World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

ASSESSMENT OF LEAD AND CADMIUM IN POWDERED EYE SHADOW SOLD IN LOCALLY MARKETED IN BAQUBA, DIYALA, IRAQ.

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Article history:

Received: February 1st 2022
Accepted: March 1st 2022
Published: April 14th 2022

Abstract:

It has been proven that many consumer products such as cosmetics are contaminated with varying levels of heavy metals. So, in this study we evaluated the level of heavy metals like lead(Pb) and cadmium(Cd) in cosmetic products sold in locally marketed in Baquba, Diyala, Iraq. The cosmetic items included Eye Shadow. The eye makeup was analyzed in triplicate using aflame atomic absorption spectrophotometer (FAAS). After they have been analyzed. The results revealed that the level of Lead in the samples ranged from (<0.01 – 8.99) ppm and cadmium ranged from (<0.01 –1.58) ppm. The results were compared with international standards and were found to be within the limits set by the cosmetic standards of WHO, Canada and US FDA. The results from this study has shown that the daily use of these eye shadow exposes users to some level of concentration of these heavy metals which could constitute potential health hazard due to their ability to bio-accumulate. Therefore, that good manufacturing practice of cosmetic products should be applied in order to ensure the absence of harmful levels of impurities in the ingredients. Antibacterial activity shows that the (un-use) intact eye shadow samples had an inhibitory effect on *Staphylococcus epidermidis* and *Escherichia coli* bacteria used, while some samples (in- use) had an effect of increasing the activity of *Staphylococcus epidermidis* bacteria, For the same brands. We conclude from this study that consumers contribute to contamination of their eye make-up, and this is what was shown by the results of biological activity.

Keywords: Environmental pollution, Eye shadow, Lead, Cadmium, Health hazards, Anti-bacteria

1. INTRODUCTION

Environmental pollution is one of the main issues facing human society and pollution with heavy metals is one of the essential problems that pose a major threat to human health and the environment [1]. There are more than twenty heavy metals, but four are namely Lead, Cadmium, Mercury, and Arsenic of particular concern to human health [2, 3]. These heavy metals are released to environmental from a number of sources, including industrial sources, like mining and smelting operations, and agricultural sources, including the use of pesticides and phosphate fertilizers containing percentages of heavy metals[4]. Colored cosmetics are considering another source that contribute to the release of heavy metals into the environment and the biological system of human [5]. Cosmetic products are divided into two types: the one which is left on the skin and the other is rinse off the skin [6]. It has been reported that cosmetics contain heavy metals like lead and cadmium as impurities[7] [8]. Most likely these impurities entered the cosmetics as a result of the use low quality ingredients, in adequate purification of raw materials, along with the pollution of the metallic devices used during the manufacturing process[9, 10]. Eye shadow is a suitable example of colored cosmetics, that is applied to the eyelid using a soft brush that gently passes over the skin [9]. Talc, mica, coloring agents, preservatives and binder are the main ingredients in eye shadow powder. Mica and talc formed about 30% of the ingredients[11]. Dermal exposure is the most important route for cosmetics, as the majority of cosmetics are applied to the skin [12]. Assessment of dermal absorption depending on several factors, including age (for example, children are exposed to heavy metal toxicity more than adults), concentration of heavy metals in the products, the quantity of product applied on the skin, the length of time left and the effect of penetration enhancers and emollients in the cosmetics[13]. Although absorption through the skin is in small quantities, the slow release of these metals causes high damage in the biological system of

