UNIVERSITI MALAYSIA KELANTAN

Determination of Heavy Metals (Cd, Pb, Hg and Zn) in Cosmetic Products using Atomic Absorption Spectrometer (AAS)

Fairus Zehan Binti Mohd Said

F15A0036

A thesis submitted in fulfilment of the requirement for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours

Faculty of Bioengineering and Technology

Universiti Malaysia Kelantan

2019

DECLARATION

I hereby declare that this thesis "Determination of Heavy Metals (Cd, Pb, Hg and Zn) in Cosmetic Products using Atomic Absorption Spectrometer (AAS)" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree.

Signature : …………………………….

Name : Fairus Zehan Binti Mohd Said

Date : …………………………….

I certify that the Report of this final year project entitled "Determination of Heavy Metals in Cosmetic Products" by Fairus Zehan Binti Mohd Said, Matric no. F15A0036 has been examined and all correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Bioindustrial Technology), Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan.

Approved by:

……………………………

Supervisor

Name : Dr. Rosmawani Binti Mohammad

Date : ………………………………

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim, In the name of Allah, the most Gracious, most Compassionate, All Praise is due to Him, the Sustainer of the Heavens and Earth and all that is within it and may His Blessing be upon the Prophet Muhammad SAW, Peace be upon him.

It is a great pleasure to address people who helped me throughout this project to enhance my knowledge and practical skills especially in this research. I would like to express my special thanks of gratitude to my beloved supervisor, Dr. Rosmawani Binti Mohammad for her inspirational moral support, undivided guidance, advice, opinion, and encouragement to all my endeavours in this research. Therefore, I managed to conduct my research smoothly, efficiently and was able to complete this thesis successfully. May Allah bless you.

I would also like to bid my gratitude to Prof. Madya Dr. Noor Azlina Binti Ibrahim, Dean of Faculty of Bioengineering and Technology (FBKT), Universiti Malaysia Kelantan, Jeli Campus, all lecturers especially from Bioindustrial Technology Programme for all their knowledge and support and also to all laboratory assistant for their help and cooperation. I gratefully thank all my wonderful friends who have given their unconditionally support and encouragement for me in completing my thesis.

Finally, my deepest appreciation is extended to my beloved mother, Puan Jauharohel Nafisah Binti Dahlan for her prayers, blessing, and love. To my wonderful family, all the support, care and helps given drove me all this while, Thank you.

.

Determination of Heavy Metals (Cd, Pb, Hg and Zn) in Cosmetic Products using Atomic Absorption Spectrometer (AAS)

ABSTRACT

Lately, there are number of cosmetics and other products were recalled and banned because of the presence of heavy metals in toxic levels to human in the products. The long term exposure of heavy metals such as mercury (Hg), lead (Pb) and cadmium (Cd) would result into numerous dangers among these; being carcinogenic, kidney dysfunction and behavioural abnormalities and skin infection. Therefore, the aim of this study was to determine the concentration of Cd, Pb, Hg and Zn in several brands of local cosmetic products in Malaysia. The concentrations of heavy metals were analysed using atomic absorption spectrometer (AAS) except for Hg where cold vapour atomic absorption spectrometer (CV-AAS) was employed. The pattern of heavy metals in several brands of local cosmetic products in Malaysia with overall mean concentration was analysed as Zn (75.563 ppm) > Cd (0.0288 ppm) > Hg (0.012 ppm) > Pb (not detectable (ND)). The highest mean concentration (ppm) of Zn was detected in Cream D (292.600 ± 1.51) which exceeded the permissible limit of WHO, followed by Cream C, Cream N and Cream Q with levels of Zn (3.6260±0.0038), 3.1620±0.0404 and 2.8630±0.0243) respectively. However, the mean concentration (ppm) of Pb was not detectable in all samples. For Cd, the mean concentrations (ppm) were obtained in the range (0.005±0.0018 – 0.09±0.0014). Meanwhile, Hg showed the level of mean concentration (ppm) is in the range (0.0002±0.00006 – 0.0312±0.0012). As a conclusion, all the local cosmetic products that have been studied are within the permissible limit except for Zn in Cream D that are exceeded the limits that was set by WHO. Hence, heavy metals concentration level in all cosmetic products must be monitored comprehensively with the respect of human health and potentially safe for use.

Keywords: Heavy Metals; Cosmetics; Atomic Absorption Spectrometer (AAS)

Penentuan Logam Berat (Cd, Pb, Hg dan Zn) dalam Produk-Produk Kosmetik menggunakan Spektrometer Penyerapan Atom (AAS)

ABSTRAK

Akhir-akhir ini, terdapat sejumlah kosmetik dan produk lain yang ditarik balik dan diharamkan kerana kehadiran logam berat dalam kadar toksik kepada manusia dalam produk. Pendedahan jangka panjang logam berat seperti merkuri (Hg), plumbum (Pb) dan kadmium (Cd) akan menyebabkan pelbagai bahaya di kalangan manusia; menjadi karsinogenik, disfungsi ginjal dan keabnormalan tingkah laku dan jangkitan kulit. Oleh itu, tujuan kajian ini adalah untuk menentukan kepekatan Cd, Pb, Hg dan Zn di dalam beberapa jenama produk kosmetik tempatan di Malaysia. Kepekatan logam berat dianalisis menggunakan spektrometer penyerapan atom (AAS) kecuali Hg di mana spektrometer penyerapan atom sejuk (CV-AAS) digunakan. Corak logam berat dalam beberapa jenama produk kosmetik tempatan di Malaysia dengan kepekatan purata keseluruhan (ppm) dianalisis sebagai Zn (75.563 ppm)> Cd (0.0289 ppm)> Hg (0.012 ppm)> Pb (tidak dapat dikesan (ND)). Kepekatan purata paling tinggi bagi Zn dikesan dalam Krim D (292.6±1.51 ppm), diikuti dengan krim C, krim N dan krim Q dengan tahap masing-masing bagi Zn (3.626±0.0038, 3.1620±0.0404 dan 2.8630±0.0243). Walau bagaimanapun, kepekatan purata (ppm) Pb tidak dapat dikesan dalam semua sampel. Bagi Cd, paras kepekatan (ppm) diperolehi dalam julat (0.005±0.0018 - 0.09±0.0014). Sementara itu, Hg menunjukkan tahap kepekatan min (ppm) dalam julat (0.0002±0.00006 - 0.0312±0.0012). Sebagai kesimpulan, semua produk kosmetik tempatan yang dikaji adalah dalam batas yang dibenarkan melainkan Zn dalam Krim D yang melebihi had yang ditetapkan oleh WHO. Oleh itu, tahap kepekatan logam berat dalam semua produk kosmetik mesti dipantau secara komprehensif dengan tahap kesihatan manusia dan ianya berpotensi untuk selamat digunakan.

Kata kunci: Logam Berat; Kosmetik; Spektrometer Penyerapan Atom (AAS)

TABLE OF CONTENTS

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 METHODOLOGY

CHAPTER 4 RESULTS AND DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Cream D	Cream *Deeja Cosmetic*	18
Cream N	Cream *Nurayysa Beauty*	18
Cream Q	Cream *Qu Puteh Beauty Care*	18
USFDA	United States Food and Drug Administration	31
ICP-MS	Inductive Coupled Plasma Mass Spectroscopy	39
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry	39
XFS	X-Ray Fluorescence Spectroscopy	39

LIST OF SYMBOLS

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Since the early of civilization, cosmetics products are considered a part of routine body care (Ullah *et al*., 2017). Cosmetics have always played an important role and gives significant effects on women's lives. Every woman deserves to be beautiful as the beauty especially facial or body beauty is the starting point of women to being confident and beautiful. 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, 2008). It is made to apply on the body or face to enhance the beauty of human which include the makeup things and skincare products. As frequently, cosmetics have always been linked to female and it seems to vary accordingly based on the age, career of people and also the geographical factors of where they are living in. Nowadays, it is a common thing or trends to use cosmetic in their daily life to be beautiful. They are using cosmetics without concerning the existence of harmful chemicals, including heavy metals inside their cosmetics which may negatively influence their body (Norudin *et al*., 2010).

In Asia, especially Malaysia, whitening, and brightening is big business in the Asian beauty industry (Norudin *et al*., 2010). Therefore, there is plenty of local product were produced by locals containing various chemical and poison including a high level of heavy metals such as mercury, cadmium, lead and zinc that have been banned for use in cosmetics. A past analysis by World Health Organization (WHO) in 2010, stated that mercury (Hg) in soaps and skin lightening creams that are commonly used in Asia and Africa are potentially hazardous when put on the face and body as they have serious adverse effects that can lead to serious health diseases.

Centers for Disease Control and Prevention (2003) stated that some cosmetics are beneficial yet others can lead to several harmful effects such as cancer, allergic reaction, mutations, respiratory problems as well as development and reproductive problems. For example, high level of concentration of cadmium leads to inhibition of DNA mismatches. Lead is a common contaminant in cosmetic products that resulting various diseases such as anemia, colic, behavioral abnormalities, learning impairment, decreased hearing and broken cognitive functions can be manifested (Nnorom *et al*., 2005). For mercury which presents in all fairness creams, it can cause kidney damage as the main adverse effect (International Programme on Chemical Safety, 2003) and may also cause discoloration and rashes of skin and scarring, as well as the level of skin's resistance to bacterial and fungal infections may be reduced (Ladizinski *et al*., 2011).

On the other hand, the World Health Organisation (WHO), has sets the limits of heavy metals in cosmetics as follows; for Cd; 0.3 ppm, Hg; 1 ppm and Pb; 10 ppm (WHO, 1995) while for Zn, there is no permissible limit of heavy metals concentration in cosmetics that currently available but levels above 40 ppm may be toxic (Al-Weher, 2008).

1.2 Problem Statement

In Malaysia, preference for lighter skin remains prevalent as they are tending to buy a cosmetic product and various beauty product without having awareness of chemicals and poison contain in it. Norudin *et al*. (2010) indicated that most of the cosmetic users in Malaysia were not concerned and seriously aware of the effect of usage of products to their skin. Most of them only focus on the immediate result to their skin appearance of being beauty instead of knowing the long-term effects on their whole body.

Nowadays, beauty products have been manufactured with local brands and sold in the market. From day cream to evening cream, there are many types of beauty products sold widely, especially in social media such as *Facebook* and *Instagram*. Recently, it is viral on social media about the beauty cream or known as '*krim timbang kilo*' story that allegedly became the choice of many 'founder' of beauty products in Malaysia to have its own brand of products. These beauty cream or '*krim timbang kilo*' are beauty creams that are produced in neighboring countries, and sell by kilograms, for buyers who want to become in an immediate skincare founder in a short time. These beauty creams were sold in a plastic tub, a random container without any protection and a proper hygiene.

