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Assessment Of Toxic Metals Health Risk In 'Ready-To-Use' Black Henna Products Collected From Different Body-Care Centers And Local Markets In Benghazi, Libya

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Abstract
Background: *Commercial henna products are available in various colors, often achieved by mixing henna with substances, such as heavy metal compounds.*
Objective: *In this study, black henna tattoo cones and black henna paste were collected from cosmetic shops and body care centers in Benghazi. The samples were analyzed using Atomic Absorption Spectroscopy to detect six toxic metals. Non-carcinogenic and carcinogenic risks were assessed by calculating the Margin of Safety (MoS), Hazard Quotient (HQ), Hazard Index (HI), and Lifetime Cancer Risk (LCR).*
Results: *Aluminum, chromium, and copper were detected in all samples, with concentrations ranging from 0.88–11.25 mg/kg for aluminum, 0.062–4.012 mg/kg for chromium, and 0.041–2.21 mg/kg for copper. Zinc and nickel were present in 77% and 69% of samples, respectively, with maximum concentrations of 19.01 mg/kg for zinc and 1.98 mg/kg for nickel. Most metal concentrations were within international safety limits, except for chromium. Lead was also found, but below the maximum allowable limit. MoS values exceeded 100, while HQ and HI were below 1. LCR values remained within permissible limits (LCR ≤ 1×10^{-4}) for all metals, except for chromium, nickel, and copper in henna paste.*
Conclusion: *the levels of the analyzed metals in the collected black henna samples were generally lower than those reported in previous studies conducted in different countries. The health risk assessment study indicated that the collected henna samples were relatively safe with minimal health risks. This study highlights the importance of monitoring and regulating metal contents to ensure the safety of Libyan women consumers.*
Keywords: *Heavy metals; "Ready to use" henna; Henna tattoo; Henna paste, Margin of safety, Cancer risk*

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I. Introduction

Throughout history, various kinds of tattoos have been employed for body decoration, including those that are permanent and temporary in nature. Permanent tattoos involve injecting colored inks into the dermis, resulting in a lifelong design [1,2]. As such, a person with permanent tattoos typically has only one design. On the other hand, temporary henna tattoos offer a convenient alternative to permanent tattoos and are widely accessible around the world [3,4]. Henna is primarily used to embellish the skin and nails in temporary tattoos, providing a limited duration of decoration. This method is painless, relatively safe, and carries virtually no risk of disease transmission [5]. In fact, henna has been recognized as a traditional cosmetic product used in various regions across the globe [6]. Henna is derived from the Lawsonia inermis plant, a flowering shrub from the Lythraceae family. The entire henna plant comprises numerous organic components [7]. The primary compound responsible for the coloration properties of natural henna is lawsone (2-hydroxy-1,4-naphthoquinone), which exists as glucoside derivatives in the henna leaves. The concentration of lawsone, and thus the intensity of henna's color, depends on factors like climate and soil conditions where it grows. The highest levels of dye content are found in henna cultivated in hot and arid climates [8]. Alongside organic components, natural henna contains elements such as potassium, magnesium, calcium, sulfur, and silicon, which are absorbed from the surrounding soil. However, henna can potentially contain elevated levels of heavy metals if it is harvested from heavily polluted soil. These toxic heavy metals have the potential to harm biological structures and cause health issues even at low concentrations [9] [9].

The process of henna painting involves applying henna paste to the body, either with fingers or with tools such as brushes, syringes, or applicator bottles. The applied paste is then left to dry for several hours [5].

As the henna paste is applied, lawsone gradually migrates from the paste into the outer layer of the skin. In a Michael addition reaction, lawsone binds directly to skin proteins, resulting in a rapid staining effect [10]. t [10].

