

Faculty of Sciences
Department of Chemistry and Forensic Sciences

**DETERMINATION OF HEAVY METALS AND HYDROQUINONE IN SKIN-
LIGHTENING PRODUCTS SOLD IN BOTSWANA**

by

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SUMMARY OF THE CONTENT

This research study investigated the contamination of some heavy metals and hydroquinone in skin lightening products that are commonly used in Botswana. Skin lightening product is any substance that is applied to the human body to cleanse, beautify and promote attractiveness. Many skin lightening products contain heavy metals such as lead, arsenic, chromium and nickel as ingredients or impurities. Hydroquinone is found as an active ingredient in skin lightening products due to its ability to stop the production of melanin in the human body leading to a fair skin tone. The presence of heavy metals and hydroquinone is very unsafe for human beings as they are toxic. It is reported that they can cause skin allergies, kidney damage, liver damage and some are carcinogenic to humans. Pregnant women are very vulnerable as lead can pass through the placenta to the fetus hence the government needs to regulate the sale of skin lightening products that contain heavy metals and hydroquinone. Currently, there exists no legislation in Botswana that regulates the use of skin lightening products sold in the Botswana market, let alone standards that set limits on the amount of toxic metals and hydroquinone found in these products. This study, being the first to be carried out in Botswana, will provide baseline data on the prevalence of counterfeit skin lightening products sold in Botswana markets and the toxic contents contained in the products.

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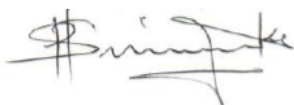
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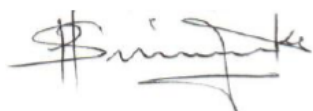
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Date: 28/02/2022

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TABLE OF CONTENTS

SUMMARY OF THE CONTENT	i
DECLARATION REGARDING THE WORK AND COPYRIGHT	ii
ANTI-PLAGIARISM REPORT	iii
CERTIFICATION BY SUPERVISOR	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	ix
LIST OF FIGURES	x
ACRONYMS	xi
Chapter 1. Introduction	1
1.1. Hydroquinone	1
1.1.1. Physical and Chemical properties of Hydroquinone.....	2
1.1.2. Natural Sources of Hydroquinone.....	2
1.1.3. Anthropogenic Sources of Hydroquinone.....	3
1.1.4. Health Effects of Hydroquinone on Human Beings.....	3
1.1.5. Hydroquinone in Skin Lightening Products.....	4
1.2. Heavy Metals Found in Skin Lightening Products	5
1.2.1. Lead (Pb).....	5
1.2.1.1. Anthropogenic Sources of Pb.....	5
1.2.1.2. Human health effects of Pb	6
1.3.2.0. Chromium (Cr).....	7
1.3.2.1. Anthropogenic Sources of Chromium	7
1.3.2.2. Human health effects of chromium.....	8
1.3.3. Nickel	9
1.3.3.1. Anthropogenic sources of Nickel.....	9
1.3.3.2. Human health Effects of Nickel	10

1.3.4.0. Arsenic (As)	10
1.3.4.1. Anthropogenic sources of Arsenic	10
1.3.4.2. Human health effects of Arsenic	11
1.4. Assessment of occurrence of restricted limits of hydroquinone and heavy metals in cosmetics.....	11
1.4. Problem Statement	12
1.5. Objectives of the study.....	12
Chapter 2. Literature Review	13
2.1. The Human Skin	14
2.1.1 The Structure of the Skin	15
2.2. Skin Bleaching Agents.....	17
2.2.1. Hydroquinone.....	17
2.2.2. Heavy metals on skin lightening products	18
2.3.0. Analytical Techniques Used for Analysis of Skin Lightening Agents	22
2.3.1. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).....	22
2.3.2. High Performance Liquid Chromatography (HPLC).....	23
Chapter 3. Methodology	25
3.1. Glassware	25
3.2. Chemicals and Reagents.....	25
3.3. Equipment's and Analytical Instruments	25
3.4. Sampling.....	25
3.5. Screening of samples using XRF for heavy metals.....	27
3.6. Digestion of Samples For determination of heavy metals	28
3.7. Extraction of Hydroquinone.....	28
3.8. Inductively Coupled Plasma Optical Emission Spectroscopy	29
3.9. Preparation of Hydroquinone standard solutions	30
3.10. High Performance Liquid Chromatography.....	30
3.11. Health Risk Assessment.....	31

Chapter 4. Results and Discussion.....	32
4.1.0 Method Validation for ICP-OES.....	32
4.1.1. Linearity	32
4.1.2. Accuracy.....	33
4.1.3. Precision	34
4.1.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)	34
4.2. Method Validation for HPLC	34
4.3. Heavy metals in cosmetics	35
4.3.1 Health Risk Assessment.....	39
4.4. Determination of Hydroquinone Content.....	42
Conclusion	44
References.....	45

LIST OF TABLES

Table 1. Examples of medicinal products that contain hydroquinone [9].	3
Table 2. Lead Compounds and their use[30]	6
Table 3. Chromium compounds and their uses[43]	8
Table 4. List of studies that have been carried out for Hydroquinone in skin lightening products	18
Table 5. Acceptable levels of heavy metals in cosmetics	21
Table 6. List of studies carried out for heavy metals in skin lightening products	21
Table 7. The origin and colour of skin lightening products sold in Botswana market	27
Table 8. Recovery studies for analyzed heavy metals	34
Table 9. Levels of heavy metals in skin lightening products sold in Botswana market	38
Table 10. Health risk assessment for Pb	39
Table 11. Health risk assessment for As	40
Table 12. Health risk assessment for Ni	41
Table 13. Health risk assessment for Cr	42
Table 14. Levels of Hydroquinone in cosmetic samples	43

LIST OF FIGURES

Figure 1. Hydroquinone	1
Figure 2. Skin pigmentation due to the use of hydroquinone [19]	4
Figure 3. Symptoms of lead poisoning in adults[29]	7
Figure 4. Effects of arsenic on the human body [74].....	11
Figure 5. The structure of the human skin [95].....	15
Figure 6. Melanogenesis process (Adapted from T.S Chang et al. (2012))	16
Figure 7. skin problem due to exposure of hexavalent chromium [126]	20
Figure 8. Schematic diagram of ICP OES	23
Figure 9. Schematic diagram of HPLC [146]	24
Figure 10. Skin lightening products.....	26
Figure 11. X-Ray Fluorescence analyzer.....	28
Figure 12. ICP OES	29
Figure 13. HPLC	30
Figure 14. Calibration curve graph for Pb	32
Figure 15. Calibration curve graph for Ni.....	32
Figure 16. Calibration curve graph for Cr.....	33
Figure 17. Calibration curve graph for As	33
Figure 18. Calibration curve for Hydroquinone.....	35
Figure 19. Concentration of Pb on cosmetic samples.....	36
Figure 20. Concentration of Ni on cosmetic samples	37
Figure 21. Concentrations of Cr on cosmetic samples.....	37
Figure 22. Concentration of As on cosmetic samples.....	38
Figure 23. Chromatogram of hydroquinone	43

ACRONYMS

HPLC- High performance liquid chromatography

ICP OES- Inductively Coupled Plasma-Optical Emission Spectrometer

US FDA- United States Food and Drug Administration

XRF- X-ray Fluorescence

MCL- Maximum Contaminant Limit

IARC-International Agency for Research on Cancer

ABSTRACT

The use of skin lightening products is a common practice among some groups of people around the world who perceive fair skin color as a feature of superiority and beauty. The use of these products poses a health hazard to people who use them. Cosmetic samples were acid-digested in a microwave digester using USEPA method 301 before analysis for Pb, As, Cr and Ni using inductively coupled plasma optical spectrometer (ICP-OES). Method validations were performed using accuracy, precision, linearity, limits of detection (LOD) and quantification (LOQ). Percent recoveries of 96-105% were achieved and %RSD for both inter-day and intra-day precisions were less than 2%. The LOD and LOQ for the analyzed metals were 0.0063 mg/kg and 0.0209 mg/kg for Ni, 0.0256 mg/kg and 0.0853 mg/kg for Pb, 0.0932 mg/kg and 0.3105 mg/kg for As and 0.0015 mg/kg and 0.0050 mg/kg for Cr respectively. The concentrations for Pb, Ni, As and Cr were 45.75 ± 0.10 mg/kg- 193.60 ± 3.58 mg/kg, 2.99 ± 0.09 mg/kg- 9.50 ± 0.45 mg/kg, 1.95 ± 0.23 mg/kg- 4.52 ± 0.22 mg/kg, and 3.32 ± 0.05 mg/kg- 7.41 ± 0.14 mg/kg respectively. Concentrations of arsenic were below 10 mg/kg. It is worth noting that the concentrations of Pb, Cr, and Ni were above the US FDA set limits of 10 mg/kg, 5 mg/kg and 5 mg/kg respectively for some of the analyzed samples. Health risk assessment was performed for heavy metals and the computed ADI concentrations for Pb, As, Cr and Ni were found to be 0.85, 0.024, 0.034 and 0.047 respectively. The hazard quotient for Pb, 64.3 % and 35.7% of samples with Ni and Cr respectively were greater than 1. The health risk index was greater than 1 for Pb, As and Ni while for Cr it was less than 1. Pb recorded the highest value of DDE than other heavy metals. This indicates that there is a potential adverse health effect.

Sixteen cosmetic products were also analyzed for hydroquinone using high-performance liquid chromatography (HPLC) equipped with ODS C18 column (4.6*250 mm, 5 μ m, the flow rate of 1.0mL/min, DAD detector(λ =292nm) and injection volume of 20 μ L. Hydroquinone was detected in four samples and the concentrations ranged between 0.067-7.65%. Out of sixteen cosmetic products analyzed only two contained hydroquinone above the set limit by the US FDA of 2 %. The occurrence of elevated concentrations of heavy metals and hydroquinone in cosmetics products sold in the Botswana market should be strictly controlled and legislative measures should be instituted that prohibit the importation of such products.

Chapter 1. Introduction

The pursuit of physical beauty by men and women has increased the use of cosmetics around the world. In particular, the use of cosmetics products, especially skin lightening cosmetics has been found to be a common practice in Africa, Asia, Middle East and Latin America countries. In the early years of cosmetics use, it was assumed that these products worked only on the surface of the skin and would not be absorbed through the skin. However, studies later established that some of the additives in these products can have systemic absorption through the skin which raised concerns about their safety.

1.1. Hydroquinone

Hydroquinone is an aromatic compound with the chemical formula $C_6H_4(OH)_2$ and a molecular mass of 110.11 g/mol. Hydroquinone is commonly derived from phenol through a catalytic hydroxylation process [1]. It is also known as benzohydroquinone, 1,4-dihydroxybenzene, quinol, 1,4-benzenediol, *p*-benzenediol, β -quinol and *p*-dioxobenzene. Hydroquinone occurs naturally as a conjugate with β -D-glucopyranoside in the barks, leaves and fruits such as blueberry, cranberry, bearberry, and cowberry. Hydroquinone and its glucose conjugate, arbutin is naturally present in most food and beverages. Arbutin hydrolyzes in dilute acidic solutions to give hydroquinone and D-glucose. Arbutin that is ingested is expected to be converted to free hydroquinone in the stomach. Hydroquinone has been detected in tea, coffee beans, milk, wines, orange juice and other fruits [2]. Hydroquinone can also be synthesized by oxidation of aniline to quinone and subsequent reduction of quinone to hydroquinone. Aniline is oxidized with sulfuric acid and manganese dioxide followed by reduction with water and iron dust. Other methods of synthesis of hydroquinone include oxidative cleavage of diisopropyl benzene. Figure 1 shows the structure of hydroquinone.

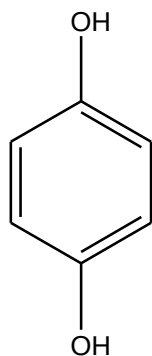


Figure 1. Hydroquinone

1.1.1. Physical and Chemical properties of Hydroquinone

Hydroquinone is an odorless, colorless crystal compound that turns brown when exposed to air due to oxidation. Hydroquinone is soluble in alcohol, water, acetone and ether and it is partially soluble in benzene at room temperature. However, hydroquinone is less soluble in water than the other two dihydroxy benzenes, implying that it has a lower affinity for hydrophilic solvents. The melting and boiling point of hydroquinone is 169-171°C and 287°C respectively [3]. Its density is 1.341 kg/m³ and its vapor pressure of 2.34×10⁻³Pa. Hydroquinone undergoes the same chemical reaction as phenols. Ring substitution can happen through sulfonation, nitration, halogenation, Friedel-Crafts acylation, alkylation and condensation with ketones or ethers. Hydroquinone also goes through oxidation in an aqueous solution and in the presence of oxygen at a rate depending on the pH of the solution. Hydroquinone can form complexes with different di- and trivalent metal ions like iron and copper. In terms of copper, the complex that is formed increases hydrogen peroxide production using hydroquinone and promotes autooxidation to benzoquinone [4].

