Original Research Article

Assessment of the Safety of Henna Cosmetics Sold in Lamu County- Kenya: Heavy Metals Components Levels with Respect to Standard Limits

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ABSTRACT

Henna painting is an art of beautification which is commonly practised by the people of Lamu County. In Lamu, henna products are sold either in powdered or paste form of different colours. However, henna user sometimes faces adverse skin reactions arising from heavy metal content of the henna products. Such metals include lead (Pb), Cadmium (Cd) and Nickel (Ni) which are toxic to both users and the environment. The level of these metals in henna products sold within Lamu County is not known. The aim of the study therefore, was to determine the level of Pb, Cd and Ni in henna cosmetic sold within Lamu County so as to assess their safety levels with respect to the standard limits. A total of 60 powdered and paste henna products from various brands were randomly selected and analyzed for heavy metals using Flame Atomic Absorption Spectroscopy (FAAS) after acid digestion of the samples. Analysis of variance (ANOVA) using SPSS version 17.0 was used to analyse the data. The lowest and highest mean levels (ppm) of heavy metals in the two categories of henna products were as follows: Powdered henna products: Pb (0.16±0.01 to 2.63±0.24), Cd (0.01±0.00 to 1.23±0.03) and Ni (0.13±0.01 to 2.73±0.09).Paste henna products: Pb (0.56±0.06 to 1.32±0.04), Cd (0.02±0.00 to 1.31±0.01) and Ni (0.14±0.02 to 3.01±0.09).Comparatively, no significant difference in the level of lead(p=0.065) and cadmium(p=0.577) was noted in paste and powdered henna brands. However, a significant difference in the level of nickel (p=0.021) was recorded in the two categories of henna. One of the paste black henna product (PABL) recorded the highest level of nickel at a concentration of 3.01±0.01ppm. Generally, the powdered henna products recorded an overall mean level of Cd and Pb below the limit of 2 ppm by KeBS as well as Ni below the limit of 1 ppm by Health Canada, hence the products are recommended for use. However, the overall mean level of Ni in paste henna products was above the recommended limit of 1 ppm by Health Canada, hence such products should not be used frequently since they expose the henna users to the toxic effects of Ni. The results of the study can be used to create awareness on the need to adopt good manufacturing practice so as to protect the henna users and the environment from the harmful effects of heavy metals. In addition, the henna users can be sensitized on the high level of exposure to heavy metals in various categories and colours of henna products with respect to the set limit by standard bodies.

Key words: KeBS, henna category, heavy metal, paste henna, powdered henna.

INTRODUCTION

Henna painting is an art which has been in practice worldwide for over 5000 years ago [1]. The ancient Egypt and its neighbouring countries practiced henna painting which later spread to Arabian countries and today, henna is used in all parts of the World [2].

Archaeological evidence indicates that henna was used in ancient Egypt to stain the toes of pharaohs prior to mummification. In countries where henna is deeply rooted in historical tradition, members of the working class more commonly applied henna for medicinal purposes as well as connection to the spirit[3]. For a long period of time, henna has been associated with special celebrations. Betrothals, weddings, birthdays and *Eids* for the Muslims are great occasions for henna use[4]. Henna has also contributed immensely in the field of medicine. Al-Arnaoutt*et al*. [3] reported in the book of “prophet medicine” that henna usage for medicinal purposes was a very common practice at the beginning of Islamic traditions. De-Almeida *et al*.[5] described naphthoquinones in henna as anti-inflammatory while other researchers described henna as fungicidal [6], virucidal [7], bactericidal[8] and anti-malarial[9].

“Henna” is English name which originated from Arabic name *hinna*. For the indigenous communities along the coastal strip of East Africa, it is known as *hina* which means life of beauty and happiness [4].

In Lamu county, henna painting forms an integral part of the historical and enriched culture which has been practiced for decades. In spite of the henna plants growing in many parts of the County, some henna users prefer the premixed henna products because they have a shorter staining period of between one to two hours compared to the traditional henna which takes longer to stain.

Henna users have different preferences for henna products depending on factors such as culture, religion and ethnicity. The natural colour of henna is red. However, in today’s World market, there are different colours of henna products such as black henna, chestnut henna and blonde henna, most of which are premixed henna[10].

Basically, henna products are sold either in powdered or paste form. The powdered forms of premixed henna contain all ingredients in the form of powder. However before using the powdered henna, it is first mixed with a solvent such as water to form a paste which helps it to stick when applied on hair, nails and skin[4].

Paste hennas are the “ready to use” type of hennas prepared by drying the henna leaves and grinding them to powder, and then this powder is mixed with oil or water to form the paste, whereas in paste henna the solvent is added by the henna manufacturer, in powdered henna the solvent is added by the henna user according to the manufacturer’s instructions [11]. The shelf life of paste henna products is usually shorter compared to powdered henna products hence paste hennas contain preservatives so that they can stay in good condition for long [4].

In addition, the different colours of premixed henna products give the henna users an opportunity to select colours of their choice [10].Some of the henna brands sold in cosmetic shops in Lamu County lack labels or may be misbranded. Such brands are suspected to be contrabands from unscrupulous henna manufacturers hence may contain toxic heavy metals which are hazardous to the henna users and the environment [12]. Some henna users within Lamu complained of itchy and painful sores on the skin after applying premixed henna products (personal communication).

