DETERMINATION OF THE LEVEL OF SOME SELECTED TOXIC HEAVY METALS (Pb, Cd AND Cr) IN SOME COMMERCIAL COSMETIC PRODUCTS AVALIABLE IN LOCAL MARKET OF ADAMA CITY

BY

ASCHALE TEREFE ALEMU

A THESIS SUBMITTED TO DEPARTMENT OF CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE

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ASCHALE TEREFE ALEMU

MAJOR ADVISOR: DEREJE TSEGAYE (PhD)

Co-ADVISOR: ESHETU BEKELE (PhD)

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We, the undersigned, members of the Board of Examiners of the final open defense by Aschale Terefe have read and evaluated his/her thesis entitled **“DETERMINATION OF THE LEVEL OF SOME SELECTED TOXIC HEAVY METALS (Pb, Cd AND Cr) IN SOME COMMERCIAL COSMETIC PRODUCTS AVALIABLE IN LOCAL MARKET OF ADAMA CITY ”** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of master in chemistry

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This is to certify that the thesis entitled "**DETERMINATION OF THE LEVEL OF SOME SELECTED TOXIC HEAVY METALS (Pb, Cd AND Cr) IN SOME COMMERCIAL COSMETIC PRODUCTS AVALIABLE IN LOCAL MARKET OF ADAMA CITY**" submitted in the partial fulfillment of the requirement of the degree of Master's in Chemistry the Graduated Program of the Department of Chemistry and has been carried out by Aschale Terefe Id. No GSU/0485/06, under our supervision. Therefore we recommended that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

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LIST OF ABBERVATIONS

AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
BIS	Bureau of Indian Standards
CFR	Code of Federal Regulation
CIR	Cosmetic Ingredient Review
CV	Coefficient variance
EU	European Union
FAAS	Flam Atomic Absorption Spectroscopy
FDA	Food and Drug Administration
FDC	Federal Drug and Cosmetic act
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
IARC	International Agency for Research on Cancer
IS	Indian Standards
ISP	International Purity Specification
KEBS	Kenya Bureau of Standards
NIOSH	National Institute for Occupational Safety and Health
RSD	Relative Standard Deviation
SD	Standard Deviation
USA	United States of America
WHO	World Health Organization

ABSTRACT

The study was aimed to determine the levels of some selected toxic heavy metals (Pb, Cd and Cr) in different commercial cosmetic products sold at different local markets of Adama city. The cosmetic items included three vaselines, three shampoos and three lipsticks of various brands. The cosmetics were digested with concentration HNO_3:$HClO_4$ in 3:1 ratio and analyzed for heavy metals (lead, cadmium and chromium) using graphite furnace atomic absorption spectroscopy and flame atomic absorption spectroscopy. The range of the concentrations in vaseline is 0.022 - 0.032 ppm, 0.0004 - 0.0006 ppm and BDL Pb, Cd and Cr respectively. The concentration range in shampoo is 0.023 - 0.036 ppm, BDL - 0.0005 ppm and 0.055 - 0.071 ppm Pb, Cd and Cr respectively. The concentration ranges in lipsticks is 0.04 - 0.063 ppm, 0.0004 - 0.0005 ppm and 0.026 - 0.034 ppm Pb, Cd and Cr respectively. The concentrations of the metal ions in some cosmetic products produced in Ethiopia were also compared with recommended maximum permissible limits according to World Health Organization (WHO), Health of Canada, International Purity Specification (IPS) for Food and Drug Administration (FDA) and Kenya Bureau of Standard (KEBS) and found that their concentration were below the permissible limits. However their prolong exposure may result in to health risk since they can be bioaccumulation. Further more comprehensive research is recommended to look into the larger picture of the source and health risk impacts of heavy metals should be conducted.

Keywords: *Heavy metals, cosmetics, GFAAS, FAAS*

1. INTRODUCTION

1.1. Background of the study

Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms (Monisha *et al*., 2014). Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure and chemical species, as well as the age, gender, genetics and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage such as damage to blood composition, lungs, kidneys, liver and other vital organs, even at lower levels of exposure (Brandi *et al*., 2002).

These metals have crucial biological functions in plants and animals; sometimes their chemical coordination and oxidation-reduction properties have given them an additional benefit so that they can escape control mechanisms such as homeostasis, transport, compartmentalization and binding to required cell constituents. These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity (Mittal *et al*., 2008). Heavy metals have been found as contaminants in many cosmetic products including sunscreen, body lotion, foundation, shampoo, nail polish, lipstick, and whitening toothpaste.

The U.S. food and drug administration (FDA) defines today's cosmetics as: 1. articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and 2. articles intended for use as a component of any such articles; except that such term shall not include soap (FDA, 2004).

A cosmetic product is any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or applied to the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly for the purpose of cleaning, perfuming, protection, changing their appearance, correcting body odors and keeping the surfaces in good condition without affecting the body's structure or functions (Sani *et al*., 2016). Cosmetics are mixtures of some surfactants, oils and other ingredients and are required to be effective, long-lasting, stable and safe to human use. The various forms of cosmetic include lipstick and lip gloss (used to color the lips), powder and rouge (used to color the face, lightening and removing flaws to produce an impression of health and youth), mascara (used to enhance the eye lashes, eye liner), eye shadow (used to color the eye lids), and nail polish (used to color the fingernails and toenails) (Adebisi *et al*., 2012).

Many cosmetic products contain heavy metals such as lead (II), cadmium (II), chromium (VI), zinc (II), arsenic (III and V), mercury (II and I), cobalt (II), and nickel (II) (Abdul *et al*., 2017). Trace amounts of potentially toxic metals can be present as ingredients or impurities in the raw materials. The key ingredients present in most cosmetics includes water, emulsifiers, preservatives (Parabens), thickeners (Cellulose, Glycol stearate**)**, moisturizers as an emollient (agar, Lecithin), colours, pH stabilizers (Sodium hydroxide) and fragrances. Ingredients can be naturally occurring or artificial, but any potential impact on our health depends mainly on the chemical compounds (Fletcher *et al*., 2008 and Ward, 2004). The use of metals as ingredients in cosmetics is prohibited in many advanced countries; metallic impurities cannot be avoided, even under good manufacturing practice, because they exist naturally in the environment (Al-Saleh and Al-Enai, 2011). These metals can easily cause many types of skin problems. The use of some heavy metals in cosmetic has been controversial due to the biological accumulation of those metals and their toxicity in the human body. Those metals can cause allergic contact dermatitis or other skin problems (Alsaffar and Hussein, 2014).

The metals of primary toxicological concern in cosmetics are lead, arsenic, cadmium, mercury and antimony (Sainio *et al*., 2000). Dermal exposure is expected to be the most significant route for cosmetic products since the majority of cosmetics are applied to the skin. Dermal absorption of heavy metals is fairly minimal, with absorption of individual elements influenced by a number of factors including physical and chemical properties of the mixtures. Oral exposure can occur for cosmetics used in and around the mouth as well as from hand to mouth contact after exposure to cosmetics containing heavy metal impurities. However, inhalation exposure is typically considered to be negligible (Shinwari *et al*., 2009). At higher concentrations, heavy metals have been shown to have negative effects. Cancerous breast biopsies show higher accumulations of chromium, cadmium, mercury and lead than non cancerous biopsies and several metals act like estrogen in the presence of some breast cancer cells (Yusuf *et al*., 2010).

