Nigerian Journal of Pharmaceutical and Applied Science Research, 8(2):120-129 May 2019 (ISSN 1485-8059 Available at www.nijophasr.com

Quantitative Assessment of Heavy Metals and Microbial Contaminants in some Lipsticks in Western Part of Nigeria

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ABSTRACT.

Lipsticks are part of cosmetics many women use to improve on their outlook but may constitute a health hazard if varying concentration of heavy metals and pathogenic microorganisms are associated with their use. This study was carried out to investigate the concentration of heavy metals and bacterial load of associated microorganisms present in some lipstick samples. Ten lipstick samples from five different brands were purchased from Ijebu-Ode, Ogun State, Nigeria. Each sample was prepared for analysis using 12.5 mls of nitric acid, 1.00 ml of sulphuric acid (H_2SO_4) and 2 ml of perchloric acid ($HClO_4$) and digested electronically .The heavy metals analysis was carried out by Flame atomic absorption spectrophotometer. The microbial loads in each lipstick samples were determined using standard procedure. The results showed that Cobalt, Lead, Nickel, Copper and Cadmium were present in all the lipstick samples with concentrations of Cobalt ranges between 29.3 ppm to 10.8 ppm while that of Lead ranges between 56.4 ppm to 11.5 ppm and Nickel ranges between 57.7 ppm to 12.8 ppm only Copper and Cadmium being within WHO permissible limits. The samples were contaminated with *Staphylococcus aureus* and *Escherichia coli* and the total aerobic bacteria counts for all samples were similar and were above WHO permissible limits. This study revealed that the use of some cosmetic product could expose the users to low concentration of toxic heavy metals and pathogenic microorganisms which constitute potential health risk.

Keywords: Lipsticks, heavy metals and pathogenic microorganisms.

INTRODUCTION

Although cosmetics, including Lipstick, were originally developed by the Egyptians as ointments and ritual oils for the dead, they eventually came to be used to soothe, adorn, accentuate and treat the skin of the living. But in recent times, they are used by over 90% of the female population for fashion and beautification. More recently, cosmetics have been associated with medicines and the products are now frequently advertised as having healing properties (Mulhern *et al*, 2003). Lipsticks in particular emerged from a jumble of primitive ingredients such as vermilion - a naturally occurring ore of mercury (red mercury (II) sulphide HgS), seaweed and mulberry, into the sophisticated products used today, containing mainly oils, fats and waxes (Cohen and Kozlowski, 1998).The choice of ingredients used to produce a cosmetic will depend on the desired colour, glossiness, and indelibility of the manufacturer. A single stick of lipstick may contain several hundreds of different chemical compounds, but there are a few substances and compounds whose inclusion is essential. A good lipstick must possess an ideal minimum and maximum thixotropy. Its viscosity must be high enough in order to produce a moulded stick product, however, being thixotropic it must undergo a reduction in viscosity when mechanically distributed via spreading on application at 32^0C (Lips temperature) to produce a smooth even layer on the lips with minimum pressure (Salvador and Chisvert, 2007). It should be of such composition as to cover only the portion of the lips up to the vermillion border and not bleed into the surrounding skin regions. Waxes, oils, pigment and dyes make up the bulk of lipstick's composition. Waxes are perhaps the most important, as they are crucial for the structure and shape of the lipstick. Waxes are water resistant materials made up of various substances including hydrocarbons (alkanes and alkenes, branched or normal), ketones, alcohols, aldehydes, sterol, esters, alkanoic acids, terpenes and monoesters with molecular chain length ranging from C_{12} to C_{38}. A range of different naturally occurring waxes can be utilized with Beeswax being commonly a major constituent.. Another type of wax used is Carnauba wax, obtained from the Brazilian Carnauba Palm. Apart from Beeswax, other waxes can include Candelilla wax obtained from the Mexican Candelilla shrub, and lanolin, a wax secreted by the glands of woolen animals. Though, they primarily provide the structure of the lipstick, these waxes can also impart other useful properties:

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they can act as emulsifying agents to help bind together the other ingredients, and can also impart glossiness on application of the lipstick (Adebajo, 2002). Another important component of lipsticks is oils. The most commonly used one is castor oil which can often comprise the largest percentage of the lipstick make-up, but others, such as olive oil, mineral, lalolin, jojoba and vegetable oil can also be used. The oils give the lipstick emollient or skin-softening properties; they also make application of the lipstick easier, and contribute glossiness to its appearance. Additionally, they act as solvents for soluble dyes used in the lipstick, or dispersing agents for any insoluble pigments. Oils in general are treated with BHT - Butylatedhydroxytoluene, an anti-oxidant used to extend the shelf life and delay rancidity of oils and fats in foods and cosmetics (Amparo and Alberto, 2007). The pigments and dyes, though they make up only a minor percentage of the lipstick's composition, are certainly the most important, as they impart the colour of the lipstick. Pigments are coloured compounds that are insoluble. Pigments give lipstick its colour and covering power. The concentration of pure pigment can vary from 1% to 10% depending on the type of product (lip gloss to a dark lipstick). The most widely used pigments are mineral (titanium and iron oxides) and organic pigments (true pigments, toners and lakes) (Amparo and Alberto, 2007), whilst dyes are more commonly either liquids themselves, or soluble. The manner in which they provide colour can also vary. Carmine red, also known as carminic acid, is a common red pigment, which is derived from cochineal bugs, a variety of scale insects that live on cacti. It is prepared by boiling the insect bodies in ammonia or sodium carbonate solution, filtering, and then adding hydrated potassium aluminium sulfate (more commonly known as alum). Another common colour imparting component is a compound called Eosin. Eosin is a fluorescein dye produced by reacting bromine with flourescein. There are two types used as pigments ; Eosin Y and Eosin B. Eosin Y is a tetrabromo derivative of fluorescein which produces a purple stain while Eosin B is a dibromodinitro derivative which produces a yellow- red stain. Eosin is a dye that actually subtly changes its colour when applied. In the lipstick, it is red, with a slightly blue tinge; when it is applied, however, it reacts with the amine groups found in proteins in the skin, and this reaction causes its colour to intensify to become a deeper red. Another benefit of this reaction is that it makes the dye indelible, or long-lasting. Of course, red is not the only lipstick colour, and in order to achieve the wide range of colours available today, other pigments and dyes are needed, of which there are a variety. Additionally, other compounds can be added in order to alter the intensity of the red coloured pigments and dyes. Titanium dioxide, a white compound in isolation, is a common addition, which can be added to red dyes in varying amounts to produce a range of pink coloured lipstick–(Amparo and Alberto, 2007).The aims of this work are to determine the concentration of heavy metals and microbial contamination in lipsticks with a view to assessing the potential risks that such cosmetics may pose to consumers and to create awareness among the consumers that some of these cosmetic products contain heavy metals that are detrimental to human health. It is also to serve as a caveat to manufacturers in placing in markets spurious cosmetic products liable to cause damage to human health when applied under normal conditions of use.

MATERIALS AND METHODS.

Sample Collection

Ten (10) different lipstick samples of the most commonly used brands of lipsticks were purchased from retail shops at New Market in Ijebu Ode, Ogun State, Western part of Nigeria. A total of ten samples from five different brands (each of two different colours) were selected for this study as shown in Table 1.

Sample digestion for Heavy Metal Analysis

This was carried out according to the method of Adepoju-Bello *et al*., (2012).

All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid for 24 hr, cleaned thoroughly with distilled water and dried in such a manner to ensure that any contamination does not occur. 1 g of each sample was carefully weighed into digestion test tube; 12.5 ml of nitric acid, 1 ml of sulphuric acid (H_2SO_4) and 2 ml of perchloric acid ($HClO_4$) was added to each sample and digested electronically using Buchi Digestion unit k-424. The digestion was carried out at low heat at first, followed by increase in temperature. Each sample was digested until the solution turns to colourless which indicate the completion of the digestion. On completion of digestion, the digested samples were allowed to cool at room temperature and the samples were filtered using Whatman number 1 filter paper into a 100 ml volumetric flask. Each filtrate was made up to 100 ml with distilled water.

Table 1 List of lipstick samples with their codes and colour.

Sample Code	Name of Lipstick	Colour
1	IB	Pink
2	IB	Red
3	Jk	Red
4	JK	Pink
5	JD	Red
6	JD	Pink
7	ML	Red
8	ML	Pink
9	TL	Pink
10	TL	Red

Quantification of heavy metals.
The concentration of heavy metals i.e. Cobalt, Lead, Cadmium, Nickel and Copper in each filtrate was measured using a Buck scientific Atomic absorption Spectrophotometer.

