

**LEVELS OF SELECTED HEAVY METALS IN *ALOE VERA*  
BRANDED SKIN COSMETICS**

**BY**

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**DECLARATION**

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

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**DEDICATION**

This work is dedicated to my husband David and our children Donlin, Adah, Olga and Amber.

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**ABBREVIATIONS AND ACRONYMS**

AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
BLD	Below Limit of Detection
CV-AAS	Cold Vapour Atomic Absorption Spectroscopy
KEBS	Kenya Bureau of Standards
MPL	Maximum Permissible Limit
RPE	Research Planning and Extension
SNK	Student Newman Keul's
WHO	World Health Organization

## ABSTRACT

Over years, it has been reported that long term exposure of heavy metals such as mercury(Hg), lead (Pb) and cadmium (Cd) would result into numerous dangers among these; being carcinogenic, sterility and behavioural abnormalities. While there are various sources of heavy metals, both herbal (those made from *Aloe vera*, neem and olive oil among others) and synthetic cosmetics and toiletries are equally feared as, would be sources especially if levels are above those set by the World Health Organisation (WHO). In China for example, 60 percent of cosmetics and other product were recalled because of the presence of heavy metals in levels toxic to human in the products. In particular, fears arise when labels on these products fail to indicate either the presence or the levels of heavy metals despite certification by bodies such as the Kenya Bureau of Standards (KEBS) in Kenya. While the use of heavy metals as deliberate cosmetics ingredients may have been given attention, heavy metals could still be found as impurities. As such dermal contact with some water-soluble toxic elements and/or their compounds would cause absorption through a moist skin and hence be toxic. The aim of this study therefore was to determine the concentration of Hg, Pb, Cd, Zn, Mn and Cr in skin cosmetics; lotions, creams and soaps made from *Aloe vera*. Cosmetics were purposively sampled from major supermarkets in Nairobi County. All the heavy metals under study were quantified using atomic absorption spectroscopy (AAS) except for Hg where cold vapour atomic absorption spectroscopy (CV-AAS) was employed. The mean levels (ppm) of heavy metals were found to fall in the following ranges:-In lotions, Hg ( $0.01\pm 0.01$ - $0.03\pm 0.01$ ), Pb ( $0.06\pm 0.02$ - $0.63\pm 0.19$ ), Zn ( $0.02\pm 0.01$ - $0.04\pm 0.01$ ), and Mn ( $0.01\pm 0.01$ - $0.06\pm 0.01$ ). In creams Hg ( $0.04\pm 0.01$ - $0.11\pm 0.01$ ), Pb ( $0.03\pm 0.01$ - $0.68\pm 0.14$ ), Cd ( $0.02\pm 0.01$ - $0.06\pm 0.01$ ), Zn ( $0.11\pm 0.01$ - $1.99\pm 0.01$ ), Mn ( $0.02\pm 0.01$ - $0.10\pm 0.01$ ), and Cr ( $0.03\pm 0.01$ - $0.08\pm 0.01$ ). In soaps, Hg ( $0.09\pm 0.01$ - $0.21\pm 0.01$ ), Pb ( $0.14\pm 0.07$ - $0.83\pm 0.01$ ), Cd ( $0.01\pm 0.01$ - $0.15\pm 0.01$ ), Zn ( $0.02\pm 0.01$ - $0.07\pm 0.01$ ), Mn ( $0.01\pm 0.01$ - $0.06\pm 0.01$ ) and Cr ( $0.03\pm 0.01$ - $0.05\pm 0.01$ ). While their presence in the cosmetics can be as an impurity, these levels were below the maximum permissible limits set by WHO and KEBS, an indication that the *aloe vera* branded cosmetics are potentially safe for use. However, significant differences ( $p < 0.05$ ) of the levels of heavy metals were found between the lotions, creams and soaps posing risks of combined and continual usage of these products. There is need therefore to indicate the levels of heavy metals on the labels of these cosmetics in order to create awareness to the users. This would as well provide insight to the fears of the dangers that may result due to the combined or long term usage of these products.

## CHAPTER ONE: INTRODUCTION

### 1.1 Background

The adverse effects of heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), manganese (Mn), zinc (Zn) and nickel (Ni) are documented (Duruibe *et al.*, 2007; Banfalvi, 2011). Heavy metals toxicity can result to damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, liver, kidneys and other vital organs (Linnila, 2000). Repeated long term contact with some of these heavy metals or their compounds may cause cancer, contact dermatitis and skin irritation which are caused by Cr, Ni and Co (Linnila, 2000; Omolayo *et al.*, 2010). Their exposure however, continues and is even increasing particularly in less developed countries (Jarup, 2003).

Cosmetics are substances that are rubbed, poured, sprinkled or introduced into the human body for purposes of cleansing, beautifying, promoting attractiveness or altering appearance (Kasture *et al.*, 2008). The use of cosmetics is widely spread for routine body care including the care of skin, hair, nails and teeth (Chauhan *et al.*, 2010). A wide range of cosmetic products exist including creams, emulsions, lotions, gels, oils, face masks, tinted bases, make up powders, toilet soaps, perfumes, shower and bath preparations, deodorants and antiperspirants, depilatories, hair care products and shaving products (Anton, 2005). Depending on the ingredients with which they are made from, cosmetics can either be herbal (those of natural origin and are made of ingredients that are gentler and less likely to be harmful) or synthetic (those which are conventional and made of

ingredients likely to be harmful) (Conors and Altshuler, 2009). Among the most commonly used skin cosmetics are those made of ingredients such as formaldehyde and formaldehyde releasing ingredients, hydroquinone, parabens and phthalates which could be harmful to human body (Baumann, 2009).

There are a number of herbal cosmetics in use with the most preferred ones being *Aloe vera*, neem and olive oil. These are more preferred because they are mild, biodegradable and have low toxicity profile (Chanchal *et al.*, 2008). Neem is the most useful traditional medicinal plant (Imam *et al.*, 2012). It contains active compounds among them alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones (Imam *et al.*, 2012). It has been found to be useful in personal care products such as skin care, nail care and hair care among others (Imam *et al.*, 2012). Other uses of neem are therapeutic and medicinal. Therapeutic uses include treating scalp conditions such as dandruff, acne, nail fungus and restoring brittle nails and also fungal infections such as ringworms, infected sores and burns. Neem also provides relieve for skin disorders such as eczema and psoriasis as well as healing of wounds, (Debjit *et al.*, 2010). Among the medicinal uses are anti-inflammatory, antifungal, antibacterial, antimalarial, antiarthritic, spermicidal, immunomodulatory, hepatoprotective and antioxidant which are brought about by compounds that have a biological activity. The compounds include salannin, volatile oils, meliantriol, nimbin, nimbinin, nimbidol and tannin (Debjit *et al.*, 2010; Imam *et al.*, 2012). Olive oil is another herb used in pharmacy, as anti-

inflammatory as well as antioxidants due to the presence of phenolic compounds, triterpenes and mannitol among others. (Pérez *et al.*, 2005)

Out of over 3,700 species of *Aloe vera*, two species (*Aloe barbadensis* Miller and *Aloe aborescens*) are most commonly used due to their medicinal nature. It plays an important role on the skin that would result into preventing penetration of UV-light, and is key in wound healing and skin repair. *Aloe vera* cosmetics are available in the market in the form of lotions, creams, soaps and shampoos (Basmatker *et al.*, 2011; Haque *et al.*, 2012). Worth noting is that like any other plant, *Aloe vera* plant will absorb heavy metals from the soil in large quantities (Rai *et al.*, 2011). For example in China 60 percent of cosmetics and other product were recalled because of the presence of heavy metals in levels toxic to human in the products (Mary, 2007). It is in light of these that products in the market undergo quality control checks to ascertain the levels of heavy metals in them (Oyelakin *et al.*, 2010). In Kenya, the productions of cosmetics undergo a quality control check by the Kenya Bureau of Standard (KEBS) but at the point of labelling, it is observed that neither the presence nor the levels of the heavy metals are indicated. KEBS has a set of the maximum permissible limits (ppm) of heavy metals in cosmetics among them: Pb (2), Cd (2) and Hg (0.1). On the other hand, the World Health Organisation (WHO), sets the limits (ppm) of heavy metals set in cosmetics as follows: Hg (1) Pb (10) and Cd (0.3) (WHO, 1995).

The maximum permissible concentration limits of heavy metals in cosmetics set by the Kenya Bureau of Standards (KEBS) and the World Health Organisation (WHO) are shown in table 1.

**Table 1** Table 1: Maximum permissible concentration limits of heavy metals set by KEBS and WHO

Heavy metal	Maximum concentration level in ppm	
	KEBS	WHO
Pb	2	10
Cd	2	0.3
Hg	0.1	1

Source (**WHO, 1995**)

Heavy metals can be absorbed into the organism through the skin and can be detected in sweat, blood and urine within periods of between six hours to 45 days of skin application (Sin and Tsang, 2003; Omolaoye *et al.*, 2010). Moist skin particularly promotes absorption of water soluble toxic elements and their compounds into the body (Omolaoye *et al.*, 2010). As such, continuous use of cosmetics may result in an increase in the heavy metal levels beyond acceptable limits (Nnorom *et al.*, 2005; Chauhan *et al.*, 2010). Due to the harmful effects of heavy metals to man, the possibility of their presence in herbal cosmetics warrant investigation (Chauhan *et al.*, 2010).

## **1.2 Problem statement and justification**

The fears of dangers of heavy metals especially if levels are above the maximum permissible WHO limits in any product continue to rise. While there are a number of sources, research points out to one possible avenue being the use of cosmetics. In China for example, cosmetics were recalled for having levels of heavy metals being higher than the permissible levels. The issue of heavy metals as deliberate cosmetics ingredients may have been given attention but these could still be found as impurities and hence cause threats as some toxic elements and/or their compounds are water-soluble and can be absorbed on moist skin. On the other hand, it is worthy to note that both herbal and synthetic skin cosmetics (lotions, creams and soaps) have gained increased use worldwide. Continuous use and as well a combination of cosmetics therefore may result into the numerous dangers of heavy metals. During growth, *Aloe vera* plant absorbs heavy metals from the soil and despite this, it is one plant that is in use in the production of herbal cosmetics. In the Kenyan market, productions of cosmetics undergo a quality control check by KEBS but a challenge is manifested on the labelling process where labels do not indicate either the presence or levels of heavy metals. Currently, there is no documentation on the levels of heavy metals in *Aloe vera* cosmetics found in the Kenyan market and therefore it is important that this is re-ascertained in the products.

