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Determination of Some Heavy Metals in Hair Gal Cosmetic Products by Atomic Absorption Spectrometer from Benghazi-Libya Markets and their Microbiological Detection

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تحديد بعض المعادن الثقيلة في منتجات تجميل الشعر (جل الشعر) باستخدام مطياف الامتصاص الذري من أسواق بنغازي، ليبيا والكشف الميكروبيولوجي لها

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Abstract:
This study investigated the concentrations of selected heavy metals-Lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc (Zn), and Manganese (Mn), in hair gel products. The samples were collected in April 2023 from markets in Benghazi, a city in eastern Libya. After wet digestion, the samples were analyzed using an atomic absorption spectrophotometer. The mean concentrations observed in the cosmetic samples of hair gel ranged from 0.04±0.03 to 0.66±0.12 mg/kg for Pb; 0.04±0.02 to 0.20±0.07 mg/kg for Cd; 0.00 to 0.15±0.10 mg/kg for Cr; 0.00 to 0.24±0.10 mg/kg for Mn; and 0.11±0.00 to 1.13±0.32 mg/kg for Zn. Statistical significance was set at $P < 0.05$. Results revealed detectable levels of all heavy metals in the products, ordered as Zn > Cd > Cr > Pb > Mn. All study samples were subjected to microorganism analysis, and the results were negative, indicating they were free of any bacterial contamination. However, the chemical analysis showed that the levels of Pb, Cd, Cr, Zn, and Mn in most study samples were lower than the permissible limits set by the World Health Organization (WHO), except for three samples that had high levels of cadmium. Although most hair metal concentrations were below reference values, the high-risk quotients (RQs) in hair creams indicate potential systemic exposure risks. These findings underscore the need for stricter regulatory oversight and routine monitoring of cosmetic products to safeguard consumer health.

Keywords: Heavy metals, hair gal, atomic absorption, spectrophotometer.

الملخص:
تناولت هذه الدراسة بحث تركيزات مجموعة مختارة من المعادن الثقيلة مثل: الرصاص والكروم والكادميوم الزنك والمنحنيزفي منتجات جل الشعر، جمعت العينات في نيسان/أبريل 2023 من الأسواق في بنغازي، وهي مدينة في شرق ليبيا. بعد التهظيم الرطب للعينات تم تحليلها باستخدام مطياف امتصاص ذري. كما خضعت جميع عينات الدراسة لتحليل الكائنات الدقيقة، وجاءت النتائج سلبية، مما يشير إلى خلوها من أي تلوث بكتيري. تراوحت التركيزات التي وجدت في العينات التجميلية من جل الشعر من 0.040-0.660 ملغم/كغم بالنسبة للرصاص؛ ومن 0.04 إلى 0.20 ملغم/كغم بالنسبة للكادميوم؛ ومن 0.00 إلى 0.15 ملغم/كغم بالنسبة للكروم؛ ومن 0.00 إلى 0.24 ملغم/كغم بالنسبة للمنجنيز ومن 0.00 إلى 0.32/ للزنك. وحُددت الأهمية الإحصائية في البرنامج الفرعي 0.05. كشفت النتائج عن مستويات يمكن اكتشافها من جميع المعادن الثقيلة في المنتجات. وأظهرت النتائج وجود مستويات قابلة للقياس من جميع المعادن الثقيلة في المنتجات، مرتبة تنازلياً حسب التركيز كالتالي: الزنك > الكادميوم > الكروم > الرصاص > المنغنيز. ومع ذلك، أظهر التحليل الكيميائي أن مستويات الرصاص والكادميوم والكروم والزنك والمنغنيز في معظم عينات الدراسة كانت أقل من الحدود المسموح بها التي وضعتها منظمة الصحة العالمية (WHO)، باستثناء ثلاث عينات احتوت علي مستويات مرتفعة من الكادميوم. ورغم أن معظم تركيزات المعادن كانت دون القيم المرجعية، إلا أن ارتفاع معامل (RQs)في المعادن الثقيلة في منتجات الشعر يشير إلى مخاطر محتملة للتعرض (التعرض الذي يؤثر على الجسم ككل). وتؤكد هذه النتائج الحاجة إلى رقابة تنظيمية أكثر صرامة ومراقبة دورية لمنتجات التجميل لحماية صحة المستهلك.

