Research Square

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Health Risk Assessment of Heavy Metals in Gazo (Oak Galls) and Shouka (Oak Manna) from Kurdistan: ICP-OES Determination and Multivariate Analysis

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Abstract

Despite their long-standing cultural significance, the safety and elemental profile of traditional oak-derived products remain poorly understood. This study evaluated the nutritional potential and toxicological risks of Gazo (oak galls) and Shouka (oak manna) collected from the Kurdistan mountains. Inductively coupled plasma-optical emission spectrometry (ICP-OES) was employed to determine the elemental composition of the samples, while human and animal health risks were assessed using the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI). The concentrations of both essential (Mg, Fe, K, Cr, Ni) and non-essential (Pb, Cd, Ba, Sr) metals were significantly higher in Gazo samples, with several elements exceeding international safety standards and World Health Organization (WHO) permissible limits. Health risk modeling indicated potential long-term non-carcinogenic risks associated with Gazo, revealing that chromium yielded a THQ > 1 and the cumulative HI significantly exceeded the safe threshold of 1. Conversely, Shouka exhibited substantially lower elemental loads, with most metals falling below maximum acceptable limits, although lead (Pb) levels remained a residual concern. Multivariate statistical analyses, including Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), revealed distinct geochemical fingerprints for Gazo and Shouka, underscoring their disparate risk profiles. Collectively, these findings suggest that Shouka is a relatively safe traditional product with low toxicological risk; however, caution is strongly advised regarding the consumption of Gazo due to its elevated heavy metal burden. These results provide essential baseline data for policymakers, consumers, and traditional healthcare practitioners to ensure the safe utilization of these culturally significant natural resources.

Highlights

1. Heavy metals in traditional Kurdistan oak products were assessed using ICP-OES.

2. Shouka (oak manna) showed low elemental loads, indicating consumption safety.

3. Gazo (oak galls) exceeded WHO permissible limits for toxic metals like Pb and Cr.

4. The Hazard Index (HI) for Gazo surpassed 1, indicating cumulative health risks.

5. PCA and HCA revealed distinct geochemical fingerprints for both oak products.

1. Introduction

Medicinal plants and herbal extracts have a long history of use for both preventative and therapeutic purposes across various civilizations, and they remain widely utilized in human and animal healthcare globally. These natural products are frequently perceived as safe and accessible alternatives or complements to conventional medication, significantly contributing to primary healthcare, particularly in regions where access to pharmaceuticals may be limited. The growing global interest in natural remedies necessitates rigorous scientific research to authenticate their historical usage, elucidate their

pharmacological mechanisms, and, crucially, assess their safety profiles (Dias et al., 2012). Among these, natural products derived from oak trees (Quercus spp.) have long been integral to Middle Eastern ethnoecological practices, local diets, and traditional medicine (Morales, 2021). Specifically, two distinct oak-derived products-Gazo (oak galls) and Shouka (oak manna)-hold significant cultural and therapeutic value. Despite their widespread consumption, few studies have comprehensively investigated their chemical safety, nutritional value, or toxicological risks, particularly concerning heavy metal contamination (Al Safı et al., 2022; Banc et al., 2023; De Nicola et al., 2015).

The term "gazo" refers to oak galls, which are aberrant hard outgrowths on the leaves of *Quercus infectoria* caused by gall wasps (Cynipidae) (Banc et al., 2023). These galls are rich in hydrolyzable tannins, specifically gallotannins and ellagitannins, and are widely employed in traditional medicine for their astringent, antibacterial, and wound-healing properties (Yazdanparats et al., 2014). Phytochemical studies have revealed the presence of gallic acid, tannic acid, and catechins, along with potent antioxidant and antibacterial activity (Darikvand et al., 2021a; Sdiq & Saeed, 2019).

