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Research article

Comprehensive health risk assessment process of trace elements in different parts of the saffron plant (*Crocus Sativus* L.): From soil to stigma

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Abstract: Saffron (*Crocus Sativus* L.), particularly its stigma, is a valuable global spice and medicinal plant. However, understanding the comprehensive process of trace element uptake and accumulation across all its plant parts, from soil to stigma, is crucial for ensuring food safety and assessing potential health risks. This study aimed to conduct a detailed health risk assessment of trace elements in different components of the saffron plant (stigma, leaves, petals, corms, stems), alongside and analysis of cultivation soil and irrigation water (total samples = 70). The investigation focused on quantifying element concentrations, evaluating their translocation dynamics within the plant, and assessing the associated human health risks through dietary exposure.

Analysis using the inductively coupled plasma optical emission spectroscopy (ICP-OES) method revealed accumulation of iron (Fe, mean 1.46 mg/kg) and aluminum (Al, mean 29.73 mg/kg) in the edible stigma, followed by zinc (Zn, 0.55 mg/kg), manganese (Mn, 0.18 mg/kg), and copper (Cu, 0.11 mg/kg). The saffron cultivation soil exhibited very high concentrations of Al (273.60 mg/kg) and Fe (275.83 mg/kg), suggesting high bioavailability and transfer to the plant tissues. Importantly, toxic metals, such as cadmium (Cd), lead (Pb), arsenic (As), nickel (Ni), and chromium (Cr), were found to be well below established safety thresholds in all analyzed plant parts. A heatmap approach provided detailed sample clustering, illustrating element distribution patterns. Furthermore, Monte Carlo simulations (MCS) for health risk assessment demonstrated that the calculated target hazard quotient (THQ) and incremental lifetime cancer risk (ILCR) values for toxic metal exposure via saffron consumption were significantly below safety limits. Both adult and child populations showed negligible non-carcinogenic risk (THQ < 1) and acceptable carcinogenic risk levels (ILCR < 10^{-6}), indicating minimal health concerns from dietary intake of saffron under the studied conditions.

This research presents a comprehensive multi-tissue assessment of essential and toxic metals in saffron, integrating data from plant tissues, soil, and irrigation water. It clarifies how trace elements move toward the edible stigma, providing key evidence for quality control and safety evaluation. The findings contribute to (i) establishing safety baselines for regulatory and quality standards, (ii) informing agricultural practices by revealing metal uptake mechanisms, and (iii) assessing consumer health risks through dietary exposure analysis. By covering the entire pathway from soil to stigma and applying advanced risk-assessment tools (THQ, ILCR, MCS), this study forms a solid foundation for future research on element management in saffron cultivation and offers valuable insights for food safety and agricultural science.

Keywords: trace elements; food analysis; spices; risk assessment; saffron

1. Introduction

Saffron (*Crocus Sativus* L.), globally recognized as the world's most precious spice, commands a significant market share, valued at US 1.07 billion in 2022 and projected to reach US 1.77 billion by 2029 [1]. Iran, the leading producer, faces dynamic cultivation landscapes influenced by agroecological conditions, irrigation strategies, and soil geochemistry [2,3]. The high value of saffron is attributed not only to its low yield and labor-intensive harvesting but also to its rich profile of unique sensory compounds (crocins, picrocrocin, safranal) and over 150 bioactive phytochemicals demonstrating antioxidant, anti-inflammatory, anticancer, and neuroprotective properties [4–6]. Optimal cultivation necessitates specific environmental parameters, including temperatures between 23–27 °C for approximately 50 days, seasonal rainfall of ~600 mm, well-drained sandy-loam soils, and low humidity to mitigate rot [1,3,7]. In response to escalating consumer interest in saffron's nutraceutical benefits and the challenges caused by climate variability (e.g., extended droughts, unpredictable weather patterns), innovative cultivation approaches, such as controlled-environment agriculture, hydroponics, and aeroponics, are emerging to stabilize global supply and reduce crop losses [8–10].

Concurrently, ensuring the quality and safety of saffron has become paramount for both public health and international trade. The accumulation and distribution of trace elements within saffron and its various plant tissues (corms, stems, leaves, petals, and the edible stigma) are intrinsically linked to soil characteristics, climatic conditions, irrigation water sources, and agricultural practices [11]. While essential elements, such as iron (Fe), zinc (Zn), and copper (Cu), are vital for plant and human health, non-essential and toxic trace metals, such as lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), arsenic (As), and chromium (Cr), can cause significant health risks even at trace concentrations [12,13]. These contaminants may enter the agricultural system through geogenic sources, fertilizers, industrial atmospheric deposition, and the use of wastewater for irrigation a common practice in water-scarce saffron-producing regions [14,15]. Consequently, commercial saffron samples have frequently been found to contain elevated levels of Pb, As, Cd, and Cr, often with distinct geographical signatures [16]. Chronic exposure to these contaminants can lead to severe multi-organ damage, including neurotoxicity and renal impairment, and significantly increases cancer risk, particularly from As and Cd accumulation [17]. Therefore, a thorough investigation into the transfer of trace elements from soil and water to the edible stigma is critical for safeguarding consumer health.

Although the corms, stems, and leaves are not directly consumed, they play a crucial role in the uptake and sequestration of trace elements and can serve as valuable bioindicators of environmental pollution. However, a significant knowledge gap exists because no study has systematically investigated the distribution and translocation dynamics of trace elements across the entire soil-plant-water continuum under field conditions, encompassing all major saffron plant parts (water → soil → corm → stem → leaves → petals → stigma). While some recent studies have explored specific aspects, such as elevated As, Cd, Cr, and Pb levels in tissues irrigated with wastewater (though still below FAO/WHO limits with THQ < 1) [1], or ecological risk assessments in farmlands indicating moderate pollution and transfer to plant tissues with low human health risks (THQ < 1) [18,19]; analyses showing trace metals below safety thresholds in other regions [20]; and impact of organic fertilizers and the weight of mother corms on saffron yield, apocarotenoid levels, and the accumulation of metal contaminants showed that the levels of key metal contaminants were below acceptable limits across all treatments, showing that none of the organic fertilizers tested led to saffron contamination [21]. These have often focused on limited plant parts or specific contaminants (Figure 1).

Furthermore, the intricate pathways of elemental translocation and their final deposition in the stigma require a more integrated and comprehensive examination. The necessity for such a study is underscored by the high global consumption of saffron as both a food additive and a nutraceutical, coupled with increasing environmental pressures and the potential for contaminant uptake [6,22]. The importance lies in establishing robust safety baselines, informing agricultural practices, and providing consumers with confidence in the safety of this valuable product. The challenges involve precise elemental analysis across diverse matrices and complex risk assessment methodologies.

Accordingly, this study aims to address these critical gaps by (i) quantifying the concentrations of a comprehensive suite of trace elements in all distinct parts of the saffron plant (stigma, petals, leaves, stems, corms), the associated cultivation soil, and irrigation water, utilizing the high-sensitivity inductively coupled plasma optical emission spectroscopy (ICP-OES) technique and (ii) assessing the non-carcinogenic and carcinogenic health risks associated with dietary exposure to these elements via saffron consumption, utilizing advanced risk assessment methods, including Monte Carlo simulations (MCS).

