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Levels of Some Heavy Metals in the Leaves of Henna Plant (*Lawsonia inermis*) within Lamu County, Kenya

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ABSTRACT

Henna plant scientifically known as *Lawsonia inermis* is an ethno botanical plant used majorly for preparation of dye called henna. The plant contains a pigment called Laws one which makes it suitable for preparation of henna used in painting or beautification of body parts. Henna painting of body parts is an art of beautification which is commonly practised by the people of Lamu County and other parts of the world. However, high levels of toxic heavy metals such as lead (Pb), Cadmium (Cd) and Nikel (Ni) commonly found in henna products pose health threat to users. One of the sources of heavy metals in cosmetic henna products is the henna leaves. The aim of the study was to determine the level of heavy metals; Lead (Pb), Cadmium (Cd) and Nickel (Ni) in henna leaves within Lamu County in order to assess their suitability as raw material for henna products with respect to the international standard limits. A total of 60 henna leaf samples were randomly selected from Mpeketoni, Amu, Pate and Kiunga sampling sites within Lamu County and analysed 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 least and highest mean levels (ppm) of heavy metals in the henna leaf samples were as follows: Pb (0.04±0.01 to 0.93±0.04), Cd (0.01±0.00 to 0.09±0.01) and Ni (0.01±0.00 to 0.34±0.02). The pattern of overall mean level of heavy metal accumulation in the henna leaves followed the order: Pb > Ni > Cd. No significant difference in the overall mean level of lead (p=0.155), cadmium (p=0.577) and nickel (p=0.355) in the henna leaves was noted. Among all the sampling sites, a significant difference in the level of nickel (p=0.021) was recorded only within Mpeketoni site. From the results of the study, it showed that henna leaves from Lamu County formed a good raw material for henna products due to their low level of lead, cadmium and nickel in comparison to the standard limits by WHO. Effective quality control measures, proper selection of raw material as well as good manufacturing practises should be enhanced so as to minimise the levels of these metals in henna products.

Key words: WHO, Heavy metal, Henna painting, Henna Products, Henna plant

INTRODUCTION

Henna plant scientifically known as *Lawsonia inermis* belongs to the plants family of Lythraceae. It grows in the tropical climates of Africa, northern Australia, and southwest Asia [1], and well known for its cosmetic and therapeutic virtues [2]. It contains a pigment called Laws one (2-hydroxy-1,4-naphthoquinone) and other compounds making leaves natural source of colour for painting/decorating body parts [3].

Henna painting is an art which has been in practise for over 5000 years ago [4]. The ancient Egypt and its neighbouring countries practised henna painting which later spread to Arabian countries and today, henna is used in all parts of the World [5]. "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 [6].

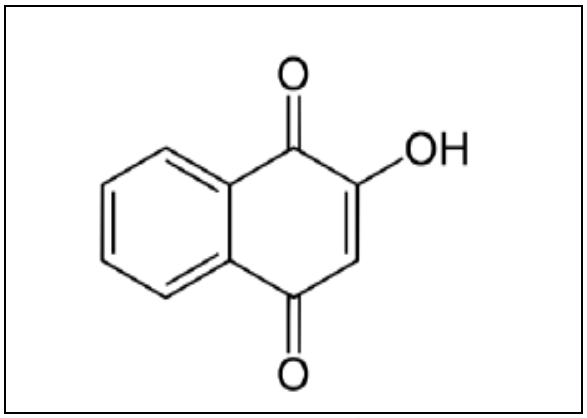

Figure 1.1: Structure of lawsone

Henna plant grows to a height of between 12 to 15 feet. The plant requires a temperature range of 10°C to 50°C. Henna plant grows well in dry soil than in damp soil. Henna leaves occur as decussate pairs which are oppositely arranged. A mature henna fruit is a dry capsule which contains many seeds [5].

Lawsone (2-hydroxy-1, 4 naphthoquinone) is the tannin product which is responsible for the henna dye. From the henna leaves, lawsone is extracted at a concentration range of 1.0% to 1.5% [7]. The structure of lawsone is shown in Figure 1.1

Lawsone is a compound which imparts the characteristic red-orange pigmentation which makes the skin, nails and hair beautiful and attractive [8].The whole henna plant consists of xanthones, coumarins (fraxetin and scopletin), flavanoides (luteolins, apigenin and their glycosides) and steroids (β-sitosterol) [8]. The structures of these organic compounds are shown in Figure 1.2.

