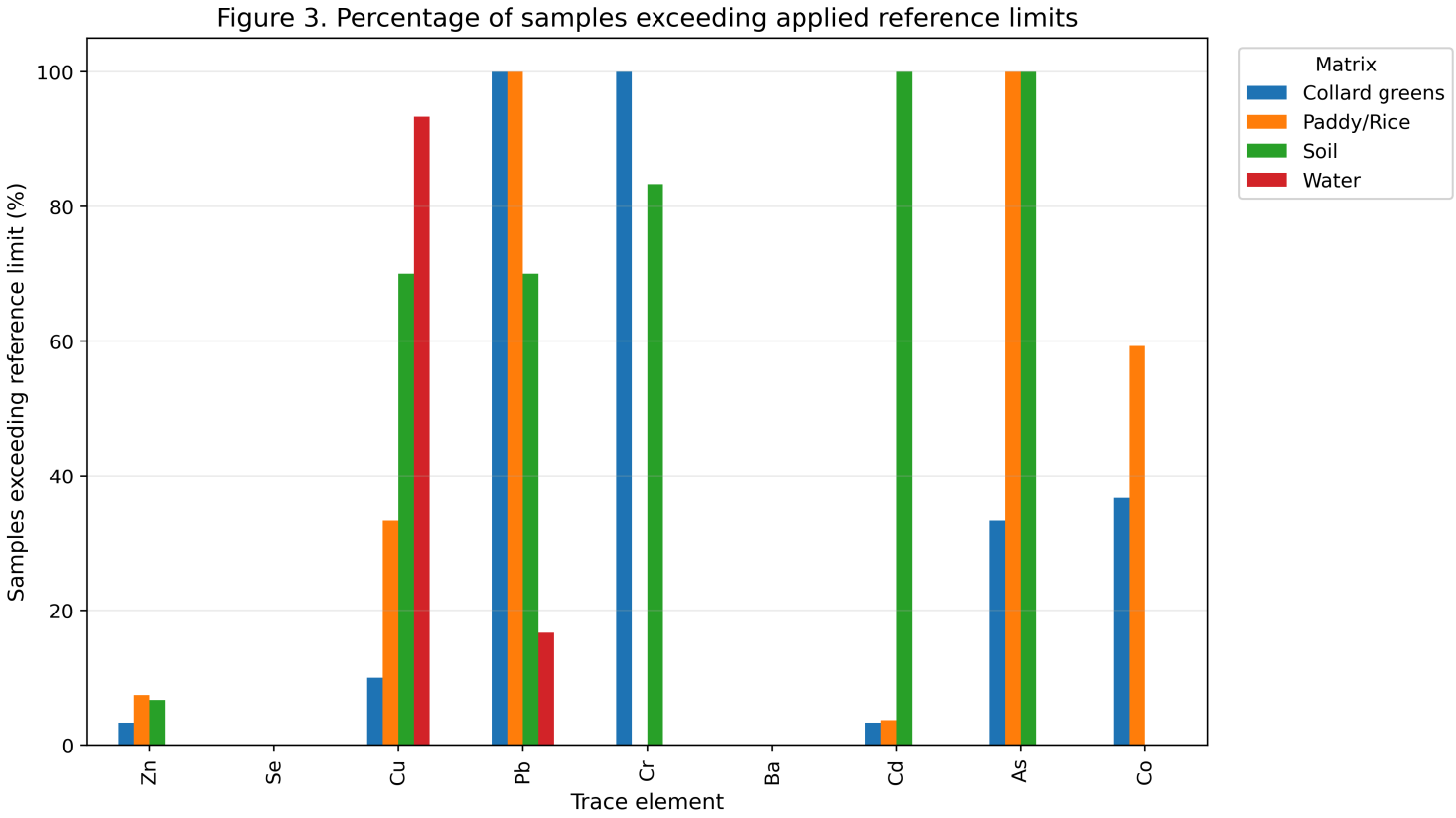


Figure 3

Percentage of samples exceeding the applied reference limit for each element and matrix.

Note: Elements without a reference limit in the workbook are omitted.