The traditional method of preparing henna paste involves mixing the powder of dried henna plant leaves with water and lemon juice [9,10]. The acidic nature of the paste allows for a gradual release of lawsone, which is the dye molecule found in henna leaves, by breaking down the cellulose. Citric acid present in the paste also helps in the penetration of lawsone by opening the cuticle pores. Additional ingredients, such as essential oils, sugar, beetroot juice, hibiscus flower juice, dried powder of indigo plant leaves, etc., have also been added to prepare henna paste [9,11]. These ingredients contribute to the stickiness, consistency, and fragrance of the henna paste, as well as darkening the resulting stain [9]. When applied to the skin, the natural henna paste with the aforementioned ingredients leaves a red-maroon stain, and a longer application time results in a deeper color. However, different cosmetics manufacturers have marketed henna products in a wide range of colors. To achieve these colors, manufacturers claim to use henna from different countries or other parts of the henna plant, such as roots and barks. In reality, colored henna products are created by incorporating metallic salts, synthetic dyes, and other plant dyes. Lead acetate, silver nitrate, and the compounds of copper, nickel, cobalt, bismuth, and iron are commonly used metallic salts [6,8]. In recent times, a trendy method known as temporary black henna tattooing has gained popularity among children, teenagers, and young adults as a form of skin decoration. These products are commonly found in the form of plastic packets or cones and are often enriched with heavy metals and PPD to intensify the color and enhance dye penetration and fixation [12]. [12].

The application of henna products to different body parts leads to the absorption of metals through the skin layers into the blood, which accumulate or replace essential elements of different biomolecules. This accumulation can disrupt various metabolic processes and trigger the unfavorable mechanisms [13,14]. Kilic S. et al. observed that direct application of cosmetics, such as henna, to hair or skin significantly enhances the absorption of both organic and metallic components into the human body [15]. This can lead to various disorders, including cancer, respiratory diseases, organ dysfunction, and developmental delays. As the inclusion of metals in cosmetic products poses significant risks to human health, these metals have been prohibited or restricted by various international health regulations in many countries. Lead and chromium are prohibited in European Union (EU) countries [6]. The World Health Organization (WHO) has established a maximum limit of 10 mg/kg for lead in cosmetic products [16]. In the United States, the Food and Drug Administration (FDA) recommends maximum permissible limits of 20 ppm for lead and one ppm for chromium in color additives [6]. The EU regulations permit the presence of nickel in cosmetics, but in very small quantities referred to as "impurities" [17]. Scientific literature suggests that a value less than 5 ppm for nickel is considered as "Good Manufacturing Practice"[18]. Therefore, it is essential to monitor commercially available cosmetics for the concentrations of these harmful trace elements to determine whether they fall within safe permissible limits. In fact, various studies have detected elevated levels of certain toxic heavy metals in henna across multiple countries [15, 19-27]. Furthermore, to the best of our knowledge, there has only been one study that assessed health risk models, including both carcinogenic and non-carcinogenic risks associated with henna products [15]. [15].

In Libya, henna and henna products are widely used for adorning women's bodies, especially during various social celebrations, regardless of age. However, due to the modern, fast-paced lifestyle, young women tend to prefer "Ready-to-Use" henna, which is either locally produced or imported from different countries and can be found in local markets. Concerningly, the Libyan Health Ministry has reported numerous cases of henna poisoning. Despite the growing risk of henna poisoning among Libyan women, only two published studies have been conducted on the determination of toxic metals in henna samples obtained from the Libyan markets [28,29]. None of these studies evaluated the health risks associated with heavy metals as impurities in cosmetics. Consequently, the aim of this research is to analyze the levels of aluminum and several heavy metals, including lead, chromium, nickel, copper, and zinc, in black henna products available at various local cosmetic shops and body care centers in Benghazi. The findings of this study will be compared to recent reference values for these metals in henna samples from different regions worldwide, as well as to the limits established by international standards. Additionally, the research will evaluate the health risks associated with the heavy metals found in the collected henna products.

II. Methodology

Sample Collection

A total of twenty-six of the common and best-selling "Ready-to-Use" black henna samples were purchased from cosmetic shops and body care centers in Benghazi. The same henna products were thoroughly mixed to get thirteen homogenous representative samples. The samples were coded as "T" (Tattoo Henna) and "P" (Paste Henna). The T samples consisted of five brands (T1-T5), which are packed in sealed metalized plastic cones. One end of the cone terminates in a point, which is cut off to allow the henna to squeeze out in line to facilitate several types of designs on the body. The information on the product container labels was carefully examined. The sample code, dates of manufacture (MF) and expiry (Exp), and the ingredients list on the labels of these products were noted and recorded in Table 1. While paste henna samples (P1-P8) purchased from the body care centers in Benghazi were packed in plastic bags and lacked labels.