However, based on chemical structure of henna dye, which belongs to a class of dyes called α-hydroxy-naphthoquinones, henna does not react to the skin except where there is naphthoquinone sensitivity [13]. That implies that the formulations of some premixed henna may consist of heavy metals and synthetic dyes such as *para*-Phenylenediamine (PPD) causing reactions to the skin. Such formulants cause toxic effects varying from mild effects such as irritation to more severe effects such as allergy which is manifested through blisters, lesions and sores on the skin [11]. Henna products have recorded different levels of heavy metals depending on their composition [14]. In Palestine, Al-Qutob and Azzatrash[15] recorded the highest cadmium level in henna products at a concentration of

13.36 ppm while Bernth et al, [16] from Denmark recorded a lower level of cadmium at a range of 0.01 ppm to 0.05 ppm. Therefore, different henna cosmetic products may have different levels of heavy metals depending on where it is manufactured.

The contamination caused by heavy metals in cosmetic products is an important environmental and health issue since the metals are toxic [17]. Some of the toxic effects of lead include haematological, cardio-vascular and neurobehavioural complications such as encephalopathy, depression and malaise [18]. Cadmium is known to trigger osteomalacia and respiratory tract effects such as pulmonary fibrosis, pneumonitis, emphysema and lung cancer [19]. Nickel is a strong allergen and it is known to be the main cause of skin sensitivity [20].

In some African countries, studies on heavy metal composition in henna products have been carried out [21], while in Kenya, limited studies have been reported. The study is necessary because henna products used in Lamu original from different parts of the world with different manufacturing processes. Hence, the research aims at finding out the levels of lead, cadmium and nickel in henna products used by Lamu residents and to compare the levels of these metals with the standard limits in order to ascertain the safety standards of henna products.

MATERIALS AND METHODS

Research design

The research adopted an experimental design which involved random sampling of paste and powdered henna products. Digestion of henna product followed a given protocol. Analysis of henna products for the level of heavy metals was done using a validated AAS instrument. Measurements in the AAS were replicated to ensure high precision. Variations in the levels of heavy metals were inferred using statistical measures. Certified values of reference material and the set limits of heavy metals by standard bodies formed the controls upon which the experimental values were compared to.

Study area

The study was done in Amu town which is located to the western part of Lamu County. Lamu is located to the north of Mombasa on latitude 2ºS and longitude 40ºE and occupies a total area of 6,474.4 km^2 that includes the mainland and over 65 islands that form the archipelago.

Sample size

A total of sixty (60) henna products were randomly selected and analyzed for lead, cadmium and nickel. The sample size was determined by an arbitrary sampling method shown in Equation 1.1.

$n=\sqrt{N}/2$……………………………………1.1

Where:

n = Sample size

N = Total population

Sampling and collection of henna products

Sixty (60) henna products were sampled out of which thirty-nine(39)were in powdered form while twenty-one (21) were in paste form which represented four (4)henna powdered brands and two (2)henna paste brands, respectively. Both the henna powder and henna paste products were purchased from four (4) randomly selected shops within Amu town. The henna products in each brand were sampled within a spectrum of 6 different colours as indicated in Table 1.1

Table 1.1: Henna products sampling

Brand of henna product	Category of henna brand	Colour of henna product
1 2 3 4	Powdered henna products (PW)	Black henna (BL) Red henna (RD) Brown henna (BR) Burgundy henna (BU) Chestnut henna (CH) Blonde henna (BD)
5 6	Paste henna products (PA)	

The henna brands were coded so as to protect the intellectual property rights of the manufacturer.

The most commonly applied henna products based on colour or categories from their respective brands were sampled under the assistance of henna pattern designer and henna shop attendant. Some colours of henna products from certain brands of henna were missing in some of the cosmetic shops. Only those henna products whose shelf life had not expired were sampled.

Reagents and solvents

All the reagents and chemical substances used were of analytical grade (AR) from Alpha chemicals. The reagents and chemical substances used were nitric acid, chloric acid, cadmium oxide, lead nitrate and nickel metal. Double distilled deionised water was used in dissolving the solids, dilution of solutions and rinsing of apparatus.

Cleaning of apparatus

The glassware, Teflon and plastic apparatus were soaked overnight in a soapless detergent in a plastic basin. The apparatus were then rinsed with double distilled deionised water and later soaked in 10% (v/v) concentrated HNO_3 for 3 days at room temperature. Thereafter, the apparatus were rinsed thoroughly with double distilled deionised water and then allowed to dry in a shaded open air space which was covered to prevent dust particles. The dried apparatus were later wrapped and stored in sealed polythene bags.

Pre-treatment of henna product

A particular henna product of the same brand, colour, category and batch number was sampled from each of the four (4) selected shops. A mass of 1.000g each of the sampled henna products from each cosmetic shop was measured to form a henna product set. In each set, the henna products were thoroughly mixed to form a homogenous mixture. For each set, a clean plastic spatula was used to scoop the henna from their packets into an air tight labeled Teflon container. All samples were stored at a room temperature, awaiting digestion.

Henna product treatment

Nitric acid-chloric acid wet digestion method was adopted to treat henna product because of its better recovery of lead, cadmium and nickel from henna products [22]. A mass of 1.000 g of henna product set was weighed using a BPS-4500-11 electronic balance from Adams equipment. To avoid cross contamination a clean plastic spatula was used each time a different set of henna sample was transferred from the Teflon containers. The weighed sample was put into a 50 ml beaker containing 6 ml of concentrated HNO_3 and the mixture was gradually heated to 80°C in a fume chamber until the mixture remained almost clear and was later cooled. Into the cooled mixture, 2 ml of 69% $HClO_4$ was added and the mixture was heated over water bath (direct heating of $HClO_4$ mixture can be explosive) and later was cooled. The process was repeated until white fumes of $HClO_4$ were produced. The sample was boiled until the final volume of the mixture was less than 5 ml. Thereafter, 10 ml of double distilled deionised water was added into the digestate and the mixture was later filtered using Whatman's No. 1 filter paper into a 50 ml volumetric flask which was then topped up to the mark. A blank digestate was prepared in the same way but without the sample.