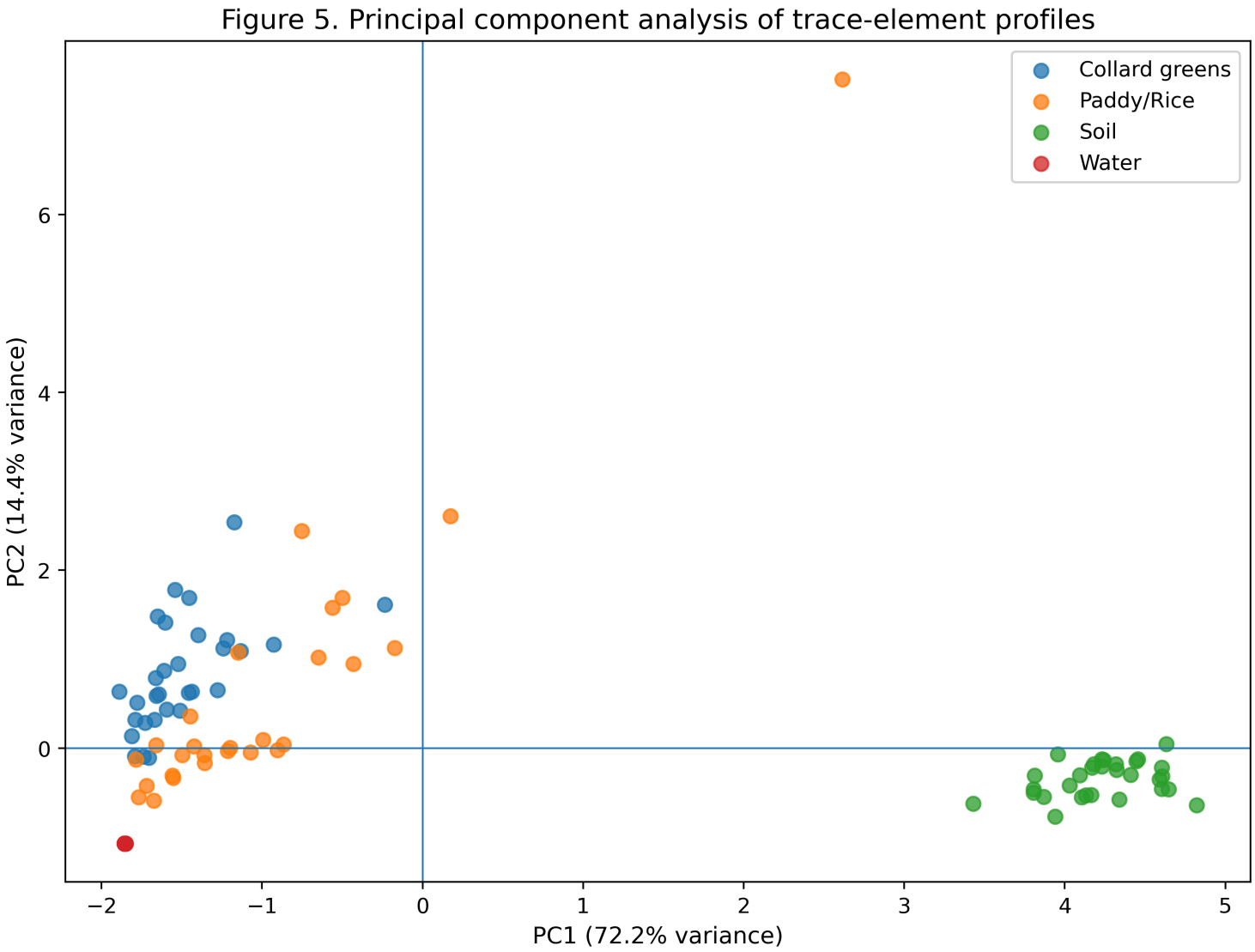


Figure 4

PCA score plot using standardized concentrations across all four sample matrices.

PC1 and PC2 together explain the proportion of variance shown on the axes.