الكلمات المفتاحية: المعادن الثقيلة، جل الشعر، الامتصاص الذري.

INTRODUCTION:

Cosmetic products have been utilized since ancient times, with each historical period showcasing its own unique characteristics. In the 20th and 21st centuries, the beauty and personal care products industry has thrived, bringing forth new and innovative items. Consequently, the cosmetics market today includes a wide range of products, such as lipsticks, mascara, eye shadows, face powders, hair products, and primarily skincare products [1]. With the mass production of cosmetics and growing demand, concerns regarding product safety have arisen. Cosmetics usually contain a long list of ingredients (organic and inorganic compounds, herbal extracts, oils), so human safety is a significant concern, especially for products applied daily or over large surfaces, as well as for products designed for infants. The issue of personal care products becoming a source of exposure to heavy metals has gained attention over the past two decades [2]. Risk assessment for human cosmetics is based on the toxicological profile of the ingredients used and their level of exposure.

The application of cosmetics to enhance the human body is a global tradition that has lasted for thousands of years. Despite difficult economic conditions, the demand for cosmetic products has risen worldwide due to people's awareness of the desire to beautify their bodies and the significant increase in advertisements for these products in both print and electronic media [3,4]. The use of cosmetics as a regular part of body care spans all income levels in society [5].

The sources of metallic impurities in cosmetic products include their use as ingredients, such as zinc oxide in sunscreen creams, inorganic mercury compounds in skin-lightening creams, lead acetate in progressive hair dyes, and aluminum and zirconium salts in antiperspirants. Additionally, these impurities can arise as contaminants during manufacturing processes [3]. The intentional use of compounds like antimony (Sb), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), and nickel (Ni) in cosmetic products is prohibited by Annex II of Directive 76/768/EEC of the European Union due to safety concerns regarding their toxicity. However, these compounds continue to be found in modern cosmetic products because of their widespread presence and contamination during production.

The presence of metals in cosmetic products raises concerns for three main reasons [5,6]: (i) the use of these products could lead to potential daily exposure for the general population, often over long periods; (ii) metals can accumulate in the body over time; and (iii) many of them are known to cause various chronic health issues, including cancer, contact dermatitis, developmental, neurological, and reproductive disorders, as well as brittle hair and hair loss. Some metals act as strong endocrine disruptors and respiratory toxins. Additionally, certain metals, such as cadmium, arsenic, lead, mercury, and antimony, are particularly toxic and can cause a wide range of chronic health effects, while chromium, nickel, and cobalt are recognized as common skin sensitizers [2,4,5]. For these reasons, the cosmetics industry is facing increased regulatory scrutiny globally, with the shared objective of ensuring safe ingredient levels in these products.

The goal is to guarantee that consumers are not exposed to the harmful effects of heavy metals, as the aim is to protect consumers from health risks [7]. Some heavy metals in hair care products have shown a correlation with the corresponding concentrations of certain metals in scalp hair and blood and/or urine. Reports demonstrate that hair has been exposed to elevated levels of arsenic, lead, mercury, cadmium, manganese, and chromium, as indicated by findings of exposure to these heavy metals. Systemic intoxication with heavy metals is also reflected not only in hair but also in blood and urine [8].

The presence of toxic metals in cosmetics has been extensively reported across the Arab world, particularly in Libya, highlighting exposure to these substances. However, there has been no investigation into heavy metal contamination in hair gel samples taken from cosmetic product shops in Benghazi, including face foundation, blusher, and face powder. Despite efforts, no attempts have been made to assess the presence of heavy metals such as lead, cadmium, iron, copper, chromium, manganese, and zinc [9-12].

Materials and Methods:

Samples Collection:

in this study, samples of different types of hair care cosmetic products were randomly selected from various local retail shops in Benghazi, Libya, during April 2023. The information on the container labels and contents was carefully examined visually, and any defects or irregularities were noted. The names of the products, dates of manufacture and expiry, as well as the ingredients list on the labels of these products, were recorded in Table 1. All the samples were transferred to the laboratory for microbiological testing and analysis of heavy metal content as soon as possible after collection.

