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Determination of Lead and Cadmium Contents and Microbial Contamination Assessment in Kohl used in Libyan Markets

Amal Belaid[1], Asma Eswayah[1], Intisar El-Sharaa[2*], Salma Shmaghi[3], Abdulkarem Tamer[4], Haya AlFassam[5], FulwahAlqahtani[6], Fadilah Aleanizy[6]
[1]*Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.*
[2*]*Department of Chemistry, Faculty of Science, University of Benghazi, Benghazi, Libya.*
[3]*Libyan food and drug administration center, Tripoli, Libya.*
[4]*Department of Microbiology, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.*
[5]*King Abdulaziz city for science and technology, Riyadh, Saudi Arabia.*
[6]*Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.*

Abstract: *This study aims to determine some heavy metals such as lead (Pb) and cadmium (Cd) content in traditional eye cosmetics known as kohl, which is available in Tripoli markets in Libya. An analytical test was performed for ten kohl samples using flame atomic absorption spectrophotometer (AA280-FS). The overall mean (n= 6) concentration for each heavy metal was analyzed. The results of nine kohl samples (2-10) showed that the concentrations of lead and cadmium in these samples were within the range of 12.92-76.14% and 0.36-16.87% respectively. These high concentrations of Pb and Cd were over the safe limits set by the world health organization standards (WHO-2012/FAO-2013). Thus the continuous use of these types/brands of kohl can increase the absorption of lead and cadmium into the human body and elevate the health hazards. However; only one kohl sample (No.1) was found to have 0.004% and 0.02% of Pb and Cd respectively. All the kohl samples were evaluated for presence of microbial contamination by culture and staining techniques. No evidence for contamination of the samples by fungi; samples 7 and 8 were free from bacterial contamination; other samples were contaminated with bacillus species which might cause ocular infection and conjunctivitis.*

Keywords: *Kohl, Lead, Cadmium, poisoning, microbial contamination*

I. Introduction

Exposure of humans to heavy metals is higher today than ever before in modern history due to continuously increasing industrialization around the globe. Industrial wastes are rich in heavy metals and these wastes are discharged near agricultural fields or mixed with soil, from where these metals are taken up by the cosmetics and are finally transported to humans. Due to this increasing threat of heavy metals contamination, it is necessary to analyze the cosmetics and kohl before consumption.

Kohl is a deeply held tradition and a culturally and religiously legitimatized practice among Arabian and Asian people. They were using many types of eye preparations for the protection and cure of eye diseases and Kohl was one of them. Thus, Kohl can be regarded as closely associated with almost all human, the word "Kohl" is Arabic in origin and actually the Arabic oculist called it as "Kahal" [1].In most cases, it is prepared at home or sold in the market in an uncontrolled way.

Many literatures for more than 20 years have included detailed case studies concentration of lead and cadmium in cosmetics especially in kohl [2].Lead and cadmium in kohl are absorbed into the body; therefore high concentration of these heavy metals is the source of poisoning in neonates [3]. Several studies have reported cases of lead poisoning or impregnation due to the use of the cosmetic product kohl [4].

Kohl appears to be a substance that may be a source of lead exposure among children and adult. In children, it can be used for eyes and/or umbilicus at birth, this application was found to be one of the causes of elevated blood lead levels in Saudi Arabian schoolgirls [5].

Lead causes a wide range of toxicity effects; one of the most serious is the teratogenic effect. Some examples of lead consequences are inhibition of the synthesis of haemoglobin; dysfunctions in the kidneys, joins and reproductive systems, cardiovascular system and acute and chronic damage to the central nervous system and peripheral nervous system. It can also cause severe and permanent brain damage and a poor development of children's brain resulting in a poor intelligent quotient [6].

Cadmium is considered as one of the most harmful metal poison since it is toxic at extremely low levels. Long term exposures results in renal dysfunctions and high inhalations of dust and fumes can cause pulmonary problems such as obstructive lung disease and cadmium pneumonitis. Cadmium is also associated with bones defects. The symptoms of toxicity depend on the seriousness of the exposure; a strong exposure may cause pulmonary odema and death[6]. Once cadmium accumulates in tissues, it cannot be removed safely by chelation therapy without causing kidney damage [7]. Cadmium affects calcium metabolism and skeletal

changes resulting from calcium loss and ends in a decrease in bone mineral density, and also cadmium causes softening of bones in animals [8;, 9].The well known health problem due to cadmium has been the itai-itai disease where a long term high level cadmium exposure caused severe damages to the Japanese population. For this reason, the World Health Organization recommended a tolerable weakly intake of cadmium of 7µg/kg body weight [10].