Table 1: List of Tattoo Henna samples collected from Benghazi City

Sample Code	Manufacturing Country	MF, Exp Dates &Other Information
T1	Pakistan	The best quality& largest selling henna in the world
T2	India	25g
T3	-	-
T4	Pakistan	MF. Date: 8-2019 Exp. Date: 7-2024
T5	Pakistan	**Ingredients**: 30g contain Henna extract, Pine oil, Terpinolene, and Preservatives MF Date: 12-2019 Exp Date: 11-2024

Chemicals and Reagents

All chemicals and reagents used in this work were of analytical grade and were obtained from well-qualified chemical companies. These chemicals included nitric acid (65%), obtained from CODEX (Milan, Italy), perchloric acid (70%), obtained from MERCK (Germany), and hydrogen peroxide (30%), obtained from HOPKIN & WILLIAMS BDH CHEMICAL Ltd (Poole, England). The standards for aluminum, chromium, copper, lead, nickel, and zinc, with a concentration of 1000 ppm, for FAAS were purchased from SCHARLAU (Spain). These standards were used to prepare calibration solutions. Deionized water was used for the preparation of all solutions.

All glassware and plastic containers were washed with liquid soap, rinsed with water, soaked in 10% nitric acid for 24 hours, cleaned thoroughly with distilled water, and dried.

Sample Digestion for Determination of Metals

Determination of Metals by Using Flame Atomic Absorption Spectrophotometer

Henna samples were wetly digested. In this process, 1.0 g of each homogeneous henna sample was placed in a 50-mL beaker. Next, it was mixed with 10 mL of an acid mixture (nitric acid and perchloric acid in a 3:1 ratio), and the mixture was heated to 60 °C for 15 minutes until it became nearly clear. The temperature was then raised to 100 °C, and the mixture was heated for an additional two hours. Following this, 5 mL of hydrogen peroxide was introduced into the digestion vessel and heated for one more hour. After the digestion process, the sample solution was allowed to cool before being transferred to a 100-mL volumetric flask. This was done by rinsing several times with deionized water, which was also used to fill the flask to the mark. The solution was then filtered through Whatman No. 41 filter paper. Additionally, a blank solution digest was performed by subjecting equal quantities of all reagents used in the henna sample digestion [30,31].

In our study, the filtered solution was analyzed for selected metals by measuring the absorbance at room temperature using a double-beam GBC model 932 Plus Flame Atomic Absorption Spectrophotometer (FAAS) (A Computer-Controlled System with GBC AA Avanta Software, version 1.33). The concentrations of metals in the henna sample solutions were determined using the calibration curve for each metal. Before conducting the analysis, standard metal solutions were utilized to calibrate the FAAS instrument, ensuring that the instrument settings and operational conditions adhered to the manufacturer's specifications. The concentration of each metal in the henna sample was calculated, using equation {(1)}.

$$Conc\ (mg/Kg) = \frac{conc\ (mg/L)}{sample\ weight} \times dilution\ factor \qquad (1)$$

Health Risk Assessment of Heavy Metals in Henna Samples

The risk of human dermal exposure to metallic impurities in black henna samples can be assessed using the Margin of Safety (MoS). The MoS is calculated as the ratio of the No Observed Adverse Effect Level (NOAEL) for the sample being studied to its Systemic Exposure Dosage (SED), following the guidelines set by the Scientific Committee on Consumer Safety (SCCS) protocol [32,33], as shown in Equation (2)4.

$$MoS = \frac{NOAEL}{SED} \qquad (24)$$

The NOAEL value was calculated by Equation (3)5.

$$NOAEL = RfD_{dermal} \times UF \times MF \qquad (35)$$

Where RfD_{dermal} represents the dermal reference doses (mg/Kkg/day) for various metals. The RfD_{dermal} values are as follows: Al = 1, Cr = 0.019, Ni = 0.0054, Pb = 0.00042, Cu = 0.04, and Zn = 0.3 [34]. The UF is the uncertainty factor, assigned a value of 1, which indicates the overall confidence in the different data sets. The MF is the modifying factor, set at a value of 100 [33,34] 100 [33,34]..