Table (1): List of cosmetics, their codes, and container label disclosures of the collected samples

Name of Cosmetic product	Sample code	Colour of product	Country of manufacture
Penduline Hair gel	G1	Crystal Clear Colourless	Egypt
Bless Hair gel	G2	Semi-Transparent	Egypt
Clary Hair gel	G3	Crystal Clear	Egypt
Bobana Styling gel	G4	Transparent Clear	Egypt
Glatt gel	G5	Transparent Blue or Standard Clear	Egypt

Reagents and Solutions:

Analytical reagent-grade chemicals (HNO_3 (65%) and H_2O_2 (30%)) and ultrapure water were used for the preparation of working reagents and for sample dilution. The glassware was cleaned and immersed in 6% nitric acid for 24 hours. Teflon beakers were treated with 6% nitric acid and then washed with ultrapure water.

Pre-treatment:

Metal standards were prepared each day. Known amounts of the certified standard stock solution (1000 ppm, Merck, Darmstadt, Germany) were added to the three probes to achieve final concentrations in the range of 0.00–2.00 ppm for the selected metals; for Pb, the range was 0.01–1.00 μg/ml. The concentration levels were determined based on apparatus specifications: 0.1, 0.20, 0.30, and 2 mg/ml. A good linear relationship was observed between absorbance and the standard concentration of the metals used. The linear correlation coefficients (r^2) for the metals ranged from 0.9856 to 1.001. The limit of detection (LOD) was estimated as the lowest concentration in the calibration for each selected metal, while the limit of quantification (LOQ) was defined as three times the LOD value calculated for each metal. The accuracy of the method was determined by measuring the recovery of metal through spiking the standard solution of each metal at three concentrations, and it was found to be in the range of 89% to 99.5%.

Samples digestion:

Wet methods were employed to prepare samples for heavy metal analysis by exposing them to the sample sections. The samples (1 g each) were digested for 3 hours on a hot plate in a gas chamber with 10 ml of a mixture of HNO_3 and HCl (3:1 v/v). If black or brown coloration developed, 3 ml of concentrated acid was added to the mixture until it produced white fumes, followed by continuous heating until the mixture was completely dry. If the mixture appeared too dry, additional acid was added until no more visible color was formed. After allowing the solutions to cool, they were poured into a calibrated flask (25 ml) and then diluted up to the mark [13,14]. Results were reported in mg/kg, and the measured absorbance was converted to concentrations using standard calibration curves. Standards of the metals of interest were obtained from Fluka Analytical (Sigma Aldrich, UK), and quantification of selected elements was performed through duplicate determinations, with the percentage of relative standard deviation (RSD) or (±Cd) calculated. The report indicated mg/kg, and standard calibration curves were utilized to convert the absorbance measured from the study results to concentrations. Quantification of the metals of interest was determined through duplicate determinations of the elements based on the obtained standards.

Media Used for Growth of Bacteria:

MacConkey and Blood agar were used for growth of bacteria provided from OXOIDE (Germany), and Sabauroud`s Dextrose agar from HIM MEDIA LABORATORIS PVT. Ltd (India) was used to grow moulds. All these media were prepared under aseptic conditions, according to the manufacture's specifications. Ethanol, from MERCK (Germany) was diluted to 70% (v/v) and used for the disinfection of the sample package [14].

Microbiology's method:

After being aseptically transferred to the microbiological laboratory, cosmetic samples were subjected to microbiological testing. Each sample was weighed at two grams, divided into 1 gram each. After being aseptically placed in a 50 ml nutrient broth contained in a bottle, the initial 1 gram was incubated at 37C° for 24 hours, during which aseptic procedures were followed. To identify bacterial and fungal contamination, we inoculated another 1 gram of the ingredients into a 50 ml Sabouraud dextrose broth contained in a bottle and incubated it at 25C° for 72 hours, observing for bacterial contamination [15,16]. Following incubation, cultures were transferred to nutrient broth, nutrient agar, MacConkey agar, and mannitol salt agar. The cultures from the Sabouraud dextrose broth were then dried onto Sabouraud dextrose agar.

