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Health Risk Assessment of Some Heavy Metals in Lipstick Brands Sold in Local Markets in Iraq

Fatima Mallalah Mohammed[1*], Maryam Abdulghafor Ahmed[2], Hadeel M. Oraibi[3]

1 Middle Technical University – Technical Institute – Baqubah – Department of Community Health Technique, Baghdad, Iraq.
2 Renewable Energy Sciences Department, College of Energy & Environmental Science, Al-Karkh University of Science, Baghdad, Iraq
3 Department of Biology, Collage of science, University of Diyala, Iraq.

Abstract: Heavy metals found in cosmetics are a safety threat to the health of consumers. Therefore, in this study, we evaluated the levels of heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), zinc (Zn), and arsenic (As) in lipstick makeup. The level of heavy metals in lipstick was discovered by using an analytical technique with high selectivity and sensitivity, namely atomic absorption spectrometry. Twenty lipsticks were selected from the same brands, yet differing in price. Ten original (expensive) and ten knockoff (cheap) lipsticks were chosen from shops in Diyala, Iraq. The detection-limit (LOD) was in between 0.01 and 0.1, the quantification-limit (LOQ) was within 0.03 and 0.33, the recovery values (Rec.%) ranged from 100.17% to 101.1%, the RE values were 0.81%, and the RSD values were 1.33%. The results also revealed that the levels of metals are in the order of Pb > Cd > Zn > Cu > As > Cr. However, the levels of heavy metals that were estimated in this study were less than the permissible limit set by the executive authorities, so there seems to be no concern associated with these heavy metals. However, the daily and frequent use of lipstick by women exposes them to low levels of toxic metals as these metals accumulate over time and pose adverse effects on the health of the users. The results of the hazard quotient (HQ) and health risk index (HI) indicate there was no harmful effect on human health related to heavy metals present in lipstick. Whereas the results of the biological activity of the samples indicated that there was no bacterial growth in expensive samples, cheap samples were contaminated with some types of organisms; this indicates poor quality.

Keywords: Cosmetics, heavy metals, toxicity effects, health risk assessment, biological activity.

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***Corresponding author. E-mail:** fm609426@gmail.com.

1. INTRODUCTION

Cosmetics are materials that are used as personal care products with the intention of coming into contact with the various outside parts of the human body like nails, hair system, epidermis, and mucous membranes of the oral cavity for cleaning, perfuming, beautifying, and changing their appearance, correcting bodily odors, and keeping the surface of the body in a good state (1). Many different products are marketed under the name “cosmetics” like shampoos, bath oils, baby products, perfumes, body lotions, personal hygiene products, and fingernail polish (2,3). To increase the elegance and glamour of ones look lipsticks are used (4). Recently, the use of lipstick has increased, resulting in their mass produce all over the world, and this industry is estimated to be in billions of dollars, with the global lipstick market expected to reach $13.4 billion by 2024 (5). Lipstick is composed of oils, waxes, dyes, moisturizers, and antioxidants. Oils make easy the application of lipstick and add glossy properties to its appearance, waxes give thermal stability to the lipstick; and dyes give multiple colors to the final product (6-8) with many other auxiliary components to enhance their desired effects depending on the manufacturers (7). Manyslipsticks have beenareported to contain many heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), and cobalt (Co) (6, 9). Either as basic ingredients or for functional reasons, they are added to cosmetics for functional reason; for example, Al, Au, Cu, and Ag are added for a wonderful metallic finish. As well , Fe, Cd, Ni, Cr, Cu, Zn, Ti, Al, Mn, Ba, and Pb, are used as dyes. However, their presence is limited to certain percentages set by the relevant authorities (10). They are considered to be just impurities in the product (11) as a result of contamination of metallic devices used during the manufacturing process, along with insufficient purification of raw materials (12,13). Thus, frequent daily use of lipstick contaminated with heavy metals is more dangerous due to direct oral ingestion (14). After that, the heavy metal ions get absorbed and form complexes with carboxylic acid (–COOH), amine (–NH_2), and thiol (–SH) of proteins; therefore, this leads to cellular malfunction or death and thus leads to a varietys of diseases (15,16). Several previous studies have been conducted to estimate some heavy metals in lipstick cosmetics, Pb is the most common metal among all other metals. Zakaria and Ho (17) found the concentration of lead in lipstick samples sold in Malaysian markets is 3.21 mg/g. Another study conducted in Saudi Arabia determined the levels of heavy metals in lipstick, the results of this study indicated the presence of high concentrations of Pb in local lipstick compared to other sources. Zainy et al. (18) found high levels of toxic metals in lipstick with dark colors compared to light colors. In addition, there are many other studies carried out to estimate the level of heavy metals in cosmetics (19-24). These studies use different techniques like UV-VIS & microfluidic Paper-based analytical device platform (µPADs) (25), inductively coupled plasma mass spectroscopy (ICP-MS) (26), inductively coupled plasma optical-emission spectroscopy (ICP-OES) (27), laser- induced breakdown spectroscopy (LIBS) (28), X-ray fluorescence (XRD) (29), graphite furnace atomic absorption spectrometry (GFAAS) (21), and including flame atomic absorption spectrometry (FAAS) (30) to evaluate the concentration of toxic metals in cosmetics. Table 1 shows a comparison between the proposed method (AAS) and other methods. Previous studies have successfully assessed the level of heavy metals in the original lipstick, however, no study has been conducted to evaluate and compare the levels of heavy metals in both the original and knockoff lipsticks sold in the local markets in Diyala/Iraq. Therefore, in this study, the level of some heavy metals in original and knockoff lipsticks was determined and compared and their health risk were assessed.

Table 1: The comparison between the proposed method (AAS) with other methods.