## **1.3 Hypothesis**

The levels of heavy metals in *Aloe vera* branded lotions, creams and soaps are below the maximum permissible limits set by KEBS and WHO.



## **1.4 Objectives**

### **1.4.1 General objective**

To determine the levels of selected heavy metals in *Aloe vera* branded skin cosmetics.

### **1.4.2 Specific objectives**

- i. To determine the levels of Hg, Pb, Cd, Zn, Mn and Cr in *Aloe vera* branded lotions.
- ii. To determine the levels of Hg, Pb, Cd, Zn, Mn and Cr in *Aloe vera* branded creams.
- iii. To determine the levels of Hg, Pb, Cd, Zn, Mn and Cr in *Aloe vera* branded soaps.

## **1.5 Significance and anticipated output**

Heavy metals or their compounds can find way into the final market products as impurities. However, labels on the packages of cosmetics sold in Kenya indicate neither the presence nor the levels of heavy metals. The strength of these findings therefore is to disseminate the fact that the *Aloe vera* branded cosmetics contain some level of heavy metals. This does not therefore rule out the same in these products sold elsewhere in the country. This information is crucial for users of these cosmetics taking note that either continual use or using a combination of cosmetics would be detrimental especially to moist skin which allows some penetration and accumulation of heavy metals. It is anticipated that cosmetic manufactures would find it necessary and important to label

whichever magnitude of level of heavy metals found in their products so that the users are well informed ahead of time in their use.

### **1.6 Scope and Limitations**

There are a number of herbal cosmetics including neem and olive oil whose heavy metal profile would be equally necessary to the users. In this study, only *Aloe vera* skin cosmetics were studied. Further, in a range of heavy metals, the study undertook to quantify mercury, lead, cadmium, zinc, manganese and chromium in the *Aloe vera* skin cosmetics. The source of raw materials and also the industrial source or matrix effect due to processing of the *Aloe vera* products were not considered.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Cosmetics

Cosmetics are substances which are applied by means of rubbing, pouring, sprinkling on the body for purposes of cleansing, beautifying, promoting attractiveness or even altering the general human appearance (Kasture, 2008). They are categorised as creams, emulsions, lotions, gels and oils for the skin. More products also exist in the form of face masks, make up powders, toilet soaps, perfumes, shower and bath preparations, deodorants and antiperspirants, depilatories, hair care products and shaving products (Anton *et al.*, 2005). More still are products for internal intimate hygiene, sunbathing products, skin-whitening products and anti-wrinkle products (Anton *et al.*, 2005). The ingredients used in cosmetics can either be herbal or synthetic (Conors *et al.*, 2009; Baumann, 2009).

Formaldehyde and formaldehyde releasing ingredients, hydroquinone, parabens and phthalates are ingredients used in synthetic cosmetics which could be harmful to human body (Baumann, 2009). Formaldehyde and formaldehyde releasing agents are used in the manufacture of water based consumer products such as soaps to prevent bacterial growth while in storage. Formaldehyde is easily absorbed by the human skin and can cause cancer. Hydroquinone is used for skin lightening. It causes decline in the production of melanin pigments. It is carcinogenic and the reduction in melanin increases the risks of cancer due to reduced protection against UV rays. Hydroquinone affects natural immunity and also brings about reproductive and developmental defects (Baumann, 2009). Parabens

are used in cosmetics to inhibit microbial growth while in storage. They are carcinogens and are easily absorbed by the skin and enter the bloodstream and the digestive system. Phthalates are used as binding agents for colour and scent in cosmetic products. They disrupt human hormonal system (Baumann, 2009). *Aloe vera*, neem and olive oil are the herbal ingredients commonly used in cosmetics and whose demand is increasing (Shweta *et al.*, 2011).

Cosmetics can either be herbal or synthetic depending on the ingredients from which they are made and either will serve the purpose of cosmetics (Kasture, 2008). The former however, are milder, biodegradable and have low toxicity profile (Chanchal *et al.*, 2008). Further, they whip up the circulation, refine the pores, refresh the skin leaving it soft and glowing and promote the skin's capacity to absorb. (Husain, 2008). Herbal cosmetics have preventive, protective, corrective and curative actions ideal for maintaining the health of the skin.

## **2.2 The *Aloe vera* plant**

*Aloe vera* plant has had its use (as an ingredient) increase tremendously in the field of 'herbal' cosmetology (Basmatker *et al.*, 2011; Sharma *et al.*, 2011). As such, it has been used in production of pills, sprays, ointments, lotions, liquids, jellies and creams. The plant belongs to the Liliaceae family, and is a shrubby (arborescent), perennial, xerophytic, succulent, pea-green colour plant (Basmatker *et al.*, 2011). It grows in warm tropical areas and is therefore commonly found in Mexico the Pacific Rim Countries,

India, South America, Central America the Caribbean, Australia and Africa (Basmatker *et al.*, 2011; Rai *et al.*, 2011). Although there are about 3,700 species *Aloe barbadensis* Miller and *Aloe aborescens* are grown commercially due to their medicinal properties (Basmatker *et al.*, 2011). Figure 2.1 shows the *Aloe vera* plant.



**Figure 1:** The *Aloe vera* plant

As a herbal skin cosmetic, *Aloe vera* has been found to be protective on the skin (Sharrif and Verma, 2011). If administered, an antioxidant protein, metallothionein, is generated in the skin which scavenges hydroxyl radicals and prevents suppression of superoxide dismutase and glutathione peroxidase in the skin (Sharrif and Verma, 2011). It reduces

production and release of skin keratinocyte-derived immunosuppressive cytokines such as interleukin-10 and hence prevents UV-induced suppression of delayed type hypersensitivity (Sharrif and Verma, 2011).

In sun protection, the enzyme bradykinase in Aloe stops the inflammatory reactions (sunburns) which are caused by an over-exposure to the sun's rays and stimulate immune system intervention (Basmatker *et al.*, 2011). Acemannan speeds up the repair phase intervening in the stimulation of macrophases and the increased production of fibroblasts and collagen (Basmatker *et al.*, 2011).

In wound healing and skin repair *Aloe Vera* plays a major role where proteolytic enzymes “digest” waste tissue, including pus, and accelerate the regenerative repair stage of tissues in the healing process (Basmatker *et al.*, 2011). In addition, the bradykinase enzyme arrests the inflammatory reactions, the barbaloin and the aloetic acid have an antibiotic and antibacterial effect while Isobarbaloin, the ester of cinnamic acid, and the salicylic acid carry out an analgesic or pain killing action (Basmatker *et al.*, 2011).

*Aloe Vera* also has skin anti-aging properties. Oligoelements present in aloe juice, manganese and selenium, constitute the enzymes superoxide dismutase and glutathione peroxidase which are anti-oxidants and cellular anti-aging agents (Basmatker *et al.*, 2011). Their high anti-oxidant properties slow down the aging process. This helps cells to become stronger in combating the negative effects caused by oxygen and broad spectrum

radiation which human skin is exposed to daily (Basmatker *et al.*, 2011). The non essential amino acid, proline, is a constituent of collagen, whose role is to ensure the perfect holding capacity and elasticity of epithelial tissues. This makes the skin smoother, hydrated and more elastic, protected from free radicals and their degenerative activity resulting in substantial anti-aging effects by constant use of *Aloe vera* (Basmatker *et al.*, 2011).

There are more than 200 compounds found in *Aloe vera*, about 75 of which have biological activity among them being anthraquinones, aloin, aloe emodine polysaccharides, enzymes, reducing sugars, organic acids and metallic cations (Saeed *et al.*, 2004; Sharma *et al.*, 2011). *A. vera* also has essential elements which account for most of the therapeutic efficiencies (Haque *et al.*, 2012). Some inorganic elements like zinc, vanadium, sodium, potassium, calcium, copper, manganese and chromium improve impaired glucose tolerance and indirectly manage diabetes mellitus. Sodium, chlorine and potassium are electrolytes that maintain normal fluid balance inside and outside cells and proper balance of acids and bases in the body and their deficiency may result in muscle cramps and hypertension. Magnesium takes part in carbohydrate and fat metabolism and its deficiency may cause diabetes mellitus because it plays a role in the release of insulin. Zinc enhances the effectiveness of insulin (Sharma *et al.*, 2011). Copper helps in the regulation of neurotransmitter levels and its deficiency can impair the function of the nervous system. Iron is essential for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats.

Chromium is an active ingredient of the glucose tolerant factor (Haque *et al.*, 2012). Other constituents are hormones, lignin, saponins, sterols and vitamins (Saeed *et al.*, 2004).

Amino acids are components of protein that are required for the functional proteins such as muscle tissues, enzymes and hormones among others. *Aloe vera* has twenty amino acids required by humans including 7 of the 8 essential ones which the body cannot synthesize (Saeed *et al.*, 2004). Anthraquinones present in *Aloe vera* act as potent antimicrobial and antiviral agents when in small quantities. In high concentration, these compounds exert a powerful purgative effect. Topically they can absorb ultraviolet light, inhibit tyronase activity and reduce the formation of melanin. *Aloe vera* provides 12 anthraquinones including aloe emodin, aloetic acid, aloin, anthracene, anthranon, barbaloin, chrysopharic acid, isobarbaloin and resistannol (Saeed *et al.*, 2004; Sharma *et al.*, 2011).

Most of the enzymes help in the breakdown of food sugars and fats. Some of the enzymes may be involved in other functions, such as carboxypeptidase, inactivates bradykinins and produces anti-inflammatory effects. There are 8 enzymes isolated from *Aloe vera*, including aliase, alkaline phosphotase, amylase, carboxypeptidase, catalase, lipase and peroxidise (Saeed *et al.*, 2004; Sharma *et al.*, 2011). Hormones are constituents that play an important role in wound healing and anti-inflammatory effects. Two hormones are known from *Aloe vera*, which are auxins and gibberllins. Lignin, on the other hand



provides penetrating power in *Aloe vera* skin penetrations, has the ability to carry other active ingredients deep into the skin to nourish the dermis (Saeed *et al.*, 2004).