Instrumentation
ASS instrument (Buck scientific Atomic absorption Spectrophotometer, Model 210/211 VGP) consisting of a hollow cathode lamp, slit width of 0.7nm and an air acetylene flame was used for heavy metal quantification in the sample. The samples were analyzed for five heavy metals namely Cobalt, Cadmium, Lead, Nickel, Copper at wavelength of 240.7nm, 228.9 nm, 283.2 nm, 341.5 nm and 324.7nm respectively.

Media Preparation for Microbiological Screening
The media used (Nutrient Agar Medium) was weighed accurately and sterilized according to the manufacturer's instruction. After sterilization, the media was transferred aseptically into a sterile disposable Petri dish and allowed to cool.

Sample Preparation for Microbiological Screening
This was carried out according to method of Kasim *et al*., (2011).

Each 1.00 g of the lipstick sample was weighed and crushed in a mortar and pestle. 1ml of distilled water was pipetted into the mortar and thoroughly mixed with the lipstick for 20 minutes in order to disperse the likely organism present in the lipstick inside the distilled water. 1ml of the water was then pipetted into the first of the 9 tubes filled with 9ml of distilled water. 1ml of solution from the first test tube was also pipette and transferred into the second test tube and mixed thoroughly. This method was applied to the remaining test tubes containing 9ml of distilled water. This method is called the serial dilution method. After the serial dilution, 0.1 ml of dilution factors of 10^3 and 10^5 were then inoculated onto the prepared agar plates and incubated for 24 hours at 37^0C. These processes were repeated for all the samples. After incubation, the number of colonies was counted and the bacterial load was expressed in terms of colony forming units (CFU) per ml. For the identification of the micro organism, Gram staining and biochemical identification tests were done.

Statistical analysis
The experimental data were presented as Mean ± Standard deviation (SD). and analyzed using the Student's 't' test and a P value $\leq$ 0.05 was considered to be statistically significant.