The SED estimates the quantity of toxic metals that enter the human body through dermal exposure. This value is calculated based on several factors, including the metal concentration in the chosen product, the amount of the product applied to the skin, the frequency of application, the surface area of the skin where the product is applied, and the average body weight [32]. The SED (mg/Kkg/day) is estimated using Equation (4)6:

$$SED = \frac{Cs \times AA \times SSA \times F \times RF \times BF}{BW} \times 10^{-3} \quad (46)$$

Where Cs is the mean metal concentration (mg/Kkg) in the henna sample and AA is the applied amount of henna product (g/cm^2). In this study with henna dyes, an amount of 1 g/cm^2 is applied for 30-45 minutes. SSA is the skin surface area onto which the henna product is applied (cm^2). The assumed surface area for the hands and feet is 2030cm^2. RF is the retention factor, which is set at 1.0 for leave-on cosmetic products. F represents the frequency of application for the finished henna product. In this study, it is assumed that one coloring event per month is a reasonable frequency for the application of the henna product. The bioaccessibility factor is represented as BF; the unit conversion factor used was 10^{-3}. In this study, all standard values of parameters, such as skin surface area, the applied amount of product, and the frequency of application, were adopted from SCCS guidelines for cosmetic products [32,33]. The term "BW" denotes the average body weight, estimated at 70 kg as reported in the study by Ali *et* al.[35] [35].

Non-Carcinogenic Health Risk Associated with Using Black Henna Products

The non-carcinogenic risk associated with dermal exposure to metals can be assessed by employing the hazard quotient (HQ) calculation, as demonstrated in Equation (57). The HQ is the ratio of systemic exposure dose of toxic metal via dermal contact (SED) to the dermal reference dose (RfD_{dermal}) of each toxic metal. If the value of HQ < 1, the exposed population is unlikely to experience obvious adverse effects. If HQ > 1, there is a potential health risk, and related interventions and protective measurements need to be taken [33,34].

$$HQ = \frac{SED}{RfD} \quad (57)$$

On the other hand, the Hazard index (HI) is the sum of the hazard quotients for all heavy metals, as explained by Equation (68) [33, 34].

$$HI = HQ_{Pb} + HQ_{Cr} + HQ_{Ni} + HQ_{Cu} + HQ_{Zn} + HQ_{Al} \quad (68)$$

Carcinogenic Health Risk Associated with Using Black Henna Products

The carcinogenic risk associated with the likelihood of developing any form of cancer over a lifetime due to exposure to a potential carcinogen is determined using the incremental lifetime cancer risk (LCR), which is calculated as illustrated in formula (79).

$$LCR = SED \times SF \quad (79)$$

where SF is the carcinogenicity slope factor in $(mg/Kkg/day)^{-1}$, it estimates the cancer risk per unit exposure dose of the carcinogenic agent to cause cancer over the average lifetime. The SF values utilized in the calculations are: Pb = 0.0085, Cr = 0.5, Ni = 0.91, Cu = 1.5, and Al = 0.0014 [33,34]. [33,34].

Statistical analysis

All analytical data for metal analysis were conducted in duplicate, and results are presented as mean (mg/Kkg) ± standard deviation (mean ±SD) alongside the ranges (min-max) for each sample. To compare metal concentrations between tattoo and paste black henna products, an independent samples t-test was employed. All the statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 19.0; IBM, Chicago, IL, USA), with a significance level set at 5% (P < 0.05). Additionally, Microsoft Excel for Windows 10 was utilized for health risk calculations.

III. Results And Discussion

Henna products and their ingredients are considered a source of significant and relatively uncontrolled human exposure to toxic metals and other harmful dyes. In this study, the concentrations of some metals in five tattoo and eight paste homogenous black henna samples that were randomly collected from local markets and beauty centers in Benghazi were analyzed using a validated FAAS method [31]. [31].

Analysis of Metals in Henna Samples

The results of this work revealed that some metals were found in extensive amounts, some in very small amounts, and some were not detected in some henna samples. The mean contents of the analyzed metals and ranges in the selected henna samples are shown in Table 24. The metal concentrations in the selected henna samples were compared with research articles on henna conducted locally in Libya and in various other countries [15, 19-27] (Table 5).