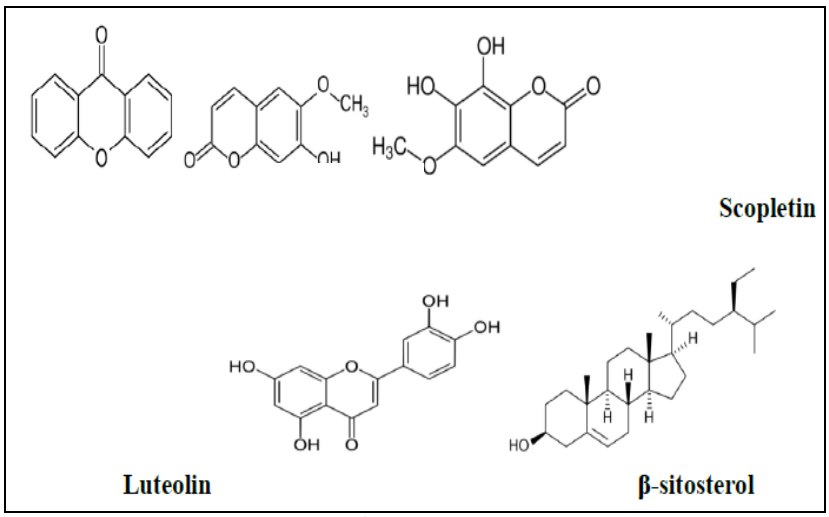

Figure 1.2: Structures of organic compounds in henna plant

In addition to the organic compounds, henna plants absorb heavy metal ions from the soil and later accumulate them in organs such as leaves [9]. Plants growing in soils which are highly polluted with heavy metals tend to accumulate higher levels of the metals compared to those growing in less polluted soils [10].In Morocco, Ghanjaouri (2014) recorded the highest level of lead in henna leaves at a concentration of 3.0 ppm while Alwakeel (2008) recorded lead level at a range of 0.011 ppm to 1.528 ppm in henna leaves sampled in Pakistan [11,12]. Hence, plants growing in different areas accumulate different levels of heavy metals depending on factors such as soil pollution level [13].

Cosmetic products are a possible source of heavy metals which may be found in the raw materials such as leaves or may be added inadvertently during the manufacturing process [14]. Henna products have recorded different levels of heavy metals depending on their composition [15]

Based on its chemical structure, henna dye molecule belongs to a class of

dyes called α-hydroxy-naphthoquinones. There are very rare reactions to lawsone compound in henna leaves and these are mainly based on naphthoquinone sensitivity [8].The formulations of some premixed henna consist of heavy metals and synthetic dyes such as *para*-Phenylenediamine (PPD). 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 [7]. Plate 2.0 shows the adverse effects of some formulants used in henna products.

Plate 1: Adverse effects of premixed henna. Henna page, Catherine (2006)

The contamination caused by heavy metals in cosmetic products is an important environmental and health issue since the metals are toxic [16]. Some of the toxic effects of lead include haematological, cardio-vascular and neurobehavioural complications such as encephalopathy, depression and malaise [17,18]. Cadmium is known to trigger oestomalacia 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]

Studies on heavy metal composition in henna leaves have been carried out in different countries. Due to the different level of soil pollution by heavy metals, the composition of heavy metals in henna leaves also differ. Various henna products are extensively used along the coastal strip of Kenya for beautification and are believed to be among the sources of heavy metals contamination which are toxic to both humans and the environment [21].The possible sources of these heavy metals includehenna leaves and poor manufacturing processes [9]. Since the level of heavy metals in henna leaves from Lamu is not known, it is difficult to control and regulate the level of heavy metals in the henna products used by the residents of Lamu. Therefore, there is need to determine the levels of heavy metals: Lead, Cadmium and Nickel inhenna leaves in order to acertain the source of heavy metalscontamination of henna cosmetic products.

MATERIALS AND METHODS

Research design

The research adopted an experimental design which involved random sampling of henna leaves. Digestion of henna leaves followed a given protocol. Analysis of henna leaf samples 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

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. Most of the henna plants growing in Lamu are in wild state and are scattered all over the County.

Sampling sites

Henna leaves were sampled from Mpeketoni, Amu, Pate and Kiunga sites.

The sites were selected based on their different anthropogenic and non-anthropogenic sources of pollution that could possibly lead to heavy metal pollution.

Mpeketoni is in the western part of Lamu County. In this site there is heavy traffic and intensive farming of cash crops such as cotton, maize and cashewnuts. As a result, agrochemicals such as fertilisers, pesticides and fungicides are highly used.

Amu was nominated by UNESCO as one of the world heritage centers. The town lacks proper waste management system. Due to poor sewerage and drainage system, waste water management is inefficient. Some henna plants grow in the pavements along the open drainage system.

Pate is located to the eastern part of Lamu County. The site receives insufficient and unreliable rainfall annually hence farming is only practised in small scale. The site is endowed with natural resources such as coal, oil and gas [22].

Kiunga boarders Garissa County to the North and Republic of Somali to the North East. Agriculture is practised in small scale due to the prevailing semi-arid conditions. The major economic activities are fishing and honey harvesting [22].

Sample size

A total of sixty (60) henna leaves were randomly selected and analysed for lead, cadmium and nickel. The sample size was determined by an arbitrary sampling method shown in Equation 3.1 [23].

$n = \sqrt{N}/2$……...3.1

Where:

n = Sample size

N = Total population

Sampling and collection of henna leaves

Based on their availability, a total of five (5) henna plants growing in different farms in each of the four (4) sites were randomly selected. Three (3) henna leaves were sampled from each henna plant. The sampling scheme for the henna leaves is shown in Figure 3.1

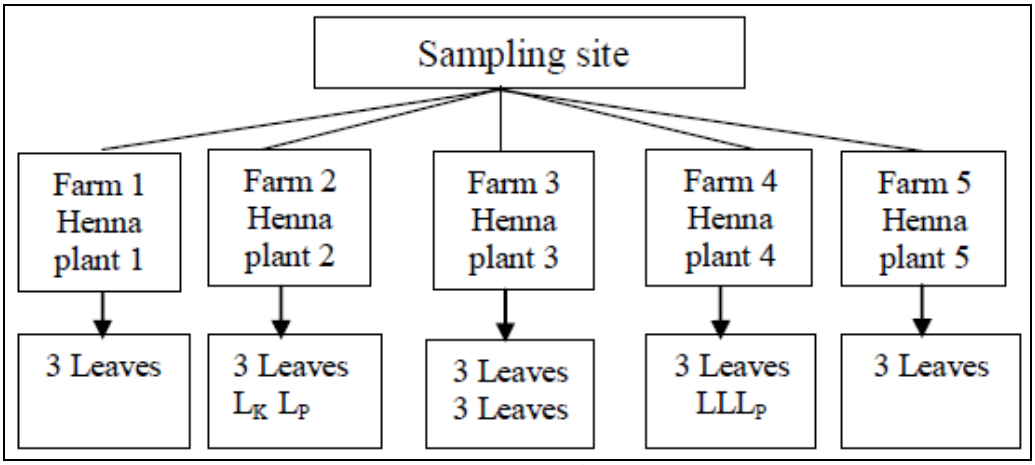

Figure 3.1: Sampling scheme for henna leaves

The sampling sites were at least 5 kilometer radius apart while the radius between the selected henna plants in each site was at least 0.5 kilometer. This was done to minimize reproducibility of results [23].The henna leaves were coded. For example, leaf code L_{M1} represents henna leaves (L) sampled from the first farm or henna plant (1) within Mpeketoni site (M) (Appendix iii).

Henna leaves were picked from the henna plants growing either in their wild or cultivated state in each of the sampling sites. The selected leaves from henna plants were of average size and age. Henna manufacturers do not prefer old henna leaves and young henna leaves due to their low lawsone and high moisture content, respectively [24]. The picked leaves were put in labeled perforated polythene bags. A

taxonomist from KEMRI assisted in the identification of the henna plants and ensured that the selected leaves were of average age.

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 leaf sample

Three (3) leaves were picked from each henna plant to form a set. The leaves were separately washed with double distilled deionised water to remove soil particles. The clean leaves were chopped into small pieces and then dried in an oven at a temperature range of 60°C to 65°C for 72 hours and later cooled [25].The dried pieces of leaves were ground using a Wiley mill to form a homogenous set of powdered henna leaves. To avoid contamination, a clean Wiley mill was used to grind a particular set of henna leaves [9]. Each set was later packed in an air tight labeled Teflon container awaiting digestion.

Henna leaf sample treatment

Nitric acid-chloric acid wet digestion method was adopted to treat henna leaf sample because of its better recovery of lead, cadmium and nickel from henna leaves [26]. A mass of 1.000 g of powdered henna leaf 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 powdered 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 Whatmans 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 [27].

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 3.2

$V_1C_1=V_2C_2$.....Eq 3.2

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

$V_1 C_1 = V_2 C_2$

V_1 x 1000 ppm = 100 ml x100 ppm;

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

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 3.2

Since, $V_1 C_1 = V_2 C_2$

V_1 x 100 ppm = 50 ml x 4 ppm;

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

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 20 ml 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 (1992) was used [28]. Its solution was prepared the same way as the henna sample.

Methods validation

Calibration curve and regression analysis

The prepared standards of each metal and their corresponding absorbencies (Appendix i) 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 3.3

$$\text{Percentage recovery} = \frac{SAS - SBS}{SS} \times 100 \quad \text{..... Eq 3.3}$$

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 [29].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) [28].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 3.4

$$y = y_B + 3_{SB} \quad \text{..... Eq 3.4}$$

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 3.2.

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

$$\text{Actual concentration} = \frac{\text{Concentration (µg/ml) x Volume digested (ml)}}{\text{Weight of sample (g)}} \quad \text{.....Eq 3.5}$$

The actual concentration of heavy metals in henna leaf samples obtained from AAS was derived using Equation 3.5

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 leaf 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 leaf henna samples. A significant statistical test was set at $p = 0.05$ ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Introduction

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

AAS validation results

Calibration curves and regression analysis

Figure 4.1 shows the standard calibration curve for lead.

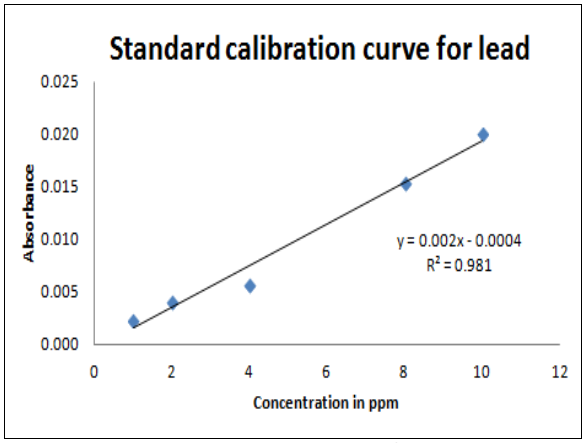

Figure 4.1: Calibration curve for lead

Figure 4.2 shows the standard calibration curve for cadmium.

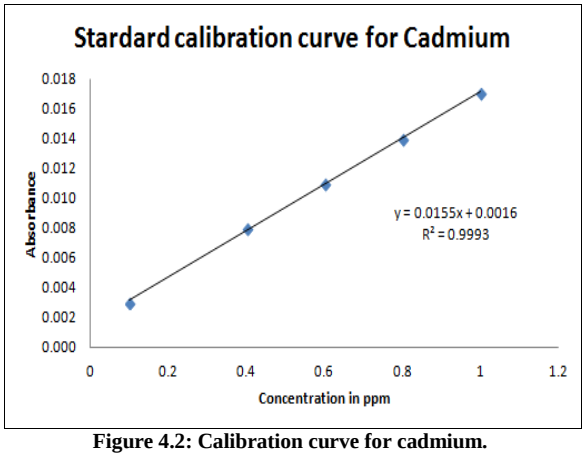

Figure 4.2: Calibration curve for cadmium.

Figure 4.3 shows the standard calibration curve for nickel.

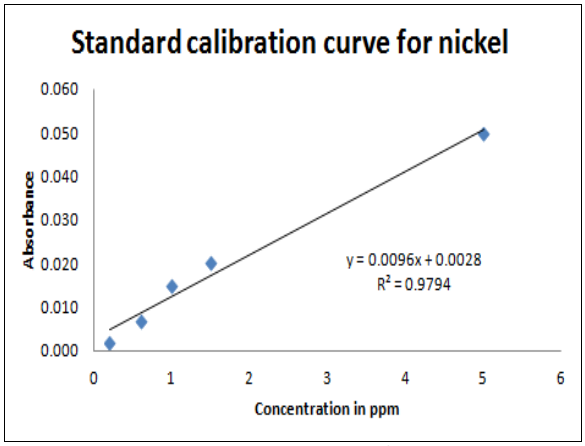

Figure 4.3: Calibration curve for nickel

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 [30]. The intercept of the curves was 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 the henna leaves [29]. 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 [31].

Standard addition

Table 4.1 shows the percentage recovery results for lead, cadmium and nickel after spiking a sample concentration with a standard solution.

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 [32].

Standard reference material

The lead, cadmium and nickel certified values of the reference material and their measured values using FAAS are given in Table 4.2.

From Table 4.2 the certified level for lead was 1.62±0.12 µg/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 showed that the

measured values were not significantly different from the certified values hence good accuracy of the instrument at 95% confidence level [29].

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

Heavy metals in henna leaves from Lamu

Level of lead, cadmium and nickel in henna leaves

Table 4.3 shows the mean level of lead, cadmium and nickel in leaves of henna plants sampled from Mpeketoni, Amu, Pate and Kiunga sites within Lamu County.

Table 4:3: Mean level of lead, cadmium and nickel in henna leaves from Lamu

Sampling site	Henna leaf code	Concentration Mean±SD (µg/g) (n=3)		
		Pb	Cd	Ni
Mpeketoni	L_{M1}	0.08±0.01[a]	0.01±0.01	0.02±0.01[a]
	L_{M2}	0.05±0.01[a]	BDL	0.06±0.01[a]
	L_{M3}	0.93±0.04[b]	0.01±0.00	0.34±0.02[ab]
	L_{M4}	BDL	0.09±0.01	BDL
	L_{M5}	0.53±0.09[b]	0.04±0.02	0.03±0.01[a]
	p-value	p=0.051	p=0.650	p=0.021
	Overall mean	**0.40±0.11**	**0.04±0.01**	**0.11±0.04**
Amu	L_{A1}	0.08±0.02[a]	0.02±0.02	0.04±0.01
	L_{A2}	0.17±0.02[b]	0.05±0.01	0.01±0.00
	L_{A3}	BDL	BDL	BDL
	L_{A4}	BDL	0.06±0.02	0.03±0.01
	L_{A5}	0.35±0.08[b]	0.03±0.01	0.02±0.01
	p-value	p=0.057	p=0.578	p=0.578
	Overall mean	**0.20±0.04**	**0.04±0.01**	**0.02±0.00**
Pate	L_{P1}	0.06±0.01[a]	0.04±0.01	0.01±0.01
	L_{P2}	BDL	BDL	BDL
	L_{P3}	BDL	BDL	0.04±0.01
	L_{P4}	0.53±0.08[b]	0.06±0.02	BDL
	L_{P5}	0.07±0.01[a]	BDL	0.02±0.01
	p-value	p=0.153	p=0.578	p=0.512
	Overall mean	**0.22±0.04**	**0.05±0.01**	**0.02±0.00**
Kiunga	L_{K1}	BDL	BDL	0.03±0.01
	L_{K2}	BDL	0.03±0.02	0.06±0.01
	L_{K3}	0.04±0.01	BDL	BDL
	L_{K4}	BDL	BDL	0.02±0.01
	L_{K5}	0.05±0.02	0.04±0.02	BDL
	p-value	p=0.736	p=0.747	p=0.612
	Overall mean	**0.05±0.00**	**0.03±0.01**	**0.03±0.01**

Mean values followed by the same small letter(s) within the same column in each sampling site do not differ significantly from one another (one-way ANOVA, SNK-test, $\alpha = 0.05$). Codes L_{M1}, L_{A1}, L_{P1} and L_{K1} represent henna leaves sampled from the first henna plant in Mpeketoni, Amu, Pate and Kiunga sites, respectively. BDL–Below detection limit.

From Table 4.3, the level of lead within Mpeketoni site ranged from 0.05±0.01 ppm in leaf L_{M2} to 0.93±0.04 ppm in leaf L_{M3}. The highest level of lead was recorded in leaf L_{A5} at a concentration of 0.35±0.08 ppm while leaf L_{A1} recorded the least level at a concentration of 0.08±0.02 ppm within Amu site. Within Pate, leaf L_{P4} recorded the highest level of lead at a concentration of 0.53±0.08 ppm which is similar to the level of lead recorded in leaf L_{M5} from Mpeketoni. Within Kiunga only two leaves, L_{K3} and L_{K5} recorded lead at a concentration of 0.04±0.01 ppm and 0.05±0.02 ppm, respectively. There was no significant difference in the level of lead in henna leaves sampled from all the sites: Mpeketoni (p=0.051), Amu (p=0.057), Pate (p=0.153) and Kiunga (p=0.736).

Lead has not been shown to perform any role in plants metabolism although it occurs naturally in plants. Compared to other heavy metals, lead forms the highest percentage in the soil [13].The level of lead in the soil as well as the form in which it occurs will determine its uptake by the plants [33].For a plant that grows in less polluted area, the level of lead is in the range of 0.1 ppm to 10 ppm [10].Since the level of lead obtained from this study falls within this range, the sites may be regarded as less polluted.

From Table 4.3, the level of cadmium within Mpeketoni ranged from 0.01±0.00 ppm in leaf L_{M1} and L_{M3} to 0.09±0.01 ppm in leaf L_{M4}. Within Amu, leaf L_{A1} recorded the least level of cadmium at a concentration of 0.02±0.02 ppm while leaf L_{A4} recorded the highest level of cadmium at a concentration of 0.06±0.02 ppm. In Pate and Kiunga, the highest level of cadmium was recorded in leaf L_{P4} and L_{K5} at a concentration of 0.06±0.02 ppm and 0.04±0.02 ppm, respectively. There was no significant difference in the level of cadmium in henna leaves sampled from all the sites: Mpeketoni (p=0.650), Amu (p=0.578), Pate (p=0.578) and Kiunga (p=0.747).

Alwakeel (2008) recorded levels of cadmium in henna leaves sampled from henna plants growing in Saudi Arabia at a concentration range of 0.016 ppm to 0.019 ppm [12] which is within the range of 0.01±0.01 ppm to 0.09±0.01 ppm obtained from this study. Although considered a non-essential element, cadmium is effectively absorbed in roots and leaves of plants [33].

From Table 4.3, the level of nickel within Mpeketoni site ranged from 0.02±0.01 ppm in leaf L_{M1} to a significant level of 0.34±0.02 ppm in leaf L_{M3}. In both Amu and Pate, leaf L_{A1} and L_{P3}, respectively recorded the highest level of nickel at a concentration of 0.04±0.01 ppm. Within Kiunga, the highest level of nickel was noted in leaf L_{K2} at a concentration of 0.06±0.01 ppm. No significant difference was noted in the level of nickel in the henna leaves sampled in Amu site (p=0.578), Pate site (p=0.512) and Kiunga site (p=0.612). However, a significant difference was noted in the level of nickel in henna leaves from Mpeketoni site (p=0.021).

Ahmad *et al*. (1994) recorded the highest nickel level in henna leaves from Faisalabad district, Pakistan at a concentration of 0.18 ppm [24] which is below the level of 0.34±0.02 ppm recorded from this study. In another study, Alwakeel (2008) analyzed nickel level in henna leaves sampled from henna plants growing in Saudi Arabia and reported a range of 0.035 ppm to 0.073 ppm nickel [12] which falls within the range of 0.01±0.00 ppm to 0.34±0.02 ppm obtained from this study.

Unlike lead and cadmium, nickel was detected in most of the sampled henna leaves. This may be due to the fact that nickel is an essential element which is important in plant's metabolism. Nickel is a constituent of metallo–enzymes such as urease which metabolizes urea into useable ammonia within the plant. Nickel is phytotoxic at higher concentration [34].

The different levels of heavy metals in the henna leaves may be attributed to different pollution levels of the soils under which the henna plant is growing [35]. Both the anthropogenic and non-anthropogenic sources of pollution contribute to the level of heavy metals in plants [34] (Ruchi *et al.*, 2014). From Table 4.3, Mpeketoni site recorded the highest overall mean level of lead and nickel which can be attributed to the use of agrochemicals such as fungicides and pesticides [36,37]. In addition, the heavy traffic in Mpeketoni as well as the effluents from car garages could contribute to the high level of lead [38]. Non-point source contamination by the waste waters from the open sewerage and drainage system could lead to a higher overall mean level of cadmium in Amu site [10]. In Pate, the highest overall mean level of cadmium might be attributed to the cadmium composition of the underlying sedimentary rock, marine phosphites and phosphates which form the basement for coal and crude

oil deposits [39]. Kiunga is a semi-arid area in which small scale farming is practised. The major economic activities include honey harvesting and fishing, which do not contribute much to pollution, hence the least overall mean level of heavy metals recorded.

Levels of accumulation of heavy metals in henna leaves

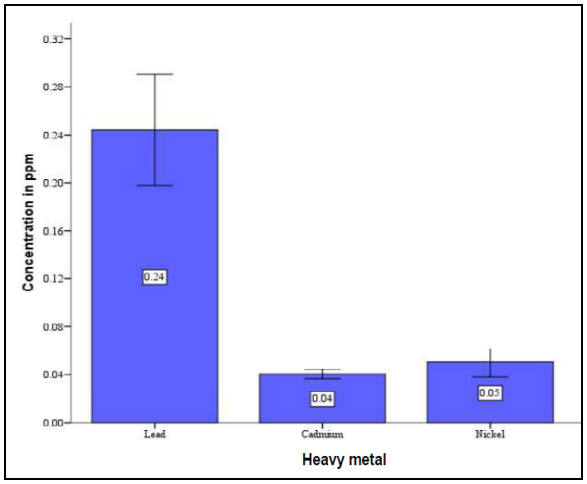

Figure 4.4: Overall mean level of lead, cadmium and nickel in henna leaves from Lamu

The overall mean levels of heavy metals in Table 4.3 were subjected to tests of significance to show the pattern of variation of heavy metals in the henna leaves as shown in Figure 4.4

From Figure 4.4, lead recorded the highest overall mean level at a concentration of 0.24 ppm while cadmium recorded the least overall mean level at a concentration of 0.04 ppm. No significant difference in the overall mean level of lead (p=0.155), cadmium (p=0.577) and nickel (p=0.355) in the henna leaves was noted. The order of accumulation of heavy metals in the henna leaves was as follows:

Lead > Nickel > Cadmium

This order is in agreement with that recorded by Ahmad *et al*. (1994) in Faisalabad district, Pakistan[24].The highest level of lead in the henna leaves may be attributed to the high pollution level of lead in soils compared to cadmium and nickel[13].The lowest level of cadmium could be due to the basic calcareous soils and limestone within Lamu[40]which could render cadmium unavailable to the roots and leaves of plants [33].

Comparison of level of heavy metals in henna leaves with WHO limits

Table 4.4 shows the overall mean level of lead; cadmium and nickel in henna leaves sampled from Lamu and the WHO recommended limits of the metals in herbal plants.

Table 4.4: Overall mean level of heavy metals in henna leaves from Lamu and WHO limits

Element	Concentration (Mean±SD) ppm in the henna leaf samples	Recommended level (ppm) by WHO (2010)
Lead	0.24±0.28 (n=36)	2.0
Cadmium	0.04±0.03 (n=36)	0.2
Nickel	0.05±0.02 (n=42)	1.68

From Table 4.4, the overall mean levels of lead, cadmium and nickel in the sampled henna leaves were lower at a concentration of 0.24±0.28 ppm, 0.04±0.03 ppm and 0.05±0.02 ppm, respectively compared to the recommended level of 2.0 ppm, 0.2 ppm and 1.68 ppm, respectively by World Health Organization [30].Due to the lower level of heavy metals, the henna leaves from Lamu form a good raw material for henna products.

Generally, the levels of heavy metals in henna leaves from Lamu are similar to those reported in other areas (section 4.3) except for a case where lead was reported to a level as high as 3 ppm [11].The study has revealed that the level of accumulation of heavy metals in henna leaves vary depending on the nature of the metal and the henna leaf sampled. Henna plants can be grown in controlled fields where the level of heavy metals in the soils is kept at a minimum level. In order to protect the henna users from the ill effects of heavy metals, the henna manufacturers should ensure that only the henna leaves with heavy metal level below the standard limit are sampled as raw material for henna

products.

DISCUSSION

From the results of this study, it showed that henna leaves from Lamu County formed a good raw material for henna products due to their low level of lead, cadmium and nickel in comparison to the standard limits by WHO. This study concurs with a study by Mamoona and Maryam (2015) which reported lower level of lead in henna leaves sampled from Pakistan at a concentration of 0.019 ppm, [41] but in contrast with a study in Morocco by Ghanjouri (2014) which recorded the highest level of lead in henna leaves at a concentration of 3.00 ppm [11] which is above the level of 0.93±0.04 ppm obtained from this study. Allergy to natural henna is not usual; however, use of poor quality raw materials and the addition of *para*-phenylenediamine (PPD) to the natural henna during processing increase the risk of allergic contact dermatitis [42]**.** Effective quality control measures, proper selection of raw material as well as good manufacturing practises should be enhanced so as to minimise the levels of these metals in henna products.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

In henna leaves, the overall mean level of heavy metals were determined and recorded as follows: Pb (0.24±0.08 ppm), Cd (0.04±0.03 ppm) and Ni (0.05±0.02 ppm). The set limit of heavy metals in herbal plants by WHO was Pb (2.0 ppm), Cd (0.2 ppm) and Ni (1.68 ppm). Therefore, henna leaves recorded lower levels of Pb, Cd and Ni compared to the limit by WHO.

In henna leaves, the overall mean level of heavy metals were determined and recorded as follows: Pb (0.24±0.08 ppm), Cd (0.04±0.03 ppm) and Ni (0.05±0.02 ppm). In henna products, the overall mean levels of heavy metals were determined and recorded as follows: Pb (0.98±0.09 ppm), Cd (0.48±0.09 ppm) and Ni (1.02±0.18 ppm). As raw materials, henna leaves recorded lower significant levels of Pb, Cd and Ni than the henna products. This showed that henna leaves were not the only source of heavy metals in the henna products but more of these metals were added during the manufacturing process

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 Kenya Bureau of Standards (Kebs) set limit of 2 ppm, generally all sampled henna products recorded lower levels Cd and Pb

Recommendations

The henna leaves sampled in Lamu County form a suitable raw material for the manufacturer of henna products because they contain lower levels of heavy metals.

Since the levels of heavy metals in henna leaves were lower compared to those in henna products, it is mostly likely that the heavy metals contamination witnessed among henna products user could be due to poor processing procedures, therefore manufacturers should improve on their methodology to safeguard the end users.

The study should be extended to determine the level of heavy metals in henna cosmetics para-phenylenediamine, common oxidative dye in premixed henna and a major cause of dermatitis.

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REFERENCES

1. Gagandeep C, Sandeep G, Priyanka P. Lawsonia inermis Linnaeus: a phytopharmacological review. Int J Pharm Sci Drug Res. 2010; 2(2):91-8.
2. Gevrenova R. Determination of natural colorants in plant extracts by high performance liquid chromatography. Journal of the Serbian Chemical Society. 2010; 75 (7):903-15.
3. El Massoudi S, Benidir M, Chabir R, Benjelloun M, El Ghadraoui L, Errachidi F. Morphological, Biochemical, and Climatological Analysis of Three Moroccan Henna Verities. The Scientific World Journal. 2019 May 27; 2019.
4. Raboobee N. Sensitisation from PPD in temporary henna tattoos and subsequent severe allergic contact dermatitis from hair dye. Curr Allergy Clin Immunol. 2004; 17:194-5.
5. Rahman AU. Studies in Natural Products Chemistry: Bioactive Natural Products (Part XII). Elsevier; 2016 Sep 6.
6. Packer, M. Henna source book: Origin of henna. Winson publishing company. http://www crystalinks.com/henna.html. Accessed 8.04.2014.
7. Deleo, Y. A. (2006). *P-Phenylenediamine*. Dermatol publishers, 4th street Manipur, India.
8. Kang IJ, Lee MH. Quantification of para- phenylenediamine and heavy metals in henna dye. Contact dermatitis. 2006 Jul; 55(1):26-9.
9. Mazoor, I. K. and Mahmoud, I. K. (2011). Study of heavy trace metals in some medicinal-herbal plants of Pakistan. *Pakistan Journal of Botany*.2011; 43: 2003-2009.
10. Chibuike GU, Obiora SC. Heavy metal polluted soils: effect on plants and bioremediation methods. Applied and environmental soil science. 2014 Oct; 2014.
11. Ghanjaoui ME, Cervera ML, El Rhazi M, de la Guardia M. Assessment Of Trace Elements In Traditional Moroccan Cosmetics By Inductively Coupled Plasma Atomic Emission Spectroscopy. International journal of scientific & technology research. 2014; 3(10):104-2.
12. Alwakeel SS. Microbial and heavy metals contamination of herbal medicines. Research Journal of microbiology. 2008 Dec 1; 3(12):683-91.
13. Khan SA, Khan L, Hussain I, Marwat KB, Akhtar N. Profile of heavy metals in selected medicinal plants. Pakistan Journal of Weed Science Research. 2008; 14(1-2):101-10.
14. Alsaffar NM, Hussein HJ. Determination of heavy metals in some cosmetics available in locally markets. IOSR J Environ Sci Toxicol Food Technol. 2014; 8(1):9-12.
15. Nnorom IC, Igwe JC, Oji-Nnorom CG. Trace metal contents of facial (make-up) cosmetics commonly used in Nigeria. African Journal of Biotechnology. 2005; 4 (10).
16. Swift JA. Fundamentals of human hair science. Weymouth, U.K: Micelle Press; 1997.
17. Eman, M., Alissa, F. and Gordon, A. (2011). Heavy metal poisoning and cardiovascular diseases. *Journal of Toxicology*.2011; 27: 76-98.
18. CDC. Centre for Disease Control and Prevention. Preventing lead poisoning in young children. Department of health and human services, Atlanta, USA.2009.
19. Kollmeier H, Seemann J, Wittig P, Rothe G, Müller KM. Cadmium in human lung tissue. International archives of occupational and environmental health. 1990 Sep 1; 62 (5):373-7.
20. CDTSC (2004). California Department of Toxic Substances Control. Review of nickel and nickel compounds.
21. Al-Dayel O, Hefne J, Al-Ajyan T. Human exposure to heavy metals from cosmetics. Oriental Journal of Chemistry. 2011 Jan; 27(1):1-1.
22. LCIDP. Lamu County Integrated Development Plan. Economic Strategies