Salicyclic acid is an Aspirin-like compound which acts as analgesic while Saponins, Glycosides which are soapy substances, general cleansers, with antiseptic properties. Sterols are anti-inflammatory agents while lupeol possesses antiseptic and analgesic properties. *Aloe vera* provides 4 main plant steroids, cholesterol, campesterol, Lupeol Beta Sitosterol (Saeed *et al.*, 2004). *Aloe vera* provides both monosaccharides (glucose and fructose) and polysaccharides (glucomannans and polymanose). Monosaccharides have anti-inflammatory action and polysaccharides possess antiviral, immunemodulating activity (Saeed *et al.*, 2004). *Aloe vera* also contains many vitamins except vitamin D. The vitamins found include vitamins A, C, E, B (Thiamine, Niacin, Riboflavin, and B12), F, Choline and Folic acid. Vitamin Bs and Choline are involved in amino acid metabolism, B12 plays an important role in production of red blood cells, and folic acid is involved in the development of red cells (Saeed *et al.*, 2004).

In Kenya, different brands of *A. vera* products are manufactured and undergo a quality control check by the Kenya Bureau of Standard (KEBS). Labelling though raises a concern, some components such as heavy metals are not included yet these are likely to be found as impurities in these products. A case in point that would support these fears is a recall of over 60% of cosmetics in China which had levels higher than the permissible

levels. As such KEBS has a set of the maximum permissible limits (ppm) of heavy metals in cosmetics among them: Pb (2), Cd (2) and Hg (0.1). On the other hand, the World Health Organisation (WHO), sets the limits (ppm) of heavy metals set in cosmetics as follows: Hg (1) Pb (10) and Cd (0.3) (WHO, 1995).

## **2.2 Heavy metals and their toxicity**

Heavy metals have densities higher than  $3\text{g/cm}^3$  and are known to cause adverse effects if present at levels beyond those that are recommended (Banfalvi, 2011; Rai *et al.*, 2011). A number of these occur as natural constituents of the earth crust including arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), manganese (Mn), zinc (Zn) and nickel (Ni) among others (Duruibe *et al.*, 2007; Banfalvi, 2011). The adverse effects occur due to their undegradable and undestructive nature. The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic (Linnila, 2000; Duruibe *et al.*, 2007). The toxicity can result to damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, liver, kidneys and other vital organs (Linnila, 2000). Through dermal contact, heavy metals can be absorbed through a moist skin (Omolaoye *et al.*, 2010). Lead for example was found in 7 out of 9 adult males to have been elevated in hair and other parts of their body after applying a hair dye containing lead acetate (Omolaoye *et al.*, 2010).

### 2.2.1 Mercury

Mercury (Hg) is a common ingredient found in skin lightening soaps and creams. In these products, it exists in two forms, organic and inorganic. While the organic form does not find its usefulness in cosmetics, the inorganic mercury compounds such as mercury chloride play a role in skin- lightening (Sin and Tsang, 2003). If absorbed through the skin or otherwise, Hg has effects ranging from renal neurological and dermal toxicity, headache, insomnia, memory loss, irritability, abdominal discomfort, nervousness, joint pain, weakness, nausea to hand tremor (Sin and Tsang, 2003). Alarming levels of Hg ranging from 878 to 36000 ppm were recorded in 6 out of 16 samples when Mexican skin lightening creams were analyzed. In 10 samples however, Hg was not detected (Peregrino *et al.*, 2011). In Saudi Arabia, skin- lightening creams were analyzed for mercury and recorded levels ranging from 0.01 to 23222 ppm. 60% of the samples analyzed contained Hg levels higher than 1 ppm (Al-Ashban *et al.*, 2006). Sin and Tsang (2003) reported the levels of Hg in urine and blood of cosmetic users in Hong Kong. The concentrations in urine were significantly higher among people who last used a beauty cream within 45 days. Blood Hg concentrations were elevated following the cream use for as short as 2 days (Sin and Tsang, 2003). The results indicated that majority of cream users had increased urine or blood Hg concentrations but remained asymptomatic, implying an incidence of overt symptomatic. Similarly dangers were noted in Kenya and Tanzania Hg where higher than the normal levels of Hg was found in urine of women who used Hg containing skin lighteners (Sukenda *et al.*, 2012). While Hg is not an authorized ingredient, Indian herbal cosmetic preparations have been found to contain Hg levels ranging from 0.041 to 2.183 ppm (Sukenda *et al.*, 2012). Of the 21 samples, 2 of them

contained 1.095 ppm and 2.183 ppm Hg which were above the WHO permissible limit of 1 ppm (WHO, 1995)

### **2.2.2 Lead**

Lead (Pb) is a common contaminant in various cosmetic products including eye and face cosmetics which have been identified as a suspected source of its exposure (Nnorom *et al.*, 2005; Chauhan *et al.*, 2010). As a result of Pb toxicity, anaemia, colic, neuropathy, nephropathy, sterility, coma, behavioural abnormalities, learning impairment, decreased hearing and impaired cognitive functions can be manifested (Nnorom *et al.*, 2005). Studies expose the risks of Pb dangers in face creams and herbal cosmetics where levels that range as low as 0.03 to 33.1 ppm, levels that are above the WHO permissible limit of 10 ppm have been reported (WHO, 1995; Gentscheva *et al.*, 2010; Chauhan *et al.*, 2010; Sukenda *et al.*, 2012).

### **2.2.3 Cadmium**

The metal cadmium (Cd) has a deep yellow to orange pigmentation that makes it find usefulness in cosmetics. Through dermal contact with cosmetics it can be absorbed into the body where it accumulates in the kidney and liver, although it can be found in almost all adult tissues (Elinder, 1985; Ayenimo *et al.*, 2010). It is toxic at extremely low levels and in humans both long term and high level exposures results in renal dysfunction, obstructive lung disease, Cd pneumonitis, bone defects, increased blood pressure and myocardic dysfunctions (Duruibe *et al.*, 2007). Chauhan *et al.* (2010) reports Cd levels in bathing soaps that ranged from 0.03ppm to 0.04ppm. Although these levels were below

the permissible limits, continued use of the soaps is feared to cause slow release into the body and result into its harmful effects (Chauhan *et al.*, 2010). However, a research carried out on Indian herbal cosmetic preparations and in Macedonia herbs recorded Cd levels of between 0.10 to 1.875 ppm, some of which were above the WHO permissible limit of 0.3 ppm (WHO, 1995; Gentscheva *et al.*, 2010; Sukenda *et al.*, 2012). The dangers of using these products are therefore evident.

#### **2.2.4 Zinc**

Zinc oxide is an ingredient in cosmetics which plays role as a sunscreen and also to whitening the skin (Butler and Poucher, 2000). While it is an essential trace element for plants, animals and humans as it forms connective tissues Zn maintains the skin nutritional requirements (Miculescu *et al.*, 2011; Sukenda *et al.*, 2012). For the skin, it helps keep it healthy by eliminating stretch marks which may result from elastin's inability to incorporate enough Zn. Zinc also acts as a mild astringent by shrinking, constructing and tightening body tissues thus helping in the treatment of acne (Null and Mc Donald, 2006). Zinc toxicity is rare but at concentrations of up to 40 mg/l (40 ppm), it may induce toxicity characterized by irritability, muscular stiffness and pain (Al-weher, 2008). Acute toxicity results in gastric distress, dizziness, nausea, vomiting and reduction in immune function (Deshpande, 2005). High levels (above acceptable limit of 170 ug/l) of Zn in brands of eye shadows have been reported in Nigeria implying continual exposure of Zn to the users (Omolaoye *et al.*, 2010). Indian herbal cosmetic preparations and some Bulgaria herbs have also been reported to contain alarming Zn with levels

ranging from 4.0 to 56.57 ppm (Gentscheva *et al.*, 2010; Sukenda *et al.*, 2012). The continuous use of these cosmetics would eventually be detrimental.

### **2.2.5 Manganese**

Manganese (Mn) is an essential element but potentially toxic in biological systems. Its overexposure causes neurological deficit in humans characterised by mental difficulties and impairment in motor skills (Yang *et al.*, 2005). Although not harmful to the skin, when ingested through hand to mouth activities and through sweating, its effects occur mainly in the respiratory tract and in the brains. Further symptoms of its poisoning are hallucinations, forgetfulness, nerve damage, can cause parkinson, lung embolism, bronchitis, impotence, permanent disability among many others (Al-weher, 2008). Mn has been reported to be found in cosmetics (Gentscheva *et al.*, 2010; Omolaoye *et al.*, 2010). Omolaoye *et al.* (2010) reported levels of Mn to be higher than 100 ppm in some eye shadows found in Nigeria. The findings led to a conclusion that the use of facial cosmetics exposes users to low levels of heavy metals but continuous use could result in an increase in the heavy metal level in human body beyond acceptable limits (Omolaoye *et al.*, 2010).

### **2.2.6 Chromium**

The oxides of Chromium (Cr), Chromium oxide and hydrated chrome oxide are the two oxides that are used in cosmetics especially soaps due to their colour (Butler and Poucher, 2000; Dayan (2001). Levels of Cr in the range of 0.50 ppm to 2.70 ppm have been found in soaps Dayan (2001). Chromium (VI) compounds are toxins and

carcinogenic. Breathing high levels of Cr can cause irritation to the lining of the nose, nose ulcers, running nose and breathing problems such as asthma, cough, shortness of breath, or wheezing (Dayan, 2001). Skin contact with Cr is reported to cause skin ulcers; allergic reactions consisting of severe redness and swelling of the skin whereas long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation (WHO, 2003).

### **2.3 Methods of analysis**

Lead, cadmium, zinc, manganese and chromium can be determined by inductively coupled plasma-Atomic emission spectroscopy (ICP-AES) (Aceto *et al.*, 2002), flame atomic absorption spectrometry (FAAS) (Bingol and Akcay, 2005), inductively coupled plasma mass spectroscopy (ICP-MS) (Horwitz, 2001) and atomic absorption spectroscopy (AAS) (Mireji *et al.*, 2007) among others. Atomic Absorption Spectroscopy was used because it is selective, highly specific and available. Cold Vapour Atomic Absorption Spectroscopy (CV-AAS) was used for the determination of mercury (Mester and Sturgeon, 2003).

#### **2.3.1 Atomic Absorption Spectroscopy**

The AAS is a method of elemental analysis that works on the principle of absorption of radiation energy by free atoms. The concentration of an element is measured by the absorption of radiation with characteristic frequency by the atoms of an element. Light of specific wavelength produced by monochromatic or hollow cathode lamp emits spectral lines corresponding to energy required for excitation of an element of interest. The

analytical signal is obtained from the difference between the intensity of the source in the absence of the element of interest and the decreased intensity obtained when the element of interest is present in the optical path. Absorption of light is associated with transition process from one steady state to another; for instance the case of a steady state of  $O$  and  $J$  where  $E_o < E_j$ , the  $O - J$  transition results in the absorption of light with the frequency given in Equation 2.1 (Skoog and Leary, 1992).



$$V_{Oj} = \frac{E_j - E_o}{h} \dots\dots\dots \text{Eq.2.1}$$

Where

$h$  = Plank's constant

$V$  = Frequency

$E_o$  = Energy at ground state

$E_j$  = Energy at the excited state

$O - J$  – the transition stimulated by absorption of external radiation.