1.1.2. Natural Sources of Hydroquinone

Hydroquinone is a naturally occurring chemical that may be found in a variety of foods (pears, beans, blueberries, broccoli, wheat and cranberries) [5]. Many marine and bacterial organisms produce it as a byproduct of metabolism. [6]. When hydroquinone is discharged into the environment, it is usually dispersed to the water compartment due to its chemical and physical characteristics. The solubility of hydroquinone in water increases as temperature rises. [7]. It is improbable that hydroquinone will be volatilized from water to air compartments. Hydroquinone in wastewater is easily biodegradable. Hydroquinone is likely to photodegrade and biodegrade in open bodies of water. Hydroquinone has a 20-hour half-life in water. Hydroquinone discharged into the soil is predicted to mineralize as organisms that are capable of degrading hydroquinone are usually found in soil and compost. The half-life of hydroquinone in soil ranges from 2 to 14 days, depending on bacterial breakdown and photooxidation. Hydroquinone in the soil can move to water and become mobile. Hydroquinone has a half-life of 4-14 days in the ground and up to a month under anaerobic conditions. Soil particles are electron donor and acceptor molecules hence they can form charge-transfer complexes with hydroquinone. It can aid in the synthesis of humic acids, which are byproducts of the polymerization of polyphenols generated during plant biodegradation. [7].

1.1.3. Anthropogenic Sources of Hydroquinone

Hydroquinone is produced industrially during benzene and phenol biotransformation. First, the benzene is metabolized by liver cytochrome P-450 monooxygenase to phenol followed by hydroxylation of phenol by cytochrome P-450 monooxygenase or with hydrogen peroxide which will result in the formation of principal hydroquinone [8]. A large amount of hydroquinone is generated during the production of cigarettes. In many countries, hydroquinone is manufactured industrially and used widely as a photographic developer, reducing agent, antioxidant for many oxidizable products, an inhibitor of stabilization and during polymerization in the presence of free radicals [9]. It is also used as a dye intermediate, as a stabilizer in motor fuel, varnishes oils and paints. Furthermore, It has been employed as an antioxidant in the food and rubber industries. Hydroquinone is also used in the production of skin lightening products and medical products as a depigmenting agent as shown in Table 1 [10]. It is also used in making plastic, black and white developers and coal tar production. Hydroquinone is the main developing agent in black and white developers [11]. Hydroquinone is released into the environment through many sources, some of which include solid castoff tar, industrial waste and paper mill [12].

Table 1. Examples of medicinal products that contain hydroquinone [13].

Product Name	Concentration of Hydroquinone (%)	Use
Admire my skin's ultra-potent brightening serum	2	• For uneven tone • To lighten dark spots
Murad Rapid Age spot and pigment lightening serum	2	• For hyperpigmentation
AMBI fade cream	2	• For discoloration

1.1.4. Health Effects of Hydroquinone on Human Beings

The toxicity of hydroquinone has been underestimated considering the results obtained from animal testing and evidence following application on human skin [14]. Hydroquinone can cause renal tubular cell tumors, cell leukemia and liver cancer [10]. Ingestion of a large amount of hydroquinone can cause dizziness, nausea, headache, vomiting, erosion of the gastric mucosa, edema of internal organs and collapse [15]. Hydroquinone also causes skin irritation

in humans as shown in figure 2. Exposure to hydroquinone dust also causes eye irritation, which can go from mild irritation to discoloration of the conjunctivae to changes in the thickness of the cornea, loss of corneal luster and diminished vision [16]. Human beings who consume water contaminated with hydroquinone are reported to have experienced abdominal cramps, diarrhea and nausea [17].



Figure 2. Skin pigmentation due to the use of hydroquinone [18]

1.1.5. Hydroquinone in Skin Lightening Products

Hydroquinone has been used as an active ingredient in skin treatment regimens for ages. Hydroquinone is a skin lightening agent that is applied topically. It is an active element in skin lightening cosmetics that are used to lighten the complexion or erase blotchy patches. [19]. Most of these bleaching products contain different kinds of harmful chemicals which affect people's health. Hydroquinone is a toxic compound but it is very useful in many skin lightening products preparations that are targeted at skin lightening by suppression of melanin production by the melanocytes in the skin [20]. Melanin is very important as it is responsible for skin pigmentation in human beings [20]. It is produced in the skin melanocytes that protect the skin from UV radiation but too much accumulation of melanin can cause cosmetic flaws such as freckles, solar lentigo, age spots and melasma [20]. Skin lightening products have been used to reduce the concentration of melanin in the skin [20].

Hydroquinone does not bleach melanin but it inhibits the production of melanin in the body [21]. Hydroquinone attaches to the histidines found on the active site of the enzyme tyrosinase resulting in a decrease in skin pigmentation in melanosis [22]. It also inhibits pigmentation by decreasing glutathione, which affects the synthesis of RNA and DNA, resulting in melanosome deterioration and melanocyte damage. It can induce persistent melanocyte damage because of oxidative damage of the membrane lipids, resulting in irreversible loss of skin pigmentation.

Furthermore, it penetrates the epidermis and enters the vascular system before being detoxified in the liver. [22].

1.2. Heavy Metals Found in Skin Lightening Products

Many skin lightening products contain heavy metals such as lead, chromium, arsenic and nickel. Some of these heavy metals are added as intentionally as ingredients while some are from the environment. The use of heavy metals in skin lightening products has been an issue due to the bioaccumulation of heavy metals and their toxicity to the human body.

1.2.1. Lead (Pb)

Lead is a heavy metal with the symbol Pb found in group 14 of the periodic table. Lead is a naturally occurring heavy metal found in the earth's crust mixed with other elements such as sulfur and oxygen. Elemental lead has atomic number 82 and mass number 207.19. It is a blue-gray, soft, ductile, corrosion-resistant and malleable metal. It constitutes about 15-20 mg/kg of the earth's crust [23]. The most important ores of lead are cerussite (PbCO_3), anglesite (PbSO_4) and galena (Pbs) [24].

1.2.1.1. Anthropogenic Sources of Pb

Lead concentrations found in the environment are a result of human activities. Anthropogenic sources of lead include mining, smelting, combustion of fossil fuels, industrial processes, and effluent from wastewater treatment [25]. Lead also enters the environment through car exhausts and larger particles will drop on the ground and pollute the surface of the water or the soil whereas smaller particles will remain in the atmosphere. Plants that are exposed to lead absorb the heavy metal through their leaves, they also take up small amounts of lead through their roots from the soil. Soils that are near smelting facilities have high concentrations of lead than soils in other areas due to their exposure to lead dust that accumulates over time [26]. Lead is combined with other metals to form alloys which are used in the production of weights in sports equipment, pipes, petrol, storage batteries, ammunition, cable covers, cosmetics, electrodes in the process of electrolysis, in candles to thread the wick and to make the glass used in television and computer screens [27]. The largest use of lead is in car batteries and in making bullets. It is widely used in bullets due to its low melting point which allows ammunition to be cast with minimal equipment. Lead is also denser than other metals, allowing for greater retention velocity in bullets. Compounds of lead are used as pigments in paints and ceramic glazes as shown in table 2. Tetraethyl and tetramethyl lead are used in the production of gasoline which is used in airplanes and off road-cars [28].

Table 2. Lead Compounds and their use[29]

Lead Compound	Use
Lead iodide (PbI_2)	Photography industry
Lead chromate (PbCrO_4)	To make paints
Lead oxide (PbO)	Production of crystal glass
Lead nitrate ($\text{Pb}(\text{NO}_3)_2$)	Making matches and special explosives
Lead arsenate (PbHAsO_4)	Insecticides and pesticides
Lead fluoride (PbF_2)	Lasers and optical glasses
Lead acetate ($(\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$)	Textile printing and dyeing

1.2.1.2. Human health effects of Pb

Even at low concentrations, lead is very dangerous to human beings, and high levels of lead are life-threatening [30]. Lead is very dangerous to children when they ingest and inhale it [29]. Children are exposed to lead through drinking water, food, lead-based paint, toys and ceramic glazes [30]. Adults are exposed to lead through the use of leaded gasoline, industrial processes like coal combustion and smelting of lead, lead pipes in water systems, cosmetics, metal cookware, medicine and lead-based paints[31]. Continual exposure to lead results in bioaccumulation of lead. When lead is taken into the body, it distributes throughout the body in the blood and accumulates in the bones [32]. The toxicity of lead comes from interference with the functions of the enzymes by binding to the sulfhydryl group [23]. It also replaces other metals which act as co-factors in enzymic activities. Some of the essential metals that interact with lead include zinc, iron and calcium [33]. Lead can cause several human health problems as shown in figure 3 and other effects include [23]:

- Rise in blood pressure
- Damage to the kidney and brain
- Decreased learning abilities in children

- Disruption to the nervous system
- Disruption of the biosynthesis of the hemoglobin

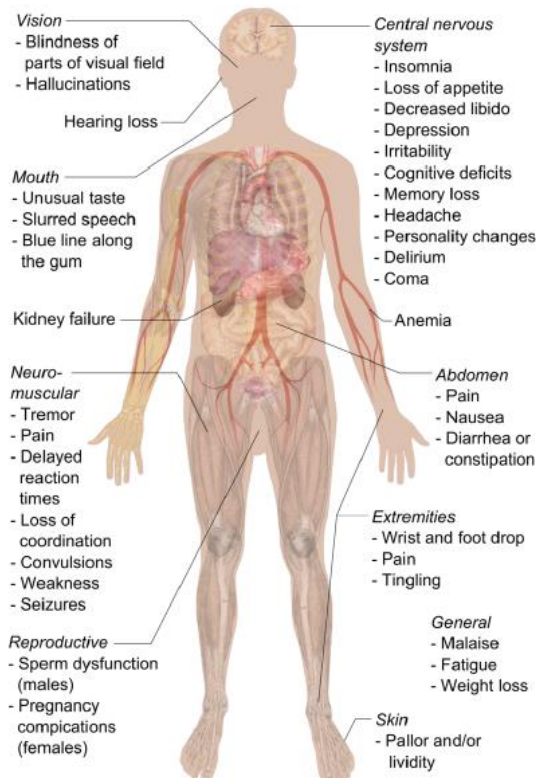


Figure 3. Symptoms of lead poisoning in adults[28]

1.3.2.0. Chromium (Cr)

Chromium is a chemical element with the symbol Cr found in group 6 of the periodic table. Chromium was discovered in 1797 by the French chemist Nicholas Louis Vauquelin [34]. It was named chromium due to its multi-colored compounds. The name chromium is derived from the Greek word “chroma” meaning color [35]. Chromium is the 21st most abundant heavy metal on the earth’s crust [36].

1.3.2.1. Anthropogenic Sources of Chromium

Human activities release large quantities of chromium into the atmosphere which amounts to about 60-70% of the total emission of atmospheric chromium [37]. Anthropogenic sources of chromium include leather tanning, electroplating and textile industries [38]. They release chromium into the atmosphere, land and surface waters. The main source of chromium in the soil is the disposal of commercial products that contain chromium and coal ash from electric utilities [39]. Chromium is mainly used in making steel and other alloys. It is added to steel to make it highly resistant to corrosion and discoloration. Chromium compounds are employed in the production of dyes and pigments, chrome plating, the preservation of wood and leather, and the treatment of cooling tower water [40]. It is also used in textiles, toners for printing machines

and drilling muds but in small amounts [41]. Other potential sources of atmospheric chromium emission include the production of cement, the wearing down of the asbestos brake lining, incineration of municipal refuse and sewage sludge, emission from the automotive catalytic converter that contain chromium, from cooling towers that used chromate chemicals as rust inhibitors [39]. Table 3 shows compounds of chromium and their uses.

Table 3. Chromium compounds and their uses[42]

Chromium Compounds	Use
Chromium sulfate ($\text{Cr}_2(\text{SO}_4)_3$)	• As a tanning agent in leather production • Brick lining • Copy machine toners
Chrome magnesite ($\text{MgO} \cdot \text{Cr}_2\text{O}_3$)	• As refractories
Sodium chromate (Na_2CrO_4)	• To make pigments for ink and paints • Wood preservative • Corrosion inhibitor
Potassium dichromate	• Used in photography • Manufacturing of waterproof glues

1.3.2.2. Human health effects of chromium

Chromium compounds are absorbed in the human body by being exposed orally, through the skin and by inhalation of chromium or chromium compounds [43]. The concentration of chromium in water and air is very low. The health effects of being exposed to chromium are dependent on the oxidation state of chromium [44]. Elemental chromium has low toxicity while the hexavalent form is very toxic. Chromium (VI) is more easily absorbed in the human body than chromium III. Chromium (VI) enters the body cell through a non-specific channel by diffusion [45]. It is more concentrated in the kidney, liver, bone and spleen than in other body parts [46]. The absorption of chromium in the human body depends on the size of the particle, solubility, interaction with biomolecules in the body and oxidation state of chromium [47]. Chromium (III) naturally occurs in many fruits, vegetables, grains, yeasts and meats [48]. Food

that is stored in cans and steel tanks may have high concentrations of chromium. Trivalent chromium is an essential nutrient for humans and lack of it can cause disruptions of metabolisms, diabetes and heart conditions [48]. High levels of chromium in the body can cause health effects. The most toxic chromium compound is chromium (VI), especially for people who work in the steel and textile industries [49]. The toxicity of chromium comes from its likelihood to be corrosive and cause allergic reactions in human beings. The metal is carcinogenic, it mostly causes lung cancer when human beings inhale it [50]. Other adverse health effects caused by chromium include:

- Skin rashes
- Ulcers and upset stomach
- Respiratory problems
- Weak immune system
- Liver and kidney damage
- Death

Health effects of hexavalent form on the skin include dermatitis, allergic skin reactions and ulcerations. Breathing in hexavalent chromium particles can result in nasal septum mucous membrane rupture, ulceration, coughing, asthmatic bronchitis, and edema. [51].