Conversely, Shouka (Kurdish: Şoka) is a sweet, syrup-like substance that, especially in the fall, forms on the surface of young acorns or twigs of species like *Quercus ithaburensis*. This phenomenon occurs sporadically, sometimes once every few years, and the product is locally consumed for its distinctive taste and alleged digestive benefits. It is likely related to sap exudation under environmental stress or insect puncture (Arshad et al., 2022; Rameshk et al., 2023; Ramezany et al., 2013). Other plants, such as *Fraxinus ornus*, also produce similar exudates called "manna" that share characteristics with Shouka. They contain mannitol, glucose, fructose, and small amounts of phenolic compounds that can help with constipation and inflammation (Arshad et al., 2022; Darikvand et al., 2021b).

While Gazo and Shouka are both culturally significant, they are wild-harvested and may be susceptible to environmental contamination, particularly by potentially toxic elements such as chromium (Cr), arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd). These metals can accumulate in these natural products through various environmental vectors, including atmospheric deposition, soil uptake, water, or insect activity (Angon et al., 2024; Mohammad et al., 2025). Serious health risks, such as hepatorenal toxicity, neurotoxicity, carcinogenicity, and immunotoxic effects, are associated with long-term exposure to such elements in both humans and animals (Jaishankar et al., 2014; Mititelu et al., 2025).

Toxic metal accumulation has received little attention in research on Quercus products, which has mostly focused on their tannin content, antioxidant activity, and ethnobotanical applications. Likewise, studies on Alhagi manna and Fraxinus have examined their sugar content and traditional medicinal uses, but they frequently lack thorough toxicological analyses (Attanzio et al., 2019; Darikvand et al., 2021a; Hussein, 2023; Kaur et al., 2008; Morales, 2021).

To the best of our knowledge, no research has examined the toxicity and nutritional contributions of trace and heavy metals found in Shouka and Gazo, which are raw materials gathered from the Kurdistan mountains. In light of these knowledge gaps, the current study aims to perform a thorough assessment of Gazo and Shouka samples, with an emphasis on elemental content analysis using inductively coupled

plasma-optical emission spectrometry (ICP-OES) and risk assessment for human and animal health using metrics like the Hazard Index (HI), Target Hazard Quotient (THQ), and Estimated Daily Intake (EDI). For local consumers, public health officials, and traditional practitioners, this study will yield vital baseline information regarding the safety and suitability of these culturally significant oak-derived products.

2. Materials and Methods

2.1 Reagents and Instrumentation Parameters

Throughout the investigation, analytical-grade reagents were used to ensure the precision and repeatability of the findings. Nitric acid (65% w/w; Merck, Germany) and hydrogen peroxide (30% w/w; BioChem, France) were utilized for sample digestion. For all dilutions, glassware rinsing, and calibration standard preparation, high-purity deionized water with a conductivity of 0.05 µS/cm was generated using a Barnstead GenPure purification system (Thermo Fisher Scientific, USA). To prevent elemental contamination, all reagents and solutions were prepared fresh and handled in a trace-metal clean laboratory setting.

Samples were digested using a microwave digestion system (Multiwave GO Plus, Anton Paar). Elemental analysis was subsequently conducted using a Thermo Scientific iCAP 7600 ICP-OES Duo equipped with Qtegra Intelligent Scientific Data Solution™ (ISDS) software. The operating parameters for the ICP-OES analysis are detailed in Table 1.

Table 1
Instrument parameters for ICP-OES.

Parameter	Setting
Pump Speed	50 rpm
Spray Chamber	Glass cyclonic
Center Tube	2 mm
Nebulizer	Glass concentric
Nebulizer Gas Flow	0.7 L·min-1
Auxiliary Gas Flow	0.5 L·min-1
Coolant Gas Flow	12 L·min-1
RF Power	1250 W
Exposure Time	UV 15 s, Vis 10 s

2.2. Sample collection

Shouka and Gazo are two traditional oak-derived products originating from the mountainous areas of Penjwin and Sharbazher in the Kurdistan Region of Iraq. Due to their extreme rarity, only four samples of Gazo and two samples of Shouka were collected. Both products are highly seasonal, harvested exclusively in the fall, which restricts their supply and elevates their cultural and economic significance.

During the fall season, specific oak trees (e.g., *Quercus ithaburensis* and *Quercus* spp.) produce a natural exudate known as Shouka. It is manually gathered from the tree surface and visually resembles sweet dew **(**Fig. 1A**).** Following collection, it is processed using traditional methods to create a concentrated sweet syrup rich in vital nutrients and bioactive substances with anti-inflammatory and antioxidant properties.