Calculation:

- **E mg/L =** (1000/V) × (X/1000) × (Vf/Va) × D.
- **E =** the element being determined.
- **V =** volume of hair sample extract analyzed.
- **X =** concentration from the standard curve.
- **Vf =** total volume of extract.
- **Va =** volume of extract aspirated.
- **D =** dilution factor, where applicable.

Statistical analysis:

The analysis of all metals was repeated using a double standard, and the analytical data were expressed as mean (mg/kg) and mean (mg/kg ± SD) for each sample. The results for each metal were presented in ranges (min-max) for their respective outcomes. The hair gel products underwent comparison using One-Way Analysis of Variance (ANOVA), which was subsequently examined using the Least Significant Difference method.

Results and Discussion:

Table 3 highlights the international standards for certain heavy metals in cosmetic products, including chromium, manganese, lead, and cadmium, which were significantly associated with the amounts of these heavy metals, as determined by a comprehensive analysis of the collected hair gel samples.

Table (3): International Standards for some heavy metals in some cosmetic products

Health international regulation	Maximum acceptable Limits (mg/kg)				Reference
	Pb	Cd	Cr	Mn	
European Union (EU)	10	5	Prohibited Annex II	Prohibited Annex II	[16,17]
Food and Drug Administration (FDA)	20	-	NE	NE	[18]
World Health Organization (WHO)	10	0.3	NE	NE	[19-20]
Health Canada	20	3.0	NE	NE	[21]

NE: Not establishes.

In Table 4, the maximum levels for each heavy metal are as follows: lead (Pb) in G4 leaves and paste was 0.66 ± 0.12 mg/kg (1.2 ± SD), while in G5 it was 0.25 ± 0.11 mg/kg. However, in G3 samples, Pb was 0.13 ± 0.05 mg/kg. G1 and G2 samples contained the lowest amounts of lead, with all measurements being lower than the international standards for lead, as shown in Table 3.

Regarding cadmium (Cd), the level in the hair gel products reached 0.20 ± 0.07 mg/kg for G3. In G1, it was 0.14 ± 0.09 mg/kg, in G4 it reached 0.04 ± 0.02 mg/kg, and in G5 it was 0.03 ± 0.01 mg/kg. According to WHO limits, samples containing high levels of Cd exceeded the threshold of 0.3 ppm, except for three samples (G1, G3, and G4), as seen in Table 3.

Table (4): Metals Concentration of gel by FAAS:

Sample of Hair gel	Pd Mean±SD	Cd Mean±SD	Cr Mean±SD	Zn Mean±SD	Mn Mean±SD
G1	0.04±0.03	0.09±0.14	±0.000.00	0.12±0.21	0.100.24±
G2	0.020.01±	±0.000.00	0.00 ±0.00	0.13±0.23	0.01±0.01
G3	0.13±0.05	0.07±0.20	0.03±0.07	0.19±0.05	±0.000.00
G4	0.66±0.12	0.04±0.02	0.13±0.06	0.01±1.11	±0.000.00
G5	0.11±0.25	0.01±0.03	0.15±0.10	0.77±0.26	0.01±0.01

For the amount of chromium, the highest level in hair gel sample G5 was 0.15 ± 0.10 mg/kg, compared to 0.13 ± 0.06 mg/kg in G4 and 0.07 ± 0.03 mg/kg for G3. Both hair gel samples G1 and G2 did not contain any detectable amount of chromium.

The presence of zinc in hair care products indicates that the product is designed to support scalp health, strengthen hair follicles, and reduce issues such as dandruff. Therefore, it is an additive that enhances the product rather than being harmful or extraneous [22]. In terms of zinc content in hair gel products, the maximum level was 1.11 ± 0.01 mg/kg in G4, followed by 0.77 ± 0.26 mg/kg in G5. The other hair gel samples contained 0.19 ± 0.05 mg/kg, 0.13 ± 0.23 mg/kg, and 0.12 ± 0.01 mg/kg for G3, G2, and G1, respectively, as shown in Figure 1.