The number of atoms in the excited state relative to the number in the ground state is given by Maxwell-Boltzmann law (Skoog and Leary, 1992), given by equation 2.2.

$$\frac{N_1}{N_0} = \frac{g_1}{g_0} \exp\left(\frac{E_0 - E_1}{KT}\right) \dots\dots\dots \text{Eq.2.2}$$

Where

$N_1$  = Number of atoms in the excited state

$N_0$  = Number of atoms in the ground state

$g_1$  and  $g_o$  – Statistical weight of excited and ground state respectively

$K$  = Boltzmann's constant

$E_o$  = Energy at ground state

$T$  = Absolute Temperature

$E_1$  = energy at the excited state

The relative fraction of atoms in excited state is dependent on temperature whereas intensity is independent of temperature. Sample solution is aspirated through nebulizer into the air/acetylene or nitrous oxide/acetylene flame (Taylor *et al.*, 2006).

An electrically heated graphite furnace is used when very high sensitivity is required. The sample solution gets dispersed mist of droplets and then evaporated into dry salt. The dry salt goes into vapour and dissociates into atoms that absorb resonance radiation from external source. The unabsorbed radiation is allowed to pass through the monochromator which isolates spectral lines. The isolated analyte line falls on the detector and the detector and the output of which is amplified and recorded. The parameter measured is absorbance ( $A$ ) and related to levels by equation 2.3.

$$A = \log I_0 / I = \epsilon cl \quad \dots\dots\dots\text{Eq.}$$

### 2.3

Where

$A$  = Absorbance

$I_0$  = Incidence radiation

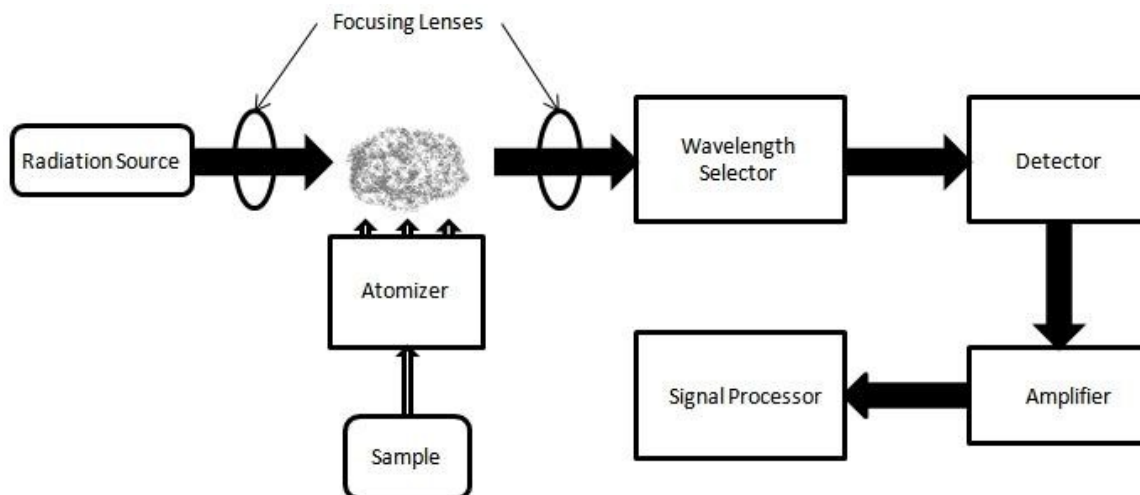
$I$  = Attenuated radiation

$\epsilon$  = Molar absorptivity ( $\text{Lmol}^{-1}\text{cm}^{-1}$ )

$c$  = Concentration ( $\text{mol dm}^{-3}$ )

$l$  = Path length (cm)

Since the relationship between absorbance ( $A$ ) and concentration ( $c$ ) is linear over a wide range concentration levels (Beers law), standards are used to obtain calibration curve from which concentration levels of analyte is established through interpolation method (Van Loon, 1980). Figure 2.2 shows a schematic diagram for the components of AAS.



**Figure 2:** Schematic diagram of AAS equipment

The most important components of atomic absorption spectroscopy (AAS) include two types of radiation sources (the continuous and line source), an atomizer, monochromator, detectors and Read out system. The continuous source gives a wide range of radiation and includes deuterium lamp and mercury vapour lamp. It is less sensitive because only a small amount of radiation passed by monochromator is absorbed while a large portion falls in the detector hence insignificant absorbance is absorbed by the metal of interest. A continuum source (deuterium lamp) is therefore not used as a light source in AAS but is merely used for background correction. The hollow cathode lamp on the other hand is the commonly used line source in AAS instrument and the hollow cathode is made up of metallic or alloy of element of interest. Hollow cathode lamp consists of a tungsten anode and cylindrical cathode sealed in a glass tube that is filled with neon or argon gas at a pressure of 1-5 torr.

The two types of atomizers are flame and electrothermal atomizers. In flame atomizer, the temperature is determined by flow rate and ratio of oxidant and fuel. In flame atomizer solvent is evaporated to produce solid molecular aerosol during dissolving process. Dissociation leads to atomic gas whereas some of the atoms ionize to give cations and electrons. In electrothermal atomizer, few molecules of the sample are first evaporated at low temperature and ashed at higher temperatures in electrically heated graphite. After ashing, the temperature is increased to 2000-3000 °C to cause atomization of the sample.

Monochromators are analyzers that present monochromatic radiation to the detector. They are made of filters, prisms or gratings that disperse or separate radiation so that selected wavelength corresponding to particular energy of the sample is transmitted. Diffraction grating is preferred to prisms as they offer accuracy over a wide range of wavelengths. Detectors convert radiation energy into electrical signal and include phototube, photomultiplier tube and photodiode array detectors. Read out system are digital and interfaced with microprocessors that allow the programming of various aspects, bringing simplicity in operation. However AAS is a single elemental method in which one element is determined in a series of samples and instrumental parameters optimized for the next element.

### **2.3.2 The Cold Vapour Atomic Absorption Spectroscopy (CV-AAS)**

In the analysis of mercury using CV-AAS, mercury contained in the sample solution is vaporised by reduction vaporization and supplied to the atomic absorption spectrophotometer. Reduction vaporization involves vaporizing the divalent mercury ions in the sample solution by reduction with stannous chloride. A certain amount of pre-treated sample is put in a reaction vessel and when a solution of stannous chloride is added to it, the mercury contained in the sample is vaporised in a short time. The vapour is lead to the gas flow cell in the measuring instrument by a pump. The amount of mercury is determined by measuring the absorption at the mercury resonance wavelength of 253.7nm (Khopkar, 2004; AOAC, 2002).

The most important components of the CV-AAS analyzer include a reaction vessel of glass with a capacity of holding 250 ml of sample, a rubber plug for reaction vessel made of silicone rubber, and a bubbler which are connected to the respective vinyl tubes coming from the front panel of the MVU-1A. Others are a magnetic stirrer used to promote the vaporization of mercury by stirring the solution and a mode cock which is a four-way cock to select the measuring mode. The “CIRCULAR” and “FLOW THRU” positions are for the circulation mode and suction mode respectively. This cock and the exhaust cock are both made entirely of Teflon. There is also an exhaust cock which is turned to “MEASURE” and “CLEAR” in each measurement after selecting the measuring mode with the mode cock. More still are stop and leakage valves used to leak in the outer air in the suction mode. With the mode cock at “FLOW THRU” (suction

mode), the stop valve is set to “OPEN” and with the mode cock at “CIRCULAR”, it is set to “CLOSE”. The leakage valve controls the leakage flow rate of outer air admitted, this indirectly controlling the vapor speed through the gas flow cell. A pump whose surfaces contain mercury vapor is made entirely of synthetic resin to prevent the production of amalgam which causes a decrease in sensitivity. The other part is U-shaped tube for desiccation packed with a desiccant (silica gel) to keep the gas flow cell dry. Granules of magnesium perchlorate of 20 to 30 meshes are most suitable as a desiccant. The U-shaped tube is softly packed with the desiccant to half the length of the tube, and then plugged both ends softly with absorbent cotton or glass wool. There is a flow meter whose switch is turned on to start the pump and a Power switch which is a bottle of polythene with 2 liters capacity. It is filled with a solution of 0.5% potassium permanganate and 5% sulphuric acid, and then the exhausted gas is passed into the solution to trap the mercury vapor there. It prevents atmospheric pollution with the harmful mercury (AOAC, 2002).

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Research design

The experimental design involved sampling of *Aloe vera* branded lotions, creams and soaps of different batches from major supermarkets in Nairobi. Laboratory analysis of heavy metals Pb, Cd, Zn, Mn and Cr using AAS and Hg using CV-AAS techniques was performed prior to data analysis by ANOVA and t-test.

### 3.2 Sampling

Purposive sampling was employed to obtain *Aloe vera* branded lotions, creams and soaps from major supermarkets in Nairobi County, Kenya. Five different brands of each cosmetic were sampled in two different batches and for each batch three samples were collected. Sampling was done twice and at different times. Those which were sampled first were coded batch 1 while those which were sampled the second time were coded batch 2. A total of ninety (90) samples were obtained. Brand names were blinded and codes: LTN1, LTN2, LTN3, LTN4 and LTN5 for lotions, CRM1, CRM2, CRM3, CRM4 and CRM5 for creams and SP1, SP2, SP3, SP4 and SP5 for soaps were used.

### 3.3 Chemicals, reagents and solvents

All chemicals, reagents and solvents used were of analytical grade and included cadmium, lead, zinc and manganese metals that were obtained from Fluka Chemie GmbH Chemical Company, inc. USA. Mercury and chromium stock solutions were purchased from kobian. Hydrochloric acid, concentrated nitric acid, hydrogen peroxide,

concentrated hydrochloric acid, sulphuric acid, stannous chloride, silica gel and potassium permanganate were sourced from Thomas Baker Chemicals Ltd Mumbai, India.