Lead, which may be an impurity, is a proven neurotoxin linked to learning, language and behavioral problems. It has also been linked to miscarriage, reduced fertility in men and women, hormonal changes, menstrual irregularities and delays in puberty onset in girls. At puberty, boys developing testis may be particularly vulnerable to lead. Pregnant women and young children are also vulnerable because lead crosses the placenta and may enter the fetal brain (Yusuf *et al*., 2010).

Cadmium target blood vessel and heart tissue as well as the kidneys, lungs, and brain, and results in heart disease, hypertension, liver damage, and suppressed immune system. Cadmium also causes bone degradation because it affects calcium metabolism (Chauchan *et al*., 2010).

Exposure to high levels of chromium has been linked not only to kidney damage but also lung and other cancers. Chromium is also linked to skin conditions such as eczema and other inflammations of the skin. Therefore, cosmetics product will have poor quality and may contain much concentration of toxic heavy metals of public health concern.

1.2. Statement of the problem

Cosmetic products are regulated for health and safety. There are concerns regarding the presence of harmful chemicals, including heavy metals in these products. There have not been many studies on presence of heavy metals in cosmetics in Ethiopia. Cosmetics have different health effects such as skin problems (skin allergies & skin discoloration), hair problems (dandruff, scalp redness, thinning of hair, and even loss of hair), eye problems (irritation, thinning of eyelashes and eye infections), nail problems (fragile), acne, infertility, premature ageing, hormonal imbalance and cancer (Oishi, 2002).

1.3. Objectives of the study

1.3.1. General objective

The main objective of this study is to determine the level of some selected toxic heavy metals (Pb, Cd and Cr) in some commercial cosmetic products available in local market of Adama city.

1.3.2. Specific objectives

The specific objectives of this study were:-

1. To determine the level of some selected toxic heavy metals (Pb, Cd and Cr) in vaseline, shampoo and lipstick collected from local market of Adama city.
2. To compare the concentration (amount) of some selected toxic heavy metals (Pb, Cd and Cr) in vaselines, shampoos and lipsticks with available standards.
3. To compare the concentration of some selected toxic heavy metals (Pb, Cd and Cr) in vaseline, shampoo and lipstick among the different cosmetic products.

1.4. Significances of the Study

Basically, the determination of some selected toxic heavy metals (Pb, Cd and Cr) in some cosmetic products, were assume to generate necessary information that help all concerned bodies to reduce the use of cosmetics products. More specifically, the researcher believed that the findings of this study have the following significances:-

- To disseminate the fact that the commercial cosmetic products (vaselines, shampoos and lipsticks) contain some level of heavy metals.
- To provide information for cosmetic users that either continual use or using a combination of cosmetics would be detrimental, especially to skin.
- It may provide information to policy makers that help them for appropriate safety control of industry products.

1.5. Delimitation of the Study

There are a number of commercial cosmetics products including body lotion, face cream and deodorant whose heavy metal profile would be equally necessary to the users. In this study, only vaseline, shampoo and lipstick commercial cosmetic products were studied. Further, in a range of heavy metals, the study undertook to quantify lead, cadmium and chromium in the vaseline, shampoo and lipstick cosmetics products. The source of raw materials and also the industrial source or matrix effect due to processing of the cosmetic products were not considered.

2. LITERATURE REVIEW

2.1. Toxic Heavy Metals

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. They may enter the human body through food, water, air or absorption through the skin. Heavy metals like arsenic, cadmium, chromium, mercury, nickel, lead, cobalt, copper and zinc can build up in the body over time and are known to cause varied health problems, which include:- cancer, reproductive and developmental disorders, neurological problems, memory loss, mood swings, nerve, joint and muscle disorders, cardiovascular, skeletal, blood, immune system, kidney and renal problems, headaches, vomiting, nausea, and diarrhea, lung damage, contact dermatitis, and brittle hair and hair loss. Many are suspected hormone disruptors and respiratory toxins and for some like lead, there is no known safe blood level (Palpandi and Kesavan, 2012).

Heavy metal toxicity to the humans is the result of long term low or high level exposure to pollutants common in our environment including in air we breathe, water, food etc. Apart from these, numerous consumer products like cosmetics and toiletries have been reported as a source of heavy metal exposure to human being. Although beauty consciousness of people has set the demand of cosmetics in market, the side effects as well as health consciousness of people has attracted the clinicians and researchers to find out the probable reason behind their side effects. Heavy metal contamination is one of the important reasons behind the same problem. Heavy metals like lead and cadmium are common contaminant in various cosmetic products (Chandak *et al*., 2014).

Cosmetic products applied to mucous membranes are more hazardous, as in lipsticks. In addition to these risks, lipsticks may also have the higher risk of direct oral ingestion, aggravating the negative effects of their chemicals (Ward, 2004 and Arsenault, 2003). In view of these reasons and concerns, the analysis and evaluation of their effects on the human body are of importance. The main concern for safety assessment of use of these products is the precise knowledge about the concentrations and native hazards of the ingredients present in these products. Some of the species absorbed from the skin are systemically transported to different organs with attendant toxicities (Vahter *et al*., 2007 and Vidal *et al*., 2002).

2.2. Sources of some heavy metals

Heavy metals occur as natural constituents of the earth crust and are persistent environmental contaminants since they cannot be degraded or destroyed. Heavy metals are considered to be one of the main sources of pollution in the environment, because of their significant effects on the ecological quality. The main sources of heavy metal pollution in the environment are man-made effects, including combustion of fossil fuels, mining activities, wastewater discharges of manufacturing industries, and waste disposal. Significant levels of heavy metals in sediments and soils may pass to the aquatic environment, groundwater, and plants through the transfer processes and uptake by animals and humans leads to accumulation. Women usually attribute their problem to face creams, deodorants, shampoos and eye cleansers whereas men blame aftershaves, deodorants and shower foam and most commonly encounter with headache, nausea and dizziness. This is despite many metals being banned as intentional ingredients in cosmetics in Canada. Moreover, they are banned as product impurities since their presence as such is considered unavoidable. Especially, an European government agency found carcinogenic impurities in 55 out of 128 products tested in 1998 (Vidal *et al*., 2002 and Khan *et al*., 2010).

Heavy metals like arsenic, cadmium, chromium, mercury, nickel, lead, cobalt, copper and zinc can build up in the body over time and are known to cause varied health problems, which include: cancer, reproductive and developmental disorders, neurological problems; memory loss; mood swings; nerve, joint and muscle disorders; cardiovascular, skeletal, blood, immune system, kidney and renal problems; headaches; vomiting, nausea, and diarrhea; lung damage; contact dermatitis; and brittle hair and hair loss. Many are suspected hormone disruptors and respiratory toxins, and for some like lead, there is no known safe blood level. Yet the awareness and exposure on the adverse effects of heavy metal research is still in its infancy (Palpandi and Kesavan, 2012).

2.2.1. Cadmium

Cadmium is a natural element in the earth's crust. Is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide). Cadmium also occurs naturally in the environment. Some of its presence in the environment is the result of natural processes such as forest fires, volcanic emissions, and weathering of soil and bedrock, but it is mostly the result of human activities, particularly metal production, fuel burning, transportation, solid waste disposal, and sewage sludge application (Gentschava et al., 2010 and Chauchan et al., 2010).

2.2.2. Lead

It can be found in all parts of our environment. Every year, industry produces about 2.5 million tons of lead throughout the world. Most of it came from human activities like mining, manufacturing and the burning of fossil fuels. Mainly in manufacturing tetraethyl lead for constructional purposes in manufacturing sulfuric acid, in manufacturing phosphoric acid, chlorination or sulfonation processes, petro refining, gas production, extraction, condensation, manufacturing various chemical equipment; pipes, valves, stirrers, coils, kettles, pumps, evaporator, condenser, for lining tanks of various electroplating solutions (Chauchan *et al*., 2010).