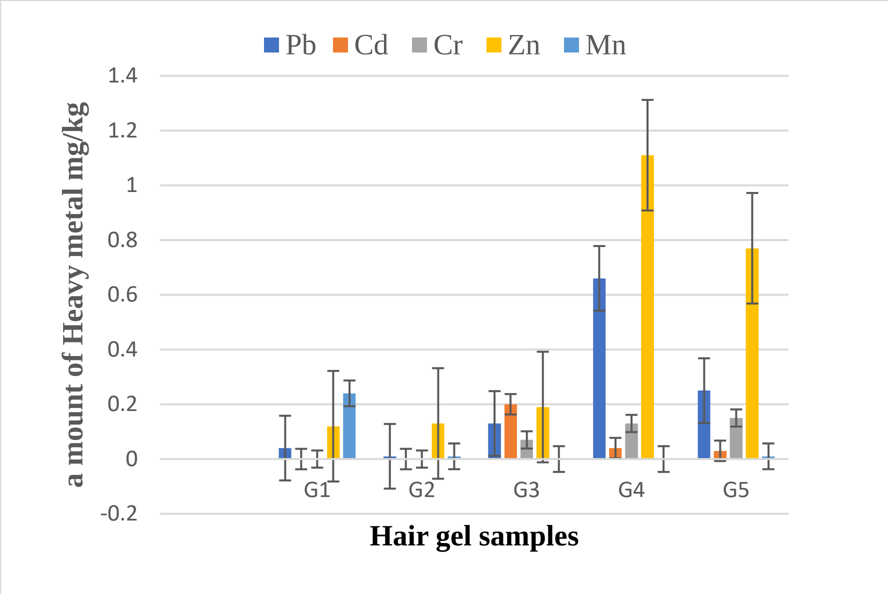

Fig (1): Amounts of heavy metals in hair gel products samples

The presence of manganese in hair care products, whether in shampoos, topical serums, or dietary supplements, means that products designed to boost hair strength and protect against aging and damage are actively supporting hair health [23]. Manganese serves as a key cofactor in activating the enzymes responsible for producing proline (an amino acid) and collagen in the body. Collagen is the structural protein that gives hair strands their elasticity and strength, protecting them from breakage and split ends [24.[

The high level of manganese in hair gel G1 was 0.24 ± 0.10 mg/kg, while hair gels G2 and G5 contained 0.01 ± 0.01 mg/kg. G3 and G4 did not have any additives of manganese. The distribution frequency of metals among the samples was in the order of Zn > Cd > Cr > Pb > Mn, which aligns with other similar reported works [25 .[

The other heavy metals do not require FDA approval before marketing, but they should be confirmed on the cosmetic labeling. The recent FDA meeting insisted on adding some GMP requirements to the old GMP guidelines issued in 2013, which were evaluated and revised this year. In the European Union, EU regulation (1223/2009) does not permit the presence of heavy metals unless in small traces that are unavoidable, which have not yet been identified. Thus, all the samples investigated exceeded European regulations because they contained potentially avoidable levels of heavy metal traces [25].

Calculation of Oxides supplements percentage:

Three metals: chromium, zinc and manganese were investigated in the samples under the study. Typically, they were present as metal oxide in the air gel cosmetic products.

Table 5, show the percentage of the compounds CrO, ZnO and MnO in the samples. The rates of metals oxides in the hair gel samples were calculated and found by applying the following equation;

$$\%MO = M \text{ results} \times \left(\frac{Mwt.MO}{At\,Wt.M}\right) \times 10^{-7}$$

Where M.wt: Molecular weight, and At wt: Atomic wight of the heavy metals, M: heavy metals (Cr, Zn or Mn).

Table (5): Metal Dioxide Percentages in hair gel products

Samples	%CrO*	%ZnO*	%MnO*
G1	0.00	0.15	0.38
G2	0.00	0.24	0.02
G3	0.21	0.14	0.00
G4	0.38	0.41	0.00
G5	0.44	0.96	0.03

*= x 10^6

Accordingly, assessing heavy metal levels is challenging due to the differing limits for each heavy metal across various guidelines and countries. Therefore, relying on individual guidelines is not practical unless the minimum levels of these heavy metals are harmonized into a single guideline that is legally applicable during cosmetic manufacturing or aftermarket quality control. Additionally, standard procedures should be established to address complaints.