Table 24: Contents of some metals in black henna samples collected from Benghazi city

Sample	Metal Concentration (mg/Kg)					
	Pb	Cr	Ni	Cu	Zn	Al
T1	0.24 ±0.003	0.13 ±0.002	1.12 ±0.005	0.56 ±0.001	12.052 ±0.002	9.52 ±0.35
T2	0.51 ±0.001	0.32 ±0.008	<LOD	0.32 ±0.004	<LOD	11.25 ±0.006
T3	0.020 ±0.001	1.35 ±0.009	1.38 ±0.2	0.19 ±0.004	7.25 ±0.007	4.99 ±0.009
T4	<LOD	1.05 ±0.006	0.45 ±0.3006	1.10 ±0.004	<LOD	10.65 ±0.004
T5	0.18 ±0.001	0.20 ±0.004	<LOD	0.32 ±0.002	8.09 ±0.004	2.011 ±0.02
Min-Max	LOD-0.51	0.13-1.35	LOD-1.38	0.19-1.10	LOD-12.05	2.01-11.25
P1	<LOD	0.062 ±0.004	0.16 ±0.006	2.21 ±0.007	19.01 ±0.001	4.018± 0.004
P2	0.31 ±0.002	2.016 ±0.005	0.22 ±0.003	0.77 ±0.003	<LOD	3.41 ±0.005
P3	0.026 ±0.003	0.25 ±0.002	<LOD	0.30 ±0.004	0.80 ±0.001	8.51 ±0.008
P4	0.42 ±0.001	1.61 ±0.008	0.093 ±0.003	1.084 ±0.007	1.11±0.002	2.61 ±0.01
P5	<LOD	0.77 ±0.006	0.027 ±0.002	0.92 ±0.002	1.11 ±0.004	0.88 ±0.003
P6	<LOD	4.012 ±0.02	0.28 ±0.005	0.041 ±0.001	2.19 ±0.003	9.12 ±0.03
P7	0.21 ±0.002	1.87 ±0.02	<LOD	1.028 ±0.001	10.078 ±0.003	3.15 ±0.002
P8	0.81 ±0.008	0.70 ±0.004	1.98 ±0.04	0.11 ±0.001	1.43 ±0.005	2.67 ±0.01
Min-Max	<LOD-0.81	0.062-4.012	<LOD-1.98	0.041-2.21	<LOD-19.01	0.88-9.12

In our study, the highest concentration of lead metal was detected at 0.51±0.001mg/Kkg in tattoo henna samples (sample T2), while the highest concentration of lead in the paste henna samples was detected at 0.81±0.008mg/Kkg in sample P8. The maximum concentration range of lead in our selected henna samples was much lower than the levels of lead metal recorded by Ajaj et al. and Bobaker *et al.* in henna samples collected from local markets and local herbalist shops in Zliten and Benghazi, Libya, respectively [28,29]. Furthermore, the highest concentration of lead in our henna samples was much lower than the lead concentration range in henna paste samples collected from Pakistan (5.5-16.0mg/Kkg) [21], Turkey (6.50-17.4mg/Kkg) (1.59-17.7mg/Kkg) [22, 23], Tunisia (1.2-8.9mg/Kkg) [25], Egypt (LOD-16.42mg/Kkg) [26], Palestine (5.35 ±1.72mg/Kkg) [27], Saudi Arabia (2.6 ±0.48mg/Kkg) [19] and Kenya (LOD-2.63mg/Kkg) (LOD-1.32mg/Kkg) [20]. In contrast, the maximum concentration range of lead metal detected in our henna samples falls within the lead concentration range of henna samples collected from Turkey (0.8-1.2mg/Kkg) (0.60-0.93mg/Kkg) [15,24] and is higher than the concentration of lead in different henna leaves collected from Kenya (0.05-0.40mg/Kkg) [20]. [20].

Substantial levels of chromium were detected in all henna samples. These concentrations ranged from 0.062±0.004mg/Kkg to 4.012±0.02mg/Kkg in paste henna samples and from 0.13±0.002mg/Kkg to 1.35±0.009mg/Kkg in tattoo henna samples (Table 2). The levels of chromium in our henna samples were much lower than the concentration range of this metal in henna samples purchased from Turkish (LOD-15.6mg/Kkg) (0.70-54.4mg/Kkg) (35.0-76.9mg/Kkg) [15,22,23] and Egyptian (LOD-15.75mg/Kkg) [26] local markets.

Lead and chromium metals can be present in cosmetic products due to the use of lead chromate and lead dichromate as pigments in the cosmetic manufacturing process. In fact, dermal contact with chromium causes skin ulcers and allergic reactions characterized by severe redness and swelling of the skin. Long-term exposure to chromium can cause skin irritation [16]. Chromium is also linked to skin effects such as eczema and other inflammations of the skin [31]. The concentrations of lead metal in the collected henna samples were less than the permissible levels for lead recommended by the WHO and FDA, while chromium concentrations in two henna tattoo samples and four henna paste samples exceeded the FDA maximum permissible limit in cosmetic [6, 16].cosmetics [6,16].