By providing a detailed characterization of trace elements throughout the saffron production continuum, from the initial environmental inputs to the final edible product, this research will establish a precise scientific basis for advanced quality control measures, ultimately contributing to the production of safer saffron products.

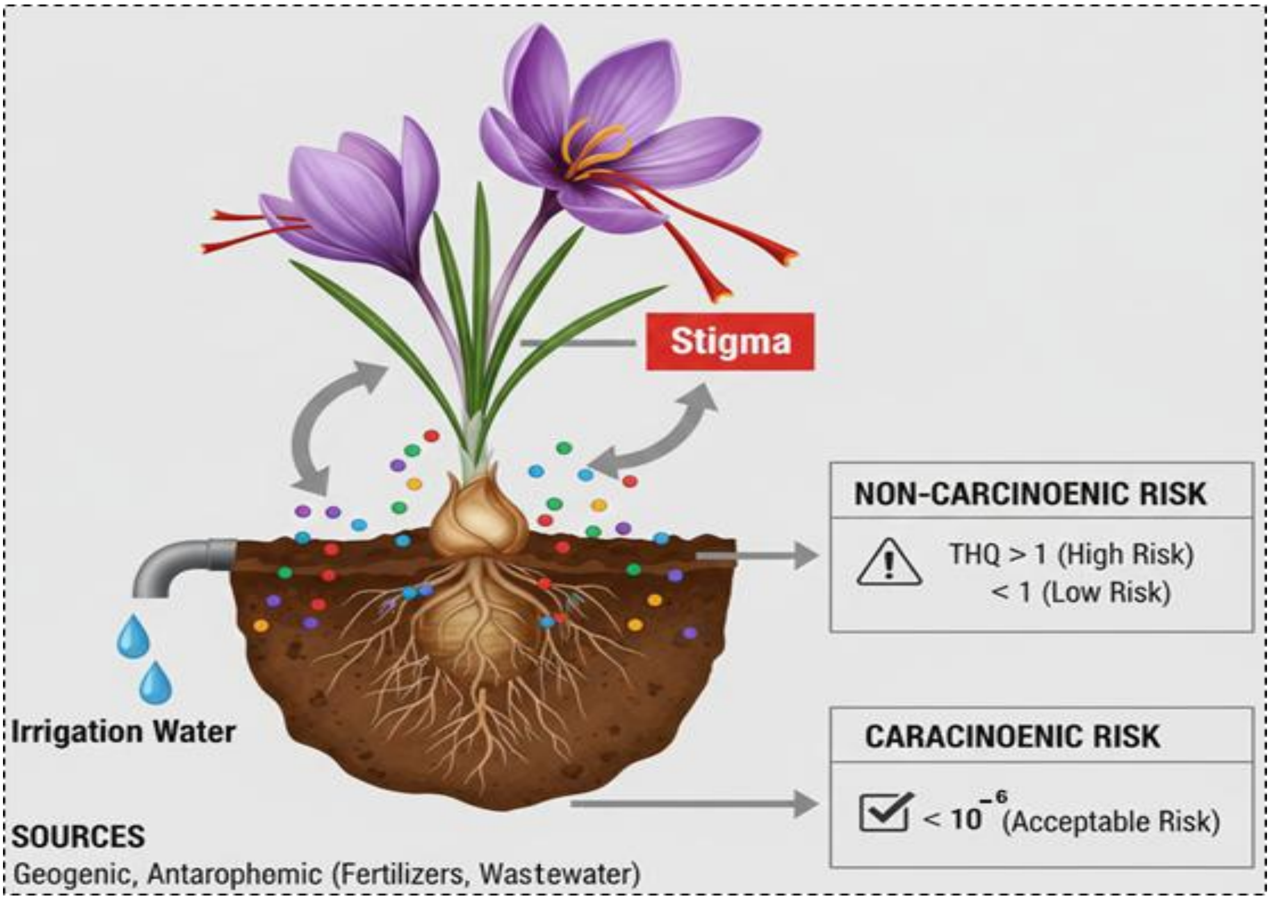

Figure 1. Schematic of the health risk assessment framework for trace elements: Illustrating the translocation pathway from irrigation water and soil through the saffron (*Crocus Sativus* L.) life cycle (corm, leaves, and petals) to the final consumer product (stigma).

2. Materials and methods

2.1. Chemicals

Nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were obtained from Tamad Kala Store, Iran originate to Merck Co., Ltd., Germany. All chemicals used were of analytical-reagent grade. The aqueous solutions used in the experiments were prepared with ultrapure water.

2.2. Sample collection

To measure the concentration of trace metals in water, soil, and different parts of the saffron, a comprehensive sampling strategy was used [23]. Samples were collected from ten different agricultural regions in Kashmar, Khorasan Razavi province include water (n = 10, from the irrigation source), soil samples (n = 10, from the rhizosphere of the saffron plants), and plant tissue samples (n = 50) such as corm, stem, leaves, petal, and stigma. In total, this resulted in the collection of 70 individual samples.

2.3. Sample digestion and analysis

Water: Water samples were first filtered through a 0.45 µm membrane filter. The filtered water (10 mL) was acidified with 9 mL of concentrated HNO_3 (65%) and H_2O_2 (1 mL) and then digested using a hot plate (Shimaz company, Tehran, Iran) at 100 °C for 2–3 h, and heating continued until obtained clear solution. The final digest was filtered, transferred to a 25 mL volumetric flask, and diluted to 25 mL with ultrapure water [24].

Soil: The samples were air-dried at 30 °C, and then passed through polyethylene sieve with mesh size No. 10 to remove stones and debris. The fine soil (1 g) was weighed and transferred into a Teflon digestion vessel contained 9 mL HNO_3 (65%) and 1 mL H_2O_2 and then digested using a hot plate at 100 °C for 2–3 h, and heating continued until obtained clear solution. The final digest was filtered, transferred to a 25 mL volumetric flask, and diluted to 25 mL with ultrapure water [24].

Saffron tissue: All plant tissues were washed and oven-dried before analysis. The different saffron parts (corm, stem, leaves, petal, and stigma) were ground to a fine powder. The dried powder (1 g) was acidified with 9 mL of concentrated HNO_3 (65%) and H_2O_2 (1 mL) and heated at 100 °C for 2–3 h, and heating continued until obtained clear solution. Finally, the digest was filtered and made up to volume (25 mL) with ultrapure water. The final solutions were kept refrigerated at 4 °C until measurement and analyzed by ICP-OES (Spectro Arcos, Spectro, Germany) [24].

2.4. ICP-OES condition

ICP-OES was carried out in RF power of 1.2 kW and flow rate of 12.0, 1.0, and 0.7 for plasma, auxiliary, nebulizer gas, respectively, and with viewing mode of axial for low-level elements (Cd, Pb, As), and radial for higher concentration elements (Fe, Zn, Cu, Mn) at replicate read time of 15 s and instrument stabilization delay of 30 s.