1.3.3. Nickel

Nickel was discovered and isolated in 1751 by the Swedish chemist from arsenosulfide ore [52]. The name nickel comes from the German word “kupfernickel” or Devil’s copper[53]. Nickel is the 5th most abundant metal on the earth’s crust. The concentration of nickel in the earth’s crust is about 80 mg/kg and the earth’s core consists mainly of nickel-iron alloy. The symbol for this chemical element is Ni, it is found in period 4 of the periodic table. Elemental nickel has atomic number 28 and mass number 58.6934.

1.3.3.1. Anthropogenic sources of Nickel

Nickel in the atmosphere results mostly from anthropogenic activities. The most common source of nickel is the emission of nickel from smelting, metal mining and refining operation from industrial activities such as the manufacturing of alloys, and land disposal of sludges and slags [54]. Other sources include the combustion of wood and waste incineration [55]. Industrial sites have high concentrations of nickel than other areas. Nickel released into the atmosphere attaches itself to small particles of dust that are taken out of the air by rain or snow

and this takes many days to happen [56]. Nickel in soil strongly attaches to particles containing manganese and iron. This metal is widely used in the production of products for industrial, customers, transport, military, aerospace and marine application [57]. It is also used in making batteries, chemicals and paints. Nickel is mostly found in iron meteorites as alloys taenite and kamacite [54].

1.3.3.2. Human health Effects of Nickel

Human beings are exposed to nickel mostly by smoking, drinking coffee, tea and eating chocolate and legumes [58]. The highest general human being exposure to nickel is seen in people who stay near nickel refineries [59]. Occupational exposure to nickel can occur by inhalation of aerosols, fumes and dust that contain nickel. Dermal exposure can occur with nickel solutions such as those used in nickel salts, alloys and electroplating [60]. If one is exposed to high concentrations of nickel by inhaling it, one may suffer severe damage to their kidney and lungs [61]. They may also vomit, feel nauseous, and have diarrhea. Chronic exposure to nickel in human beings also leads to respiratory effects such as asthma, bronchitis and decreased function of the lungs [61].

1.3.4.0. Arsenic (As)

Arsenic is a chemical element with the symbol As and belongs to group 15(V) on the periodic table. Arsenic is referred to as a metalloid because its characteristics are intermediary between metals and on-metals [62]. Arsenic exists naturally in the earth's crust; however, it is extremely rare in its free form. Arsenic was discovered in the fourth century B.C. and was isolated for the first time in 1250. The name arsenic is derived from the Persian term zarnikh, which means yellow orpiment. Arsenic is derived from the Greek word meaning "arsenikos," meaning strong [63].

1.3.4.1. Anthropogenic sources of Arsenic

Human activities such as wood combustion, coal combustion, waste incineration, non-ferrous metal mining and smelting release arsenic into the atmosphere and soil [64]. Arsenic that is released into the environment due to combustion will occur as soluble oxides. The oxide particles are dispersed by wind and return to the earth in wet deposition. Arsenic compounds are mostly used in manufacturing pesticides and herbicides, but this use is banned in most countries [65]. Arsenic is also used in leather pigments while arsanilic acid is added in animal feeds as a growth stimulant. Chromated copper and zinc arsenates are used in wood preservatives and antifungals since are strong toxins [66]. Elemental arsenic is used in making electronics and lead crystal glass [67]. It is also used commercially as an alloying agent and in

the production of lasers, semiconductors, transistors, ammunition, textiles, paper, glass and metal adhesives [65]. Arsenic trioxide is effective in making medicines used to treat people with promyelocytic leukemia [63].

1.3.4.2. Human health effects of Arsenic

Human beings are exposed to arsenic mainly through ingestion and inhalation [68]. However, dermal exposure can occur. Human beings also consume foods (fish, meat and poultry) that contain arsenic [69]. Very small amounts of arsenic can enter the human body from the soil, air and drinking water. The major source of inhaled arsenic in the atmosphere comes from cotton gins, smelters, tobacco smoke and manufacturing pesticides and glass [70]. High levels of inorganic arsenic can cause nausea, vomiting, diarrhea, dehydration, and shock [71]. Long-term exposure to inorganic arsenic has been linked to an increased risk of diabetes, hypertension, skin disorders, and various cancers [72]. Other adverse effects of arsenic on human health are shown in figure 4 below.

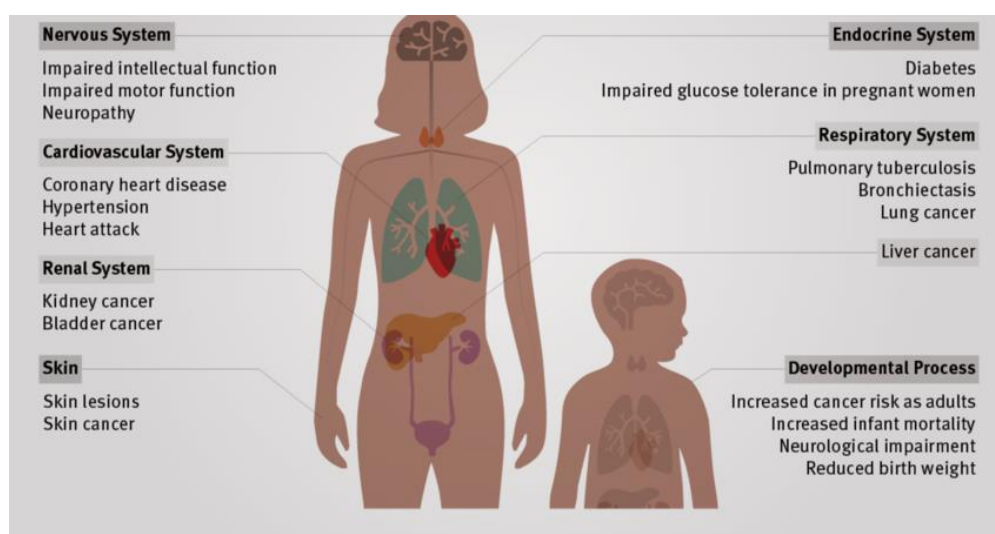


Figure 4. Effects of arsenic on the human body [73]

1.4. Assessment of occurrence of restricted limits of hydroquinone and heavy metals in cosmetics

Most of the raw materials that are used in manufacturing skin lightening products contain a significant amount of hydroquinone and heavy metals. Many countries have banned the use of hydroquinone and heavy metals in skin lightening products as ingredients. They have implemented regulatory measures in order to reduce the use of harmful compounds in the manufacturing skin lightening products., however, some manufacturing companies have

devised means to bypass these regulatory measures. The US FDA has set the limit on the amount of hydroquinone and heavy metals that can be used as ingredients in skin lightening cosmetics [74]. These compounds can cause skin problems and severe diseases such as cancer. For instance, it is mandatory for manufacturing companies to list all the ingredients they used to make for the consumers. However, a number of them play tricks to avoid meeting these requirements. Some print the ingredients list on the insert label using small eligible font sizes while others leave out ingredients in an effort to withhold information from customers.

1.4. Problem Statement

People around the world especially women use cosmetic products to lighten their skin complexion. Some of these products contain heavy metals and hydroquinone which are very toxic and have been banned from use in cosmetic preparation. The toxic effects of heavy metals and hydroquinone can be observed or experienced even at lower concentrations. Despite these side effects, creams that contain these harmful chemicals are still sold to the public in developing countries like Botswana. In addition, not all the skin lightening products sold in Botswana have insert labels that provide a full, detailed list of all the active ingredients used during manufacturing. It is also very important to make people aware of the dangers of using some of these cosmetic products as they may contain toxic heavy metals and hydroquinone. Furthermore, there is little or no data/work done on the levels of heavy metals and hydroquinone in skin lightening products sold on the Botswana market. These can be achieved by determining the level of these active ingredients in skin lightening cosmetics and sensitizing the public.

1.5. Objectives of the study

- To analyze for the first time in Botswana, the presence of heavy metals and hydroquinone content in some skin lightening creams and cosmetic soaps bought in the local market.
- To evaluate the exposure risk associated with the heavy metals and hydroquinone content in skin lightening products.
- To raise public awareness on the need for more stringent measures for controlling the use and distribution of skin lightening products to prevent possible long-term adverse health effects in Botswana.

Chapter 2. Literature Review

Skin lightening or skin bleaching involve the use of chemical agents to lighten the complexion of one's skin. It can also be referred to as skin bleaching. It has no limit and extends across all age groups, races and sex. In most societies, having beautiful blemish free and even toned skin is everybody's dream. This has led to many people using skin lightening products to bleach their skin [75]. Skin lightening practice is believed to have originated from countries like India and Egypt [76]. In these societies, skin color was one time used to define ethnic grouping, race and cultural group [77]. They used either locally available or sophisticated imported products depending on their affordability and availability. To fulfill the desire for lighter skin, they prepared a mixture of herbs, mud and caustic noxious chemicals in clay pots over the open fire [75]. They also used oil with essence and ointments to soften their skin, while natural paints and dyes were used for coloring faces during religious and ceremonial occasions [76].

Currently, skin bleaching is practiced worldwide and it is one of the fastest growing industries in the world. It is estimated that by 2024, the industry would have a net worth US\$ 31.2 billion [78]. Skin lightening practice is more common in Asian, African and Caribbean nations [79]. Notwithstanding, in North America and Europe, skin lightening products are mostly used by dark skinned populations [79]. In Asian countries, people with a lighter and even toned skin are regarded as more attractive and desirable, hence they have social privileges like better paying jobs [78]. In countries like Japan, India, China and Korea even toned and beautiful skin is seen as a cultural symbol for wealth, class and social status [80]. In India, there are many skin lightening creams and lotions and the market is estimated to be worth US\$ 450-535 million [81]. The use of skin lightening products has been there in many African countries (South Africa, Zimbabwe, Senegal, Nigeria, Kenya and Ghana) for over centuries [82]. The use of skin lightening products for cosmetic reasons was reported to be high and very common in Africa [83]. A prevalence of 20% was reported in Zimbabwe among university students [84]. A study in Tanzania also revealed that most females use skin lightening to appeal to their partners [84].

Most women like to keep their skin beautiful and even-toned and this has led them to use skin lightening products which ultimately pose a potential threat to their health [83]. Majority of these products contain many different chemicals, some of which are harmful and could have a negative effect on their health [85]. These products can contain one or two active ingredients; hydroquinone. Other agents that promote complexion include lactic acid, L-ascorbic acid, α -arbutin, vitamin C and kojic acid [86]. In addition, there have been some reports showing the

presence of toxic heavy metals such as Cr, Cd, Ni, Pb and As in different skin lightening products[87]. Bleaching agents are not only used for cosmetic use, but they are also used for medical purposes for example; in the treatment of melasma and post-inflammatory hyperpigmentation [88]. They can also be used as an alternative to treat solar lentigines, ephelides and nevi [88]. An ideal bleaching agent should lead to permanent removal of unwanted pigment on the skin and carry no short- or long-term side effects. The majority of bleaching chemicals that are commonly used cause temporary elimination of hyperpigmentation, which reappears following withdrawal [89].

Most of the skin lightening creams are imported from the USA, Europe, and Cote d'Ivoire. According to research, it was recorded that 75% of the population in Lagos, Nigeria [90]; 53% of the population in Dakar, Senegal, 35% in Pretoria, South Africa use skin bleaching products [91]. It is also estimated that in Cote d'Ivoire eight out of ten women with fair skin complexion use skin lightening products regularly [91]. Among Zambian women that are aged between 30 and 39, it is reported that 60% of them use skin lightening creams regularly [91].

2.1. The Human Skin

The skin is the largest organ of the human and forms its external surface .It has a surface area of about 1.7 m² which is approximately 10% of the total body mass of an average person [92]. The skin is made up of about 70% water, 25% protein and 3% lipids [93]. Other components include nuclei acids, minerals, glycosamines and other chemicals. The functions of the skin include: [92]

- Serving as a barrier between the body and its surroundings, protecting it from ultraviolet (UV) radiation, bacteria, chemicals, allergies, and the loss of nutrients and moisture
- Keeping the body temperature and blood pressure in check. It serves as a key sensory organ in contact with the environment, sensing stimuli such as pressure, pain, and temperature.
- Serving as a site for the delivery of therapeutic compounds for local and systemic effects by providing a substantial barrier to the penetration of most substances.
- Maintaining the body's homeostasis and takes part in the excretion of toxic substances from the body.