Preparation of stock and standard solutions

Stock solutions of 1000 ppm of lead, cadmium and nickel were prepared from their analytical grade metal salt, metal oxide and pure metal, respectively[23].

Lead stock solution and its standards

Lead stock solution (1000 ppm) was prepared by dissolving 1.598 g of $Pb(NO_3)_2$ into 1litre of double distilled deionised water. Lead standard solutions of concentration range of 1.0, 2.0, 4.0, 8.0 and 10.0 ppm were prepared by serial dilution of the stock solution using the formula shown in Equation 1.2

$V_1C_1=V_2C_2$.. Eq 1.2

Where:

V_1– Initial volume

C_1 – Initial concentration

V_2– Final volume

C_2 – Final concentration

For instance, to prepare 50 ml of 4 ppm from the stock solution of lead, a working solution of 100 ml of 100 ppm lead solution was prepared using the formula given in Equation 3.2

$\mathbf{V_1 C_1 = V_2 C_2}$

V_1 x 1000ppm = 100ml x100ppm;

$$\text{Hence } V_1 = \frac{100\text{ml x } 100\text{ppm}}{1000\text{ppm}} = 10\text{ml}$$

The 10 ml of the lead stock solution (1000 ppm) obtained was diluted to 100 ml so as to prepare a working solution of 100 ppm lead solution. Using the working solution, the 50 ml of 4 ppm standard lead solution was prepared in the same way using Equation 1.2

Since, $\mathbf{V_1 C_1 = V_2 C_2}$

V_1 x 100ppm = 50ml x 4ppm;

$$\text{Hence} V_1 = \frac{50\text{ml x } 4\text{ppm}}{100\text{ppm}} = 2\text{ml}$$

The 2 ml of 100 ppm working solution of lead was diluted to 50 ml to prepare the 50 ml of 4 ppm standard solution of lead. Similar procedure was used to prepare the rest of the standard solutions of lead.

Cadmium stock solution and its standards

Cadmium stock solution (1000 ppm) was prepared by dissolving 1.142 g of cadmium oxide in 20ml of concentrated nitric acid and then topped up to 1litre. Cadmium standard solutions of concentration range of 0.1, 0.4, 0.6, 0.8, and 1 ppm were prepared by serial dilution of its stock solution using the same procedure as the lead standards.

Nickel stock solution and its standards

Nickel stock solution (1000 ppm) was prepared by dissolving 1.000 g of nickel in 20 ml hot nitric acid and then topped up to 1litre. Nickel standard solutions of concentration range of 0.2, 0.6, 1.0, 1.5 and 5.0 ppm were prepared by serial dilution of its stock solution using the same procedure as for the standard solutions of lead.

Preparation of standard reference material (SRM)

A certified standard reference material No. 1572 citrus leaves (CL) from NIST[24] was used. Its solution was prepared the same way as the henna sample.

Calibration curve and regression analysis

The prepared standards of each metal and their corresponding absorbencies were used to plot calibration curves. The product-moment correlation coefficient factor and the equation for the line of regression were derived from calibration curve of each metal.

Standard addition

The AAS was validated by spiking the samples with known standards and obtaining the percentage recovery as shown in Equation 1.3

$$\text{Percentage recovery} = \frac{SAS - SBS}{SS} \text{ x } 100 \text{........................ Eq 1.3}$$

Where:

SAS – Sample concentration after spiking

SBS – Sample concentration before spiking

SS – Standard used for spiking

Equal volumes of different concentrations of the sample solution were prepared and separately spiked with different amounts of the standard [25]. The absorbencies of all the sample solutions were measured in triplicate before and after spiking in order to obtain the percentage recovery.

Standard reference material

The accuracy of FAAS was tested using a certified standard reference material No. 1572 citrus leaves (CL) [24]. The absorbencies of the reference material were run in triplicate. The t-test critical values were compared with the calculated values so as to test the validity of the FAAS instrument.

Analysis of the sample by FAAS

Before the analysis, the FAAS was set at its optimum conditions. The blanks were aspirated into the flame and their absorbencies recorded. The limit of detection (LOD) was calculated by obtaining a mean of ten blanks plus three times the blank's standard deviation for each metal as shown in Equation 1.4

$y = y_B + 3s_B$.. Eq 1.4

Where:

y – Limit of detection

y_B – Blank signal

s_B – Standard deviation of the blank.

The standard solutions and sample solutions were separately nebulised into the flame and their absorbencies were recorded in triplicate. The absorbencies obtained with the samples were used to calculate the unknown concentrations of the metals by interpolation of their absorbencies on the calibration curve for each metal. The blank, standard and sample solutions were run in the AAS in an alternating manner so as to monitor the performance of the instrument.

FAAS instrumentation

The model of FAAS used in the analysis was Buck Scientific Model 210VGP. The optimised operational conditions of the machine are given in Table 1.2.