Calibration curves (at least six points) were constructed in the range 0.01-10 mg/L depending on the element, with correlation coefficients ≥ 0.9995. Samples were introduced in triplicate, and the mean value was reported.

2.5. Exposure and risk assessment

The risk due to consumption of elements in Saffron was estimated using a strategy of probabilistic risk assessment with MCS. The risk was estimated in two scenarios; non-carcinogenic risk which is expressed as the target hazard quotient (THQ) and a carcinogenic risk which is expressed as the incremental lifetime cancer risk (ILCR) as follows [18,25]:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}} \tag{1}$$

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{ED} \times \mathrm{EF} \times \mathrm{IR}}{\mathrm{BW} \times \mathrm{AT}} \tag{2}$$

$$\mathrm{HI} = \sum \mathrm{THQ} \tag{3}$$

$$\mathrm{ILCR} = \mathrm{CSF} \times \mathrm{EDI} \tag{4}$$

Where EDI: estimated daily intake, RfD: oral reference dose, CSF: cancer slope factor, and HI: hazard index. The EDI (mg/kg/day of body weight) was calculated using (Eq. 2), in which the oral reference doses for Ba, Mn, Hg, Cd, Ni, As, and Cr were based on 0.2, 0.14, 0.0001, 0.001, 0.02, 0.00006, and 0.003 mg/kg/day, respectively [26], C represents the mean concentration of metals in the Saffron (mg/kg); IR is ingestion rate (2 g/person by day) (U.S. EPA, 1990); EF is exposure frequency (365 days/year) [27]; ED is exposure duration (70 years) (U.S. EPA, 1990); BW is the body mass, adult (70 kg) [28]; and AT is averaging time for noncarcinogens (365 days/year× number of exposure years [29].

ILCR is the possibility of developing cancer of Cr, Cd, and As, and CSF relates to the carcinogenic slope factor of 0.5 (mg/kg/day) for Cr, 1.5 (mg/kg/day) for As, 0.38 (mg/kg/day) for Cd recommended by United States Environmental Protection Agency (USEPA).

The values of CSF related to the carcinogenic slope factor used for Cr was 0.5 (mg/kg/day), As = 1.5 (mg/kg/day), and Cd = 0.38 (mg/kg/day). Based on EPA, $>10^{-4}$ means high cancer risk, $10^{-6}<$ ILCR $<10^{-4}$ signifies acceptable or low cancer risk, and $<10^{-6}$ means negligible cancer [18,24]. The MCS was performed with the Crystal Ball using 10,000 iterations in the variables and 5000 iterations in the uncertainty.

2.6. Statistical analysis

The results of research were shown as mean ± standard deviation (SD), and data elements in the Saffron sample were evaluated for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene's test) using the program of Statistical Package for the Social Sciences (SPSS Inc., USA, version 24.0). A heat map was employed to analyze (clustering method: average linkage; distance method: Pearson) the relationship between elements in saffron at online https://biit.cs.ut.ee/clustvis/. An MCS was used to the uncertainty risk assessment by using the Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Denver, CO, USA).

3. Results and discussion

This section presents and interprets the findings concerning the concentration of trace elements in various parts of the saffron plant (from soil to stigma) and evaluates the associated human health risks.

3.1. Trace elements analysis

The statistical evaluation of trace element concentrations in soil, irrigation water, and saffron plant components (corms, leaves, petals, stems, and stigmas) revealed clear and distinguishable patterns among both essential and non-essential elements (Table 1). Safe limits for certain trace metals in edible stigma (mg/kg), according to Food and Agriculture Organization (FAO)/World Health Organization WHO) and United States Department of Agriculture (USDA) standards, are as follows: As (0.50), Cd (0.10), Cr (1.0), Pb (0.30), Hg (0.50), Ni (1.50), Cu (20.0), Fe (425.50), Mn (30.0), Zn (5.0) [23].

Based on mean concentrations (Table 1), the essential elements showed the following order: Fe > Mn > Zn > Cu. Fe exhibited the highest mean concentration among essential nutrients (43.78 mg/kg), demonstrating uptake and translocation within the saffron plant. This elevated absorption is consistent with the plant's high requirement for Fe in chlorophyll biosynthesis, electron transport, and pigment formation in the stigma, which may explain the high Fe content observed in both vegetative and reproductive tissues [30,31]. The accumulation of Fe in the stigma, the directly consumed part, can be considered a beneficial nutritional source [6,22], provided it does not exceed permissible limits.

Zn presented a mean concentration of 2.61 mg/kg, falling within the optimal physiological range for *Crocus Sativus*. This concentration reflects its essential role in enzymatic activation, protein synthesis, and regulation of secondary metabolites that influence saffron quality attributes such as aroma and coloring strength [22,32,33]. The moderate but sufficient Zn content suggests that soil Zn availability was adequate and that the plant maintained efficient homeostatic control over Zn uptake [11,17].

Mn showed a mean concentration of 3.46 mg/kg, considered normal for saffron metabolic activity. The observed values align with Mn's role in photosystem II stabilization, antioxidant defense mechanisms, and regulation of carbohydrate metabolism in corms [22,34]. The consistency of Mn levels across plant tissues suggests controlled uptake mechanisms that prevent both deficiency and toxicity.

Cu had the lowest mean concentration among essential elements (0.50 mg/kg) but remained within acceptable and physiologically functional levels. Cu's involvement in phenolic metabolism, oxidative enzyme activity, and crocin biosynthesis [30,35], explains why even low concentrations are sufficient to meet metabolic demand. The relatively low Cu content may result from limited soil availability or adsorption to soil organic matter and clay minerals, which typically reduce Cu mobility and plant uptake [11,35].

Among all analyzed elements, Al showed the highest mean concentration (44.926 mg/kg), exceeding even the essential nutrients. Although Al is not required for plant growth, its elevated presence likely reflects its naturally high abundance in the regional soils and the acidic conditions that enhance Al solubility and root uptake [11]. The substantial translocation of Al into different plant parts, including the edible stigma, suggests that saffron can tolerate elevated Al levels, potentially due to internal detoxification mechanisms, such as organic acid exudation and compartmentalization. However, the high concentration observed raise concerns regarding long-term plant health and possible implications for food safety.

Regarding toxic heavy metals, the mean concentration of Pb was 0.24 mg/kg, remaining below the maximum permissible limits for saffron established by WHO/FAO and the European Spice Association (0.3 mg/kg) [1]. The presence of Pb at this level may be attributed to low-level geogenic background sources or minor anthropogenic inputs such as atmospheric deposition from vehicle emissions or dust particles. The limited mobility of Pb in soil due to its strong adsorption to clay minerals and organic matter likely restricted its uptake by the saffron plant, resulting in values within safe dietary limits [22].

Cd was detected at a very low mean concentration (0.002 mg/kg), far below international safety thresholds. Cd's low concentration can be explained by its limited natural abundance in the regional soils and the absence of major anthropogenic sources such as industrial effluents or phosphate fertilizers, which are typically the primary contributors to Cd accumulation in crops. Additionally, saffron possesses selective uptake mechanisms that restrict Cd translocation from roots to aerial tissues, further reducing its concentration in edible parts [22].