RESULTS

Standard Calibration Curves

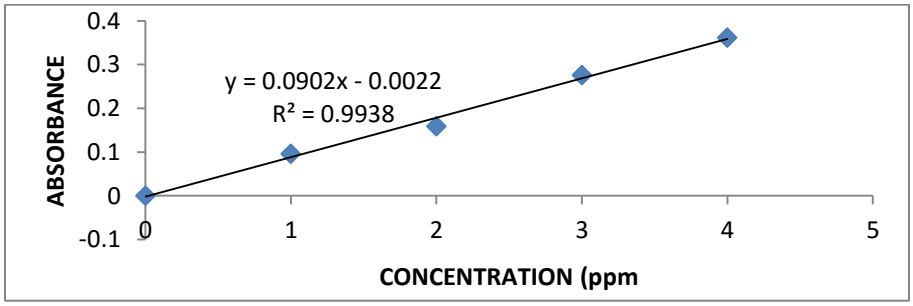

Fig. 1. Calibration Curve for Cobalt

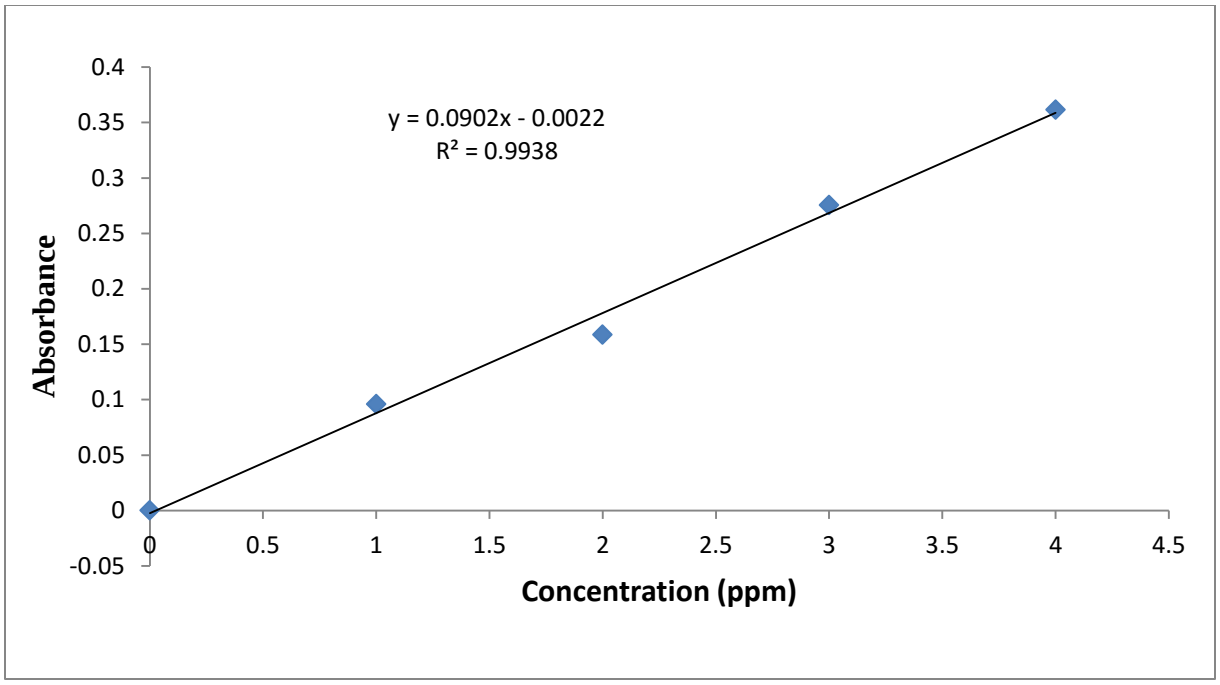

Fig.2.Calibration Curve for Lead

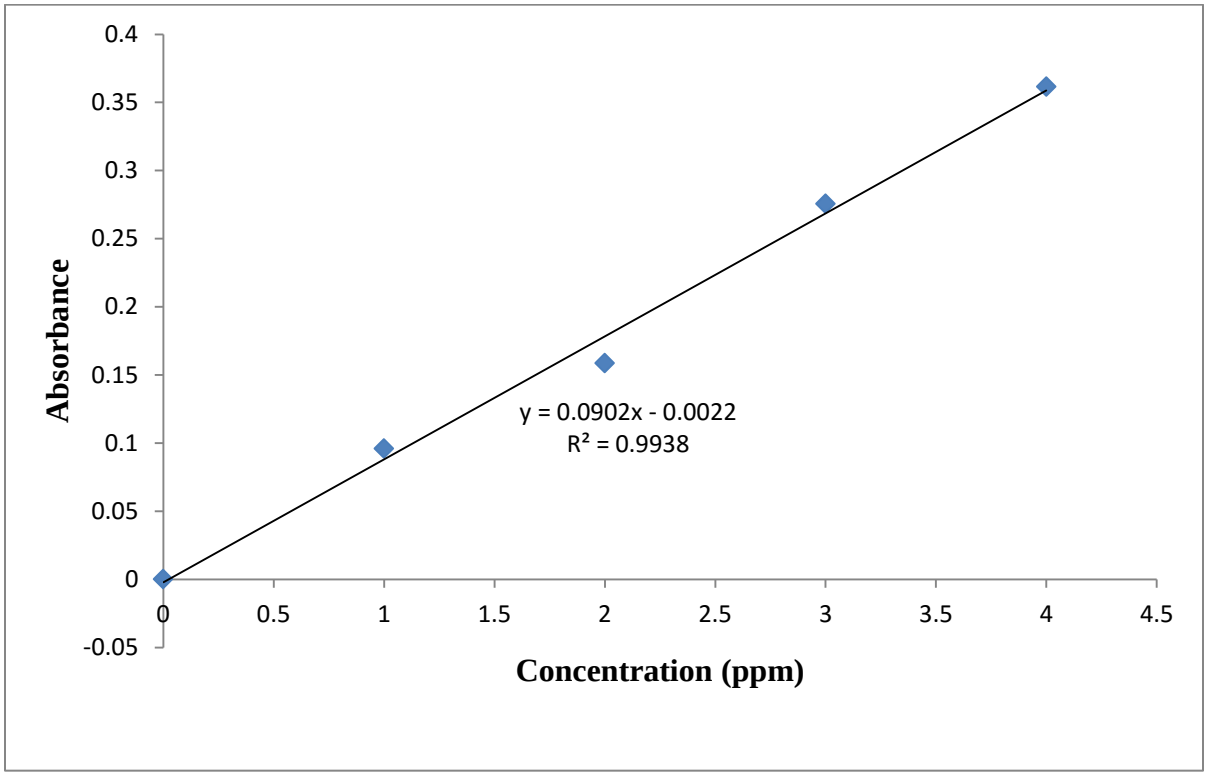

Fig. 3 Calibration Curve for Nickel