### **3.4 Cleaning of apparatus**

Plastic containers and glassware were washed with liquid detergent and warm water and rinsed with tap water. They were soaked overnight in 10% analytical grade nitric acid and then rinsed with distilled water. The glassware was dried in an oven at 105°C for 24 hours.

### **3.5 Preparation of standard stock solutions and working standards**

Stock solutions were prepared from which working standards were freshly prepared by serial dilution. The stock solutions of mercury and chromium were obtained already prepared. Five serial standards of each element were prepared for the calibration. The final acid concentration was maintained at about 1% during serial dilution and subsequent dilution of stock solutions to keep the metal in free ion state

Appropriate weighing of metals was done prior to dissolving them in acids to make 1000 ppm of stock solutions. For Pb, 1.000 gram of lead metal, was dissolved in 250ml of 1% v/v HNO<sub>3</sub> and diluted to 1 litre with 1% v/v HNO<sub>3</sub>. Cd, 1.000 gram of the metal, was dissolved in 250ml HCl and then diluted to 1 litre with 1% v/v HCl. Zn, 1.000 gram of Zn metal, was dissolved in 250ml of HCl and diluted to 1 litre with 1% v/v HCl. Mn



1.000 gram of the metal, was dissolved in 250ml HNO<sub>3</sub> and diluted to 1 litre with 1% v/v HCl.

Following appropriate dilutions, serial standard were prepared in the following ranges in ppm; Hg (0.01-0.06), Pb (1.0-5.0), Cd (0.2-1.0), Zn (0.4-1.6), Mn (0.5-2.5) and Cr (1.0-1.5). The serial standards were aspirated into the instruments. The absorbances were plotted against their concentrations to obtain calibration curves. The correlation coefficients were calculated to and used to express the performance of the instruments.

### **3.6 Digestion of samples and blanks**

The digestion procedure employed for the elements (except Hg) was as described by Mester, 2003, 1.000 gram of lotions was accurately measured into a conical flask. 15ml concentrated nitric acid was added followed by 5ml 30% hydrogen peroxide and then 5ml concentrated hydrochloric acid. The flask was closed and left for 15 minutes to ensure complete reaction. The mixture was heated at 150° C until no more brown fumes were produced. The sample solution was then cooled and 20ml of deionised water added. The solution was filtered through Whatman paper number 1 into a 50ml volumetric flask and diluted to volume with deionised water before aspiration into the instrument. Aspirations were performed in triplicates in each case to ensure precision. For mercury analysis, micro-wave assisted digestion was used. The above procedure was repeated for digestion of creams.

The procedure according to Oyelakin. *et al* (2010) was adopted for digestion of soaps. 0.500 grams of the soap sample was measured into a pre-weighed Telfon Vials and 10 ml of digest acid comprising 70% nitric acid and 30% sulphuric acid was added. It was capped tightly and placed in an oven at 60° C for 12 hours. 30 ml of deionised water was added when the digested sample had cooled (Oyelakin *et al.*, 2010). In order to account for the background effects from the acids and to correct for changes resulting from digestion procedures, the above procedure was used to digest six blanks.

### 3.7 Investigating accuracy of instruments

The accuracy of CV-AAS and AAS were investigated by spiking samples with a known amount of standards. Triplicate analysis was performed in each case. The concentration of unspiked and spiked sample as well as that of the standard added used were determined.

**Table 2: Mean concentration (ppm) of standards of heavy metals before and after spiking**

Heavy metal	Concentration (Mean ± SE)		
	Unspiked sample	Standard added to sample	Spiked sample.
Hg	0.010±0.01	0.010	0.0197± 0.01
Pb	0.440±0.01	1.000	1.430±0.02
Cd	0.150±0.00	0.200	0.348±0.01
Zn	0.400±0.01	0.400	0.790±0.02
Mn	0.100±0.01	0.500	0.599±0.00
Cr	0.070±0.01	1.000	1.069±0.02

The percentage recoveries for the metals after digestion were calculated using the equation 3.1 (Al Weher, 2008).

$$\%R = \left( \frac{a-b}{c} \right) \times 100 \dots\dots\dots \text{Eq3.1}$$

Where:

R-recovery

a- Concentration of the sample after spiking

b- Concentration of the sample before spiking

c- Concentration of standard used for spiking

### **3.8 Measurements of levels of heavy metals**

The digested samples were aspirated in triplicates with regular intercepts of standards to maintain a check on the instrument stability. Air/Acetylene flame and oxidant flow of 4.51/min was used for Pb, Cd, Cr, Zn and Mn while for Hg, N<sub>2</sub>O/Acetylene flame was used. Other operating conditions for the instruments are given in table 2.

**Table 3: CV-AAS and AAS operating conditions**

Metal	Wavelength (nm)	Slit width (nm)	Sensitivity (ppm)	Lamp current (mA)	Detection limit (ppm)	Optimum working range (ppm)
Hg	253.7	0.7	-	-	0.0002	80-200
Pb	217.0	0.7	0.5	10.0	0.0001	5-25
Cd	228.8	0.7	0.025	2.0	0.0005	0.2-2.0
Zn	213.9	0.2	0.02	7.0	0.0005	0.1-1.5
Mn	278.5	0.7	0.05	10.0	0.0001	1.0-4.0
Cr	357.9	0.7	0.25	2.0	0.0005	1.0-5.0

### 3.9 Data analysis

The replicate values for levels of the heavy metals in *Aloe vera* skin cosmetics were compared by One-Way ANOVA at 95% confidence level using SPSS 18 for windows. Significant differences were taken for  $p < 0.05$ . Whenever a significance difference was found, the means were separated by standard error (Sawyer and Beebe, 2007). The student t-test was used to compare the means in levels of the heavy metals between any two products.

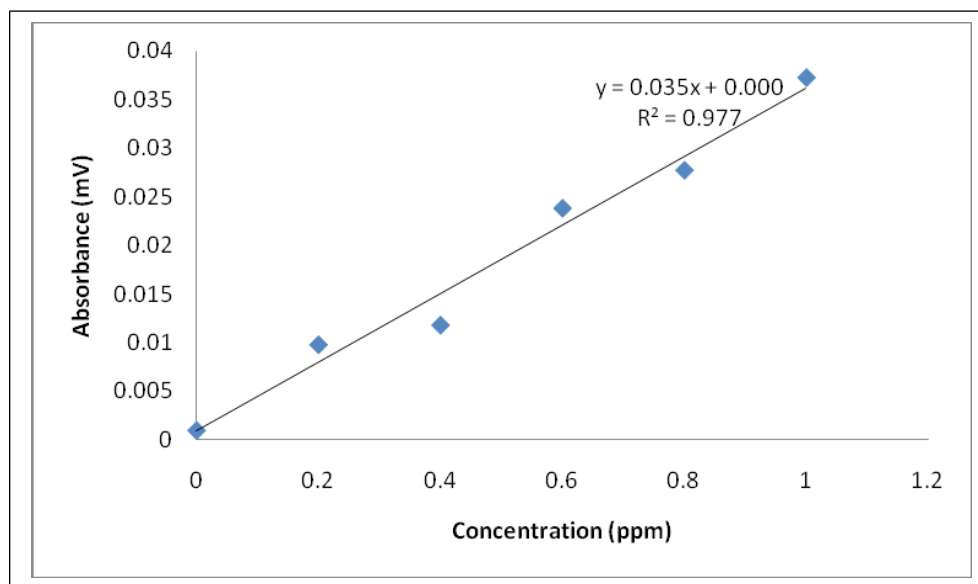
## CHAPTER FOUR: RESULTS AND DISCUSSION

### 4.1 Introduction

The levels of heavy metals Hg, Pb, Cd, Zn, Mn and Cr in *Aloe vera* branded lotions, creams and soaps were determined using CV-AAS for Hg and AAS for Pb, Cd, Zn, Mn and Cr. The results obtained are presented in tables prior to their discussions.

### 4.2 Instrument calibration

Calibration curves were plotted for each metal. The calibration curve for Cd is presented in figure 1. Calibration curves for Hg, Pb, Zn, Mn and Cr are presented in the appendix (Fig 2-6). The equations of the best line of fit and the value of correlation coefficient are presented in table 4.1.



**Figure 3:** Calibration curve for cadmium standard

**Table 4: Correlation coefficient and equations of lines of best fit for metals by CV-AAS and AAS instruments**

Instrument	Metal	Correlation coefficient ( $r^2$ )	Regression equation
CV-AAS	Hg	0.978	$y=0.219x - 0.000$
AAS	Pb	0.978	$y=0.001x + 0.000$
	Cd	0.977	$y=0.035x + 0.000$
	Zn	0.991	$y=0.076x + 0.003$
	Mn	0.987	$y=0.031x - 0.002$
	Cr	0.985	$y=0.016x + 0.003$

The correlation coefficient values were above 0.977, thus implying linearity (Mendham *et al.*, 1999).

#### 4.3 Accuracy of instruments

The percent recoveries obtained as explained in chapter three section 3.7 were as presented in table 4.2.

**Table 5: Percentage recoveries for metals**

analyte	Concentration (Mean $\pm$ SE)			% recovery
	Unspiked sample	Standard sample	added to Spiked sample.	
Hg	0.010 $\pm$ 0.01	0.010	0.0197 $\pm$ 0.01	97.00
Pb	0.440 $\pm$ 0.01	1.000	1.430 $\pm$ 0.02	97.73
Cd	0.150 $\pm$ 0.00	0.200	0.348 $\pm$ 0.01	98.66
Zn	0.400 $\pm$ 0.01	0.400	0.790 $\pm$ 0.02	97.50
Mn	0.100 $\pm$ 0.01	0.500	0.599 $\pm$ 0.00	98.00

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Cr	0.070±0.01	1.000	1.069±0.02	98.57
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The values obtained were above 97% implying that the method had a conventionally acceptable precision and accuracy (Taylor *et al.*, 2006).

#### **4.4 Mean levels of heavy metals in *Aloe vera* branded cosmetics**

The mean levels of Hg, Pb, Cd, Zn, Mn and Cr in two batches of five brands of *Aloe vera* branded lotions, creams and soaps are presented in tables 4.3-4.5.