Nickel metal was detected in nearly 69% of the collected samples. The highest concentrations of nickel metal were detected at 1.38 ±0.2mg/Kkg and 1.98 ±0.04mg/Kkg in tattoo and paste henna samples, respectively. Human bodies require a trace amount of nickel metal to aid prolactin production and glucose breakdown [36]. Nickel metal causes allergic contact dermatitis and hand eczema when applied to the skin [18]. In our work, the concentrations of nickel in the selected tattoo and paste black henna samples were lower than the highest concentration of nickel metal detected in henna powder (<LOD-1.59mg/Kkg), henna paste (<LOD-3.01mg/Kkg) [20], (2.2-4.0mg/Kkg) [15], and temporary black henna tattoo (1.13-2.20mg/Kkg) [23] collected from Kenyan [20] and Turkish markets. However, the highest level of nickel in our samples was much lower than the concentrations reported by Ibrahim et al. and Al-Qutob et al. for henna samples collected from Egyptian (<LOD-223.11mg/Kkg) and Palestinian (29.65±3.53mg/Kkg) [26, 27] local markets, respectively. In contrast, the maximum concentrations of nickel metal in our samples were higher than the concentrations of nickel metal detected in henna leaves (0.02-0.11mg/kg) and in green and black henna samples (0.49-1.06mg/Kkg) collected from Kenya [20] and Turkey [24], respectively. In this study, there were no henna samples containing a concentration of nickel greater than 5ppm [18].5ppm [18].

In the present work, different levels of copper were detected in all the henna samples (Table 2). These levels ranged from 0.19±0.004mg/Kkg to 1.10±0.004mg/Kkg for henna tattoos and from 0.041±0.001mg/Kkg to 2.21±0.007mg/Kkg for paste henna samples. It was found that the maximum concentrations of copper detected in henna samples from Turkey (2.70-67.6mg.kg-1) [22], Egypt (<LOD-118.9mg/Kkg) [26], and the mean concentration of copper detected in henna samples from Palestine (95.92±4.02mg/Kkg) [27] were much higher than the concentration range of copper in our samples.

Zinc is one of the minor essential nutrients for the human body. Zinc is a constituent of enzymes and insulin, which are required for healthy growth of skin and hair [36, 37]. In cosmetic manufacturing, zinc is commonly used as a white pigment in ultraviolet filters. Moreover, zinc oxide and silver nanoparticles form the main ingredients of chemically modified clays because of their microbicidal activity [6]. Because of the shiny nature of zinc compounds, cosmetic manufacturers add unregulated amounts of zinc to various cosmetic products [38]. As shown in Table 2, zinc metal was found at detectable levels in all henna samples, except for three samples (T2, T4, and P2), where the zinc concentration was below the detectable limit of the analysis method. However, the lowest zinc concentration in the paste henna samples was detected in sample P3 at 0.80±0.001mg/Kkg and the highest concentration was detected in sample P1 at 19.01±0.001mg/Kkg. In tattoo henna samples, the lowest zinc concentration (7.25±0.007mg/Kkg) was detected in sample T3, and the highest concentration (12.052±0.002mg/Kkg) was detected in sample T1. The concentration ranges of zinc in the analyzed samples were much lower than the values of zinc concentration ranges reported for henna analysis in different countries, including Tunisia (3.7-90.0mg/kg) [25], Egypt (<LOD-996.3 mg/Kkg) [26], and Palestine (89.93±3.43mg/Kg) [27]. 43mg/kg) [27].

