DETERMINATION OF LEAD, CADMIUM AND NICKEL IN HENNA LEAVES AND COSMETIC HENNA PRODUCTS WITHIN LAMU COUNTY, KENYA

BY

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree or any other award in any other university.

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This thesis has been submitted to Kenyatta University graduate school for examination with our approval as University supervisors

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DEDICATION

I dedicate this piece of work to ALLAH and His Prophet Mohammad (P.B.U.H).

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
ALAD	Aminolevulinic Acid Dehydrogenase
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BDL	Below Detection Limit
DEPA	Danish Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectroscopy
KEMRI	Kenya Medical Research Institute
KeBS	Kenya Bureau of Standards
LLB	Level of Lead in Blood
PPD	*Para*-Phenylenediamine
SNK	Student-Newman-Keul *test*
UNESCO	United Nations Educational, Scientific and Cultural Organisation

OPERATIONAL DEFINITION OF TERMS

Black henna	A type of henna which stains black because it contains synthetic dye, *para*-Phenylenediamine.
Henna category	Powdered or paste henna brand.
Henna product	A premixed henna.
Henna sample	Henna leaf or henna product.
Neutral henna	A dye which does not change the colour of hair but imparts brilliant shiny look and enhances hair manageability.
Sensitivity	Allergic reaction.
Traditional henna	Natural henna which is basically prepared from henna leaves, however other plant products may be added to improve its efficacy in terms of staining.

ABSTRACT

Lawsonia inermis Linn, commonly known as henna, is an ethno botanical plant which has been used since antiquity for cosmetic and medicinal purposes. Henna painting is an art of beautification which is commonly practised by the people of Lamu County. Heavy metals such as Pb, Cd and Ni are toxic and commonly found in cosmetic products such as henna. The level of these metals in henna leaves and henna products from Lamu is not known. The aim of the study was to determine the level of Pb, Cd and Ni in henna leaves from Lamu so as to identify the source of these metals in the henna products used by the residents of Lamu and in addition to assess the suitability of using the paste and powdered henna products based on their heavy metal content with respect to the standard limits. A total of 120 henna samples were randomly selected 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 lowest and highest mean levels (ppm) of heavy metals in the henna samples were as follows: Henna leaves: 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). 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). From the results, henna leaves recorded the lowest significant levels of heavy metals compared to both powdered and paste henna products with a significant difference noted in the level of Ni ($p=0.035$) at 95% confidence level. Apart from the henna leaves, the results indicate that more heavy metals were added into the henna products either inadvertently or as formulants. Generally, the powdered henna products recorded their 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 minimize or stop the use of heavy metals as formulant in henna products and to reduce the addition of the metals into the products inadvertently during or after the manufacturing process. The henna users can be sensitised 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.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Henna painting is an art which has been in practise for over 5000 years ago (Catherine, 2006). 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 (Elsevier, 2012). “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 (Packer, 2009).

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 (Elsevier, 2012). Plate 1 shows a henna plant.

Plate 1:1 *Lawsonia inermis* Linn (Henna plant). Source: Yamasaki (2002)

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% (Singh *et al.,* 2007). The structure of lawsone is shown in Figure 1.1

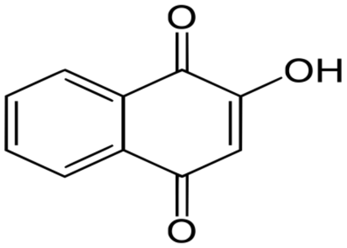
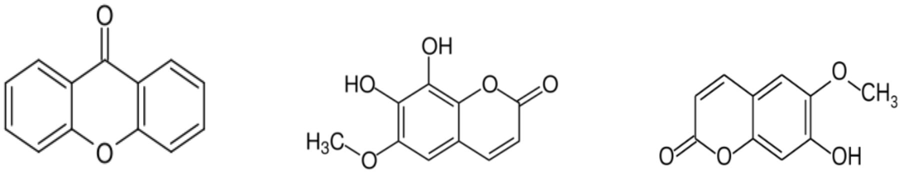
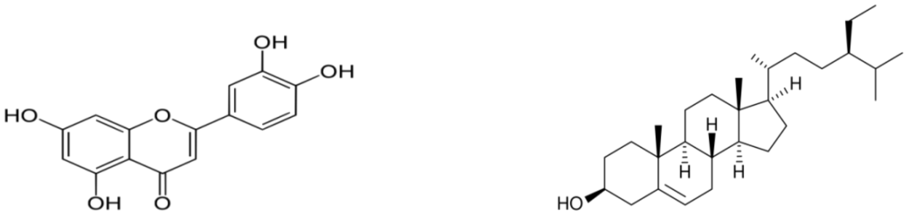


Figure 1.1: Structure of lawsone

Lawsone is a compound which imparts the characteristic red-orange pigmentation which makes the skin, nails and hair beautiful and attractive (Deleo, 2006). The whole henna plant consists of xanthones, coumarins (fraxetin and scopletin), flavanoides (luteolins, apigenin and their glycosides) and steroids (β-sitosterol) (Singh *et al.,* 2007). The structures of these organic compounds are shown in Figure 1.2.



Xanthone **Fraxetin** **Scopletin**



Luteolin **β-sitosterol**

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 (Mazoor and Mahmoud, 2011). 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 (Raven and Johnson, 1998). 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. Hence, plants growing in different areas accumulate different levels of heavy metals depending on factors such as soil pollution level (Shad *et al*., 2008).

Cosmetic products are a possible source of heavy metals because the metals may either form part of the formulation or may be added inadvertently during or after the manufacturing process (Marcovecchio *et al*., 2007). Henna products have recorded different levels of heavy metals depending on their composition (Nnorom *et al.*, 2005). In Palestine, Al-Qutob and Azzatrash (2013) recorded the highest cadmium level in henna products at a concentration of 13.36 ppm while DEPA (2005) from Denmark recorded a lower level of cadmium at a range of 0.01 ppm to 0.05 ppm.

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 (Singh *et al.*, 2007). 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 (Deleo, 2006). Plate 2.0 shows the adverse effects of some formulants used in henna products.

Plate 2: 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 (Vodela *et al*., 1997). Some of the toxic effects of lead include haematological, cardio-vascular and neurobehavioural complications such as encephalopathy, depression and malaise (Eman *et al*., 2011; CDC, 2009). Cadmium is known to trigger oestomalacia and respiratory tract effects such as pulmonary fibrosis, pneumonitis, emphysema and lung cancer (Kollmeier *et al*., 1990). Nickel is a strong allergen and it is known to be the main cause of skin sensitivity (CDTSC, 2004).

Studies on heavy metal composition in henna leaves and henna products have been carried out separately in different countries. It is therefore difficult to relate and compare the levels of heavy metals in henna leaves and henna products because the analyses were not carried out in tandem in one particular area. Since the henna products are manufactured from henna leaves, almost similar levels of heavy metals in henna leaves and henna products are expected. Hence there is need to compare the levels of heavy metals in henna leaves and henna products in order to identify the source of these metals in the henna products and also to determine whether henna products are fit for use based on the limits of heavy metals by standard bodies.

1.2 Statement of the problem

Cosmetic products are extensively used in the coastal areas of Kenya including Lamu for beautification. However, these products are known to be among the sources of heavy metals which are toxic both to humans and the environment (Al-Dayel *et al*., 2011). The possible sources of heavy metals in cosmetic products include the raw materials used and poor manufacturing process (Mazoor and Mahmoud, 2011). 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. In addition, the level of heavy metals in henna products used in Lamu is not known hence such products may contain heavy metal levels beyond tolerable limits set by standard bodies therefore posing a health risk to the henna users. Heavy metals such as lead and cadmium are potentially hazardous as a result of their bioaccumulative effect while nickel is a strong allergen which causes sensitivity reactions (Tsankov *et al.,* 2010). Therefore, there is need to determine the levels of heavy metals in henna leaves and henna products in order to identify the source of the metals and to protect the henna users from the toxic effects of heavy metals.

1.3 Justification

Henna painting forms an integral part of the historical and enriched culture which has been practised for decades by the residents of Lamu County. 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. In addition, the different colours of premixed henna products give the henna users an opportunity to select colours of their choice (Deleo, 2006). 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 harzadous to the henna users and the environment (Al-Dayel, 2011). Some henna users within Lamu complained of itchy and painful sores on the skin after applying premixed henna products (personal communication). Studies on heavy metal composition in henna leaves and henna products have been carried out in African countries like Nigeria (Chukwuma, 1997) while in Kenya, limited studies have been reported. The study is necessary because the environmental condition under which the henna plant is growing and the type of henna products used by the residents of Lamu differ from other regions. Hence, the research aims at finding out the source 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.

1.4 Hypotheses of the study

i. Henna leaves and henna products within Lamu County do not contain high levels of heavy metals, lead, cadmium and nickel.
ii. The levels of lead, cadmium and nickel do not differ significantly in henna leaves and henna products within Lamu County.

1.5 Objectives

1.5.1 General objective

The purpose of the study was to determine the levels of selected heavy metals in henna leaves and cosmetic henna products within Lamu County.

1.5.2 Specific objectives

1. To determine the levels of lead, cadmium and nickel in henna leaves sampled from Mpeketoni, Amu, Pate and Kiunga sites in Lamu County.
2. To determine the levels of lead, cadmium and nickel in paste henna and powdered henna products applied by henna users within Lamu County.

1.6 Significance of the study

Information on the level of lead, cadmium and nickel in henna leaves will assist in the selection of henna leaves with low heavy metal content. In addition, the results will be used to sensitize on the need to minimize the use of heavy metals as formulants or reduce the addition of the metals inadvertently during henna manufacturing. The study will also create awareness to the henna users on the high level of exposure to lead, cadmium and nickel in various henna products used within Lamu County. The research will be instrumental to the quality control body to effectively monitor and control the level of heavy metals in henna products.

1.7 Scope and limitation

i. Only lead, cadmium and nickel were analysed as they are heavy metals which are highly toxic and commonly found in cosmetic products.
ii. The ingredients used in henna products were not investigated.
iii. The factors affecting the absorption of heavy metal cations by henna plants were not studied.
iv. The organic compounds in henna leaves were not analysed.
v. The levels of lead, cadmium and nickel in soils where henna plant grew were not determined.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

A cosmetic product is a substance which is applied onto the human body so as to enhance attraction by way of cleaning and making the body beautiful (SCCNFP, 2002). As a cosmetic product, henna is popular among ladies who use it for beautifying their hair, hands and feet in an artistic way (Neeraj, 2007).

2.2 The origin and use of henna

The history and origin of henna is hard to trace with centuries of migration and cultural interaction, it is difficult to determine where particular traditions began. However, historians point out that henna has been used for at least 5,000 years ago. Some researchers revealed that henna originated from ancient India while others are of the opinion that it was brought to India by Egyptian moguls in the 12th century (Elizabeth, 2002).

Archaeological research 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 (Al-Arnaoutt *et al.*, 1987). For a long period of time, henna has been associated with special celebrations. Betrothals, weddings, the eighth month of pregnancy, birthdays and *Eids* for the Muslims are great occasions to be hennaed (Packer, 2009). Henna has also contributed immensely in the field of medicine. Al-Arnaoutt *et al*. (1987) reported in the book of “prophet medicine” that henna usage for medicinal purposes was a very common practise at the beginning of Islamic traditions. De-Almeida *et al*. (1990) described naphthoquinones in henna as anti-inflamatory while other researchers described henna as fungicidal (Gafner *et al*., 1996), virucidal (Heinrich *et al*., 2004), bactericidal (Bintu *et al*., 1996) and anti-malarial (De-Moura *et al*., 2001).

2.3 Henna cosmetic products

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 (Neeraj, 2007).

Basically, henna products are sold either in powder 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 (Packer, 2009).

Paste hennas are the "ready to use" type of hennas in which the ingredients are premixed with a certain solvent selected by the manufacturer. 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 (Elizabeth, 2002). 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 (Packer, 2009).

In cosmetic shops, henna products are sold under different brand names based on the manufacturing company. The demand for henna in the market depends on its quality which is associated with the brand name of the henna product. However, due to stiff competition for henna products, some unscrupulous henna manufactures practice misbranding whereby they sell their products under the brand names of other henna manufacturers. Such a practise is unhealthy to the henna users because it may expose them to the toxic effects of heavy metals through henna products which contain the metals beyond the recommended limit **(**Catherine, 2006).

The henna products in the cosmetic shops in Lamu County were sold under brand names of different henna manufacturing companies. From a particular brand, there were different colours of henna products such as red, black, brown and chestnut (Deleo, 2006). The henna users in Lamu County had preferences for certain colours of henna products hence such products were commonly used (Neeraj, 2007). Some henna products sold in the cosmetic shops in Lamu lacked labels to indicate the country of origin and the ingredients used **(**Catherine, 2006). Such products only displayed colourful illustrations of henna patterns on the package just to attract the henna user while no information about the composition of the henna product was given (Elizabeth, 2002).

2.4 Ingredients used in traditional and premixed henna

The traditional way of preparing henna is by drying the henna leaves which are later ground and sieved to form a powder. In order to form a paste, the powder may either be mixed with oil or water (Elizabeth, 2002). Further treatments on the henna paste are done so as to improve the quality of henna stain depending on individual's preferences. Table 2.1 shows some common ingredients used in the preparation of traditional henna.

Table 2.1: Common ingredients used in traditional henna

Ingredient	**Role**
Citric acid	It digests the cellulose in henna leaves so as to release the dye molecule, lawsone. It also opens up the pores of the cuticle for the penetration of the dye molecule.
Sugar	It sticks the dye molecule onto skin or fur. It prevents cracking and drying of the henna paste which makes it silky smooth and creamy.
Essential oil	Gives henna paste its fragrance and makes the stain dark. It also opens the pores of skin and stimulates heat release.
Henna seed	Adds fragrance, stickiness and stringiness to henna paste.
Water	It adds moisture to the henna. It also promotes exfoliation.
Tea	Adds tannins which increase acidity and together with heat helps in dye release. It also adds moisture, fragrance and texture to henna paste.
Okra	Adds stickiness and stringiness to the henna paste. It also slows the drying and reduces cracking of the dried paste.
Clove	It darkens henna stain and adds fragrance to the henna paste.
Honey	It is a preservative. It smoothens the henna paste and adds stickiness to it.
Eucalyptus oil	It acts as a fragrance. It opens the skin pores and stimulates circulation of heat.
Coffee	Adds moisture and fragrance. It also improves the texture of henna paste.

Source: Ogunwande and Olawore (2006)

Unlike traditional henna, the formulation of premixed henna product may be very extensive. The formulation may include: petroleum jelly, ammonia, camphor, peroxides, detergents, flammable liquids, dyes, alpha hydroxyl, metal and metallic compounds (Ogunwande and Olawore, 2006). Henna ingredients may be used as pre-treatment or post-treatment agents, which help to give out the best quality results of the henna stain (Packer, 2009). Table 2.2 shows the role of treatment agents used in the manufacture of henna products.

Table 2.2: Henna treatment agents

Treatment Agents	Role
Sealer	It is applied as a post-treatment to darken the henna design and improve skin texture. It also helps to speed up the darkening of the henna design.
Fader	It is applied as a post-treatment to speed up exfoliation process. It ensures that henna design fades evenly and removes unwanted designs.
Oxidiser	It helps to darken the stain by oxidising the lawsone while it is on the skin.
Exfoliate	It removes the upper dead layer of skin cells which contain the henna stain.
Moisturiser	It is applied as a pre-treatment to enhance turgidity of the skin cells hence making the skin layer more transparent and thus improves the outline of the henna design.
Colorant releaser	It releases the lawsone from henna so that it can be attached on to the skin.
Texturizer	It helps to treat henna paste so as to suite a particular use in order to achieve the best results. Different textures exist such as smooth, flexible, stringent and sticky depending on the use of that henna paste.

Source: Elizabeth (2002)

2.5 The dyeing process of henna

2.5.1 Henna dye chemical compounds

A number of naphthoquinones including 2-methoxy-3-methyl-1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone (lawsone) are natural pigments which are present in the leaves of henna plants (Singh *et al.,* 2007). Naphthoquinones are highly reactive organic compounds which are used as natural or synthetic dyes whose colours range from yellow to red. Lawsone is the principle secondary metabolite which produces the characteristic red-orange pigmentation whose intensity is enhanced by the conjugation between carbonyl and double bonds to give rise to 1,4-naphthoquinone (Lamoureux *et* *al.*, 2008) as shown in Figure 1.1. The concentration of lawsone in henna leaves vary depending on soil properties, cultivation practises and climatic conditions. The level of lawsone in young henna plant which lacks spines is relatively lower compared to mature henna plant with spines. The highest lawsone content is in the new growth of henna leaves following a period of heat and drought, then a brief flush of rain (Catherine, 2006).

2.5.2 Mechanism of henna dyeing process

Generally, naphthoquinones act as electrophiles which interact with nucleophillic functions in arylation process based on Micheal's addition reaction. Figure 2.2 shows the mechanism of dyeing process.

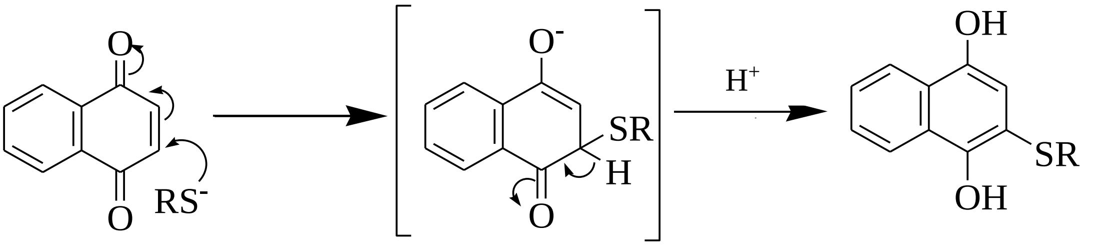


Figure 2.1: Mechanism of lawsone dyeing process

Naphthoquinones are considered as Michael's acceptors because they possess the electrophillic α-β-unsaturated carbonyl system and also due to their ability to form covalent bonds with nucleophillic groups which are the sulphide (RS^{-}) and amide (RNH^{-}) functions of the keratin. Lawsone has a strong affinity for keratin and interacts with it directly in a non-oxidative process which is directed towards retention of electrons through Michael's addition reaction.

Due to steric hindrance, the nucleophile prefers the β-carbon of the lawsone molecule which is far away from hydroxyl group to form lawsone-sulfide complex as shown in

Figure 2.2. Depending on the nucleophillic group, the complex formed in Figure 2.2 is either a sulphide or amide complex (Kumagai *et al*., 2011). The nucleophiles may also attack the keto-carbon in lawsone where an imine or schiffs base complex is formed as shown in Equation 2.1

$$C_9H_5O_2\text{-}C{=}O + H_2N\text{-Keratin} \longrightarrow C_9H_5O_2\text{-}C{=}N\text{-Keratin} + H_2O\text{....................Eq 2.1}$$
(Lawsone) (Amine-keratin) (Schiff's base)

A similar reaction involving sulphide functional group of keratin could also occur as shown in Equation 2.2.

$$C_9H_5O_2\text{-}C{=}O + HS\text{-Keratin} \xrightarrow{H^+} C_9H_5O_2\text{–}C{=}S\text{-Keratin} + H_2O\text{....................Eq 2.2}$$
(Lawsone) (Sulphide-keratin) (Schiff's base)

2.5.3 Fixation of the dye molecule on keratin

The chemistry of bonding of dyes to fibres involves complexation process in which hydrogen bonding and hydrophobic interactions occur. Keratin retains the lawsone through hydrogen bonding between polypeptide linkages and the dye (Kumagai *et al*., 2011). During the dyeing process, lawsone anions interact with protonated groups of keratin through electrovalent bonds which fix the dye firmly onto the keratin. Lawsone has high affinity for keratin hence making henna stain permanent (Tsankov *et al*., 2010).

Henna products contain natural and synthetic substances which perform specific roles such as to increase the intensity of the henna stain or to alter stain colour (Guareschi *et al*., 2007). Natural dyes such as lawsone require metal salts as mordants to alter light absorption characteristics of tannins and in addition to acquire washing fasteness. Metal salts such as nickel salts help to fix dyes to the keratin (Al-Saleh and

Coate, 2006). In addition, heavy metals in decorative cosmetic products act as colouring agents. For example, lead salts such as lead sulphide, lead oxide or lead acetate help to impart red pigmentation in henna products (Elizabeth, 2002). Through redox cycling, lawsone can chelate with heavy metal cations leading to their bioaccumulation (Tsankov *et al*., 2010). Since henna is applied on the skin, dermal exposure forms the vital route through which ions of heavy metals get into the body. When heavy metal ions come into contact with skin, they form complexes with carboxylic acid (-COOH), amine ($-NH_2$) and thiol (-SH) of protein resulting in malfunction or death of cells (Al-Saleh *et al*., 2009).

2.6 Heavy metals

Heavy metals are commonly defined as those having a specific density of more than 5 g/cm^3 (Hu, 2000). Factors affecting the absorption of heavy metal cation by a plant include, cation mobility, level of the cation in the soil and chemical properties of the metal (Ruchi *et al*., 2014). Some essential heavy metals such as nickel help to regulate and control biological functions within a plant while non-essential heavy metals such as lead and cadmium do not perform any physiological role (Tsankov *et al*., 2010). In medicinal plants such as henna plant, heavy metal cations play a vital role in therapeutic mechanism of the plant (De-Almeida *et al*., 1990).

The type and quantity of heavy metals used in henna products depend on the propriety of the henna manufacturer (Marcovecchio *et al*., 2007). Lack of and or ineffective quality control measures and proper legal framework on heavy metals used in henna products pose a health risk to the henna users (ACS, 2014). This is due to the toxic effects associated with the heavy metals (Vodela *et al*., 1997).

2.7 Lead

2.7.1 Toxic effects of lead in the body

In biochemical processes, lead mimics the action of calcium which results in osteomalacia (ATSDR, 2005). Exposure to lead may decrease the cognitive function in children leading to encephalopathy. Neurobehavioural effects of lead poisoning on adults such as irritability and lethargy have been reported in adults with lead levels in blood (LLB) ranging from 40 to 80mg/dL (CDC, 2009). Lead inhibits the action of δ-aminolevulinic acid dehydrogenase (ALAD) and ferrochelatase which interferes with heme biosynthesis and insertion of iron in protoporphyrin 1X ring, respectively, leading to anaemia (Eman *et al*., 2011). Lead poisoning may result in nephrotoxicity (ATSDR, 1999). Sperm count may decrease at higher LLB (Alexander *et al*., 1996).

2.7.2 Levels of lead in plants

In medicinal herbal plants growing in Pakistan, Sumayya *et al*. (2010) reported lead levels at a concentration of 4.81 ppm and 5.16 ppm in *Azadirachta indica* and *Helanthus annus,* respectively. Comparatively, the reported levels of lead were above the WHO (2010) recommended limit of 2 ppm in herbal plants. In another study, Moses *et al*. (2012) recorded levels of lead in leaves of *Physalis peruviana* growing in Kisii, Kenya at a concentration of 0.41 ppm.

Using AAS, Alwakeel (2008) and Mamoona and Maryam (2015) reported levels of lead in leaves of henna plants growing in Pakistan at a concentration of 0.011 ppm to 1.528 ppm and 0.019 ppm, respectively. Using ICP-AES, Ghanjaouri (2014) reported the highest lead levels in leaves of henna plants growing in Morocco at a concentration of 3 ppm.

2.7.3 Levels of lead in cosmetic products

Nnorom *et al*. (2005) reported the highest levels of lead in facial cosmetics commonly used in Nigeria at a concentration of 131.0 ppm, 13.4 ppm and 14.6 ppm in eye liners, eye pencils and lipstick respectively. These levels of lead were above the tolerable limits of 10 ppm and 2 ppm by WHO (1995) and KeBS respectively. The levels of lead in *Aloe vera* cosmetic products used in Nairobi, Kenya ranged as follows: 0.06 ppm to 0.63 ppm in lotions and 0.03 ppm to 0.68 ppm in creams (Onyambu, 2014).

A study done by Chukwuma (1997) on sampled cosmetic henna products sold in Nigeria recorded the highest level of lead at a concentration of 4.53 ppm. A similar study on imported henna products in Denmark by DEPA (2005) recorded lead at a concentration range of between 0.89 ppm to 2.0 ppm.

2.8 Cadmium

2.8.1 Toxic effects of cadmium in the body

Cadmium fumes cause cadmium fever (“Cadmium blues”) which is characterised by flu-like symptoms. Overexposure to cadmium fumes may lead to respiratory tract effects such as bronchitis, pneumonitis and pulmonary oedema (Roels *et al*., 1989). Cadmium increases low molecular weight proteins such as 2-microglobulin and retinol binding proteins in urine, a condition called proteinuria (ATSDR, 2012). Chronic cadmium exposure may lead to glomerular dysfunction (Iwata *et al*., 1992). Cadmium related effects on skeletal tissue may lead to osteoporosis (Kido *et al*., 1990), teeth discolouration and loss of sense of smell (OSHA, 1992). In 1950, farmers in Toyama (Japan) developed “itai itai” (“ouch ouch”) disease after consuming cadmium contaminated rice from Jinzu river (Nogowa *et al*., 2004). Lung cancer and other types of tumour may be induced through cadmium exposure (IARC, 1987).

2.8.2 Levels of cadmium in plants

A study on the levels of cadmium in leaves of medicinal herbs growing in Pakistan reported the following concentrations: 32.18 ppm Cd in *Azadirachta indica* and 34.48 ppm Cd in *Helanthus annus* (Sumayya *et al*., 2010). These concentrations were extremely higher than the WHO (2010) recommended limit of 0.2 ppm. In another study, Mitei (1996) reported levels of cadmium in leaves of tobacco plants cultivated in Kenya at a concentration range of 0.5 ppm to 2.33 ppm.

Using AAS, Alwakeel (2008) reported cadmium level in leaves of henna plants growing in Saudi Arabia at a concentration range of 0.016 ppm to 0.019 ppm. Using ICP–AES, Ghanjaouri (2014) reported the highest cadmium level in leaves of henna plants from Morocco at a concentration of 0.170 ppm.

2.8.3 Levels of cadmium in cosmetic products

The levels of cadmium in facial cosmetics commonly used in Nigeria were reported at a concentration of 1.30 ppm in eyeliners, 0.80 ppm in eye pencils and 1.10 ppm in lipsticks (Nnorom *et al*., 2005). These levels of cadmium were above the threshold limit of 0.3 ppm (WHO, 1995). However, compared to the set limit of 2 ppm by KeBS, these levels of cadmium were lower.

A study carried out to determine the levels of heavy metals in henna products sold in Palestine using ICP-MS (Agilent 7500) showed a remarkable level of cadmium detected at a concentration of 13.36 ppm (Al-Qutob and Azzatrash, 2013). This level was extremely high compared to the set limit of 2 ppm by KeBS. DEPA (2005) investigated heavy metals in selected henna products sold in Denmark and recorded cadmium level at a concentration range of 0.01 ppm to 0.05 ppm.

2.9 Nickel

2.9.1 Toxic effects of nickel in the body

In 2008, nickel was declared the “allergen of the year” due to many cases of skin sensitisation reactions caused by nickel in cosmetics. Contact dermatitis is the most commonly reported adverse effect associated with nickel (ATSDR, 1988). Exposures to nickel carbonyl ($Ni(CO)_4$) can cause headache, nausea, vomiting and breathing problems and in case of high exposure, it may result in pneumonia and death (CDTSC, 2004). Inhalation of nickel compounds such as nickel sulphate ($NiSO_4$), nickel subsulphide (Ni_3S_2) and nickel oxide (NiO) may trigger non-cancerous lung inflammation (IPSC, 1991) as well as cancer of the lungs, nose and sinuses (ATSDR, 1988). Chronic non-cancer health effects may result from long-term exposure to relatively low concentration of nickel (IPSC, 1991). As a carcinogen, nickel subsulphide combines with the nucleus of lung epithelial cells to form macrocyclic nickel complexes, $[NiCR]^{2+}$ and $[Ni(CR–2H)]^{2+}$ which may cause inheritable changes in chromosomes (Aleksandra and Ursuzula, 2008).

2.9.2 Levels of nickel in plants

Shad *et al*. (2008) investigated levels of nickel in selected medicinal plants growing in Pakistan and reported the following concentration: 4.35 ppm in *Artemisia vulgaris*, 4.16 ppm in *Galium aparine* and 2.36 ppm in *Stevia rebaudiana* all of which had their nickel levels above the WHO (2010) recommended limit of 1.68 ppm. In another study, Moses *et al*. (2012) reported the highest level of nickel in *Bidens pilosa* growing in Kisii, Kenya at a concentration of 1.16 ppm.

Using AAS, a study done by Alwakeel (2008) on the level of heavy metals in leaves of henna plants growing in Saudi Arabia reported a concentration range of between

0.036 ppm to 0.073 ppm of nickel. Ahmed *et al*. (1994) recorded nickel at a level of 0.18µg/g in henna leaves sampled from henna plants growing in Faisalabad District, Pakistan.

2.9.3 Levels of nickel in cosmetic products

Onwordi *et al*. (2013) analysed some toxic metals in body creams sold in Lagos, Nigeria and noted the levels of nickel in skin lightening creams and moisturizers at a concentration of 5.09±0.202 ppm and 3.61±3.46 ppm, respectively. From the study, the level of nickel from these products was above the tolerable limit of 1 ppm by Health Canada (2011). Using AAS, Chung (2006) analysed heavy metals found in henna products sold in Seoul Korea and noted the level of nickel at a concentration range of 2.5 ppm to 3.96 ppm. DEPA (2005) reported nickel level in selected henna products sold in Denmark at a concentration range of 1.7 ppm to 4.0 ppm. From these studies, the levels of nickel in henna products were above the tolerable limit of 1 ppm by Health Canada (2011).

From the studies of heavy metals in plants, similar or different species of plants recorded different levels of heavy metals. This could be as a result of different pollution levels of the soils among other factors (Raven and Johnson, 1998). The studies also showed that similar or different cosmetic products recorded different levels of heavy metals depending on factors such as raw materials and the manufacturing process (Mazoor and Mahmoud, 2011). Since henna users have different preferences, the types or brands of henna products used are equally different. As a result, different henna products from various henna brands available in the market have different levels of heavy metal exposure since their composition is different **(**Catherine, 2006).

2.10 Methods of analysis

Various analytical methods can be employed to analyse the presence and levels of heavy metals in cosmetic products. Such methods include flame atomic absorption spectroscopy (FAAS), inductively coupled plasma mass spectroscopy (ICP-MS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), energy dispersive x-ray (EDX), elemental analyser (EA) and neutron activation analysis (NAA). Heavy metals in cosmetic products can best be determined using atomic absorption spectroscopy (AAS) (Skoog *et al.*, 1992). More than 62 different metal ions in aqueous state can be analysed using AAS. The advantages of AAS over other analytical methods include higher sensitivity, greater limit of detection and minimal interference effect. AAS can directly analyse some types of liquid samples and in addition it only requires a small sample size (Skoog and Leary, 1992). In this study FAAS was used to analyse heavy metals in henna samples.

2.10.1 Principle involved in FAAS

The principle in AAS involves absorption of light by free atoms of an element at a specific wavelength to that element. An aqueous sample containing the metal analyte is aspirated into an air-acetylene flame, causing evaporation of the solvent and vaporisation of the free metal atoms. This process is called atomization. A line source operating in the UV-visible spectral region is used to pass radiation through the flame where the free atoms absorb the characteristic wavelength of radiation. When light is absorbed, a transition process occurs whereby atoms move from one energy level to another. For the case of steady state 0 and 1(where 0 is the ground state and 1 is the excited state), with their corresponding energies E_0 and E_1, where $E_1>E_0$, the $0\rightarrow1$ transitions result in the absorption of light with frequency shown in Equation 2.3

$$V_{01} = \frac{E_1 - E_0}{h} \quad \text{.......Eq 2.3}$$

Where:

V_{01} – Frequency
h – Planks constant
E_0 – Energy at ground state
E_1 – Energy at excited state

External radiation from hollow cathode tube is responsible for the stimulation of $0 \rightarrow 1$ transition. Upon excitation, an outer electron will be promoted to a higher energy which is less stable excited state (Skoog and Leary, 1992).

When a parallel beam of light at a resonance wavelength strikes a cell unit containing N atoms, the amount of light absorbed can be calculated. Consider light intensity P_0 entering a cell, the intensity remaining after absorption is given by Equation 2.4

$$P = 1 – P_0 \exp^{[-kl]} \quad \text{.......Eq 2.4}$$

Where:
P – unabsorbed radiation
l – path length
P_0 – Incident radiation
k – absorption coefficient

When applying logarithm, Equation 2.4 reduces to Equation 2.5

$$kl = \log P_0/P \quad \text{.......Eq 2.5}$$

The product kl is proportional to the number of atoms in the cell. The expression $\log P_0/P$ is called absorbance and is used in AAS. However, absorbance recorded in AAS relates with the concentration of the analyte as shown in the Beers-Lamberts Law in Equation 2.6

$A = \varepsilon bc$...Eq 2.6

Where:
A – absorbance (no units, since $A=\log_{10}P_0/P$).
ε – the molar absorptivity with units in L mol^{-1} cm^{-1}.
b – the path length of the sample that is, the path length of the cuvette in which the sample is contained. It is expressed in centimetres.
c – the concentration of the compound in solution, expressed in mol/L.

From the Beers-Lamberts law it shows that absorbance is directly proportional to concentration (mol/L) and path length (cm) (Dean, 1995).

2.10.2 Instrumentation of Atomic Absorption Spectroscopy(AAS)

Basically, AAS consists of five main components: radiation source, atomizer, wavelength selector, detector and readout device. Figure 2.3 shows the basic components in AAS.

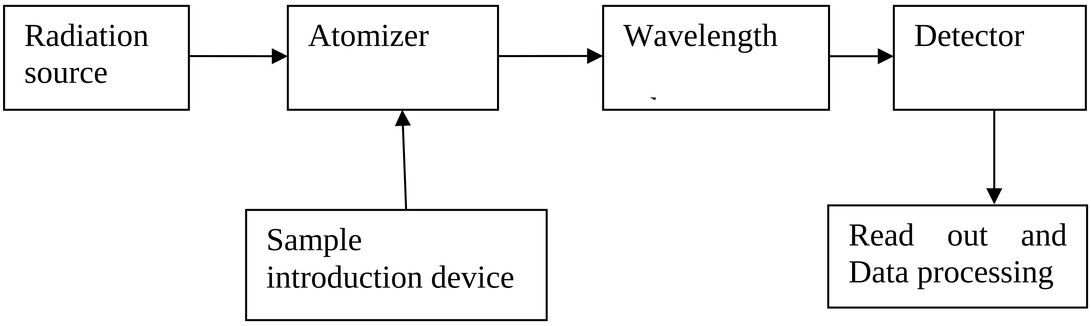


Figure 2.1: Block diagram of an AAS. Source: Beaty and Kerber, (1993)

The common radiation sources used in AAS are hollow cathode lamp and electrode discharge lamp. The radiation source is operated to give narrow and intense light from the element that forms the cathode. There are two types of atomizers, flame and electro thermal atomizer. Atomizers produce the ground state free gas phase atoms which are necessary for the AAS process to take place. Monochromator is used as a wavelength selector which separates the absorption line of interest from other spectral lines emitted by hollow cathode lamp. Monochromators include filters, prisms and gratings, the later being commonly used in AAS. Detectors used include phototube, photomultiplier and photodiode. The most common detector used in AAS is photomultiplier detector. Readout system simplifies operations through programming of various functions through microprocessors and microcomputers (Beaty and Kerber, 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

The research adopted an experimental design which involved random sampling of henna leaves, paste henna products and powdered henna products. Digestion of henna sample followed a given protocol. Analysis of henna 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.

3.2 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.

3.3 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 used.

Amu was nominated by UNESCO as one of the world heritage centers. Amu town is where most of the major cosmetic henna shops are located. The other parts of the County get their henna supply from Amu town. The town lacks proper waste management system. Due to poor sewerage and drainage system, waste water management is inefficient. Some henna plants grow 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 (LCIDP, 2013).

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 (LCIDP, 2013).

3.4 Sample size

A total of one hundred and twenty (120) henna samples 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 (UNODC, 2009).

$$n = \sqrt{N}/2 \qquad \text{……………………………………………………………………..…3.1}$$

Where:
n = Sample size
N = Total population

3.4.1 Sampling and collection of henna products

Sixty (60) henna products were sampled out of the total sample size of one hundred and twenty (120). Thirty nine (39) henna products were in powdered form while twenty one (21) were in paste form which represented four (4) henna powder 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 3.1.

Table 3.1: Henna products sampling

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

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

The most commonly applied henna products based on their category and colour from their respective brands were sampled from cosmetic shops 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.

3.4.2 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. Out of the total sample size of one hundred and twenty (120), sixty (60) samples were henna leaves picked from all the selected henna plants. The sampling scheme for the henna leaves is shown in Figure 3.1

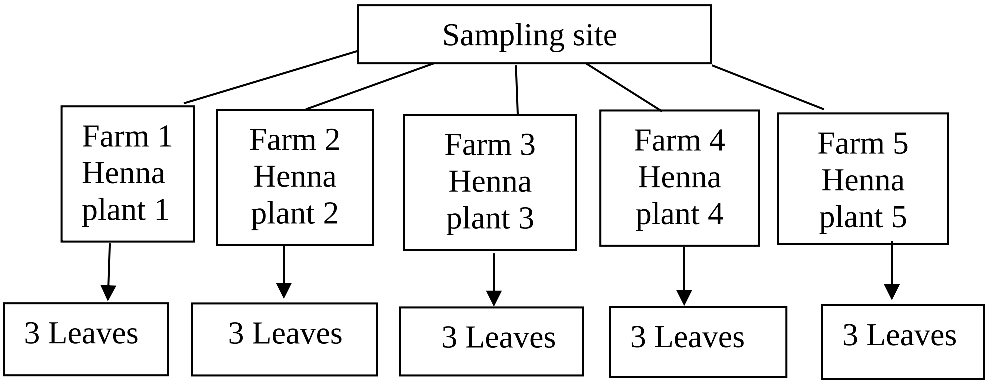


Figure 3.1: Sampling scheme for henna leaves

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 (UNODC, 2009). 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 (Ahmed *et al.*, 1994). 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.

3.5 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.

3.6 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.

3.7 Pre-treatment of henna sample

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 1.000 g 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.

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 (Ogunwade and Olawore, 2006). 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 (Mazoor and Mahmoud, 2011). Each set was later packed in an air tight labeled Teflon container awaiting digestion.

3.8 Henna sample treatment

Nitric acid-chloric acid wet digestion method was adopted to treat henna sample because of its better recovery of lead, cadmium and nickel from henna leaves and henna products (Umar and Caleb, 2013). A mass of 1.000 g of either henna product set or 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 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.

3.9 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 (Skoog and Leary, 1992).

3.9.1 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

$\mathbf{V_1 C_1 = V_2 C_2}$..Eq 3.2

Where:
$\mathbf{V_1}$ – Initial volume
$\mathbf{C_1}$ – Initial concentration
$\mathbf{V_2}$ – Final volume
$\mathbf{C_2}$ – 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

$\mathbf{V_1 C_1 = V_2 C_2}$

$\mathbf{V_1}$ x 1000 ppm = 100 ml x100 ppm;

Hence $\mathbf{V_1} = \frac{100 \text{ ml x } 100 \text{ ppm}}{1000 \text{ ppm}} = 10$ 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, $\mathbf{V_1 C_1 = V_2 C_2}$

$\mathbf{V_1}$ x 100 ppm = 50 ml x 4 ppm;

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

3.9.2 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.

3.9.3 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.

3.10 Preparation of standard reference material (SRM)

A certified standard reference material No. 1572 citrus leaves (CL) from NIST (1992) was used. Its solution was prepared the same way as the henna sample.

3.11 Methods validation

3.11.1 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.

3.11.2 Standard addition

The AAS was validated by spiking the samples with known stapndards 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 (Miller and Miller, 1988). The absorbencies of all the sample solutions were measured in triplicate before and after spiking in order to obtain the percentage recovery.

3.11.3 Standard reference material

The accuracy of FAAS was tested using a certified standard reference material No. 1572 citrus leaves (CL) (NIST, 1992). 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.

3.12 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

$$y = y_B + 3s_B \quad \text{Eq 3.4}$$

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.

3.13 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.

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

3.14 Calculation of concentration of heavy metals in henna samples

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

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

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

3.15 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 $p = 0.05$ ($\alpha = 0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

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

4.2 AAS validation results

4.2.1 Calibration curves and regression analysis

Figure 4.1 shows the standard calibration curve for lead.

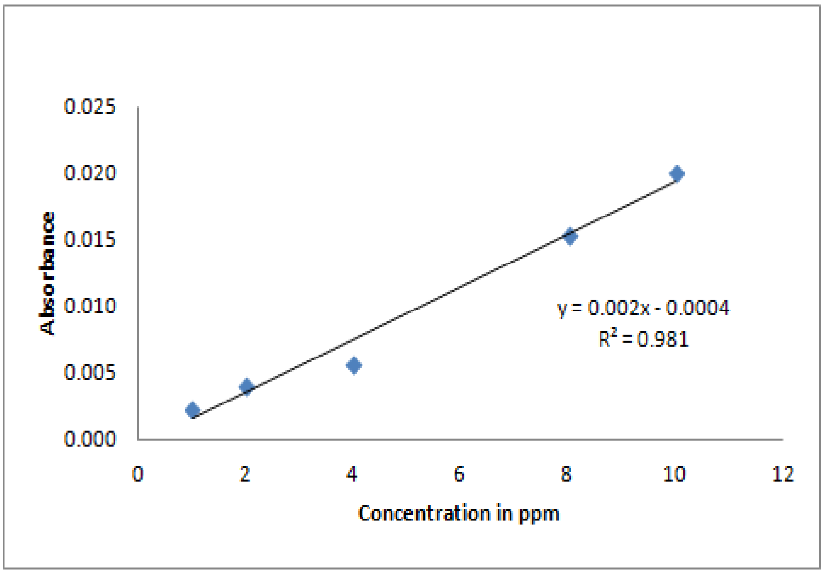


Figure 4.1: Calibration curve for lead

Figure 4.2 shows the standard calibration curve for cadmium.

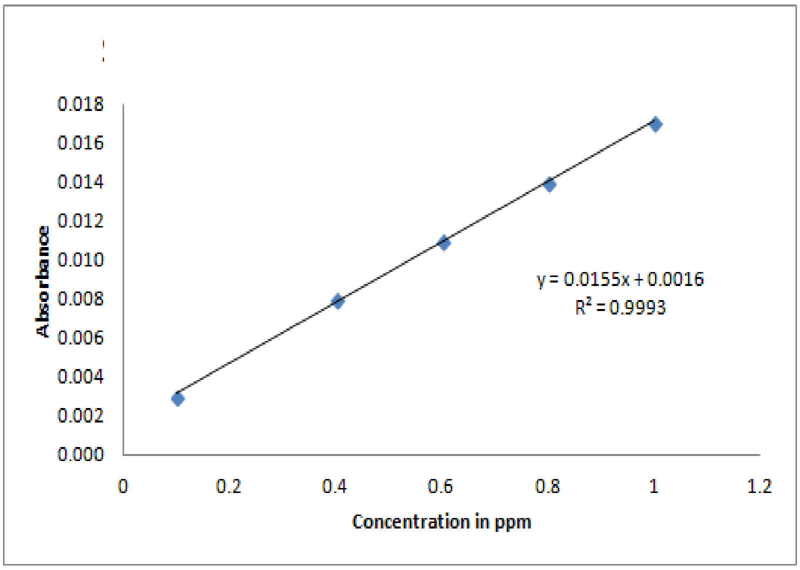


Figure 4.2: Calibration curve for cadmium.

Figure 4.3 shows the standard calibration curve for nickel.

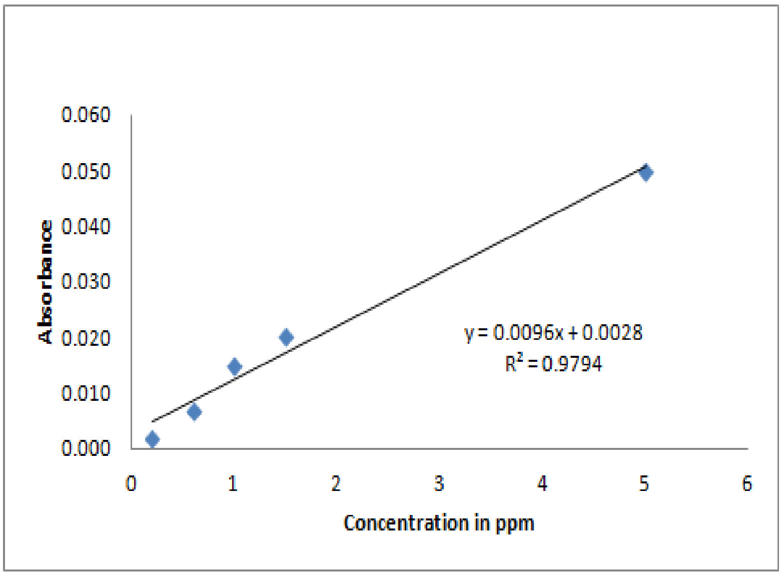


Figure 4.3: Calibration curve for nickel

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 (Mendham *et al*., 1999). 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 the henna leaves and henna products (Miller and Miller, 1988). 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 (Sawyer and Beebe, 2007).

4.2.2 Standard addition

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

Table 4.1: 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 (Kings and Burgess, 1998).

4.2.3 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.

Table 4.2: 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 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 shows that the measured values were not significantly different from the certified values hence good accuracy of the instrument at 95% confidence level (Miller and Miller, 1988).

4.3 Heavy metals in henna leaves from Lamu

4.3.1 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).

In Morocco, Ghanjouri (2014) recorded the highest level of lead in henna leaves at a concentration of 3.00 ppm which is above the level of 0.93±0.04 ppm obtained from this study. However, Mamoona and Maryam (2015) reported lower level of lead in henna leaves sampled from Pakistan at a concentration of 0.019 ppm.

Lead has not been shown to perform any role in plant's metabolism although it occurs naturally in plants. Compared to other heavy metals, lead forms the highest percentage in the soil (Shad *et al.*, 2008). The level of lead in the soil as well as the form in which it occurs will determine its uptake by the plants (Kabata and Pendias, 1985). For a plant that grows in less polluted area, the level of lead is in the range of 0.1 ppm to 10 ppm (Raven and Johnson, 1998). 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 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 (Kabata and Pendias, 1985).

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 which is below the level of 0.34±0.02 ppm recorded from this study. In another study, Alwakeel (2008) analysed 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 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 (Ruchi *et al.*, 2014).

The different levels of heavy metals in the henna leaves may be attributed to different pollution levels under which the henna plant is growing (Al-Saleh *et al*., 2009). Both the anthropogenic and non-anthropogenic sources of pollution contribute to the level of heavy metals in plants (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 (Kaara, 1992; Mitei, 1996). In addition, the heavy traffic in Mpeketoni as well as the effluents from car garages could contribute to the high level of lead (Mutua, 2013). 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 (Raven and Johnson, 1998). 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 (WHO, 1992). 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.

4.3.2 Levels of accumulation of heavy metals in henna leaves

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

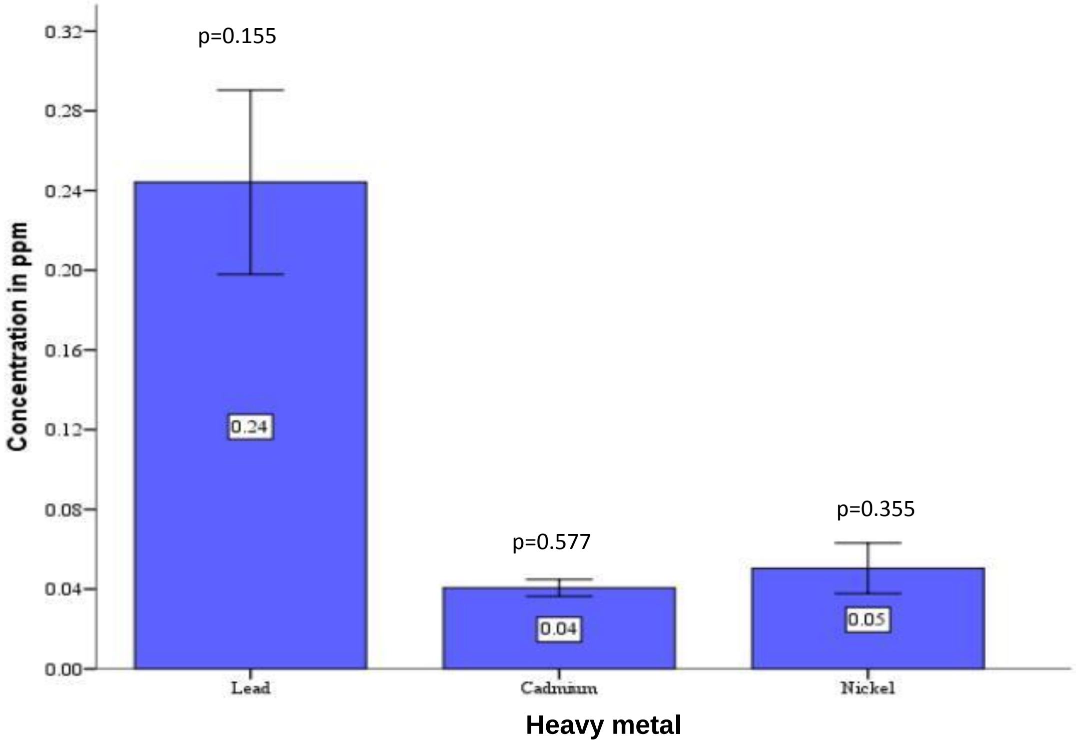


Figure 4.4: Overall mean level of lead, cadmium and nickel in henna leaves from Lamu

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. 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 (Shad *et al*., 2008). The lowest level of cadmium could be due to the basic calcareous soils and limestone within Lamu (Muchena and Ekirapa, 1992) which could render cadmium unavailable to the roots and leaves of plants (Kabata and Pendias, 1985).

4.3.3 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 WHO (2010). 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 (Ghanjaouri, 2014). Hence, 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.

4.4 Heavy metals in henna products from Lamu

4.4.1 Level of lead, cadmium and nickel in powdered henna brands

Table 4:5: 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 4.5, 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 brand 2 (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 (1997) 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±0.03 ppm. 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 (2005) in Denmark. Among other roles, cadmium may be added to cosmetic products to give deep yellow to orange pigmentation (Elizabeth, 2002). 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 (Iwata *et al*., 1992).

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 (2006) 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.

4.4.2 Level of lead, cadmium and nickel in paste henna brands

Table 4:6: 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

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 4.6, henna product PABR and PARD of brand 5 and 6 recorded the highest level of lead at a concentration of 1.32±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 BDL to 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.

Al-Qutob and Azzatrash (2013) 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 analysed, 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 from 0.14±0.02 ppm to 3.01±0.01 ppm.

Tables 4.5 and 4.6 showed spurious levels of lead, cadmium and nickel in the henna products sampled from different brands (Neeraj, 2007). Since the metals were not indicated on the label of 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 limit or was not aware of these metals in the products since the metals were added inadvertently. Differences in the level of heavy metals between the powdered henna products and paste henna products were evident.

4.4.3 Comparison of heavy metal level in powdered and paste henna brands

Results from Table 4.5 and 4.6 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 4.5

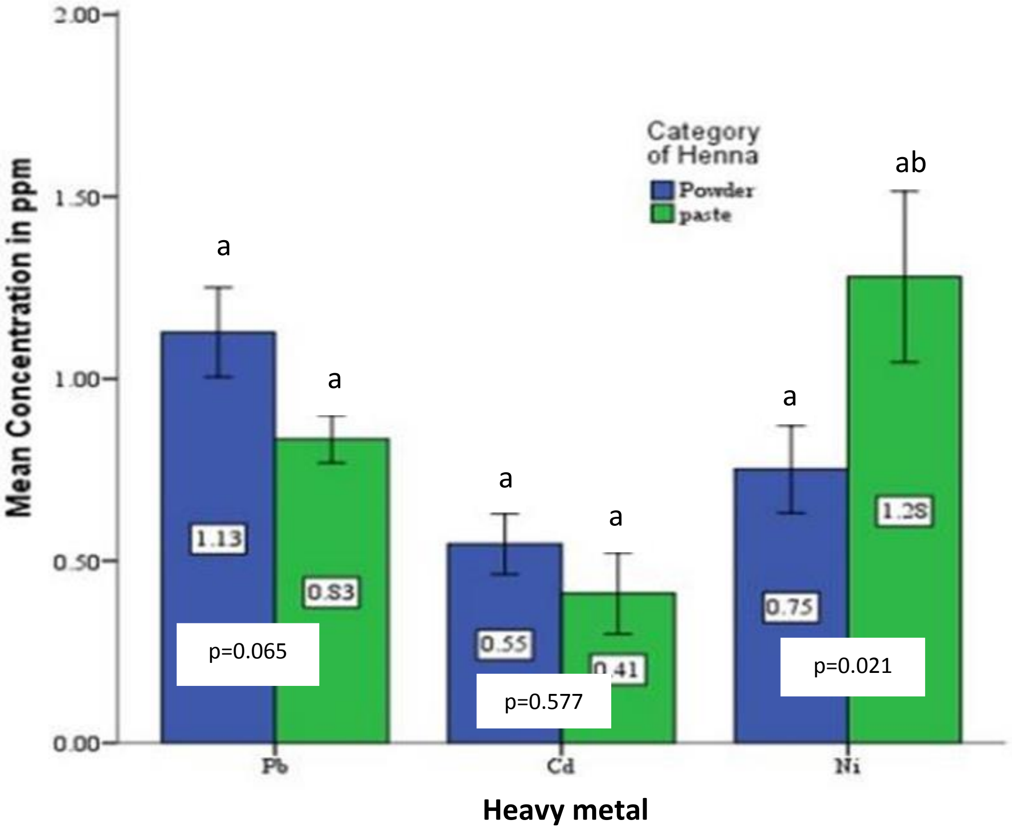


Figure 4.5: Overall mean level of lead, cadmium and nickel in powdered and paste henna brands from Lamu

From Figure 4.5, 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.