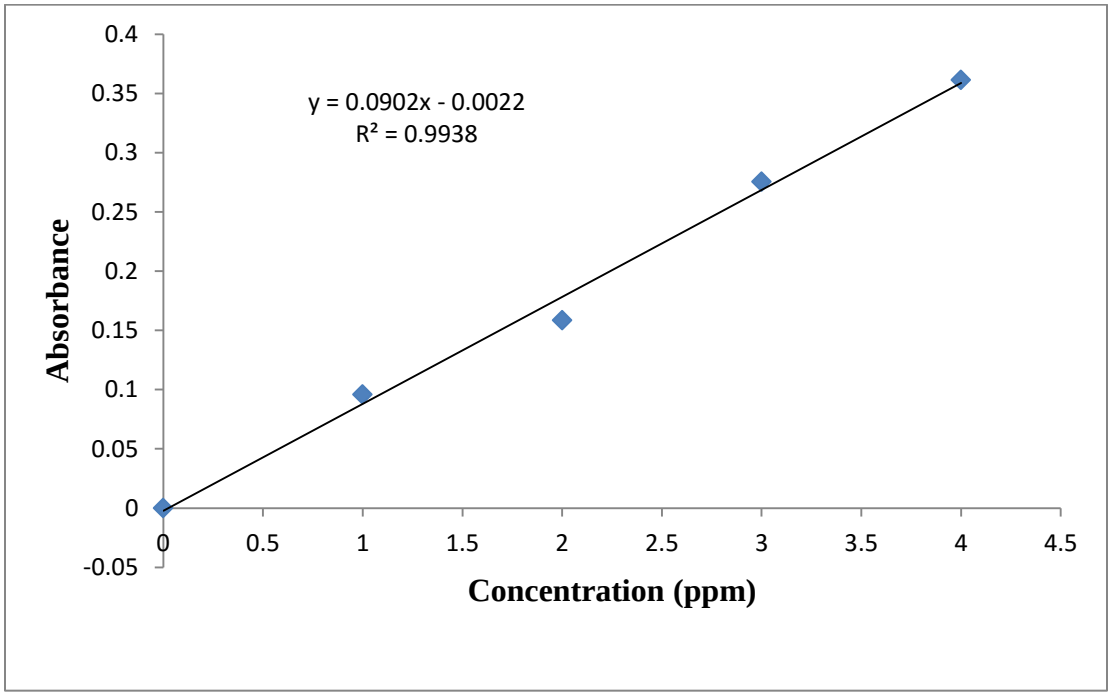

Fig. 4 Calibration Curve for Copper

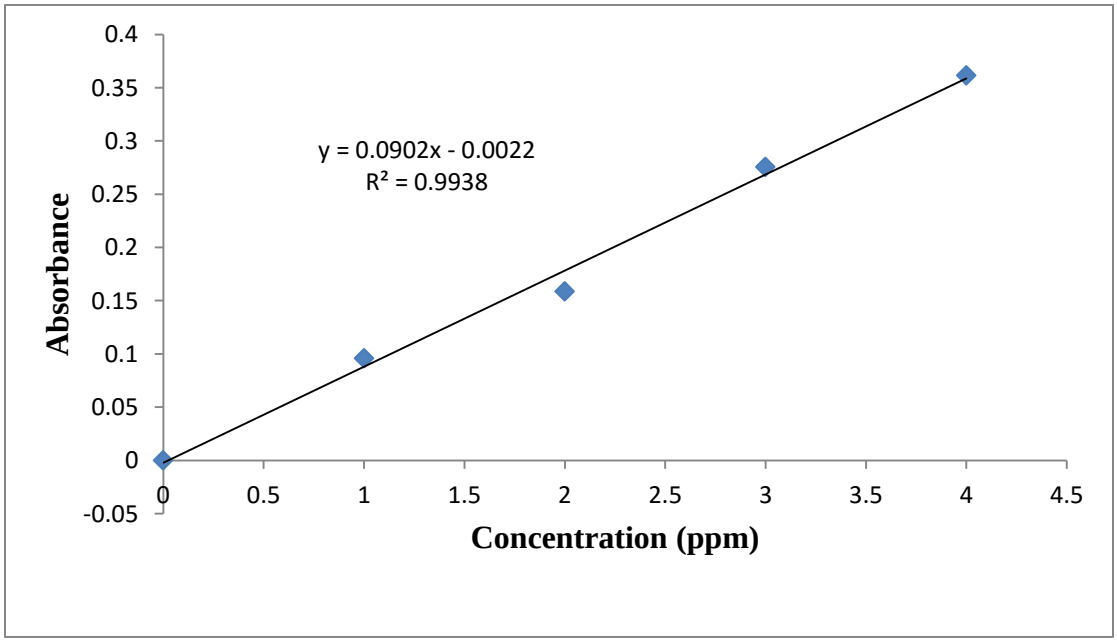

Fig. 5 Calibration Curve for Cadmium

Table ; 2. The Mean ± SD. of heavy metal concentrations in red and pink lipsticks.