This research was done to focus in determining the concentration of heavy metals using Atomic Absorption Spectrometer (AAS) of cadmium (Cd), mercury (Hg), lead (Pb) and zinc (Zn) that may present in cosmetic products collected from several local markets in Malaysia such as *Deeja Cosmetic*, *Nurraysa Beauty*, *Qu Puteh Beauty Care* and *Chriszen* which are widely used by local people on Malaysia using Atomic Absorption Spectrometer (AAS). The aimed of this study is to know whether these metals concentrations are within the permissible limits that had been set by WHO.

1.3 Objectives

The main objectives of this study are:

i. To measure the concentration of heavy metals (Cd, Hg, Pb, and Zn) present in Malaysia cosmetic products (*Nurraysa Beauty, Deeja Cosmetic*, *Chriszen, and Qu Puteh Beauty Care*) using Atomic Absorption Spectrometer (AAS).

ii. To investigate whether the concentration level of these heavy metals present in cosmetic products is within the permissible limit for human consumption according to the World Health Organisation (WHO).

1.4 Scope of Study

This research was focused on determining the concentration of heavy metals present in the cosmetic product in Malaysia such as Cd, Hg, Pb, and Zn. In this study, the only cream form of skin cosmetics that is produced by local entrepreneur was used such as *Deeja Cosmetic*; turmeric night cream, *Chriszen*; moist foundation, *Nurraysa Beauty*; UV cream, and *Qu Puteh Beauty Care*; cream foundation. These determination of heavy metals in these samples were determined by Atomic Absorption Spectrometry (AAS).

1.5 Significance of Study

The importance of this study is to investigate the concentration of heavy metals that may contain in cosmetic products that have been consumed by our people in Malaysia. Therefore, from this study, people in Malaysia could emphasize awareness on what ingredient contained inside the cosmetic beauty product that they put on their face. It is important among Malaysian to taking note regarding their choices of cosmetics as either continual use or combination use of cosmetics would be detrimental to skin and even to their body.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metals

Heavy metal is referred as metals that has a relatively high density and is toxic or poisonous at low concentrations (Tchounwou *et al*., 2012). Banfalvi (2011) reported that, heavy metals that have densities higher than 3g/cm^3 are known to cause dangerous effects if exists at certain levels and exceed the permissible limit than recommended. A number of these occur due to the existence of natural components of the earth crust such as 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.

Generally, Cd, Pb, Hg, and Zn have been listed by WHO as 10 major chemicals and metals of concern that lead to hazard thus causing negative effects to human health and environment (WHO, 2010). These can occur if it does not properly manage, followed by air pollution, asbestos, benzene, dioxin, inadequate or excess fluoride and highly hazardous pesticide (WHO, 2010) These effects could lead to toxicity, neurotoxicity, carcinogenicity, mutagenicity, and teratogenicity toward nature (Linnila, 2000; Duruibe *et al*., 2007). Linnila (2000) reported that the toxicity of heavy metals will give negative impact towards human as it can cause a damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, liver, kidneys and other crucial parts of organs. Heavy metals could be penetrated through moist skin through dermal or skin contact (Omolaoye *et al*., 2010).

2.2 The Determination of Heavy Metals in Cosmetic Products and its Toxicological Effects towards Human

2.2.1 Cadmium (Cd)

Cd is a soft, malleable, ductile, bluish-white divalent metal. Godt *et al*. (2006) stated that the use of Cd in cosmetics products is because of its color property of deep yellow to orange pigmentation as a color pigmentation usage in many industries. Cd compound is highly carcinogenic, mutagenic and toxic to human health. In 2013, The United States Environment Protection Agency (USEPA), had banned and restricted the standards for Eco-labels products from cosmetics, toys, plastics and building materials. Cd compound can penetrate throughout the body through dermal contact with cosmetics where it accumulates in the human liver and kidney, although it can be discovered in almost all adult human tissues (Elinder, 1985; Ayenimo *et al*., 2010).

In addition, an exposure to Cd can lead to kidney dysfunction and high levels of Cd exposure can severely cause death. The kidney is the targeted organ in humans when exposed chronically to cadmium by ingestion (Adekunle *et al*., 2014). It is extremely toxic even in extremely low levels of concentration of cadmium in the human body for a long-term effect. Other than that, high level of Cd exposures results in bone defects, renal dysfunction, Cd pneumonitis, obstructive lung disease, increased blood pressure and myocardial dysfunctions (Duruibe *et al*., 2007). Chauhan *et al*. (2010) reports that Cd level in bathing soaps is ranged from 0.03 ppm to 0.04 ppm. Although these levels were below the permissible limits proposed by WHO, the continuous usage of the soaps is feared to lead a harmful effect by slowly release into the body (Chauhan *et al*., 2010). However, some Indian herbal cosmetic and Macedonia herbs were prepared in a research recorded that the Cd levels contained was in range of 0.10 to 1.875 ppm, where some of which of the cosmetic products were above the WHO permissible limit of 0.3 ppm (WHO, 1995; Gentscheva *et al*., 2010; Sukenda *et al*., 2012).

2.2.2 Lead (Pb)

According to Chauhan *et al*. (2010), 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. The continuous use of these cosmetics through dermal cosmetic absorption can increase the absorption of heavy metals especially Pb in the body (Nourmoradi *et al*., 2013).

As a result of lead toxicity, anemia, colic, neuropathy, nephropathy, sterility, coma, behavioral abnormalities, learning impairment, decreased hearing and impaired cognitive functions can be manifested (Nnorom *et al*., 2005). There are plenty of studies that expose the risks of Pb in face creams and herbal cosmetics where levels of Pb concentration that range as low as 0.03 to 33.1 ppm, 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). Lead can cause a serious brain and kidneys damage and even death. Based on Rudolph (2003) report, Pb can cross the blood-brain barrier by mimicking thus reducing the myelin sheaths of neurons, reduces their numbers, interferes with neurotransmission routes, and decreases neuronal growth.

2.2.3 Mercury (Hg)

Mercury (Hg) is a heavy metal that only exists in a liquid state at a room temperature and pressure. Hg in cosmetic is acted as thiomersal as it widely used in the manufacture of cosmetics. According to Paul (2008) the first state in the United States (US) of Minnesota is banning the products such as mascara, eye liners and skin-lightening creams that intentionally adding add mercury in cosmetics, allowing a tougher standard than the federal government.

It is common to find Hg in any skin lightening soaps, creams, and various lotions. 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. 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, analysis of Hg in skin lightening creams was recorded within the range levels from 0.01 ppm 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 used for as short as 2 days. The results indicated that the 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 whereby Hg were higher than the normal levels of Hg were found in the 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 a*l., 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).

2.2.4 Zinc (Zn)

Zinc oxide is an inorganic compound in cosmetics that plays the roles as a sunscreen and also to whiten the skin (Butler and Poucher, 2000). It also acts as an essential trace element for plants, animals, and humans as it forms connective tissues that maintains the skin nutritional requirements (Miculescu *et al*., 2011). Zinc helps to keep a healthy skin by eliminating stretch marks and scars which may result from elastin's inability. Null and Mc Donald (2006) reported that zinc acts as a mild or normal astringent by shrinking and tightening body tissues thus assisting in the acne treatment. However, though Zn toxicity is uncommon, at concentration levels of Zn of up to 40 ppm, it may cause various toxicity such as muscular pain, skin irritability and muscular stiffness (Al-Weher, 2008).

Severe toxicity may be resulted in gastric distress, dizziness, nausea, vomiting, and reduction in immune function (Deshpande, 2005). In Nigeria, applying cosmetic with high level of Zn above the acceptable limit of 170 µg/L, had induce the toxicity by using cosmetics such as eyeshadows and skin lightening cream where it implying continual exposure of Zn to the users (Omolaoye *et al*., 2010). Moreover, there are some Indian herbal cosmetic preparations and Bulgaria herbs that have also been reported to contain alarming Zn with levels ranging from 4.0 to 56.57 ppm (Gentscheva *et a*l., 2010; Sukenda *et al*., 2012). The continuous use of these cosmetics would eventually be detrimental to humans. There are potential consequences that shows an excessive manifestation to Zn would induce toxicity at several levels in cosmetic products. Though Zn toxicity is rare, but the continuous exposure would still be dangerous particularly if taken orally and also through dermal absorption.

2.3 Method of Analysis

Cadmium (Cd), lead (Pb) and zinc (Zn) can be determined using Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer (AAS) (Mireji *et al*., 2007) controlled by the proven WinLab 32 ™ AAS computer software that was fast and easy to get from sample to results. AAS was used because it is selective, highly specific and available. Cold Vapour Atomic Absorption Spectrometer (CV-AAS) was used for the determination of mercury (Mester and Sturgeon, 2003). The PinAAcle spectrometer was coupled to a Flame Atomic Absorption Spectrometer (FAAS) system that incorporates two peristaltic pumps, a 5-port flow injection valve and a regulated gas supply (Hineman, 2011).

2.3.1 Atomic Absorption Spectrometer

(i) Flame Atomic Absorption Spectrometer

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 the energy required for excitation of an element of interest (Taylor *et al*., 2006). 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. (Skoog and Leary, 1992). Figure 2.1 shows a schematic diagram for the components of AAS.

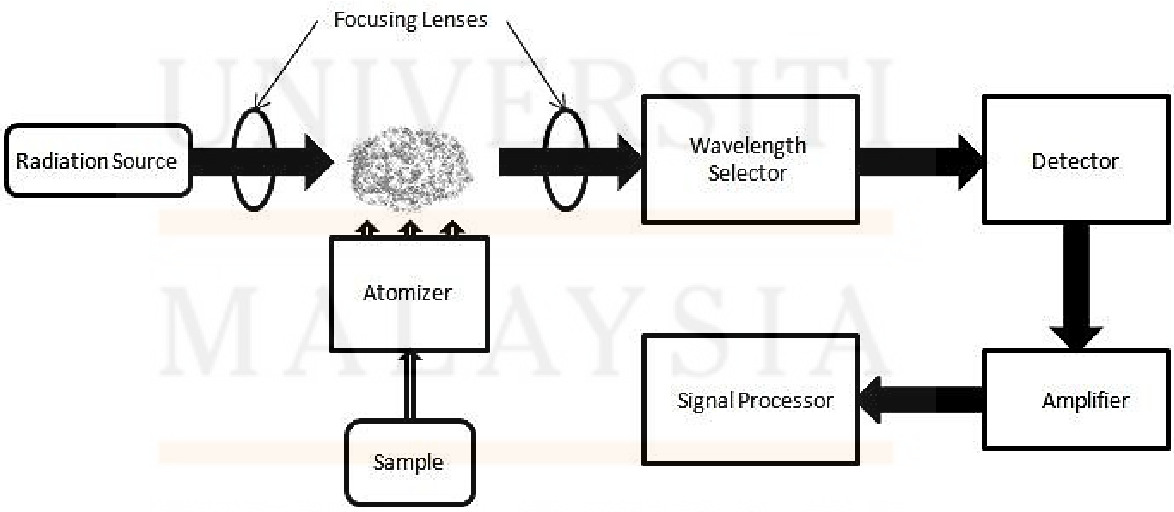


Figure 2.1: A Schematic Diagram of AAS instrumentation

(Source: Onyambu, 2014)

Onyambu (2014) reported that the most crucial parts of the atomic absorption spectrometer (AAS) are including the two types of radiation sources which are the continuous and line source, an atomizer, monochromatic, detectors, and the readout system. A wide range of radiation that continuously giving source is including deuterium lamp and mercury vapor lamp. A little amount of radiation absorbed by monochromatic lead to less sensitivity while a large part is falling in detector gives insignificant absorbance by the metal. Before this, the continuum source that called deuterium lamp is not used as a light source in AAS but is only used for background correction. The one that commonly used in AAS instrument is the hollow cathode lamp that made from metallic or alloy. This hollow cathode lamp is including of a tungsten anode and cylindrical cathode sealed in a glass tube that is filled with certain gas such as neon or argon gas at a pressure of 1-5 torr.

