





Determination of Some Heavy Metals in Eye Shadows Cosmetics

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Minor quantities of several heavy metals have been documented in cosmetic products as impurities which may lead to various types of skin allergies through percutaneous absorption. This study determined the concentration of heavy metals present in the various samples of eye shadows available in Riyadh markets of Saudi Arabia. A total of 21 popular international eye shadow brands from China, France, Italy and Ireland were tested for the presence of some toxic heavy metals. Eye shadows were categorized in three different types (cheap, medium and expensive) on the base of their price. Eye shadow samples were acid digested in a microwave and contents of lead, cadmium and arsenic were measured by graphite furnace atomic absorption spectroscopy (GFAAS), while mercury was quantified by flame atomic absorption spectrometry (FAAS). Results showed that the mean concentration of mercury, arsenic and cadmium were more or less similar in all the three categories (cheap, medium and expensive) of eye shadow samples, whereas the concentration of cadmium was fairly high in cheap eye shadow samples as compared to medium and expensive priced quality. Lead concentration was higher in expensive eye shadows samples than medium and cheap priced samples. This study showed that the mean concentration of toxic heavy metals in all the eye shadow samples were below the US FDA and SFDA permissible limits for cosmetic products with the exception that mercury content in eye shadows samples was higher than SFDA.

Keywords: Eye shadows, Heavy metals, Atomic absorption spectroscopy.

INTRODUCTION

Most of the cosmetic products used by both male and female community has been shown to contain various types of heavy metals in different proportions [1]. Eye shadows are usually available as compressed powders, anhydrous creams, emulsions, sticks, and pencils and are frequently used by women of all ages. Compressed powder type of eye shadows are the most used ones and are usually applied to the eyelid by lightly stroking a soft sponge tipped applicator across the skin. Its main ingredients are talc with pigments [2,3].

Eye shadows contain particles of heavy metals such as Cd, Co, Cr, Ni, As, Hg and Pb as impurities in the pigments added during the manufacturing process or released by the metallic devices used during the manufacturing of cosmetics. Both kohl and surma have been identified as a major source of Pb exposure to the ocular system in adults and children [4-6].

Heavy metals like arsenic, cadmium, lead and mercury have been described as highly toxic and carcinogens, exhibiting wide-ranging toxic effects on many bodily systems, including

the nervous, endocrine, renal, musculoskeletal, immunological, and cardiovascular systems [7]. On the other hand, copper, nickel, chromium and iron have been classified as micronutrients and are essential in very low concentration for the survival of all forms of life, but when present in higher concentrations can cause various types of metabolic anomalies [8]. Eye shadows and other similar cosmetics containing toxic heavy metals are applied onto the human skin thereby increasing the risk of direct local exposure of humans to these poisonous and carcinogenic chemicals.

The eye-lids can, therefore, be mechanically broken thus enabling the penetration of pigment and toxic metals into the body. Metals are also absorbed into the organism through the healthy skin [9]. Although absorption of metals through the skin is often ignored, studies have shown that lead actually can be absorbed through the skin [10].

The skin is considered a protective barrier, but continuous use of cosmetics over the skin of face, lips and eyes may enhance systemic exposure and the absorption of toxic heavy metals, especially Cd and Pb, during facial makeup, eating

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and sweating [11,12]. Mercury compounds are readily absorbed through the skin on topical application and have the tendency to accumulate in the body. They may cause allergic reactions, skin irritation or neurotoxic manifestations [13].

Lead is a toxic element and can affect many organs in the human body [14]. Its continuous and long-term exposure through dermal, environmental or other sources may cross the allowable limits for the human body and could reach the level of significant toxicity thus causing serious health problems of acute or chronic nature [15-17].

Lead and cadmium, considered as the most dangerous contaminants to arise in human civilization, are distributed in the environment as polluting elements [18,19]. An increase in Pb and Cd level may cause acute and chronic poisoning, multiorgan pathological changes causing cardiovascular, renal, bone and liver diseases. These elements can also cause cancer owing to excessive accumulation in the human body [17,19].