		RED ppm	PINK ppm
Cobalt	IB	14.7±0.6*	10.8 ± 0.1*
	MI	15.6±0.6*	29.1 ± 0.3*
	Jk	23.6±0.6*	20.3 ± 0.2*
	JR	21.7±0.4*	29.3 ± 0.2*
	TL	16.7 ± 0.6*	21.6 ± 0.2*
Lead	IB	55.6±12.4*	42.7 ± 0.2*
	MI	9.7±0.7*	11.5 ± 0.1*
	Jk	13.7±0.2*	37.2 ± 1.5*
	JR	27.3± 1.6*	23.3 ± 0.4*
	TL	56.4 ± 0.8*	55.9 ± 1.3*
Nickel	IB	31.6 ± 0.9*	57.7 ± 0.6*
	MI	12.8 ± .2*	24.8 ± 0.6*
	JK	51.7 ± 0.3*	44.0 ± 0.1*
	JR	39.4 ± 1.1*	48.7 ± 0.8*
	TL	56.2 ± 0.9*	44.3 ± 1.3*
Copper	IB	15.8 ± 0.1	17.9 ±0.4
	MI	21.8 ± 0.4	19.8 ± 0.5
	JK	18.6 ± 0.3	18.4 ± 0.3
	JR	18.9 ± 0.3	17.6 ± 0.8
	TL	20.8 ± 0.6	25.0 ± 0.6
Cadmium	IB	0.5 ± 0.1	2.0 ± 0.1
	MI	0.9 ± 0.1	1.6 ± 0.1
	Jk	2.1 ± 0.1	1.9 ± 0.1
	JR	4.0 ± 0.3	1.0 ± 0.1
	TL	1.0 ± 0.1	1.6 ± 0.1

Keys;* = Significant $P \leq 0.05$; SD = Standard Deviation

Results of Microbial screening of the Lipstick samples After 24 hours of incubation at 37^0C, all the inoculated plates showed signs of growth.

Table 3; Total Bacterial Load of Micro organism Isolated.

Sample Code	Name of Lipstick	Colour	No of Colonies		Total Bacteria Load	
			10^1	10^2	10^1	10^2
1	IB	Pink	50	30	5.0×10^3	3.0×10^4
2	IB	Red	62	40	6.2×10^3	4.0×10^4
3	JK	Red	60	37	6.0×10^3	3.7×10^4
4	JK	Pink	52	35	5.2×10^3	3.5×10^4
5	JD	Red	78	40	7.8×10^3	4.0×10^4
6	JD	Pink	68	30	6.8×10^3	3.0×10^4
7	ML	Red	60	41	6.0×10^3	4.1×10^4
8	ML	Pink	59	38	5.9×10^3	3.8×10^4
9	TL	Pink	70	32	7.0×10^3	3.2×10^4
10	TL	Red	72	40	7.2×10^3	4.0×10^4

Table 4; Biochemical analysis of isolates

Sample Code	Name of Lipstick	Colour	Catalase Test	Indole Test	*MR	*VP	*TSIAIA	Citrate	Gas Production	Micro organism Confirmed
1	IB	Pink	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
2	IB	Red	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
3	JK	Red	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
4	JK	Pink	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
5	JD	Red	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
6	JD	Pink	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
7	ML	Red	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
8	ML	Pink	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*E. coli*
9	TL	Pink	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*
10	TL	Red	+ve	-ve	+ve	+ve	+ve	-ve	+ve	*S.aureus*

Keys;

* Triple sugar iron agar (TSIA) Test; * Methyl Red Test (MR); * Vogues-Proskauer Test(VP Test) +ve = positive, -ve =negative

DISCUSSION

Cosmetics are seen as one of the most important sources of releasing heavy metals into the environment and the human biological system. Following such 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 exposure to low levels of heavy metals can cause certain kinds of cancers (Henson and Chedrese, 2004).. Also, the possibility of skin allergy and contact dermatitis may increase due to the presence of heavy metals in cosmetics (Reed, 2007).

