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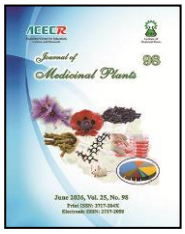

Research Article

Heavy metals in henna leaves (*Lawsonia inermis*) from Sistan and Baluchestan Province, Iran: dermal exposure and toxicological risk assessment

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ABSTRACT

Background: *Lawsonia inermis* L., commonly known as henna has been used for cosmetic and medicinal purposes since ancient times. **Objective:** This study evaluated the potential health risks associated with arsenic (As), lead (Pb), and zinc (Zn) in henna leaves collected from Dalgan and Iranshahr, Sistan and Baluchestan Province, Iran. **Methods:** Twenty samples were collected from each site and analyzed by inductively coupled plasma–optical emission spectrometry (ICP-OES; PerkinElmer Optima DV 2000). Hazard quotient (HQ), hazard index (HI), and carcinogenic risk (CR) values for dermal exposure were calculated, and statistical analysis performed using SPSS ($P < 0.05$). **Results:** Heavy metal concentrations were higher in samples from Iranshahr compared to Dalgan. Zinc levels reached 41.9 ± 18.10 µg/g in Iranshahr and 33.2 ± 8.98 µg/g in Dalgan, while lead concentrations were 1.05 ± 0.62 µg/g and 0.7 ± 0.30 µg/g, respectively. Mean arsenic concentrations exceeded international standards, measuring 6.2 ± 2.51 µg/g in Iranshahr and 3.07 ± 1.39 µg/g in Dalgan. All henna samples exceeded the WHO recommended limit for arsenic, with 45% of Dalgan and 90% of Iranshahr samples surpassing the Canadian threshold. Nevertheless, dermal HQ values for all metals and the total HI remained below 1, indicating low non-carcinogenic risk, and dermal CR values for arsenic and lead were negligible. **Conclusion:** Although henna use poses minimal immediate health risks, prolonged exposure to elevated arsenic, particularly in Iranshahr, may be detrimental. Quality control measures are therefore essential to ensure that heavy metal concentrations in medicinal plants remain within regulatory limits.

1. Introduction

Herbal medicines are used therapeutically by approximately 70-80 % of the world's population [1, 2], and numerous plant species also have cosmetic applications [3]. *Lawsonia inermis* L., or henna, is a medicinal and industrial plant belonging to the Lythraceae family [4]. The material used as henna is obtained from finely powdered dried leaves, which contain lawsone (2-hydroxy-1,4-

Abbreviations: WHO, World Health Organization; IARC, International Agency for Research on Cancer; FAD, Food and Drug Administration; USEPA, United States Environmental Protection Agency; CDI, Chronic daily intake; HQ, Hazard Quotient; HI, Hazard Index; CR, Carcinogenic risk

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naphthoquinone), the principal bioactive pigment responsible for the characteristic red-brown color [5, 6]. The plant grows mainly in tropical and subtropical areas, including North Africa, the Middle East, South Asia, and the Arabian Peninsula [7, 8]. In Iran, it is cultivated extensively in the southern regions, particularly in the Kerman and Sistan and Baluchestan Provinces, where it is valued for cosmetic and therapeutic uses [9, 10]. A substantial portion of henna produced in the Sistan and Baluchestan and Kerman Provinces is exported, contributing to an estimated global trade exceeding 10,000 tons annually. Iran, Pakistan, Sudan, India, and Egypt are the leading exporters, whereas major demand originates from the Middle East, North Africa, Western Europe, and North America [8]. Natural henna is generally regarded as safe due to its diverse bioactive compounds, which confer antibacterial, antifungal, anti-inflammatory, analgesic, antiproliferative, and hepatoprotective effects [11, 12].