Although the European Union law banned the use of lead and lead containing compounds in cosmetics since 1976, however, trace amounts of this element are unavoidable under conditions of good manufacturing practice [20]. The present study was designed and conducted to determine contents of lead, cadmium, arsenic and mercury in popular and commonly used eye shadow brands available in Riyadh, Kingdom of Saudi Arabia.

EXPERIMENTAL

Microwave digestion – System, high performance from (ETHOS ONE). Atomic absorption spectrometer 240 FS, from the Agilent Technologies with (Graphite Furnace) GTA 120) as PSD"120" programmable sample dispenser and carrier gas was argon. Mettler analytical balance, Milli-Q system, Millipore, Bedford, MA

All reagents used in this study were of analytical grade. 69 % HNO₃ (CL CHEM-LAB, Belgium), 48 % HF, 36 % HCl (Sigma-Aldrich, Germany) and 35 % H₂O₂ (Riedel-de Haen, Germany), were used for microwave digestion.

Single element stock standard solutions of Cd, Pb, As and Hg (Panreac Quimica SA, European Union Brand), having a ready-made concentration of 1.0 mg/mL, were purchased from the local market. Samples and stock standard solutions of the elements were appropriately diluted with high-purity deionized water (Milli-Q system, Millipore, Bedford, MA) to generate a linear calibration curve. Orthophosphoric acid was used (1.0 mg/mL) as a chemical modifier for the analysis of Pb and Cd, whereas 5 % Ni solution was used as a modifier to facilitate the analysis of Hg.

Eye shadow products were purchased from different cosmetic stores in Riyadh city. Eye shadows were categorized according to their price range: "low" (\leq 60 SR), "medium" (61-130 SR) and "high" (> 130 SR). In all, 21 different brands of eye shadows were collected and analyzed.

Sample digestion: Eye shadow samples were digested by using ETHOS One™ Microwave Digestion System (Milestone Inc., USA) according to the procedure described by Piccinini [21]. Briefly, 0.25 g of eye shadow sample was weighed in a microwave PTFE-vessel liner and 8.0 mL of 69 % HNO₃, 1.0 mL of 35 % H₂O₂, 1.0 mL of 48 % HF and 1.0 mL of 36 % HCl were added to the sample.

Digested eye shadow samples were filtered to remove undigested particles (wax, glitters, *etc.*) using Whatman 40 filter paper. After cooling, the extracted samples were transferred to a 50 mL volumetric flask and adjusted with deionized water.

The operating conditions during the analysis of heavy metals are listed in (Table-1). Clear solutions of the digested samples were analyzed for Pb, Cd and As used argon gas (GTAAS) spectrometry. Hg analyzed using (FAAS) with airacetylene (model: AAS 240 FS), the Agilent Technologies Company, America, by the standard calibration technique. All measurements were run in triplicates for the samples and standard solutions and the results reported as the mean ± standard deviation

Quality control: Quality control (QC) is a procedure or set of procedures intended to ensure that a sample analysis adheres to a defined set of quality criteria, as the limit of detection (LOD) and limit of quantification (LOQ) according to USEPA [22]. LOD and LOQ were calculated according to the procedure reported by Khan *et al.* [23].

Statistical analysis: The data were analyzed using package for the social science (SPSS) VERSION 17. Descriptive statistical parameters such as mean and standard deviation (SD) were used to describe the heavy metal concentration in the eye shadows. One way analysis of variance (ANOVA) was used to determine the difference of the heavy metals concentration among different price categories of eye shadows at a significance level of p < 0.05.