**Table 6: Mean levels (ppm) of heavy metals in brands of Aloe vera branded skin lotions**

Mean levels (ppm) in different brands (Mean±SE; n=9)							
Parameter	batch	LTN1	LTN2	LTN3	LTN4	LTN5	p-value
Hg	1	0.03±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.04±0.01 <sup>b</sup>	<0.001
	2	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.233
p-value		0.618	0.662	0.949	0.893	0.134	
Pb	1	0.60±0.01 <sup>b</sup>	0.63±0.19 <sup>b</sup>	0.49±0.10 <sup>b</sup>	BLD	0.31±0.07 <sup>b</sup>	<0.001
	2	0.44±0.17 <sup>b</sup>	0.32±0.12 <sup>a</sup>	0.08±0.04 <sup>a</sup>	0.06±0.02 <sup>a</sup>	BLD	0.008
p-value		0.384	0.185	0.001	-	-	
Cd	1	BLD	BLD	BLD	BLD	BLD	
	2	BLD	BLD	BLD	BLD	BLD	
Zn	1	0.10±0.01 <sup>c</sup>	0.03±0.01 <sup>a</sup>	0.09±0.01 <sup>c</sup>	0.04±0.01 <sup>a</sup>	0.07±0.01 <sup>b</sup>	<0.001
	2	0.40±0.01 <sup>c</sup>	0.02±0.01 <sup>ab</sup>	0.03±0.01 <sup>a</sup>	0.05±0.01 <sup>a</sup>	0.05±0.01 <sup>a</sup>	<0.001
p-value		<0.001	<0.001	<0.001	0.130	0.167	
Mn	1	0.05±0.01 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.04±0.01 <sup>b</sup>	0.04±0.01 <sup>b</sup>	<0.001
	2	0.05±0.01 <sup>b</sup>	0.06±0.01 <sup>b</sup>	0.03±0.01 <sup>ab</sup>	0.06±0.01 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.001
p-value		0.758	<0.001	<0.001	0.038	0.277	
Cr	1	BLD	BLD	BLD	BLD	BLD	-
	2	BLD	BLD	BLD	BLD	BLD	-



BLD- Below Limit of Detection

Means followed by same letter(s) within the same row are not significantly different (SNK,  $\alpha=0.05$ ).

**Table 7: Mean levels (ppm) of heavy metals in five brands *Aloe vera* branded skin creams**

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Mean levels (ppm) in different brands (Mean $\pm$ SE; n=9)

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Parameter	Batch	CRM1	CRM2	CRM3	CRM4	CRM5	p-value
<b>Hg</b>	1	0.04±0.01 <sup>a</sup>	0.05±0.01 <sup>ab</sup>	0.07±0.00 <sup>a</sup>	0.10±0.01 <sup>c</sup>	0.09±0.01 <sup>c</sup>	<0.001
	2	0.04±0.01 <sup>a</sup>	0.06±0.00 <sup>a</sup>	0.05±0.01 <sup>a</sup>	0.09±0.00 <sup>b</sup>	0.11±0.01 <sup>b</sup>	0.002
<b>p-value</b>		0.673	0.609	0.180	0.491	0.261	
<b>Pb</b>	1	0.01±0.01 <sup>c</sup>	0.37±0.13 <sup>ab</sup>	0.33±0.06 <sup>ab</sup>	0.34±0.09 <sup>ab</sup>	0.68±0.14 <sup>b</sup>	0.01
	2	0.31±0.15 <sup>ab</sup>	0.29±0.10 <sup>ab</sup>	0.16±0.05 <sup>ab</sup>	0.40±0.02 <sup>b</sup>	BLD	-
<b>p-value</b>		0.069	0.646	0.042	0.511	-	
<b>Cd</b>	1	BLD	BLD	0.03±0.01 <sup>a</sup>	0.06±0.01 <sup>b</sup>	0.03±0.01 <sup>a</sup>	<0.001
	2	BLD	BLD	0.03±0.01 <sup>a</sup>	0.02±0.01 <sup>b</sup>	0.02±0.01 <sup>b</sup>	<0.001
<b>p-value</b>		-	-	1	<0.001	1	
<b>Zn</b>	1	0.37±0.01 <sup>b</sup>	0.57±0.02 <sup>c</sup>	1.99±0.01 <sup>d</sup>	0.37±0.01 <sup>b</sup>	0.16±0.01 <sup>a</sup>	<0.001
	2	0.45±0.01 <sup>c</sup>	0.49±0.01 <sup>c</sup>	1.87±0.01 <sup>d</sup>	0.38±0.01 <sup>b</sup>	0.11±0.01 <sup>a</sup>	<0.001
<b>p-value</b>		<0.001	<0.001	<0.001	<0.001	<0.001	
<b>Mn</b>	1	0.07±0.01 <sup>a</sup>	0.04±0.01 <sup>a</sup>	0.06±0.01 <sup>a</sup>	0.07±0.02 <sup>a</sup>	0.10±0.01 <sup>b</sup>	0.01
	2	0.08±0.01 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.07±0.01 <sup>b</sup>	<0.001
<b>p-value</b>		0.380	0.033	<0.001	0.014	0.002	
<b>Cr</b>	1	BLD	BLD	0.04±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.06±0.01 <sup>b</sup>	<0.001
	2	BLD	BLD	0.04±0.01 <sup>a</sup>	0.08±0.01 <sup>c</sup>	0.06±0.01 <sup>b</sup>	<0.001
<b>p-value</b>		-	-	1	<0.001	0.868	

Mean values within the same row followed by the same letters are not significantly different (SNK,  $\alpha=0.05$ ).

BLD- Below Limit of Detection

**Table 8: Mean levels (ppm) of heavy metals in five brands of *Aloe vera* branded skin soaps**

Mean levels (ppm) in different brands (Mean±SE; n=9)							
parameter	Batch	SP1	SP2	SP3	SP4	SP5	p-value
Hg	1	0.09±0.01	0.17±0.02	0.40±0.28	0.15±0.01	0.21±0.01	0.500
	2	0.11±0.01 <sup>a</sup>	0.14±0.01 <sup>b</sup>	0.17±0.01 <sup>c</sup>	0.16±0.01 <sup>c</sup>	0.21±0.01 <sup>d</sup>	<0.001
p-value		0.08	0.222	0.496	0.077	0.077	
Pb	1	0.42±0.16 <sup>b</sup>	0.12±0.06 <sup>a</sup>	0.71±0.01 <sup>c</sup>	0.16±0.06 <sup>a</sup>	0.77±0.01 <sup>c</sup>	<0.001
	2	0.14±0.07 <sup>a</sup>	0.33±0.07 <sup>b</sup>	0.74±0.01 <sup>c</sup>	0.66±0.10 <sup>c</sup>	0.83±0.01 <sup>c</sup>	<0.001
p-value		0.132	0.045	0.120	0.001	<0.001	
Cd	1	BLD	0.01±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.77±0.01 <sup>c</sup>	<0.001
	2	0.01±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.06±0.01 <sup>b</sup>	0.83±0.01 <sup>c</sup>	<0.001
p-value		-	0.706	0.326	<0.001	<0.001	
Zn	1	0.05±0.01 <sup>b</sup>	0.14±0.01 <sup>d</sup>	0.03±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.07±0.01 <sup>c</sup>	<0.001
	2	0.02±0.01 <sup>a</sup>	0.06±0.01 <sup>c</sup>	0.05±0.01 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	<0.001
p-value		0.063	<0.001	<0.001	0.137	<0.001	
Mn	1	0.01±0.01 <sup>a</sup>	0.04±0.01 <sup>bc</sup>	0.04±0.01 <sup>b</sup>	0.03±0.01 <sup>b</sup>	0.06±0.01 <sup>c</sup>	<0.001
	2	0.05±0.01 <sup>ab</sup>	0.06±0.01 <sup>b</sup>	0.06±0.01 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.03±0.01 <sup>ab</sup>	0.010
p-value		0.022	0.154	0.006	0.238	<0.001	
Cr	1	BLD	BLD	0.03±0.01	BLD	BLD	-
	2	BLD	BLD	0.05±0.01	BLD	BLD	-

Mean values within the same row followed by the same letters are not significantly different (SNK,  $\alpha=0.05$ ).

BLD- Below Limit of Detection

#### 4.4.1 Mercury

Mercury was detected in the *Aloe vera* branded lotions ranging between  $0.01\pm 0.01$  to  $0.04\pm 0.01$  ppm (**Table 4.3**). There were significant differences noted between different brands of lotions in batch 1 ( $p < 0.05$ ) level of significance. In *Aloe vera* branded creams Hg was detected in all the samples (**Table 4.4**). The mean levels ranged from  $0.04\pm 0.01$  to  $0.11\pm 0.01$  ppm. The levels were found to differ significantly ( $p < 0.05$ ) between the brands. The mean levels of Hg ranged from  $0.09\pm 0.01$  to  $0.21\pm 0.01$  ppm in *Aloe vera* branded soaps (**Table 4.5**). The levels were found to differ significantly ( $p < 0.05$ ) between the brands for soaps in batch 2.

The presence of Hg in the cosmetics can be explained as it is a common ingredient in cosmetics with its role being to lighten the skin (Sin and Tsang, 2003; Sukenda *et al.*, 2012). The levels of Hg in the lotions, creams and soaps were all below the maximum WHO recommended limit of 1ppm (WHO, 1995). While statistical differences observed can be explained to arise from compositional differences of products and environmental conditions of the plant, this however was not investigated. The difference in levels of Hg between brands raises concern for users on the choice of cosmetic. Although the levels found may imply safety, caution should be taken in continual use of these products. This follows alarming results where levels of Hg in urine and blood of cosmetic users have been reported (Sin and Tsang, 2003). In comparison to Hg levels found in Indian herbal cosmetic preparations (0.041 to 2.183 ppm), the present findings show lower levels but agree to the risks that could result to the use of these products since Hg has adverse

effects if absorbed through the skin or otherwise (Sin and Tsang, 2003; Sukenda *et al.*, 2012).

#### 4.4.2 Lead

Lead was detected in the *Aloe vera* branded lotions ranging between  $0.06\pm 0.02$  to  $0.63\pm 0.19$  ppm except in LTN 4 batch 1 and LTN 5 batch 2 (**Table 4.3**). There were significant differences noted between batches for lotion LTN 3 ( $p < 0.05$ ). Similarly the brands of lotions had significant differences of the levels of lead for both batches. In creams, lead was detected in all except CRM5 batch 2 (**Table 4.4**). The mean levels ranged from  $0.03\pm 0.01$  to  $0.68\pm 0.14$  ppm. The levels however were found to differ significantly between the brands ( $p < 0.05$ ). All the *Aloe vera* branded soaps analyzed were found to contain lead whose mean levels ranged from  $0.14\pm 0.07$  ppm to  $0.83\pm 0.01$  ppm (**Table 4.5**). The levels differed significantly between the brands in batch 2 ( $p < 0.05$ ) level of significance.