Despite its widespread use, concerns persist regarding contamination with toxic metals, pesticides, and microbial agents due to industrial exposure. The World Health Organization (WHO) recommends monitoring medicinal plants for such pollutants, as heavy metal contamination can result from irrigation, soil quality, fertilizers, pesticides, and industrial emissions [13]. Zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) are required in trace amounts, whereas lead (Pb), arsenic (As), chromium (Cr), cadmium (Cd), and mercury (Hg) can exert toxic effects even at relatively low concentrations [14]. Arsenic and mercury typically originate from industrial sources, whereas lead and cadmium contamination are often linked to fertilizers [15]. Heavy metals exhibit strong reactivity with cellular proteins and membranes, rendering them nonbiodegradable and persistent environmental pollutants [1, 16]. Excess Zn accumulation may cause adverse effects such as gastrointestinal and renal disturbances, nausea, vomiting, and diarrhea [16]. Lead and arsenic, also recognized by WHO as critical public health concerns, pose additional risks via dermal absorption [3]. The International Agency for Research on Cancer (IARC) classifies inorganic arsenic compounds and lead as carcinogenic to humans [17]. Reports indicate that lead can enter the bloodstream through skin contact, with elevated levels detectable in sweat, blood, and urine within six hours of exposure. Arsenic readily binds to keratin and may accumulate in hair and nails, while acute exposure may cause dermatological symptoms [7]. The US Food and Drug Administration (FDA) specifies limits of 20 µg/g for lead and 3 µg/g for arsenic in henna products when used as color additives in cosmetic formulations [7]. Given henna's widespread use for both medicinal and cosmetic purposes, along with Iran's substantial contribution to global exports, possible health consequences of metal contamination warrant investigation. Prolonged application may increase dermal absorption, potentially leading to toxic effects. This study investigates the concentrations of lead, arsenic, and zinc in henna samples sourced from Sistan and Baluchestan province. By comparing these levels against international cosmetic and health standards, this study is the first to assess the non-carcinogenic and carcinogenic risks from dermal exposure in adult and child consumers.

2. Materials and methods

2.1. Study area

Dalgan (27° 25′35″ N, 59° 40′24″ E) and Iranshahr (27° 12′9″ N, 60° 41′5″ E) are located in southeastern Iran, in Sistan and Baluchestan

Province. The administrative center of Dalgan is Golmorti. Dalgan is bordered by Kerman Province to the west, northwest, and southwest, and by Iranshahr to the northeast and east. Iranshahr, centrally located within the province, borders Dalgan to the west and Kerman Province to the northwest (Fig. 1).

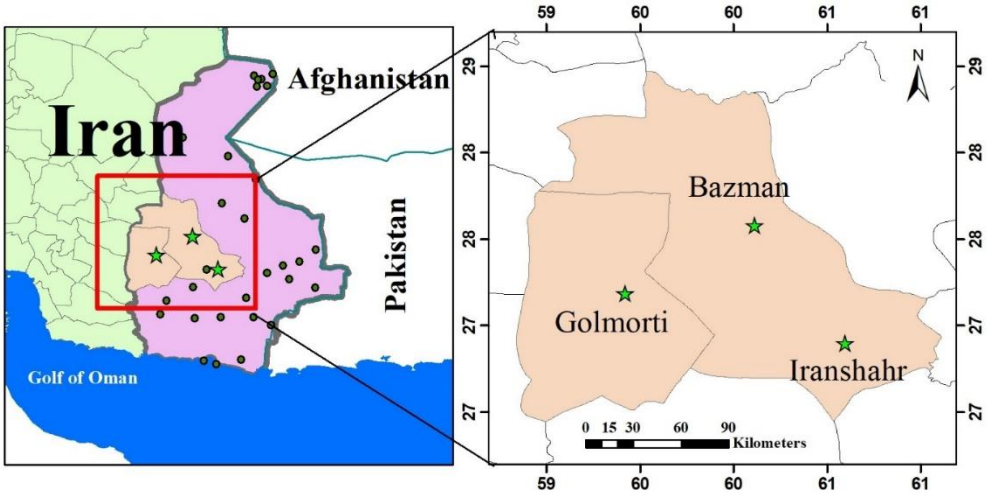

Fig. 1. Location of the sampling sites in Sistan and Baluchestan Province, southeastern Iran, focusing on the administrative centers and districts of Dalgan and Iranshahr counties.

2.2. Chemical and Reagent

All reagents were analytical grade and used without additional purification. Merck (Darmstadt, Germany) supplied nitric acid (HNO3, 65 % w/v) and hydrochloric acid (HCl, 37 % w/v). Ultrapure water with a resistivity of 18.2 MΩ·cm was used to prepare solutions. Calibration solutions for lead, zinc, and arsenic were obtained by dilution of 1000 mg/l Merck stock standards. To minimize contamination, glassware and digestion vessels were first washed with detergent, immersed overnight in 5 % (v/v) HNO3, rinsed thoroughly, soaked in ultrapure water for 24 h, dried, and kept in sealed polypropylene containers.