As exhibited a trace mean level of 0.09 mg/kg, which is consistent with its generally low solubility and strong binding affinity to iron oxides in soil. This binding significantly limits As mobility and plant availability. The low values also suggest minimal influence from irrigation water, as groundwater is often the dominant pathway for As contamination in crops [22].

Cr was detected at a mean concentration of 0.26 mg/kg. This level likely reflects the presence of naturally occurring Cr (III) in soils, which is the stable and less toxic form of chromium. Cr (III) has low bioavailability due to its tendency to form insoluble complexes, resulting in restricted uptake by root tissues. The absence of major anthropogenic Cr sources (e.g., industrial discharge, leather tanning waste) in the study area further supports the low concentrations observed [22].

Ni showed a mean concentration of 0.83 mg/kg, falling within acceptable limits for edible plant products. Moderate Ni levels are common in soils derived from ultramafic or metal-rich parent materials, which may partially explain the values detected. Despite being more mobile than Pb or Cr, Ni uptake by saffron appears biologically regulated, preventing excessive accumulation in the stigma [1,22,35].

Overall, the toxic metals Cd, As, Cr, and Ni were present at minimal to low concentrations, indicating limited environmental contamination and effective biological restriction by the saffron plant. However, the comparatively higher mean values of Al and Pb, although still within permissible limits, highlight the need for careful soil assessment, monitoring of irrigation water quality, and the adoption of agronomic practices aimed at reducing heavy metal availability. These measures are essential to ensure the continued production of safe, high-quality saffron suitable for both domestic consumption and international markets.

The mean concentrations of the analyzed trace elements in different parts of saffron, soil, and irrigation water revealed distinct distribution patterns (Table 2). Fe showed the highest levels in soil (275.83 mg/kg) and was also elevated in the stigma (1.46 mg/kg), followed by leaves (6.13 mg/kg), indicating active uptake and translocation from soil to the economically important reproductive tissue. Al was similarly abundant in soil (273.60 mg/kg) and exhibited considerable accumulation in the stigma (29.73 mg/kg) and corms (1.32 mg/kg), suggesting efficient transfer of Al from soil into both storage and edible organs despite its non-essential nature.

Among essential micronutrients, Zn concentrations ranged from 0.55 mg/kg in stigma to 2.38 mg/kg in leaves and 2.69 mg/kg in stems, remaining within expected physiological limits and reflecting normal nutrient homeostasis. Mn was highest in soil (21.13 mg/kg) but appeared at much lower levels in saffron tissues, particularly stigma (0.18 mg/kg), consistent with its regulated uptake to avoid phytotoxicity. Cu displayed moderate concentrations across plant parts (0.11–0.54 mg/kg), with the highest value measured in soil (1.50 mg/kg). These observations suggest that saffron maintains controlled uptake of micronutrients, absorbing sufficient quantities for metabolic functions while preventing excessive accumulation [17,22].

Regarding toxic heavy metals, Pb and Cd concentrations in the stigma were low (0.09 and 0.001 mg/kg, respectively), remaining well below international safety limits. As and Cr were similarly low in the stigma (0.02 and 0.05 mg/kg), indicating limited transfer from soil to edible tissues. Irrigation water contained negligible levels of all monitored trace elements, confirming that soil was the primary source influencing elemental distribution within the plant.

Overall, while the essential elements displayed balanced and physiologically appropriate distribution within plant tissues, the substantial transfer of certain elements particularly Al from soil to the stigma warrants attention due to potential implications for product safety and long-term soil management in saffron-producing regions.

The concentrations of trace elements observed in the saffron stigma in the present study are in good agreement with those reported by previous investigations on saffron stigma from various producing regions of India [1,16], Ukraine [36], Iran [18,37], and Morocco [5], indicating consistent micronutrient accumulation patterns in *Crocus Sativus* regardless of geographical origin and differing soil/water characteristics.

For example, Abou Fayssal et al. (2024) investigated health risk assessment of trace metals in saffron (*Crocus Sativus* L.) cultivated in domestic wastewater and lake water irrigated soils in India [23]. The study compared the effects of domestic wastewater (DW), Sarbal Lake water (SLW), and borewell water (BW) on soil characteristics and saffron cultivation in Pampore, India. DW irrigation produced the strongest significant improvements ($p < 0.05$) in soil physicochemical and nutrient properties, followed by SLW and BW. DW irrigation resulted in higher concentrations of trace metals in saffron corms, petals, and stigmas, with observed ranges (mg/kg) in corms: As (0.21–0.40), Cd (0.04–0.09), Cr (0.16–0.41), Cu (7.31–14.75), Fe (142.38–303.15), Pb (0.18–0.31), Mn (15.26–22.81), Hg (0.18–0.25), Ni (0.74–1.18), and Zn (3.44–4.59). Comparatively, the stigma part was characterized by the lowest levels of trace metals (mg/kg): As (0.12–0.24), Cd (0.01–0.04), Cr (0.09–0.27), Cu (4.16–9.57), Fe (105.04–210.67), Pb (0.11–0.24), Mn (10.38–17.22), Hg (0.12–0.20), Ni (0.41–0.75), and Zn (2.33–3.78). Despite these increases, all values remained below FAO/WHO permissible limits. Health risk assessments also indicated no potential hazard for consumers of saffron irrigated with DW or SLW. The results of trace metal concentrations in stigma show that the levels of certain elements, such as Fe, Mn, Zn, and Cu, are high, similar to what is reported in our results.

In another study, health risk assessment of trace metals in saffron samples in Gonabad, Iran was reported by Taghavi et al. (2024) [18]. Quantities of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in saffron samples varied from 0.01 to 0.36 (mean 0.17) mg/kg, 0.01–1.31 (mean 0.37) mg/kg, 0.01–0.28 (mean 0.10) mg/kg, 0.86–2.31 (mean 1.32) mg/kg, 4.98–42.04 (mean 21.20) mg/kg, 92.530–740.66 (mean 237.23) mg/kg, 14.80–26.28 (mean 17.67) mg/ kg, 0.66–1.78 (mean 1.05) mg/kg, 0.07–0.84 (mean 0.25) mg/kg and 24.32–56.71 (mean 37.23) mg/kg, respectively. The mean concentrations of trace metals in saffron samples were in order of Fe>Zn>Cu>Mn>Cr>Ni>Cd> Pb>As>Co. The higher quantities of Fe were also observed in soil samples taken from the same saffron farmlands in a recent study [19].

Table 1. The statistical analysis of the elements in total samples (mg/kg).