RESULTS AND DISCUSSION

The use of cosmetics is becoming almost everybody's daily habit today applying one or more of facial cosmetic creams, powders, eyeliners, foundations, concealers, eye shadows, mascaras and lipsticks or glosses and viewed as one of the most important sources of releasing heavy metals into the environment and the human biological system. Following such

TABLE-1 STANDARD OPERATING PARAMETERS OF THE ELEMENTS ANALYZED									
Parameters	Pb	Cd	As	Hg					
Wavelength (nm)	283.3	228.8	197.2	253.7					
Slit width (nm)	0.5	0.5	0.5	0.5					
Lamp current (mA)	10	4	10	4					
Sensitivity (mg/kg) at 0.2 absorbance	27 μg/kg	1.00 μg/kg	50 μg/kg	70 mg/kg					
Detection limit	8.22 μg/kg	0.23 μg/kg	4.9 µg/kg	1.87 mg/kg					
Quantification limit	24.9	0.69	14.85	5.68					
Optimum working rang	24.9-50.00 μg/kg	0.69-3.00 µg/kg	14.85-300 μg/kg	5.68-30.00 mg/kg					
Instrument	GTAAS	GTAAS	GTAAS	FAAS					

observation, there is an increasing need to investigate the concentration of toxic metals in some commonly used cosmetic products. It is known, for instance, that high doses of heavy metals can be deadly and that even long-term exposure to low levels of heavy metals can cause certain cancers [24]. In this study, the different categories (different price) of eye shadows cosmetic products were tested for the presence of lead, cadmium, arsenic and mercury.

Heavy metals' concentration in the eye shadows samples of different price, categories is summarized in (Table-2). The recoveries for Pb, Cd, As and Hg was 99.89, 99.53, 99.68 and 99.07 %, respectively (Table-3). The correlation coefficients (R²) 0.9986, 0.999, 0.9995 and 0.9993 for Pb, Cd, As and Hg, respectively, which lie in an acceptable range. The average concentration of Pb, Cd, As and Hg, were varied through a wide range of values in the eye shadows samples, in price categories I (cheap price) from 19.52 (sample 6), to 96.06 (sample 2), 0.53 (sample 6) to 3.92 (sample 2), 52.01 (sample 1), to 529.80 (sample 4) µg/kg and 0.32 (sample 4) to 7.42 (sample 3) mg/kg, respectively. While the average concentration of lead, cadmium, arsenic and mercury, were varied through a wide range of values in eye shadows samples, in price categories II (intermediate price) from 39.48 (sample 7) to 97.87 (sample 2), 0.66 (sample 4) to 3.25 (sample 2), 45.62 (sample 4), to 190.44 (sample 2) µg/kg and 2.94 (sample 5) to 8.24 (sample 2) mg/kg, respectively.

The average concentration of Pb, Cd, As and Hg varied through a wide range of values in eye shadows samples, in price categories III (expensive price) from 44.09 (sample 1), to 181.90 (sample 2), 0.48 (sample 1) to 3.46 (sample 2), 49.64 (sample 1), to 460.28 (sample 3) µg/kg and 1.83 (sample 3) to 10.00 (sample 2) mg/kg, respectively (Table-2).

Lead and cadmium are two potentially harmful metals that have aroused considerable interest. Particularly, lead has been described as the most harmful environmental contaminant to arise in human civilization and has been shown to impair renal, homopoietic and the nervous system with different reports linking it to the deficiency in cognitive functioning [25]. The presence of lead in cosmetics has also been reported and thus the European Union (EU) law for cosmetic banned lead and lead compounds in cosmetics since 1976 and strict adherence to good quality control is essential in ensuring that lead contamination in cosmetic products is prevented [26]. Consistent with the above, results showed that the concentration of lead in this study was higher than allowed (Nill) according to US FDA [27] and in the permissible limits (20 mg/kg), that was established as the maximal lead limit as impurities in colour additives in the cosmetics for external use, formulated following the good manufacturing practices [27]. The campaign for safe cosmetics published a report on heavy metals in face paints, 10 products studied contained lead [28], 4 contained nickel, 2 contained cobalt and 5 con-