Proposed method (AAS)	**Other methods (ICP-MS, ICP-OES, LIBS, XRD, UV-Vis, µPADs, etc.)**
• Techniques based on atomic absorption spectrometry (AAS) are FAAS and GFAAS. It is an analytical technique widely used to determine the level of heavy metals in several types of samples, such as cosmetics, food, water, drugs, soil, and nanomaterials (31). • It has a very high sensitivity, so it can measure very low concentrations of up to 1000 ppm. • High selectivity and detection limit than other techniques, so that a particular element in the sample can be measured out of all the other elements present. • Accuracy in results. • It requires the use of a very small amount of sample, which reduce residue generation • Low spectral interference • Less contamination of samples. • Digestion time is shorter (32,33). • Quick nature.	▪ These analytical techniques (UV-Vis, µPADs, ICP-MS, ICP-OES, LIBS) are used to determine heavy metals in various samples, including cosmetics. • ICP-MS and ICP-OES: the advantages of these techniques are their wide linear range and low detection limits; the disadvantages of these techniques are the presence of spectral and other non-spectral interferences, and the method of sample digestion is very important in order to obtain samples with low carbon content and the least amount of suspended solids, in order to avoid clogging the nebulizer system and carbon deposition on the interface of the equipments (34). • LIBS and XRD are techniques for direct analysis of heavy metals in cosmetics without the need for a sample preparation step. However, these techniques have some disadvantages represented by spectral overlaps, poor accuracy, and high standard deviation values (35). • The UV-Vis and µPADs techniques need to perform color reactions, which means consuming a quantity of reagents and needing a longer time, and the results are less accurate compared to the atomic absorption technology. Also, the µPADs technique is not widely available in laboratories

	(36,37).
• One of the disadvantages of this technique is the overlap of some atomic lines, which can be reduced or overcome.	• Some of these techniques, such as ICP-OES, ICP-MS, and LIBS, require trained people to work on them in addition to being difficult procedures.

1.1 Toxicological Effects of Some Heavy Metals

Cosmetics (such as lipstick) are one of the most significant sources of toxic heavy metal release into the environment and the biological systems of humans compared with other sources (water, air, and food) (1,38). Due to the cumulative properties of these elements in the human body, they are a concern for consumers. Cd, Cr, Cu, Pb, Zn, and Ni are heavy metals that preoccupy the minds of lipstick users because of their negative effects on human health (39).

1.1.1. Cd

Cd is one of the heavy metals that is dangerous, and cadmium compounds are present in lipstick to give different pigments from yellow to deep orange (40,41). Cadmium selenide and cadmium sulfide are used for yellow and green colors, respectively, and can produce a wide range of colors when other metals are added (42). In general, in cosmetics, cadmium should not exceed the permissible limit of 3 ppm, the limit set by regulatory authorities (43). The exposure to cadmium through lipstick ingestion can cause severe stomach irritation, vomiting, low blood pressure, and diarrhea, while long-time exposure to low concentrations can cause bone deformation (the ability of bones to break easily because of calcium metabolism), hepatic damage, and renal damage (44).

1.1.2. Cr

Cr is an essential nutrient and plays a significant role in cholesterol and glucose metabolism (45). In contrast, chromium (VI) is a very toxic metal that spreads easily in the body and is considered a carcinogen for humans according to IARC (International Agency for Research on Cancer) (46, 47). Chromium (VI) is banned in cosmetic products, whereas Cu, $Cr(OH)_3$, and Cr_2O_3 are allowed as cosmetic colorants (48). According to the EPA the safe level of chromium in cosmetics is 1 ppm (49). When exposed to chromium (VI) by ingestion, it can cause problems with the kidneys, liver, and stomach (50).

1.1.3. Cu

Cu is an essential trace metal in the human body, and it is used in many industries, including the cosmetics industry (51). Copper is used in cosmetics as coloring pigments or to block UV rays (52). The permissible limit for copper in cosmetics when used as a color additive is 50 µg/g (53). However, it may have harmful effects at high levels (54). It may cause nose and throat irritation and even dermatitis. Chronic exposure to Cu can result in numerous physiological and behavioral disturbances, which include acute brain damage, cirrhosis of the liver, aggressive behavior, and hemolytic anemia, as well as psychiatric disturbances such as depression (11).

1.1.4. Pb

Like cadmium, lead is a heavy metal and one of the most toxic chemical pollutants in human history. It is used in lipstick to give a synthetic pearlescent pigment, which increases the shimmer effect of product (7). Lead in lipstick can be ingested when licking the lips, eating, or drinking. Thus, lead will accumulate in the body over time, even if the permissible limit does not exceed (10 to 20 ppm), the limits set by Health Canada and the US Food and Drug Administration. Pb is an impurity found in cosmetics (55). When excessive Pb accumulates in the human body, it can cause many harmful effects, including acute and chronic poisoning, pathological changes in organs, diseases of the cardiovascular system, kidney, bone, and liver, and even cancer (56).

1.1.5. Zn

Zinc is a metal of great importance to humans and is considered to have no significant toxicological effect. The presence of zinc in cosmetics has several possibilities depending on the type of compound; some zinc salts make it easier to apply cosmetics to the skin (57). However, the presence of an excess of Zn causes neurological diseases and gastrointestinal disorders (58). and can cause other health effects such as stomach cramps, vomiting, nausea, skin irritation, and anemia (55).

1.1.6 As

As is one of the most common heavy metals found in lipstick products, which are added as impurities (12). It can have many negative effects on human health, such as elevated blood pressure, melanosis, and gangrene (59). A combination of heavy metals like Hg, Pb, Cd, and As can generate synergistic effects that lead to dysfunction and cognitive damage (60). Chronic exposure to As can result in macrophage dysfunction due to impairments in the immune system. Ultraviolet light (UV) and arsenic are the major risk factors that contribute to squamous cell carcinoma and basal cell carcinoma (61). As a result of these health effects, the concentration of arsenic in cosmetics should not exceed 3 ppm (47).