	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Sb	Se	Sn	V	Zn
Min	ND	ND	0.02	0.01	ND	ND	0.03	ND	ND	ND	ND	ND	0.00	ND	ND	ND	ND	0.00	0.00
Max	0.01	327.43	0.54	4.64	0.029	0.94	1.78	1.76	276.21	ND	24.11	0.011	6.02	1.66	ND	0.15	0.07	1.69	11.01
Mean	0.001	44.92	0.09	0.69	0.002	0.13	0.26	0.50	43.78	ND	3.46	0.006	0.83	0.24	ND	0.05	0.02	0.22	2.61
Median	ND	2.38	0.03	0.10	0.001	0.01	0.04	0.42	3.49	ND	0.52	0.006	0.12	0.04	ND	0.03	0.02	0.01	2.43
SD	0.000	0.98	0.01	0.13	0.000	0.03	0.05	0.04	0.97	ND	0.75	0.000	0.17	0.05	ND	0.00	0.00	0.05	0.20

ND: Not detected, SD: Standard deviation, Ag: Silver, Al: Aluminum, As: Arsenic, Ba: Barium, Cd: Cadmium, Co: Cobalt, Cr: Chromium, Cu: Copper, Fe: Iron, Hg: Mercury, Mn: Manganese, Mo: Molybdenum, Ni: Nickel, Pb: Lead, Sb: Antimony, Se: Selenium, Sn: Tin, V: Vanadium, Zn: Zinc.

Table 2. The statistical analysis of the elements in different segments of Saffron, soil and irrigation water (mg/kg).

		Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	Sn	V	Zn
Leaves	Min	ND	2.285	0.022	0.186	0.001	0.010	0.038	0.345	4.999	0.496	0.007	0.100	0.038	0.124	0.038	0.009	1.993
	Max	0.017	3.343	0.035	0.217	0.003	0.013	0.055	0.467	7.511	0.595	0.011	0.128	0.053	0.158	0.063	0.015	3.880
	Mean	0.002	2.773	0.030	0.202	0.001	0.011	0.044	0.405	6.136	0.543	0.009	0.110	0.046	0.142	0.048	0.012	2.386
	Median	ND	2.787	0.030	0.204	0.001	0.011	0.043	0.400	6.073	0.540	0.009	0.108	0.046	0.138	0.047	0.012	2.156
	SD	0.000	0.280	0.004	0.011	0.000	0.001	0.005	0.033	0.647	0.029	0.001	0.008	0.006	0.011	0.006	0.002	0.571
Petal	Min	ND	2.381	0.029	0.086	ND	0.012	0.033	0.475	3.363	0.434	0.006	0.173	0.045	0.023	0.012	0.011	2.553
	Max	0.009	22.976	0.038	0.139	0.003	0.018	0.072	0.599	5.710	0.824	0.011	0.218	0.107	0.061	0.025	0.014	3.942
	Mean	0.001	6.915	0.034	0.111	0.002	0.016	0.056	0.541	4.825	0.653	0.009	0.202	0.066	0.042	0.018	0.012	3.485
	Median	ND	3.552	0.035	0.105	0.002	0.016	0.057	0.540	4.814	0.659	0.009	0.208	0.060	0.043	0.017	0.012	3.531
	SD	0.000	0.655	0.003	0.019	0.001	0.002	0.012	0.040	0.778	0.121	0.002	0.017	0.021	0.014	0.004	0.001	0.368
Soil	Min	ND	213.427	0.362	3.034	ND	0.665	1.142	1.138	275.32	16.818	ND	3.793	1.125	ND	ND	1.167	4.673
	Max	0.013	327.433	0.548	4.643	0.029	0.947	1.786	1.760	276.21	24.116	ND	6.025	1.669	ND	ND	1.698	11.015
	Mean	0.003	273.605	0.463	3.952	0.005	0.834	1.504	1.536	275.83	21.131	ND	4.950	1.417	ND	ND	1.446	6.297
	Median	ND	289.700	0.466	4.100	0.004	0.875	1.566	1.582	275.90	22.231	ND	5.110	1.478	ND	ND	1.500	5.943
	SD	0.000	0.391	0.063	0.572	0.000	0.094	0.188	0.205	0.303	2.421	ND	0.619	0.185	ND	ND	0.191	1.769

Continued on the next page

		Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	Sn	V	Zn
Corm	Min	ND	0.888	0.030	0.239	0.002	0.009	0.032	0.331	1.841	0.386	ND	0.094	0.022	0.089	0.023	0.007	1.548
	Max	ND	1.683	0.038	0.379	0.007	0.014	0.054	0.510	2.910	0.634	0.003	0.157	0.063	0.136	0.078	0.014	3.421
	Mean	ND	1.325	0.035	0.325	0.003	0.012	0.043	0.421	2.377	0.527	0.001	0.112	0.037	0.116	0.042	0.010	2.299
	Median	ND	1.405	0.035	0.342	0.003	0.013	0.044	0.423	2.411	0.554	0.001	0.113	0.039	0.117	0.043	0.011	2.174
	SD	ND	0.276	0.002	0.048	0.001	0.002	0.008	0.061	0.371	0.092	0.000	0.019	0.011	0.016	0.015	0.002	0.618
Stigma	Min	ND	2.753	0.021	0.041	0.001	0.008	0.037	0.096	1.132	0.104	0.002	0.123	0.023	ND	0.033	0.000	0.511
	Max	0.011	107.068	0.033	0.099	0.001	0.009	0.061	0.156	2.006	0.340	0.003	0.180	0.261	ND	0.060	0.007	0.624
	Mean	0.003	29.732	0.024	0.061	0.001	0.008	0.050	0.118	1.460	0.181	0.002	0.142	0.090	ND	0.046	0.002	0.551
	Median	0.001	10.591	0.022	0.052	0.001	0.008	0.052	0.110	1.316	0.124	0.002	0.136	0.047	ND	0.045	0.001	0.544
	SD	0.000	0.441	0.005	0.024	0.000	0.001	0.010	0.025	0.341	0.010	0.001	0.023	0.010	ND	0.010	0.000	0.046
Stem	Min	ND	1.090	0.022	0.037	0.001	0.006	0.032	0.317	2.459	0.221	0.005	0.084	0.016	0.032	0.030	0.004	2.009
	Max	ND	1.772	0.035	0.057	0.002	0.011	0.052	0.521	4.097	0.343	0.008	0.133	0.036	0.056	0.069	0.007	3.168
	Mean	ND	1.522	0.025	0.048	0.001	0.010	0.042	0.442	3.412	0.295	0.006	0.118	0.030	0.044	0.044	0.006	2.695
	Median	ND	1.507	0.024	0.049	0.001	0.010	0.042	0.452	3.464	0.296	0.006	0.121	0.031	0.043	0.042	0.006	2.755
	SD	ND	0.217	0.004	0.006	0.000	0.001	0.006	0.064	0.473	0.035	0.001	0.014	0.006	0.007	0.010	0.001	0.332
Water	Min	ND	ND	0.021	0.012	ND	ND	0.038	ND	ND	ND	0.003	0.001	ND	ND	ND	0.004	0.009
	Max	ND	ND	0.024	0.017	ND	0.003	0.054	0.002	0.182	0.007	0.005	0.181	0.003	ND	0.002	0.026	0.241
	Mean	ND	ND	0.022	0.014	ND	0.001	0.046	0.000	0.043	0.001	0.004	0.044	0.000	ND	0.000	0.021	0.079
	Median	ND	ND	0.022	0.014	ND	ND	0.046	ND	0.029	0.000	0.004	0.003	ND	ND	ND	0.022	0.071
	SD	ND	ND	0.001	0.001	ND	0.001	0.004	0.000	0.005	0.000	0.001	0.007	0.000	ND	0.000	0.006	0.006
Sig.		0.02	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

ND: Not detected, SD: Standard deviation, Ag: Silver, Al: Aluminum, As: Arsenic, Ba: Barium, Cd: Cadmium, Co: Cobalt, Cr: Chromium, Cu: Copper, Fe: Iron, Hg: Mercury, Mn: Manganese, Mo: Molybdenum, Ni: Nickel, Pb: Lead, Sb: Antimony, Se: Selenium, Sn: Tin, V: Vanadium, Zn: Zinc.