Table 1.2: Working parameters for FAAS

Operational conditions	Element		
	Lead	Cadmium	Nickel
Lamp current (mA)	5 to 10	7 to 15	5 to 10
Slit width (nm)	1.0	0.7	0.2
Wavelength (nm)	283.3	324.8	232.0
Flow rate (litres/mm)	1.5	1.5	1.5
Flame temperature (°C)	2300	2300	2300
Detection limit (µg/g)	0.01	0.001	0.005

Calculation of concentration of heavy metals in henna samples

The actual concentration of heavy metals in henna product obtained from AAS was derived using Equation 1.5

$$\text{Actual concentration} = \frac{\text{Concentration } (\mu g/ml) \times \text{Volume digested } (ml)}{\text{Weight of sample } (g)} \text{..........Eq 1.5}$$

For dilutions, the actual weight was obtained by multiplying the readout results from AAS with the dilution factor.

Data analysis

From the FAAS, concentrations of the heavy metals were recorded as triplicate measurement of their mean values with their corresponding standard deviations (SD). The variation of levels of lead, cadmium and nickel in the various henna samples was determined by a one-way ANOVA at 95% confidence level. In SPSS (Statistical package for social sciences) version 17.0, SNK was used to compute the variation of the levels of heavy metals in henna samples. A significant statistical test was set at $p = 0.05$ ($\alpha = 0.05$)

RESULTS AND DISCUSSION

The analytical results on the levels of heavy metals in henna products were subjected to statistical tests of significance and summarised in tables, linear and bar graphs.

AAS validation results

Calibration curves and regression analysis

Figure 1.1 shows the standard calibration curve for lead.

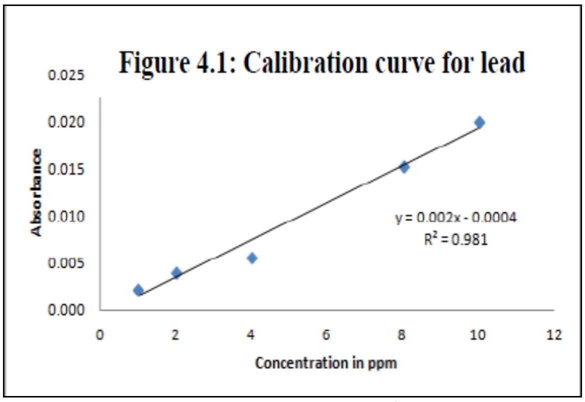

Figure 1.1: Calibration curve for lead

Followed by the Figures 4.1, 4.2 and 4.3, a positive correlation of absorbance versus concentration was observed. The correlation coefficient (R^2) values were more than 0.977 indicating linearity on the calibration curves hence good instrumental stability which is necessary for analyte determination [26]. The intercept of the curves were close to zero indicating minimum matrix interference and their slopes were above zero meaning that the instrument was sensitive. Followed by the Figures 4.1, 4.2 and 4.3, the curves were used to quantify the heavy metals in henna products [25].Results from the blanks recorded detection limits for lead, cadmium and nickel at 0.02 ppm, 0.005 ppm and 0.009 ppm, respectively. These limits were below 0.1 ppm hence appropriate for the analysis of heavy metals [27].

Figure 1.2 shows the standard calibration curve for cadmium.

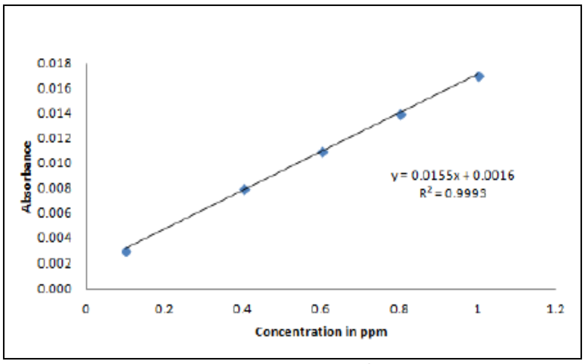

Figure 1.2: Calibration curve for cadmium.

Figure 1.3 shows the standard calibration curve for nickel.

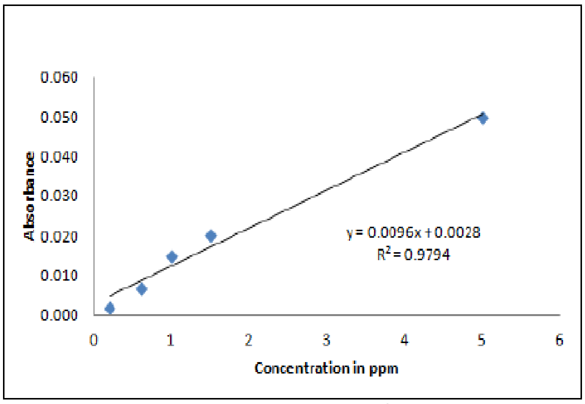

Figure 1.3: Calibration curve for nickel

Standard addition

Table 1.3 shows the percentage recovery results for lead, cadmium and nickel after spiking a sample concentration witha standard solution.

Table 1.3: Results for standard addition method

Element	Sample concentration before spiking. Mean±SD (μg/ml) (n=3)	Standard used for spiking (μg/ml)	Sample concentration after spiking Mean±SD (μg/ml) (n=3)	Percentage recovery (%)
Lead	2.12±0.02	50	51.90±0.02	99
Cadmium	0.3±0.01	30	29.89±0.01	98
Nickel	1.0±0.03	20	20.80±0.03	99

For lead, there was 99% recovery after spiking 2.12±0.02 μg/ml with 50 μg/ml of lead. For cadmium, there was 98% recovery after spiking 0.3±0.01 μg/ml with 30 μg/ml of cadmium. For nickel, there was 99% recovery after spiking 1.0±0.03 μg/ml with 20 μg/ml of nickel. Since the percentage recovery for all the metals were within the acceptable range of 96% to 105%, this indicated that the analytical method was accurate and reproducible.