This work aimed to determine the concentration of some heavy metals like Cd, Cr, Cu, Pb, Zn, and As in lipstick brands by using flame atomic absorption spectrophotometer (FAAS) technique, and the levels of heavy metals were compared to those of original and copied lipstick brands. Moreover, estimation of bacterial contamination and knowledge of the type of organism present in the lipsticks of original (expensive) and knockoff (cheap) brands sold in local markets in Diyala, Iraq.

2. METHODOLOGY

2.1. Collection of Samples

Twenty lipstick samples (ten originals and ten fake (knockoff) samples) were purchased from the local markets in Diyala, Iraq. The lipsticks used in this study have the same brands, characteristics, and colors, but they differ in price. The expensive (original) lipstick was imported from several countries (Ireland, Türkiye, China, France, USA), as written on the labels, while the cheap (knockoff) lipstick is mostly of the same origin. Some of them are of different origins, and some are of unknown origins. Table 2 lists information on expensive and cheap lipsticks.

Table 2. Information on the lipstick samples.

Knockoff lipstick (n = 10)			**Original lipstick (n = 10)**		
Origin	**Color**	**Samples code**	**Origin**	**Color**	**Samples code**
Unknown	Violet -Red	**1A**	Ireland	Violet -Red	**A1**
	Maroon	**2A**		**Maroon**	**A2**
Türkiye	Pink	**1B**	Türkiye	Pink	**B1**
	Brown	**2B**		**Brown**	**B2**
Unknown	Red	**1C**	China	Red	**C1**
	Chocolate	**2C**		**Chocolate**	**C2**
USA	Purple	**1D**	USA	Purple	**D1**
China	Orange	**1E**	China	Orange	**E1**
France	Pink	**1F**	France	Pink	**F1**
China	Red	**1G**	USA	Red	**G1**

2.2. Reagents and Chemicals

Samples were prepared by using analytical grade $HClO_4$ (purity 70%, Sigma Aldrich, USA) and concentrated HNO_3 (purity 69.5%, BDH, England). Also, all samples were diluted with distilled water.

2.3. Optimal Conditions of Digestion Method (hydrogen function, Temperature, Time of Heating, and Order of addition).

To verify the method used, the effects of some variables were studied: the effect of *hydrogen function*, temperature (50-250 °C), time of heating (15, 30, 60, and 120 minutes), and order of addition.

2.3.1. Hydrogen function influence

Acids play a key role in the digestion of cosmetic samples, especially HNO_3, which is used alone or with other acids. The main function of these acids is as oxidizing agents by which inorganic metal oxides can be dissolved in cosmetic samples (62). The preparation of solutions containing a mixture of concentrated acids $HClO_4$:HNO_3 of different proportions (1:1,1:2, 1:3,1:4, 2:1,3:1, and 4:1). The result of this step shows that 1:3 is optimal.

Influence of Heating Time: Heating the reaction components for different times (15, 30, 60, 120, and 150) minutes. 120 minutes were enough to complete the reaction.

2.3.2. Influence of temperature

In general, temperatures help to get rid of steam and organic impurities by converting organic carbon into carbon dioxide gas (CO_2). High temperatures cause the loss of heavy metals to be determined from the samples, while low temperatures cause incomplete oxidation of the materials. The reaction mixture was heated to different temperatures (50, 100, 150, 200, and 250 °C). The results of this step indicated that heating to 100 °C for two hours was good.

2.3.3. Order of addition

The change in reaction components' addition order; the sample (A) first, then the acid mixture (B), and finally distilled water (C) was the most appropriate. Figure 1 shows the results of the optimal conditions for the method used for the determination of heavy metals in lipsticks.