There are two types of atomizers which are flame and electro-thermal atomizers. The one that has been used in the experiment are flame atomizer which able to detect various heavy metals such as Cd, Pb and Zn except for Hg. Flame Atomic Absorption Spectrometer (FAAS) is the instrument in which the temperature is determined by flow rate and the ratio of oxidant and fuel. The solvent used in flame atomizer is evaporated in order to produce a solid molecular aerosol during the dissolving process. Cations and electrons are given by the ionization of atoms from the dissociation lead to the atomic gas. This technique had very limited applications due to its poor detection limit for determine the Hg content. FAAS method can only shows a minimal absorption at certain concentration for detecting the Hg (Matusiewicz and Krawczyk, 2008). Therefore, to detect the levels of Hg in real and certified reference materials, Cold vapor atomic absorption spectrometry (CV-AAS) were employed in this experiment.

(ii) The Cold Vapour Atomic Absorption Spectrometer (CV-AAS)

Mercury is an element with very special physical and chemical properties, it cannot be measured very sensitively by flame AAS. Therefore, in order to determine the mercury content of cosmetic, CV-AAS was employed as the sample were use vaporization method by reduction and supplied to the atomic absorption spectrometer as it is more successful way to precisely analyze traces of mercury. CV-AAS is the most efficient and widely used analytical technique for the determination of mercury due to its simplicity and good reproducibility. The mercury can remain in a specified temperature in its atomic state at room temperature. This method is using vapour that pressure the mercury at 20 ºC is 0.16 Pa that correlate to 14 $mg \cdot m^{-3}$ mercury concentration in air dissimilar with FAAS (Neetu *et al*., 2010).

Atomic mercury can be dispensed out from its digested samples by using reduction process. The most commonly used reducing agents are $SnCl_2$/HCl or $KMnO_4$/NaOH. After volatilization, mercury vapor is released out of the solution and move through to the absorption cell by an inert gas. The mercury absorption line in this research is at 253.65 nm. The magnitude of the signal shows the quantity of mercury exist in the samples loaded. The most definite method for mercury determination was completely examined by Hatch and Ott (1968) as shown in Figure 2.2.

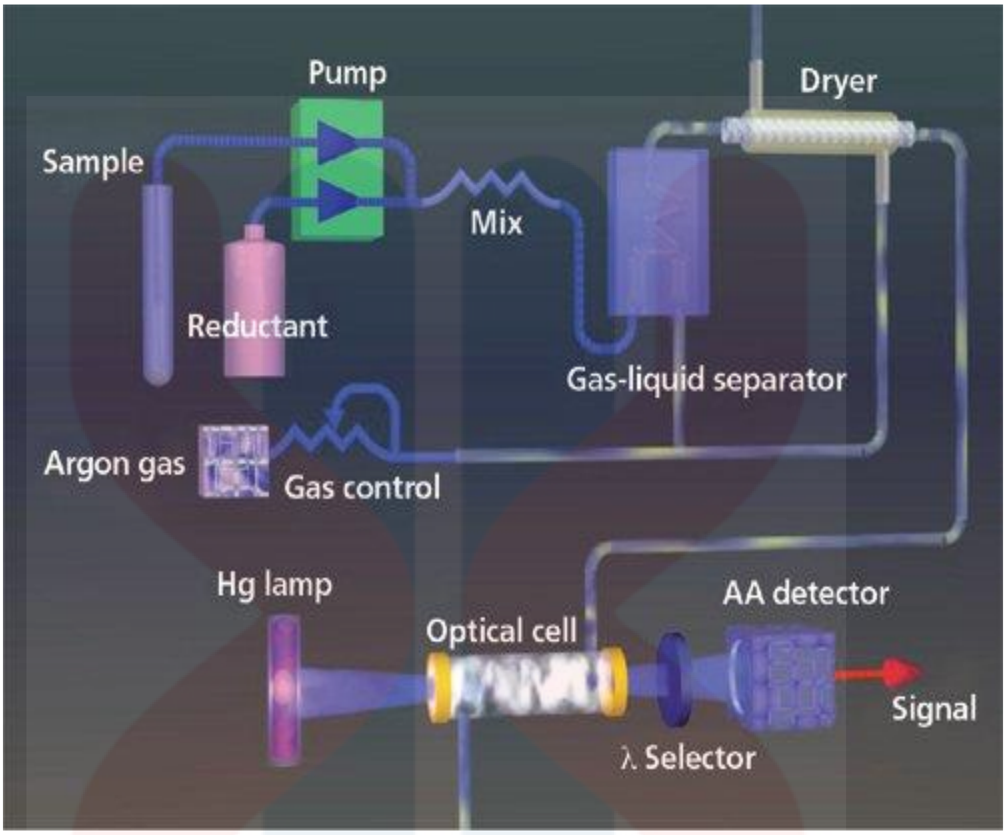


Figure 2.2: An overview of cold vapor atomic absorption

(Source: Pfeil, 2011)

Figure 2.2 display an overview of a Cold Vapor Atomic Absorption (CV-AAS) system. Typically, in CV-AAS instrument system, a peristaltic pump is employed to promote sample and stannous chloride ($SnCl_2$) into a gas–liquid separator where a stream of pure, dry gas is bubbled through the mixture to release mercury vapor. The Hg is then transported via carrier gas through a dryer and then into an atomic absorption cell. Mercury absorbs 254 nm light in proportion to its concentration in the sample.

CHAPTER 3

METHODOLOGY

3.1 Apparatus

The apparatus that are used in the laboratory were listed in Table 3.1. The apparatus was handled and measured carefully to reduce the error and obtain a precise result.

Table 3.1: List of Apparatus

No.	Apparatus
1	Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer
2	WinLab 32 TM AAS Computer Software
3	Hollow Cathode Lamp: Cd, Hg, Pb, and Zn
4	Whatman No.40 filter paper
5	0.45 µm syringe filter
6	Weighing machine
7	Fume hood chamber
8	Hotplate
9	Porcelain crucible
10	Spatula
11	Erlenmeyer flask
12	25 mL and 50 mL of Volumetric flask

13	10 mL of measuring cylinder
14	Chemical glass bottle
15	100 mL of beaker
16	15 mL and 50 mL of Falcon tube
17	Dropper

3.2 Chemicals and Reagents

All chemicals that are used in this study are analytical grade. The chemicals and reagent used in this study were listed in Table 3.2.

Table 3.2: List of chemicals and reagent

No.	Chemicals and Reagent
1	Nitric acid, HNO_3 (1.00 M)
2	Concentrated hydrochloric acid, 37% HCl
3	Concentrated hydrochloric acid, 3% HCl
4	Potassium permanganate ($KMnO_4$)
5	Distilled water

3.3 Collection of Cosmetic Products' Samples

Different types of local cosmetic brands and samples were purchased from several local markets in Selangor. These samples are all in the form of cream but different brand

and functions. The list of cosmetic brand and type that were used in this research was listed in Table 3.3.

Table 3.3: List of Cosmetic Brand and Type

No.	Brand of Cosmetic	Type of Cream	Sample Name
1	*Deeja Cosmetic*	Turmeric night cream	Cream D
2	*Nurraysa Beauty*	UV Cream	Cream N
3	*Chriszen*	Moist Foundation	Cream C
4	*Qu Puteh Beauty Care*	Cream Foundation	Cream Q

3.4 Cleaning of Apparatus

All apparatus used were washed with liquid detergent, warm water and rinsed with tap water. They were soaked overnight in 10% analytical grade nitric acid (HNO_3) and then rinsed with distilled water. The glassware was dried out in an oven at 105 ºC for 24 hours (Onyambu, 2014).

3.5 Instrumentation

Precise determination of heavy metals levels in cosmetic products is quite important because there is a narrow margin of safety between the adequate amount and over consumption (Lloyd, 2000). Thus, analysis Cd, Hg, Pb and Zn was carried out by using

Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer (AAS) with a WinLab 32 ™ AAS Computer Software system.

3.6 Preparation of Standard Solutions

The standard solutions of Cd, Pb and Zn were prepared in the range of 0.3 to 0.9 µg/L for Cd, 2.5 to 7.5 µg/L for Pb and 0.25 to 0.75 µg/L for Zn set in AAS method. A certain amount of these heavy metals were then weighted and dissolved in distilled water. Thus, the standard solutions were measured using AAS for preparation of a standard calibration curve.

For Hg, the chemical used for standard preparation is potassium permanganate ($KMnO_4$) solution. 0.5g of $KMnO_4$ crystals were weighed into 50 mL falcon tube and then mark-up with distilled water. 10 ppm Hg solution was prepared as an intermediate stock solution by pipetting 1 mL of 1000 ppm Hg stock into 100 mL volumetric flask then top-up to the mark with 3% hydrochloric acid (HCl). The $KMnO_4$ used was acts as reducing agent to stabilize the sample solution.

3.7 Digestion of Samples and Blanks

Cream and face foundation cosmetics at the formula of a semi-solid structure were employed using acid digestion procedure for the elements described by Massadeh (2017). 2.0 g of each cosmetic sample was be accurately weighed and dissolved in a mixture of 6 mL of nitric acid (HNO_3) (1.00 M) and 4 mL concentrated hydrochloric acid into a porcelain crucible.

The porcelain crucible contains sample was heated on a hotplate to near dryness. An aliquot of 15 mL nitric acid (HNO_3) (1.00 M) was added to the digested sample and filtered through a Whatman No. 40 filter paper. The digested sample were then transferred quantitatively into a 25 mL volumetric flask and diluted with distilled water. Each digested sample were evaporated at 70ºC to about 1 mL and transferred into an Erlenmeyer flask and diluted with 25 mL distilled water. Then, four times concentration of serial dilution was performed by transferring 1.5 mL of the pure sample to 15 mL falcon tube in each cosmetic sample at 10^{-1}, 10^{-2},10^{-3} and 10^{-4} and 13.5 mL of distilled water was added to fill the falcon tube.