The result of heavy metal analysis in the Ten lipstick samples (5 red and 5 pink) were presented in Table 2. The concentrations of Cobalt in red and pink lipstick were as shown table 2. The concentration of cobalt in pink lipstick was found to follow this trend; JD (29.3)>ML (29.1)>TL (21.6)>JK (20.3)>IB (10.8) ppm while that of red lipstick followed this trend; JK (23.6)>JD (21.7)>TL (16.7)>ML (15.6)>IB (14.7) ppm as shown in Table 2 which all exceed the permissible limits for Cobalt which is 5ppm as stated by the International Purity Specifications (IPS) of the Food and Drug Administration (FDA). JD was found to have the highest concentration of Cobalt in the pink lipstick samples while JL was found to have the highest concentration of cobalt in the red lipstick samples. IB was also found to have the lowest level of cobalt in both the red and pink lipsticks.

The concentration of Lead in pink coloured lipstick samples follow this trend; TL (55.9)>IB (42.7)>JK (37.2)>JD (23.3)>ML (11.5) ppm as shown in Table 2 while the concentration of Lead in red coloured lipstick samples follow this trend; TL (56.4)>IB

(55.6)>JD (27.3)>JK (13.7)>ML. It was notable that across the brands, there were significant variations P ≤ 0.5 in the concentration of Lead. ML showed far lower concentration in both the red and pink lipstick samples which is within the permissible limit while others exceeded the permissible limit for lead which is 20 ppm as stated by the International Purity Specifications (IPS) of the FDA. This means that while some brands are doing exceptionally well in keeping down Lead concentration levels in their products, other brands are doing little in this respect. Higher levels of Pb have been reported in cosmetics by some researchers. Ulah*et al*, (2013) discovered ≤ 42.03 ppm of Pb in lipsticks samples; Sah in 2012 also discovered ≤ 145 mg/kg in lipstick samples. Odoemelam and Ibezim (2010) reported higher level of Pb in lipsticks procured from markets in Nigeria to be 14.2 – 369.9 mg/kg while Ullah *et al. in* 2013 also found lipsticks samples in Pakistan to contain higher level of Pb (141.6 mg/kg) also Popoola *et al*. (2013) likewise discovered low level of Pb in lipstick samples which was 3.74mg/kg. The concentration of Nickel in red and pink coloured lipstick samples were presented in as shown in Table 2. The concentration of Nickel in red coloured samples follows this trend; TL (56.2)>JK (51.7)>JD (39.4)>IB (31.6)>ML (12.8) while the level of Nickel in pink coloured lipstick samples follows this trend; IB (57.7)>JD (48.7)>JK(44.0)>TL (44.3)>ML (24.8) as shown in Table 2, which all exceeded the permissible limits for Nickel which is 5ppm as stated by the international purity specifications (IPS) of the FDA. TL was found to have the highest concentration of Nickel in the red lipstick samples while IB was found to have the highest concentration of Nickel in the pink lipstick samples. ML was found to have the lowest level of Nickel in both the red and pink lipsticks but all exceed the permissible limit. Different researchers have also reported varying levels of Nickel in lipstick samples for example Faruruwa *et al* in 2014 reported Nickel to be present in cosmetic samples from Kaduna metropolis. Ullah*et al* in 2013 also reported detectable amount of Nickel in lipsticks.

. The concentration of Copper in red coloured samples follows this trend; ML (21.8)>TL (20.8)>JD (18.9)>JK (18.6)>IB (15.8) while the concentration of Copper in pink coloured samples follows this trend; TL (25.0)>ML (19.8)>IB (17.9)>JK (18.4)>JD (17.6) as shown in Table 2. None of the brands tested have copper beyond the permissible limit of 50ppm as stated by the international purity specifications (IPS) of the FDA which means that most brands have managed to successfully eliminate this toxic metal from their products. The study reveals that there are variations across brands in meeting the permissible limit target for heavy metals. The result of the level of Copper in lipstick samples were similar to the result of Ullah*et al* in 2013 who reported 26.62mg/kg of Copper in lipstick samples. The concentration of Copper in all the samples analyzed though a bit high are below the permissible level (50ppm) set by US FDA has reported by Ulah*et al* in 2013.