2.1.1 The Structure of the Skin

The human skin is made up of the epidermis, dermis and subcutaneous layer as shown in figure 5. The epidermis is the outermost layer of the skin and it is about 50 - 100 μm thick [94]. The viable epidermis is composed of Langerhans, Merkel cells and keratinocytes [95]. Keratinocytes rise from the stratum basal and it go into differentiation while migrating towards the stratum corneum. Keratinocyte's differentiation is observed as an increase in the number of keratinocytes due to the formation of intracellular networks of keratin fibres. Keratinocytes differentiation maintains the stratum corneum by restoring stratum lipids lost via desquamation [96]. The epidermis does not have blood vessels therefore the epidermal cells source nutrients and removes waste through diffusion across the epidermal-dermal layer to cutaneous circulation in the dermis. The epidermis protects the body from the environment [97].

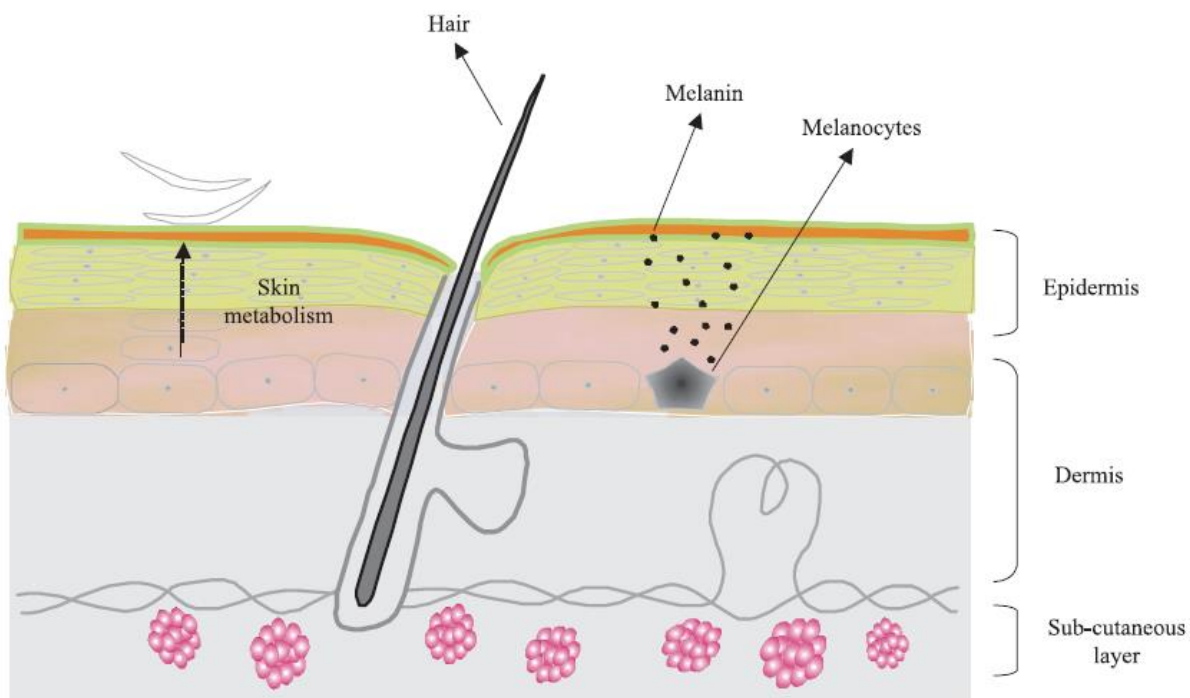


Figure 5. The structure of the human skin [98]

Skin lightening products are applied to the epidermis which contains cells called the melanocytes [99]. Melanocytes are cells that produce the skin pigment (melanin) [100]. Skin color is determined by the amount of melanin that the body produces. Melanin is a dark, sulphur containing pigment which is found in the skin, eyes, and hair [101]. It is produced by a process called melanogenesis which takes place in a special organelle called the melasomes [101]. Melasomes are located in the melanocytes. During melanogenesis, two types of melanin are

synthesized in the melanosomes i.e pheomelanin and eumelanin (figure 6) [102]. Pheomelanin is a pale red-yellow soluble polymer containing sulfur, whereas eumelanin is a dark brown black polymer that is insoluble [22]. The biosynthesis of pheomelanin and eumelanin is catalyzed by tyrosinase, an enzyme that contains copper. The enzyme catalyzes the hydroxylation of the L-tyrosine to L-dopaquinone. L-dopaquinone is then converted to dopachrome and dopa by auto oxidation. Dopa is a substrate of the enzyme tyrosinase, which is then oxidized again to dopaquinone. Eumelanin is produced from dihydroxyindole-2-carboxylic and dihydroxyindole by oxidation of the dopaquinone [102]. Pheomelanin is formed by the conversion of dopaquinone to cysteinyl dopa in the presence of L-cysteine [103] .

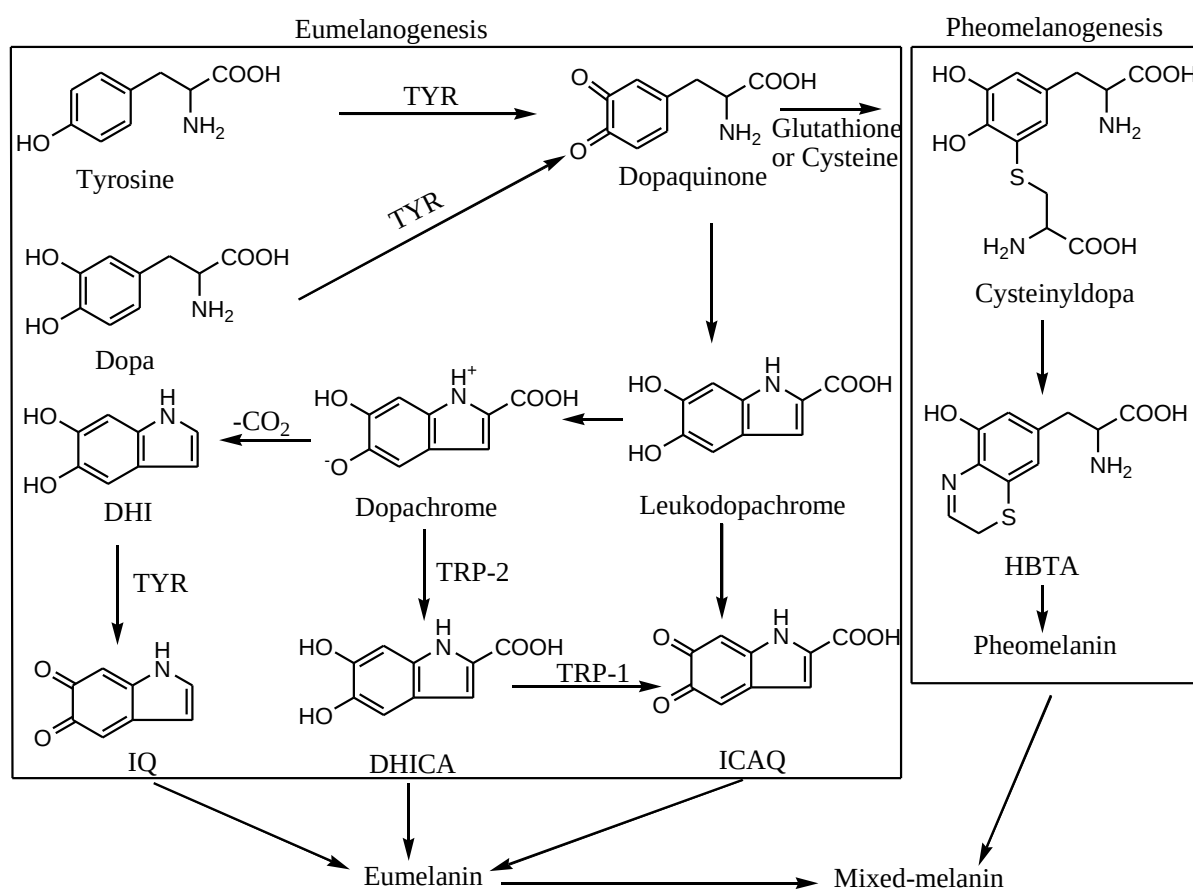


Figure 6. Melanogenesis process (Adapted from T.S Chang et al. (2012))

In a region of a body where the concentration of melanin in the melanocytes is high, the skin is darker than the normal background color [104]. This area of hyperpigmentation is normally referred to as age spots, brown spots, or liver spots [104]. Bleaching agents found in cosmetics are capable of reducing melanin concentration in the melanocytes of the skin [98]. Skin bleaching agents are absorbed in the lower layer of the skin so that they stop the production of

melanin in the melanocytes by reducing oxidized dopaquinone, reacting with copper ion at the active site of the enzyme, damaging the DNA, suppressing tautomerization of dopaquinone to DHICA and inhibiting the maturation of the melanosome cells [105].

Visible pigmentation in human beings come from synthesis and distribution of melanin pigment in the skin [98]. Melanin also plays a crucial role in absorption of free radicals that are generated in the cytoplasm [102]. They also help in shielding the skin from ionizing radiations. However, dark skin that is caused by accumulation of melanin is not cosmetically pleasing to many people. High concentration of melanin on the skin can also lead to a great number of skin diseases such as solar lentigines, post-inflammatory hyperpigmentation and melasma [106].

2.2. Skin Bleaching Agents

2.2.1. Hydroquinone

Skin lightening products contain several chemicals of which some are considered hazardous to human being. Despite the serious health side effects caused by using these products, men and women continue using them. Some of the bleaching agents that are used mostly contain: hydroquinone, mercury, kojic acid, arbutin and shea butter [107]. Most skin lightening products contain 8% to 15% of hydroquinone, however the maximum allowable dose of hydroquinone is 2% which is approved by United States Food and Drug Administration (FDA) in United states [108]. Hydroquinone has been used for many years to cure hyperpigmentation and it has been very successful when treating melanosis [105]. As a bleaching agent, hydroquinone prevents the transformation of dopa to melanin by inhibiting the tyrosinase enzyme [109]. Hydroquinone inhibits melanogenesis when the active tyrosinase is present however, it cannot change the colour of the melanin which is previously present in the epidermis and dermis [105]. Hydroquinone also interferes with melanosome formation or degradation by inhibiting DNA and RNA synthesis within melanocytes [105].

It was discovered that 2% hydroquinone causes a reduction in hyperpigmentation which was considered as good in 14-70% of treated individuals [89]. High levels of hydroquinone proved to be more effective, although they were associated with increased adverse effects such as irritation at the application site [89]. Hydroquinone is carcinogenic hence it is banned for use in cosmetic products in many regions such as Japan, Europe, several African countries, UK and Australia [110]. Research has shown that topical application of hydroquinone has a negative impact on human beings especially when used in high concentrations. Use of high concentrations of hydroquinone frequently can cause skin irritation. Prolonged application of

hydroquinone containing skin lightening products has been associated with exogenous ochronosis of which by 2007 about 789 cases had been reported worldwide [111]. In the US, 2-4% of hydroquinone is only allowed in skin lightening products as prescription and less than 2% in cosmetic as an active ingredient [112]. Dermal absorption of hydroquinone has been studied and it was observed that 24h application of hydroquinone, was enough for this chemical to be absorbed by human beings with bioavailability of $45.3 \pm 11.2\%$ [113]. Researchers from the university of Amsterdam (Netherlands) have reported that the use of cosmetic products that contained hydroquinone can lead to kidney damage and blood cancer (Leukaemia)[114]. A total of 22 cosmetic samples were analysed in Pakistan using high performance liquid chromatography for hydroquinone [88]. Eleven samples showed the presence of hydroquinone in range of 0.002%- 0.092% [88].

Table 4. List of studies that have been carried out for Hydroquinone in skin lightening products

Country of Study (Year)	Concentration of Hydroquinone	Times greater than the set limit	Reference
Pakistan (2021)	0 -7.14 %	3.57	[110]
Nigeria (2014)	0.288- 2.35%	1	[115]
Canada (2016)	0-6%	3	[116]
Ghana (2016)	0.243%	0	[86]

2.2.2. Heavy metals on skin lightening products

Skin lightening products contain many chemical substances. Some of these are heavy metals such as chromium, lead, nickel and arsenic [87]. These heavy metals have different uses in the skin lightening products industry, for example they are used as pigments in coloured skin lightening products and to filter ultraviolet in the body or face [117]. Skin lightening products can also contain heavy metals from using apparatuses that are made from metals during production as contaminants [117]. When essential metals such as nickel are present at high levels, they are toxic to the human body. When heavy metal ions are absorbed into the body they form complexes with thiol, carboxylic acid and amine of proteins which leads to malfunction or damage to the cells leading to variety of diseases [118]. Lead is a contaminant of skin lightening products [119]. Even in low levels, lead is toxic to human beings and fatal

in high concentrations. In skin lightening products, there is no level of lead permitted to be used as ingredient in manufacturing cosmetics. It is rather found as an impurity in cosmetics. The toxicity of lead was reported in Nigeria with concentrations of lead in cosmetics ranging from 6.1 to 45.9 mg/kg [120]. H. Nourmoradi and co-workers (2013) also performed a research study where they analysed 50 samples of lipstick and eye shadows [121]. The analysed samples had levels of lead ranging from 0.08-5.2 µg/g in lipstick and 0.85- 6.90 µg/g in eye shadows. These levels have been proven to be detrimental to human health, if people continue to use these products, the absorbance of the heavy metals in the body could cause serious health problems [121].