In contrast, Gazo (oak galls) consists of aberrant hard outgrowths that form on *Quercus infectoria* leaves. As previously detailed, these structures are induced by the activity of gall wasps (Cynipidae) during the fall season. Gazo is manually harvested and subsequently processed into a powdered form for traditional applications **(**Fig. 1B**).**

To prevent external contamination, all samples were collected directly from their natural sources using sterile, inert containers. Prior to laboratory analysis, each sample was appropriately labeled with the collection date and location and stored in a controlled environment.

2.3. Sample Preparation

Samples were placed in a laboratory oven at 105°C for two hours to dry to a constant weight. Subsequently, one gram of each dried sample was weighed into a PTFE-TFM digestion vessel. A customized acid mixture consisting of nine milliliters of concentrated nitric acid (65% w/w) and one milliliter of hydrogen peroxide (30% w/w) was added to each vessel. The vessels were sealed and processed using a microwave digestion system. The heating program consisted of a temperature ramp to 200°C over 20 minutes, followed by a 20 minute holding time at 200°C. After the vessels were allowed to cool to room temperature, they were carefully opened, and the inner walls were rinsed with high-purity deionized water to ensure complete sample transfer. The resulting digests were filtered through Whatman No. 41 filter paper to remove any residual particulates and quantitatively diluted to a final volume of 50 mL with deionized water in a volumetric flask.

2.4. Analytical Procedure

Elemental concentrations in the Shouka and Gazo samples were determined using a Thermo Scientific iCAP 7600 ICP-OES Duo, operated under the parameters detailed in Table 1. External calibration curves were established using multi-element standard solutions prepared across the expected concentration ranges for each analyte. High-purity deionized water was utilized for all dilutions. Instrument calibration was verified using independent quality control standards, and method blanks were analyzed periodically to monitor for potential background contamination and memory effects. Each sample digest was analyzed in triplicate, and the final quantitative results were expressed in mg/kg on a dry weight basis.

2.5. Health risk assessment

The potential human health risks associated with the consumption of Gazo and Shouka were evaluated using standard toxicological indices: Estimated Daily Intake (EDI) (Demir & Ağaoğlu, 2023), Target Hazard Quotient (THQ) (Barone et al., 2015), and the Hazard Index (HI) (Ferreira et al., 2023; Storelli, 2008).

The EDI for each metal was calculated according to the equation:

$$EDI = \frac{C\,x\,IR}{BW}$$

where C represents the determined concentration of the metal in the sample (mg/kg, dry weight), IR is the estimated average daily ingestion rate (0.003 kg/day, corresponding to 3 g/day), and BW is the average body weight of an adult, standardized to 70 kg.

The THQ for each element was determined using the formula:

$$THQ = \frac{EDI}{RfD}$$

where *RfD* is the established oral reference dose for each specific element (mg/kg/day). The RfD values were acquired from published toxicological guidelines provided by the United States Environmental Protection Agency (USEPA) (Mills, 2002), the World Health Organization (WHO) (*Joint FAO/WHO Expert Committee on Food Additives (JECFA) Guidance Document for the Establishment of Acute Reference Dose (ARfD) for Veterinary Drug Residues in Food Geneva*, n.d.), and the Agency for Toxic Substances and Disease Registry (ATSDR) (Atsdr, n.d.; Taylor et al., 2023; Wong et al., 2022). Elements without an established oral RfD (e.g., Na, K) were excluded from the THQ calculations. A THQ value of less than 1 indicates that adverse non-carcinogenic health effects are unlikely, whereas a value equal to or greater than 1 signifies a potential health risk associated with chronic exposure to that specific metal.

To evaluate cumulative risk, the Hazard Index (HI) was calculated as the sum of all individual THQs for a given sample:

$$HI = \sum_{i=1}^{n} THQ$$

An HI value of less than 1 indicates no significant combined toxicological risk from exposure to multiple metals. Conversely, an HI greater than or equal to 1 suggests a potential for cumulative adverse health effects, warranting precautionary measures. All calculations were executed independently for each sample, and the resulting concentrations were systematically compared against the relevant human and animal toxicity thresholds defined by international guidelines.