Followed by Figure 4.5, the paste henna brands were more vulnerable to nickel exposure because their overall nickel content of 1.28 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 determine their level of vulnerability which differ with the state of henna matrix. The type of preservative used and the binding power in henna may determine the type of heavy metal used in a given henna matrix. Hence, 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.

4.5 Comparison of heavy metal level in henna products with standard limits

Table 4.7 compares the level of lead, cadmium and nickel in henna products sampled from Lamu with the standard limit by KeBS, WHO and Health Canada.

Table 4.7: 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

In Table 4.7, 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 (1997) 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.

The type and level of heavy metals in some cosmetic products depend on the colour that they impart (Packer, 2009). The study showed that the deeper the red colour of henna the higher the lead content (Elizabeth, 2002). This is shown in Table 4.7 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 4.7 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.

4.6 Comparison of levels of heavy metals in henna leaves, powdered and paste henna products from Lamu

Results from Table 4.3, 4.5 and 4.6 were used to derive the overall mean levels of heavy metals in henna leaves, powdered and paste henna products. The overall mean levels of heavy metals were subjected to tests of significance to show the pattern of variation of heavy metals in henna leaves, powdered and paste henna products from Lamu is indicated in Figure 4.6

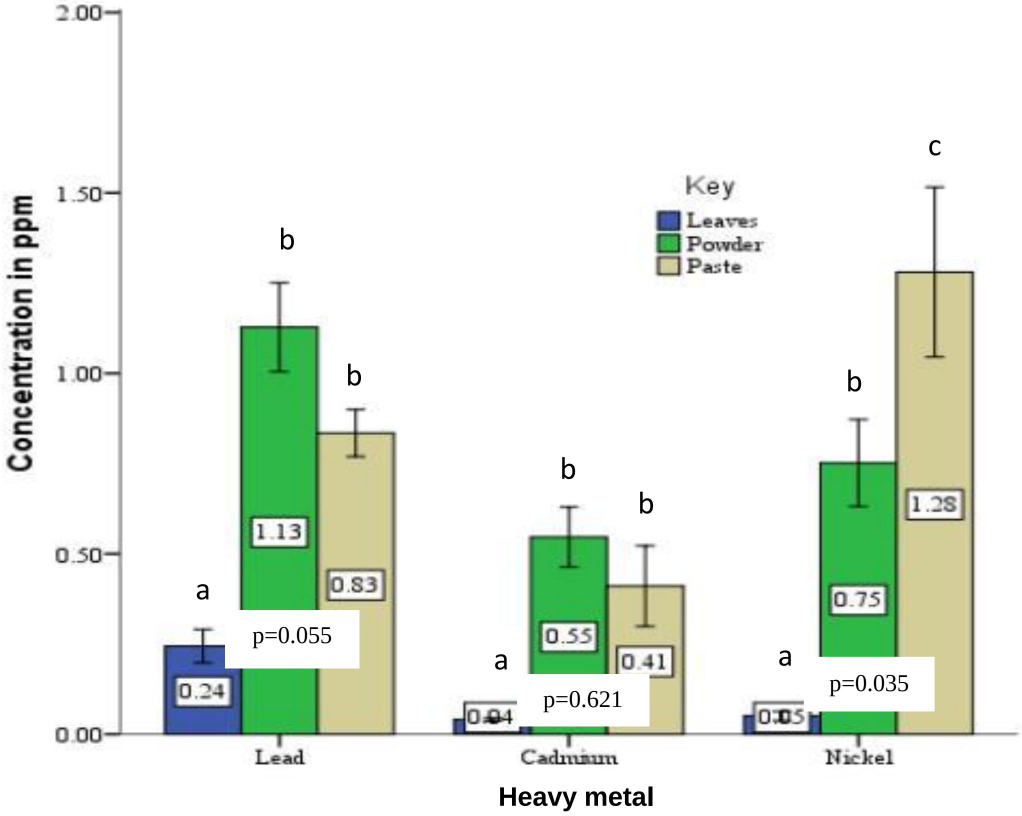


Figure 4.6: Overall mean level of lead, cadmium and nickel in henna leaves, powdered and paste henna products from Lamu

From Figure 4.6, henna leaves recorded the least significant overall mean levels of lead, cadmium and nickel at a concentration of 0.24 ppm, 0.04 ppm and 0.05 ppm, respectively compared to both powdered and paste henna products. Powdered henna products recorded the highest overall mean level of lead at a concentration of 1.13 ppm while paste henna products recorded the highest significant overall mean level of nickel at a concentration of 1.28 ppm. Generally, the overall mean level of lead, cadmium and nickel in all the powdered and paste henna products was recorded at a concentration of 0.98±0.09 ppm, 0.48±0.09 ppm and 1.02±0.18 ppm, respectively. A significant difference was noted in the overall mean level of nickel (p=0.035) while lead (p=0.055) and cadmium (p=0.621) did not differ significantly among the henna leaves, powdered and paste henna products.

From the study, the level of lead, cadmium and nickel in henna leaves did not match with the levels in the henna products. This showed that the source of heavy metals in henna products was not from the henna leaves only but more of these metals were added either during or after the manufacturing process of henna products. During the manufacturing process, these metals could have been added either inadvertently or compounded as formulants. After the manufacturing process, poor handling during packaging, storage and transport of henna products was another possible source of the metals. Effective quality control measures, proper selection of raw material as well as good manufacturing and analytical skills should be put in place so as to minimise the levels of heavy metals in henna leaves and henna products.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i. 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.

ii. 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.

iii. 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.

iv. The levels of heavy metals in all henna products were determined and compared with standard limit. In comparison to KeBS set limit of 2 ppm, generally all sampled henna products recorded lower levels Cd and Pb.

5.2 Recommendations

5.2.1 Recommendations from this study

i. 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.

ii. Since the levels of heavy metals in henna leaves were lower compared to those in henna products, the henna manufacturers should minimise the use of heavy metals in henna products as formulants and or reduce the addition of these metals inadvertently.

iii. 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.

iv. Application of henna products that contain heavy metals should be minimised because the metals are toxic and bioaccumulative.

5.2.2 Recommendations for further study

i. The study should be extended to analyse *p*-PD, a common oxidative dye in premixed henna and a major cause of contact dermatitis.

ii. Other toxic heavy metals in henna products such as arsenic and chromium should be analysed.

iii. The level of lawsone in henna leaves of different age should be investigated.

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APPENDICES

Appendix I: 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

Appendix II: Codes for henna brands and henna products based on their categories and colours

Henna brand	Category	Colour	Sample code
1	Powder	Red	PWRD
	Powder	Brown	PWBR
	Powder	Red	PWRD$_1$
	Powder	Black	PWBL
2	Powder	Brown	PWBR
	Powder	Burgundy	PWBU
	Powder	Chestnut	PWCH
	Powder	Blond	PWBD
3	Powder	Red	PWRD
	Powder	Black	PWBL
4	Powder	Chestnut	PWCH
	Powder	Red	PWRD
	Powder	Red	PWRD$_1$
5	Paste	Blonde	PABD
	Paste	Brown	PABR
	Paste	Black	PABL
	Paste	Red	PARD
6	Paste	Brown	PABR
	Paste	Burgundy	PABU
	Paste	Red	PARD$_1$

Appendix III: 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}