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

the human body if it is allowed to accumulate. These metals can accumulate in the body due to their long half-life and have a negative effect[1, 12]. Like lead which is a dangerous environmental contaminant and it is found as impurities in cosmetics. Some manufactures would add lead compounds because it make the skin smoother and shinier[3]. And it has a harmful effects on adult females, pregnant and lactating women [14]. Increased levels of lead in adult females due to hormonal changes and infertility, in Pregnant women can pass through the umbilical cord and pass to the fetus, causing birth defects, baby low weight, premature birth, and in extreme cases, miscarriage. While in lactating women, it can be passed on to newborns by breastfeeding and retained in the bones, causing osteoporosis, increased nervous behavior, and affecting intelligence quotient (IQ), impaired vitamin D metabolism, anemia, kidney damage and lack of attention capacity [15-17]. And it may even cause cancer when excessive lead accumulates in the human body[18]. Similarly to lead, cadmium is one of the most toxic metals[16]. Found as impurities in cosmetics. And there is no safe level of cadmium exposure, it has many health effects as Spartan abdominal irritation, diarrhea and vomiting, while contact to low levels for an extensive time can cause bone deformity, kidney damage, and the capability of bones to breakdown smoothly[19]. Because its affects calcium metabolism [2] .While excessive exposure to cadmium (Cd) would affect lung function and increase the risk of lung cancer[20]. It is considered to be "carcinogenic to humans" by the IARC(International Agency of Research on Cancer)[21]. In addition to the above, recent research has indicated that approximately 8 to 15% skin diseases such as eczemas and allergic contact dermatitis are caused by the use of cosmetics contaminated with heavy metal[22, 23]. Therefore, there is need for moderation in the topical application of these products to avoid dermal absorption and bioaccumulation to harmful levels.

This work was aimed to determine the concentration of (Pb) and (Cd) in different brands of eye shadow, and study the anti-bacterial activity (intact and in-use) of the same brands that were purchased from the local markets from Baquba, Diyala, Iraq.

2. MATERIAL AND METHODS

2. 1 . Collection of Samples

Forty samples of eye shadow powdered with different colors were purchased from shops in Baquba, Diyala, Iraq. its divided into eight groups, which represented eight different brands (HUDA BEAUTY, NUDE, CaTRICe, Diamond Beauty, MAC, Flormar, NOTE, NARS) samples were imported from China, P.R.C, USA, and Turkey. as written on the label. The information of these items used in this study is summarized in Table 1.

Table .1: *information of eye shadow*

No.	Samples Code	Brand name of eye shadow	Origin country	Color
1	A1	HUDA BEAUTY	China	Purple
2	A2			Pink
3	A3			Black
4	A4			Rose
5	A5			Golden
6	B1	NUDE	China	White
7	B2			Yellow
8	B3			Crimson
9	B4			Violet
10	B5			Brown
11	C1	CaTRIce	P.R.C	Light Brown
12	C2			Dark Purple
13	C3			Green
14	C4			Dark magenta
15	C5			Dark Blue
16	D1	Diamond Beauty	China	Mauve
17	D2			Plum
18	D3			Orange

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

19	D4			Violet Red
20	D5			Chocolate
21	E1	MAC	USA	Gray
22	E2			Brown
23	E3			Pink
24	E4			White
25	E5			Rose
26	F1	Flormar	Turkey	Green
27	F2			Violet
28	F3			Dark Red
29	F4			Chocolate
30	F5			Orange
31	G1	NOTE	Turkey	Silver
32	G2			Black
33	G3			Plum
34	G4			Blue
35	G5			White
36	H1	NARS	China	Turquoise
37	H2			Pink
38	H3			Blue
39	H4			White
40	H5			Salmon

2.2. Preparation of standard solution

Standard solutions of Lead (Pb) and Cadmium (Cd) were prepared from 1000 ppm Standard Stock Solution of GFS Fishers' AAS Reference Standard. The stock solutions were serially diluted to give concentrations of calibration curve for lead into the range of 0, 0.5, 1, 2,4,6,8 to 10 ppm while 0, 0.25,0.5,1, 1.5, to 2 ppm for cadmium[12].

2.3. Preparation and digestion of samples

In acid digestion for the determination of lead and cadmium according to the method described by Muhamad *et al.*[5], approximately 0.50 g of sample was weighed using sensitive balance was digested with 10 ml of mixture of concentrated nitric acid (HNO3): 1:3 (HCL) Hydrochloric acid. Then heated for half hour on a hot plate. After digestion, the samples were allowed to cool to room temperature and 10 mL deionized water was added, mixed well, and made a volume up to 50 mL in volumetric flask then the samples were filtered (use whatman filter paper No.41) and the sample solutions were analyzed for Pb and Cd using FAAS (model: AA-7000 (SHIMADZU-Japan)). The parameters of the instrument were listed in Table 2.