TABLE-2 CONCENTRATION OF HEAVY METALS IN THE EYE SHADOWS SAMPLES										
Price category	Code	Country of production	Pb (μg/kg) Mean ± SD	DF	Cd (µg/kg) Mean ± SD	DF	As (μg/kg) Mean ± SD	DF	Hg (mg/kg) Mean ± SD	
	C 1	China	72.95 ± 0.003	2.14	0.93 ± 0.001		52.01 ± 0.001		2.47 ± 0.002	
	C 2	China	96.06 ± 0.001	2.14	3.92 ± 0.002	2.14	142.82 ± 0.002		7.07 ± 0.001	
	C 3	China	92.97 ± 0.001	2.14	3.40 ± 0.001	2.14	122.59 ± 0.003		7.42 ± 0.003	
I	C 4	China	39.12 ± 0.002		0.99 ± 0.002		529.80 ± 0.002	2.14	0.32 ± 0.001	
	C 5	China	45.54 ± 0.002		0.87 ± 0.001		99.53 ± 0.004		2.90 ± 0.002	
	C 6	China	19.52 ± 0.001		0.53 ± 0.002		288.39 ± 0.003		0.24 ± 0.003	
	C 7	China	29.01 ± 0.001		0.42 ± 0.002		335.42 ± 0.002	2.14	0.89 ± 0.001	
	L 1	Italy	75.25 ± 0.001	2.14	0.81 ± 0.001		76.84 ± 0.002		4.51 ± 0.002	
	L 2	Italy	97.87 ± 0.001	2.14	3.25 ± 0.002		190.44 ± 0.003		8.24 ± 0.001	
	L 3	America	66.24 ± 0.003	2.14	0.90 ± 0.001		88.98 ± 0.003		4.04 ± 0.001	
II	L 4	Italy	29.15 ± 0.001		0.66 ± 0.001		45.62 ± 0.002		4.31 ± 0.002	
	L 5	France	55.60 ± 0.001		0.80 ± 0.002		68.10 ± 0.004		2.94 ± 0.002	
	L 6	Ireland	65.19 ± 0.001	2.14	1.07 ± 0.002		75.67 ± 0.002		3.92 ± 0.001	
	L 7	France	39.48 ± 0.001		1.00 ± 0.002		102.11 ± 0.003		3.48 ± 0.003	
	E 1	France	44.09 ± 0.002		0.48 ± 0.001		29.64 ± 0.003		4.97 ± 0.002	
	E 2	France	181.90 ± 0.003	4.00	3.46 ± 0.001	2.14	184.08 ± 0.001		10.00 ± 0.003	
	E 3	France	18.55 ± 0.001		1.18 ± 0.003		460.28 ± 0.001	2.14	1.83 ± 0.001	
III	E 4	America	97.00 ± 0.002	2.14	1.07 ± 0.001		60.21 ± 0.004		5.45 ± 0.002	
	E 5	France	90.14 ± 0.001	2.14	1.34 ± 0.001		65.77 ± 0.000		4.06 ± 0.001	
	E 6	France	59.46 ± 0.002		1.23 ± 0.002		77.08 ± 0.001		3.62 ± 0.001	
	E 7	France	89.96 ± 0.001	2.14	1.28 ± 0.001		57.84 ± 0.001		4.76 ± 0.001	
*DF was c	*DF was dilution factor									

TABLE-3 RECOVERY AND SPIKING OF MICROWAVE DIGESTED SAMPLES CONCENTRATION Un-spiked Standard added Spiked Correlation Elements Recovery (%) $(Mean \pm SD)$ to sample $(Mean \pm SD)$ coefficient (R²) Pb (µg/kg) 2.8 ± 0.01 8 10 ± 0.02 0.9986 99.89 Cd (µg/kg) 0.7 ± 0.00 10 11 ± 0.01 0.9990 99.53 0.3 ± 0.01 2 2.4 ± 0.01 0.9995 99.68 As (µg/kg) 0.3 ± 0.01 1.4 ± 0.02 0.9993 99.07 Hg (mg/kg)

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tained chromium (ranging from 1.6 to 120 mg/kg), the levels found in all the products exceeded the recommendation of several industries [29], which agree with the present study.