. The level of Cadmium in the red coloured lipstick follows this trend; JD (4.0)>JK (2.1)>TL (1)>ML (0.9)>IB (0.5) while that of pink lipstick samples follow this trend; IB (2.0)>JK (1.9)>TL (1.6)>ML (1.6)>JD (1.0) as shown in Table 2. Cadmium was detected in trace amount in some samples. None of the brands tested have Cadmium beyond the permissible limit of 15 ppm as stated by the international purity specifications (IPS) of the FDA which means that most brands have managed to successfully eliminate this toxic metal from their products. The concentration of Cadmium was found to be the lowest in all the lipstick samples. This result was not in accordance with the findings of Odoemelam and Ibezim (2010) who reported higher level of Cadmium in lipsticks samples. This result was similar to the reports of many researchers who reported lower levels of Cadmiun in lipsticks samples ;(Popooola *et al*. 2013, Faruruwa *et al*. 2014; Muhammed and Hussein, 2014).

The heavy metals found in some of the products tested were categorized as unintentional contaminant. These metals were not intentionally added to the formulation but were simply impurities in the product and were therefore not required to be listed on the labels. An impurity is a substance not intentionally added to a product, but rather was either a byproduct of the manufacturing process, formed by the breakdown of ingredients or an environmental contaminant of raw ingredient (Ayenimo*et al*, 2010). The metals analyzed in this study were not listed as ingredients on any of the products. Due to lack of manufacturer testing and regulatory oversight, it is possible that the companies were not even aware that the products were contaminated with these toxic metals. These contaminants were likely to have gained entrance into the cosmetic products when poor ingredients were used.

Moreover, these toxic metals could have been contaminants from one or more of the inorganic base materials used in the manufacturing process.

Tables 3 and 4 above showed the result obtained for the microbiological screening of lipsticks. Table 3 reports the microbial counts of the Nutrient agar plate that were inoculated with aliquots of dilution factor 10^1 and 10^2. Visible colonies were counted on each plate after 24 hours after which the total microbial count was calculated. Results showed that Petri plate

of sample 4 had the highest number of colonies while Petri plate of sample 8 had the lowest number of colonies. The total aerobic bacteria counts for all the samples were similar which ranges between 10^3 - 10^4cfu/ml which was above the acceptable limit which is 10^3 as stated with the findings of Sawant and Varsha (2015). The isolation of *S. aureus* as the most predominant contaminant also tallies with the findings of Anavella (2004).

Based on the findings of this research work, the lipsticks analyzed were contaminated with bacteria. *Staphylococcus aureus* and *Escherichia coli* that are not allowed to be found in cosmetics as shown in Table 4 . and this can pose a serious health risk for consumers and causes spoilage of the product. Out of the 10 samples of lipstick analyzed, *S. aureus* was isolated from 9 (90%) of the samples while *E. coli* was isolated only in one of the lipstick samples (ML red). In a similar study, Sawant and Varsha (2015) reported the presence of bacterial contamination than fungal contamination. Several studies have also been done to investigate the presence of bacterial contamination in lipstick. Anavella in his study in 2004 found out that 34 samples out of 81 samples were found to be contaminated and the total aerobic bacteria count were between 10^4- 10^6.Anavella (2004) also discovered that the cosmetic samples had pathogenic micro organism and contained more than 10^3cfu/ml which could be deleterious to human health when consumed. Likewise, Omurdagand Abbasoghu, (2010) in their study observed the presence of microbial contamination in 5 samples of cosmetics, the total number of aerobic bacteria were found to be above the limit specified in monograph. They also observed that *Candida Spp*, *S. aureus* and *E. coli* that were not allowed to be found in cosmetics were found in them.

According to FDA data, most cases of contamination are due to manufacturers' using poorly designed, ineffective preservative systems and not testing the stability of the preservatives during the products customary shelf life and under normal condition for use. To control microbial growth and to stabilize any cosmetic product, some form of preservative should be added in order to inhibit the growth of contaminating micro organism during manufacturing, storage and use by consumers (Campana*et al*, 2006).

CONCLUSION

The study showed that some of the popular lipstick samples available in the market contain high levels of heavy metals (like Pb, Ni and Co), and were of poor microbiological quality since their bacterial load were above standards and also there were presence of microorganisms such as *S. aureus* and *E. coli* which may cause different kinds of disabilities and health problems in man on usage.

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