3. Results and Discussion

3.1. Metals in Gazo and Shouka Samples

The concentrations of essential and non-essential metals in Gazo and Shouka samples collected from multiple sites were determined and evaluated against established human and animal safety thresholds (Fig. 2). This comparison provides critical insights into both the nutritional contributions and the potential health risks of these traditional products. As illustrated in Fig. 2A, several essential elements in the Gazo samples significantly exceeded permissible limits for both humans and animals. Most notably, potassium (K), magnesium (Mg), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), and nickel (Ni) were found at elevated levels, while copper (Cu) exceeded only the animal safety limits.

All measured non-essential and potentially toxic elements in Gazo-specifically barium (Ba), lead (Pb), cadmium (Cd), and strontium (Sr)-surpassed both human and animal safety thresholds. Of particular concern, elements such as Mg, Fe, Ni, Ba, Cd, and Sr consistently exceeded WHO permissible limits for humans (*Joint FAO/WHO Expert Committee on Food Additives (JECFA) Guidance Document for the Establishment of Acute Reference Dose (ARfD) for Veterinary Drug Residues in Food Geneva*, n.d.), as well as established animal feed guidelines, highlighting a significant toxicological risk. Overall, Fig. 2A demonstrates that while certain elements like Cu and Zn pose minimal risk, the hazard profile is predominantly driven by heavy metals such as Pb, Ni, Cd, and elevated transition metals like Fe. These elemental exceedances are frequent and severe, particularly under animal exposure scenarios, directly aligning with the elevated THQ and HI values calculated for Gazo. Figure 2B presents the elemental profiles of the Shouka samples relative to the same toxicity thresholds. Overall, Shouka exhibited markedly lower elemental concentrations compared to Gazo, with the vast majority of values remaining well below established safety limits. Among the essential elements, only potassium (K) and magnesium (Mg) approached or marginally exceeded animal limits, though they remained safely within human permissible ranges. Chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), and zinc (Zn) were present at concentrations far below both human and animal thresholds, indicating negligible toxicological concern for these metals. Regarding non-essential elements, Ba, Cd, and Sr were detected merely at trace levels, strictly within safe consumption ranges. However, lead (Pb), while substantially lower than the levels observed in Gazo, still exceeded WHO permissible limits for humans and select animal thresholds, underscoring a residual contamination risk. These findings suggest that, unlike Gazo, Shouka poses minimal hazard based on its overall metal content, with potential health risks being strictly limited to Pb exposure.

3.2. Health risk assessments

To evaluate the potential health risks associated with the consumption of Gazo and Shouka, the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) were determined for all detected metals (Fig. 3A–C). As illustrated in Fig. 3A, the EDI values for the majority of the evaluated elements were significantly higher in Gazo than in Shouka, reflecting an overall enrichment of both major and trace elements in the Gazo samples. Although the dietary exposure for several metals in both

groups falls within low ranges, the elevated EDI of Mg, Fe, Sr, and specific trace metals in Gazo indicates a substantially greater daily intake burden for consumers.

In Fig. 3B, the THQ values demonstrate that most metals remain safely below the non-carcinogenic threshold (THQ < 1). However, chromium (Cr) in the Gazo samples significantly exceeded this threshold, signaling a clear potential non-carcinogenic health risk from chronic exposure. By contrast, all evaluated metals in Shouka remained well below the THQ safety threshold, underscoring its comparatively low-risk profile.