According to the Directive 2008/105/EC, the environmental quality standard for cadmium system in surface waters or heavily modified water bodies depends on the hardness of the water. The most restrictive value is less than 0.08 g/ dm3 (2008/105/EC).

II. Materials And Methods

1.1. Chemicals and reagents:

All reagents used were of analytical reagent grad (Merck). Deionized water was used for the preparation of all solutions. Standard solutions for lead are (0.00-15.00 mg /L) and (0.00 – 1.00 mg /L) for lead were purchased from FlukaChemie GmbH Aldrich chemical company, INC, USA.

1.2. Sample collection:

Total ten samples of the most popular brands of Kohl in its natural form and processed into powder or cream were purchased from the various local market and cosmetic shops in Tripoli, Libya. The samples were collected separately in sterilized stoppered glass vials. The collected different brands of Kohl coded (1-10) were prepared for lead and cadmium determination (Figure S1).

Kohl was sold in powder form in black, grey, brown, reddish brown and silver color, and it was also available in natural unprocessed form as pieces of kohl stone. Kohl in Libya mainly originates from commercial manufactures from Saudi Arabia, India, Yemen and Pakistan. The information about kohl products tested in present study is summarized in table 2.They were tested for lead and cadmium contents as well as the microbial contamination.

1.3. Digestion of Kohl samples:

100mg of Kohl sample was dissolved in 50 mL aqueous nitric acid solution (2M) and the resulting suspension was stirred for 2h at room temperature. It was then filtered, the filtrate was transferred to the volumetric flask and the measurements were carried out[11].

1.4. Apparatus:

Lead and cadmium analysis were performed using a flame Atomic Absorption Spectrometer type AA280-FS with a hallow cathode lamp, the optimized heating programs followed for the analysis of lead and cadmium were that described by the instrument manufacturer. Table 1 shows the instrumental conditions of both lead and cadmium. Calibration lead standards were prepared each day using a manual standard addition procedure where kohl samples were divided into four equal portions. Known amounts of aqueous lead solutions were added to these to give final concentrations in the range of 0.00-15.00 ppm. There was a good linear relation between absorbance and standard concentration of lead. Linearity was evaluated by calculating the linear correlation coefficients (r) for 4 runs of kohl, which was 0.9985 ± 0.0001. While the linear correlation coefficients of cadmium was 0.9992± 0.0021. FiguresS2 and S3show the standard curve of both Pb and Cd. Detection limits calculated following the recommendation of International Atomic Energy Association were 1.0 mg/g.

1.5. Quality control:

The certified reference material IAEA- MESL-2013 -02 PT/TM sample from the IAEA for quality control and proficiency test was used for the purpose of method validation, the certified values are 35.5 ± 1mg/kg, and the result obtained using the analytical procedure was 35.3 ±0,5 mg/kg.

1.6. Microbiological analysis:

All culture media (nutrient broth medium, sabouraud dextrose broth with chloramphenicol, mannitol-salt agar medium, nutrient agar medium, MacConkey agar medium, sabouraud dextrose agar with chloramphenicol) were purchased from Oxoid, HiMedia and Bacto.

The collected kohl samples were aseptically transferred to microbiological lab; two weights of 1 gram for each sample were measured. The first 1 gram were aseptically placed into 50ml of nutrient broth containing duram bottle and incubated at 37C for 24hours. Other 1 gram were placed into 50ml of sabaroud dextrose broth containing duram bottle and incubated at 25◦C for 72 hours in order to detect bacterial and fungal contamination respectively [12]. After incubation, cultures in nutrient broth were streaked onto nutrient agar, macConkey agar

and mannitol salt agar while the cultures from sabouraud dextrose broth were streaked onto sabouraud dextrose agar.

1.7. Statistical Analysis:

The values of %Pb and %Cd were expressed as Mean ±standard deviation (SD). Statistical analyses were carried out using Duncan Multiple Range test[13]. In all cases probability level of 95% was taken as significant.

III. Results And Discussion

In this study, ten kohl samples were tested for lead and cadmium contents, one of them was found to contain more than 70% lead, four samples contained more than 50% lead, six samples contained more than 30%, three contained less than 20% lead, and just one sample was free of lead as shown in table 2. These results showed that the kohl samples contained high levels of lead. None of the samples had labeling that indicated a list of ingredients or lead content. Without laboratory analysis, it is impossible for the consumer to distinguish kohl products with a high content of lead from those with a low content when purchasing these products.