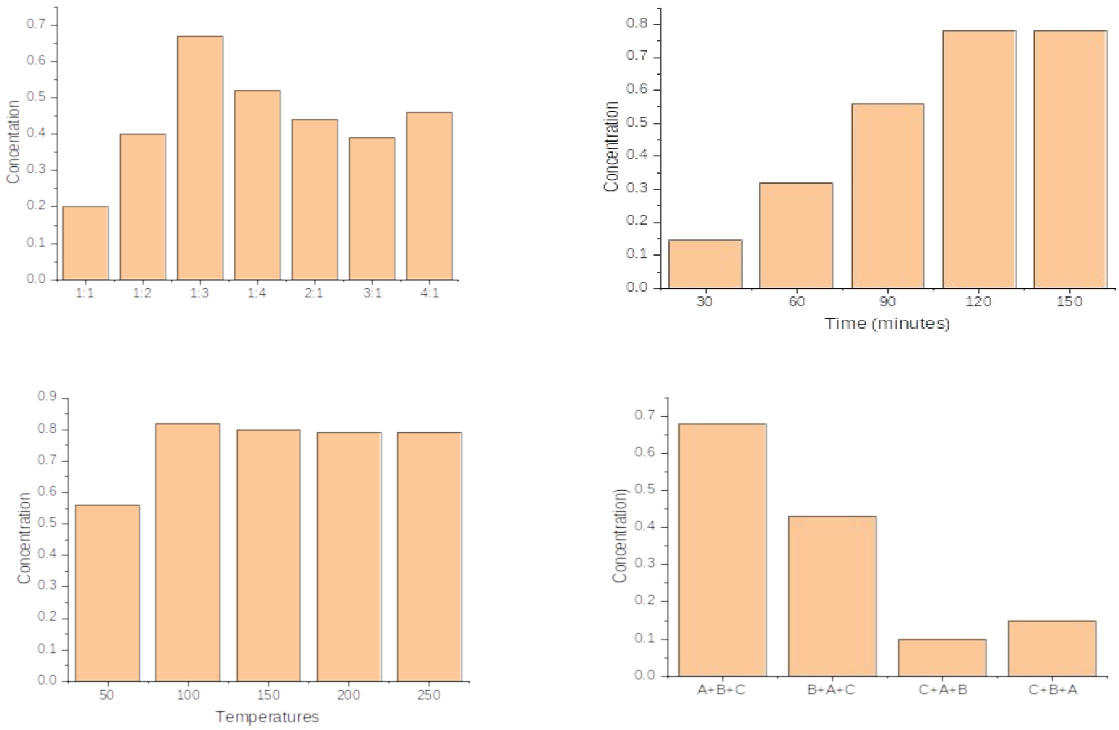


Figure 1. Influence of (a) hydrogen function (1:1,1:2, 1:3,1:4, 2:1,3:1, and 4:1), (b) temperature (50-250) °C, heating time (15, 30, 60, 120, and 150) minutes, and (d) order of addition ((A) lipstick sample, (B) acid mixture, (C) water).

2.4. Optimum Digestion Method of Samples

In this work, wet acidic digestion was used for the determination of a quantity of heavy metals like Cd, Cr, Cu, Pb, Zn, and As by the method adopted by Sani et al. (2016) (3). With some modifications, 0.5 g of each lipstick was weighed using an electronic balance and placed in a conical flask, followed by 5 mL of a concentrated acid mixture $HClO_4$:HNO_3 (1:3), which was then heated for 2 hours on a hot plate at 100 °C. Then 3 mL of the concentrated mixture was added, and the solution was heated again for two hours to complete the digestion process. The digested samples were cooled to room temperature and diluted to 25 mL with deionized water. The solution was filtered through filter paper (Whatman No. 41) to remove unwanted components, and the pure solution was used for metal determination.

2.5. Analysis of Heavy Metals by FAAS

The quantities of Cd, Cr, Cu, Pb, Zn, and As in lipstick samples were determined by using flame atomic absorption. Table 3 shows the operating parameters of AAS.

Table 3: Operating parameters of the Atomic Absorption Spectrophotometer(AAS) used in the heavy metal analysis.

Operating parameters	As	Zn	Pb	Cu	Cr	Cd
Wavelength (nm)	193.7	213.9	283.30	324.80	357.90	228.80
Slit width (nm)	0.50	0.70	0.70	0.70	0.70	0.70
Detection limit (mg/L)	0.05	0.03	0.08	0.005	0.04	0.01
Lamp current (mA)	5.00	5.00	5.00	1.50	2.00	2.00
Flame Type (Color)	Air-Acetylene /(lean/ Blue)	Air-Acetylene/ (lean/Blue)	Air-Acetylene/ (lean/Blue)	Air-Acetylene/ (lean/Blue)	Air-Acetylene/ (rich/ Yellow)	Air-Acetylene/ (lean/Blue)

2.6. Method Validation

2.6.1. Linearity

To ensure the reliability of the results. The quantification of the concentration of the metals was carried out using a five-point calibration curve for each of the metals used in the study. The calibration was accomplished by adding the standards prepared in concentrations of 1, 5, 10, 25, and 50 ppm from the 1000 ppm standard stock solution. The analytical procedure validation for quantitative analysis of heavy metals in cosmetics products was performed using linear ranges, detection limit, quantification limit, precision, accuracy, and recoveries of spiked standards in the defined calibration ranges were calculated. Analytical method validation for heavy metals analysis was applied in-accordance with Eurachem guide.

2.6.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection was estimated as the mean plus three times the standard deviation (SD) of a blank sample, and it was determined following the equation below:

$$LOD=3\frac{SD}{b} \quad \text{(Eq. 1)}$$

$$LOQ=10\frac{SD}{b} \quad \text{(Eq. 2)}$$

where, SD is the standard deviation- of the blank (based on three independent analyses of sample blank). b is the calibration-graph slope (21).

2.6.3. Accuracy

Because of the unavailability of certified material for lipstick, the accuracy of the method was obtained by adding the true values of Cd, Cr, Cu, Pb, Zn, and As to the cosmetics matrix. The same analytical procedure was applied for test samples, and the percentages of relative error (%RE) and analyte percentage relative recovery (%Recovery) were calculated by the following equation:

$$\%RE=\left(\frac{found-true}{true}\right)\times 100 \quad \text{(Eq. 3)}$$

$$\%Recovery=\left(\frac{found}{true}\right)\times 100 \quad \text{(Eq. 4)}$$

found:- result value, true:- real value.

The analytical recovery figures for spiked lipstick are shown in Table 4.

2.6.4. Precision

The precision of method was determined by relative standard deviation (%RSD). It is studied by using three concentrations of heavy metals, which are calculated using the following equation:

$$\%RSD=\left(\frac{SD}{x}\right)\times 100 \quad \text{(Eq. 5)}$$

$$SD=\left[\sum\frac{(x_i-x)^2}{(n-1)}\right]^{0.5}, x=\sum\frac{x_i}{n} \quad \text{(Eq. 6)}$$

Where SD: standard deviation, x: the average of the samples.

2.7. Health Risk Assessment

Exposure routes: A lipstick may enter a human body through ingestion; this intake can be calculated using the following equation:

$$\boldsymbol{ADDing=\frac{C\times IR\times EF\times ED}{BW\times AT\times C}F} \quad \text{(Eq. 7)}$$

ADDing is the average ingested daily dose (mg/kg day); C is the concentration of heavy metals; IR is the intake rate (40 mg/day); EF is the exposure frequency (260 days/years); ED is the exposure duration (35 years); BW is the body weight (57.9 kg); AT is the average time (calculated by ED × 365 days/year); and CF is the conversion factor (10^{-3}).