Table . 2: *Operating parameters of the atomic absorption spectrophotometer used in the heavy metal analysis.*

Operating parameters	Metals	
	Lead (Pb)	Cadmium (Cd)
Wavelength (nm)	283.30	228.80
Slit width (nm)	0.70	0.70
Sensitivity check (mg/L)	10.00	0.75
Lamp current (mA)	5.00	2.00
Flame type	Air-Acetylene	Air-Acetylene

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Volume-9, April 2022
ISSN: 2749-3644

3. ANTI-BACTERIAL ASSAY.

To examine the anti-bacterial activity of eye shadow from microbial contamination were selected by Gram positive *(Staphylococcus epidermidis)* and Gram negative *(Escherichia coli)* bacteria. The bacteria *(S. epidermidis)* was selected because it is the most species of bacteria found on the skin of the eyelid[24]. Antibacterial activity was determined for eight samples of (intact) eye shadow. which were used in this study as a one sample of each brand and compared with samples of (in-use) eyes shadow for the same brands, by agar well diffusion method described by Parekh et al. [25].In this method, pure isolate of 24hour to growth was cultured in Muller-Hinton Agar plate (HiMedia) by using sterile swab to achieve a confluent growth. A cork-borer of diameter (5.0) mm was used to make well in each agar plates. Each well was filled with eye shadow suspension volume (100 µl) from each sample by micro-pipette into (HiMedia). The plates were incubated for 24hour at 37°C. To determine the antibacterial activity of each material by measuring the zone of inhibition by millimeters. Each experiment was performed in a triplicate, then mean value was used.

4. RESULTS AND DISCUSSION

This study showed the concentration of heavy metals (lead and cadmium) in the lipstick samples in average of three repetitions are given in Table 3. The results are reported as the concentration for each metal.

Table. 3: *Concentrations of (Pb) and (Cd) in eye shadow.*

Samples	Lead (ppm)	Cadmium (ppm)
A1	ND	0.410
A2	0.660	0.091
A3	2.501	0.521
A4	0.918	ND
A5	3.221	1.101
B1	ND	0.921
B2	0.901	0.551
B3	0.777	0.087
B4	ND	0.560
B5	5.331	0.077
C1	0.431	0.055
C2	0.995	0.661
C3	1.448	0.701
C4	0.741	0.333
C5	ND	1.580
D1	2.234	0.882
D2	3.086	0.802
D3	8.990	ND
D4	1.125	0.504
D5	0.99	0.760
E1	2.281	1.009
E2	6.001	0.856
E3	0.651	0.091
E4	ND	1.033
E5	1.455	0.099
F1	2.003	0.583
F2	2.414	0.312
F3	3.898	0.593
F4	0.702	1.111
F5	7.810	0.087
G1	0.585	ND
G2	3.313	1.022

World Bulletin of Public Health (WBPH)
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Volume-9, April 2022
ISSN: 2749-3644

G3	0.603	0.909
G4	2.046	1.201
G5	1.956	0.966
H1	ND	0.777
H2	2.002	0.502
H3	0.623	1.372
H4	ND	0.055
H5	1.334	0.989

*ND: not detected

Lead and cadmium are the most heavy metals detected in cosmetics. These minerals are just impurities that are not intentionally added to the product[15]. Therefore, the World Health Organization (WHO), Germany, Canada Heath and United States Food and Drug Administration (US FDA), warned against excessive exposure to these metals as a result of everyday use of cosmetics. To this end, limits have been set to control the presence of lead and cadmium in cosmetics. table 4 shows the various regulatory bodies as well as the corresponding limits for lead and cadmium metals.

Table 4: *- International permissible limits of the heavy metals in cosmetic products[18, 26, 27].*

Country	Lead (ppm)	Cadmium (ppm)
WHO	2.00	2.00
Germany	0.500	0.100
Canada	10.00	3.00
(US FDA)	20.00	3.00

Lead (Pb) is a heavy metal that is not supposed to have it in our body[10]. The level of lead in eye shadow samples used in our study ranged between (<0.01 – 8.99) ppm. Seven of the samples were not detected with lead (Pb). This conforms with the European union act which prohibits the preserve of lead in cosmetics. While the highest concentration of lead (8.99, 7.81) ppm was found in orange shades (D3, F5) they were from Diamond beauty and Flormar brand. This is due to the use of a large amount of lead chromate as a pigment in this samples [28]. While cadmium (Cd) is also harmful metal[29]. Its concentration ranged between (<0.01 – 1.58) ppm. Cadmium in three samples was not detected. This is consistent with the European Union prohibits the presence of cadmium in cosmetics[30]. While the highest concentration of cadmium (1.58) ppm was found in the dark blue eye shadow (C5) of the CaTRIce brand[28]. T-test was performed at (95%) confidence. To find out the effects of eye shadow color on the amount of metal used. The results were indicating t_{stat}(3.72) is greater than t_{crit} (2.02), and this indicates that the colors depended on the presence of a particular metal[31, 32].