The results in Table 2 revealed that aluminum was detected in both tattoo and paste henna samples. The concentrations of aluminum in tattoo henna samples ranged from 2.011±0.02mg/Kkg to 11.25±0.006mg/Kkg. In the paste henna samples, the highest aluminum mean concentration (9.12±0.03mg/Kkg) was detected in sample P6, and the lowest aluminum concentration (0.881±0.003mg/Kkg) was detected in sample P5. However, aluminum is not needed in the human body. This metal is characterized by high toxicity during prolonged exposure. Borowska and Brzoska mentioned that the presence of aluminum in herbal plants, and therefore in herbal cosmetics, was due to the increasing absorption of large amounts of aluminum compounds from the earth`s crust during soil acidification and then by plants [6]. In cosmetics, aluminum and its compounds are used as abrasive, absorbent, anticaking, bulking, and pacifying agents [39]. In antiperspirant products, aluminum tends to form a physical plug of aluminum-proteoglycan precipitate on the cells at the surface of the duct, which prevents sweat from escaping onto the body surface [6]. When aluminum enters the body, it has the ability to accumulate in the organs, where it begins to compete for important minerals like calcium [40]. The maximum aluminum concentration in our henna samples was much lower than the aluminum concentration in henna samples collected from Libya (Zliten) (53.8-496.9mg/Kkg) [28], Egypt (10.63-19681.5mg/Kkg) [26], and Palestine (142.1 ±1.52mg/Kkg) [27]. Conversely, the maximum concentration of aluminum in our henna samples was higher than the maximum aluminum concentrations in henna samples collected from Turkey (0.122-5.22mg/Kkg) [22]. The safety assessment by the Cosmetic Ingredient Review (CIR) does not include aluminum as a cosmetic ingredient [39]. [39].

Finally, there was no statistically significant difference ($P > 0.05$) between tattoo and paste black henna samples in all examined metal concentrations, including lead, nickel, copper, zinc, and aluminum, with the exception of chromium metal. The variation in chromium content between the two henna samples could mean that, during the preparation process, industrial chemical additions cause chromium metal to infiltrate into the henna paste [26]. [26].

Human health risk assessment of toxic metals

Exposure assessment stands as a critical element in risk evaluation, essential for determining the likelihood and extent of individuals' exposure to a chemical substance and investigating the probability and scale of people's exposure to a chemical agent [41]. The current study encompassed a survey on both the non-carcinogenic and carcinogenic effects of dermal exposure to certain toxic heavy metals. Within this study, terms such as SED, MoS, HQ, HI, and cancer risk were utilized to assess the potential health hazards linked to dermal exposure to heavy metals in the collected henna samples. The calculated SED and MoS values at 100% bioaccessibility for the analyzed metals were presented in Table 63. The SED values varied from 1.84×10^{-4} to 2.13×10^{-4} mg/Kkg/day for lead, 5.90×10^{-4} to 1.36×10^{-3}mg/Kkg/day for chromium, 3.31×10^{-4} to 5.68×10^{-4} mg/Kkg/day for nickel, 4.80×10^{-4} to 7.80×10^{-4} mg/Kkg/day for copper, 4.31×10^{-3} to 5.29×10^{-3} mg/Kkg/day for zinc, and 4.15×10^{-3} to 7.42×10^{-3} mg/Kkg/day for aluminum, in both black henna samples.

Based on the SED values, the MoS values for lead and aluminum exceed 100 and 10,000, respectively, while the majority of MoS values for the other analyzed metals surpass 1000 (Table 36). Indeed, the MOS values presented in Table 3 indicate no evident risk for humans [33,42].

Table 36: The values of systematic exposure dose (SED) and margin of safety (MoS) of some heavy metals detected in black henna samples, using 100% bioaccessibility

Henna sample	The analyzed metals					
Systemic Exposure Dose (SED)						
	Lead	**Chromium**	**Nickel**	**Copper**	**Zinc**	**Aluminum**
Tattoo	1.84×10^{-4}	5.9×10^{-4}	5.68×10^{-4}	4.8×10^{-4}	5.29×10^{-3}	7.42×10^{-3}
Paste	2.13×10^{-4}	1.36×10^{-3}	3.31×10^{-4}	7.8×10^{-4}	4.31×10^{-3}	4.15×10^{-3}
Margin of Safety (MoS)						
Tattoo	2.28×10^{2}	3.22×10^{3}	9.51×10^{2}	8.33×10^{3}	5.67×10^{3}	1.35×10^{4}
Paste	1.96×10^{2}	1.40×10^{3}	1.63×10^{3}	5.13×10^{3}	6.96×10^{3}	2.41×10^{4}