After calculating6the average daily dose, the hazard quotient-(HQ) for non-carcinogenic health effects was calculated using the following formula:

$$HQ=\frac{ADDing}{Rf\ \ D} \quad \text{(Eq. 8)}$$

Where, R*f* D (Reference Oral Dose) is the specific reference dose (mg/kg/day) that varies for all metals (17), R*f* Ds used for the hazard assessment were 0.001 for Cd, 0.003 for Cr, 0.04 for Cu, 0.004 for Pb, 0.3 for Zn, And 0.001 for As (24, 63).

When the ADDing value is less than the R*f* D, there will be no health effect, but if the ADDing value is greater than the R*f* D, there may be noncarcinogenic health effects.

HQ < 1 indicates no adverse health effects, while HQ ≥ 1 indicates likely adverse health effects. The health risk index (HI) was used to calculate the total risk effect of all the elements studied. This index was calculated using the following formula:

$$\boldsymbol{HI=\sum HQ} \quad \text{(Eq. 9)}$$

$$\boldsymbol{HI=\sum HQ=HQCd+HQCr+HQCu+HQPb+HQZn+HQAs}$$

2.8. Study of Biological Activity

To detect the antibacterial activity, two groups of lipstick samples were used, the first group consisted of 10 samples of the original (expensive), and the second group also used 10 samples of knockoff (cheap). In this study, a range of different gram-negative and gram-positive bacteria were selected. The isolates of gram-negative bacteria included (*E.coli, Pseudomonas aeruginosa)* while gram-positive bacterial include *(Staphylococcus aureus, Staphylococcus epidermidis*) isolate. The microbial activity of different lipsticks was determined by the (agar, well, diffusion) method (64). In this method, growth was cultivated on a Muller-Hinton Agar plate (HiMedia, Mumbai, India). After the plates had dried, one well was drilled into each of the agar plates using a sterile cork borer with a diameter of 5.0 mm. A micropipette was used to dispense 80 μL of lipstick suspension from each sample into each well of a Muller-Hinton agar plate. After standing for at least an hour to allow pre-diffusion to occur, the plates were incubated for 48 hours at 37 °C. In millimeters, the zone of inhibition was measured. Three duplicates of each experiment were carried out.

2.8.1. Statistical analysis

All results are expressed as the mean±SD, and all data were analyzed by t- test, which was used for general comparison between two groups of lipstick using SPSS software.

3. RESULTS AND DISCUSSION

The analytical producer validation for quantitative analysis of heavy metals in cosmetics products was performed using linear ranges, ranging from 1 to 50 ppm, coefficients of correlation ranged from 0.9983 to 0.9995, LOD ranged from (0.01 to 0.1), and LOQ was set from(0.03 to 0.33), which indicates a selective and sensitive method. Table 4 shows the results obtained in this study.

Table 4: Method validation results of the heavy metals studies.

LOQ (ppm)	LOD (ppm)	R^2	Linear range	Regression Equation	Metals
0.33	0.1	0.9983	1-50	y = 0.0293x + 0.1421	**Cd**
0.1	0.03	0.9985	1-50	y = 0.0243x + 0.0199	**Cr**
0.17	0.05	0.9989	1-50	y = 0.0261x + 0.0354	**Cu**
0.33	0.1	0.9995	1-50	y = 0.0299x + 0.0431	**Pb**
0.07	0.02	0.9979	1-50	y = 0.0244x + 0.0151	**Zn**
0.03	0.01	0.9992	1-50	y = 0.0179x + 0.0778	**As**

For accuracy and precision, spiking was performed using the calibration-standard solution in three fortification levels (1, 5, and 25 ppm) of the linear range as the sample matrix for accuracy and precision calculations, using Equations 3, 4, and 5 to calculate the values of percentage of relative error, percentage of recovery, and relative standard deviation. The results (Table 5) were presented as mean data, indicating the recovery values (from 100.17% to 101.1%), RE values of 0.81%, and RSD values of ≤ 1.33 %, confirming good accuracy and precision**.**

Table 5: Accuracy and precision of studied metals.

*(%RSD)	*(%RE)	*(% Recovery)			Metals
		25 ppm	5 ppm	1 ppm	
1.32	0.71	100.23 ±0.67	101.2±1.76	100.7±1.53	**Cd**
1.21	0.46	100.06±0.38	100.4±2.11	100.9±1.15	**Cr**
1.1	0.54	100.57±0.70	100.87±2.05	100.17±0.55	**Cu**
0.78	0.58	100.3±0.36	100.5±0.71	100.43±1.55	**Pb**
1.33	0.53	99.7±1.11	100.8±1.23	101.1±1.66	**Zn**
0.65	0.81	101.43±0.76	100.7±0.72	100.3±0.46	**As**

*Average of three replicates.

3.1. Concentration of Heavy Metals in Lipstick Samples

In this study, twenty samples of different brands of lipstick were investigated. The concentration of each heavy metal in the lipstick samples is given in Table 6.

The acceptable limit of heavy metal content in cosmetics was set by (USFDA) in 2007, in particular 3 ppm for cadmium, 1 ppm for chromium, 50 mg/g for copper, 10 ppm for lead, and 3 ppm for arsenic. Based on Table 3, the concentrations of Cd in the studied brands of lipstick were from 0.11 to 0.56 ppm in the original brand and 0.21 to 0.88 in knockoff brand; these values did not exceed the legal threshold of 3 ppm set by the US Food and Drug Administration and Health Canada (41).

In a study reported by Saleh et al. to determine the level of cadmium in lipsticks sold at different prices, the results showed that the level of cadmium ranged from 0.03 to 0.07 ppm (41). Previous studies have shown different concentrations of cadmium in lipstick samples. Nourmoradi et al. detected that the cadmium concentration in some brands of lipstick was within the range of 4.08 to 60.20 mg/g (1). Another study reported by Iwegbue et al. found that the concentration of cadmium ranged between 0.34 and 37.3 (46), 0.01 to 0.06 (65), and 0.77 to 1.19 (66). A study in Jordan found the levels of Cd ranged from 0.12 to 2.72 (67). However, the level of cadmium in the present study was lower than that in other studies conducted by Nourmoradi et al. and Iwegbue et al. (1, 42).

The concentration of Cr in the original and knockoff brands of lipstick samples was not detected. This is consistent with the European Union's prohibition on the presence of chromium in cosmetics (17). Different studies in many countries have shown different ranges for chromium in lipstick. In Iran, Naalband et al. found the concentration of Cr in lipstick ranged between 0.06 and 0.75 (40). In Malaysia, Zakaria et al. found that the Cr ranged between 0.24 and 2.25 (17). In Portugal and Brazil, Pinto et al. reported that the concentration of Cr ranged between 2.26 and 2.28 (68). In another study conducted by Sani et al., the researchers determined the levels of chromium in lipsticks with varying prices. The results showed the levels of chromium ranged from 0 to 0.05 ppm (3). The concentration values of Cr obtained in this study were lower than the values in the other study mentioned above.

Table 6: Concentrations (ppm) of heavy metals in original (expensive) and knockoff (cheap) lipsticks.

	Original			Knockoff		
Metals	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**Min**	**Max**
Cd	0.34±0.23	0.11	0.56	0.55±0.18	0.26	0.88
Cr	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Cu	0.12±0.07	0.014	0.28	0.22±0.13	0.06	0.39
Pb	0.68±0.19	0.5	1.06	0.98±0.17	0.76	1.26
Zn	0.19±0.18	0.026	0.5	0.33± 0.31	0.067	0.77
As	0.04±0.03	0.012	0.098	0.1±0.07	0.034	0.28

*values are expressed as an, average for, three determinations.

The concentration of copper (Cu) in the original and knockoff brands of lipstick ranged from 0.014 to 0.28 and 0.06 to 0.39, respectively. In other studies, the levels of copper in lipstick have been reported. For example, researchers found the level of copper in some brands of lipstick ranged between 0.0 and 75.92 (57). Iwegbue CM et al. conducted a study to estimate several heavy metals, like copper (Cu), in 160 samples of facial cosmetics, including lipstick, sold in southern Nigeria. The results showed that the concentration of copper ranged from 1.1 to 135.4 ppm (42). A study published by Chauhan SB et al. found that the concentration of Cu in lipstick ranged between 0.0498 and 7.0782 (69), as well as 1.86 and 21.72 (24). The results showed that the level of this heavy metal in the current study was comparable to that found in the study conducted by Chandak et al. (2014), while the levels of Cu in this study were less than the values obtained in other studies (24,42,57). However, the results show that the concentration of copper was less than (50 mg/g), the standard allowed by the regulatory authorities for copper in cosmetics (6).

Pb is found naturally in the earth's crust. Lead was detected in all brands of lipstick used in this study; the levels of Pb ranged from 0.5 to 1.06 ppm in the original brand and 0.76 to 1.26 ppm in the knockoff brand. Pb levels in lipstick were measured in ppm in various studies. In a study conducted by Nnorom in 2005, the levels of Pb ranged between 87 and 123 ppm (50); in 2012, they ranged between 5.5 and 47.8 (66); and in 2013, they ranged between 0.58 and 3.36 (70). In 2016, they ranged between 0.18 and 0.8 (40), and in 2020, they ranged between 0.286 and 6.234 (24). The observed levels of Pb in this study are similar to those reported in this study by Nourmoradi et al. (2013), and Kamarehie et al. (2020), but they are lower than those in the previous studies. The lead values used in this study, however, did not exceed the permissible limit for lead as an impurity in cosmetics of 20 ppm and 10 ppm, respectively, as set by the US Food and Drug Administration and Health Canada (71).

Zn was used as a pigment in cosmetics; the average concentration of Zn in the studied brands ranged between 0.0026 and 0.5 ppm, 0.067 and 0.77 ppm, and the means were 0.19 and 0.33, in the original and knockoff brands, respectively. In other countries, different ranges of Zn in lipstick brands have been reported. For example, in a study from Iran, Ghaderpoori, M. et al. reported concentrations ranging between 3.64 and 216.53 ppm (72). In Pakistan, a range between 0.4757 and 6.7694 ppm was reported by Kamarehie et al. 2020 (73). In Khyber, the range was between 0.696 and 1.610 ppm (15). In Nigeria, the level of Zn ranged from 2.23 to 3.01 ppm was reported by Okol et al(6). In Poland, it ranged from 1.73 to 488.13 ppm (57). When the Zn range values in this study were compared to other studies in the aforementioned countries, the level of Zn in this study was found to be similar to those in the Khyber City study reported by Ullah et al., while being lower than the other studies mentioned above. However, the mean concentrations of Zn were higher than the LOD.

The concentration of As in different brands of lipstick used in this study ranged from 0.012 to 0.098 and 0.034 to 0.28 ppm. Different studies conducted in different times reported the levels of As in lipstick. In 2014, Ouremi et al. reported levels of As ranging between 0.8 and 3.0 ppm (47). In 2015, they ranged from 0.11 to 0.43 ppm (74). In 2019, it ranged from 0.990 to 9.235 ppm (21). In 2020, it ranged 0.29 to 4.83 ppm (75). The level of As in the present study was lower than in the above mentioned studies. The level of arsenic in the studied samples was less than (3 ppm), which is the permissible limit for As in cosmetics, according to Health Canada (76).

In this study, the amount of heavy metals was less than the limit allowed by the executive authorities, and in addition, the original brand of lipstick gave a better result than the imitation. When comparing the results of this study with those of previous studies, it was found that the amount of heavy metals in the present study was similar to the values reported in the literature for lipstick products. The concentrations of the heavy metals analyzed are in the following order: Pb > Cd > Zn > Cu > As > Cr. Lead has the highest concentration, while chromium has the least (25). The results in Table 4 confirm the proposed method's accuracy and precision in heavy metals' determination. A t-test was conducted to compare expensive and cheap lipstick products. The results of the test showed that there were no statistically significant differences in the concentrations of heavy metals. Among the expensive and cheap cosmetics.

3.2. Risk Assessment

Heavy metals in cosmetics may seem like a small proportion of the sources that threaten human health in comparison to air, food, and water. Therefore, its harmful effects must be avoided. In this part of the study, to determine the non-carcinogenicity risk of contact with cosmetics products, the hazardous quotient (HQ) and hazardous index (HI) were estimated from Equations 7 and 8. Table 8 shows the results for HQ and HI as calculated. According to Table 8, the amount of HQ in all lipstick brands examined was below 1, indicating there was no significant non-carcinogenic health risk for lipstick users. The highest amount of HQ was found in lead (6.3E-01), which was observed in the sample (1B), but the lowest amount of HQ was found in zinc (4.3E-05), which was detected in the sample (A1). According to HI, the amount of HI for all samples used in our study was lower than 1. This indicates that the consumer was at the safety limit.

3.3. Anti-bacterial Activity

The results indicated that there was no bacterial growth in the original brand of lipstick, and this is in accordance with the laws of the US Food and Drug Administration. While in the case of knockoff brands (1A, 2A, 1B, 2B, E, 1C, 2C, 1D, 1E, 1F, and 1G), these samples showed the most antimicrobial effects on gram-negative and gram-positive bacterial isolates. This contradicts what the US Food and Drug Administration law stipulates that "cosmetics are not required to be sterile, but rather, they must not be contaminated with microorganisms that may cause disease, and cosmetics are required to remain in this state even when used before consumers" (77). Figure 2, shows the anti-bacterial activity in knockoff lipstick samples.

These results are in agreement with many studies. According to a recent study conducted by Siya using thermal sequencing analysis, on 20 lipstick samples, the results indicated that the samples were contaminated with 105 bacterial genera, including Streptococcus, *Staphylococcus*, *Pseudomonas,* and *Escherichia* (78). Another study reported by Vassoler, M., et al. analyzed the microbiological quality of 30 lipstick samples sold in Brazil; the results indicated the presence of bacterial contamination with different types of bacteria, including *S. aureus* and *S. epidermidis* (79).

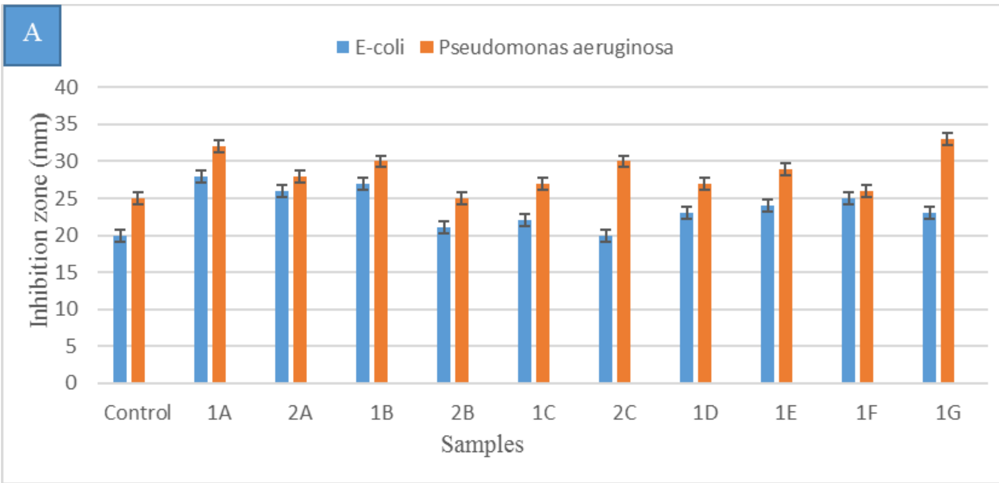
Table 7: Estimated adding of selected metals found in the original and knockoff lipstick brands.

Original Lipstick						
Code	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**	**As**
A1	1.6E-04	0	7.1E-06	4.3E-04	1.3E-05	1.1E-05
A2	2.8E-04	0	5.2E-05	3.8E-04	4.6E-05	1.3E-05
B1	1.1E-04	0	5.5E-05	2.5E-04	4.3E-05	1.5E-05
B2	2.2E-04	0	4.3E-05	2.7E-04	1.5E-05	1.4E-05
C1	1.5E-04	0	5.3E-05	4.1E-04	2.2E-05	4.5E-05
C2	2.2E-04	0	5.7E-05	3.3E-04	1.2E-04	2.4E-05
D1	2.8E-04	0	1.4E-04	3.0E-04	2.2E-04	1.9E-05
E1	2.3E-04	0	6.3E-05	2.5E-04	4.1E-05	0.5E-05
F1	1.5E-04	0	7.3E-05	2.4E-04	1.6E-04	4.9E-05
G1	5.3E-05	0	4.3E-05	5.3E-04	2.5E-04	2.4E-05

Knockoff Lipstick						
Code	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**	**As**
1A	2.3E-04	0	3.1E-05	5.1E-04	3.3E-05	2.1E-05
2A	3.3E-04	0	9.3E-05	4.4E-04	1.6E-04	2.4E-05
1B	1.9E-04	0	1.5E-04	6.3E-04	4.2E-05	2.5E-05
2B	3.4E-04	0	4.6E-05	4.1E-04	6.1E-05	5.4E-05
1C	3.1E-04	0	2.3E-04	4.6E-04	5.2E-05	5.5E-05
2C	1.3E-04	0	1.1E-04	5.3E-04	1.3E-04	5.4E-05
1D	4.4E-04	0	1.9E-04	4.7E-04	2.9E-04	3.1E-05
1E	3.4E-04	0	6.8E-05	3.8E-04	4.8E-05	1.7E-05
1F	2.3E-04	0	8.3E-05	3.2E-04	4.4E-04	1.4E-04
1G	2.2E-04	0	8.4E-05	6.2E-04	3.5E-04	5.4E-05

Table 8:- Health risk assessment of heavy metals (HQ and HI) in original and knockoff lipstick brands.

Original Lipstick							
Code	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**	**As**	**Total HQ or HI**
A1	0.16	ND	0.0002	0.43	0.000043	0.011	0.601
A2	0.28	ND	0.0013	0.38	0.00015	0.013	0.674
B1	0.11	ND	0.0014	0.25	0.00014	0.015	0.372
B2	0.22	ND	0.0011	0.27	0.00005	0.014	0.505
C1	0.15	ND	0.0013	0.41	0.00007	0.045	0.606
C2	0.09	ND	0.0014	0.33	0.00004	0.024	0.445
D1	0.28	ND	0.0035	0.30	0.00073	0.019	0.603
E1	0.23	ND	0.0015	0.25	0.00014	0.005	0.487
F1	0.15	ND	0.0018	0.24	0.00053	0.049	0.441
G1	0.053	ND	0.0011	0.53	0.00083	0.024	0.609
Knockoff Lipstick							
1A	0.23	ND	0.0008	0.51	0.00011	0.021	0.762
2A	0.33	ND	0.0023	0.44	0.00053	0.024	0.797
1B	0.19	ND	0.0038	0.63	0.00014	0.025	0.849
2B	0.34	ND	0.0012	0.41	0.00020	0.054	0.805
1C	0.31	ND	0.0058	0.46	0.00017	0.055	0.831
2C	0.13	ND	0.0028	0.53	0.00043	0.054	0.717
1D	0.44	ND	0.0048	0.47	0.00097	0.031	0.947
1E	0.34	ND	0.0017	0.38	0.00016	0.017	0.739
1F	0.23	ND	0.0021	0.32	0.00147	0.14	0.694
1G	0.22	ND	0.0021	0.62	0.00117	0.054	0.897



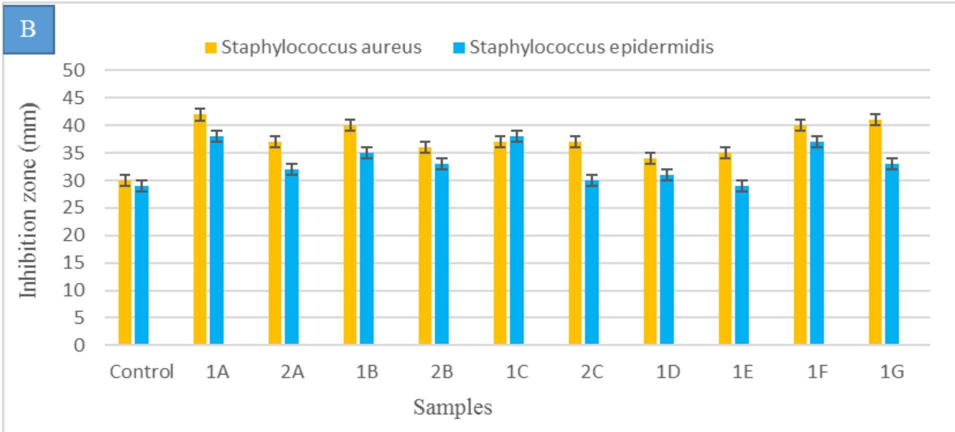


Figure 2: Antimicrobial-activity of knockoff lipstick samples. (A) Inhibition of different gram-negative bacteria isolates by lipsticks samples, (B) Inhibition of different gram-positive bacteria isolates by lipstick samples.

4. CONCLUSION

The primary components present in lipstick are oils, waxes, dyes, and alcohols. Even though heavy metals are not an essential component of lipsticks, they are in cosmetics as impurities. And even with good manufacturing processes, the presence of heavy metals is unavoidable in cosmetics. Therefore, the executive authorities have set values for heavy metals in cosmetics that should not be exceeded. FAAS is a simple and rapid analytical method used to estimate the quantity of cadmium, chromium, copper, lead, zinc, and arsenic in two groups of lipstick samples used in this study. The results showed that the samples used in this study contained all the metals that were analyzed. Except for chromium, which was not detected in all the studied samples, whether expensive or cheap, while the levels of lead were higher than all other metals, they were less than the permissible limit set by the executive authorities. The results of the THQ showed that the values were below 1 for all tested samples, meaning that the detected heavy metals in lipstick did not pose a dangerous health risk to consumers, and the original brand of lipstick showed better results than knockoff brands. However, there are concerns that daily and repeated use of cosmetics contaminated with toxic heavy metals may lead to biological accumulation in body tissues and cause negative health effects on consumers. The results of the biological activity show that there was no bacterial growth in the original lipstick samples while there was contamination with organisms in the cheap samples.

5. CONFLICT OF INTEREST

The researchers declare that there are no conflicts of interest regarding the current manuscript.

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