3.2. Estimated daily intake (EDI)

The EDI (percentile 95%) of molybdenum (Mo), barium (Ba), Hg, Cd, Ni, As, Mn, Cr, Al, cobalt (Co), Pb, Cu, Fe, tin (Sn), vanadium (V), and Zn estimated from the saffron samples for Iranian person are reported in Table 3. This approach allows us to estimate potential exposure levels to this element based on typical dietary habits. The results showed that the daily intake was below the recommended daily dietary and upper tolerable values allowances all saffron samples can be consumed safely by both adults and children.

Vanadium had the lowest daily intake, while Al had the highest daily intake in saffron samples. A detailed comparison of the recommended dietary allowance (RDA), tolerable upper intake levels (UL), and estimated daily intake (EDI) of heavy metals in the Saffron samples is presented in Table 3.

Table 3. Uncertainly analysis for the estimated daily intake (EDI) of elements in saffron samples by MCS (mg/kg/day).

Trace element		EDI							
	RDA*/AI*/UL values/ TWI and ADI* values (mg/kg/day)	Adults Percentiles				Children			
		5%	50%	75%	95%	5%	50%	75%	95%
Mo	4.50E-02	6.69E-10	9.80E-10	1.16E-9	1.46E-9	2.29E-9	3.50E-9	4.14E-9	5.20E-9
Ba	0.002	3.62E-8	5.31E-8	6.27E-8	7.85E-8	1.22E-7	1.83E-7	2.17E-7	2.79E-7
Hg	-	1.38E-10	2.06E-10	2.43E-10	3.07E-10	4.90E-10	7.21E-10	8.47E-10	1.08E-9
Cd	0.001	6.40E-10	9.55E-10	1.12E-9	1.43E-9	2.23E-9	3.32E-9	3.91E-9	4.91E-9
Ni	0.002	4.73E-8	7.07E-8	8.30E-8	1.08E-7	1.64E-7	2.45E-7	2.96E-7	3.69E-7
As	0.0003	3.33E-9	4.91E-9	5.82E-9	7.40E-9	1.12E-8	1.71E-8	2.03E-8	2.58E-8
Mn	2.3	1.09E-7	1.64E-7	1.93E-7	2.42E-7	3.77E-7	5.74E-7	6.80E-7	8.68E-7
Cr	0.003	5.19E-8	7.67E-8	8.93E-8	1.11E-7	1.77E-7	2.68E-7	3.17E-7	4.13E-7
Al	1.00E+00	1.79E-6	2.74E-6	3.25E-6	4.23E-6	6.61E-6	9.66E-6	1.12E-5	1.43E-5
Co	0.005	3.99E-9	6.13E-9	7.19E-9	9.13E-9	1.43E-8	2.09E-8	2.48E-8	3.16E-8
Pb	-	2.52E-8	3.80E-8	4.50E-8	5.74E-8	8.88E-8	1.36E-7	1.58E-7	1.99E-7
Cu	0.9	7.56E-8	1.13E-7	1.31E-7	1.67E-7	2.57E-7	3.90E-7	4.58E-7	5.80E-7
Fe	18	1.25E-6	1.85E-6	2.16E-6	2.73E-6	4.40E-6	6.57E-6	7.59E-6	9.81E-6
Sn	14	4.50E-9	6.82E-9	8.03E-9	1.03E-8	1.58E-8	2.38E-8	2.84E-8	3.49E-8
V	1.8	6.29E-11	9.43E-11	1.12E-10	1.44E-10	2.18E-10	3.30E-10	3.87E-10	4.90E-10
Zn	11	4.20E-7	6.30E-7	7.36E-7	9.33E-7	1.44E-6	2.21E-6	2.57E-6	3.31E-6

AI: Adequate Intake. UL: Tolerable Upper Intake Level. RDA: Recommended Dietary Allowance, TWI: Tolerable Weekly Intake.

3.3. Estimation of THQ and HI

In this research, MCS was employed to quantify the uncertainty associated with input parameters, analyze the probability distributions of exposure, and evaluate both non-carcinogenic and carcinogenic risks. The results of the health risk assessment are summarized in Table 4. It should be mentioned that the ranking of HI indices at the 95% confidence level of heavy metal exposure for children was As(4.70E-4) > Cr(1.11E-4) > Hg(8.36E-5) > Ni(4.42E-5) > Mn(5.66E-6) > Cd(5.17E-6) > Mo(2.81E-6) >

Ba(1.66E-6) and for adults was As (1.33E-4) > Cr (3.21E-5) > Hg(2.35E-5) > Ni(1.29E-5) > Mn(1.56E-6) > Cd(1.47E-6) > Mo(7.81E-7) > Ba(4.96E-7).

The cumulative non-carcinogenic effects of multiple metals were evaluated using a HI, calculated by summing the THQs for each individual metal. The HI values for the 95th percentile exposure scenario for adults and children obtained were 2.06E-4, and 7.25E-4, respectively.

In various investigations, the evaluated dietary exposure to heavy metals from food consumption showed different trends. In this regard, the results of Mahmoud Taghavi et al. (2024) study demonstrated that the HQ value for metalloids in saffron grown in Gonabad was less than 1 for children, teenagers, and adults, representing non-carcinogenic adverse effects. Also, the values of ILCR for metalloids from long-term consumption of saffron were among the studied groups was low and negligible [18].

Table 4. Uncertainly analysis for THQ of heavy elements exposure to saffron samples by MCS.