In table4 show the concentration of lead(Pb) and cadmium(Cd) in different brand of eye shadow as mean± SD.

Table. 4: *Concentrations (mean ± SD) of lead and cadmium in different brands of eye shadow.*

Brand of eye shadow	Mean±SD (Pb)	Mean±SD (Cd)
HUDA BEAUTY(A)	1.46±1.35	0.42±0.44

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

NUDE (B)	1.40±2.23	0.44±0.36
CaTRIce (C)	0.72±0.55	0.67±0.57
Diamond Beauty (D)	3.29±3.30	0.59±0.36
MAC (E)	2.08±2.35	0.62±0.48
Flormar (F)	3.37±2.73	0.54±0.38
NOTE (G)	1.7±1.14	0.82±0.47
NARS (H)	0.79±0.87	0.73±0.49

Data presented in table 4 reveals the mean ± SD of lead and cadmium content in different brands of powdered eyes shadow samples. For lead, it is turned out that the lowest mean of lead was detected in CaTRIce brand, while the highest mean of lead was detected in Flormar brands of eye shadows. However, lead values used in this study did not exceed the health Canada 10ppm and US FDA 20ppm permissible limit for lead as impurities in cosmetics. While 77% of the samples exceeded the Germany limits for cosmetics.

As for cadmium where it turned out that the lowest mean of cadmium was detected in HUDA BEAUTY brand, while the highest mean of cadmium was detected in NOTE brands of eye shadows .However cadmium levels used in this study did not exceed 3 ppm which is the permissible cadmium level in cosmetics according to health Canada and (US FDA) establishment. While 65% of the samples exceeded the Germany limits for cosmetics. In generally, in all brand of eye shadow used in this study levels of lead were higher than levels of cadmium but, it is still less than the permissible limit.

Though the lack of detection of Pb and Cd in some samples because of analytical detection limit, but this does not mean the absence of these metals at all. Previous studies show different levels of lead and cadmium in eye shadow cosmetics. Nourmoradi et al. found the level of lead in some brand of eye shadow ranged of 0.085 to 6.90 ppm[33]. Al-Saleh et al. conducted a study on lead in eye shadows and found that the level of lead ranged between 0.42 to 58.7ppm. Only one eye shadow product exceeded the 20 ppm limit permitted by the US Food and Drug Administration for lead as an impurity[10]. Sainio et al. detected the content of lead was less than (20 ppm) in all brands of eye shadow [34]. Riyadh Jihad. reported that the content of lead in eye shadow range of 6.8 to 9.68 mg/kg[35]. These studies indicate the importance of determining the level of heavy metals in cosmetic. other study conducted on 40 samples of eye shadows of different brands using (FAAS) technique, found the levels of lead ranged from (0.0 to 25.57 ppm). Four samples contained lead at levels above 10 ppm, and two samples had lead at levels above 20 ppm. Which is the permissible limit for lead as impurities in cosmetics by Health Canada and US FDA respectively. These study reported by Nibras et al [12]. The found of cadmium in cosmetics such as eye cosmetics, has many harmful effects on the human body as a result of the absorption of cadmium by the skin through the union of cadmium ions with the sulfhydryl radical in the epidermal keratin [36, 37]. Nourmoradi et al. reported that concentration of cadmium in some brands of eyeshadow range of (1.54–55.59) ppm[33]. Riyadh Jihad. reported that concentration of cadmium in eye shadow range of (0.25 to 0.31) mg/kg[35]. other study conducted on 40 samples of eyes shadow of different brands using (FAAS) technique, found the levels of cadmium ranged from (0.0 to 8.14) ppm. five samples were higher than 3ppm permissible limit for cadmium as impurities in cosmetics by health Canada and US FDA .These study reported by Nibras et al [12]. When comparing our current study with previous

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

studies, it was found that the amount of lead and cadmium in our current study is similar to the values found in the literature. In general, the results of our current study were less than the international standard for heavy metals in cosmetics.