2.3. Sample Collection

Forty henna samples were collected in July 2023 from farms located in Golmorti and Dalgan (Dalgan County), as well as Iranshahr and Bazman (Iranshahr County), in Sistan and Baluchestan Province, Iran. To ensure reliability, sampling points were spaced at least 0.5 km apart. The samples were individually coded and placed in polyethylene zip-lock bags pre-cleaned with 5 % (v/v) HNO3 and ultrapure water. For species identification, the samples were transferred to the Research Institute of Medicinal Plants, University of Sistan and Baluchestan, where they were authenticated and assigned the herbarium code (Lyt328) and (Lyt329) for the Dalgan and Iranshahr samples respectively. In the laboratory, adhering soil was removed with a brush, the leaves were washed with tap water, and excess moisture was removed with clean paper towels at room temperature. The Samples were subsequently kept in a dry environment until analysis (Fig. 2).

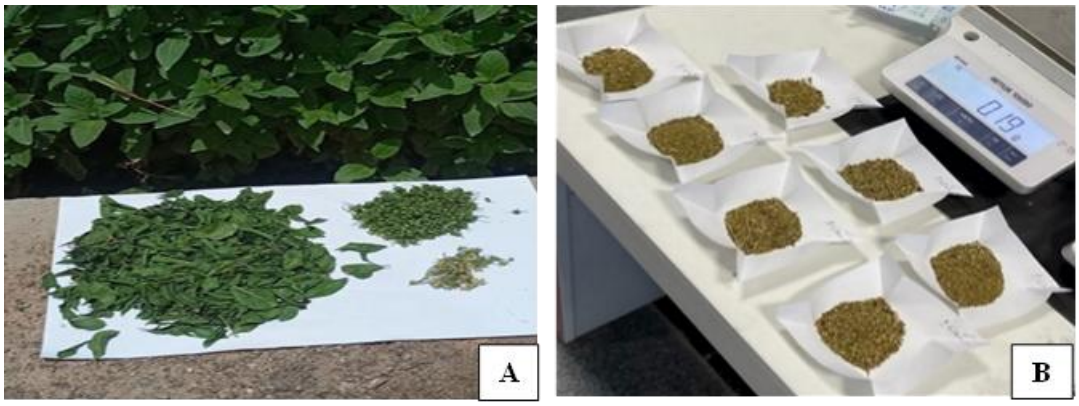

Fig. 2. Henna plant and its processing. A: Henna plants (*Lawsonia inermis* L.) and Henna leaves. B: Processed Henna.

2.4. Preparation of samples and analysis

After drying, the henna material was pulverized using a porcelain mortar and prepared for acid digestion. A 0.5-g portion of each sample was then accurately weighed into a cast-iron digestion vessel, followed by the addition of 9 mL of 10 M HNO3 and 3 mL of 10 M HCl (v/v, 3:1). The vessels were sealed and shaken at room temperature for 15 min [18]. Microwave digestion was subsequently performed at 200 °C for 1 h using a Milestone ETHOS 1000 W system, until a clear digest was obtained. After cooling, each digest was passed through a 0.45 µm Millipore syringe filter, transferred into a 50 ml volumetric flask, and diluted to volume with distilled water. The prepared samples were stored at 4 °C and analyzed within one week.

Heavy metal concentrations (Pb, As, and Zn) were determined using ICP-OES (PerkinElmer Optima DV 2000). Because a certified reference material specific to henna was unavailable, analytical accuracy was assessed through recovery experiments. For each analyte, calibration curves were prepared from standards of 0.1, 0.2, 0.3, and 0.5 mg/L. Procedural blanks were included to ensure analytical accuracy, and final concentrations were corrected accordingly. All measurements were performed in triplicate. The limits of detection (LODs) were 0.1 µg/g for As and 0.05 µg/g for Pb and Zn (dry weight). Recovery rates ranged from 98.4 % to 99.9 %, confirming the reliability of the analytical method. All measurements were performed at the Central Laboratory of the University of Sistan and Baluchestan.