Cadmium poisoning symptoms range from the renal and myocardic dysfunction, obstructive lung disease, bone defects [30,31]. The levels of cadmium in the studied samples are higher than allowed and is not safe, according to the Food and Drug of Saudi Arabia (Nill) [32] and studies conducted by Muhamad *et al.* [24], they proved that the levels of cadmium are generally low, being much less than 3 mg/kg. According to Environmental Protection in United States (0.5 mg/kg bw/day) it was within the allowable limit, hence unlikely to represent a health risk.

As poisoning includes the damage of tissues, organs, chromosomes, immune and urinary system [32]. Arsenic was generally low to the extent of being smaller than the allowed limit < 3 mg/kg, US FDA [27] and in line with the government of the Federal German (5 mg/kg) and also consistent with a study conducted by Sainio *et al.* [33], they determined arsenic in 88 colours of eye shadows (2.3 mg/kg), but was in varies with the Food and Drug of Saudi Arabia (Nill), US FDA [27].

Mercury a widely used element in most cosmetics as eye shadows Lipetsk, skin lightening creams and as antiseptic creams or ointments has been detected in the studied samples. Mercury poisoning ranges from insomnia, dermal toxicity, nervousness, lack of coordination in taste, touch and sight [34,35]. The mercury level in the three categories of samples in cosmetics eye shadows, was higher than allowed and not safe and this varies with the Food and Drug of Saudi Arabia (Nill). According to the US Food and Drugs Administration (FDA), cosmetic products should not contain mercury as an ingredient (FDA) < 1 mg/kg [36,37] and agree with Al-Saleh, who reported that, mercury content was more than six times that found in other reports described in the literature [3], such as those reported by Uram *et al.* [38].

On the other hand, based on the guidelines of control of cosmetic products in Saudi Arabia, which is prepared in accordance with the ASEAN cosmetic directive, lead, cadmium, mercury and arsenic are included in the list of substances which must not form part of the composition of cosmetic products as reported [39]. These heavy metals should not be added to cosmetics during the manufacturing process as an ingredient formula.

The concentrations of four metals Pb, Cd, As and Hg were determined in 21 samples from 3 different brands of eye shadows. Results were determined as mean \pm SD of dry weight from three replicates in each test. All the eye shadows monitored in this study contained detectable contents of lead, cadmium, arsenic and mercury.

Analysis of variance (ANOVA) was done on each brand of eye shadows to find out if there is significant variation in the concentrations of heavy metals in a different category of price. The results of ANOVA showed that there is no significant difference in the levels of lead content among the eye shadows in price categories I, II and III, where p > 0.05 [p = 0.386, F (2.18) = 1.005]. The high and medium price have highest concentrations of lead (83.01 and 61.25 μ g/kg), while low price has the lowest lead content (56.45 μ g/kg) (Table-4).

The results of ANOVA showed that, p > 0.05 for cadmium level this means that no significant difference in the levels of cadmium content among the eye shadows in price categories I, II and III, [p = 0.829, F (2.18) = 0.189]. The high and low price have highest concentrations of cadmium (1.43 and 1.58 μ g/kg), while medium price has the lowest cadmium content (1.21 μ g/kg) (Table-5).

Similarly, there was no significant difference of arsenic content among the eye shadows in price categories I, II and III, where p > 0.05, [p = 0.199, F(2.18) = 1.77]. The low and high price have highest concentrations of arsenic (224.38 and 133.56 µg/kg), while medium price has the lowest arsenic content (92.54 µg/kg) (Table-6).

On the other hand, the results of ANOVA test for Hg showed that p > 0.05, indicating there was no significant difference of Hg content among the eye shadows in price categories I, II and III, [p = 0.348, F(2.18) = 1.121]. The high and medium price have highest concentrations of mercury (4.96 and 4.49 µg/kg), while low price has the lowest mercury content (3.04 mg/kg) (Table-7). The results of this study were comparable to the study by Piccinini *et al.* [22].