2.2.3. Chromium

Chromium (Cr) is a naturally occurring element present in the earth's crust, with oxidation states (or valence states) ranging from chromium (II) to chromium (VI). Chromium enters into various environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources with the largest release coming from industrial establishments.

Industries with the largest contribution to chromium release include metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production. The increase in the environmental concentrations of chromium has been linked to air and wastewater release of chromium, mainly from metallurgical, refractory, and chemical industries. Chromium released into the environment from anthropogenic activity occurs mainly in the hexavalent form Cr (VI) (Barsanti *et al*., 2007).

Hexavalent chromium Cr (VI) is a toxic industrial pollutant that is classified as human carcinogen by several regulatory and non-regulatory agencies. The health hazard associated with exposure to chromium depends on its oxidation state, ranging from the low toxicity of the metal to the high toxicity of the hexavalent form. All Cr (VI) containing compounds were once thought to be man-made, with only Cr (III) naturally ubiquitous in air, water, and soil and biological materials. Chromium is widely used in numerous industrial processes and as a result, is a contaminant of many environmental systems (Ghani, 2011 and Costa, 2006).

2.3. Toxicity of cosmetic products

Cosmetics are one of the most important sources of releasing heavy metals into the environment and the human biological system. There is an increasing need to investigate the concentration of toxic metals in some commonly used cosmetic products. It is known for instance that high doses of heavy metals can be deadly and that even long-term exposure to low levels of heavy metals can cause certain cancers. There is also a growing concern about the physiological and behavioral effects of toxic metals on human population in general. The toxicity of lead at high concentrations of exposure is well documented but a major concern in recent time is the possibility that continual exposure to even relatively low levels of these toxic metals in cosmetic products may pose potential health risks (Omar *et al*., 2011).

The amount of toxins in the environment has reached a level where the US FDA now has designated permissible levels of dioxin and other harmful chemicals in the environment, food and cosmetics. According to national geographic society, USA, studies, various chemicals in foods and environment indicate that man contributes 700,000 tons of pollutants into the air every day, ranging from everyday household cleaners to cosmetics and hair dyes. The cosmetics are one of the main sources of toxins in environment. Today over 300 chemical toxins including dioxin were detected in human tissues that were not reported before the food and drug administration issued an unprecedented warning to the cosmetics industry stating that the agency is serious about enforcing the law requiring companies to inform consumers that personal care products safety has not been documented, but the safety of the ingredients in these products is looked into almost exclusively by a manufacturer-controlled safety committee called the cosmetic ingredient review (CIR) panel (Sabah *et al*., 2013).

Cosmetics, especially the skin lightening types are used widely in most African countries, especially by women. Since these products are used for a long time, on a large body surface and under hot humid conditions, cutaneous absorption is enhanced (Soldownik *et al*., 2008). The complications of these products can be very serious. Some studies have indicated an association between some ingredients of cosmetics and a variety of health problems (Toutain *et al*., 2010).

The pattern of accumulation of these metals in female body can be altered by some physiological changes. Most of the metals act as endocrine disrupters interfering with the hormonal system (loppi *et al*., 2007).

The usage of cosmetics is an important experience in life and leadership fashion for modern females as students, employees (larger consumer) as well as housewives. Whoever, scientists are, now raising concerns about the damage those chemicals in these cosmetics can cause to their bodies (Amit *et al*., 2010). Several ways, were mentioned by which makeup can contaminate human bodies, (Mahe *et al*., 2003) in sharing makeup (during use), low quality makeup (use of cheap, impure raw materials), poor handling procedures during manufacturing, especially for those, which are not following good laboratory practices and inadequate storage conditions (more likely for bacteria to grow).

The study reported by Anandhan *et al*. (2017) a total of 4 lipsticks were tested to determine the concentration of aluminum, chromium, copper, iron, lead and zinc. The level of iron was found to be higher when compared with all other metals tested. Aluminum was found to be available in all branded lipsticks in the concentration range of 0.09 - 0.82 ppm. The metal Iron was in the concentration range of 0.091-1.64 ppm respectively. Chromium and copper were detected only in sample III and in a concentration of 0.04 ppm, 0.01 ppm respectively. The heavy metals Pb and Zn were not found in any of the lipsticks.

Table 1: Some selected toxic heavy metals (Pb, Cd and Cr) concentration of cosmetic product performed by other researchers

Cosmetics item	Method	Concentration of heavy metal (ppm)			Reference
		Pb	Cd	Cr	
Lipstick	AAS with Wet digestion	6.35 ± 0.01	0.69 ± 0.09	-	(Amaechi and Nasirudeen, 2015)
Lipstick		0.286 - 6.234	0.2 - 0.5	0.222 - 5.43	(Riaz *et al*., 2013)
Shampoo		1.3 ± 0.16	4.17 ± 1.37	0.34 ± 0.04	(Umar and Caleb, 2013)
Lipstick		0.18 - 0.8	0.01 - 0.05	0.06 - 0.75	(Akari et al., 2016)
Lipstick		0.03 - 0.65	0.2 - 0.5	ND	(Bukhari *et al*., 2013)
Shampoo		1.782 - 3.322	0.058 - 0.203	ND	(Amir *et al*., 2017)
Lipstick		2.58 - 11.33	0.20 - 0.43	ND - 0.77	
Lipstick		0.08 - 5.2	4.08 - 60.20	-	(Foroghi *et al*., 2013)

2.4. Health impact of heavy metals in cosmetics

A variety of chemicals are used in cosmetics as ingredients and some are used as preservatives. These chemicals have different health effects.

2.4.1. 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 (Dayan and Paine, 2001). People are generally exposed to lead by three major ways such as inhalation, ingestion and absorption. Routes of exposure to lead include contaminated air, water, soil, food, and consumer products. The more toxicologists and other researchers investigated the health effects of lead, the more they realized that even very low levels of lead exposure were hazardous (Gimba *et al*., 2010).

Lead may cause neuro developmental effects in children (Garg *et al*., 2010). Other effects include cardiovascular, renal, gastrointestinal, hematological and reproductive effects (Sujit and

Roop, 2012). Lead poisoning, especially among children can lead to damages of the central nervous system, causing mental impairment, affecting oxygen transport in the body and causing digestive problems, long term exposure to lead can cause coma, or death. Lead is a poisonous metal that can damage nervous connections especially in young children and cause blood and brain disorders (ATDR, 2007). Adults that are exposed to a dangerous amount of lead can experience anemia, nervous system dysfunction, weakness, hypertension, kidney problems, decreased fertility and increased level of miscarriages, and low birth weight and premature deliveries. Lead poisoning has been a recognized health hazard for more than 2,000 years. Characteristic features of lead toxicity, including anemia, colic, neuropathy nephropathy, sterility and coma. Exposure to low levels of lead has also been associated with behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals (Gondal *et al*., 2016).

2.4.2. Cadmium

Cadmium is present in many cosmetics products but mostly present in lipsticks and face powders. It is used as color pigment in many cosmetics industries. Cadmium absorption through the skin is very low (0.5%). Oral limited value for cadmium is 0.09 ppm to 3 ppm was given by USP for nutritional supplements. The threshold value for dietary products exposure is below 2.5 ppm per body weight in a week. Cardiovascular system is also affected at the low level of cadmium exposure. Diabetes and hypertension are also associated with its exposure (Chauchan *et al*., 2010).