Next, each of the cosmetic samples was filtered using nylon 0.45 µm syringe filter to filter out the aqueous solution before performing AAS. This action was performed to avoid any residues stuck during AAS process. The filtration was started from least concentration samples then followed with the highest concentration which is from 10^{-4} to 10^{-1}.

Blank were treated in the same procedure without adding the cosmetic sample. The reagent blank solution was prepared by using 6 mL of nitric acid (HNO_3), 4 mL concentrated hydrochloric acid 37% HCl and diluted with distilled water in a 25 mL volumetric flask. The flowchart of this research is shown in Figure 3.1.

Collection of cosmetic samples

↓

Preparation of chemicals and reagents

↓

Preparation of instrumentation

↓

Sample preparation using acid digestion procedure:

↓

2.0 g of each cosmetic sample was dissolved in a mixture of 6 mL HNO_3 and 4 mL HCl in a porcelain crucible

↓

The sample was then heated on a hotplate to near dryness

↓

An aliquot of 15 mL HNO_3 (1 M) was added to the digested sample and filtered through a Whatmann No. 40 filter paper

↓

The digested sample was transferred quantitatively into a 25 mL volumetric flask with distilled water

↓

Each digested sample was evaporated at 70ºC to about 1 mL and transferred into a polyethylene flask and diluted with 25 mL distilled water

↓

Blank was treated in the same procedure

↓

Construction of calibration curves

↓

Data collection

↓

Analysis of Data

Figure 3.1: Research Flowchart for Experimental Methodology

(Source: Modified from Massadeh *et al*., 2017)

3.8 Calculation and Evaluation of Result

As stated by AOAC (2002), the concentration (C) of each heavy metal in each test samples were calculated according to Equation 3.1:

$$C = \frac{(a - b) \times V}{m \times 1000} \tag{3.1}$$

Where;

C = Concentration in the test sample (µg/L)

a = Concentration in the test solution (µg/L)

b = Average of concentration in blank solution

V = Volume of test solution (mL)

m = The weight of test portion (g)

CHAPTER 4

RESULTS AND DISCUSSIONS

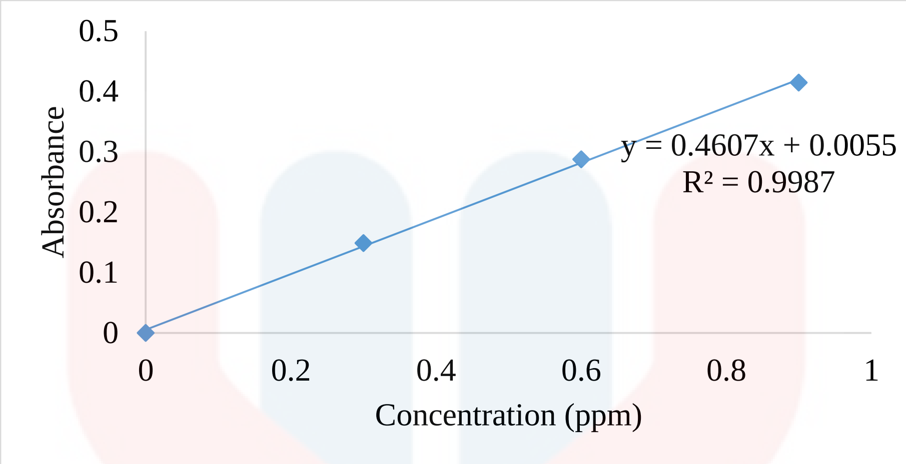
4.1 Introduction

In this research, the levels of heavy metals Cd, Pb, Hg and Zn in various local cosmetic creams were determined using Atomic Absorption Spectrometer (AAS). The data obtained are presented in tables prior to their discussions as shown in Appendix A.

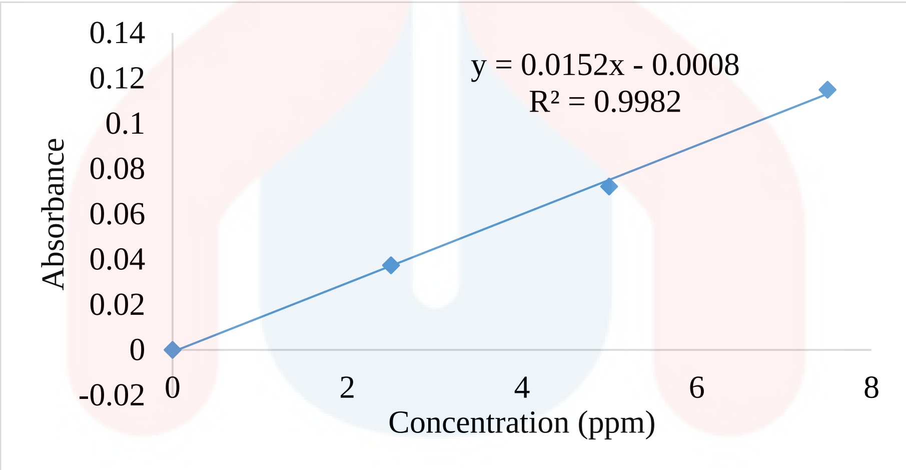
4.2 Standard Calibration Curve

The standard calibration curves for each heavy metal is plotted using AAS. The measurement of the blank solution gave the reading of 0.00 mg/L. This indicated that there were no impurities or error in the blank solution and instrument used.

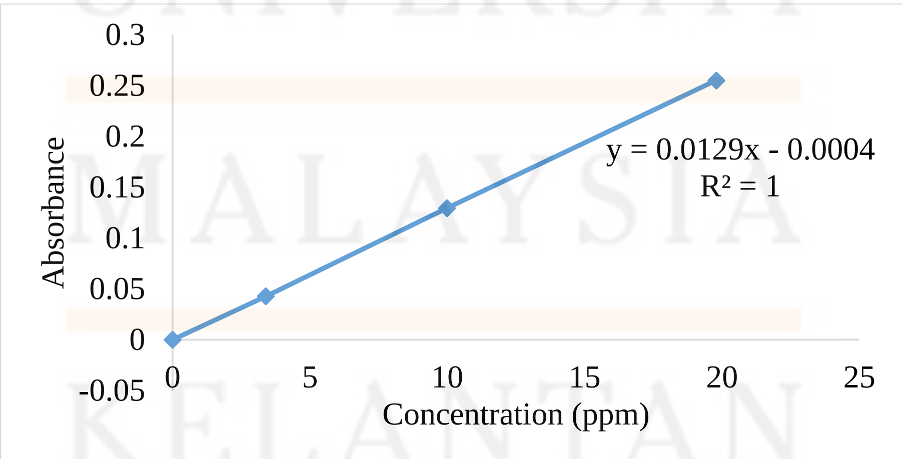
Figure 4.1 shows the linear calibration curve from AAS where the standard solutions of heavy metals studied were prepared in the range of 0.3 - 0.9 µg/L for Cd, 2.5 - 7.5 µg/L for Pb, 0.25 - 0.75 µg/L for Zn and 0.2 - 1 µg/L for Hg.


0.5
0.4
0.3
0.2
0.1
0
Absorbance
0
0.2
0.4
0.6
0.8
1
Concentration (ppm)
y = 0.4607x + 0.0055
R² = 0.9987

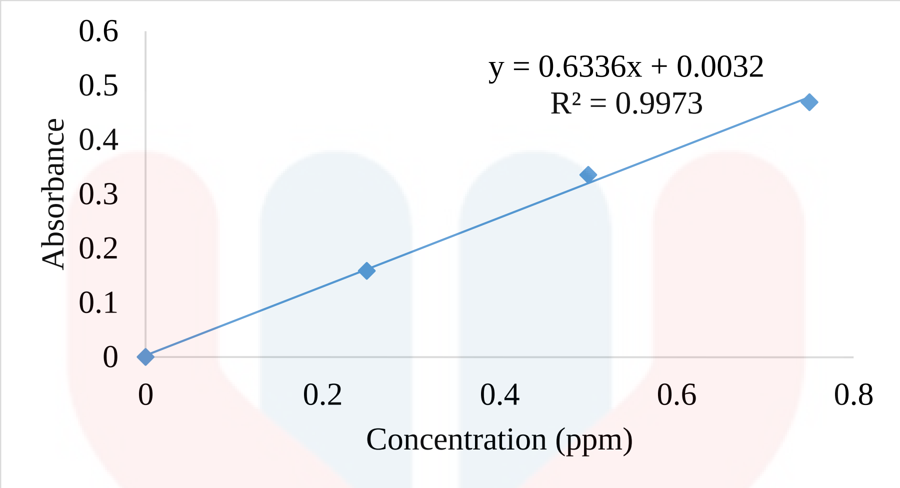
(a)


0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
Absorbance
0
2
4
6
8
Concentration (ppm)
y = 0.0152x - 0.0008
R² = 0.9982

(b)


0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
Absorbance
0
5
10
15
20
25
Concentration (ppm)
y = 0.0129x - 0.0004
R² = 1

(c)



(d)

Figure 4.1: Linear calibration curve for Cadmium (a), Mercury (b), Lead (c) and Zinc (d) using AAS

4.3 Heavy Metals Concentrations in Cream Cosmetic Products

The guideline of the World Health Organization (WHO) was adopted in this study for comparison of these heavy metals concentrations in selected cream cosmetics in Malaysia. The WHO has recommended the permissible limit of heavy metals concentrations for cosmetic products as shown in Table 4.1.

Table 4.1: The recommended permissible limit for cosmetic products by WHO

Metal	Permissible limit (ppm)
Cd	< 0.3
Pb	< 1.0
Hg	< 10.0
Zn	< 40.0

4.3.1 Cadmium (Cd)

Figure 4.2 shows the mean concentration of Cd in all brands of local cream cosmetic products. The concentration of Cd obtained for all cosmetic samples were in the range of 0.005 to 0.010 ppm. The highest concentrations of Cd were detected in Cream N's UV cream samples which are 0.09 ppm followed by Cream D and Cream Q cream foundation with an average concentration of 0.010 ppm and 0.010 ppm respectively. The lowest concentration of element among four cosmetic samples was Cream *C* which is 0.005 ppm.