Standard reference material

The lead, cadmium and nickel certified values of the reference material and their measured values using FAAS are given in Table 1.4.

Table 1.4: Results for standard reference citrus leaf (CL) material

Reference material	Element (n=3)		
	Lead Mean±SD (μg/g)	Cadmium Mean±SD (μg/g)	Nickel Mean±SD (μg/g)
Certified value (μg/g)	1.62±0.12	0.23±0.12	3.04±0.64
Measured value (μg/g)	1.59±0.27	0.21±0.35	2.98±0.42
Calculated t-test (|t|)	0.005	0.004	0.015
Critical value (υ=2)	4.303	4.303	4.303

From Table 1.4 the certified level for lead was 1.62±0.12μg/g while the measured value was 1.59±0.27 μg/g. The calculated |t| value for 2 degrees of freedom was 0.005, which was less than the critical value of |t| which was 4.303 at p=0.05. For cadmium and nickel, there was also no significant difference between the certified values and the values obtained using this method since the calculated |t| values were lower than the critical values at 95% confidence level. Since the t-test critical values were more than the calculated values it shows that the measured values were not significantly different from the certified values hence good accuracy of the instrument at 95% confidence level [25].

Heavy metals in henna products from Lamu

Level of lead, cadmium and nickel in powdered henna brands

Table 1.5: Mean level of lead, cadmium and nickel in powdered henna products from Lamu

Henna brand and the country of origin	Henna product	Concentration Mean±SD (µg/g) (n=3		
		Pb	Cd	Ni
1 (Indian origin)	PWRD	0.17±0.02[a]	0.06±0.01[a]	1.00±0.02[b]
	$PWRD_1$	1.30±0.02[b]	0.91±0.02[a]	BDL
	PWBR	1.01±0.03[b]	0.67±0.02[a]	0.79±0.03[a]
	PWCH	NA	NA	NA
	PWBD	NA	NA	NA
	PWBU	NA	NA	NA
	PWBL	0.17±0.02[a]	1.06±0.02[b]	0.13±0.01[a]
	p-value	p=0.057	p=0.065	p=0.577
2 (unknown)	PWRD	NA	NA	NA
	$PWRD_1$	NA	NA	NA
	PWBR	1.41±0.03[b]	BDL	0.39±0.08
	PWCH	0.91±0.03[b]	BDL	0.51±0.02
	PWBD	1.38±0.04[b]	0.01±0.00	0.63±0.02
	PWBU	0.16±0.01[a]	0.02±0.01	0.21±0.02
	PWBL	NA	NA	NA
	p-value	p=0.055	p=0.747	p=0.578
3 (unknown)	PWRD	2.02±0.02	0.85±0.02[a]	0.46±0.0[a]
	$PWRD_1$	NA	NA	NA
	PWBR	NA	NA	NA
	PWCH	NA	NA	NA
	PWBD	NA	NA	NA
	PWBU	NA	NA	NA
	PWBL	BDL	1.23±0.03[b]	1.59±0.0[b]
	p-value	-	p=0.065	p=0.055
4 (Pakistan origin)	PWRD	0.83±0.13[a]	0.16±0.01[b]	2.73±0.09[b]
	$PWRD_1$	2.63±0.24[c]	1.02±0.01[c]	0.40±0.01[a]
	PWBR	NA	NA	NA
	PWCH	1.55±0.08[b]	0.02±0.00[a]	0.17±0.01[a]
	PWBD	NA	NA	NA
	PWBU	NA	NA	NA
	PWBL	NA	NA	NA
	p-value	p=0.021	p=0.021	p<0.001

Mean values followed by the same small letter(s) within the same column in each brand do not differ significantly from one another (one-way ANOVA, SNK-test, α = 0.05). PW-Powdered henna. Henna colour codes RD-red, RD_1-deep red, BR-brown, CH-chestnut, BD- blonde, BU-burgundy and BL-black. NA-Not available. BDL-Below detection limit.

From Table 1.5, the level of lead in brand 1 ranged from 0.17±0.02 ppm in henna product PWRD and PWBL to a level of 1.30±0.02 ppm in henna product $PWRD_1$. In brand 2 the least level of lead was recorded at a concentration of 0.16±0.01 ppm in henna product PWBU while the highest level of 1.41±0.03 ppm was noted in henna product PWBR. In brand 3, only one henna product, PWRD, recorded lead at a concentration of 2.02±0.02 ppm. The highest significant level of lead was recorded at a level of 2.63±0.24 ppm in henna product $PWRD_1$ of brand 4. No significant difference in the level of lead was noted in henna products of brand 1 (p=0.057) and brand2 (p=0.055). However, a significant difference in the level of lead was noted in henna products of brand 4 ($p=0.021$).