Cadmium is absorbed into the body, accumulating in the kidney and the liver, although it can be found in almost all adult tissues. The total amount absorbed by humans has been estimated to be between 0.2 and 0.5 µg/day with absorption via skin estimated to be 0.5 per cent (Health Canada, 2010). Cadmium is classified as a human carcinogen by the national institute for occupational safety and health (NIOSH) (international agency for research on cancer (IARC, 1997) and by the United States department of health and human services. Cadmium binds to epidermal keratin when applied topically, thus explaining the limited dermal absorption observed. However, significant dermal exposure can cause irritant dermatitis (Gentscheva *et al*., 2010).

2.4.3. Hexavalent chromium (Cr^{+6})

Hexavalent chromium (Cr^{+6}) is corrosive and allergic to the skin. Cr^{+6} compounds are enlisted as carcinogens by the IARC. Adverse effects of the Cr^{+6} on the skin may include ulcerations, dermatitis, and allergic skin reactions. Inhalation of Cr^{+6} compounds can result in ulceration and perforation of the mucous membranes of the nasal septum, irritation of the pharynx and larynx, asthmatic bronchitis, bronchospasms and edema. Respiratory symptoms may include coughing and wheezing, shortness of breath, and nasal itch (Dayan and Paine, 2001).

2.5. Regulations for Cosmetics

2.5.1. India

Cosmetics products in India are regulated under the drugs and cosmetics act 1940 and rules 1945 and labeling declarations by Bureau of Indian standards (BIS). BIS sets the standards for cosmetics for the products listed under cosmetics rules 194. BIS has provided the specification for skin creams and lipstick in the Indian standards (IS) 6608:2004 and 9875:1990 respectively. IS 6608:2004 says that if all the raw materials requiring test for heavy metals have been so tested and comply with the requirements, then the manufacturer may not test the finished cosmetic for heavy metals and arsenic. The dyes colors (pigments lakes) if used in the manufacture of skin creams and lipstick shall comply with IS 4707 (part I) subject to the provision of schedule Q of drugs and cosmetics act and rules, issued by the government of India, and as amended from time to time. Other ingredients shall comply with the provisions of IS 4707 (Part 2). The permitted synthetic organic colors and natural organic colors used in the cosmetic shall not contain more than:

- 2ppm (parts per million) of arsenic calculated as arsenic trioxide.
- 20ppm of lead calculated as lead.
- 100ppm of heavy metals other than lead calculated as the total of the respective metals.

2.5.2. European Union

European Union (EU) cosmetic legislations are based on council directive 76/768/EEC of 11 July 2013 on the approximation of the laws of the member states relating to cosmetic products (cosmetics directive). Manufacturers are responsible for ensuring that cosmetic products comply with the law before they are marketed. The manufacturer or importer of cosmetics is responsible for demonstrating that the product is safe for its intended use. Regulations are enforced at the national level and each country in the EU has an authoritative body that is responsible for upholding compliance. EU has banned about 1,328 chemicals in cosmetics. The chemicals prohibited in cosmetic products are listed. Arsenic, lead cyanide, human tissue etc are prohibited in cosmetics (EU legislation, 2012).

2.5.3. USA

In the US, cosmetics are regulated by Federal Food, Drug and Cosmetic act. It is the role of the FDA to oversee the compliance with these regulations. However, as opposed to drugs, cosmetic products and ingredients are not subject to FDA premarket approval with the exception to color additives. The regulations published by the FDA are all codified in title 21, code of federal regulations (21 CFR) applicable to cosmetics 4 stated at 21 CFR, parts 700 to 740 (21 CFR 700 to 740) defines ingredients as any single chemical entity or mixture used as a component in the manufacture of a cosmetic product. The FD&C act prohibits any product or ingredient that is a poisonous or deleterious substance which may render the product injurious to users under the conditions of use prescribed in the labeling. FDA addresses the presence of trace impurities in cosmetics on a case by case basis. Requirements for specific cosmetics are listed in 21 CFR parts 700. In addition, FDA has established specifications for lead, arsenic, and mercury in most color additives. A few more heavy metal specifications have been established for certain color additives.

2.5.4. Canada

The Canadian food and drug act stipulates that no person shall sell any cosmetic that:-

- has or on it any substance that may cause injury to the health of the user when the cosmetic is used,
- consists in whole or in part of any filthy or decomposed substance or of any foreign matter,
- was manufactured, prepared, preserved, packaged or stored under unsanitary condition”

In December 2008, health Canada released draft guidance on heavy metal impurities in cosmetics for lead, arsenic, cadmium, mercury, and antimony (Health Canada, 2011).

2.6. Analytical techniques to asses toxic heavy metals in cosmetic products

2.6.1. Graphite furnace atomic absorption spectroscopy (GFAAS)

GFAAS is an appropriate atomization technique used to determine analytes concentrations in samples with an acceptable limit of precision at the parts per billion (ppb) levels. In this technique, samples are mixed with modifiers prior to the atomization processes and dispensed into a graphite tube (atomizer) which is programmed to be heated in series of steps, including drying, ashing, and atomizing to remove the solvent and matrix components and to atomize the remaining sample. The atomized sample is retained within the tube and the light path for a prolonged period of time, which leads to an improvement in sensitivity. Chemical interference is relatively low due to the use of chemical modifiers to stabilize the analyte and make the matrix more volatile; hence, it contributes in further improvement to the sensitivity of this technique. GFAAS has a very good detection limits for majority of elements, as a small sample size of 20 µL is required for analysis (Abbas *et al*., 2016). The advantages of GFAAS are solid samples can be analyzed in addition to samples in solution, it shows greater sensitivity than FAAS (Flame atomic absorption spectroscopy), smaller quantities of sample are required (normally 5-60 µL), the atomization process is more efficient comparing to FAAS (Flame atomic absorption spectroscopy) (Garcia and Baez, 2012).

2.6.2. Flame atomic absorption spectroscopy (FAAS)

FAAS is a suitable technique for determining metals at the part per million (ppm) concentration levels with good precision for many elements. FAAS offers air-acetylene and/or nitrous oxide flame atomizer. Samples are introduced into the atomizer as an aerosol by the nebulizer. FAAS technique provides fast analysis of 10-15 s per sample, with very good precision (repeatability), moderate interferences that can be easily corrected, and relatively low cost. FAAS was successfully applied for the determination of heavy metals in various matrices (Abbas *et al*., 2016). The main advantages of FAAS with atomization in a widespread air acetylene flame are easy to use, very fast, low operational costs and reasonably good analytical performance (Stratis *et al*., 2005).

3. MATERIALS AND METHODS

3.1. Collection of Samples

Samples of commercial cosmetic products were collected from the various retail shops from local market of Adama city. In this study, a total of 9 samples (3 samples of each brands) were collected. Three samples of different brands of each cosmetic viz. vaseline, shampoo and lipstick were purchased separately and transported to Kotebe Metropolitan University analytical chemistry laboratory for digestion of cosmetic samples. The digested samples were transported to Bless Agri Food Laboratory service PLC, Addis Ababa, for GFAAS and FAAS determination of heavy metals.

Table 2: List of Cosmetic samples and code name

No	Brand name	Sample Code	Remark
1	vaseline (nunu petroleum jell)	V1	Local
	vaseline (solo)	V2	Local
	vaseline (petroleum jell)	V3	Local
2	shampoo (organza)	S1	Local
	shampoo (aloevera)	S2	Local
	shampoo (GST)	S3	Local
3	lipstick (carmex)	L1	Local
	lipstick (magic)	L2	Local
	lipstick (romantic)	L3	Local

3.2. Materials and Apparatus

3.2.1. Apparatus and Equipment

The laboratory apparatus that are used during the study include:-different sizes of beakers, measuring cylinders, Pyrex vials, digestion flask, spatula, funnel, filter papers, pipettes, different size volumetric flasks, electronic analytical balance (Mttlertoleadop, model AG 204, Switzerland) and hot plate, GFAAS (Agilent Technology, model 240ZAA, Malaysia) and FAAS

(Agilent Technology, model 240FSAA, Malaysia) were used for analysis. All the glassware's were cleaned with detergent and 10% nitric acid and rinsed thoroughly with distilled water before use.