In another study carried out on different cosmetic products sold at a shopping mall in Kano Metropolis (Nigeria), the range of concentration of heavy metals in skin lightening products was 3.68-11.03 mg/kg, 0-0.05 mg/kg and 0.05-0.14 mg/kg for Ni, Cr and Pb respectively [122]. Other studies carried out on heavy metals in skin lightening products are shown in table 6. Saudi Arabia recorded the highest concentration of Pb which was 14 times more than the acceptable level set by USFDA. The acceptable level of contamination is 5 ppm for chromium and nickel [123] while US food and drug administration (USFDA) fixed a maximum content for Pb and As at 10 mg/kg as shown on table 5 [74].

Hexavalent chromium is very corrosive and can cause allergic reactions when comes in contact with the skin (figure 7). Chromium compounds are also listed as carcinogenic by the International Agency for Research on Cancer (IARC) [44]. The US Food and drug administration recommends a daily intake of 120mcg of chromium while National Academy of sciences committee in the US recommends 25 mcg and 35 mcg for women and man respectively [44]. Dermal exposure of chromium can lead to dermatitis, allergic skin reactions, eczema, ulcers and skin burns. It has been reported that dermatitis develops in four phases [124]:

-Phase 1(Refractory phase): There is no skin inflammation that occurs however chromium hapten is absorbed into the skin and conjugates with specific protein in the epidermal.

-Phase 2 (induction phase): The conjugate chromium hapten reacts with T-lymphocytes

-Phase 3(Elicitation phase): Secondary chromium exposure activates the effector cells which releases the cascade of mediators leading to skin inflammation.

-Phase 4(persistence phase): recognition of the chromium hapten conjugate continues by the effector lymphocytes.



Figure 7. skin problem due to exposure of hexavalent chromium [125]

Cosmetic goods in India are governed by the Drugs and Cosmetics Act 1940 and Rules 1945, as well as labelling declarations issued by the Bureau of India Standards (BIS). The Bureau of India Standards is in charge of establishing standards for cosmetics items classified in Schedule "S" of the Drugs and Cosmetics Rules 1945. The Indian Standard (IS) 6608:2004 for skin creams and 9875:1990 for lipstick have been issued by BIS. According to IS 6608:2004, if all raw materials needing heavy metal testing have been tested and meet the standards, the producer may not test the final cosmetic for heavy metals and arsenic [126][127].

The cosmetics Directive European union was adopted in 1976. It was then re-evaluated in 2009 to allow for harmonization. The principle of the Cosmetics Regulations is that if a product fits all the regulations' requirements, it should have instant market access and be able to be marketed freely throughout the European Union. According to the rules, the producer is solely responsible for the product that is placed on the market. It is the obligation of the maker to ensure that the product is safe and complies with all cosmetics requirements [128].

Table 5. Acceptable levels of heavy metals in cosmetics

Elements	US FDA
Pb	10 mg/kg
As	10 mg/kg
Ni	5 mg/kg
Cr	5 mg/kg

Table 6. List of studies carried out for heavy metals in skin lightening products

Country of study (Year)	Heavy metal studied	Concentration (mg/kg)	Times above Limit	Reference
Pakistan (2020)	- Pb - Ni - Cr - As	0.2997-4.7287 0.7487-1.5958 1.0453-2.7455 ND	0 0 0 0	[129]
Bangladesh (2019)	- Pb - Cr	14.38 – 50.39 0 -2.82	5 0	[87]
India (2018)	- As - Pb	1.897-8.783 11.07-23.317	0 2	[130]
Nigeria (2015)	- Pb - Cr - Ni	0.5-4.5 4.25-8.0 0.03-1.65	0 0 0	[131]
Nigeria (2013)	- Pb - Ni - Cr	1.2 -45.9 1.1- 9.2 ND	4 1 0	[120]
Saudi Arabia (2016)	- Pb - As	1.20-143 1.0-12.6	14 1	[132]

2.3.0. Analytical Techniques Used for Analysis of Skin Lightening Agents

Many analytical techniques have been used utilized for analysis of heavy metals and hydroquinone in skin lightening products, but the most common are ICP OES and HPLC.

2.3.1. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

ICP OES is an analytical instrument with high sample throughput and multi-element options[133]. It is used to analyse for metals and heavy metals in water, soil, cosmetics, plants, toys and hair samples [134] . It allows for analysis of liquid samples hence solid samples such as skin lightening cosmetics are first digested using acids before they are injected into the instrument. The instrument consists of (figure 8):

- Sample introduction system (nebulizer)
- ICP torch
- High frequency generator
- Spectrometer
- Detector
- Data processing unit

When argon gas is passed through the quartz torch and electric current is applied to the coil at the end of the torch tube, plasma is generated. The plasma is formed when argon gas is ionized by the electromagnetic field produced in the torch by the high frequency current. The temperature of the plasma can reach highs of 10000K. The high temperature in the plasma is enough to break down samples into atoms and provide energy for ionization and excitation [135].

The liquid sample is introduced by the peristaltic pump into the nebulizer, which converts the liquid sample into an aerosol using argon gas [136]. The aerosol is then passed to the spray chamber where large particles of the samples are removed and the remaining sample is passed to the plasma for atomization. The plasma removes the solvent by evaporating it and breaks down the molecules of the samples into ions and atoms. Excited electrons migrate to a lower energy level, where light is released at a different wavelength based on the elements and energy level. The optical spectrometer's components guide the light generated by ions and atoms in the plasma to the instrument's spectrometer, where it is separated and the intensity of the light is measured by the detector. The information collected by the instrument is fed to the controlling computer. A specialized instrument software calculates the concentrations of each element in the sample and a report is generated [137].

The analysis of heavy metals in skin lightening products can be done using ICP OES, as samples can be digested with acids such as nitric acid, hydrochloric acid and hydrofluoric acid [134]. For quantitative analysis of lead, arsenic, nickel and chromium, standard solutions are prepared with known concentration and run on the instrument to obtain a calibration curve. A calibration curve is a plot of intensity against concentration that allows for determination of the concentration of an unknown sample [138]. The advantages of using ICP OES include the ability to quantify and identify most elements on the periodic table, low detection limit for most elements within a range of 1- 100g/L and multielement analyses which can be completed in short period of time while consuming small amount of volume [139] .

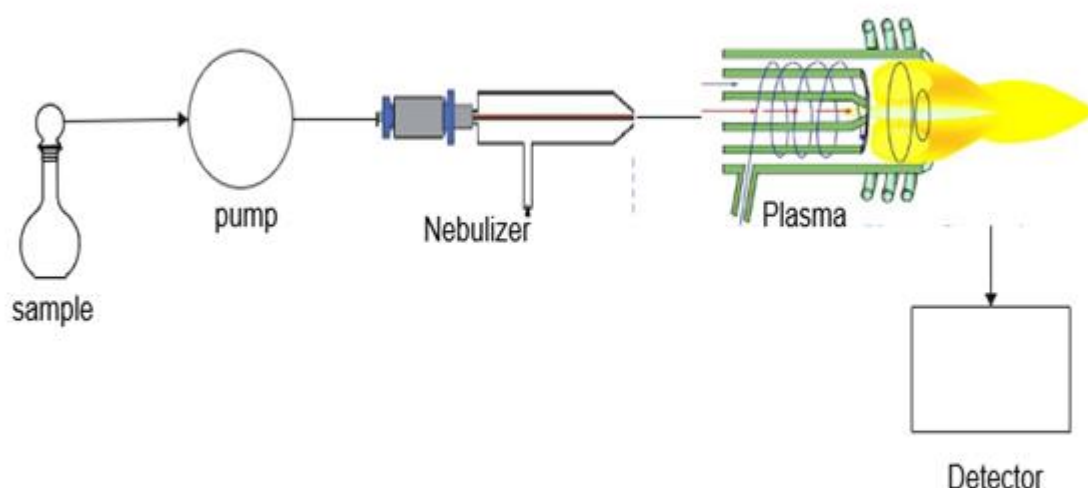


Figure 8. Schematic diagram of ICP OES

2.3.2. High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is an analytical method that is used to separate, identify, and quantify a wide variety of constituents in liquid samples [140]. Its operation is based on the propulsion of sample dissolved in a mobile phase through a stationary phase in a process called elution. Each constituent in the sample interacts differently with the stationary phase, resulting in a variable flow rate and component separation. High performance liquid

chromatography is used in research areas, industries and for medical purposes [141]. HPLC can be used for both qualitative and quantitative analysis [142]. Figure 9 is a diagram showing the important components of HPLC.

Many research studies in the literature report the investigation of hydroquinone levels in cosmetics using HPLC. The analysis is possible after extraction of hydroquinone using a suitable solvent such as methanol, ethanol, hexane and acetone. The dissolved cosmetic sample injected into the instrument where it is carried by the mobile phase through the column for separation. Separation is determined by solute-stationary phase interaction which includes liquid- solid adsorption, liquid-liquid partitioning and solute-mobile phase interaction. In liquid-solid adsorption, the stationary phase is solid while the mobile phase is liquid and separation is by adsorption. The stationary phases that are normally used are made from silica and alumina [143]. In liquid-liquid chromatography, both the mobile and the stationary phases are liquid but the stationary phase is made more polar than the mobile one [144].

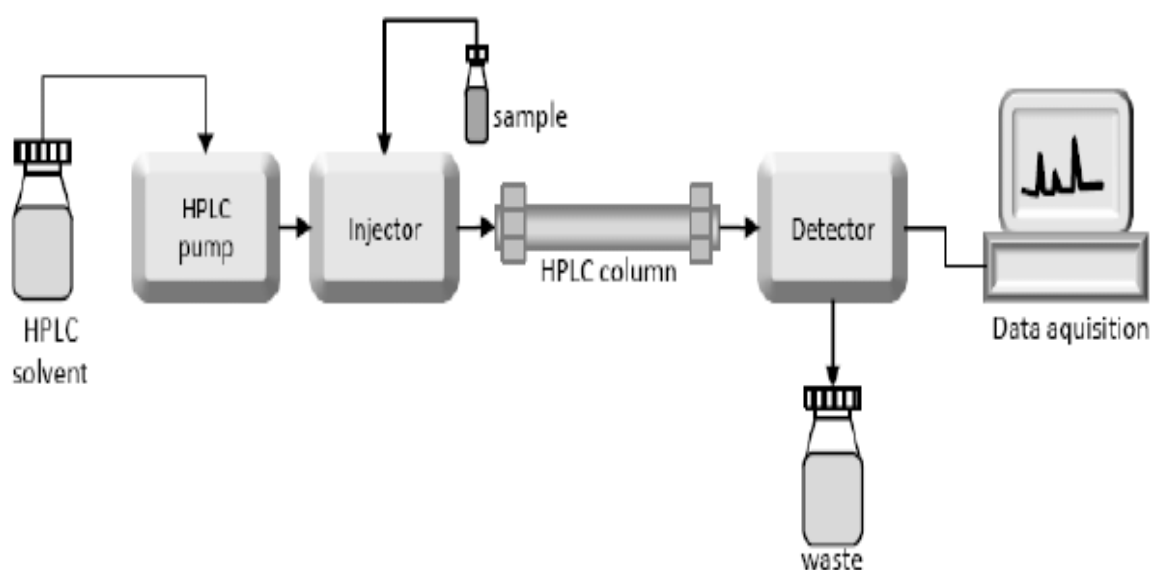


Figure 9. Schematic diagram of HPLC [145]

- **Reservoir:** Holds the mobile phase (solvent)
- **An injector port:** introduces liquid sample into the HPLC column
- **Column:** it contains the stationary phase used for separation of sample constituent
- **Detector:** Used to show the separated compounds bands as they elute the HPLC column
- **Data processing unit:** calculates the concentration of the detected component from the area or height of corresponding peak, and reported.

Chapter 3. Methodology

3.1. Glassware

All the glassware (beakers, pipette, measuring cylinders, funnels, volumetric flasks, and burettes) used was washed with liquid soap and rinsed with distilled water. They were then soaked in 2% nitric acid overnight and rinsed again with distilled water before use.

3.2. Chemicals and Reagents

All reagents used in this project were of analytical grade. Hydrochloric acid 35%, Nitric acid 65%, Hydrofluoric acid and HPLC grade methanol were purchased from Sigma Aldrich (St. Louis, USA). Hydroquinone standard was purchased from Sigma Aldrich. Double distilled water was used for dilution of samples that were analysed with ICP OES.

3.3. Equipment's and Analytical Instruments

Inductively coupled plasma Optimal Emission spectrometer 7000 series fitted with an autosampler was purchased from Thermo scientific. A handheld X-ray fluorescence analyser was purchased from Olympus scientific. A vortex mixer SA8 was purchased from Stuart equipment. Microwave Reaction system MARS 6 was from CEM corporation, USA. Reversed phase HPLC was carried out using Agilent high performance liquid chromatography 1200 infinity. It was fitted with analytical reverse phase column for separation. A syringe of capacity of 50 μ L was used for injection of samples. The mobile phase was a mixture of water and methanol (45: 55) pumped at flow rate of 1.0 mL/min. The UV detector was operated at a wavelength of 292nm with a sensitivity of 0.50. Standard solution were prepared by dissolving a weighted amount reference standard in a mixture of methanol and water and injected to obtain a calibration curve.

3.4. Sampling

A total of sixteen samples of skin lightening products were analysed for heavy metals and hydroquinone. All available brands of skin lightening products in beauty aid stores in local market of Gaborone, Botswana were purchased. Samples were stored at room temperature until analysis. They are shown on figure 10 below. Table 7 shows the manufacturing countries and the colour of the cosmetic products used in this study.



Figure 10. Skin lightening products

Table 7. The origin and colour of skin lightening products sold in Botswana market

Sample id	Country	Colour
S1	Guangzhou	black
S2	Italy	colourless
S3	South Africa	white
S4	Italy	colourless
S5	Italy	colourless
S6	South Africa	white
S7	Switzerland	yellow
S8	Guangzhou	black
S9	South Africa	Pink
S10	Spain	white
S11	Guangzhou	white
S12	RDC	orange
S13	Guangzhou	white
S14	Guangzhou	white

3.5. Screening of samples using XRF for heavy metals

The purchased skin lightening products were pre-screened for metal content using a handheld X-ray fluorescence analyzer. The following table shows the number of samples that contained the element. Figure 11 is an illustration of the analyzer used.



Figure 11. X-Ray Fluorescence analyzer

3.6. Digestion of Samples For determination of heavy metals

Prior to analysis for heavy metals, 1.0 g of each sample was weighed accurately into a Teflon microwave digestion vessel. A 10 mL mixture of Nitric acid and hydrofluoric acid in the ratio 4: 1 was added to the samples. The mixture was closed and swirled to homogenize the sample with the acid. The mixture was digested at 200 °C in a microwave digestion system for 15 minutes to obtain a clear solution. The solution was allowed to cool to room temperature and transferred to a 100 mL volumetric flask and topped to mark with distilled water.

3.7. Extraction of Hydroquinone

Solvent extraction method was used to extract hydroquinone from cosmetic samples. About 0.5 g of cosmetic samples was weighed accurately into a beaker and 10 mL of the methanol and water mixture was added to the sample then mixed until homogenous. The mixture was then transferred to a 25 mL volumetric flask and vortexed for 1 minute. The flask was placed in a water bath at 60 °C for 15 minutes. After cooling the mixture to room temperature, the flask was topped to the mark with the mobile phase (methanol: water) and filtered through a 0.45 µm membrane filter.

3.8. Inductively Coupled Plasma Optical Emission Spectroscopy

Figure 12 shows the picture of ICP-OES used for analysis of heavy metals in skin lightening products.



Figure 12. ICP OES

The analysis for the heavy metals was performed using ICP-OES equipment as per conditions shown below.

- RF power: 1150 W
- Pump rate: 50rpm
- Aux.Gas flow: 0.5 L/min
- Nebulizer gas flow: 0.5 L/min
- Sample flash time: 20 sec

3.9. Preparation of Hydroquinone standard solutions

Standard solutions of hydroquinone were prepared by dissolving 5 mg of hydroquinone in a mixture of methanol and water in a 25 mL volumetric flask and filled to the mark with solvent mixture. Various concentrations (0.08, 0.12, 0.16 and 0.2 mg/mL) were prepared by diluting aliquots of the stock solution with the mixture.

3.10. High Performance Liquid Chromatography

Figure 13 below illustrates the HPLC instrument used to analyse for hydroquinone in cosmetic samples



Figure 13. HPLC

HPLC analysis of the samples for hydroquinone was performed using the conditions shown below.

- Column: 4.6* 250 nm, 5 μ m ODS-C18
- Column temperature: 25°C
- Flow rate: 1.0 mL/min
- Signal: wavelength(λ)= 292 nm, 4nm ref= 360,100
- Injection Vol: 20 μ L
- Elution: Gradient
- Mobile phase: methanol: water (55:45)

3.11. Health Risk Assessment

The health risk assessment was calculated involving the hazard quotient (HQ), daily dermal exposure (DDE), average daily intake (ADI) and the health risk index basing on the concentrations of heavy metals detected on the samples. The hazard quotient of different metals in the samples was calculated using the ratio of the measured concentration of the metal to the set limit of the metal and expressed as

$$\text{Hazard Quotient} = \frac{\text{Concentration of the metal}}{\text{Maximum Contaminant limit}}$$

The daily dermal exposure (DDE) of the substance applied on the skin was estimated using the following equation:

$$\text{DDE (mg/day)} = C(\%) \times 10^{-2} \times F(\text{day}^{-1}) \times A(\text{g/application}) \times 10^3$$

Where C (%) is the concentration of the metal detected on the sample, F is the frequency of application of the cosmetic product and A is the amount of the cosmetic product that is applied. The risk calculation of Averagely daily intake for exposure dose was estimated for all samples using equation:

$$\text{ADI (mg/day)} = \frac{C \times SA \times AF \times ABS \times EF \times ED}{BW \times AT}$$

Where C(mg/kg) is the measured concentration of the analyte of interest, SA (cm²) is the exposed skin area, AF (mg/cm²/day) is the adherence factor, ABS is the dermal absorption factor, EF (day/year) is the frequency of the exposure, ED (year) is the exposure duration, BW (kg) is the body weight of a human being and AT (day) is the averaging time. The health risk index was estimated using the average daily intake of the metals (ADI) and oral reference dose (Rf_D). The oral reference dose is estimated per day exposure of metals to the human body that has no hazardous effect during life time. HRI is estimated using the following equation

$$\text{HRI} = \frac{\text{ADI}}{\text{Rf}_D}$$

Chapter 4. Results and Discussion

4.1.0 Method Validation for ICP-OES

4.1.1. Linearity

Aliquots of working standard solutions were prepared in 100 mL volumetric flasks. The volume was made up to the mark with double distilled water to obtain concentrations of 5, 10, 15, 20 and 25 mg/kg. A calibration curve was created by plotting the average response intensity vs concentration, and the regression equation was calculated. The linear regression equation and R^2 for each metal is shown on graphs below:

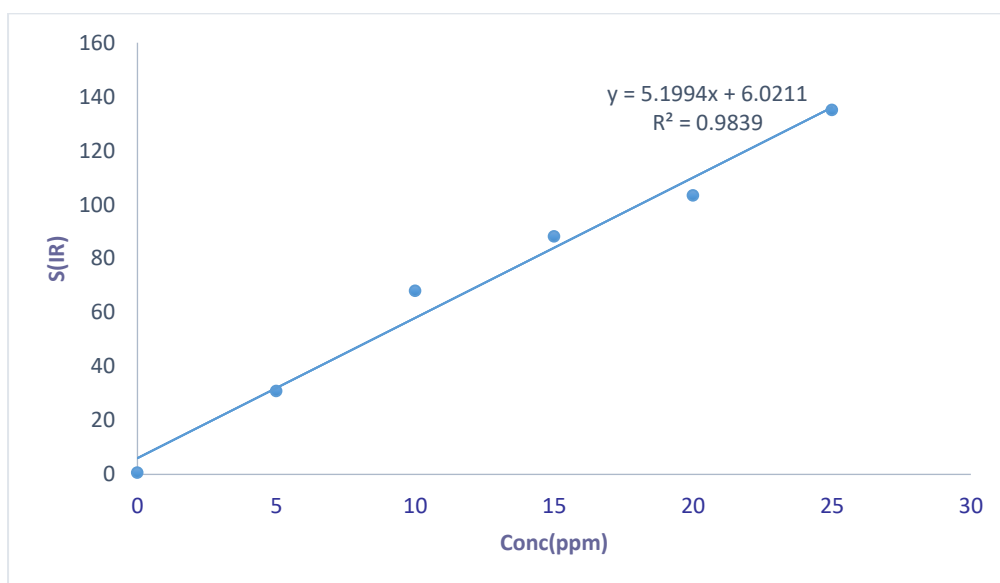


Figure 14. Calibration curve graph for Pb

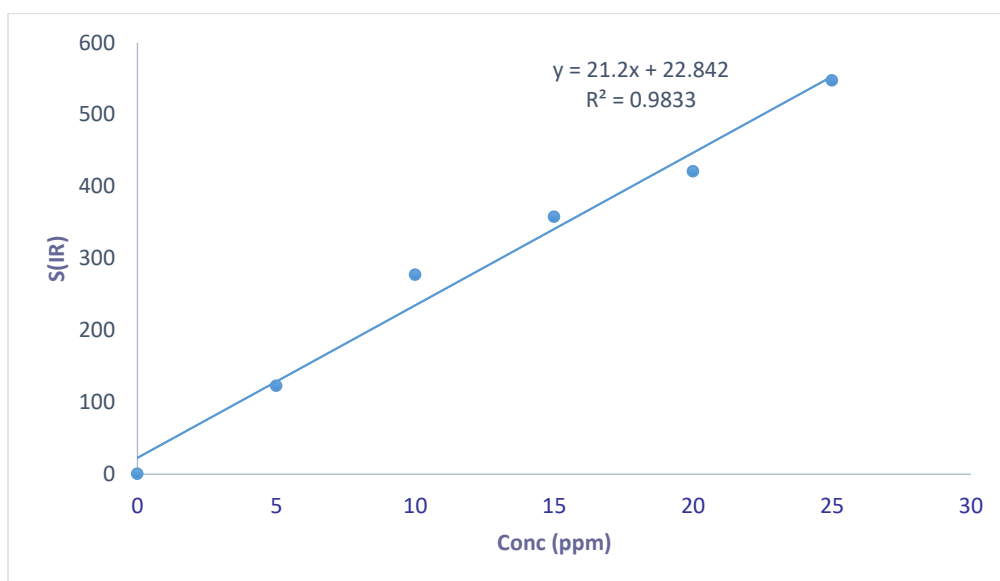


Figure 15. Calibration curve graph for Ni

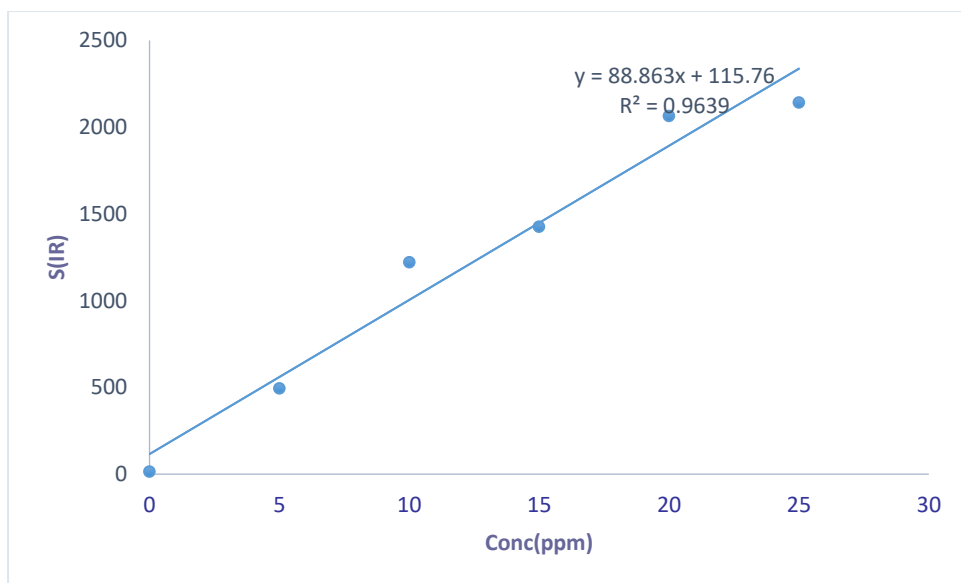


Figure 16. Calibration curve graph for Cr

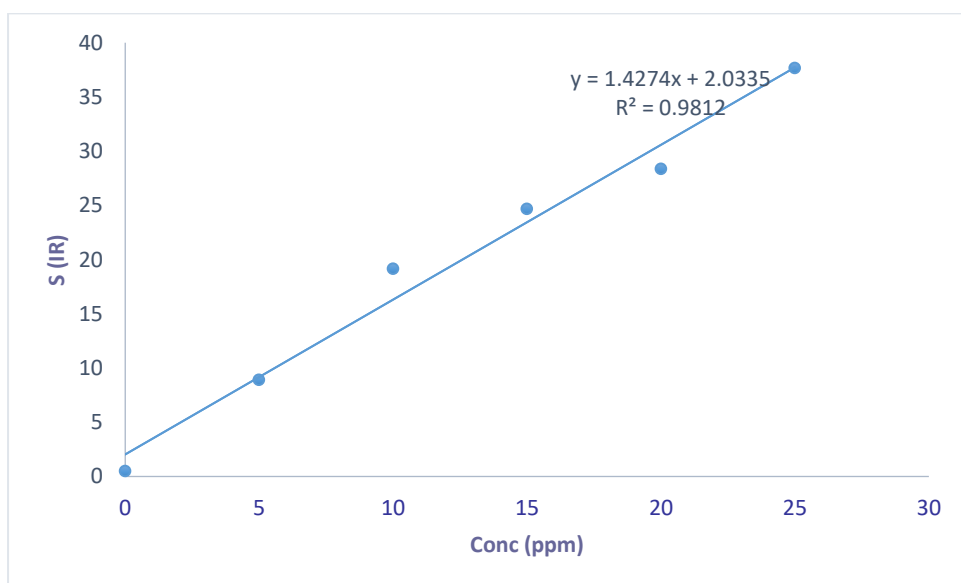


Figure 17. Calibration curve graph for As

4.1.2. Accuracy

Accuracy was obtained by analysis of certified reference materials. Recoveries for each element were then computed as percentages. The results from recovery analysis are presented in table 8 below. The values obtained from the method were in good agreement with the recovery ratios ranging from 96%-105%.

Table 8. Recovery studies for analyzed heavy metals

Heavy metals	Percentage Recovery
Pb	96.3
As	105.2
Cr	104.6
Ni	96.7

4.1.3. Precision

For intraday precision, 5 mg/L multi-elemental standard solution was analysed under optimized conditions for five times twice in a day and response concentrations were recorded. For inter day precision, 5 mg/L multi-elemental standard solution was analysed five times for three consecutive days under optimized conditions and the response concentration was recorded. The calculated %RSD values for both inter-day and intra-day were less than 2% indicating that the data set are closer to each other.

4.1.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the formula $\frac{3SD}{S}$ for LOD and $\frac{10SD}{S}$ for LOQ. The value was calculated from the standard deviation (SD) of response and the slope of the curve (S). The LOD and LOQ after analysis were found to be 0.0063 mg/kg and 0.0209 mg/kg for Ni, 0.0256 mg/kg and 0.0853 mg/kg for Pb, 0.0932 mg/kg and 0.3105 mg/kg for As and 0.0015 mg/kg and 0.0050 mg/kg for Cr respectively.

4.2. Method Validation for HPLC

Calibration curve for hydroquinone was prepared by using standard solutions 0.08, 0.12, 0.16 and 0.2 mg/mL concentrations as shown in figure 18. These concentrations were calculated when preparing a stock solution of 0.2 mg/mL. The linearity was confirmed by correlation coefficient (R^2) which was 0.9942 for hydroquinone. Four different standards solutions were used to draw up the calibration curve in order to calculate the regression equation.

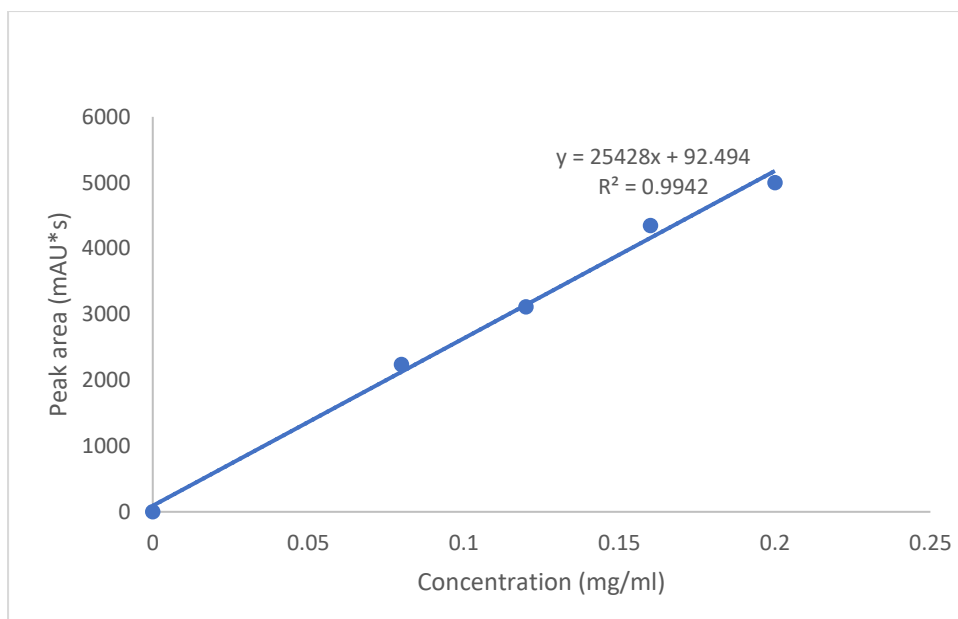


Figure 18. Calibration curve for Hydroquinone

4.3. Heavy metals in cosmetics

All samples were imported from different parts of the world and were creams and lotions of different colour. The package of the samples had no information on heavy metals and hydroquinone. The fourteen samples of skin lightening products were pre-screened with a handheld XRF analyzer and analysed for Pb, As, Cr and Ni using ICP-OES. All heavy metals were able to be detected using the handheld XRF analyzer. The results are illustrated in Table 9, fig 19, fig 20, fig 21 and fig 22 respectively.

According to figure 19 a significant level of lead is found in all skin lightening products samples ranging from 45.75 mg/kg – 193.60 mg/kg. The highest concentration of lead was recorded in sample S8 at 193.60 mg/kg. This value exceeded the maximum contaminant limit of 10 mg/kg set by US FDA. The results also indicate that sample S13 has the lowest concentration of lead amongst the fourteen samples. As shown in figure 20 nickel was also detected in all samples, with concentration ranging from 2.99 mg/kg- 9.9.50 mg/kg. The highest concentration of nickel was detected at 9.50 mg/kg in sample S8. Similarly, figure 21 shows that the total concentration of chromium in cosmetic samples ranged from 3.34 mg/kg- 7.41 mg/kg. The lowest concentration of chromium was found in sample S9 and the highest was in sample S1 as shown in figure 21. The concentration of nickel in the studied cosmetic samples are much higher than the concentrations of skin lightening products analysed in Nigeria [131]. Figure 22 indicates that sample S5 recorded the lowest concentration of arsenic with 1.95 mg/kg while sample S4 had the highest concentration of 4.32 mg/kg. The results obtained in this study are similar to other studies carried on other parts of the world. Most

manufacturing companies do not inform customers on the presence of heavy metals in cosmetics instead they omit the information on the labels.

Heavy metals such chromium and nickel in small concentrations play an important role in organisms, however toxic effects are experienced at high concentrations and if one is exposed to low concentrations for a long time. Side effects such as liver and kidney damage, respiratory problems and lung cancer have been reported with nickel and chromium exposure [146]. Health effects that are associated with chromium depend on the oxidation state of the metal. Chromium (VI) compound is considered carcinogenic to human beings whereas chromium III is not carcinogenic to humans [147].

All heavy metals analysed can lead to reduced or damaged central nervous system, kidney, liver, lungs and other important organs. The results show that toxic heavy metals such as lead and arsenic were detected in all samples. US FDA has set the maximum contaminant limit for Pb in cosmetic products at 10 mg/kg. Table 9 below is a summary of the results and it shows that all samples exceed the maximum contamination limit for Pb set by US FDA. All samples that were analysed for As are below the recommended maximum contaminant limit by US FDA. Nonetheless, heavy metals have bioaccumulative properties in the human body hence skin lightening products can be regarded as substantial sources of heavy metals. The use of skin whitening products containing heavy metals has been discovered to have serious consequences for humans, particularly small children and pregnant women. Pb may pass the placenta and poison the unborn child [121].