2.5. Statistical Analysis

Statistical analyses were conducted using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). Results are reported as mean ± standard deviation (SD). Differences in Pb, As, and Zn concentrations among the henna samples were examined using an independent t-test and one-way analysis of variance (ANOVA), with $P < 0.05$ considered statistically significant.

2.6. Health risk assessment

2.6.1. Estimated chronic daily intake of heavy metals

Carcinogenic and non-carcinogenic health risks were assessed using models developed by the United States Environmental Protection Agency (USEPA). Human exposure to heavy metals can occur through ingestion, inhalation, and dermal absorption [19]. Toxic effects depend on the magnitude of daily intake [19]. Since henna is primarily applied to the hair,

nails, and skin for cosmetic purposes, this assessment focused on dermal exposure. The chronic daily intake (CDI) through dermal absorption was estimated using the following equation [20-22]:

$$CDI(dermal) = \frac{CS \times SA \times AF \times ABS \times EF \times ED \times CF}{BW \times AT} \quad (1)$$

Where CS denotes the exposure-point concentration (mg/kg), SA is the exposed skin area (2800 cm^2 for children and 5700 cm^2 for adults), AF represents the adherence factor (0.07 mg/cm^2), ABS is the dermal absorption fraction (0.001), EF is exposure frequency (350 days/year), ED is exposure duration (6 years for children and 30 years for adults), BW is body weight (15 kg for children and 70 kg for adults), AT is the average time for non-carcinogens, ED×365 days and CF is the conversion factor (10^{-6} kg/mg) [20-23].

2.6.2. Non-carcinogenic risk

The non-carcinogenic effect of heavy metal exposure is quantified by the hazard quotient (HQ) [19, 23], which is defined as the ratio of the estimated exposure to the chronic reference dose (RfD, mg/kg/day) and is calculated using equation 2.

$$HQ = CDI(dermal)/RfD(dermal) \quad (2)$$

The dermal reference doses for Pb, As, and Zn are 0.04, 0.0003, and 0.3 mg/kg/day, respectively [24, 25]. An HQ < 1 indicates safety, whereas an HQ > 1 signals risk. The combined effect of exposure to all three metals was evaluated using the hazard index (HI), which was calculated as the sum of the individual HQ values [18, 26].

$$HI = \sum HQ = HQPb + HQAs + HQZn \quad (3)$$

An HI value below 1.0 indicates no apparent health risk; values above 1.0 may indicate a potential health impact. An HI greater than 10.0 has been interpreted as suggesting a serious chronic health effect [18, 21].

2.6.3. Carcinogenic risk

Carcinogenic risk (CR) estimates cancer likelihood due to chemical exposure [19] and is calculated as follows:

$$CR = CDI(dermal) \times SF \quad (4)$$

Here, CDI is the chronic daily intake (mg/kg/day) whereas SF denotes the slope factor for the hazardous substances $(mg/kg/day)^{-1}$. Dermal SF values are 0.0085 for Pb [20, 24, 26] and 1.5 for arsenic [24], but unavailable for zinc. Regulatory tolerable risk ranges from 10^{-6} to 10^{-4}; CR values below 10^{-6} are considered negligible, whereas values above 10^{-4} indicates significant risk [21, 23].

3. Results

3.1. Concentrations of toxic metals in henna samples

Table 1 presents Pb, As, and Zn concentrations (µg/g) in henna leaves. The mean heavy metal concentrations followed the order Zn > As > Pb. In Iranshahr and Dalgan, the mean Pb levels were 1.05 ± 0.62 µg/g (range: 0.5–2.9 µg/g) and 0.7 ± 0.30 µg/g (range: 0.4–1.4 µg/g), respectively. The mean As levels were 6.2 ± 2.51 µg/g (range: 2.3–10.5 µg/g) in Iranshahr and 3.07 ± 1.39 µg/g (range: 1.1–5.2 µg/g) in Dalgan. Zn concentrations were 41.9 ± 18.10 µg/g (range: 21.7–83.9 µg/g) in Iranshahr and 33.2 ± 8.98 µg/g (range: 19.3–51.0 µg/g) in Dalgan.

Heavy metal accumulation was higher in Iranshahr. The mean As concentration differed significantly between the two locations (P = 0.03), whereas the differences in Pb (P = 0.20) and Zn (P = 0.15) were not statistically significant (Table 2).