Finally, Fig. 3C illustrates the cumulative Hazard Index (HI), which aggregates the individual metal THQs to estimate total exposure risk. The HI value for Gazo was markedly higher than 1, confirming a significant potential for cumulative adverse health effects. Conversely, the HI for Shouka remained strictly below 1, suggesting a negligible combined toxicological risk. Together, these results indicate that while both products contribute to dietary heavy metal exposure, the Gazo samples pose a substantially greater health concern due to higher overall metal burdens and the specific exceedance of Cr-based toxicological thresholds. This integrated assessment highlights the critical importance of monitoring heavy metal accumulation in culturally valued natural products and provides a robust quantitative basis for evaluating consumer safety. Figure 3. Health risk assessment of heavy metal exposure from Gazo and Shouka consumption. (A) Estimated Daily Intake (EDI) of analyzed metals, demonstrating higher ingestion burdens for Gazo. (B) Target Hazard Quotient (THQ) values; notably, only Cr in Gazo exceeds the safety threshold of 1. (C) Hazard Index (HI) evaluating cumulative exposure, indicating a significant potential health risk for Gazo (HI > 1) but a negligible risk for Shouka.

3.3. Multivariate statistical analysis

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are robust multivariate statistical tools utilized to reduce data dimensionality, reveal latent patterns, and classify samples based on compositional similarities in complex datasets (Hossain et al., 2011; Stanford, 2012; Yi et al., 2015). Application of PCA to the elemental composition data revealed a distinct separation between the Gazo and Shouka samples, underscoring their unique geochemical signatures (Fig. 4A). PC1, which accounted for an overwhelming 89.39% of the total variance, served as the primary axis of separation, providing compelling evidence of their geochemical divergence. The clustering of the Gazo samples on the positive side of PC1 reflects their relative enrichment in major and trace elements such as K, Mg, Fe, and Sr, which dominate the compositional variability. In contrast, the Shouka samples plot negatively along PC1, which is consistent with their systematically depleted elemental profiles. This stark contrast suggests fundamentally different geochemical environments, biological accumulation pathways, or source materials between the two products. The Gazo enrichment pattern may reflect derivation from highly mineralized bedrock, enhanced weathering inputs, or potential anthropogenic influence, whereas the depleted Shouka signatures point to either a geologically impoverished substrate or selective biological exudation processes that naturally limit trace element transport.

The minor variation along PC2 (6.20% of variance) indicates that intra-site heterogeneity is limited compared to the dominant between-product differences. However, subtle divergences-particularly within the Shouka samples-may signal localized environmental or physiological effects. Importantly, the non-overlapping confidence ellipses underscore the statistical robustness of the separation, confirming that the two groups represent discrete geochemical populations rather than overlapping variations of a single source. From a broader perspective, these results highlight the efficacy of multivariate statistical approaches, particularly PCA, for discriminating geochemical fingerprints in complex botanical matrices. The clear distinction between Gazo and Shouka reinforces the potential of such methods to unravel provenance, environmental processes, and resource characteristics in geoscientific and food safety investigations.

Hierarchical Cluster Analysis (HCA) was employed to further explore the geochemical relationships among the samples and to independently validate the groupings observed in the PCA. HCA classifies samples based on their Euclidean distance in terms of elemental composition, producing a dendrogram that illustrates relative similarities among groups. The resulting clustering pattern (Fig. 4B) perfectly mirrors the PCA separation, with all Gazo samples forming a coherent, tightly grouped cluster, while the two Shouka samples are grouped distinctly apart. Within the Gazo cluster, Gazo-1 and Gazo-4 display the highest similarity, followed by Gazo-3 and Gazo-2, indicating only minor intra-group variability. By contrast, the Shouka samples cluster tightly together but remain highly dissimilar from the Gazo group, strongly reinforcing their geochemical distinctiveness. Together, the PCA and HCA analyses provide powerful, complementary evidence that the two traditional products represent statistically and chemically discrete populations.

4. Conclusion

This study provides the first comprehensive evaluation of the elemental composition and associated health risks of Gazo and Shouka, two culturally significant oak-derived products widely consumed in the Kurdistan region of Iraq. The findings demonstrate that Shouka is comparatively safe for human and animal consumption; the vast majority of its metal concentrations remain well below international safety thresholds, with the exception of a modest residual risk linked to Pb contamination. In stark contrast, Gazo presents substantial toxicological concerns. Multiple elements-most notably heavy metals like Cr and Pb, alongside elevated levels of Mg and Fe-exceeded permissible exposure limits, ultimately contributing to a cumulative Hazard Index (HI) significantly greater than 1. Furthermore, multivariate statistical analyses (PCA and HCA) confirmed that the two products are geochemically distinct, reflecting fundamentally different environmental sources, biological origins, and elemental accumulation pathways. These insights emphasize the urgent need for the routine monitoring of Gazo, the development of localized safety regulations, and increased public health awareness regarding its potential heavy metal risks. Conversely, Shouka demonstrates strong potential as a promising and safe natural sweetener or nutraceutical resource. Future research should expand the sample size, investigate seasonal and geospatial variability, and explore processing mitigation strategies to reduce