The concentration of cadmium in kohl samples ranges between 0.02 %-16.87% for samples 1 to 4; generally the concentration of cadmium in kohl samples is much less than lead concentration as illustrated in table 2 and figure 1. It was generally noticed that samples of kohl collected from different markets in Tripoli contained a very high concentration of lead reach to more than 76%, while the concentration of cadmium reach to about 17%. As shown in figure 3, sample 10 has the highest concentration of lead (76.41%, powdered stone sample), while the highest concentration of cadmium has found in sample 4 (16.87%). Figure 3 showed that samples 1, 2, 5 and 8 contain traces of cadmium. All samples have high concentration of lead except just one sample comparing with[14, 15].

The results presented in table 3 indicated that there is no evidence for contamination of kohl samples by fungi. Samples 7, 8 were free from bacterial contamination while other samples on nutrient agar visually confirm presence of large, spreading, dry and irregularly shaped colony that further evaluated by staining techniques and identified as gram positive rod shaped arranged in chains, spore forming which indicates presence of bacillus spp.

According to microbiological analysis, presence of bacillus spp associated with these kohl products could be attributed to their natural source. These kohl products applied onto eyes; therefore presence of those bacteria or their metabolites could constitute a health hazard and might cause ocular problems with repetitive use.

IV. Conclusion

In this study, we affirm the presence of trace of lead and cadmium elements in some kohl products sold in Tripoli markets in Libya. The results of this study showed that the concentration of lead and cadmium in investigated kohl samples are high which might be a one of the major public health hazards especially for women, neonatal and children. The high levels of lead in most tested kohl samples can cause lead poisoning or impregnation of regular kohl users. In addition, our results indicate a need for establishing safe intake values of heavy metals in cosmetic products and point out the importance of their analysis before they reach the consumer.

Concerning the microbial contamination screening, for 10 kohl products analyzed in this study, 8 were generally found to be contaminated with gram positive *bacillus* species.

Acknowledgment

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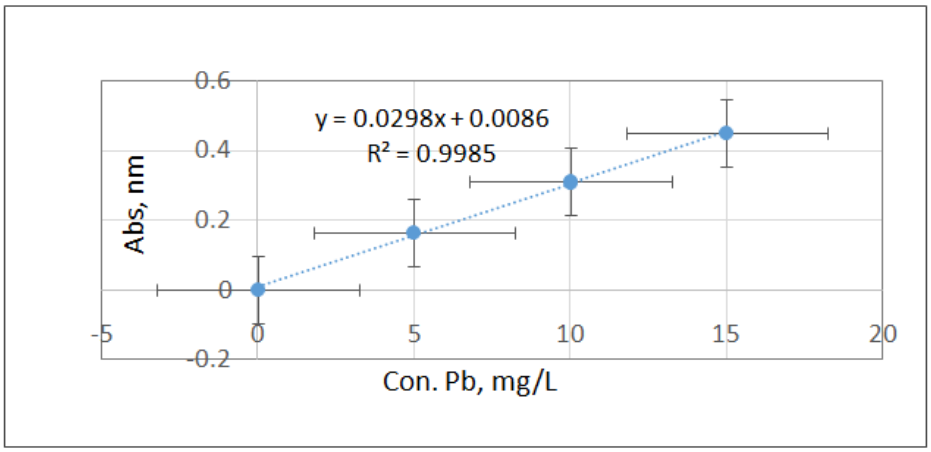
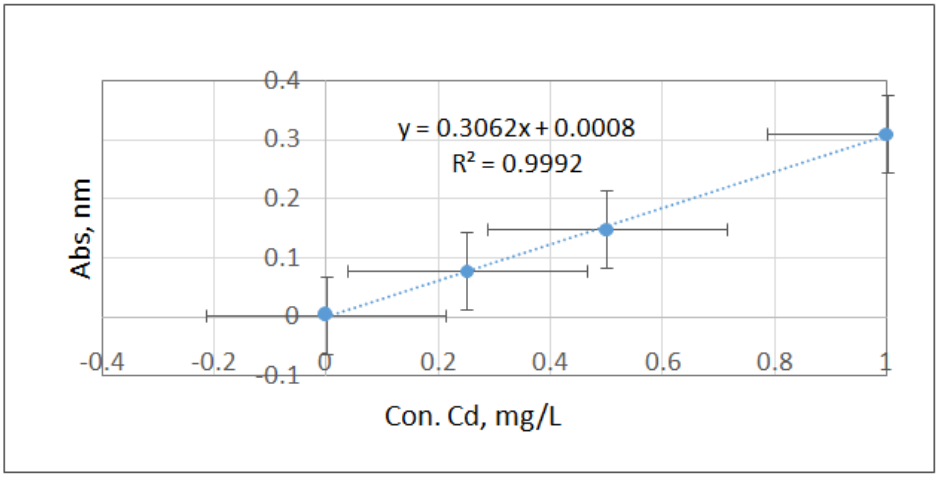
Supplementary

Figure S1: Different brands of kohl samples used in this study.