Lead concentrations in all samples were below the FDA (20 µg/g), WHO (10 µg/g), and Canadian government (10 µg/g) limits. In contrast, all samples exceeded the WHO limit for As of 1 µg/g. Additionally, As levels in 9 Dalgan and 18 Iranshahr samples exceeded the FDA and Canadian government limits of (3 µg/g). Overall, the mean As concentrations at both sites exceeded regulatory limits, indicating a potential health risk for henna users.

Table 1. Concentrations of Pb, As, and Zn (µg/g dry weight) in henna leaf samples (n = 40). HD: henna samples from Dalgan, HI: henna samples from Iranshahr

Samples from Dalgan	Pb	As	Zn	Samples from Iranshahr	Pb	As	Zn
HD-1	0.4	1.1	25.0	HI-1	0.6	5.9	25.4
HD-2	0.5	1.9	30.1	HI-2	0.5	2.3	27.6
HD-3	0.6	4.8	48.5	HI-3	0.6	3.8	31.2
HD-4	1.4	3.7	46.2	HI-4	0.5	4.6	29.9
HD-5	0.7	2.1	35.4	HI-5	0.5	6.5	30.7
HD-6	1.4	5.7	33.8	HI-6	0.9	5.8	26.5
HD-7	0.7	3.4	19.3	HI-7	0.7	6.3	23.9
HD-8	0.5	2.9	51.0	HI-8	0.5	4.8	40.9
HD-9	0.4	4.1	30.8	HI-9	0.7	8.5	40.6
HD-10	0.4	5.2	37.2	HI-10	1.1	5.3	42.9
HD-11	0.5	1.4	35.4	HI-11	1.1	3.6	42.6
HD-12	1.1	2.7	31.2	HI-12	0.8	4.9	49.1
HD-13	0.6	1.4	34.1	HI-13	1.1	6.7	39.4
HD-14	0.8	2.3	34.2	HI-14	1.0	2.6	42.1
HD-15	0.5	1.6	45.5	HI-15	0.9	10.2	30.5
HD-16	0.4	4.7	27.3	HI-16	1.2	9.8	83.9
HD-17	1.0	3.2	29.6	HI-17	1.4	9.4	21.7
HD-18	0.6	4.6	24.9	HI-18	1.9	10.5	74.3
HD-19	0.8	2.4	22.7	HI-19	2.1	4.7	70.5
HD-20	0.7	2.2	21.9	HI-20	2.9	8.8	65.9

Table 2. Levels of heavy metals (µg/g) in henna leaves samples collected from Iranshahr and Dalgan

Heavy metal	Location	Mean±S.D	Min–max	p-value
Pb	Iranshahr	1.05 ± 0.62	0.5 - 2.9	0.20
	Dalgan	0.7 ± 0.30	0.4 - 1.4	
As	Iranshahr	6.2 ± 2.51	2.3 - 10.5	0.03
	Dalgan	3.07 ± 1.39	1.1 - 5.2	
Zn	Iranshahr	41.9 ± 18.10	21.7 - 83.9	0.15
	Dalgan	33.2 ± 8.98	19.3 - 51.0	

3.2. Health risk assessment

For adults and children, the chronic daily intake (CDI) of Pb, As, and Zn was estimated based on the respective mean metal concentrations (Table 3). The calculated CDI values decreased in the order Pb > Zn > As, indicating that lead had the highest estimated exposure among the analyzed elements.

3.2.1. Non-carcinogenic risk assessment

The Hazard Quotient (HQ) for individual heavy metals and the Hazard Index (HI) for the combined heavy metal exposure in henna samples from Iranshahr and Dalgan were estimated separately (Table 4). All dermal HQ and HI values were below 1, indicating that,

under the exposure assumptions used in this assessment, the studied henna samples were not associated with an apparent non-carcinogenic risk for either adults or children.

3.2.2. Carcinogenic risk assessment

Because a dermal slope factor (SF) was not available for Zn, carcinogenic risk (CR) was calculated only for Pb and As (Table 5). For both metals, the estimated CR values were below 10^{-6}, indicating that dermal exposure to Pb and As in the studied henna samples was not associated with an apparent significant cancer risk for adult and child consumers.