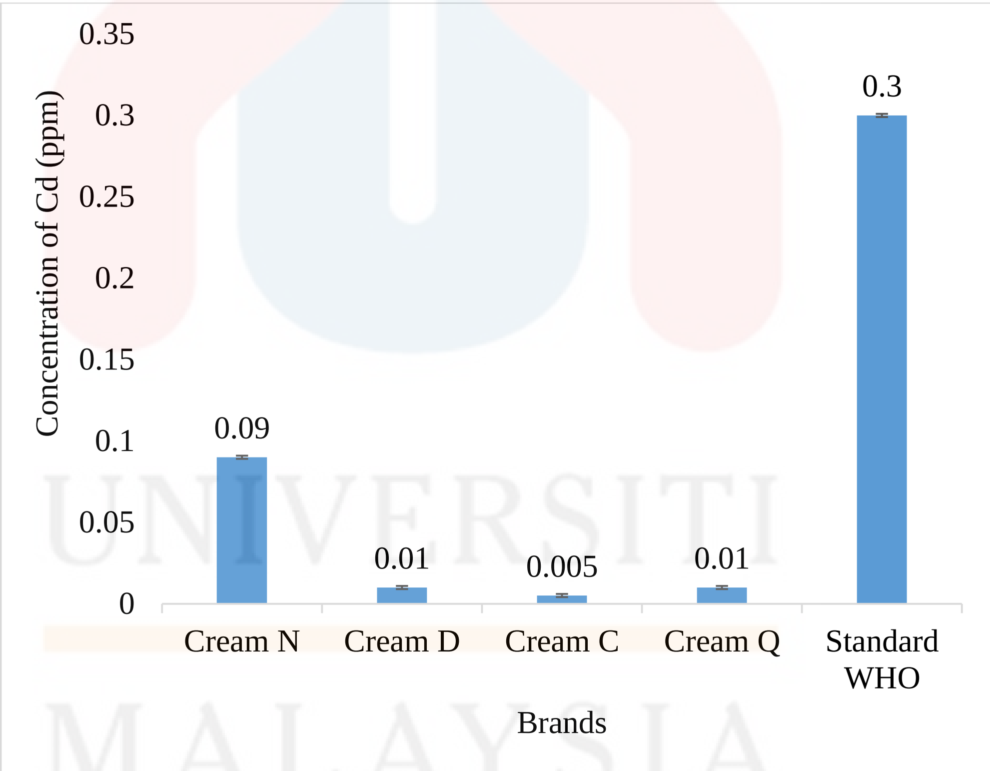


Figure 4.2: Concentration of Cd (ppm) in different brands of local cream cosmetic products

It was observed that the concentrations of Cd in all cream cosmetic samples from four different local brand analyzed are below the permissible limit which is 0.3 ppm as stated in Table 4.1. Hence, the concentrations of Cd in all cosmetic samples seems to be at the safe level. Although the value of heavy metals in these cosmetics is minimal, they cannot be ignored.

Cream N shows a higher concentration of Cd compared to other brands may due to the type of cream studied. The type of cream that has been investigated was UV cream which functions as sunscreen. Sunscreen is being beneficial in protecting skin from the sun's harmful effect. However, Zachariadis and Sahanidou (2009) have reported that sunscreen in the market was present of toxic metals that have been added to the product such as UV filter. This UV filter has no availability for standardized analysis for regulators or quality controllers, though there are regulations that limit the maximum amounts of each type of UV filter.

The concentration of Cd was also reported in several international cosmetic products. Chauhan (2010) reported that Cd level in bathing soap is ranged from 0.03 to 0.04 ppm is within the permissible limits recommended by WHO, though, continuous usage of the soaps may lead to harmful effects by slowly release inside the body. Other than that, some Indian herbal cosmetic and Macedonia herbs were recorded to have Cd concentration in the range between 0.10 to 1.875 ppm. These past studies imply that they are still in a safe level as it were below the WHO permissible limit of 0.3 ppm (WHO, 1995; Gentscheva *et al*., 2010; Sukenda *et al*., 2012).

The presence of Cd in the samples, such as cosmetic products, has adverse effects on the human body. This metal is toxic even at very low levels. In humans, long-term exposure results in renal dysfunction. Tumors on the skin and lesions in the scrotum were observed in the rats following dermal application of Cd (Fasanya-Odewumi *et al*., 1998). Two mechanisms were proposed that facilitate Cd absorption by the skin include binding of free Cd ions to sulfhydryl radicals of cysteine in epidermal keratins or complexing and induction with metallothionein. Nevertheless, absorption of Cd by the skin as low as 0.5% would be a concerned where the concentrated solution was exposed to the skin for several hours (Guy *et al*., 1999). Therefore, due to the long-term exposure of cosmetic product with skin, the absorption of Cd through these products becomes extremely significance towards the user (Muhammed and Hamsa, 2017).

4.3.2 Lead (Pb)

Table 4.2 shows that the concentration of Pb was not detected for all cosmetic samples. The concentration of heavy metals obtained for this study is in negative values in the range of -0.224 to -0.154 (ND) ppm which were below the limit of detection. The highest level of Pb concentration is in cream D which is -0.154 ppm and lowest levels are cream C which is -0.243 ppm. Hence, the concentrations of Pb in all cosmetic samples completely seem to be at the safe level.

Table 4.2: Concentration of Pb (ppm) in different types of local cream cosmetic products

Brands	Concentration (ppm)	WHO permissible limit (ppm)
Cream N	ND	
Cream D	ND	
Cream Q	ND	1.0
Cream C	ND	

After analyzed the samples using AAS, all of the 4 different local cosmetic brands collected in Malaysia were not containing any lead that can affect our health if exposed on the skin. However, Gondal *et al*. (2012) revealed that in a 40 samples consisting of 10 different types of talcum powder facial commonly used in Nigeria, the concentration of lead was found in the range of 15 ppm to 20 ppm which shows a high concentration of Pb than the limitation of heavy metals proposed by WHO (1.0 ppm). This is because of the addition of external additives like perfume during manufacturing and the addition of shining properties used to enhance the beautification (Gondal *et al*., 2009). Morais and Garcia stated that, Pb is the most significant toxin of heavy metal elements with no essential function to human.

However, previous study by Orisakwe (2013) have reported that the levels of lead that have conducted in 28 body creams and lotions, 10 powders, 3 soaps, 5 eye make-ups, and 4 lipsticks widely available on Nigerian markets shows increases over suggested or limited levels of lead in creams and lotions ranged from 6.1 to 45.9 ppm and from 1.2 mg/kg to 9.2 mg/kg when compared with Cosmetic Ingredients Review Expert Panel 2007 and German safe maximum permissible limit of lead in cosmetics, respectively.

Therefore, the level of concentration in the local market of cosmetic products in Malaysia is in below the detectable limit compared with Nigerian markets. This may be due to the high demand for beauty products especially the skin lightening types which are used widely in most African countries, especially by women. The metal absorption efficiency through the skin is also affecting by the application site of cosmetic product and some of these metals such Pb can cause different long-health effects, like cancer; hormonal disruptors and organs damage which is highly toxic (Pratchyapruit, 2007).

These studies show the importance of discovering the levels of concentration of heavy metal in daily used cosmetic samples. In other recent studies, higher levels of Pb have been detected in foundation cream by using FAAS which 7.8 to 32.9±1.4 µg/g that exceeded the USFDA maximum permissible concentration of 10 µg/g in cosmetics (Brandao *et al*., 2012).

4.3.3 Mercury (Hg)

Figure 4.3 shows that all brands analysed by this study on Hg concentrations are under the acceptable limits for heavy metals in cosmetics which is 1.0 ppm according to standard limit of heavy metals concentration by World Health Organization (WHO). Even though all the cream samples collected did not show any declaration on the packaging that they contain mercury or mercury complexes, but the concentration of Hg still existed in the samples even in small amount. The least concentration level of heavy metals is in cream C which is only 0.00491 ppm followed by Cream Q and Cream D with concentrations of 0.0071 ppm and 0.0743 ppm respectively. Meanwhile, the highest mean concentration of Hg is Cream N with 0.3124 ppm.

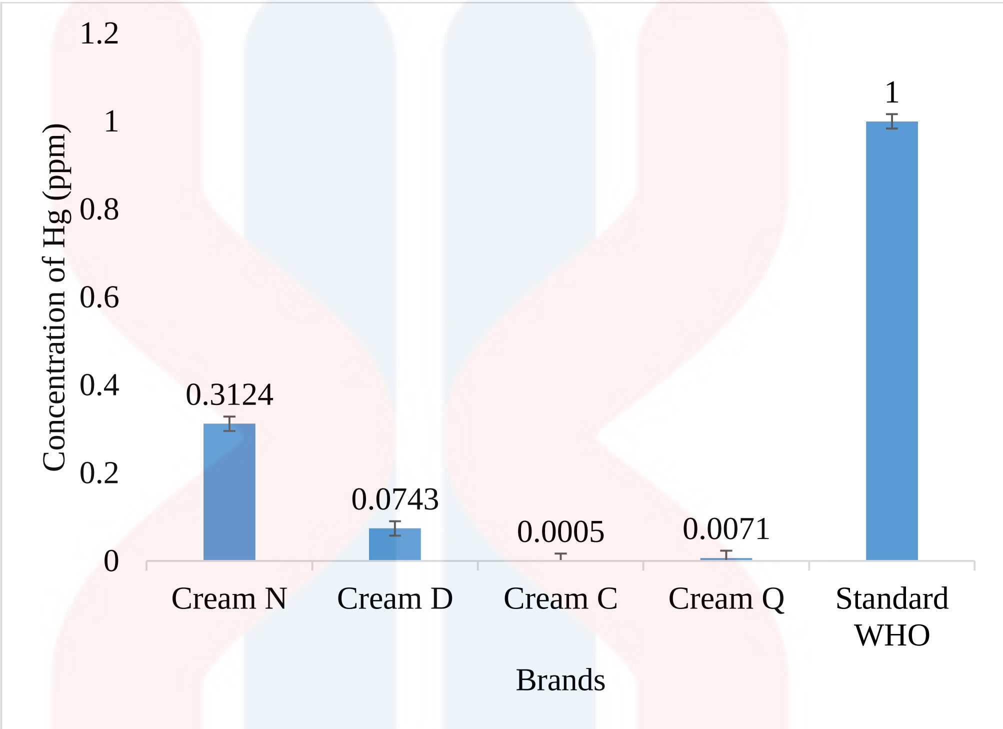


Figure 4.3: Concentration of Hg (ppm) in different types of local cream cosmetic products.

Mercury is a common element needed in a skin-lightening product. Even though in this research, mercury in all sample analysis from local cosmetic is safe and under the permissible limit, but the continually use of the products that contained mercury could be detrimental to the skin and even body (Palaniyandi, 2016). A study in Hong Kong by Soo *et al*. (2003) shows that a 34-year-old woman diagnosed with the neuropathic syndrome due to membranous neuropathy after continually been using a skin-lightening product containing 0.18% mercury for five years.

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 cream cosmetics obtained in Malaysia were all below the WHO recommended a limit of 1 ppm (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 the 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 that shows concentration of heavy metals ranging between 0.041 ppm to 2.183 ppm, the past findings show low level of Hg concentration agree that risks could resulted 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).

4.3.4 Zinc (Zn)

Figure 4.3 shows the concentration of Zn (ppm) in selected cosmetic samples. The Zn was detected in all cosmetic samples and the mean levels of concentrations are ranged from 2.863 to 292.6 ppm. Cream D had the highest of Zn content at 292.6 ppm which exceeded the permissible limit by WHO at 40 ppm. Cream D's turmeric night cream shows an extremely high level of Zn concentration. This may show that the type of turmeric used in cream cosmetic contributes to its heavy metal content (Bazargani and Pajohi, 2017).