contamination, thereby ensuring the safe integration of these traditional natural products into modern dietary and therapeutic practices.

Declarations

Competing Interests:

The authors declare that they have no competing interests.

Ethics Approval:

Not applicable. This study involved the chemical analysis of plant-derived materials and did not involve human participants or live animal experiments.

Consent to Participate:

Not applicable.

Consent for Publication:

Not applicable.

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Author Contribution

H.J.H. contributed to the study conceptualization, methodology, and wrote the original draft of the manuscript. H.A.I. conducted the formal data analysis, data curation, and contributed to writing the original draft. A.H.A. supervised the research project and contributed to reviewing and editing. M.S.M. performed the investigation, validated the data, and prepared the figures and visualizations. All authors reviewed the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

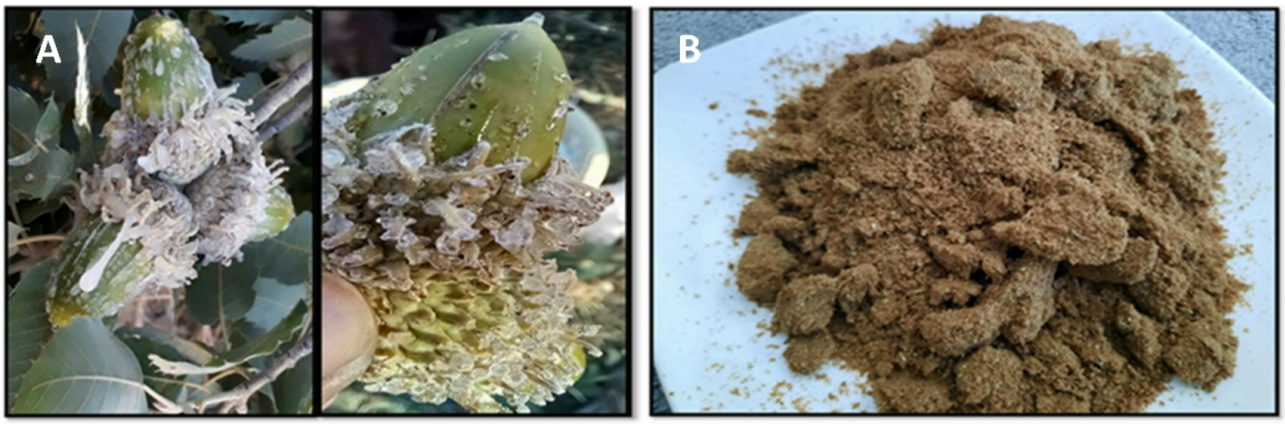


Figure 1

(A) Shouka, a natural exudate that forms on the surface of oak trees, and (B) Gazo, a powdery deposit found on the leaves of Quercus infectoria.