Figure 6. Distribution of selected trace elements across sample matrices

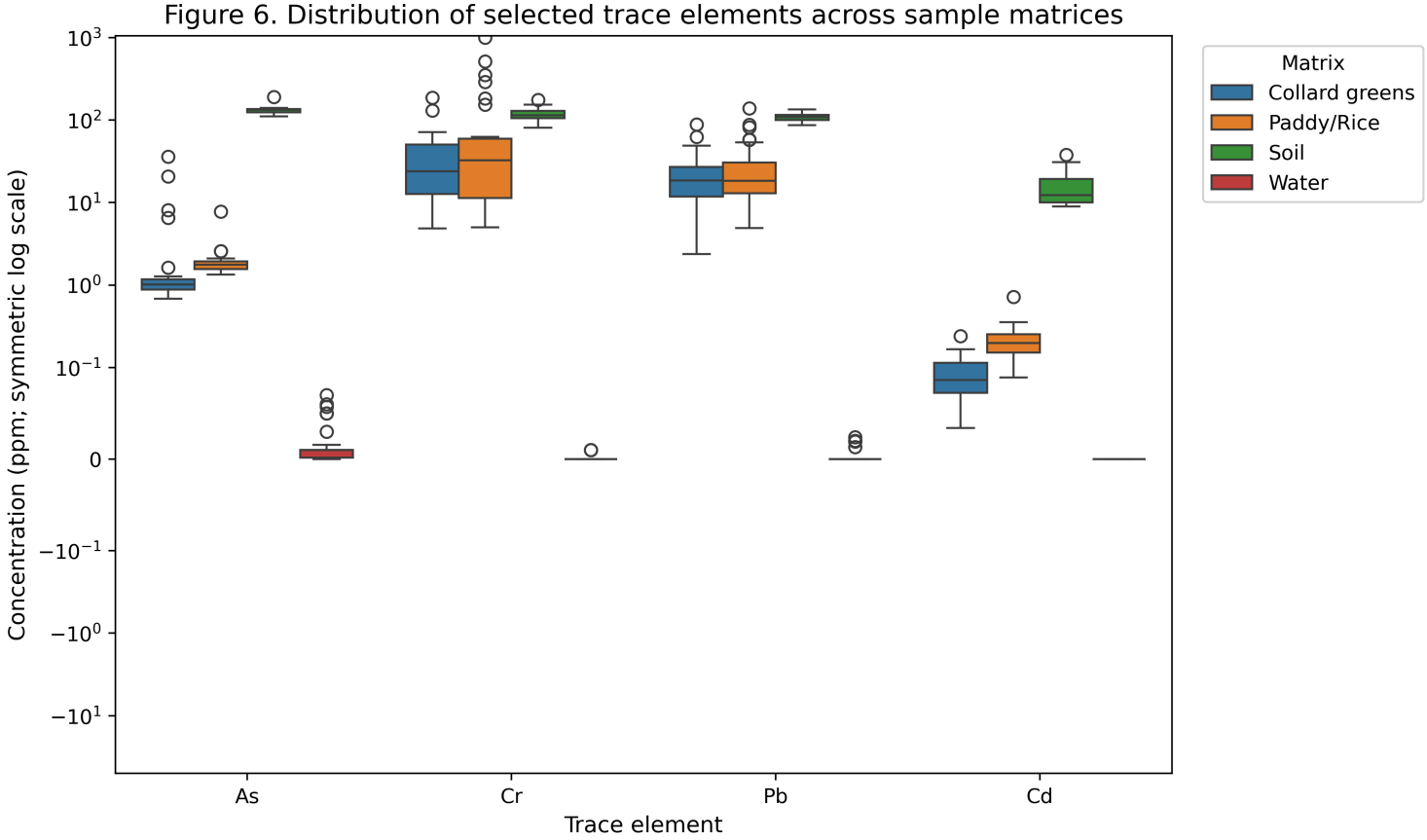


Figure 5

Distribution of selected elements across sample matrices. A symmetric-log y-axis is used because of the large concentration range.

Note: Although nine trace elements were analyzed, Figure 6 presents boxplots for four (As, Cr, Pb, Cd), selected due to their carcinogenicity relevance and clearer visualisation of sample types.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx