

DETECTION AND SAFETY AWARENESS OF HEAVY METALS IN COSMETIC PRODUCTS FREQUENTLY USED IN SAUDI ARABIASamia S. Barghash^{*1}, Hatem Abdel Moniem Ahmed² and Modhi K. Al-Baker³¹Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine for Girls Al- Azhar University, Cairo, Egypt.²Department of Forensic Chemistry, College of Forensic Sciences, Naif Arab University for Security Sciences, Riyadh, Saudi Arabia.³College of pharmacy, Qassim University.***Corresponding Author: Dr. Samia S. Barghash**

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ABSTRACT

Background: Heavy metals contamination in cosmetic products is becoming an important health problem in both worldwide and locally at the level of the Kingdom of Saudi Arabia (KSA). The aim of this study was to detect the concentration of heavy metals in the most commonly used eyeliners purchased in Saudi Arabia. Also, to assess safety awareness of toxic elements of most concern heavy metals in cosmetics. **Methods:** A cross-sectional survey was designed and distributed electronically in Saudi Arabia in November, 2015. The questionnaire was conducted on 769 Saudi women aged from 15-60 years old that is using cosmetic products. Secondly, 10 samples from 10 different types of eyeliners frequently used in Saudi Arabia were digested. The digested samples were analyzed for lead, cadmium and mercury using graphite furnace - atomic absorption spectrometry (GF-AAS). **Results:** The majority of cosmetic types used by participants were eye-liner "kohl" (73.9%). About (34.59 %) of participants were not aware about heavy metals contents in cosmetics. The heavy metals, cadmium, lead and mercury were detected in all samples with varying concentrations. The level of lead was the highest. There was a significant difference of cadmium, lead and mercury concentration $p < (0.05)$ in different brands of eyeliner. There was statistically significant difference between cadmium and lead also between mercury and lead $p < 0.05$ of different eyeliner brands. **Conclusion:** It was found that the majority of participants expected that expensive cosmetics are safe and they preferred international brands which is not necessary to be "safe".

KEYWORDS: Cosmetics, Heavy metals, Eyeliner "kohl", Lead, Cadmium, Mercury.**1. INTRODUCTION**

Cosmetics are products applied to the body for the purpose of cleaning, beautifying or improving appearance.^[1] One of the Ancient Egyptians wide ranges of make-up tools is Kohl (Sormeh), which was used to outline the eyes. Kohl is made up of lead, copper, burned almonds, soot and other ingredients.^[2] In Nigeria very high level of trace metals was reported in locally produced facial makeup.^[3] Some of the cosmetics used contain varying components elemental silicon or talc hematite, organic compounds and even heavy metals such as lead.^[4] The skin of the eyelid is the most susceptible to eczemas, irritant and allergic contact dermatitis. Heavy metal contamination is one of the important reasons behind the same problem.^[5] Main human problems that occurred due to lead toxicity are stomach pain, unconsciousness, anemia, infertility; nervous system disorders.^[6] Heavy metals (e.g., lead) can be absorbed by children's and women's skin through using cosmetic products.^[7] Kohl, a type of customary

cosmetic product used for eyeliner in the Middle East, contains more than 50% of lead.^[8] The traditional eye cosmetic to be put around the eyes is commonly known as kohl. Other names may be used such as Kajal, al-Kohl or Surma. In Western cultures, the name eye liner may be more common, although names as kohl and Kajal often are included in the product name.^[9] Kohl is often mixed with other chemical substances and is applied to eyebrows, skin area around the eyes.^[10] There are some reports for the determination of heavy metals in cosmetic samples. For example, cheaper brands of lipsticks and eye shadows imported from countries with poor safety, regulatory and manufacturing practice, but sold in riyal stores in Saudi Arabia were analyzed. Lead was found in the range 0.42-58.7 ppm for eye shadow.^[11] Cadmium is present in many cosmetics products but mostly present in lipsticks and face powders. It is used as a color pigment in many cosmetics industries.^[12] The cardiovascular system is also affected by the low level of cadmium exposure. Diabetes

and hypertension are also associated with its exposure.^[13] There are currently no international standards for impurities in cosmetics. Cosmetic products and ingredients are not subject to FDA premarket approval authority. The aim of this study was to detect the concentration of heavy metals in the most commonly used eyeliners (kohl) purchased among females in Saudi Arabia, and safty awareness of toxic elements of most concern heavy metals in cosmetics.

2. MATERIAL AND METHODS

A cross-sectional survey was designed and distributed electronically in Saudi Arabia in November; 2015. The questionnaire was conducted on 769 Saudi women aged from 15-60 years old that is using cosmetic products.

2.1. Chemicals and reagents

All reagents must be of analytical grade (Nitric acid, Hydrochloric acid, Hydrogen peroxide 30% v/v, Reductant: For Hg either, 1.1 % w/v stannous chloride in 3%v/v hydrochloric acid or 0.2 % w/v sodium borohydride in 0.05% sodium hydroxide, 50% w/v Magnesium nitrate, Deionized water, resistivity 18.2 Mohm). Standard calibration solutions: Cd, Pb and Hg standard stock solutions conc. 1000 g/ml. Modifier for Pb and Cd: Mix 1:1 of 0.2% w/v Mg (NO₃) 2.6H₂O in 0.5% v/v nitric acid and 0.2 % w/v NH₄H₂PO₄ in 0.5% v/v nitric acid.

2.2. Instruments

Microwave Digestion – System, High Performance from (ETHOS ONE), Atomic Absorption Spectrometer 240FS AA, from Agilent Technologies with (Graphite Furnace) GTA 120 (PSD 120 Programmable Sample Dispenser, and carrier gas was Argon.

2.3. Sample collection

A total of 10 samples of 10 different types of eyeliners (kohl) of moderate price frequently used among females in Saudi Arabia were used in this study for detection of lead, cadmium and mercury contents. Commonly used kohl products will be purchased from superstores, open markets.

2.4. Digestion Method

The digestions of organic material in the samples carried out using microwave digestion device and determine the concentration of cadmium and leads by using graphite furnace - atomic absorption spectrometry (GF- AAS). Weight 0.25 grams of the sample into the tube (50 ml) of high pressure resistance microwave Teflon vessels. Then add 8 ml of concentrated nitric acid and 1 ml (30%) of hydrogen peroxide using a pipette and 1 ml of hydrofluoric acid. After cooling at room temperature, added 20 ml of deionized water to digest sample. Then filtering the solution through a filter paper, then transfer the solution to a 50 ml volumetric flask and dilute the solution to the mark with deionized water.

2.5. Preparation of standard stock solutions and working standards

Stock solutions were prepared from which working standards were freshly prepared by serial dilution. The stock solutions of mercury and chromium were obtained already prepared. Five serial standards of each element were prepared for the calibration. The final acid concentration was maintained at about 1% during serial dilution and subsequent dilution of stock solutions to keep the metal in a free ion state appropriate weighing of metals was done prior to dissolving them in acids to make 1000 ppm of stock solutions. Serial standard solutions were prepared in the following ranges in ppm ; Hg (5, 10, 20, 30, 40 mg/L), Pb (10, 20, 30, 40, 50 µg/L), Cd (0.5, 1, 1.5, 2, 2.5 µg/L). The serial standards were aspirated into the instruments. The absorbance was plotted against their concentrations to obtain calibration curves. The correlation coefficients were calculated to and used to express the performance of the instrument.

2.6. Cosmetic Samples Digestion Protocol

According to ASEAN method (ASEAN Association of South East Asian Nations). The digested samples were aspirated in triplicates with regularly intercepts of standards to maintain a check on the instrument stability. Air/Acetylene flame and oxidant flow of 4.51/min was used for Pb, Cd while for Hg; N₂O/Acetylene flame was used.

2.7. Statistical analysis

Data will be collected, tabulated and analyzed using the SPSS Version 21.0. To find out that there is a statistically significant difference between the concentrations of the elements, we applied One Way ANOVA. The concentrations of three elements: (cadmium, lead, mercury) entered as dependent variables and different eyeliner samples entered as independent variable, the test applied on the concentrations of elements in different kohl samples. Multiple comparisons with post hoc test, that conducted because we found a statistically significant result in the ANOVA test.

3. RESULTS

From 769 women who completed the questionnaire. The range of participants age 15-60 (mean ± S.D = 31±12). The majority of them (41.6 %) were employees. The greatest proportion of the participants (43.2%) were in the age group of 20-30 years, and 60.7% of participants were single (Table1).

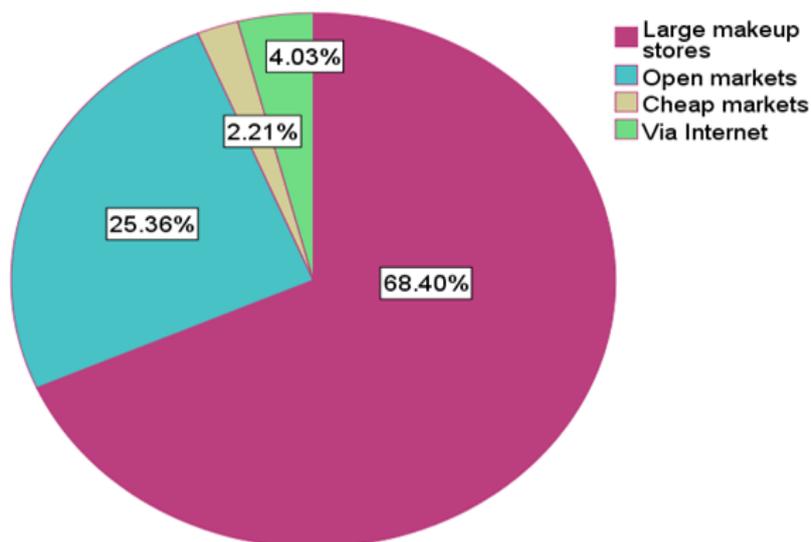
Table 1: Demographic characteristics of participants (N= 769).

Items	Frequency	Percent (%)
Age		
• < 20 years	84	10.9
• 20-30 years	332	43.2
• 30-40years	187	24.3
• > 40 years	166	24.3
Total	769	100.0
Employment Status		
• Student	228	29.6
• Student and Part-Time employee	9	1.2
• Employee	320	41.6
• Housewife	212	27.6
Total	769	100.0
Marital status		
• Married	467	60.7
• Single	302	39.3
Total	769	100.0

Results are expressed by number N, and percentage (%).

Most of the participants (68.40%) were purchased cosmetics from large makeup stores, while the lowest

percentage (4.03%) was of participants purchased via internet (Figure 1).

**Figure 1: The most preferred places of purchasing cosmetics among participants.**

The highest percentages of participants (38.9%), (34.2%) were affected by recommendation from friends and type of brand respectively (Table 2).

Table 2: Important factors affect the participant's decision while buying cosmetics products.

Factors	Frequency	Percent (%)
Price	207	26.9
Recommendation from friends or relatives	299	38.9
Type of brand	263	34.2
Total	769	100.0

Results are expressed by number N, and percentage (%)

The highest percentage (52.93%) of participants expected that expensive cosmetics products are safe and free from heavy metal (Figure 2).

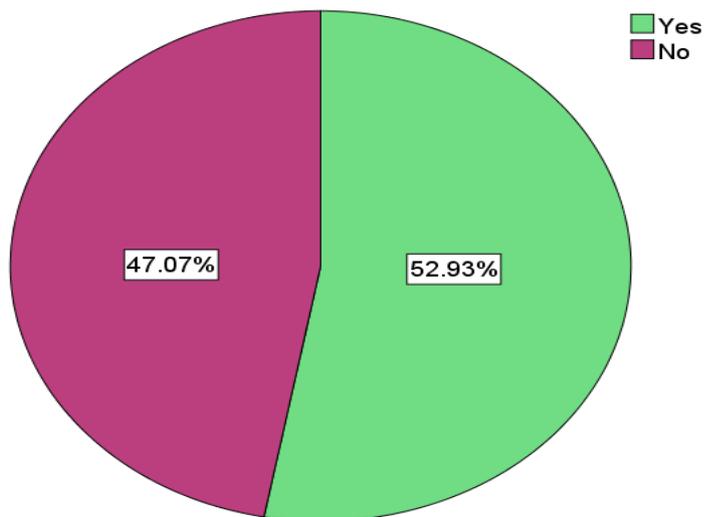


Figure 2: The safety of usage of expensive cosmetics among participants.

The highest percentages of participants (34.7% & 31.2%) were used cosmetics daily and two or three times per week respectively (Table 3).

Table 3: The number of times of cosmetics use per month among participants.

Number of times per month	Frequency	Percent (%)
Daily	267	34.7
Two to three times per week	240	31.2
Once per week	131	17.0
Two to three times per month	79	10.3
Once per month	52	6.8
Total	769	100.0

Results are expressed by number N, and percentage (%).

Most of the participants (65.41%) were aware about cosmetics ingredients which may contain heavy metals (Figure 3).

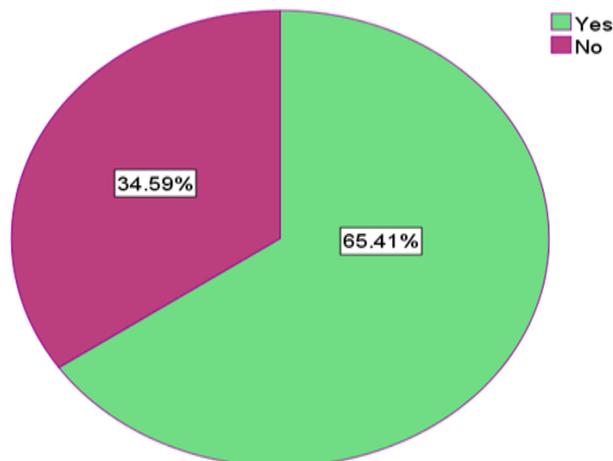


Figure 3: Awareness of participants about cosmetics which may contain heavy metals.

We noticed that the majority of participants (63.8%) didn't have any side effects of using cosmetics

and approximately (35.4%) were experienced side effects of using cosmetics (Figure 4).

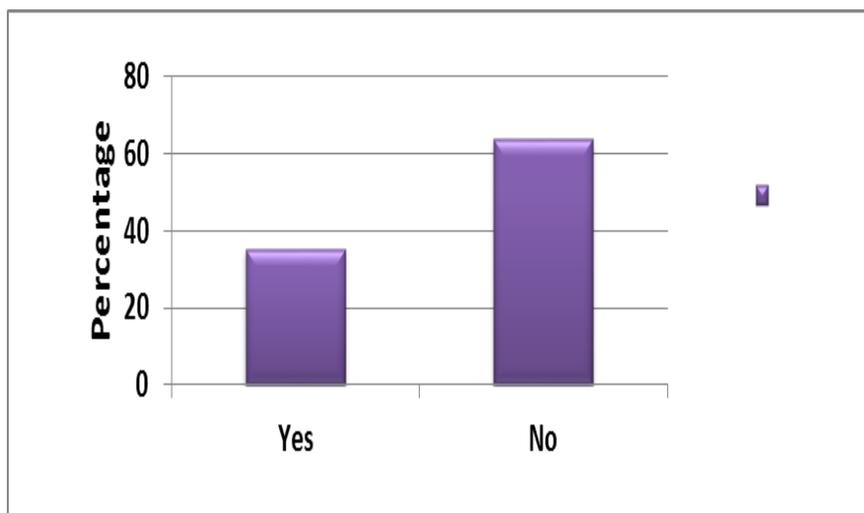


Figure 4: The side effects of using cosmetics among participants

The greatest proportion of cosmetic type used by participants was eye-liner (73.9%), followed by mascara (73.6%), while the lowest type (28.6%) used was eye shadow (Table 4).

Table 4: Common types of cosmetic products usually used among participants.

Cosmetic products	N	(%)
Foundation	390	50.7%
Face -Powder	236	30.7%
Lipstick	339	44.1%
Nail-polish	254	33%
Eye-linear	568	73.9%
Eye-shadow	220	28.6%
Eyebrows-dye	320	41.6%
Mascara	566	73.6%

Results are expressed by number N, and percentage (%).

The majority of participants (41.8%) were started using cosmetics in the age below 15 years (Table 5).

Table 5: Age group of start using cosmetics among participants.

Time period	N	Percentage %
< 15 y	46	41.8
15-20	36	32.7
20-25	22	20
> 25	6	5.45
Total	110	100

Results are expressed by number N, and percentage (%)

The number of investigating eyeliner samples was ten from different brands, colors and country of origin (Table 6).

Table 6: The most common brands of eyeliner samples.

Sample Code	Color	Country of Origin
S.1	Blue	Italy
S.2	Black	China
S.3	Black	Germany
S.4	Black	France
S.5	Black	Italy
S.6	Black	Turkey
S.7	Black	Germany
S.8	Black	China
S.9	Green	China
S.10	Black	Germany

The heavy metals, cadmium and lead were detected in all ten samples with varying concentrations ($\mu\text{g/l}$), while mercury was detected with varying concentrations (mg/l). The concentration range of cadmium was 0.25-1.69 $\mu\text{g/l}$, the concentration range of lead was 5.36 - 20.93 $\mu\text{g/l}$ and the concentration range of mercury 0.16-2.91 mg/l in the eyeliner samples. The concentration of lead was generally higher compared to the other metals. The highest concentration of lead observed (20.93 and 20.52 $\mu\text{g/l}$) in sample 1 and 10 respectively, while the lowest concentration (5.356 and 5.85 $\mu\text{g/l}$) detected in sample 2 and 4 respectively. The overall levels of cadmium determined were much lower than lead. The highest concentrations of cadmium observed (1.69, 1.15 $\mu\text{g/l}$) in sample 1 and 10 respectively, while the lowest concentration (0.25 $\mu\text{g/l}$) was observed in sample 4. The highest concentrations of mercury (2.91 and 2.26 mg/l) were observed in sample 7 and sample 10 respectively, while the lowest level (0.16 mg/l) was observed in sample 4. So sample 4 contained the lowest concentration of cadmium, lead and mercury (0.25, 5.36 $\mu\text{g/l}$ and 0.16 mg/l) so it's considered the safest sample (Table 7).

Table 7: Heavy Metals Concentrations (mg/L) in Eyeliner samples.

Sample Code	Cadmium (Cd)	Lead (Pb)	Mercury (Hg)
S.1	1.69	20.93	0.85
S.2	0.49	5.85	0.62
S.3	0.46	12.25	0.58
S.4	0.25	5.36	0.16
S.5	0.48	6.786	0.97
S.6	0.50	8.41	0.75
S.7	0.91	13.72	2.91
S.8	0.76	15.26	2.16
S.9	0.33	14.12	1.09
S.10	1.15	20.52	2.26

The mean \pm standard of Cd, Pb and Hg in the ten samples were 0.70 ± 0.44 , 1.23 ± 5.69 and 1.23 ± 0.89 respectively (Table 8).

Table 8: The mean & standard deviation for heavy metals concentration in eyeliner samples.

Heavy metal	Range	Mean \pm Standard deviation	Variance
Cd	1.44	0.70 ± 0.4	0.19
Pb	15.57	1.23 ± 0.2	32.38
Hg	2.75	1.23 ± 0.89	0.79

Results are expressed as mean \pm SD.

There was a significant difference of cadmium, lead and mercury concentration in different brands of eyeliner at the $p < (0.05)$ (Table 9).

Table 9: The Results of One Way Anova test to determine the relation between and different eyeliner samples.

	Sum of Squares	Degrees of freedom (df)	Mean Square	Ratio of the mean squares(F)	Sig.
Between Groups	862.07	2	431.03	38.81	0.001
Within Groups	299.887	27	11.11		
Total	1161.96	29			

Results are expressed as mean.

*. The mean difference is significant at the 0.05 level.

Table 10 showed that, factors 1, 2 and 3 are for cadmium, lead and mercury respectively. The test indicated that the mean score (M) between cadmium and lead (M= -11.616) was a statistically significant

difference at the level $p < 0.05$ of different eyeliner brands. The mean score between mercury and lead (M = -11.109) was statistically significant different at the level.

Table10: The Result of Post Hoc Test for multiple comparisons between cadmium, lead and mercury concentrations in different eyeliner samples.

Factor(I)	Factor(J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1-(Cd)	2-(Pb)	-11.616*	1.490	0.001	-14.674	-8.558
	3-(Hg)	-0.5062	1.490	0.737	-3.564	2.552
2-(Pb)	1-(Cd)	11.6161*	1.490	0.001	8.558	14.6742
	3-(Hg)	11.1099*	1.490	0.001	8.052	14.168
3-(Hg)	1-(Cd)	0.5062	1.490	0.737	-2.552	3.564
	2-(Pb)	-11.1099*	1.490	0.001	-14.168	-8.052

Results are expressed as mean.

* The mean difference is significant at the P value < 0.05 .

4. DISCUSSION

The awakening of female consumer's consciousness during the recent years leads to, alteration of consumer behavior, and influences the woman's usual conception for pursuing fashion and cosmetics application.^[14] In the present study, from 769 women, the mean \pm S.D of age was mean \pm S.D 31 \pm 12. AlGamdi study, conducted a questionnaire on the use of topical bleaching agents among women, the questionnaire was distributed to 620 women. In total, 509 women aged 10-15 years (mean \pm SD 29.22 \pm 9.07).^[15]

The highest percentage of the participants (43.2%) was in the age group of 20-30 years. This agreed with AlGamdi study who found the highest percentage of participants (42.90%) was in the age of 20-29.^[15] Another study reported the majority of the participants (65%) who consumed cosmetic products are in the age of teenagers.^[16] Since the majority of the participants in the present study (41.6 %) were employees. Nilesh study, found that the highest percentage of participants (55%) and (23.50%) were students and employed respectively.^[16]

In the present study, concerning marital status, the highest percentages of participants who consumed cosmetics products (60.7%) were married. This result agreed with AlGamdi study who found that the most of participants (60.80%) were married^[15] While in Nilesh study who found that the most of the respondents (60.8%) were unmarried.^[16]

The highest percentages of the participants (68.40%) were purchased cosmetics from large stores and international brands, while the lowest percentage (4.03%) of the participant was purchased via internet. This agreed with Nilesh study, who found that the most preferred place to buy cosmetic products among respondents (53%), (36%) were shopping mall and tradition shop respectively.^[16] Abdullah study, reported that the most preferred place to buy cosmetic products (41%) was from a cosmetic store.^[17] Another study found that the most of the respondents (87.32%) got information through the Internet, being the most accessible medium nowadays.^[18] In the present study the highest percentage of participants (38.9%) and (34.2%) were affected by recommendation from friends or relatives and the type of brand. Nilesh study, found that the most of the respondents (4.37%) and (2.89%) take quality as a most important factor to purchase cosmetic products and packaging as a least important factor for purchasing cosmetic products respectively.^[16] In our study, the highest percentage (52.93%) of participant expected that expensive cosmetics are safe and free from harmful ingredients such as heavy metals. Abdullah study, found that there is an increase in awareness related to cosmetic products. Female consumers prefer cosmetic products which are made from natural ingredients.^[17] May study, reported that the majority of respondents would use cosmetics that were

readily available (58.12%) and safe (49.68%). However, most of the respondents' preferred brands contain harmful cosmetic ingredients. The result supported the almost low score in the knowledge section. Also May found a huge number of respondents got information through mass media newspapers (70.42%), magazines (69.01%) because most of the respondents also write for magazines, television (50.7%) and radio (23.94%).^[18] In the present study, the highest percentage of cosmetic type used among participants was eye-liner "kohl" (73.9%), followed by mascara (73.6%). May study, who reported for skincare (and facial-care) brands, the respondents listed down their preferred brands of soap, deodorant, lotion, sunscreen, lip care and face powder.^[18] In our study, the number of investigating eyeliner samples was ten from different brands, colors and country of origin. The concentration range of cadmium was 0.25 - 1.69 μ g/l, the concentration range of lead was 5.36 - 20.93 μ g/l and the concentration range of mercury 0.16 - 2.91 mg/l in the eyeliner samples.