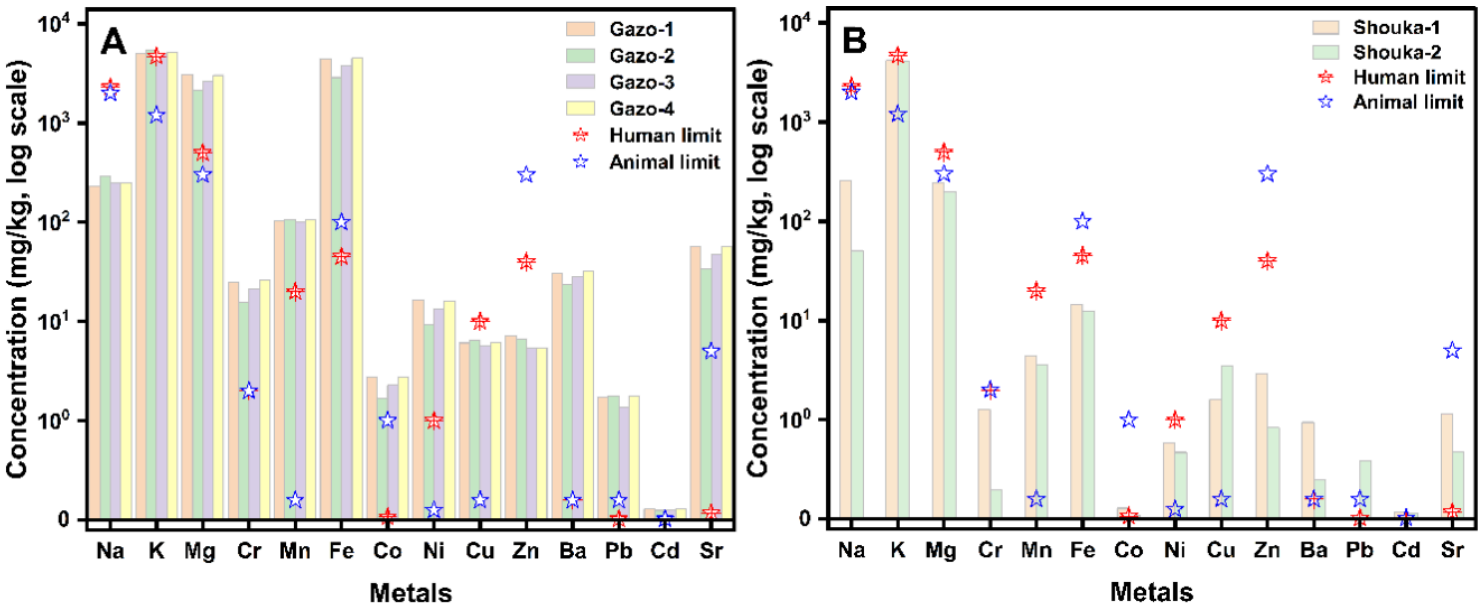


Figure 2

Essential and non-essential elements content in different samples of Gazo and Shouka and their maximum allowable limit for both human and animal.