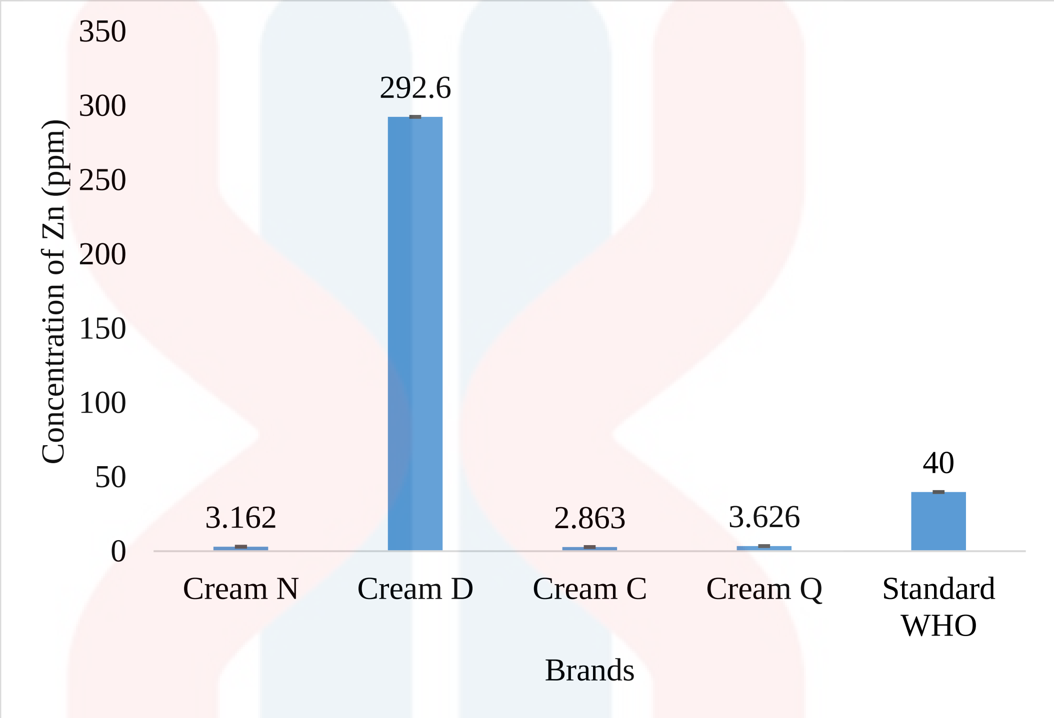


Figure 4.3: Concentration of Zn (ppm) in different types of local cream cosmetic products

The least of the concentration of Zn is in Cream C at 2.863 ppm followed by Cream N and Cream Q at 3.162 ppm and 3.626 ppm respectively. These studies show that Cream N, Cream C, and Cream Q cosmetic is safe to be used except for Cream D. Even though there is no permissible limit for Zn in cosmetics currently available but levels above 40 ppm may be toxic (Al-Weher, 2008).

Zinc oxide (ZnO) is an inorganic compound that in the form of white powder is frequently used as an ingredient in sunscreens or UV blocker. Recent studies in animal models show that of ZnO indicated that this compound is virtually non-toxic. However, it was reported that ZnO with nanoparticle size of 40 nm, lead to significant changes in anemia-related hematologic parameters and mild to moderate pancreatitis in male and female Sprague-Dawley rats at 536.8 mg/kg/day in a 13-week oral toxicity study. These shows that ZnO is non-carcinogenic and the ZnO penetration into the skin is low (Kim *et al*., 2017).

However, even though zinc toxicity in human is rare, but at concentrations of up to 40 ppm, it may induce toxicity characterized by irritability, muscular stiffness and pain (Al-Weher, 2008).

4.4 Comparison between the overall mean concentration of Heavy Metals in different local Cosmetic Products

The data that shows the overall mean concentration of heavy metals in different local cosmetic products in Malaysia is shown in Table 4.3. The pattern of heavy metals' level of concentration was analysed using the mean and standard deviation formula. Values in the tabulated shown is form as mean ± SD with ND as not detectable.

Table 4.3: Overall Mean Concentration of Heavy Metals from mean values (ppm)

Overall mean concentration from Mean (ppm) ± SD; (n=4)					
Heavy Metals	**Mean (ppm) ± SD**				**Overall mean concentration ± SD**
	Cream N	**Cream D**	**Cream Q**	**Cream C**	
Cd	0.0900±0.0014	0.010±0.0020	0.0100±0.0003	0.0050±0.0018	0.0288±0.0010
Pb	ND	ND	ND	ND	ND
Hg	0.0312±0.0012	0.0074±0.0003	0.0071±0.0001	0.0005±0.00006	0.012±0.0004
Zn	3.1620±0.0404	292.600±1.51	2.8630±0.0243	3.6260±0.0378	75.563±0.6391

Note: ND- not detected

Based on Table 4.3, it can be analysed that the pattern of heavy metals in different local cosmetic brands in Malaysia with overall mean concentration is Zn (75.563 ppm)> Cd (0.0288 ppm)> Hg (0.012 ppm)> Pb (not detectable (ND)). The highest mean concentration (ppm) of Zn was detected in Cream *D* (292.600±1.51), followed by cream C, Cream N and Cream Q with levels of Zn (3.6260±0.0378, 3.1620±0.0404 and 2.8630±0.0243) respectively. However, the mean concentration (ppm) of Pb was not detected is all cosmetic's samples. For Cd, the mean levels (ppm) were obtained in the range of 0.0050±0.0018 – 0.0900±0.0014. Meanwhile, Hg showed the highest of average concentration (ppm) in Cream N which is 0.0312±0.0012 followed by Cream Q, Cream D and Cream C with Hg levels at 0.0071±0.0001 ppm, 0.0074±0.0003 ppm and 0.0002±0.00006 ppm respectively.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research, the mean concentration of all heavy metals; Cd, Hg, Pb and Zn found in selected cosmetic samples; *Nurraysa Beauty* (Cream D), *Deeja Cosmetic* (Cream D), *Chriszen* (Cream C), and *Qu Puteh Beauty Care* (Cream Q) are within the permissible limit. However, the mean concentration of Zn found in Cream D is extremely high than the permissible limit proposed by WHO. The pattern of heavy metals in different local cosmetic brands in Malaysia with overall mean concentration was analysed as Zn (75.563 ppm) > Cd (0.0289 ppm) > Hg (0.012 ppm) > Pb (ND).

To be concluded, the concentration of all heavy metals that present in Malaysia cosmetic product is successfully measured by using Atomic Absorption Spectrometer (AAS). In addition, throughout this study, it can be concluded that all heavy metals were found in cosmetics as impurities in many cases, they were not added to them on purpose, in result the consumer does not find them on the product's labels (Ayenimo, 2010).

Bocca *et al*. (2014) concluded that the amounts of heavy metals in cosmetic products can be considered "technically avoidable" but many cosmetics did not match with the impurities legislation as this may leads to necessary to know the differences about the metal level between safe cosmetic product and technically avoidable. Expensive cosmetics are not necessary to be "safe" about the problem of heavy metals; the consumers must be notified about the general harmful effects of cosmetics, without any attention to the product's cost (Faruruwa and Bartholomew, 2014). Metal absorption efficiency through the skin is also affecting by the application site of cosmetic product (Pratchyapruit, 2007).

Some of these metals can cause different long- health effects, like cancer; hormonal disruptors and organs damage (highly toxic) like Cd, Hg, Pb, and Zn (Thyssen and Menñe, 2010). All these cosmetics must be nontoxic, nonirritant and safe because of the daily use by consumers who do not have a sufficient awareness about these products and their side effects on health (Amasa *et al*., 2012). These cosmetics applied to healthy skin (essentially the face), nail or hair but they caused allergy dermatitis when applied to damaged skin (White and Groot, 2001).

Women are putting cosmetics every day without knowledge on eyes, face, and lips, this might be small amounts but via the cosmetics yet the exposure is increased and accumulated and constantly use of them make these levels behind acceptable limits (Godt, 2006). This multiple using of cosmetics involving heavy metals considered as a supplementary source for toxic chemicals and metals (Borowska and Brozóska, 2015).

In this study, toxic heavy metals Cd, Hg and Zn were detected in all the investigated cosmetic products. However, this the cosmetic products do not exceed the maximum permissible limit values for heavy metals content in cosmetic products except for Zn (292.6 ppm) that exceed 40 ppm. However, Cd is prohibited in any amount in cosmetics. The presence of heavy metals in cosmetics can cause serious problems to the consumer as they can cause premature aging of the skin, skin allergies, and skin cancer.

Further, toxic metals have a role to set up conditions that lead to inflammation in arteries and tissues, results in osteoporosis (Omolaoye, 2010). Thus, there is an urgent need for constant quality assessment of cosmetic products in the market in order to ensure the safety of consumers. To achieve this, regulatory bodies and the government sector should implement the stringent policies to regulate and monitor the standards of herbal products manufactured, advertised, sold, and used. At the same time, the scientific community should develop simple and convenient analytical methods.

5.2 Recommendation

In this research, the concentration of Pb is negative because maybe it is absent in the samples or may due to the amount of Pb in the samples is below the detection level. It may due to the instruments used to detect Pb is not suitable. There are other techniques or instruments that are more effective to analyze and trace the amount of heavy metals in cosmetic samples. The lower Pb concentration achieved in this research, acquire more effective instrument such as Inductive Coupled Plasma Mass Spectroscopy (ICP-MS) which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{15} ppq on non-interfered low-background isotopes and able to produce an intense emission for almost all elements. This ICP-MS is highly recommended to be employ as it can produce a high productivity and even at a very low detection limits (John, 1991).

Other than AAS, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), and X-ray Fluorescence Spectroscopy (XFS) methods also can be employed especially for lower concentration in the sample. However, these ICP-MS, ICP-AES, and

XFS are usually costlier, and need more training to handle and the maintenance and operation is far more complicated than AAS. In this study, a simple, reliable, sensitive and convenient AAS method has been developed for quantitative estimation of trace metals and heavy metals which can conveniently be utilized for the quality control of herbal cosmetic preparations at the industrial level (Hamid, 2011).

REFERENCES

Adekunle, A. S., Oyekunle, J. A. O., Baruwa, S. O., Ogunfowokan, A. O., and Ebenso, E. E. (2014). Speciation study of the heavy metals in commercially available recharge cards coatings in Nigeria and the health implication. *Toxicology Reports*, *1*, 243–251.

Al-Ashban, R.M., Barratt, D.A. and Shah, A.H. (2006) Mercury Contents of Skin-Lightening Creams Marketed in Saudi Arabia. Journal of Saudi Chemical Society, 10, 383-388.

Al-Weher, M. (2008). Levels of heavy metals Cd, Cu, and Zn in three fish species collected from the Northern Jordan Valley. *Jordan Journal of Biological Sciences*; 6: 47-52.

Amasa, W., Santigo, D., Mekonen, S. and Ambelu, A. (2012). Are cosmetics used in developing countries safe? Use and dermal irritation of body care in Jimma town, southwestern Ethiopia. Journal of Toxicology: 8pp.

AOAC International. (2002). AOAC Official Method 999.10, Lead, Cadmium, Zinc and Iron in Foods Atomic Absorption Spectrometry after microwave digestion, AOAC, International.

Ayenimo, J. G., Yusuf, A. M. and Adekunle, A. S. (2010). Heavy metal exposure from personal care products. *Bulletin of Environmental Contamination and Toxicology;* 84**:** 8-14.

Balarastaghi, S., Zahra, K., Pouran, M., Seyedeh, H. A., Zohreh, S. M., Kobra, S. and Gholamreza, K. (2018). Determination of toxic element levels (lead and cadmium) in commonly used cosmetic products in Iran, Toxin Reviews, 37:2, 117-122.

Banfalvi, G. (2011). Cellular effects of heavy metals. Springer UK. Pp. 6-13.

Bazargani, G. B and Pajohi, A. M. (2017). Evaluating of Heavy Metal Contaminations in the Most Applicable Food Spices and Flavors in Hamedan City. Arch Hyg Sci 2017;6(3):268-275.

Bocca, B., Pino, A., Alimonti, A. and Forte, G. (2014). Toxic metals contained in cosmetics: a status report. Regulatory and pharmacology, 68: 447- 467.

Borowska, S. and Brozóska, M. M. (2015). Metals in cosmetics: implications for human health. J. Appl. Toxicol. 35: 551- 572.

Brandao, J, Okonkwo, O., Sehkula, M. and Raseleka, R. (2012). Concentrations of lead in cosmetics commonly used in South Africa. Toxicol Environ Chem 94:70–7.

Butler, H. and Poucher, W. A. (2000). Poucher's perfumes, cosmetics, and soaps. Kluwer Academic Publishers, Dordrecht, Netherlands. Pp. 484.

Centers for Disease Control and Prevention (CDC), 2003. Second National Report on

Human Exposure to Environmental Chemicals. Retrieved at http://www.cdc.gov/exposurereport/2nd.

Chauhan, A. S., Bhadauria, R., Singh, A. K., Lodhi, S. S., Chaturvedi, D. K. and Tomar, V. S. (2010). Determination of lead and cadmium in cosmetic products. *Journal of Chemical and Pharmaceutical Research*; 2**:** 92-97.

Deshpande, (2005). Handbook of Food Toxicology. *Taylor and Francis e-Library, UK*. Pp.179.

Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N. (2007). Heavy Metal Pollution and Human Biotoxical Effects. *International Journal of Physical Sciences*. 2. 112 - 118.

Elinder, C.-G. (1985). Normal values for cadmium in human tissues, blood, and urine in different countries. *Cadmium and Health: A Toxicological and Epidemiological Appraisal;* 1: 81-102. and human biotic effects. *International Journal of Physical Sciences*; 2: 112-118.

Faruruwa, M. D. and Bartholomew, S. P. (2014). Study of heavy metals content in facial cosmetics obtained from open markets and superstores within Kaduna metropolis, Nigeria. *American Journal of Chemistry and Application*, 1(2): 27- 33.

Fasanya-Odewumi, C., Latinwo, L. M., and Ikediobi, C. O. (1998). The genotoxicity and cytotoxicity of dermally-administered cadmium: effects of dermal cadmium administration. *International Journal of Molecular Medicine*. 1:1001–6

Gentscheva, G. D., Stafilov, T. and Ivanova, E. H. (2010). Determination of some essential and toxic elements in herbs from Bulgaria and Macedonia using Atomic Spectrometry. *Eurasian Journal of Analytical Chemistry;* 5:104-111.

Godt, F. S. J., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A. and Groneberg, D. A. (2006) The toxicity of cadmium and resulting hazards for human health, *Journal of Occupational Medicine and Toxicology*. 1, 1–6, 2006.

Gondal, M. A., Dastageer, M. A., Naqvi, A. A., Isab, A. A., and Maganda, Y. W. (2012). Detection of toxic metals (lead and chromium) in talcum powder using laser-induced breakdown spectroscopy. Applied Optics, 51(30), 7395.

Gondal, M. A., Seddigi, Z. S., Nasir, M. M. and Gondal, B. (2009) Spectroscopic detection of health hazardous contaminant in lipstick using Laser induce breakdown Spectroscopy. Journal of Hazardous Materials. 2010;175(1):726–732

Guy, R., Hostynek, J. J., Hinz, R. S. and Lorence, C. R., (1999). Metals and the skin: Topical effects and systemic absorption. New York: CRC Press, Marcel Dekker.

Hamid, G. A (2011). Comparison Between Inductively Coupled Plasma ICP and Atomic Absorption [Powerpoint Slides]. Retrieved from https://www.slideshare.net/GamalAbdulHamid/icp-aas-comparison-74892920.

Hatch, W. R. and Welland L. O. (1968). Determination of Submicrogram Quantities of Mercury by Atomic Absorption Spectrophotometry. *Analytical Chemistry*. 40(14): p. 2085-2087.

Hineman, A. (2011). Determination of As, Se and Hg in Waters by Hydride Generation/ Cold Vapor Atomic Absorption Spectroscopy. Application Note Atomic Absorption, 009831A (01).

International Programme on Chemical Safety, IPCS (2003). Elemental mercury and inorganic mercury compounds: human health aspects. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document 50; http://www.who.int/entity/ipcs/publications/cicad/en/cicad50.pdf

John, W. O. (1991). Elemental analysis using ICP-OES and ICP/MS. *Analytical Chemistry*. 63 (1), 12A-21A.

Kasture, P. V. (2008). Practical Pharmaceutics-1. Nirali Prakashan, India. Pp. 30.

Kim, K. B., Kim, Y. W., Lim, S. K., Roh, T. H., Bang, D. Y., Choi, S. M. and Lee, B. M. (2017). Risk assessment of zinc oxide, a cosmetic ingredient used as a UV filter of sunscreens. *Journal of Toxicology and Environmental Health*, Part B, 20(3), 155–182.

Ladizinski B, Mistry, N. and Kundu, R. V. (2011). Widespread use of toxic skin lightening compounds: medical and psychosocial aspects. Dermatologic Clinics, 29:111–123.

Linnila, P. M. (2000). Zinc, lead and cadmium speciation in deeper water bodies. *Lakes and Reservoirs: Research and Management*; 5: 261-270.

Lloyd, D. D. (2000). AAS Mercury Cold Vapor and Hydride Generation Atomic Absorption: Mercury and Arsenic, *Chem. Dept, The University of the West Indies, St. Augustine campus The Republic of Trinidad and Tobago*.

Massadeh, A. M., El-khateeb, M. Y., and Ibrahim, S. M. (2017). Evaluation of Cd, Cr, Cu, Ni, and Pb in selected cosmetic products from Jordanian, Sudanese, and Syrian markets. *Public Health*, *149*, 130–137.

Matusiewicz, H. and M. Krawczyk, Determination of Total Mercury by Vapor Generation in Situ Trapping Flame Atomic Absorption Spectrometry. Chem. Anal. (Warsaw), 2008. 53: p. 905-925.

Mester, Z. and Sturgeon, R. (2003). Sample preparation for trace element analysis. *Elsevier*, Netherlands. Pp. 212-213.

Miculescu, F., Miculescu, M., Ciocan, L., Ernuteanu, A., Antoniac, I., Pencea, I. and Matei, E. (2011). Comparative studies regarding heavy elements concentration in human cortical bone. *Digest Journal of Nanomaterials and Biostructures*; 6: 1117-1127.

Mireji, P. O., Keating, J., Hassanali, A., Mbogo, C. M., Nyambaka, H., Kahindi, S., and Beie, J. C. (2007). Heavy metals on mosquito larval habitat in urban Kisumu and Malindi, Kenya, and their impact. *Toxicology and Environmental safety*; 10: 101.

Morais, S., and Garcia, F. (2010). Heavy Metals and Human Health. Environmental Health Emerging Issues and Practice.

Muhammed N. A. A. and Hamsa, T. A. A. (2017). Detection of Cadmium and Chromium in some Facial Cosmetics. *Iraqi Journal of Science*, Vol. 58, No.1C, pp: 408-416.

Neetu, B., James, V., Amiel, B., and Tony. L. (2010). The Determination of Trace Mercury in Environmental Samples: A Review. *Rio Tinto Alcan* Technology QRDC. Queensland Alumina Limited, Australia.

Nnorom, I. C., Igwe, J. C. and Oji-Nnorom, C. G. (2005). Trace metal contents of facial (Make-up) cosmetics commonly used in Nigeria. *African Journal of Biotechnology*; 4**:** 1133-1138.

Norudin, M., Desnika, M. A. and Rafi, M. (2010). Cosmetic usage in Malaysia, understanding the major determinants affecting the users. *International Journal of Business and Science*, *1*(3), 273.

Nourmoradi, H., Muhamad, F. and Mohamad, V. D. (2013). Assessment of Lead and Cadmium Levels in Frequently Used Cosmetic Products in Iran. Retrieved December 5, 2018, from https://www.hindawi.com/journals/jeph/2013/962727/citations/

Null, G. and Mc Donald, A. (2006). Get healthy now: A complete guide to prevention. pp 65.

Omolaoye, J. A., Uzairu, A. and Gimba, C. E. (2010). Heavy metal assessment of some eyeshadow products imported to Nigeria from China. *Archives of Applied Science Research*; 2: 76-84.

Onyambu, M. Z. (2014). *Levels of Selected of Heavy Metals in Aloe Vera Branded Skin Cosmetic. A thesis submitted in partial fulfillment of the requirements for the awards of the degree of Master of Science (Applied Analytical Chemistry).* In the School of Pure and Applied Science of Kenyatta University.

Orisakwe, O. E. and Otaraku, J. O. (2013). Metal Concentrations in Cosmetics Commonly Used in Nigeria; *The Scientific World Journal of Chemical Technology*, 14(1), 50–56, 2012. Volume 2013, Article ID 959637, 7 pages. Hindawi Publishing Corporation.

Palaniyandi, S. (2016). *Determining mercury and selenium in vitro bioaccessibility in country foods collected from Nunavik, Québec (Degree dissertation).* A thesis presented to the University of Waterloo in fulfillment of the thesis requirement for the degree of Master of Science in Public Health and Health Systems.

Paul, T. (2008). "Mercury in your eye?". Cidpusa organization. Archived from the original on 5 January 2010. Retrieved 5 December 2018 at http://www.cidpusa.org/mercury.htm.

Peregrino, C. P., Moreno, M. V., Miranda, S. V., Rubio, A. D., and Leal, L. O. (2011). Mercury levels in locally manufactured Mexican skin lightening creams. *International Journal of Environmental Research and Public Health*; 8: 2516-2523.