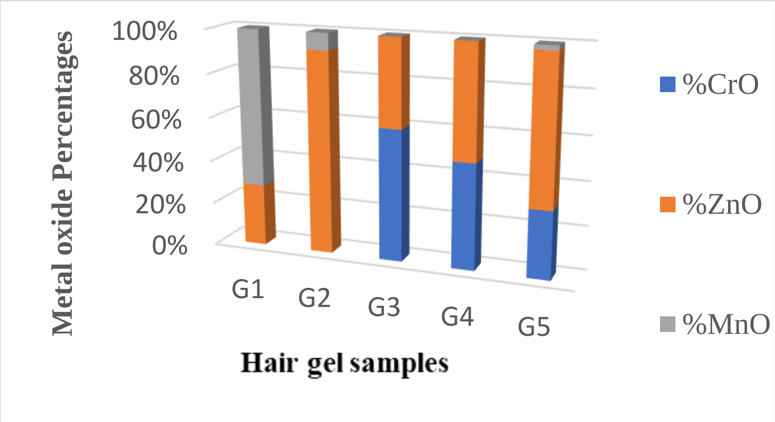

Fig (2): Percentages of supplement of metals oxides in hair gel samples

Trace heavy metals are still present in even the highest quality products, despite the application of Good Manufacturing Practices (GMP) by some manufacturers, as determined and quantified in this study under possible quality control measures. The results highlight the need to minimize or eliminate the use of certain heavy metals in cosmetic products. Contamination also arises from environmental sources across different temperate zones and from industrial pollution. This study aligns with previous research conducted in various parts of Libya [23], which concluded that these products pose a potential risk of heavy metal poisoning due to their widespread use and the increased absorption of heavy metals through the skin or inhalation. The application of international guidelines with varying heavy metal limits in this study underscores the necessity for harmonized acceptable levels of heavy metals that can be applied globally. The findings suggest that continuous monitoring of metallic content or impurities in these products, as well as in other facial cosmetics, should be taken seriously.

As shown in Table 5, both hair gel cosmetic samples G1 and G2 exhibited lower levels of heavy metal contamination compared to other samples, while samples G4 and G5 showed high concentrations of Pb, Cd, Cr, and Zn (see Table 4 and Figure 2).

Microbiology Analysis of Cosmetics Samples results:

The microbiological analysis of all cosmetic samples, including those for hair gel, revealed no bacterial or fungal growth in any of the studied hair gel samples. The FDA, JSMO, and Egyptian Standards state that cosmetic tests should indicate that products contain only trace amounts of pathogenic microorganisms [26-29].

Cconclusion:

European regulations define cosmetic products as substances or combinations intended for use in contact with the external parts of the human body. Examples include leave-on products that remain on the skin after application and rinse-off products that are cleaned from the skin after being used for a brief time. Additionally, the regulations discuss one possible future.

Directives and permissible metal concentrations for cosmetics contain limits for heavy metal contamination, which is regulated by authorities around the globe. The objective is to protect consumers from potential health risks posed by heavy metal exposure. Evidence suggests that the consequences have been underestimated. Hair care products can include certain heavy metals in their formulations, which can accumulate in blood, urine, and hair, resulting in correlations with the concentrations of these metals. Reports of elevated levels of arsenic, lead, and cadmium in hair highlight the evident connection between heavy metal exposure and increased hair shedding.

Heavy metal intoxication, indicated by elevated levels in blood, urine, and hair, suggests systemic intoxication not only of these metals in the bloodstream but also throughout the body. The use of cosmetic products made from chemical substances requires that metals be processed and handled similarly to other bio-indicators like hair, teeth, skin, and nails.

The results of heavy metal analysis for Pb, Cd, Cr, Zn, and Mn in five hair gel cosmetic samples sold in Benghazi markets in Libya, using a flame atomic absorption spectrophotometer (FAAS), showed that the concentration of cadmium in the investigated hair gel samples was high, which could be a major public health hazard. Specifically, high levels of cadmium were found in three of the tested samples. In addition, our results indicate a need for establishing safe intake values for heavy metals in cosmetic products and emphasize the importance of their analysis before they reach consumers. Regarding microbial contamination screening, the results for the cosmetic products analyzed in this study showed no biological growth.

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