Treace elements	Adults				Children			
	Percentiles							
	5%	50%	75%	95%	5%	50%	75%	95%
Mo	3.17E-7	5.11E-7	6.07E-7	7.81E-7	1.19E-6	1.82E-6	2.15E-6	2.81E-6
Ba	1.92E-7	3.09E-7	3.74E-7	4.96E-7	7.09E-7	1.09E-6	1.30E-6	1.66E-6
Hg	9.89E-6	1.53E-5	1.84E-5	2.35E-5	3.48E-5	5.41E-5	6.63E-5	8.36E-5
Cd	6.11E-7	9.52E-7	1.15E-6	1.47E-6	2.13E-6	3.29E-6	3.99E-6	5.17E-6
Ni	5.17E-6	8.15E-6	9.66E-6	1.29E-5	1.83E-5	2.82E-5	3.36E-5	4.42E-5
As	5.54E-5	8.68E-5	1.04E-4	1.33E-4	1.94E-4	3.01E-4	3.61E-4	4.70E-4
Mn	6.61E-7	1.03E-6	1.21E-6	1.56E-6	2.38E-6	3.70E-6	4.42E-6	5.66E-6
Cr	1.32E-5	2.09E-5	2.46E-5	3.21E-5	4.61E-5	7.10E-5	8.58E-5	1.11E-4

3.4. Carcinogenic risk analysis

The distribution of the ILCR for both children and adults is depicted in Figure 2. An ILCR exceeding 1×10^{-4} indicates a significant cancer risk due to exposure, whereas an ILCR below 1×10^{-6} suggests an insignificant risk. For saffron consumption, the 95% ILCR values for the 95th percentile exposure scenario for adults was 5.85E-08 (As) > 4.48E-08 (Cr) > 5.57E-10 (Cd), and for children it was 2.09E-07 (As) > 1.66E-07 (Cr) > 1.92E-09 (Cd) (Table 5). Overall, these levels suggest a low likelihood of carcinogenic effects. The combined ILCR for all trace metals was estimated at 3.77E-07 for children and 1.04E-07 for adults. According to Figure 2, arsenic did not pose a significant cancer risk, as its ILCR remained below the threshold of 1×10^{-6}.

Non-carcinogenic and carcinogenic risk assessments for food often evaluate factors like as heavy metal levels, frequency of consumption, and how readily metals are absorbed by the body [38,39]. Research often aims to identify potential health effects associated with common dietary items [40]. Elevated heavy metal levels are often found in vegetables and fruits grown in areas with contaminated soil or water, which can threaten food safety. Even at moderate metal levels, vegetables and fruits eaten regularly may increase health risk indices [41]. The origin of the produce and farming methods significantly impacts metal contamination. Regular consumption of vegetables and fruits with high health risk indicators could pose long-term health concerns, since cumulative exposure to multiple metals may elevate overall risk [42].

Table 5. The 95% ILCR values for the 95th percentile exposure scenario by saffron consumption.

Percentiles	Adults			Children		
	Cd	As	Cr	Cd	As	Cr
5%	2.38E-10	2.48E-8	1.92E-8	7.88E-10	8.60E-8	6.77E-8
50%	3.54E-10	3.83E-8	3.07E-8	1.26E-9	1.33E-7	1.08E-7
75%	4.29E-10	4.50E-8	3.68E-8	1.49E-9	1.61E-7	1.30E-7
95%	5.57E-10	5.85E-8	4.48E-8	1.92E-9	2.09E-7	1.66E-7

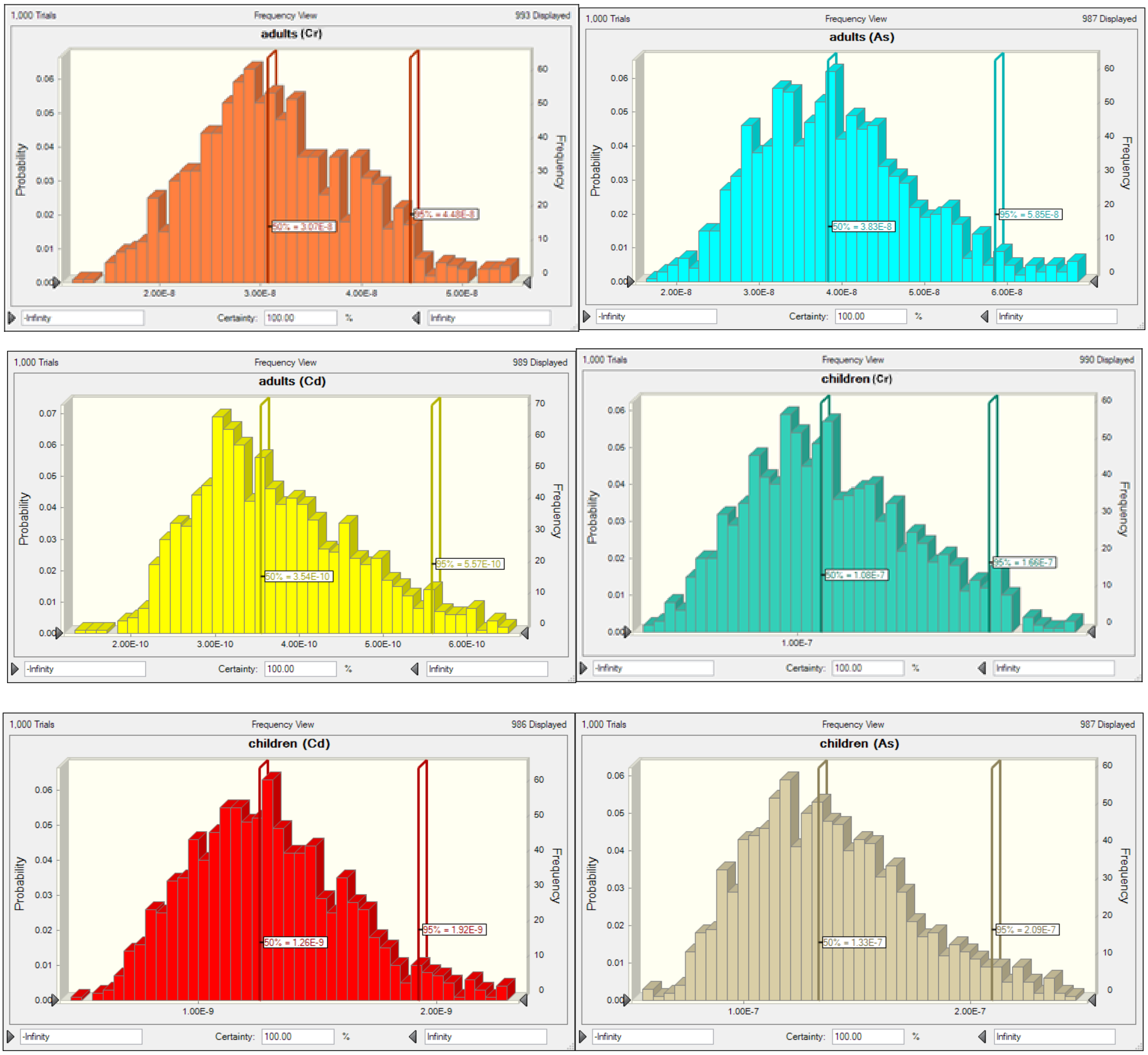

Figure 2. The ILCR of elements in saffron samples by MCS.