within Lamu County, Kenya. 2013.
23. UNODC. United Nations Office on Drugs and Crime. Guidelines on representative drug sampling. United nations publications. ISBN 978-92-1-148 241-6. New York.2009.
24. Ahmed, I., Siddiqui, M. and Khan, Z. The major, minor and trace elements in henna leaves. *Journal of Chemistry Society in Pakistan*.1994; 16: 29-35.
25. Ogunwande IA, Olawore NO. Volatile fractions from the leaf and flowers of "African Marigold", Tagetes erecta Linn from Nig. J. Essent. Oil Res. 2006; 18:366-8.
26. Umar MA, Caleb H. Analysis of metals in some cosmetic products in FCT-Abuja, Nigeria. International Journal of Research in Cosmetic Science. 2013; 3(2):14-8.
27. Skoog, D. A. and Leary, J. J. Principles of analytical chemistry. (4^{th} edition). Harcourt Brace College publishers, Fort Worth. Philadelphia.1992.
28. NIST. National Institute of Standard and Technology, Standard Materials Programme, Gaithersburg M.D, U.S.A.1992.
29. Miller, J. C. and Miller, J. N. Statistics for analytical chemistry. (2^{nd} edition) Ellis horwood limited. Chichester, west sussex, po19 IEB, England.1998.
30. Mendham, R. C., Renny, J. O. and Barnes, K. Quantitative chemical analysis. (6^{th} edition). Prentice Hall, U.K. 1999.
31. Sawyer, W. and Beebe, A. Chemistry experiment for instrumental methods. Wiley, New York.2007.
32. Kings, F. and Burgess, A. (1998). Nutrition for developing countries: A critical role on health. (2^{nd} edition). New York: Oxford University Press Inc. 1998.
33. Kabata, A. and Pendias, H. (1985). Trace elements in soils and plants. CRC press, Inc. Florida
34. Ruchi, B. S., Deepak, K.S. and Catherine, C. J. *Lawsonia inermis L*. (Henna). Ethnobotanical, phytochemical and pharmacological aspects. *Journal of Ethnopharmacology*, 2014 Aug 8; 155(1):80-103.
35. Al-Saleh I, Al-Enazi S, Shinwari N. Assessment of lead in cosmetic products. Regulatory Toxicology and Pharmacology. 2009 Jul 1; 54(2):105-13.
36. Kaara, J. N. Determination of heavy metals in sewage sludge, sewage effluent, garden soils and food crops grown in ordinary sewage-sludge amended soils, Masters dissertation, Kenyatta University, Nairobi, Kenya (published).1992
37. Mitei, Y. C. Determination of heavy metals in Kenyan cigarettes, tobacco leaves and intercropped plants by atomic absorption spectroscopy, M.Sc Thesis, Kenyatta University, Nairobi, Kenya (published). 1996.
38. Mutua, S. M. Lead, cadmium and zinc speciation in garage soils, their levels in Kales and water along Katothyani stream, Machakos town, Kenya, M.Sc Thesis, Kenyatta University, Nairobi, Kenya (published). 2013.
39. Muchena, F. N. and Ekirapa, A. E. Soils of the Magarini land settlement scheme (Kilifi District). Kenya soil survey. Site evaluation report No. P63. National Agricultural Research Laboratories.1992
40. WHO (2010). World Health Organisation. Exposure to lead: A major public health concern, Geneva, Switzerland.
41. Mamoona, N. and Maryam, N. Determination of metal content in *Lawsonia inermis* Linn by flame atomic spectroscopy. *International Journal of Healthcare Sciences*. 2015; 3: 54-58.
42. Al-Suwaidi A, Ahmed H. Determination of para-phenylenediamine (PPD) in henna in the United Arab Emirates. International Journal of Environmental Research and Public Health. 2010 Apr; 7(4):1681-93.

APPENDICES

Appendix I: Sampled farms and henna leaf codes

Sampling site	Sampled farm	Henna plant number	Henna leaf code
Mpeketoni	*Mkunumbi*	Henna plant 1	L_{M1}
	Mapenya	Henna plant 2	L_{M2}
	Hongwe	Henna plant 3	L_{M3}
	Baharini	Henna plant 4	L_{M4}
	Kibaoni	Henna plant 5	L_{M5}
Amu	*Pamba roho*	Henna plant 1	L_{A1}
	Uyoni	Henna plant 2	L_{A2}
	Idabo	Henna plant 3	L_{A3}
	Matondoni	Henna plant 4	L_{A4}
	Langoni	Henna plant 5	L_{A5}
Pate	*Siyu*	Henna plant 1	L_{P1}
	Nyabwogi	Henna plant 2	L_{P2}
	Kandaani	Henna plant 3	L_{P3}
	Mbwajumwali	Henna plant 4	L_{P4}
	Faza	Henna plant 5	L_{P5}
Kiunga	*Dederi*	Henna plant 1	L_{K1}
	Kangwe	Henna plant 2	L_{K2}
	Kiduruni	Henna plant 3	L_{K3}
	Kombokombo	Henna plant 4	L_{K4}
	Kwaiyuu	Henna plant 5	L_{K5}

Appendix II: Standards against absorbency for lead,cadmium and nickel

Metal	Standards	Absorbances			Mean absorbance
Lead	1.0	0.002	0.002	0.003	0.0023
	2.0	0.004	0.004	0.004	0.0040
	4.0	0.006	0.006	0.005	0.0056
	8.0	0.016	0.015	0.015	0.0153
	10.0	0.020	0.020	0.020	0.0200
Cadmium	0.1	0.002	0.002	0.002	0.0020
	0.4	0.009	0.010	0.010	0.0096
	0.6	0.011	0.012	0.012	0.0116
	0.8	0.012	0.012	0.012	0.0120
	1.0	0.017	0.017	0.017	0.0170
Nickel	0.2	0.002	0.002	0.002	0.0020
	0.6	0.007	0.006	0.007	0.0066
	1.0	0.015	0.015	0.015	0.0150
	1.5	0.020	0.021	0.020	0.0203
	5.0	0.050	0.049	0.050	0.0496

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