3.2.2. Chemicals and Reagents

All Reagents and chemicals used were of analytical grade, unless otherwise stated. Nitric acid (65% Sigma Aldrich, Europe), perchloric acid (70% Sigma Aldrich, Germany) and distilled water were used for sample preparation. The water used for all the experiments was distilled water.

3.3. Sample preparation

3.3.1. Sample preparation for lead, cadmium and chromium determination

Vaseline, shampoo, and lipstick were analyzed for lead, cadmium and chromium. Sample preparation for the determination of lead, cadmium and chromium ions was done by adapted the method of (Chauhan *et al*., 2010). About 1.0 g of each sample was digested in 5.0 mL mixture of concentrated acid HNO_3:$HClO_4$ (3:1) for 2-3 hours on a hot plate at 90^0C. Then 3.0 mL of acid mixture was again added and then again heated for 2-3 hours to complete the digestion. The digested samples were cooled and about 5.0 mL distilled water was added and mixed well and the volume made up to 25 mL in volumetric flask. The final solution was then filtered through whatman filter paper (number 42). The clear solution was used for metal quantification.

3.4. Preparation of standard solution

The determination of a given metal concentration in the experimental solution was based on its respective calibration curve. In plotting the calibration curves for lead, cadmium and chromium a stock solution of each metal ion of (1000 ppm sigma Aldrich, Europe) was purchased. The selected heavy metals were lead, cadmium and chromium. Calibration curves for lead and chromium metals were prepared using five standard solutions while cadmium metal was prepared using four standard solutions. Five and four standard solutions were prepared by serial dilution of each stock solution. Calibration curves were prepared to determine the concentration of the metals in the sample solution. The instrument was calibrated using five series of working standards for Pb and Cr and four for Cd respectively. The working standard solutions of each metal were prepared from the 20 mg/L intermediate standard solutions of their respective metals. Calibration curve for each metal ion to be analyzed was prepared by plotting the absorbance as a function of metal ion standard concentration. These standards prepared by dilution from 1000 ppm stock solution were as follows: 0.01 ppm, 0.02 ppm, 0.03 ppm, 0.04 ppm and 0.05 ppm for Lead, 0.8 ppm, 1.6 ppm,2.0 ppm and 2.4 ppm for cadmium and 0.5 ppm 1.0 ppm, 1.5 ppm, 2.0 ppm, and 2.5 ppm for chromium. Calibration curves were drawn for Pb, Cd and Cr by plotting absorbance versus metal ion concentration.

3.5. Statistical analysis

Data for metal estimation was tabulated and the results were analyzed statistically and expressed as mean ± SD.

Analysis of variance (ANOVA) is statistical technique which can be used for the separation and estimation of the different causes of variation of more than two means obtained from different experiments (Kurichi and Sonnad, 2006). For this study, the significance of variation between samples was analyzed using ANOVA.

In Spike/Recovery test, known amounts from stock solution of each metal element were spiked into a samples and run in the GFAAS and FAAS. The percentage recovery was calculated by using the formula given below (Fong *et al.*, 2006).

$$\%\ \text{Recovery} = \frac{\text{C (spike)} - \text{C (non spike)}}{\text{C (added)}} \times 100$$

Where C (spike) is metal content of the spike sample

C (non spike) is metal content of non spiked sample

C (added) is metal content of metal added

3.6. Sample Analysis

3.6.1. Sample analysis for lead, cadmium and chromium determination

Concentration of the metal ions present in the sample was determined by reading their absorbance using GFAAS, FAAS and comparing it on the respective standard calibration curve. Then Pb and Cd were analyzed with GFAAS, while Cr was analyzed with FAAS equipped with deuterium arc back ground corrector and standard air acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wave length adjustment) were optimized for minimum signal intensity of the instrument. Three replicate determinations were carried out on each sample. The metals were determined by concentration mode and the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in digested blank solutions and for the spiked samples.

Table 3: Instrumental Conditions for determination of concentration of some selected toxic heavy metals (Pb, Cd and Cr) using GFAAS and FAAS

Metals	Wave length(nm)	Slit width(nm)	Lamp current(mA)	IDL(µg/L)	MDL(mg/L)
Pb	283.3	0.5	10	0.002	0.003
Cd	228.8	0.5	10	0.000007	0.008
Cr	357.9	0.2	7	0.006	0.007

4. RESULTS AND DISCUSSION

4.1. Calibration of the instrument

After making sure that the instrument was properly calibrated, concentration of metals in each sample was measured. The correlation coefficient (r^2) of the calibration curves of some selected toxic heavy metals were determined by plotting prepared standards concentration versus their corresponding absorbance.

A concentration of working standard solutions of each metal is presented in Table 4. The calibration curve of each metal analyzed is shown in Figure (1-3) of Appendix A1.

Table 4: Concentration values of working standard solutions and correlation coefficients of the calibration graphs for some selected toxic heavy metals (Pb, Cd and Cr)

Metals	Concentration of working standards (mg/L)	Absorbance of working standards	Correlation coefficient of working standards (mg/L)	Regression equation
Pb	0.01,0.02, 0.03,0.04, 0.05	0.0076, 0.0182, 0.0241, 0.0315, 0.0396	0.993	Y=0.001X+ 0.001
Cd	0.0008, 0.0016, 0.002, 0.0024	0.0394, 0.0752, 0.0976, 0.1296	0.980	Y=0.055X- 0.008
Cr	0.5, 1.0, 1.5, 2.0, 2.5	0.0166, 0.0316, 0.0467, 0.0635, 0.0759	0.998	Y=0.03X + 0.002

4.2. Method validation

4.2.1. Method Detection Limit

Detection limit of an analytical method refers to the minimum concentration of a substance that can be reported with 99% confidence to be greater than zero (Daniel, 2007). In other words, method detection limit is the lowest analyte concentration that produces a response detectable above the noise levels of the system, typically three times the noise level but not necessarily as an exact value (Butcher and Sneddon, 1998). In the present study, method detection limit for each metal was estimated by digesting three analytical blanks. Triplicate analyses of three blank samples for three metals were performed and the standard deviation of the three blank reagents was calculated. The detection limits were obtained by multiplying the standard deviation of the reagent blank (S blank) by three (MDL = 3xS blank, n = 3).

4.2.2. Accuracy

The accuracy of wet digestion procedure, recovery tests were performed using spiked samples with known concentrations. As shown in Table 5 the results of percentage recoveries for the studied metals were all ranged between 90.6 - 100.0 %. The results were obtained through calculation. Generally good recoveries were obtained for all metals. The results of the recovery tests for samples were within the acceptable range for most metals 80 - 120 % (Mico *et al*., 2006) verifying the validity of the wet digestion method for the cosmetic samples.

Table 5 Recovery test for cosmetic sample

Metals	Concentration of spike (μg/L)	Concentration of added (μg/L)	Concentration of un spike (μg/L)	Recovery (%)
Pb	101.8	60	44.5	95.5
Cd	1.4	1.3	0.1	100.0
Cr	1449.4	1600	0.00	90.6

In general, the agreement of the Method Detection Limit (MDL) and Instrumental Detection Limits (IDL) and the acceptability of the recovery tests for almost all heavy metals under consideration show the appropriateness of the overall methods, procedures and instruments used in this study.