Exposure to heavy metals like Cd, Pb, and As is a known risk factor for cancer and other diseases [43]. Saffron, while typically safe in culinary use, can absorb these metals from contaminated soil. Consequently, long-term consumption of saffron with elevated heavy metal concentrations could pose a health risk. Existing research on this topic is scarce. Although one study found no significant health risk from heavy metals in Iranian saffron [44], other researchers have highlighted the potential for soil contamination to increase heavy metal uptake by saffron plants, raising concerns about human exposure [18]. Over time, regular intake of saffron containing elevated metal concentrations might contribute to chronic disease development. Current research remains limited regarding health risk assessments of metal contaminants in saffron, especially in crops irrigated through diverse water systems. A notable investigation by Mohammadi et al. analyzed Iranian Sohan saffron and concluded that there were minimal health risks from consumption. However, recent findings by Taghavi et al. [18] highlight that multiple pollution sources in saffron-growing soils could enhance heavy metal transfer to plants, subsequently increasing human exposure risks. This underscores the necessity for systematic monitoring of heavy metal levels in saffron cultivated with domestic water and saline wastewater irrigation systems to mitigate potential long-term health hazards.

3.5. Multivariate analysis

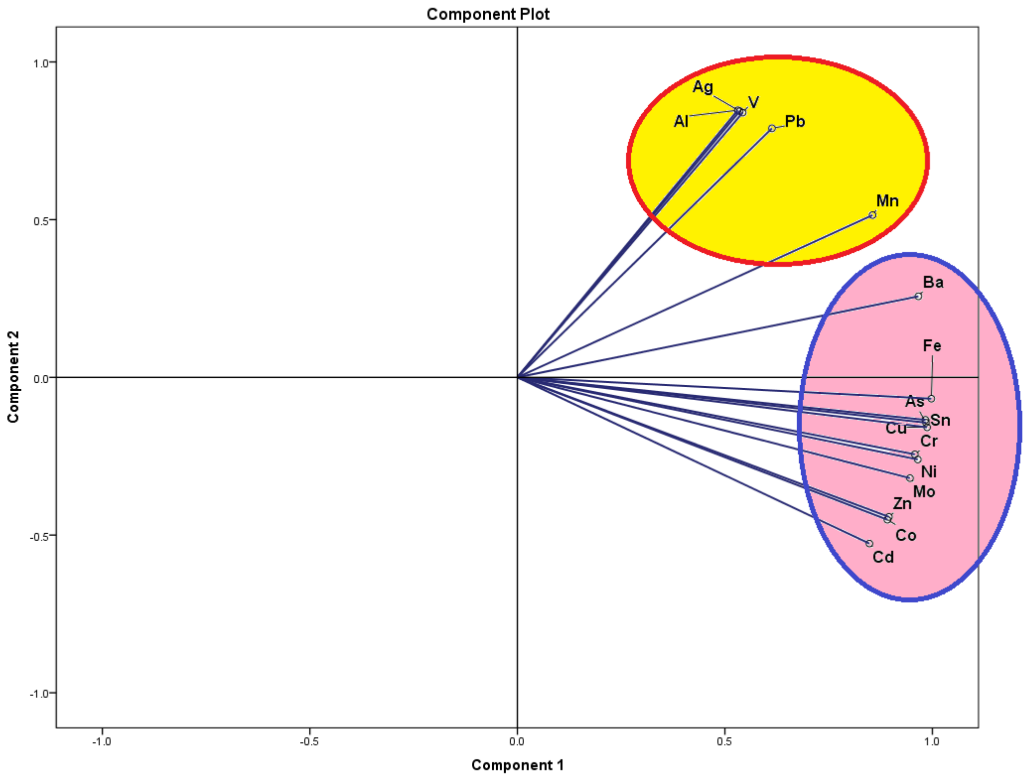

Figure 3. PCA of elements in saffron samples.

A principal component analysis (PCA) was used to analyze the relationships between metal concentrations in saffron samples (Figure 3) [38]. This visualization revealed distinct patterns in metal distribution, grouping similar metals and samples together. The heat map analysis effectively classified the metals, showing statistically significant differences between these classifications. In particular, the data was categorized into two main components PC1 (variance: 74.18%) and PC2 (variance: 25.39%). The saffron samples are clustered into two main groups (Figure 3): one primarily containing Ag, V, Al, and Pb. The vector length of different metal concentrations showed that there were significant similarities among As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, and Zn clustered most closely, suggesting similar concentration trends across the different samples.

4. Conclusions and future research directions

This study comprehensively evaluated the trace element composition of saffron from the investigated region, alongside its soil and irrigation water. Our findings indicate that saffron cultivated in this area generally exhibits adequate concentrations of essential elements, namely Fe, Zn, Mn, and Cu. These levels are sufficient to support both optimal plant development and contribute positively to the nutritional profile of the spice. Al accumulation, a non-essential element, warrants attention from a food safety perspective. To mitigate potential risks and ensure the production of high-quality saffron for both domestic consumption and international markets, it is recommended to implement soil remediation strategies, careful selection of irrigation water sources, and consistent monitoring of heavy metal content.

Furthermore, the application of PCA effectively illustrated the relationships and differences among various metals, providing clear insights into contamination patterns. MCSs provided a robust assessment of human health risks, indicating that exposure to heavy metals through saffron consumption led to negligible non-carcinogenic (THQ < 1) and carcinogenic (ILCR < 1E-6) risks for both adult and child populations. However, to ensure the broad applicability and robustness of these conclusions, validation through multiple growing seasons and diverse geographical locations is essential.

Based upon the current findings and recommendations, several areas warrant further investigation from a public health and agricultural sustainability standpoint:

(i) Comprehensive food basket analysis: Extend risk assessment methodologies to include other main food products commonly consumed alongside saffron or within the same dietary patterns. This includes analyzing trace element levels in whole-grain cereals, fruits, vegetables, and other dietary components prevalent in the region. A “total diet” approach, rather than focusing on a single food item, will provide a more holistic understanding of dietary exposure to trace elements and potential cumulative health risks or benefits.

(ii) Long-term health impact studies: Conduct longitudinal epidemiological studies to monitor the health outcomes of populations with varying levels of saffron consumption, particularly in relation to potential cumulative exposure to elements like Al. While current risk assessments suggest negligible risks, long-term, real-world health impacts associated with dietary exposure to specific elements over decades are complex and require dedicated study.

(iii) Optimization of soil and water management: Research and evaluate the efficacy of specific soil changes (e.g., organic matter, specific mineral additives) and water treatment techniques designed to reduce Al bioavailability and uptake by saffron plants without negatively impacting the absorption of essential nutrients. Developing practical, cost-effective solutions for managing soil Al is crucial for safeguarding saffron quality and consumer health.

(iv) Comparative multi-locational and multi-annual validation: Replicate this study across a wider range of geographical locations with differing soil types, climatic conditions, and agricultural practices and conduct the study over several years. This will confirm the generalizability of the findings regarding essential element adequacy, Al accumulation, and heavy metal safety, providing a more definitive basis for regional or international quality standards. By addressing these future research directions, it can further refine our understanding of saffron's elemental profile, enhance food safety protocols, and promote sustainable saffron cultivation practices.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

M.A.S & N.Sh (Conceptualization- Methodology, Investigation- review and editing), M.A & A.Kh (Data curation- formal analysis- writing- original draft preparation). All authors have read and agreed to the published version of the manuscript.

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