5. ANTIBACTERIAL EFFECT

The antibacterial effect was examined for eight samples eye shadow used in this study, one sample for each brand against two species of bacteria by using agar well-diffusion method

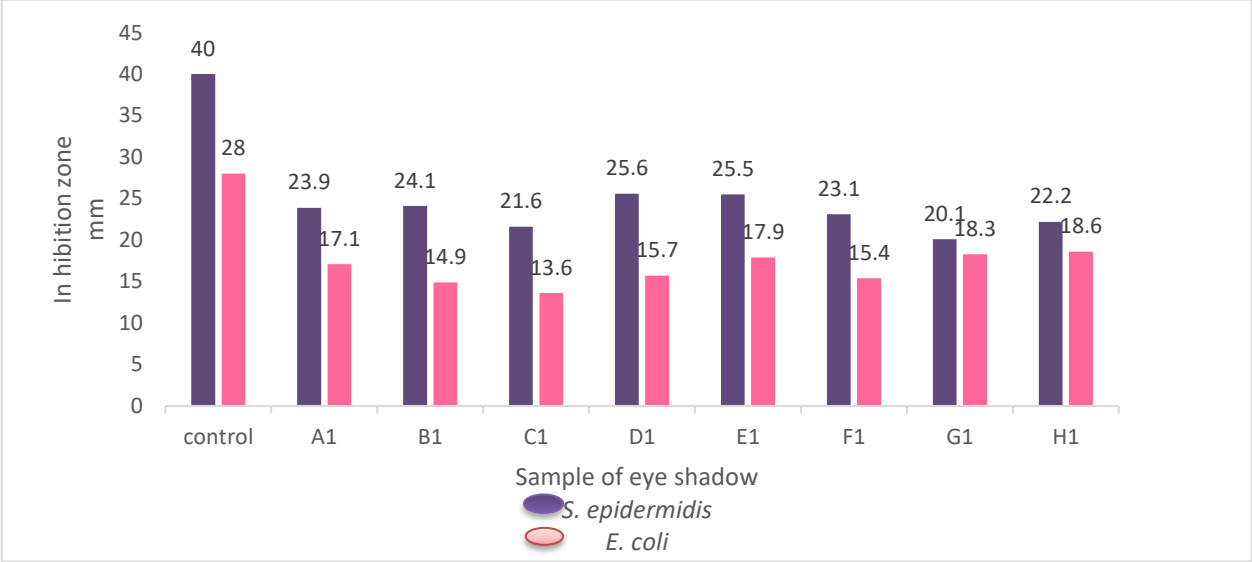

Figure 1:- Antibacterial activity for eight (intact) eyes shadow samples

The result of the antibacterial activity test shown in the figure 1 that all samples (A1-H1) of (intact) eye shadow had an inhibitory effect on the growth of *E-coli* and *S- epidermidis* bacteria. The reason for this is due to its eye shadow composion that contains preservatives such as parabens as one of its main ingredients, and preservatives are added to cosmetics to prevent contamination with microorganisms, keep products in good condition and increase their shelf life[38]. The results of biological activity were compared with other samples(a-h) for same brands of eyes shadow (in-use) by some subjects to estimate the antimicrobial effect.