4.2.3. Precision

The precision of an analytical procedure expresses the closeness or agreement between replicate measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (repeatability or reproducibility) (James and Jane, 2005). The common terms used to measure variability is the coefficient of variation (CV) or relative standard deviation (RSD). Which may also be expressed as a percentage and it is a parameter of choice for expressing precision in analytical sciences (Mitra, 2003). In this study, the precision of the results were evaluated by the relative standard deviation of the results of triplicate measurements. Triplicate measurements of each sample (n=3) were used for the analysis of some selected toxic heavy metals (Pb, Cd and Cr) in cosmetic samples. The relative standard deviations of some selected toxic heavy metals (Pb, Cd and Cr) in most samples were less than 20 % of mean concentration of metals except samples of V1, S1, S2 and L2 in lead metal and V3, S1, S3 and L3 in cadmium metal respectively. The percent relative standard deviation values for some selected toxic heavy metals are summarized in (Table 6). The greater value indicates that random error was occurred during the doing of experiments or metal quantifications process.

4.3. Heavy metals contents of studied cosmetic samples

The concentrations of some selected toxic heavy metals (Pb, Cd and Cr) in nine different cosmetic samples which were collected from local market of Adama city were determined by using GFAAS and FAAS. The result showed that the selected toxic heavy metal contents in different cosmetic samples are different from one another. However, both lead and cadmium determined appeared to have values beyond their detection limit in most studied samples and chromium metal was below detection limit in most cosmetic products which indicated that there are no minimum sufficient amount chromium ions in all cosmetic samples except sample S2 and L1. The results obtained as such are summarized in Table 6 below**:-**

Table 6: Mean level concentration and standard deviation of some selected toxic heavy metals (Pb, Cd and Cr) in cosmetic products

Sample code	Metal concentrations (mean ± SD) (µg/L)		
	Pb	Cd	Cr
V1 % RSD	20.15 ± 0.13 0.63	BDL ----	BDL ----
V2 % RSD	48.14 ± 15.01 31.17	0.90 ± 0.10 10.91	BDL
V3 % RSD	12.23 ± 0.94 7.66	0.07 ± 0.04 49.28	BDL ----
S1 % RSD	38.35 ± 10.70 27.91	0.04 ± 0.03 72.22	BDL ----
S2 % RSD	26.68 ± 5.85 21.93	0.38 ± 0.05 12.11	62.73 ± 7.78 12.59
S3 % RSD	23.46 ± 2.02 8.59	0.51 ± 0.53 104.54	BDL ----
L1 % RSD	86.88 ± 14.18 16.32	0.39 ± 0.06 16.62	29.93 ± 3.82 12.71
L2 % RSD	39.70 ± 14.69 37.00	0.48 ± 0.07 16.67	BDL ----
L3 % RSD	28.89 ± 5.9 20.4	0.50 ± 0.11 21.56	BDL -----

Key: BDL= below detection limit

4.3.1. Determined some selected toxic heavy metal concentration in each cosmetic samples

The metal concentrations of Pb, Cd and Cr were observed in varying concentrations. Each cosmetic sample analyzed was found to have different metal contents. Generally, lipstick cosmetic sample was being the highest heavy metal contents followed by shampoo and vaseline cosmetic samples, respectively. The results of this study also indicated that mean concentration of lead is the highest followed by chromium and cadmium. The concentrations of the metals in each cosmetic sample described clearly in Table 6.

4.3.1.1. Lead

The overall mean concentration range of lead in nine analyzed cosmetic samples were varied from 12.23 ± 0.13 to 86.88 ± 15.01 µg/L (Table 6). In other words the minimum concentration of lead (12.23 ± 0.13 µg/L) was detected in the vaseline sample which was coded by V3 and the highest level of lead (86.88 ± 15.01 µg/L) observed in lipstick samples that assigned by L1. The levels differed significantly between vaseline and lipstick, lipstick and shampoo samples for ($p<0.05$) level of significance. The detectable concentrations were found in 100% of the analyzed samples. Based on the mean concentration of lead in each groups of samples were arranged as follows:

For vaseline samples V2 $>$ sampleV1 $> sampleV3$

For shampoo sample S1 $>$ sampleS2 $>$ sampleS3

For lipstick sample L1 $>$ sampleL2 $>$ sample L3

Lead has been found to be a common contaminant in various cosmetics (Chauchan *et al*., 2010). In this study to compare the concentration of lead in different analyzed samples, the amount of lead in sample V3 is one-fourth of lead content in sample V2 and it was greater than half of sample V1, but sample V2 was contained less level of lead than in sample L1. The mean concentration of lead in each vaselines, shampoos and lipsticks were below the recommended permissible limit (Table 9) and other research (published) values (Table 8).

4.3.1.2. Cadmium

Cadmium was found to be contained most samples next to lead. The mean concentration of cadmium range from BDL to 0.90 ± 0.53 µg/L (Table 6) with the highest concentration observed in sample V2 (solo) and the lowest concentration observed in sample V1 (nunu) was below detection limit. The mean concentrations of each analyzed sample were below recommended permissible limit of cadmium (Table 9) and other research values (published) Table (8). The level has no significant difference between three different cosmetic brands ($p < 0.05$) level of significance. The detectable concentrations of Cd were observed in 88.89 % of the analyzed samples. Based on the mean concentration of cadmium contents in each group of samples were arranged as follows:

For vaseline sample V2 > sample V3 > $sample\ V1$

For shampoo sample S3 > sample S2 > sample S1

For lipstick sample L3 > sampleL2 > sample L1

4.3.1.3. Chromium

Chromium was detected only in the two analyzed cosmetic samples. The mean concentration of chromium for carmex lipstick 0.03 ± 0.004 ppm and aloevera shampoo 0.06 ± 0.008 ppm. The mean concentration of chromium in carmex lipstick and aloevera shampoo was below recommended permissible limit of the International Purity specifications (IPS) of US FDA (Table 9). This reveals that the industry in general has succeeded in maintaining the levels of chromium in their products. However, considerable variation was observed across the industry with brands like aloevera (0.055 – 0.071 ppm) and carmex (0.026 – 0.034 ppm). The detectable concentrations of Cr were observed in 22.22% of the analyzed samples and the rest 77.78% of the analyzed samples were observed below detection limits. The levels differed significantly between the shampoo and lipstick brand ($p < 0.05$) level of significance.

Table 7: Mean level concentration of some selected toxic heavy metals (Pb, Cd and Cr) in three brands of cosmetics

Cosmetics item	Mean concentration (ppm)		
	Pb	Cd	Cr
Vaseline	0.022 - 0.032	0.0004 - 0.0006	BDL
Shampoo	0.023 - 0.036	0.0001 - 0.0005	0.055 - 0.071
Lipstick	0.040 - 0.063	0.0004 - 0.0005	0.026 - 0.034

Table 7 show the lead, cadmium and chromium levels in vaseline, shampoo and lipstick in commercial cosmetic products. As chromium concentrations in the vaseline cosmetic product was below the detection limit. The concentration of lead ranged from 0.022 to 0.032 ppm and cadmium ranges from 0.0004 to 0.0006 ppm. The concentration of lead and cadmium in vaseline cosmetic product is below the recommended permissible limit (Table 9). The concentration of lead, cadmium and chromium ranges from 0.023 to 0.036 ppm, 0.0001 to 0.0005 ppm and 0.055 to 0.071 ppm respectively. The concentration of lead, cadmium and chromium in shampoo cosmetic products were below the recommended permissible limit (Table 9) and other related published values (Table 8). The lead, cadmium and chromium concentration ranges from 0.040 to 0.063 ppm, 0.0004 to 0.0005 ppm and 0.026 to 0.034 ppm respectively. The concentration of lead, cadmium and chromium in lipstick cosmetic product was below recommended permissible limit (Table 9) and other related research values (published) (Table 8).