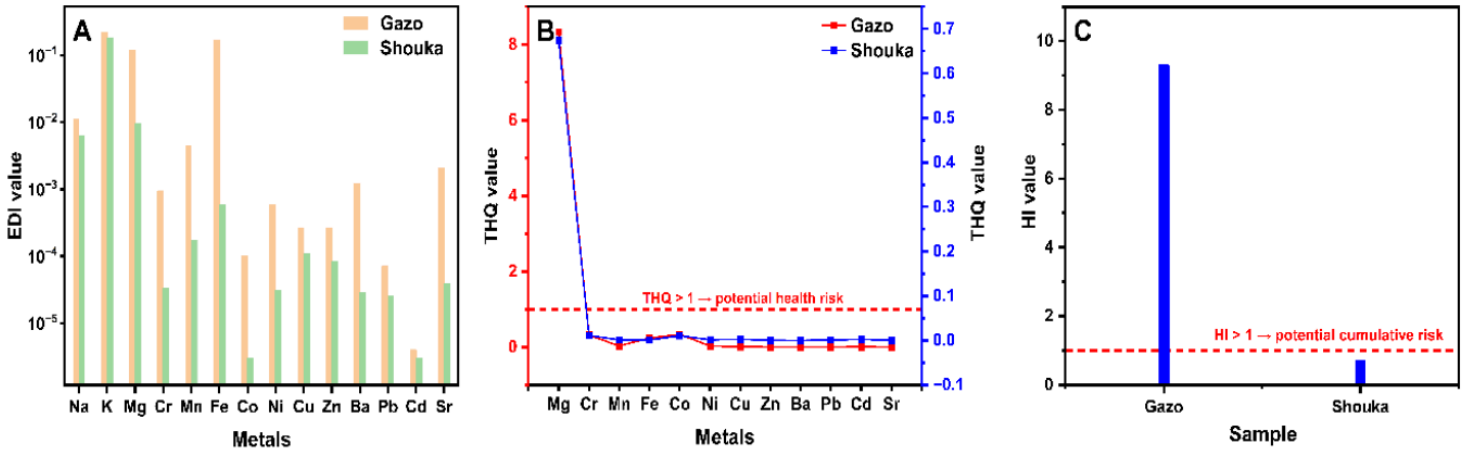


Figure 3

Health risk assessment of metal exposure from Gazo and Shouka. (**A**) EDI of metals, higher for Gazo. (**B**) THQ values; only Cr in Gazo exceeds safety limit. (**C**) HI indicates potential cumulative risk for Gazo (HI > 1), negligible for Shouka.

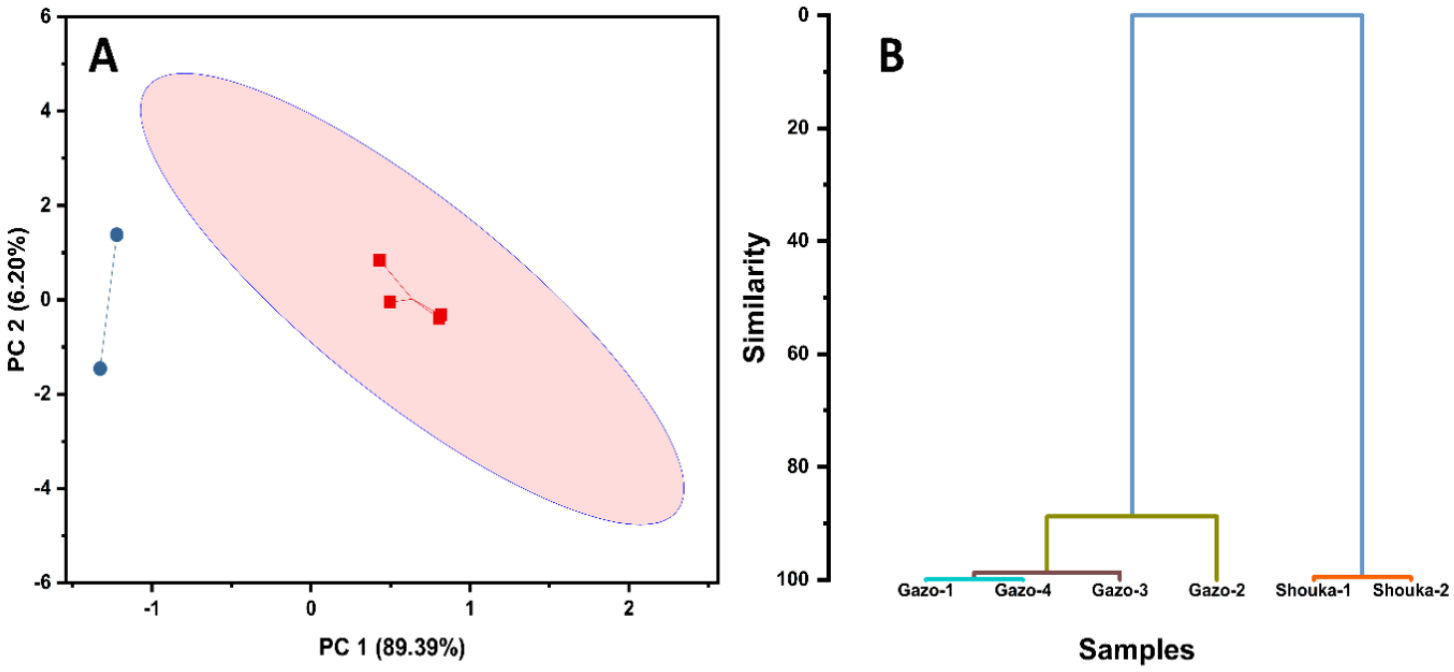


Figure 4

(**A**) PCA score plot showing distinct separation between Gazo and Shouka samples. (**B**) HCA dendrogram confirming the grouping, with Gazo and Shouka forming two geochemically distinct clusters.