The German Federal Government studies, determined that heavy metal levels in cosmetic products above the values listed below are considered technically avoidable 25: Lead: 20 ppm, Arsenic: 5 ppm, Cadmium: 5 ppm, Mercury: 1 ppm.^[19] Because of the lack of governmental and international rules associated with the maximum permissible content of lead in cosmetics, the Campaign for Safe Cosmetics (CSC) has set 0.1 mcg / g for lead in cosmetics such as lipstick. This rule has been assigned on the basis of the maximum allowable lead concentration in candy, because it has been assumed that lipstick may be directly taken in via the mouth.^[20] However the Canadian Government has taken a bold step by having a draft regulation which proposes a maximum limit for some of these heavy metals in cosmetics which include lead-10 ppm, Cadmium-3 ppm, Arsenic-3 ppm, Mercury-3 ppm and Antimony-5 ppm.^[21] Saudi Food and Drug Authority (SFDA) prohibited heavy metals as ingredients in cosmetic products in Saudi Arabia, and as impurity in eye products.^[22] So, according to Saudi Food and Drug Authority (SFDA) all samples in our study were not safe, however, according to Canada all samples had mercury and cadmium within the permissible limit but six samples from ten had lead above the permissible limit.

In the present study, the concentration of lead is generally higher compared to the other metals. The highest concentration of lead observed (20.93 and 20.52 μ g/l) in sample 1 and 10 respectively, while the lowest concentration (5.36 and 5.85 μ g/l) detected in sample 2 and 4 respectively. The overall levels of cadmium determined were much lower than lead. The highest concentrations of cadmium observed (1.69, 1.15 μ g/l) in sample 1 and 10 respectively, while the lowest concentration (0.25 μ g/l) was observed in sample 4. Nour study, investigated fifty samples of lipstick (35 samples) and eye shadow (15 samples). The samples analyzed showed that lead and cadmium were detected in

all brands of the cosmetics with varying concentrations. The eye shadow samples had also a lead level of 0.85–6.90 mcg/g and a cadmium level of 1.54–55.59 mcg/g.^[23] Nnorom found that, the average cadmium concentrations: eyeliner (1.0 µg/g) and eye pencil (0.7µg/g).^[24] Amit study, detected the presence of lead and cadmium in powder sample and recorded the highest values for Pb to be 0.38 µg/g and 0.02 µg/g for Cd.^[25] Another study reported that the average of cadmium levels in several facial cosmetics (eye cosmetics, lipsticks, and lip gloss) was approximately 1 mcg/g.^[26] In the present study, the highest concentrations of mercury (2.91 and 2.26 mg/l) were observed in sample 7 and sample 10 respectively, while the lowest level (0.16 mg/l) was observed in sample 4. We observed that the sample 4 contained the lowest concentration of cadmium, lead and mercury (0.25, 5.36 and 0.16 mg/l respectively) so it's considered the safest sample. AL-Dayel study, reported the concentration of twenty eight elements on the Mascara and Eye Shadow samples from the Saudi market. The study found that lead, arsenic, cadmium, mercury and antimony levels in the samples were within the normal level.^[19]

In our study, we indicated that, there was a significant difference of cadmium, lead and mercury concentration in different brands of eyeliner at the $p < (0.05)$. Nour study, found that the cadmium content in both cosmetic products was higher than lead content ($p < 0.04$). There was a significant difference between the average of the lead content in the different brands of eye shadows ($p = 0.02$). There was not significant difference between cadmium content for various brands of the eye shadows ($p > 0.05$).^[26]

5. CONCLUSION

The most common cosmetic type used by Saudi women was eyeliner "kohl". There was a lack of awareness about heavy metal presence in cosmetics in 34.59% of participants. The majority of participants expected that expensive cosmetics are safe and free from heavy metals and they preferred international brands which is not necessary to be "safe" in term of heavy metals content. All samples contained heavy metals with varying concentrations. Efforts should be made to increase the awareness of the cosmetic users and the general public of the harmful consequences of cosmetics, regardless of the product cost. Major quality controls are recommended for products designed to direct contact with the human body for long time period.

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