Lead has been found to be a common contaminant in various cosmetics (Chauhan *et al.*, 2010). The levels of lead in the lotions, creams and soaps, however, were below the WHO maximum recommended limits of 10 ppm (WHO, 1995). The explanation behind the differences is stated in section 4.4.1 (Chauhan *et al.*, 2010; Sukenda *et al.*, 2012). While some of the values found in this study were lower than those reported in literature, some were higher (Gentscheva *et al.*, 2010; Sukenda *et al.*, 2012). Gentscheva *et al.*, 2010 reported levels ranging from 0.40-1.40 ppm in herbs. Sukenda *et al.* (2012),

however, recorded levels much higher ranging from 1.470 to 33.1 ppm. Despite the compositional differences that could explain these differences, studies have revealed that lead can be absorbed through the skin and would be the cause for anaemia, colic, neuropathy, nephropathy, sterility, coma, behavioural abnormalities, learning impairment among others (Nnorom *et al.*, 2005; Chauhan *et al.*, 2010).

#### 4.4.3 Zinc

Zinc was detected in all the lotions and the mean levels ranged from  $0.02 \pm 0.01$  to  $0.04 \pm 0.01$  ppm (**Table 4.3**). For both batches 1 and 2, the levels were found to differ significantly between the brands ( $p < 0.05$ ) while for lotions LTN1, LTN 2 and LTN 3, the different batches showed significant differences in the Zn levels ( $p < 0.05$ ) level of significance. Zinc was found to be contained in all the *Aloe vera* branded creams analyzed. The mean levels ranged from  $0.11 \pm 0.01$  to  $1.99 \pm 0.01$  ppm (**Table 4.4**). The levels differed significantly between the brands and also between batches ( $p < 0.05$ ) level of significance. Zinc was also detected in all the soaps analyzed and recorded mean levels ranging from  $0.02 \pm 0.01$  to  $0.07 \pm 0.01$  ppm (**Table 4.5**).

To play a role as a sunscreen and also to whiten the skin, zinc oxide is used as an ingredient in cosmetics (Butler and Poucher, 2000). The levels of zinc as found in this study were lower than those reported in literature where zinc has been detected to levels as high as 56.57 ppm (Gentscheva *et al.*, 2010; Sukenda *et al.*, 2012). This together with the difference in the mean levels may be attributed to compositional differences of

products as well as environmental conditions where constituent plant was grown (Sukenda *et al.*, 2012). While it is an essential element in both plants animals and humans, levels above 40 ppm may be toxic (Al-weher, 2008). Zinc toxicity is characterised by irritability, muscular stiffness and pain (Deshpande, 2005; Al-weher, 2008).

#### 4.4.4 Manganese

Manganese was found to be in all the lotions with mean levels ranging from  $0.01\pm 0.01$  to  $0.06\pm 0.01$  ppm (**Table 4.3**). The levels differed significantly between the brands while in lotions, LTN 2, LTN 3 and LTN 4 showed significant differences in levels of manganese between batches ( $p < 0.05$ ) level of significance. Manganese was also detected in all the creams with mean levels ranging from  $0.02\pm 0.01$  to  $0.10\pm 0.01$  ppm (**Table 4.4**). The levels differed significantly between the brands and also between batches ( $p < 0.05$ ) level of significance. Like in lotions and creams, manganese was detected in all the soaps. The mean levels ranged from  $0.01\pm 0.01$  ppm to  $0.06\pm 0.01$  ppm (**Table 4.5**). The levels differed significantly between the brands and also between batches in SP1, SP3 and SP5 ( $p < 0.05$ ) level of significance.

The presence of manganese in these cosmetics may be attributed to the Aloe vera plant which absorbs metal ions from the soil among them manganese (Rai *et al.*, 2011). The levels obtained were low compared to those recorded by (Gentscheva *et al.*, 2010) which ranged from 65 to 717 ppm. This may be due to compositional differences of products (Sukenda *et al.*, 2012). Manganese is an essential but potentially toxic trace metal in

biological systems. Overexposure to manganese causes neurological deficit in humans characterised by mental difficulties and impairment in motor skills (Yang *et al.*, 2005). Manganese is not harmful to the skin but when ingested through hand to mouth activities and sweating, its effects occur mainly in the respiratory tract and in the brains. Manganese poisoning is characterised by hallucinations, forgetfulness nerve damage and bronchitis (Al-weher, 2008). While long term exposure causes impotence in men, chronic manganese poisoning may result in permanent disability whose symptoms include languor, sleepiness, weakness, emotional disturbances, spastic gait, recurring leg cramps, and paralysis (Al-weher, 2008).

#### **4.5.5 Cadmium**

The levels of cadmium in the *Aloe vera* branded skin lotions in both batches were below the limit of detection (**Table 4.3**). Cadmium was found to be contained in all the creams except in CRM1 and CRM2 which were both below the limit of detection. The mean levels of cadmium ranged from  $0.02 \pm 0.01$  to  $0.06 \pm 0.01$  ppm (**Table 4.4**). The levels differed significantly between the brands and also between batches for CRM4 ( $p < 0.05$ ) level of significance. In soaps cadmium was detected in all samples except SP1 batch 1 with concentration below the limit of detection. The mean levels ranged from  $0.01 \pm 0.01$  to  $0.15 \pm 0.01$  ppm (**Table 4.5**) and differed significantly between the brands ( $p < 0.05$ ) level of significance.



In cosmetics cadmium find usefulness due to its deep yellow to orange pigmentation. The levels were however below the maximum recommended limits of 10 ppm by WHO (WHO, 1995. Sukenda *et al.*, 2012 recorded slightly higher levels ranging from 0.623 to 1.875 ppm. Low levels ranging from 0.10 to 0.46 ppm were also recorded by (Gentscheva *et al.*, 2010). Apart from the environmental conditions where constituent plant was grown, the difference in the levels of cadmium recorded in this study may be attributed to the compositional differences of products (Sukenda *et al.*, 2012). Through dermal contact with cosmetics cadmium can be absorbed into the body where it accumulates in major body organs among them the kidney and the liver (Elinder, 1985). While high exposure can lead to obstructive lung disease, cadmium pneumonitis, bone defects, increased blood pressure and myocardic dysfunctions, Cadmium is toxic at extremely low levels and in humans long term exposure results in renal dysfunction (Duruibe *et al.*, 2007).

#### **4.5.6 Chromium**

The level of chromium in the *Aloe vera* branded skin lotions were below the limit of detection. Chromium was detected in all the creams except in CRM1 and CRM2 whose concentration levels were below the limit of detection. The mean levels ranged from  $0.03\pm 0.01$  to  $0.08\pm 0.01$  ppm (**Table 4.4**). The levels differed significantly between the brands and between batches in CRM4 ( $p < 0.05$ ) level of significance. In soaps chromium was detected in SP3 with mean levels ranging from  $0.03\pm 0.01$  to  $0.05\pm 0.01$  ppm (**Table 4.5**).

In cosmetics the oxides of Chromium (Cr), Chromium oxide and hydrated chrome oxide are used especially in soaps due to their colour (Butler and Arthur, 2000; Dayan 2001). The low levels recorded in this study are comparable to the levels recorded by (Dayan, 2001) which ranged from 0.50 ppm to 2.70 ppm. The difference in the levels of chromium may be attributed to the difference in the environmental conditions where the constituent plant was grown (Sukenda *et al.*, 2012). Chromium (VI) compounds are toxins and carcinogenic. Breathing high levels of Cr can cause irritation to the lining of the nose, nose ulcers, running nose and breathing problems such as asthma, cough, shortness of breath, or wheezing (Dayan, 2001). Skin contact with chromium is reported to cause skin ulcers; allergic reactions consisting of severe redness and swelling of the skin whereas long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation (WHO, 2003). While breathing high levels of chromium can cause irritation to the lining of the nose, nose ulcers, running nose and breathing problems such as asthma, cough, shortness of breath, or wheezing, Chromium (VI) compounds are known to be toxins and human carcinogens (Dayan, 2001).

#### **4.5 Comparison of mean levels of heavy metals in *Aloe vera* branded skin cosmetics**

Statistical analysis performed to compare the levels of heavy metals between the cosmetics; lotions, creams and soaps is presented in table 8.

**Table 9: Comparison of the mean levels of heavy metals in *Aloe vera* skin cosmetics**

Concentration (ppm) ; Mean±SE(n=18)				
Metal	Lotions	Creams	Soaps	p-value
Hg	0.02±0.01 <sup>a</sup>	0.07±0.01 <sup>b</sup>	0.16±0.01 <sup>c</sup>	<0.001
Pb	0.49±0.05 <sup>a</sup>	0.47±0.04 <sup>a</sup>	0.62±0.03 <sup>b</sup>	<0.001
Cd	BLD	0.04±0.01 <sup>a</sup>	0.07±0.01 <sup>b</sup>	<0.001
Zn	0.09±0.01 <sup>a</sup>	0.68±0.07 <sup>b</sup>	0.05±0.01 <sup>a</sup>	<0.001
Mn	0.05±0.01 <sup>a</sup>	0.06±0.01 <sup>b</sup>	0.04±0.01 <sup>a</sup>	<0.001
Cr	BLD	0.05±0.01 <sup>b</sup>	0.04±0.01 <sup>a</sup>	<0.001

ND- Not detected; Mean values followed by small letters within the same row are not significantly different (SNK,  $\alpha=0.05$ ).

The results showed that the skin cosmetics had significantly different amounts of the levels of the heavy metals. In particular, soaps recorded the highest levels of mercury, lead and cadmium while creams were found to contain the highest mean levels of zinc, manganese and chromium. This may be due to compositional differences of products and environmental conditions where constituent plant was grown and possibilities of their occurrence as impurities (Chauhan *et al.*, 2010; Sukenda *et al.*, 2012). Although the levels of mercury, lead and cadmium are below the maximum recommended limits by WHO, 1ppm, 10ppm and 0.3ppm respectively, continuous use of *Aloe vera* branded cosmetics may be feared as long term exposure to these harmful heavy metals (Chauhan *et al.*, 2010). Further the combined use of these cosmetics may aggravate the dangers.

## CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

The results obtained in this study show evidence of the presence of heavy metals in *Aloe vera* branded cosmetics; lotions, creams and soaps. The levels however were lower than those set by WHO and KEBS. These results do not warrant safety though for use of these products since it is evidenced from literature that continuous use of cosmetics would be detrimental to health.