Table 3. Estimated chronic daily intake (CDI) of heavy metals via the dermal consumption of henna samples in adults and children

CDI of metals (mg/kg/d)	Age group of consumers	Samples from Dalgan	Samples from Iranshahr
Pb	Adults	3.82×10^{-6}	5.73×10^{-6}
	Children	8.77×10^{-7}	1.31×10^{-8}
As	Adults	1.67×10^{-8}	3.38×10^{-8}
	Children	3.84×10^{-8}	7.76×10^{-8}
Zn	Adults	1.81×10^{-7}	2.29×10^{-7}
	Children	4.15×10^{-7}	5.24×10^{-7}

Table 4. Non-carcinogenic risk (HQ_{dermal} and HI) of heavy metals in collected henna sample

Samples	Age group of consumers	HQ_{Pb}	HQ_{As}	HQ_{Zn}	HI
Samples from Dalgan	Adults	9.55×10^{-8}	5.56×10^{-5}	6.03×10^{-7}	5.62×10^{-5}
	Children	2.19×10^{-7}	1.28×10^{-4}	1.38×10^{-6}	1.29×10^{-4}
Samples from Iranshahr	Adults	1.43×10^{-7}	1.12×10^{-4}	7.63×10^{-7}	1.12×10^{-4}
	Children	3.27×10^{-7}	2.58×10^{-4}	1.74×10^{-6}	2.59×10^{-4}

Table 5. Carcinogenic risk (CR) of heavy metals detected in collected henna sample.

Samples	Age group of consumers	CR (Pb)	CR (As)	CR (Zn)
Samples from Dalgan	Adults	3.24×10^{-11}	2.50×10^{-8}	NA
	Children	7.45×10^{-11}	5.76×10^{-8}	NA
Samples from Iranshahr	Adults	4.87×10^{-11}	5.07×10^{-8}	NA
	Children	1.11×10^{-10}	11.64×10^{-8}	NA

NA-not available

4. Discussion

Henna has traditionally been used for hair dyeing and body art in various cultures and has recently gained popularity in Europe [11, 27]. However, herbal materials are not always sourced from uncontaminated environments, increasing the risk of heavy metal contamination and associated health hazards [1]. Despite the protective barrier function of the skin, cosmetic applications may introduce chemical compounds capable of causing irritation or allergic reactions [6]. The extent to which metals accumulate in plant tissues depends on plant characteristics, contamination sources, environmental conditions, and the mechanisms governing metal uptake [4, 28]. Understanding these factors is essential for evaluating the safety of henna products.

Zinc is an essential nutrient required for normal growth, enzymatic activity as a cofactor, and numerous critical cellular processes. Emerging evidence suggests a potential role for

zinc in cancer development and progression [29]. While zinc deficiency is associated with various physiological disorders, excessive intake may result in acute renal and gastrointestinal toxicity [30]. Zinc is also necessary for plant growth, however, concentrations above the physiological levels can interfere with plant metabolism and development [31].

Mean Zn concentration was higher in the Iranshahr samples than in those from Dalgan, although the difference was not statistically significant. The concentrations measured at both sites fell within the permissible background range for plants (27–150 µg/g) [4]. Previous research conducted in Tunisia reported a mean Zn concentration of 26.6 ± 0.2 µg/g in henna leaves, with values ranging from 3.7 to 90.0 µg/g. Although the mean value was lower than that observed in our study, it remained within the established background range [32]. Another Tunisian investigation reported means of 46.5 to 118.5 µg/g, which were higher than the values in our study but still within the acceptable plant background range [4]. In contrast, Rubio et al. documented Zn concentrations in commercial henna samples ranging from 12 to 30 µg/g, which are lower than those reported here [6]. Similarly, henna samples obtained from herbal markets in Riyadh, Saudi Arabia, exhibited a mean Zn concentration of 0.46 µg/g (0.3–0.6 µg/g), substantially lower than our findings and below typical background levels for plants [33]. Egyptian black henna showed a much wider Zn range of 1.67-996.3 µg/g, with many reported values exceeding standard background levels [5].

The variability in Zn concentrations across different studies likely reflects differences in geographical origin, environmental conditions, soil composition, and agricultural practices. Importantly, our assessment of non-carcinogenic risks associated with dermal Zn exposure indicates that henna use collected at the study sites does not constitute a significant health risk to either children or adults.