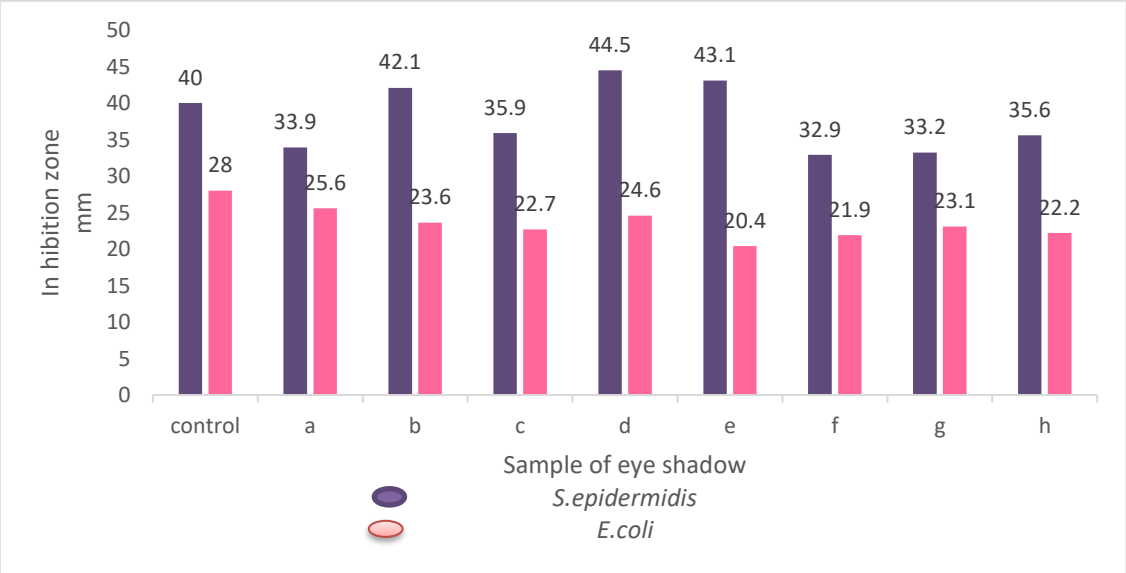

Figure 2:- Antibacterial activity for eight (in-use) eyes shadow samples

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

The results show in figure 2 indicated that all samples (a-h) of eye shadow (in used) had an inhibitory effect on the growth of *E- coli* bacteria. While in the case of bacteria *S-epidermidis*, most of the samples had an inhibitory effect, while the samples (b, d, e) had increase in the activity of bacteria. This is due to several factors that contributed to the introduced of pollutants into cosmetics, including that Eye shadow contains many inorganic and organic ingredients, and this makes it a suitable environment for the growth of microbes especially when preservatives have become damaged[39]. Talc is considered one of the main ingredients in eye shadows. When the product is exposed to air, it absorbs ammonia and carbon dioxide gases, which are nutrients for bacteria[11]. Also consumers have a role in contaminating their eye cosmetics during use. This is due to the use of contaminated brushes or as a result of using a contaminated finger to apply eye shadow on the eyelid, which leads to contamination of the eye shadow box. As a result, the eye suffers from serious infections. According to a study conducted by Notoma et al. to determine the microorganisms activity of 15 brands (approximately 1345 samples) of in-used eyes shadows , the results showed that 67% of the samples. It was contaminated with one or more species of microorganisms including the Micrococcus, Staphylococcus, Corynebacterium[39]. Other study was conducted by El-Bazza et al. to assess the bacterial contamination of eye shadows. In this study, forty samples (intact and in-use) were used. It was found that 26 samples were contaminated with different species of bacteria Staphylococcus aureus (55.3) %, Staphylococcus epidermidis (13.2)%, Bacillus megaterium (21.1)% and Klebsiella pneumoniae (10.5)% [40].

6. CONCLUSION

The application of the flame atomic absorption spectroscopy (FAAS) technique allowed the quantification of heavy metals in eye shadow imported from different brands . That is sold in locally marketed in in Baquba, Diyala, Iraq. The results obtained in this study show in some samples, lead and cadmium were not detected due to the detection limit of the analytical procedure this does not mean that these metals were completely absent, and in the other samples, different brands, orange eye shadows were found to have the highest concentration of lead, while blue eye shadows had the highest concentration of cadmium. Although the levels of these heavy metals in the tested samples are not on the extreme, frequent use of this eye shadow could result in the bioaccumulation of the heavy metals on the skin, leading to health hazards especially for those metals causing allergic contact dermatitis. As a result, it is important that these heavy metals be reduced to the barest minimum or removed from eye shadow products totally where possible as they have little or no benefits to human health. Thus, Careful selection of the raw materials can contribute to reducing the proportion of heavy metals in cosmetics and this, in turn increases their safety and reduces their environmental impact. The results of the bacterial activity were indicating an inhibition in the growth of bacteria when using unused eye shadow samples. While in the case of eye shadow samples under use, there was an activation in the activity of some types of bacteria.

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World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-9, April 2022
ISSN: 2749-3644

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