Generally, the level of lead in powdered henna products ranged from BDL to 2.63±0.24 ppm. In brand 4, the level of lead in deep red-orange henna product $PWRD_1$ was significantly higher than in red henna product PWRD. However, the highest level of lead at a concentration of 2.63±0.24 ppm recorded from this study is lower than the level recorded by Chukwuma[21]in Nigeria at a concentration of 4.53 ppm. In Denmark, DEPA (2005) recorded the level of lead in powdered henna products at a concentration range of between 0.89 ppm to 2.0 ppm. This range almost compares to the range of BDL to 2.63±0.24 ppm of lead obtained from this study. From Table 4.5, the level of cadmium in brand 1 ranged from 0.06±0.01 ppm in henna product PWRD to a significant level of 1.06±0.02 ppm in henna product PWBL. In brand 2 only two products, PWBU and PWBD recorded cadmium at a level of 0.02±0.01 ppm and 0.01±0.01 ppm, respectively. Similarly, in brand 3, only two products were available and one of the products, PWBL recorded a significant level of cadmium at a concentration of 1.23±0.03 ppm. Brand 4 recorded cadmium level ranging from 0.02±0.0 ppm to a significant level of 1.02±0.00 ppm in the product PWCH and $PWRD_1$, respectively. The level of cadmium in henna products of brand 1($p=0.065$), brand 2 ($p=0.747$) and brand 3 ($p=0.065$) did not differ significantly while a significant difference in cadmium level was evident in henna products of brand 4 ($p=0.021$).

Generally, the level of cadmium in powered henna products ranged from BDL to 1.23±0.03ppm. The highest level of cadmium recorded at a concentration of 0.02±0.01 ppm in brand 2 falls within the range of 0.01 ppm to 0.05 ppm cadmium in powdered henna products recorded by DEPA [28]in Denmark. Among other roles, cadmium may be added to cosmetic products to give deep yellow to orange pigmentation. The biocumulative effect of cadmium results in impairment of the functions of major organs such as kidney and liver. This occurs after prolonged exposure to cadmium[29].

From Table 4.5, the level of nickel in brand 1 ranged from BDL to a significant level of 1.00±0.02 ppm in henna product PWBL and PWRD, respectively. In brand 2, henna product PWBU recorded the lowest nickel level at a concentration of 0.21±0.02 ppm while henna product PWBD recorded the highest level of nickel at a concentration of 0.63±0.02 ppm. In brand 3 only two henna products, PWRD and PWBL were available in the shops and their level of nickel were recorded at a concentration of 0.46±0.00 ppm and 1.59±0.00 ppm, respectively. In brand 4,the highest significant level of nickel at a concentration of 2.73±0.09 ppm was recorded in henna product PWRD. No significant difference in the level of nickel was noted in henna products of brand 1 ($p=0.577$), brand 2 ($p=0.578$) and brand 3 ($p=0.055$).However, the level of nickel differed significantly in products of brand 4 ($p<0.001$). Generally, the level of nickel in powdered henna brands ranged from BDL to 2.73±0.09 ppm. In Seoul Korea, Chung [30] analysed heavy metals in powdered henna products and recorded the highest level of nickel at 3.96 ppm which is slightly below the level of 2.73±0.09 ppm nickel obtained from this study.

Level of lead, cadmium and nickel in paste henna brands

Mean values followed by the same small letter(s) within the same column in each brand do not differ significantly from one another (one-way ANOVA, SNK-test, $\alpha = 0.05$).PA-Paste henna. Henna colour codes RD-red, RD_1-deep red, BR-brown, CH-chestnut, BD-blonde, BU-burgundy and BL-black. NA-Not available. BDL-Below detection limit.

From Table 1.6, henna product PABR and PARD of brand 5 and 6 recorded the highest level of lead at a concentration of1.32±0.04 ppm and 0.81±0.01 ppm, respectively. The level of lead in henna products of brand 5 (p=0.135) and brand 6 (p=0.578) did not differ significantly. Generally, the level of lead in paste henna products ranged from BDLto 1.32±0.04 ppm.

In brand 6, the highest significant level of cadmium was recorded in henna product PARD at a concentration of 1.31±0.01 ppm. In brand 5 (p=0.571) the level of cadmium in the henna products did not differ significantly while in brand 6 (p=0.021) there was a significant difference in the cadmium level. Generally, the level of cadmium in paste henna products ranged from BDL to 1.31±0.01 ppm.

Table 1.6: Mean level of lead, cadmium and nickel in paste henna products from Lamu

Henna brand and the country of origin	Henna product	Concentration Mean±SD (µg/g) (n=3)		
		Pb	Cd	Ni
5 (Indian origin)	PARD	1.03±0.04[b]	0.07±0.01	0.18±0.02[a]
	$PARD_1$	NA	NA	NA
	PABR	1.32±0.04[b]	0.21±0.02	1.79±0.01[b]
	PACH	NA	NA	NA
	PABD	BDL	BDL	0.55±0.02[a]
	PABU	NA	NA	NA
	PABL	0.56±0.06[a]	0.15±0.01	3.01±0.01[c]
	p-value	p=0.135	p=0.571	p<0.001
6 (Morocco origin)	PARD	0.81±0.01	1.31±0.01[ab]	2.42±0.02[b]
	$PARD_1$	NA	NA	NA
	PABR	0.64±0.01	0.70±0.01[a]	0.14±0.02[a]
	PACH	NA	NA	NA
	PABD	NA	NA	NA
	PABU	0.65±0.14	0.02±0.00[a]	0.88±0.01[a]
	PABL	NA	NA	NA
	p-value	p=0.578	p=0.021	p<0.001

Al-Qutob and Azzatrash [15] recorded the highest level of cadmium in paste henna products sampled from Palestine at a concentration of 13.36 ppm. This level was very high compared to the level of cadmium obtained from this study at a concentration of 1.31±0.01 ppm.