Statistical analysis of experimental data showed evidence that the levels of selected heavy metals under study in the *Aloe vera* branded skin cosmetics (lotions creams and soaps) were significantly different at 95% confidence level. This may raise alarm especially where users apply a combination of these cosmetics.

### 5.2 Recommendations

#### 5.2.1 Recommendations from the study

It is recommended that precaution be taken on the choice of skin cosmetic to be used. Further cosmetic users are called upon to be aware that continual use of *Aloe vera* branded lotions, creams and soaps could result in an increase in the level of heavy metal in their body to levels above the WHO and KEBS maximum permissible limits.

It is important on the other hand for KEBS to include levels of heavy metals on the labels of the skin cosmetics in the market.

**5.2.2 Recommendations for further work**

- i. Other *Aloe vera* cosmetics apart from those for the skin should be studied.
- ii. It is important that other herbal cosmetics including those made from neem and olive oil should be investigated for the levels of heavy metals to document the entire profile for herbal cosmetics.
- iii. Synthetic cosmetics also need to be assessed for heavy metals levels.
- iv. Determine other heavy metals in *Aloe vera* skin cosmetics not covered in the study.

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## APPENDIX

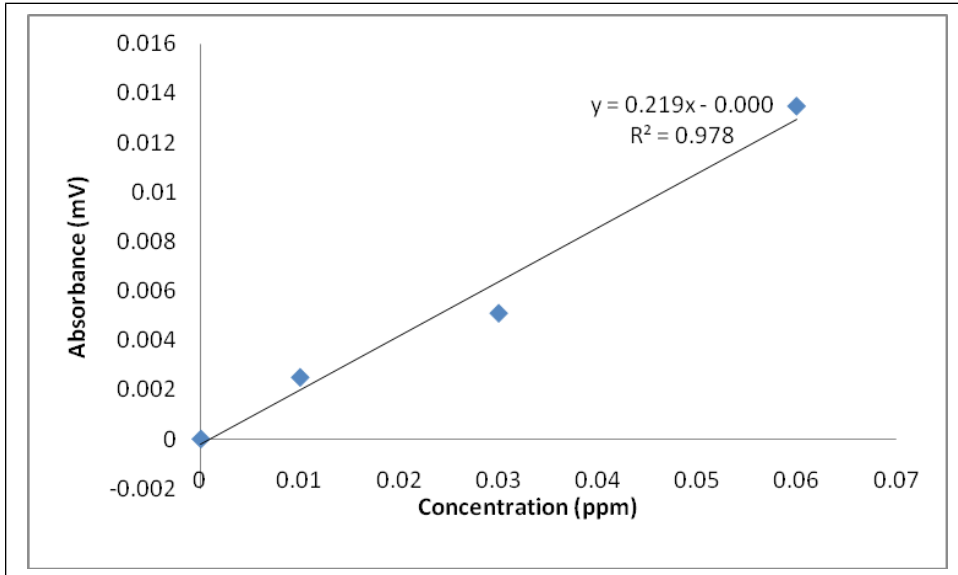


Figure 4: Calibration curve for Mercury standards

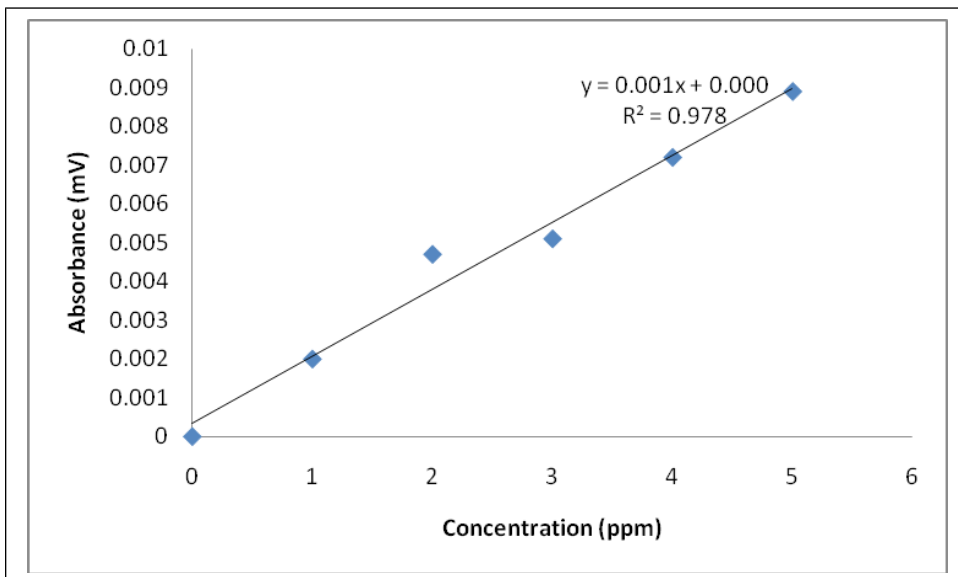


Figure 5: Calibration curve for lead standards

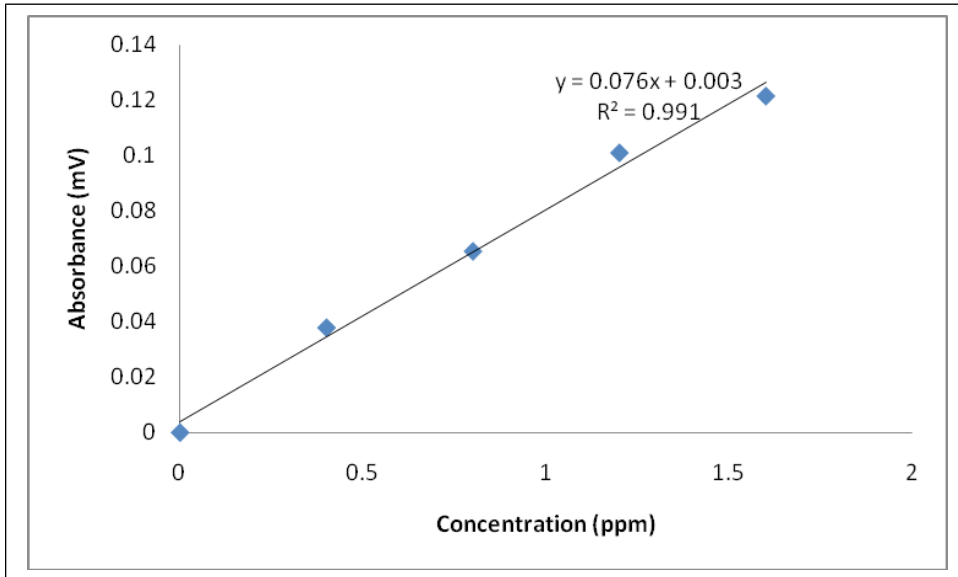


Figure 6: Calibration curve for Zinc standards

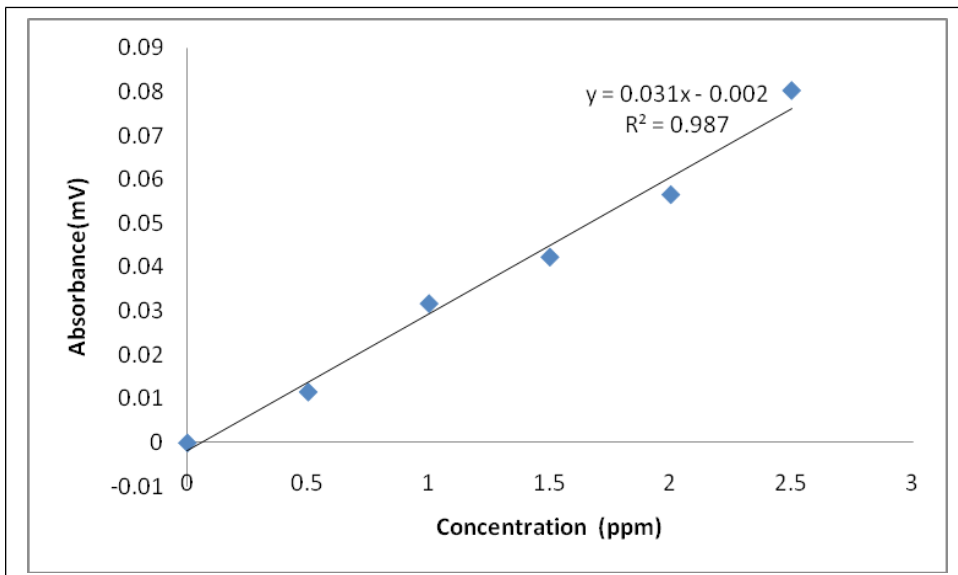


Figure 7: Calibration curve for Manganese standards

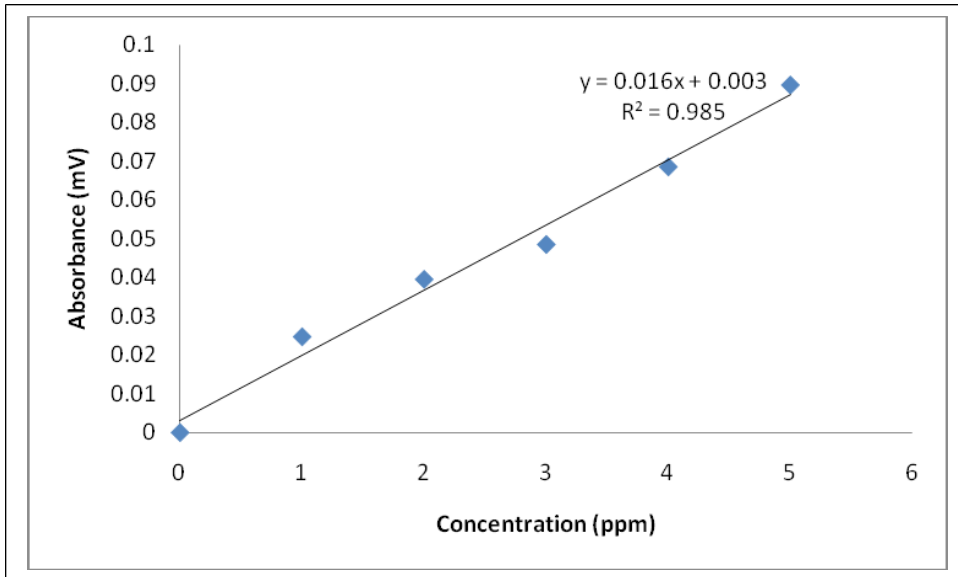


Figure 8: Calibration curve for Chromium standards