In our study, we examined the non-carcinogenic effects of the investigated heavy metals through HQ and HI calculations. The HQ and HI values for both henna samples, assessed at 100% bioaccessibility, are recorded in Table 7. It is noteworthy that the individual HQ values of the analyzed metals in both henna samples, utilizing dermal RfD for adults, were below 1. This indicates that adult females in Benghazi city are unlikely to face significant health risks through dermal absorption of heavy metals from the studied black henna samples. Furthermore, the HI values for the black henna samples were recorded at 0.61 and 0.68 for the tattoo and paste henna samples, respectively (Table 4.7). As indicated in Table 4, lead contributes to around 70% and 75% of the HI values in tattoo and paste henna samples, respectively. Nevertheless, the HI values, in general, indicate that the probability of non-cancer and cancer risks arising from dermal exposure to these metals at these concentrations is unlikely [43].y [43].

Table 74: The Hazard Quotient (HQ) and Hazard Index (HI) of the analyzed metals in Henna samples

Henna Sample	HQ_{Pb}	HQ_{Cr}	HQ_{Ni}	HQ_{Cu}	HQ_{Zn}	HQ_{Al}	HI
Tattoo	4.37×10^{-1}	3.10×10^{-2}	1.05×10^{-1}	1.20×10^{-2}	1.76×10^{-2}	7.42×10^{-3}	0.61
Paste	5.08×10^{-1}	7.16×10^{-2}	6.14×10^{-2}	1.95×10^{-2}	1.44×10^{-2}	4.15×10^{-3}	0.68

The US-EPA has identified the cancer risk values less than 1×10^{-6} as insignificant and of no consequence. Conversely, cancer risk values greater than 1×10^{-4} have been deemed significant enough to necessitate remediation measures [44]. In this study, the carcinogenic risk of dermal exposure to lead, chromium, nickel, copper, and aluminum was calculated at 100% bioaccessibility, and the results were presented in Table 85. In this study, our results show that the cancer risks of the toxic metals in tattoo black henna samples varied between 3.45×10^{-9} and 5.79×10^{-5}. These values indicate that the cancer risk associated with skin exposure to heavy metals in tattoo samples is very small. Conversely, in the paste henna samples, the lifetime cancer risks associated with chromium, nickel, and copper were found to surpass permissible levels, as indicated in Table 85. Due to their long half-lives, these metals can accumulate in the body; thus, ongoing use of these henna products may lead to harmful levels of these elements [44].

Table 58: Cancer risk values of some carcinogenic metals in Henna samples using 100% bioaccessibility

	Lifetime Cancer Risk (LCR)	
Metal	**Tattoo Henna Sample**	**Paste Henna Sample**
Lead	3.45×10^{-9}	1.81×10^{-6}
Chromium	9.17×10^{-6}	6.80×10^{-4}
Nickel	4.75×10^{-6}	3.01×10^{-4}
Copper	5.79×10^{-5}	1.17×10^{-3}
Aluminum	1.35×10^{-6}	5.81×10^{-6}

IV.Conclusion

This work shows the contents of six metals in two types of "ready-to-use" black henna products that are frequently purchased by local inhabitants in Benghazi, Libya. According to the elemental analysis, aluminum, chromium, and copper were found in every sample of henna that was collected, whereas zinc, nickel, and lead metals were detected in 77%, 69%, and 69% of the investigated samples, respectively. The concentration variations of the analyzed metals among the samples may be attributed to differences in chemical and coloring additives that were added during the preparation and manufacturing processes.

All levels of lead detected in henna samples were below the maximum allowed limits set by different international regulations for toxic metals in cosmetic products. Conversely, the levels of chromium detected in 54% of the analyzed henna samples were above the maximum allowed limits set by the FDA. The internationally acceptable maximum limits for aluminum, zinc, and copper in cosmetic products are not available. The contents of the analyzed metals in the collected henna samples were mostly lower than the results reported by previous studies conducted internationally in different countries.

The contents of metals detected in henna samples show MoS values higher than 100, indicating that there is no toxicological concern for systemic toxicity following dermal exposure to henna products. Furthermore, the values of HQ and HI were within the permissible limit (<1) for all henna samples. However, the LCR values were within the permissible limit for all metals in tattoo and paste henna samples, except for chromium, nickel, and copper metals in paste samples.

As toxic metals tend to accumulate in the human body, continuous exposure to these products may cause cumulative effects, and the health effects remain a concern. This study recommends educating the users about the presence of toxic metals and the potential toxic effects of accumulated metals and other polluting substances in henna products.

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