The highest significant level of nickel was recorded in henna product PABL of brand 5 and henna product PARD of brand 6 at a concentration of 3.01±0.01 ppm and 2.42±0.02 ppm, respectively. Another product, PABR of brand 5 also recorded a high level of nickel at a concentration of 1.79±0.01 ppm. Among all the paste henna products analyzed, none of them recorded nickel level below the detection limit. There was a significant difference (p<0.001) in the level of nickel in both brands 5 and 6.Generally, the level of nickel in paste henna products ranged from0.14±0.02 ppm to 3.01±0.01 ppm.

Tables 1.5 and 1.6 showed spurious levels of lead, cadmium and nickel in the henna products sampled from different brands. Since the type and levels of the metals were not indicated on the henna package, it is possible that the henna manufacturer was either avoiding the risk of a ban for his products since the metals were above the standard limit or was not aware of these metals in the products since they were added inadvertently. Differences in the level of heavy metals between the powdered henna products and paste henna products were evident.

Comparison of heavy metal level in powdered and paste henna brands

Results from Table 1.5 and 1.6 were used to derive the overall mean levels of heavy metals in paste and powdered henna brands. The overall mean levels were subjected to statistical tests of significance to show the pattern of variation of lead, cadmium and nickel in the two categories of henna brands as indicated in Figure 1.5

From Figure 1.4, the powdered henna brands recorded a higher overall mean level of lead at a concentration of 1.13 ppm compared to a level of 0.83 ppm recorded in paste henna brands.

However, paste henna brands recorded a higher significant overall mean level of nickel at a concentration of 1.28 ppm compared to 0.75 ppm recorded in powdered henna brands. In the two categories of henna, the levels of lead (p=0.065) and cadmium (p=0.577) did not differ significantly while the level of nickel (p=0.021) differed significantly.

The paste henna brands were more vulnerable to nickel exposure because their overall nickel content of1.28 ppm was above the recommended limit of 1 ppm (Health Canada) compared to the overall nickel level of 0.75 ppm recorded in powdered henna brands. Hence paste henna should not be used frequently since they expose one to the toxic effects of nickel.

The level of heavy metals in henna products vary with the state of henna matrix as indicated in the two categories of henna. The specific role of a heavy metal as a mordant in the henna product may depend on the powder or paste state of the matrix. For instance, the type of preservative used and the binding power in henna may determine the type of heavy metal used in a given henna matrix.

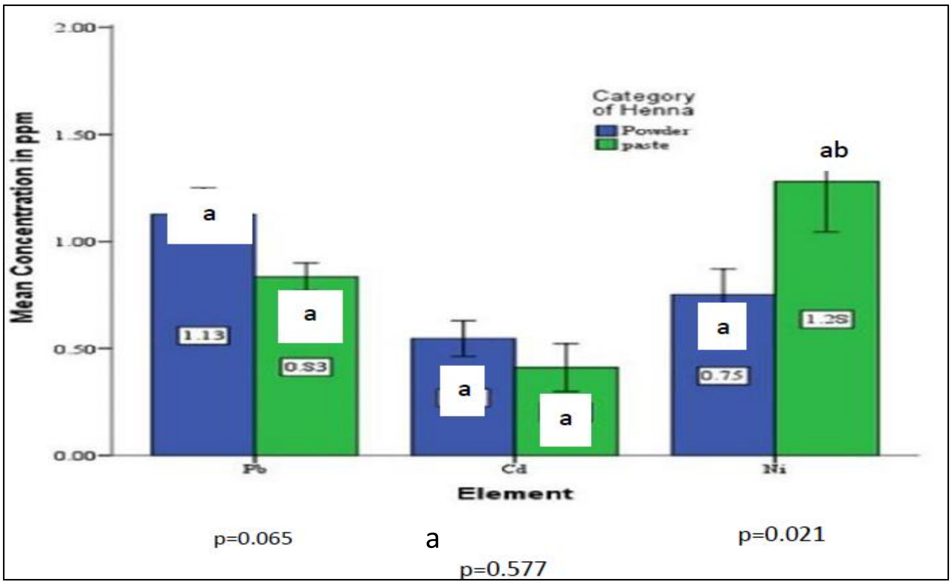

Figure 1.4: Overall mean level of lead, cadmium and nickel in powdered and paste henna brands from Lamu Heavy metal

Comparison of heavy metal level in henna products with standard limits

Table 1.7 compares the level of lead, cadmium and nickel in henna products sampled from Lamu with the standard limit by KeBS, WHO and Health Canada.

In Table 1.7, the level of lead in all the sampled henna products was below the limit of 10 ppm (WHO). With an exception of a deep-red henna product $PWRD_1$ of brand 4, the level of lead in all henna products was below the limit of 2 ppm (KeBS).A red henna product sampled in Nigeria by Chukwuma[21]recorded a higher level of lead at a concentration of 4.53 ppm compared to a level of 2.63±0.24 ppm obtained from this study. In comparison to other brands, brand 3 recorded the highest overall mean level of lead at a concentration of 2.02±0.01 ppm.

All sampled henna products recorded cadmium level below the tolerable limit of 2 ppm (KeBS).However, compared to WHO limit of 0.3 ppm, products PWRD and PABL of brand 3, product $PWRD_1$ of brand 4, and products PARD and PABR of brand 6 recorded a higher level of cadmium. The highest level of cadmium was in product PARD of brand 6 at a level of 1.31±0.01 ppm while the highest overall mean level of cadmium was recorded in brand 3 at a concentration of 1.04±0.08 ppm.