FigureS2: Standard curve of lead in kohl samples



FigureS3: Standard curve of cadmium in kohl samples

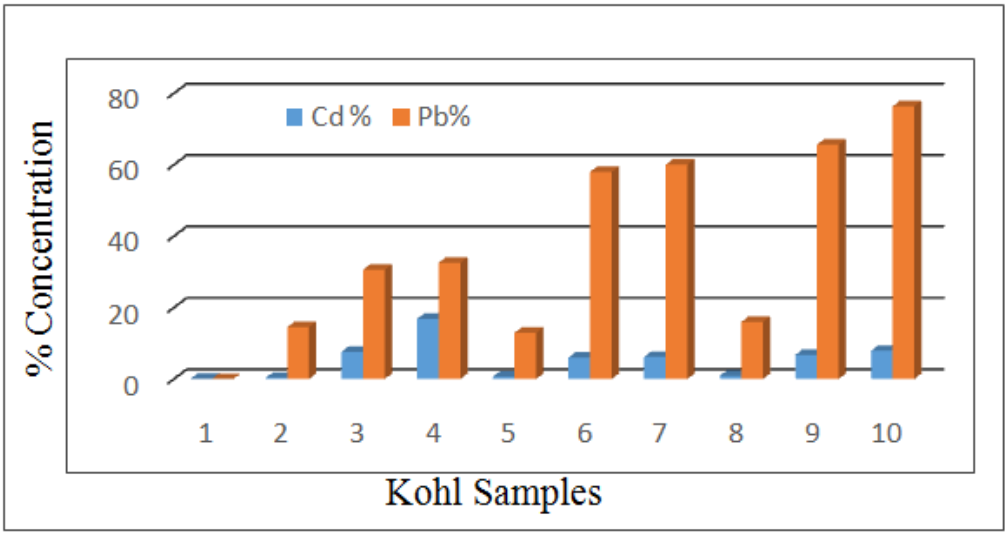


Figure 1: Concentrations of lead and cadmium in kohl samples

Table 1: Instrumental conditions for determination of lead and cadmium by FAAS

Parameter	***Pb***	***Cd***	***Parameter***	***Pb***	***Cd***
Wavelength (nm)	217.0	228.8	Lamp current (mA)	10.0	4.0
Slit width(nm)	1.0	0.5	Lamp type	Pb, HCl	Cd, HCl
Flame composition	C_2H_2/Air	C_2H_2/Air	Burner height (mm)	13.5	13.5
Burner type (mm)	50	50	Background correction	D_2-lamp BC on	D_2-lamp

Table 2: Percent contents of lead and cadmium in the tested kohl samples

Sample No.	**Appearance/ texture**	**Color**	**Origin**	**Pb % content in 1g kohl**	**Cd % content in 1g kohl**
1	powder	pale grey	Pakistan	0.004±0.006	0.02±0.04
2	powder	black	India	14.57±0.18	0.36±0.13
3	powder	grey	Saudi Arabia	30.60±1.20	7.65±0.88
4	powder	brown	Yemen	32.59±1.62	16.87±1.34
5	powder	reddish brown	Saudi Arabia	12.92±0.98	0.65±0.44
6	powder	grey	Unknown	58.00±2.45	6.00±1.32
7	powder	black	Pakistan	60.03±2.78	6.21±1.45
8	sticky paste	black	Pakistan	16.00±1.43	0.87±0.32
9	powder	dark grey	Saudi Arabia	65.64±3.11	6.79±2.10
10	powdered stone	silver	Unknown	76.41±4.21	7.91±2.31

Values mean (n=6) + S.D

Table 3: Identification and isolation of microorganisms present in tested kohl samples

Sample No	Sabouraud dextrose agar	Mannitol-salt agar	Mac-conkey agar	Nutrient agar	Isolated microorganism
1	-ve	-ve	-ve	+ve	*Bacillus spp*
2	-ve	-ve	-ve	+ve	*Bacillus spp*
3	-ve	-ve	-ve	+ve	*Bacillus spp*
4	-ve	-ve	-ve	+ve	*Bacillsspp*
5	-ve	-ve	-ve	+ve	*Bacillus spp*
6	-ve	-ve	-ve	+ve	*Bacillus spp*
7	-ve	-ve	-ve	-ve	*No growth*
8	-ve	-ve	-ve	-ve	*No growth*
9	-ve	-ve	-ve	+ve	*Bacillus spp*
10	-ve	-ve	-ve	+ve	*Bacillus spp*