Table 8: Comparison of the concentration of some selected toxic heavy metals (Pb, Cd and Cr) in cosmetic sample with the research value (published).

Cosmetics item	Metal concentration in (ppm)			Reference
	Pb	Cd	Cr	
Vaseline	0.022 - 0.032	0.0004 - 0.0006	BDL	This study
Shampoo	0.023 - 0.036	0.0001 - 0.0005	0.055 - 0.071	This study
Lipstick	0.040 - 0.063	0.0004 - 0.0005	0.026 - 0.034	This study
Lipstick	6.36 - 6.36	0.6 - 0.78	-	(Amaechi and Nasirudeen, 2015)
Lipstick	0.286 - 6.234	0.2 - 0.5	0.222 - 5.43	(Riaz et al., 2013)
Shampoo	1.14 - 1.46	2.8 - 5.54	0.3 - 0.34	(Umar and Caleb, 2013)
Lipstick	0.18 - 0.8	0.01 - 0.05	0.06 - 0.75	(Akari et al, 2016)
Lipstick	0.03 - 0.65	0.2 - 0.5	ND	(Bukari et al., 2013)
Shampoo	1.782 - 3.322	0.058 - 0.203	ND	(Amir et al., 20170)
Lipstick	2.58 - 11.33	0.2 - 0.43	ND - 0.77	

Key: ND= not detected

Table 9: Comparison of the concentration of some selected toxic heavy metals (Pb, Cd and Cr) in cosmetic sample with recommended permissible limit

Cosmetics	Metal concentration in (ppm)			Reference
	Pb	Cd	Cr	
Vaseline	0.022 - 0.032	0.0004 - 0.0006	BDL	This study
Shampoo	0.023 - 0.036	0.0001 - 0.0005	0.055 - 0.071	This study
Lipstick	0.040 - 0.063	0.0004 - 0.0005	0.026 - 0.034	This study
Cosmetics	10	0.3	-	WHO
Cosmetics	10	3	-	Health Canada
Cosmetics	20	15	2	IPS of US FDA
Cosmetics	1	0.1	-	Germany
Cosmetics	1	0.08	-	USA
Cosmetic	0.5	0.5	-	EU
Cosmetics	2	2	-	KEBS

4.4. Statistical analysis of some toxic heavy metal contents of the cosmetic samples

In order to check whether there is significant difference or not in between groups and within groups of the metal concentration of the three different cosmetic brands (nine different cosmetic samples), one-way ANOVA was applied. The result of the analysis is described in different ANOVA output were attached at Appendix A2.

The significant difference ($p < 0.05$) in mean concentrations of Pb, Cd and Cr at 95% confidence level between the three different cosmetic brands (nine different cosmetic samples) have been summarized from ANOVA result as follow. ANOVA is used to test hypothesis about differences between two or more means. For the present study, the significance of variation between samples has been studied using one-way ANOVA. One-way ANOVA showed that there were significant differences ($P < 0.05$) among the mean concentrations of Pb metal in the V1 and V2, V2 and V3, S1 and S3, L1 and L2, L3 and L1 between samples (Appendix A2). However, no significant differences ($P > 0.05$) in the Pb concentrations were observed in V1 and V3, S1 and S2, S2 and S3, L2 and L3 between samples (Appendix A2).

The level found to differ significantly ($p < 0.05$) between samples of V2 and V3 among the mean concentrations of cadmium but were not significantly different ($P > 0.05$) in the mean concentrations of Cd metal in the S1 and S2, S1 and S3, S2 and S3, L1 and L2, L1 and L3, L2 and L3 between samples from the statistical analysis results (Appendix A2).

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this work, a study of the levels of some selected toxic heavy metals (Pb, Cd and Cr) in nine commercial cosmetic products obtained from local market of Adama city. The concentration of some selected toxic heavy metals (Pb, Cd and Cr) has been analyzed by GFAAS and FAAS. The wet digestion methods were used in this study. A recovery study of the analytical procedure was carried out by spiking known amounts of standard solutions of the metals with the analyzed samples. The ranges of recoveries obtained were 90.6 - 100 % and are in a good agreement with the expected values. This indicated that the procedures for determining them were reliable. Calibration curves were plotted with five points for Pb and Cr while four points for Cd metal standards using absorbance against concentrations and the calibration curves obtained. Concentrations of the studied metals between the group and within the samples were also compared using one-way ANOVA. The results obtained in the present study, the concentration of some selected toxic heavy metals are also compared with some guidelines and other review literatures. Generally the highest content of Pb was found in carmex lipstick cosmetic sample, whereas Cr concentration was the lowest below detection limit. In the present work all concentration of some selected toxic heavy metals (Pb, Cd and Cr) analyzed in cosmetic samples below safe permissible limit of WHO, IPS US FDA, Health Canada, Germany, USA, EU and KEBS. The continued use of products contaminated with such heavy metals may cause slow release of these metals into the human body and thus show their harmful effects. So the extensive uses of such products should be avoided.

5.2. Recommendations

The following recommendations are made as a result of this study:-

- It is recommended that precaution be taken on the choice of skin cosmetics to be used.
- Other cosmetics apart from those for the skin should be studied.
- Determine other heavy metals in vaseline, shampoo and lipstick cosmetics not covered in this study.
- Monitoring of the levels of heavy metals in commercially available cosmetic products should be encouraged.