Henna product PWBL of brand 3, PWRD of brand 4, PABL and PABR of brand 5 as well as PARD of brand 6 recorded nickel levels above the limit of 1 ppm (Health Canada).The highest level of nickel was recorded in black paste henna product, PABL of brand 5 at a concentration of 3.01±0.01 ppm. A black henna product from Denmark recorded the highest nickel level at a concentration of 4.0 ppm (DEPA, 2005), which is above the level of 3.01±0.01 ppm obtained from this study. Brand 5 recorded the highest overall mean level of nickel at a concentration of 1.38±0.34 ppm.

Table 1.7: Mean level of lead, cadmium and nickel in henna products from Lamu and the standard limits

Henna brand and the country of origin	Henna product	Concentration Mean±SD(ppm) (n=3)		
		Pb	Cd	Ni
1 (Indian)	PWRD	0.17±0.02	0.06±0.01	1.00±0.02
	$PWRD_1$	1.30±0.02	0.91±0.02	BDL
	PWBR	1.01±0.03	0.67±0.02	0.79±0.03
	PWBL	0.17±0.02	1.06±0.02	0.13±0.01
	Overall mean level	0.66±0.15	0.67±0.12	0.64±0.13
2 (unknown)	PWBR	1.41±0.03	BDL	0.39±0.08
	PWCH	0.91±0.03	BDL	0.51±0.02
	PWBD	1.38±0.04	0.01±0.00	0.63±0.02
	PWBU	0.16±0.01	0.02±0.01	0.21±0.02
	Overall mean level	0.97±0.15	0.02±0.00	0.44±0.05
3 (unknown)	PWRD	2.02±0.02	0.85±0.02	0.46±0.0
	PWBL	BDL	1.23±0.03	1.59±0.0
	Overall mean level	2.02±0.01	1.04±0.08	1.03±0.25
4 (Pakistan)	PWRD	0.83±0.13	0.16±0.01	2.73±0.09
	$PWRD_1$	2.63±0.24	1.02±0.01	0.40±0.01
	PWCH	1.55±0.08	0.02±0.00	0.17±0.01
	Overall mean level	1.67±0.27	0.40±0.16	1.10±0.41
5 (Indian)	PARD	1.03±0.04	0.07±0.01	0.18±0.02
	PABR	1.32±0.04	0.21±0.02	1.79±0.01
	PABD	BDL	BDL	0.55±0.02
	PABL	0.56±0.06	0.15±0.01	3.01±0.01
	Overall mean level	0.97±0.11	0.14±0.02	1.38±0.34
6 (Morocco)	PARD	0.81±0.01	1.31±0.01	2.42±0.02
	PABR	0.64±0.01	0.70±0.01	0.14±0.02
	PABU	0.65±0.14	0.02±0.00	0.88±0.01
	Overall mean level	0.70±0.04	0.68±0.19	1.15±0.33
Standard limit of heavy metals by standard bodies (ppm)	KeBS	2	2	-
	WHO (1995)	10.0	0.3	-
	Health Canada (2011)	-	-	1.0

The type and level of heavy metals in some cosmetic products depend on the colour that they impart [4]. The study showed that the deeper the red colour of henna the higher the lead content. This is shown in Table 1.7 whereby the deep red henna product ($PWRD_1$) always recorded a higher level of lead than the red henna product (PWRD) in all the brands that the two products were available. On the other hand, black paste henna products were vulnerable to nickel exposure since the products recorded the highest level of nickel in all the brands that the product was available.

The use of henna products from henna brands which are unlabeled may endanger the health of the henna user. Since no information is given about the country of origin and the ingredients used, such products may be sold as contrabands and may predispose one to the toxic effects of heavy metals due to the high heavy metal content in them. In support of this is brand 3 (unlabeled) as shown in Table 1.7 which recorded the highest overall mean level of lead and cadmium compared to all the other brands.

From the results, the henna products which recorded their heavy metal levels above the set limit by the standard bodies should not be used frequently because they predispose the henna users to the toxic effects of the metal. Although most of the henna products analysed from this study recorded heavy metal levels below the tolerable limits, frequent use of these products may pose a health hazard to the henna users since the metals are biocumulative. Due to the ubiquitous nature of heavy metals, it is difficult to label a particular brand, colour or category of henna product as absolutely safe or unsafe. Good manufacturing and analytical practises should be adopted in order to reduce the levels of heavy metals in henna products.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The overall mean level of Ni in powdered henna products was 0.75 ppm while paste henna products recorded a significant overall mean level of Ni at 1.28 ppm. Hence the results showed that paste henna products contain higher level of Ni compared to powder henna products. The levels of heavy metals in all henna products were determined and compared with standard limit. In comparison to KeBSset limit of 2 ppm, generally all sampled henna products recorded lower levels Cd and Pb.

Recommendations

The henna manufacturers should minimise the use of heavy metals in henna products as formulants and or reduce the addition of these metals inadvertently. Henna users should be sensitised on the high content of nickel in paste hennas which could expose them to the toxic effects of the metal such as skin sensitivity. Application of henna products that contain heavy metals should be minimised because the metals are toxic and bioaccumulative. The study should be extended to analyse *p*-PD, a common oxidative dye in premixed henna and a major cause of contact dermatitis. Other toxic heavy metals in henna products such as arsenic and chromium should be analyzed.

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How to cite this article: Mohammed TK, Paul JO. Assessment of the safety of henna cosmetics sold in Lamu County- Kenya: heavy metals components levels with respect to standard limits. *International Journal of Science & Healthcare Research.* 2020; 5(4): 317-330.

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