Pfeil, P. (2011). Measurement Techniques for Mercury: Which Approach Is Right for You? Spectroscopy. Volume 26, Issue 9. Retrieved December 2, 2018, from http://www.spectroscopyonline.com.

Pratchyapruit, W., Kikuchi, K., Gritiyarangasan, P., Aiba, S. and Tagami, H. 2007. Functional analysis of the eyelid akin constituting the softest and smooth area on the face: contribution of its remarkably large superficial coenocytes to the effects of water- holding capacity of the stratum corneum. TOC. 13(2): 169- 175.

Sanders, T., Liu, Y., Buchner, V. and Tchounwou, P. B. (2009). Neurotoxic Effects and Biomarkers of Lead Exposure: A Review. *Reviews on Environmental Health*, 24(1), 15–45.

Sin, K. W. and Tsang H. F. (2003). Large-scale mercury exposure due to a cream cosmetic: community-wide case series. *Hong Kong Medical Journal*; 9: 329-334.

Skoog, D. A. and Leary, J. (1992). Principles of instrumental analysis, 4th edition. *Saunders publications*, Fort Worth. Pp 162-163.

Soo, Y. O. Y., Chow, K. M. and Lam, C. W. K. (2003). A Whitened Face Woman with Nephrotic Syndrome. *American Journal of Kidney Diseases* 41 (1): 250-3.

Sukenda, K., Jaspreet S., Sneha, D. and Munish, A. (2012). AAS estimation of heavy metals and trace elements in Indian herbal cosmetic preparations. *Research Journal of Chemical Sciences;* 2**:** 46-51.

Taylor, A., Branch, S., Day, M. and White, M. (2006). Atomic spectrometry update. *Journal of Analytical Atomic Spectrometry*; 21: 114-121.

Tchounwou, P. B, Sanders, T., Liu, Y., Buchner, V. and (2009). Neurotoxic Effects and Biomarkers of Lead Exposure: A Review. *Reviews on Environmental Health*, 24(1), 15–45.

Thyssen, J. and Menñe, T. (2010). Metal allergy to exposures, penetration, genetics, prevalence, and clinical implications. *Chemical Research in Toxicology*. 23(2): 309- 318.

Ullah, H., Noreen, S., Fozia, Rehman, A., Waseem, A., Zubair, S. and Ahmad, I. (2017). Comparative study of heavy metals content in cosmetic products of different countries marketed in Khyber Pakhtunkhwa, Pakistan. *Arabian Journal of Chemistry*.

United States Environment Protection Agency (USEPA). 2013. Survey of Cadmium and Cadmium compounds. Environmental project No. 1471. Washington. DC.

White, I. R. and Groot, D. (2001). Cosmetics and skin care products. In: Contact Dermatitis. Rycroft, R. J. G.; Menne, T.; Frosch, M. P. and Lepoittevin, J. P. (eds.) 3rd ed. Springer- Verlag Berlin in Heidelberg. New York. pp: 394- 509.

WHO (1995). Environmental health criteria. *International programme on chemical safety*. Geneva. World Health Organization. Pp 165.

WHO (2010). Chromium, zinc, lead, in drinking water. *Background document for preparation of WHO Guidelines for drinking-water quality*, Geneva, World Health Organization (WHO/SDE/WSH/03.04/4).

Zachariadis. G., and Sahanidou, E. (2009). Multi-element method for determination of trace elements in sunscreens by ICP-AES. *Journal of Pharmaceutical and Biomedical Analysis*. 50, 342.

APPENDIX A

A. Data for Heavy Metals (Cd, P, Hg and Zn) using AAS

i) Data for Cd obtained from AAS analysis.

Cosmetic brands	Types	Reading 1 (ppm)	Reading 2 (ppm)	Reading 3 (ppm)	Mean (ppm)	SD (ppm)
Nurraysa Beauty	UV cream	0.010	0.010	0.008	0.090	0.0014
Deeja Cosmetic	Turmeric night cream	0.008	0.011	0.011	0.010	0.0020
Qu Puteh Beauty Care	Cream foundation	0.010	0.010	0.010	0.010	0.0003
Chriszen	Moist foundation	0.004	0.007	0.004	0.005	0.0018

ii) Data for Pb obtained from AAS analysis.

Cosmetic brands	Types	Reading 1 (ppm)	Reading 2 (ppm)	Reading 3 (ppm)	Mean (ppm)	SD (ppm)
Nurraysa Beauty	UV cream	-0.252	-0.200	-0.219	-0.224	0.0261
Deeja Cosmetic	Turmeric night cream	-0.200	-0.145	-0.117	-0.154	0.0427
Qu Puteh Beauty Care	Cream foundation	-0.267	-0.220	-0.206	-0.231	0.0320
Chriszen	Moist foundation	-0.290	-0.203	-0.237	-0.243	0.0436

iii) Data for Hg obtained from AAS analysis.

Cosmetic brands	**Types**	**Reading 1 (ppm)**	**Reading 2 (ppm)**	**Reading 3 (ppm)**	**Mean (ppm)**	**SD (ppm)**
Nurraysa Beauty	UV cream	32.49	31.05	30.18	31.24	1.166
Deeja Cosmetic	Turmeric night cream	0.786	0.730	0.713	0.743	0.0380
Qu Puteh Beauty Care	Cream foundation	7.083	6.971	7.187	7.080	0.1080
Chriszen	Moist foundation	0.426	0.543	0.505	0.491	0.0599

iv) Data result of Zn from AAS analysis.

Cosmetic brands	**Types**	**Reading 1 (ppm)**	**Reading 2 (ppm)**	**Reading 3 (ppm)**	**Mean (ppm)**	**SD (ppm)**
Nurraysa Beauty	UV cream	3.118	3.197	3.172	3.1620	0.0404
Deeja Cosmetic	Day cream	291.1	292.7	294.1	292.60	1.5100
Qu Puteh Beauty Care	Cream foundation	2.867	2.837	2.886	2.8630	0.0243
Chriszen	Moist foundation	3.614	3.669	3.596	3.6260	0.0378

APPENDIX B

B. Calculation of Preparing 1M of Nitric Acid (HNO_3)

Preparing 1.00 M concentration of nitric acid (HNO_3);

$$M = \frac{1000 \times Density \times Percentage\ of\ Purity}{Molar\ Mass}$$

$$M = \frac{1000 \times 1.51\ g/cm^3 \times 0.63}{63.01\ g/mol}$$

M = 15.1 M

By using equation $M_1V_1 = M_2V_2$;

(15.1) (V_1) = (1M) (100mL)

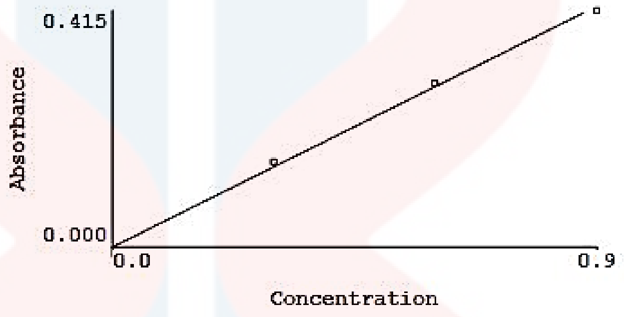
$$V_1 = \frac{100\ mL}{15.1\ M}$$

V = 6.6225 mL of HNO_3 need to be added.

APPENDIX C

C. Standard calibration curve for Cd, Pb, Hg and Zn

(i) Cadmium (Cd)

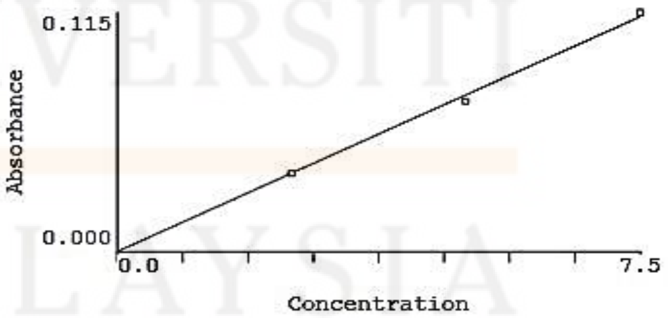


Calibration data for Cd 228.80 — Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	0.00
Calib Std 1	0.1491	0.3	0.318	0.00	0.19
Calib Std 2	0.2874	0.6	0.613	0.00	0.46
Calib Std 3	0.4146	0.9	0.884	0.00	0.62

Correlation Coef.: 0.997957 Slope: 0.46876 Intercept: 0.00000

(ii) Lead (Pb)

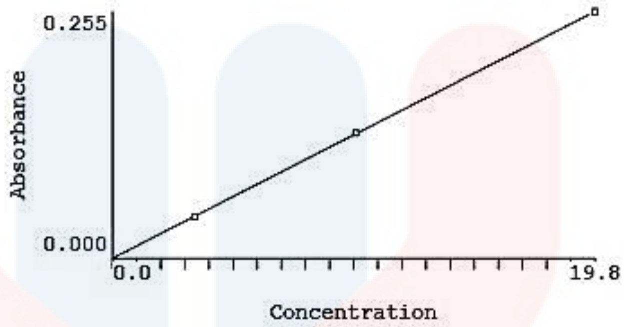


Calibration data for Pb 217.00 — Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	22.44
Calib Std 1	0.0374	2.5	2.484	0.00	0.63
Calib Std 2	0.0722	5.0	4.796	0.00	0.44
Calib Std 3	0.1150	7.5	7.634	0.00	0.90

Correlation Coef.: 0.997606 Slope: 0.01506 Intercept: 0.00000

(iii) Mercury (Hg)

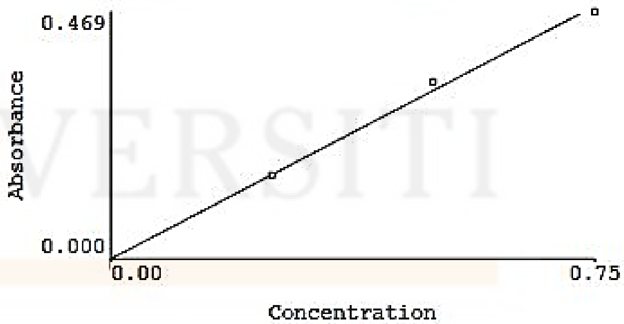


Calibration data for Hg 253.65 Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. ug/L	Calculated Conc. ug/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.0427	3.4	3.311	0.00	0.10
Calib Std 2	0.1295	10.0	10.041	0.00	0.36
Calib Std 3	0.2552	19.8	19.794	0.00	0.37

Correlation Coef.: 0.999965 Slope: 0.01289 Intercept: 0.00000

(iv) Zinc (Zn)



Calibration data for Zn 213.86 Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1588	0.25	0.248	0.00	0.28
Calib Std 2	0.3354	0.50	0.524	0.00	0.74
Calib Std 3	0.4691	0.75	0.733	0.00	0.94

Correlation Coef.: 0.996488 Slope: 0.63978 Intercept: 0.00000