TABLE-4
IADLE-4
ONE WAY ANALYSIS OF VARIANCE (ANOVA) OF LEAD CONCENTRATION IN DIFFERENT PRICE OF EYE SHADOWS SAMPLES
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	N	Mean	Std. Deviation	Std. Error	95 % Confidence	e interval for mean	Minimum	Maximum
	in Mea	Mean	Std. Deviation		Lower bound	Upper bound		
Low	7	56.4529	30.85193	11.66093	27.9196	84.9861	19.52	96.06
Medium	7	61.2543	22.78052	8.61023	40.1858	82.3228	29.15	97.87
High	7	83.0143	52.11934	19.69926	34.8119	131.2166	18.55	181.90
Total	21	66.9071	37.36852	8.15448	49.8972	83.9171	18.55	181.90

TABLE-5 ONE WAY ANALYSIS OF VARIANCE (ANOVA) OF CADMIUM CONCENTRATION IN DIFFERENT PRICE OF EYE SHADOWS SAMPLES

N Mean	N	Maan	Std. Deviation	Std. Error	95 % Confidence	e interval for mean	Minimum	Maximum
	Mean	Stu. Deviation	Std. Error	Lower bound	Upper bound	Millillulli	Maximum	
Low	7	1.5800	1.44400	0.54578	0.2445	2.9155	0.42	3.92
Medium	7	1.2129	0.90847	0.34337	0.3727	2.0531	0.66	3.25
High	7	1.4343	0.93861	0.35476	0.5662	2.3024	0.48	3.46
Total	21	1.4090	1.07766	0.23517	0.9185	1.8996	0.42	3.92

TABLE-6 ONE WAY ANALYSIS OF VARIANCE (ANOVA) OF ARSENIC CONCENTRATION IN DIFFERENT PRICE OF EYE SHADOWS SAMPLES

	N	Mean	Std. Deviation	Std. Error	95 % Confidence	e interval for mean	Minimum	Maximum
	IN IVIE	Mean	Std. Deviation		Lower bound	Upper bound		
Low	7	224.3800	169.31025	63.99326	67.7941	380.9659	52.01	529.80
Medium	7	92.5371	46.58067	17.60584	49.4572	135.6171	45.62	190.44
High	7	133.5571	152.20425	57.52780	-7.2083	274.3226	29.64	460.28
Total	21	150.1581	139.23788	30.38420	86.7778	213.5384	29.64	529.80

TABLE-7 ONE WAY ANALYSIS OF VARIANCE (ANOVA) OF MERCURY CONCENTRATION IN DIFFERENT PRICE OF EYE SHADOWS SAMPLES

N Mean	N	Maan	n Std. Deviation	Std. Error	95 % Confidence	interval for mean	Minimum	Maximum
	Mean	Std. Deviation	Std. Elloi	Lower bound	Upper bound	Minimum	Maximum	
Low	7	3.0443	3.04496	1.15089	0.2282	5.8604	0.24	7.42
Medium	7	4.4914	1.73423	0.65548	2.8875	6.0953	2.94	8.24
High	7	4.9557	2.51940	0.95224	2.6257	7.2858	1.83	10.00
Total	21	4.1638	2.50674	0.54701	3.0228	5.3049	0.24	10.00

Conclusion

The overall results of the present study indicate that lead and mercury content were extremely high. The content of cadmium and arsenic was appropriate in some references, but not safe with the Food and Drug of Saudi Arabia. It is feared, however, that the continuous use of eye shadows products contaminated with such heavy metals may, however, cause a slower release of these metals into the human body and cause harmful effects to the consumers over time. To safeguard consumer health, our research calls for an immediate mandatory regular testing program to check these metals in whitening eye shadows and other cosmetic products that are being marketed and consumed in Saudi Arabia.

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