Lead, despite its relatively low bioavailability, can exert toxic effects even at low concentrations [4]. Chronic exposure in humans has been associated with gastrointestinal disturbances, appetite suppression, weight loss, sleep disorders, fatigue, and persistent headaches [2]. Of particularly concerning is the heightened vulnerability of fetuses, infants, and children due to lead's neurotoxicity, which can interfere with nervous system development [6]. Although ingestion remains the primary route of lead entry, dermal absorption is also significant [7]. The dermal absorption rate of lead is approximately 0.3 %, reinforcing concerns regarding transdermal exposure [7]. Regulatory authorities have prohibited the use of lead in cosmetic and healthcare products due to its cumulative toxicity [5]. The bioaccumulative properties of lead pose a long-term exposure risk, as systemic retention can result in substantial concentrations over time [3]. Long-term henna use has been associated with increased lead accumulation in hair samples [34].

The Iranshahr samples had a higher mean Pb concentration than the Dalgan samples, although the difference was not statistically significant. A Saudi Arabian study reported a mean of 0.625 µg/g in henna samples [33]. Similarly, henna samples from Turkey [35] and Europe [6] had Pb concentrations ranging from 0.60–0.93 µg/g and 0.28–0.90 µg/g, respectively, which were comparable to those observed in the Dalgan samples. In contrast, Pb concentrations in henna samples from both Iranshahr and Dalgan were considerably lower

than those reported in Iran [3], Egypt [5], Libya [7], Serbia [34], and two studies from Tunisia [4,32], but remained higher than the Pb concentrations reported in Kenya [36].

The lower Pb concentrations detected in field-grown henna suggest that cultivation areas in southeastern Iran are unlikely to be the primary source of lead contamination. Instead, the observed variations may be attributed to multiple factors, including differences in plant uptake and translocation of metals, which are affected by soil pH, organic matter, cation-exchange capacity, redox conditions, and other physicochemical properties. In addition, atmospheric deposition, which depends on proximity to industrial areas and traffic-related emissions, as well as contamination introduced during harvesting, transportation, storage, mixing, or processing of commercial henna products, may further increase Pb concentrations. Furthermore, differences in analytical methodologies may also contribute to the variability in reported Pb concentrations among studies [3, 37]. For henna used exclusively as a hair dye, the SCCS (Scientific Committee on Consumer Safety) has established permissible Pb impurity levels at 1.04 ppm [6]. In accordance with these guidelines, lead concentrations in all Dalgan henna samples remained within acceptable limits, whereas 15 % of the samples from Iranshahr exceeded this threshold. However, Pb levels in all analyzed samples complied with the regulatory limits established by the FDA, WHO, and Canadian authorities. Comparative assessments with international studies further support these findings. Studies conducted in Tunisia and Turkey reported that Pb concentrations in all tested henna samples were below WHO and FDA-approved limits [32, 38]. In contrast, studies conducted in Libya indicated that 66.7 % and 60 % of henna samples contained Pb levels exceeding WHO and FDA recommendations, respectively [7]. Furthermore, a previous study of commercially marketed henna in Iran reported that 53.85 % of market-purchased samples exceeded the WHO safety threshold, whereas all samples remained below the FDA limit [3]. In the present study, the Pb HQ was below 1 in both adults and children, indicating negligible non-carcinogenic risk. The estimated CR was below 10^{-6} for all age groups, indicating that dermal application of the studied henna is unlikely to pose carcinogenic or non-carcinogenic health hazards to consumers.

Arsenic is broadly distributed in the environment, and food and drinking water are important routes of human exposure [39]. Classified as a human carcinogen, chronic arsenic exposure is associated with dermal, vascular, neurological disorders, as well as various cancers [40]. Sustained exposure to high concentrations may produce skin irritation, nail striping, and hair loss [41], whereas acute toxicity can cause gastrointestinal distress, diarrhea, vomiting, and abdominal pain [3]. In environmental contexts, arsenic exists in three primary forms, with inorganic arsenic species (As III) demonstrating significantly higher toxicity compared to As V and organic arsenic. Nonetheless, total arsenic concentrations are commonly reported in plant-based and cosmetic products. The dermal absorption rate of arsenic through human skin is approximately 1.9 % [42].