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7. APPENDIX

APPENDIX A1 CALIBRATION CURVE

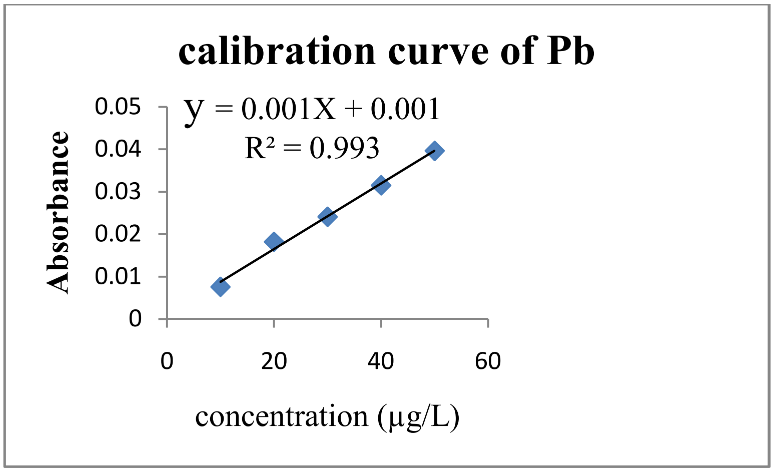

Figure 1 Calibration curve of Pb

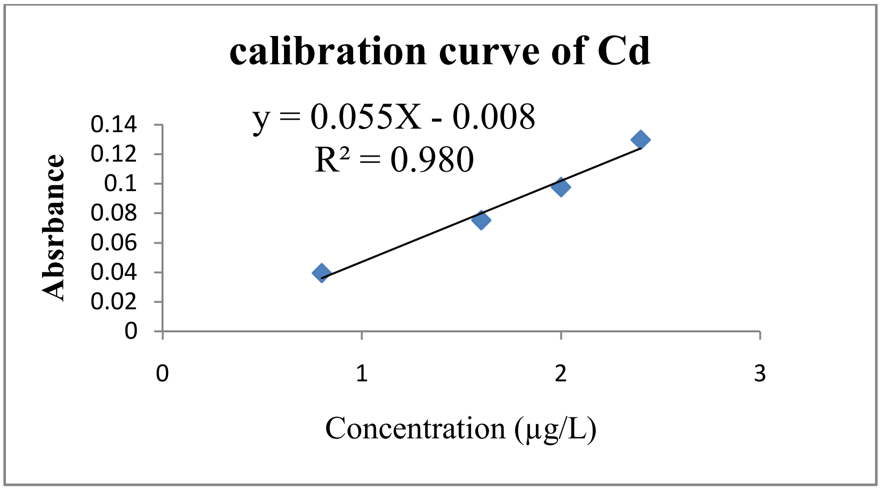

Figure 2 Calibration curve of Cd

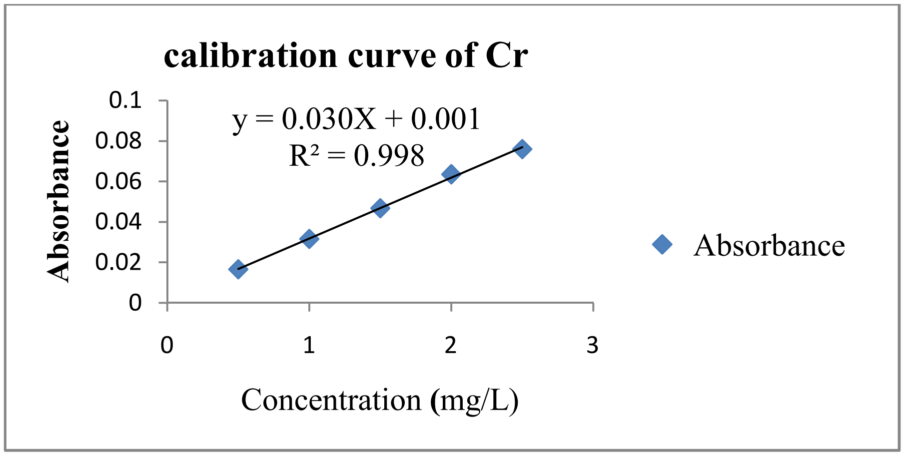

Figure 3 Calibration curve of Cr

APPENDIX A2 ANOVA TABLE

ANOVA Table of lead

Oneway

ANOVA

the concentration of lead in selected cosmetic samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3389.167	2	1694.584	4.121	.029
Within Groups	9869.532	24	411.231		
Total	13258.699	26			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: the concentration of lead in selected cosmetic samples

LSD

(I) code of cosmetic samples	(J) code of cosmetic samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
VSLN	LPST	-24.9843222*	9.5595270	.015	-44.714216	-5.254428
	SHMP	-2.6586000	9.5595270	.783	-22.388494	17.071294
LPST	VSLN	24.9843222*	9.5595270	.015	5.254428	44.714216
	SHMP	22.3257222*	9.5595270	.028	2.595828	42.055616
SHMP	VSLN	2.6586000	9.5595270	.783	-17.071294	22.388494
	LPST	-22.3257222*	9.5595270	.028	-42.055616	-2.595828

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: concentration of lead in vasline

LSD

(I) code of vasline samples	(J) code of vasline samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
vsln1	vsln2	-27.9899000*	7.0882658	.008	-45.334262	-10.645538
	vsln3	7.9221000	7.0882658	.306	-9.422262	25.266462
vsln2	vsln1	27.9899000*	7.0882658	.008	10.645538	45.334262
	vsln3	35.9120000*	7.0882658	.002	18.567638	53.256362
vsln3	vsln1	-7.9221000	7.0882658	.306	-25.266462	9.422262
	vsln2	-35.9120000*	7.0882658	.002	-53.256362	-18.567638

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: concentration of lead in shampoo samples

LSD

(I) code of the shampoo samples	(J) code of the shampoo samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
smp1	smp2	11.6668667	5.8272712	.092	-2.591952	25.925686
	smp3	14.8846333*	5.8272712	.043	.625814	29.143452
smp2	smp1	-11.6668667	5.8272712	.092	-25.925686	2.591952
	smp3	3.2177667	5.8272712	.601	-11.041052	17.476586
smp3	smp1	-14.8846333*	5.8272712	.043	-29.143452	-.625814
	smp2	-3.2177667	5.8272712	.601	-17.476586	11.041052

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: the concentration of lead in lipistck samples

LSD

(I) code of lipistck samples	(J) code of lipistck samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
lpst1	lpst2	47.1732000*	10.0186180	.003	22.658525	71.687875
	lpst3	57.9851333*	10.0186180	.001	33.470458	82.499809
lpst2	lpst1	-47.1732000*	10.0186180	.003	-71.687875	-22.658525
	lpst3	10.8119333	10.0186180	.322	-13.702742	35.326609
lpst3	lpst1	-57.9851333*	10.0186180	.001	-82.499809	-33.470458
	lpst2	-10.8119333	10.0186180	.322	-35.326609	13.702742

*. The mean difference is significant at the 0.05 level.

ANOVA Table for cadmium

-

[DataSet0]

ANOVA

Concentration of cadmium metal in selected cosmetic sampls

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.144	2	.072	.743	.488
Within Groups	2.036	21	.097		
Total	2.180	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Concentration of cadmium metal in selected cosmetic sampls

LSD

(I) the code of selected cosmetic samples	(J) the code of selected cosmetic samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
vsln	lspt	.0274889	.1641180	.869	-.313813	.368791
	shmp	.1750000	.1641180	.298	-.166302	.516302
lspt	vsln	-.0274889	.1641180	.869	-.368791	.313813
	shmp	.1475111	.1467916	.326	-.157759	.452781
shmp	vsln	-.1750000	.1641180	.298	-.516302	.166302
	lspt	-.1475111	.1467916	.326	-.452781	.157759

ANOVA

concentration of cadmium in vasline samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.027	1	1.027	189.760	.000
Within Groups	.022	4	.005		
Total	1.049	5			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: concentration of cadmium in in shampoo samples

LSD

(I) code of shampoo samples	(J) code of shampoo samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
shmp1	shmp2	-.3483333	.2503634	.214	-.960951	.264284
	shmp3	-.4706667	.2503634	.109	-1.083284	.141951
shmp2	shmp1	.3483333	.2503634	.214	-.264284	.960951
	shmp3	-.1223333	.2503634	.642	-.734951	.490284
shmp3	shmp1	.4706667	.2503634	.109	-.141951	1.083284
	shmp2	.1223333	.2503634	.642	-.490284	.734951

Post Hoc Tests

Multiple Comparisons

Dependent Variable: concentration of cadmium in lipstick samples

LSD

(I) code of lipistck samples	(J) code of lipistck samples	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
lpst1	lps2	-.0985000	.0683486	.200	-.265743	.068743
	lps3	-.1163333	.0683486	.140	-.283576	.050910
lps2	lpst1	.0985000	.0683486	.200	-.068743	.265743
	lps3	-.0178333	.0683486	.803	-.185076	.149410
lps3	lpst1	.1163333	.0683486	.140	-.050910	.283576
	lps2	.0178333	.0683486	.803	-.149410	.185076

ANOVA Table for chromium

ANOVA

the concentration of chromium in selsectd cosmtic samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	1	.002	42.972	.003
Within Groups	.000	4	.000		
Total	.002	5			