The mean As concentration was significantly greater in the Iranshahr henna than in the Dalgan samples. Moreover, the mean arsenic concentrations measured in both regions exceeded the maximum permissible limits established by the FDA, (3 µg/g), WHO, (1 µg/g) and the Canadian Government (3 µg/g)

[7]. These findings indicate that henna cultivated in the study area, particularly in Iranshahr, may represent a potential source of arsenic exposure following repeated dermal application. Although the extent of dermal arsenic absorption depends on several factors, including skin condition, exposure duration, and arsenic species, chronic use of contaminated henna could contribute to cumulative arsenic exposure, especially among individuals who frequently apply henna for cosmetic purposes, such as women and children.

Comparison with previous studies revealed substantially lower arsenic concentrations in henna from other regions. For instance, Libyan henna contained an average As concentration of 0.68 ± 0.33 µg/g, a value below international safety limits and well below the concentrations observed in the present study [7]. European commercial henna products contained 0.06–0.53 µg/g [6], whereas commercially marketed henna from Iran had a reported mean of 0.51 ± 0.23 µg/g [3]. Collectively, these findings suggest that arsenic contamination in farm-collected henna analyzed in the present study were markedly higher than those reported for commercially available henna products from other geographical regions. These differences may be associated with regional geological conditions, irrigation water quality, soil characteristics, agricultural practices, or other environmental factors. However, further studies integrating environmental monitoring of soil, irrigation water, and cultivation practices are required to identify the primary sources of arsenic contamination.

The present findings are in agreement with studies of medicinal plants in Iran showing that metal accumulation varies among plant species and geographical regions due to differences in soil characteristics, geological background, irrigation water quality, agricultural practices, and other environmental factors [43, 44]. Consequently, comparisons of metal concentrations across studies should be interpreted cautiously, as differences in plant species, cultivation conditions, sampling strategies, and analytical methods can influence the reported values. These observations support continued monitoring of cultivation soils, irrigation water, and herbal products as part of consumer-safety surveillance. The present study provides baseline data on heavy metal concentrations in farm-collected henna from southeastern Iran, offering insight into contamination occurring at the cultivation stage rather than that potentially introduced during commercial processing.

To our knowledge, few peer-reviewed English-language studies have reported arsenic concentrations in henna, and the present study represents the first documented arsenic assessment in henna leaves collected directly from cultivation farms in Iran. Given that the measured arsenic concentrations exceeded several international permissible limits, both non-carcinogenic (HQ_{dermal}) and carcinogenic risks were evaluated for adults and children. Despite the elevated arsenic concentrations, the calculated HQ_{dermal} hazard index (HI), and CR values remained below internationally accepted safety thresholds, suggesting that dermal use of henna from the investigated farms is unlikely to produce a significant non-carcinogenic or carcinogenic risk under the exposure assumptions applied here.

The analysis measured total arsenic rather than individual arsenic species. Since

inorganic arsenic is considerably more toxic than organic forms, the conservative assumptions used in the risk assessment may lead to an overestimation of actual risk. Future investigations should therefore include arsenic species in henna to improve exposure estimates and provide a more precise assessment of consumer safety.

5. Conclusion

This study evaluated Pb, As, and Zn contamination in cultivated henna collected directly from agricultural production areas from Sistan and Baluchestan, Iran, highlighting the presence of lead, arsenic, and zinc and their potential health implications. while zinc is an essential trace element, excessive accumulation in henna plants necessitates consideration of long-term exposure effects. Although Pb levels remained within global safety limits, its bioaccumulative nature underscores the need for continued monitoring. Arsenic concentrations, particularly in Iranshahr, exceeded the FDA, WHO, and Canadian regulatory thresholds, likely due to regional water scarcity and the use of urban wastewater for irrigation. The risk assessments suggest minimal carcinogenic and non-carcinogenic hazards from dermal exposure; however, as this study measured total arsenic rather than its more toxic inorganic fraction, potential risks may be overestimated. Comparisons with previous studies also revealed considerable regional variability in heavy metal concentrations, highlighting the importance of monitoring cultivation soils, irrigation water, and henna products. This study expands the limited literature on henna safety and underscores the necessity for continuous surveillance and preventive measures to mitigate health risks for consumers.

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Authors' contributions

R K carried out sample collection, sample preparation steps for laboratory measurements, and data analysis. M M conceptualized and designed the work, data analysis and wrote the manuscript. All the authors proofread the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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