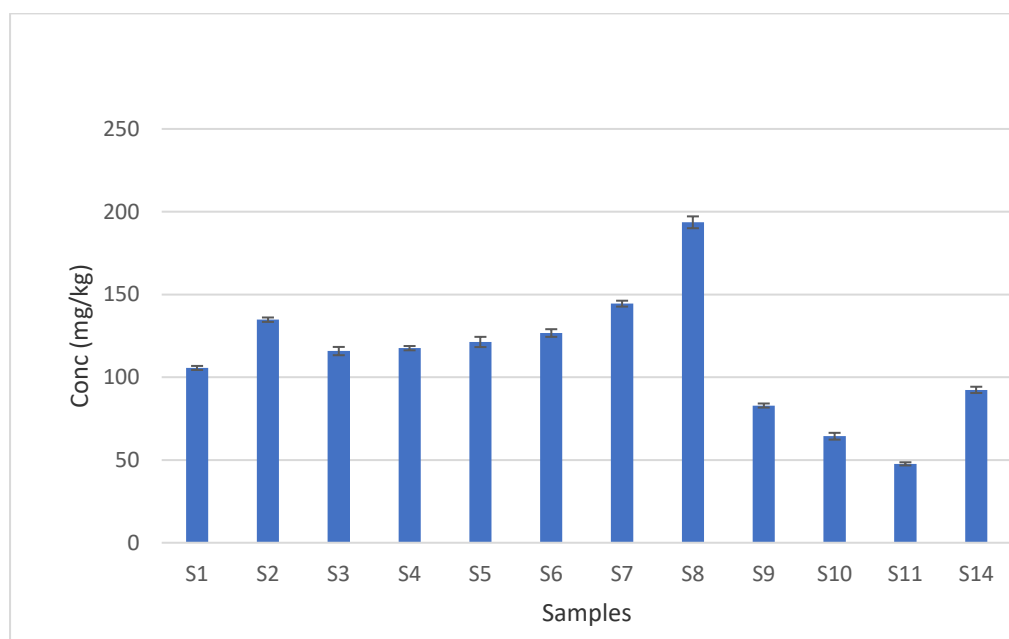


Figure 19. Concentration of Pb on cosmetic samples

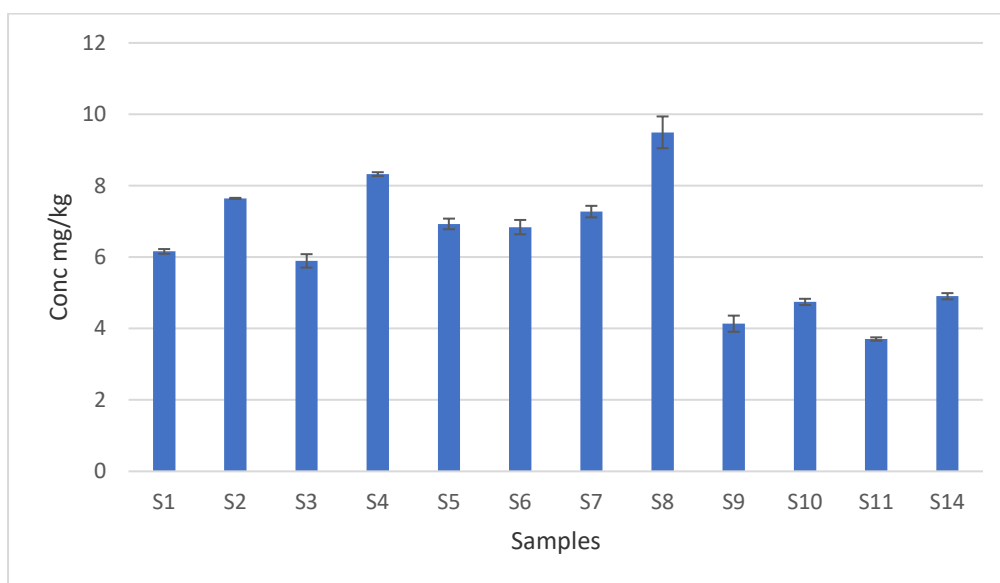


Figure 20. Concentration of Ni on cosmetic samples

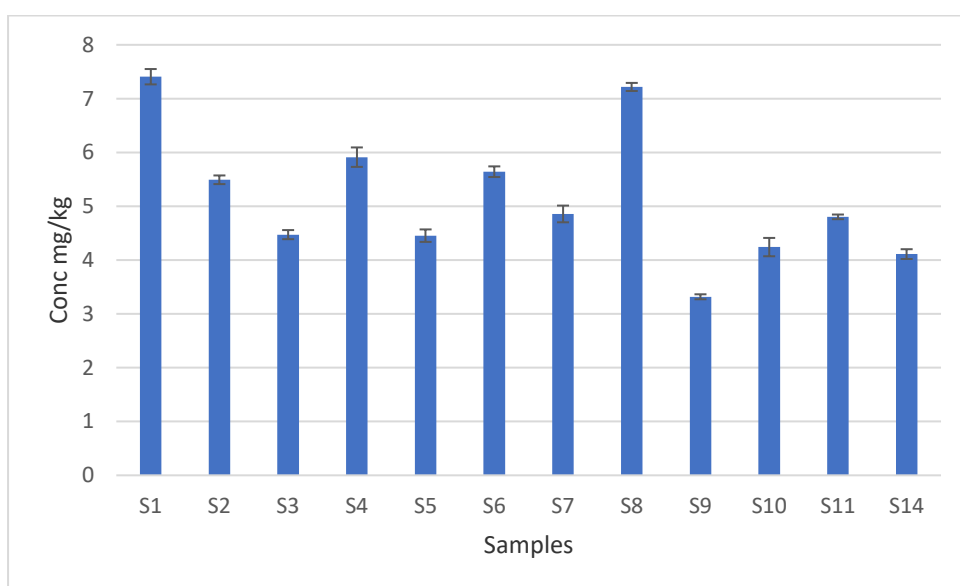


Figure 21. Concentrations of Cr on cosmetic samples

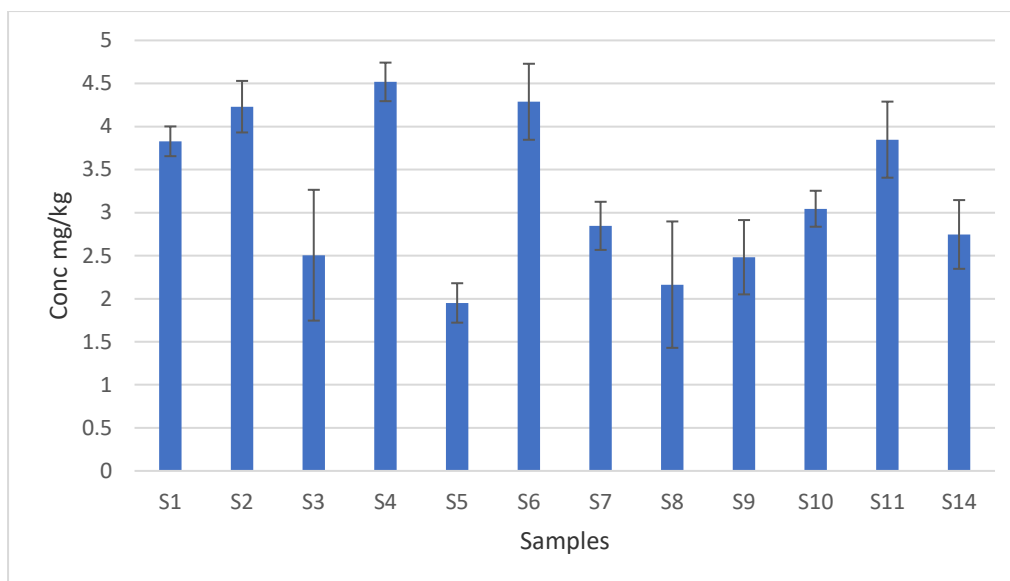


Figure 22. Concentration of As on cosmetic samples

Table 9. Levels of heavy metals in skin lightening products sold in Botswana market

Sample ID	Pb(mg/kg)±SD	As(mg/kg)±SD	Ni(mg/kg)±SD	Cr(mg/kg)±SD
S1	105.60±1.22	3.83 ±0.17	6.16±0.07	7.41±0.14
S2	134.75±1.34	4.23±0.30	7.65±0.01	5.49±0.08
S3	115.80±2.50	2.51±0.0.76	5.90±0.19	4.47±0.08
S4	117.55±1.29	4.52±0.22	8.33±0.05	5.91±0.18
S5	121.30±3.09	1.95±0.23	6.93±0.15	4.45±0.12
S6	126.70±2.32	4.29±0.44	6.84±0.20	5.64±0.90
S7	144.45±1.78	2.85±0.28	7.28±0.16	4.86±0.16
S8	193.60±3.58	2.16±0.73	9.50±0.45	7.22±0.07
S9	82.90±1.23	2.48±0.43	4.14±0.23	3.32±0.05
S10	64.35±2.05	3.04±0.21	4.75±0.09	4.24±0.17
S11	47.65±0.96	3.85±0.44	3.71±0.05	4.80±0.04
S12	92.35±1.89	2.75±0.40	4.91±0.09	4.11±0.09
S13	45.75±0.10	2.66±0.30	2.99±0.09	3.72±0.01
S14	148.95±1.97	3.13±0.09	7.22±0.21	4.26±0.04

4.3.1 Health Risk Assessment

A health risk assessment was performed by computing the hazard quotient, DDE and ADI and the results are shown in the tables below. The findings show that the hazard quotient for As for all samples was below 1 indicating that the exposure is likely to cause no adverse health effects. However, the hazard quotient for Pb for all samples in this study was greater than 1 indicating the exposed population is likely to experience adverse health effect. On the other hand, 35.7% and 64.3% of samples analysed for Cr and Ni respectively had hazard quotient greater than 1. The calculated values for DDE for the different metals are presented on table 10, 11, 12 and 13 below. Pb had the highest values of DDE values than other metals ranging from 70 461.16 – 229 381.40 mg/kg. The toxicity of heavy metals in human beings is dependent on the average daily intake of the metal. The mean ADI concentrations of Pb, As, Cr and Ni were calculated as 0.85, 0.024, 0.039 and 0.047 mg/kg respectively. Pb had the highest value of ADI and As had the lowest ADI value. The health risk index was greater than 1 in samples that had concentrations of Pb, As and Ni but it was lower than 1 for chromium. If health risk index is greater than it indicates a potential health risk and protective measures are to be taken.

Table 10. Health risk assessment for Pb

Lead				
Sample ID	HQ	DDE (mg/day)	ADI (mg/kg)	HRI
S1	10.56	162622.46	0.82	204.33
S2	13.47	207513.46	1.04	260.73
S3	11.58	178330.46	0.89	224.06
S4	11.75	181025.46	0.91	227.45
S5	12.13	186800.46	0.94	234.71
S6	12.67	195116.46	0.98	245.15
S7	14.44	222451.46	1.12	279.50
S8	19.36	298142.46	1.50	374.60
S9	8.29	127664.46	0.64	160.40
S10	6.43	99097.46	0.50	124.51
S11	4.76	73379.46	0.37	92.20
S12	9.23	142217.46	0.71	178.69
S13	4.58	70461.16	0.35	88.53
S14	14.89	229381.46	1.15	288.21

Table 11.Health risk assessment for As

Arsenic				
Sample ID	HQ	DDE (mg/day)	ADI (mg/kg)	HRI
S1	0.38	5895.67	0.030	98.77
S2	0.42	6514.75	0.033	109.14
S3	0.25	3858.25	0.019	64.64
S4	0.45	6958.27	0.035	116.57
S5	0.20	3004.32	0.015	50.33
S6	0.43	6602.53	0.033	110.61
S7	0.28	4383.39	0.022	73.43
S8	0.22	3331.57	0.017	55.81
S9	0.25	3822.06	0.019	64.03
S10	0.30	4689.08	0.024	78.55
S11	0.38	5924.93	0.030	99.26
S12	0.27	4229.39	0.021	70.85
S13	0.27	4094.64	0.021	68.60
S14	0.31	4817.67	0.024	80.71

Table 12.Health risk assessment for Ni

Nickel				
Sample ID	HQ	DDE (mg/day)	ADI (mg/kg)	HRI
S1	1.23	9486.40	0.048	2.38
S2	1.53	11773.30	0.059	2.96
S3	1.18	9078.30	0.046	2.28
S4	1.67	12820.50	0.064	3.22
S5	1.39	10672.20	0.054	2.68
S6	1.37	10533.60	0.053	2.65
S7	1.46	11203.50	0.056	2.82
S8	1.90	14622.30	0.073	3.67
S9	0.83	6367.90	0.032	1.60
S10	0.95	7308.07	0.037	1.84
S11	0.74	5706.47	0.029	1.43
S12	0.98	7553.70	0.038	1.90
S13	0.60	4600.75	0.023	1.16
S14	1.44	11111.10	0.056	2.79

Table 13. Health risk assessment for Cr

Chromium				
Sample ID	HQ	DDE (mg/day)	ADI (mg/kg)	HRI
S1	1.48	11407.24	0.057	0.038
S2	1.10	8458.14	0.043	0.028
S3	0.89	6888.11	0.035	0.023
S4	1.18	9104.94	0.046	0.031
S5	0.89	6858.85	0.034	0.023
S6	1.13	8689.14	0.044	0.029
S7	0.97	7480.24	0.038	0.025
S8	1.44	11114.64	0.056	0.037
S9	0.66	5109.41	0.026	0.017
S10	0.85	6531.60	0.033	0.022
S11	0.96	7396.31	0.037	0.025
S12	0.82	6330.63	0.032	0.021
S13	0.74	5731.57	0.029	0.019
S14	0.85	6567.79	0.033	0.022

4.4. Determination of Hydroquinone Content

The determination of hydroquinone content of the samples was performed using RP-HPLC and the chromatogram of hydroquinone is shown on figure 23 below. The concentrations ranged from 0.06 to 7.65 %. Two of the skin lightening creams (S13 & S14) had concentrations of hydroquinone higher than the 2% set by the US FDA [16]. These results do not vary much from those obtained in other studies which reported hydroquinone contents ranging from 0-6% [116]. Even though these values appear low there is high health risk associated with the sale of skin lightening products, since some of them exceed the maximum contaminant limit set by US FDA. 12.5 % of the samples analysed contained concentration of hydroquinone higher than 2% limit set by US FDA while 8% of samples analysed for hydroquinone in Nigeria were found to exceed the 2%. They recorded the concentration of hydroquinone ranging from 0.001 to 3.45% in 50 cream samples [2]. The application of skin lightening cosmetics may result in renal damage as well as blood malignancies such as leukaemia. When hydroquinone-containing skin lightening cosmetics are applied to the skin, hydroquinone is absorbed into the circulatory system and eventually eliminated via the kidney.

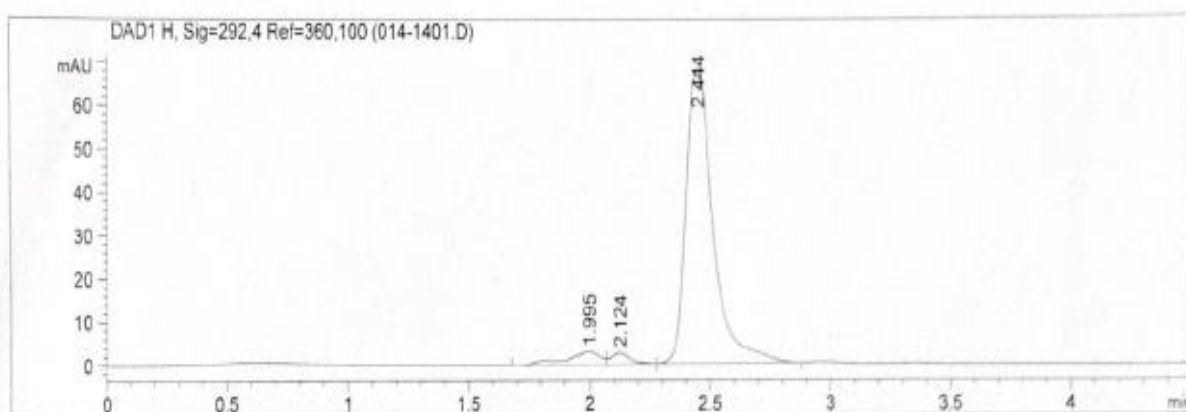


Figure 23. Chromatogram of hydroquinone

Table 14. Levels of Hydroquinone in cosmetic samples

Sample ID	HQ (%)
S1	ND
S2	ND
S3	ND
S4	ND
S5	ND
S6	ND
S7	ND
S8	0.06
S9	0.21
S10	ND
S11	ND
S12	ND
S13	5.27
S14	7.65
S15	ND
S16	ND

ND= Not Detected

Conclusion

A number of skin lightening products that are commonly available in Botswana market were investigated in this study. None of the skin lightening creams are manufactured in Botswana. The skin lightening creams were imported from Italy, Spain, south Africa, Guangzhou, Switzerland and DRC. A total of fourteen creams were analyzed for heavy metals using ICP OES and sixteen creams were analyzed for hydroquinone using HPLC.

All the skin lightening creams were screened for Pb, As, Cr and Ni using X-ray Fluorescence analyzer which showed the presence of all the heavy metals in the skin lightening creams. The samples were further analyzed with ICP OES for quantitative analyses of Pb, Ni, As and Cr. The correlation coefficient for different heavy metal calibration curves were 0.9839, 0.9812, 0.9833 and 0.9639 for Pb, As, Ni and Cr respectively. The concentration of Pb, Ni, As and Cr in the samples ranged from 45.75 mg/kg-193.60 mg/kg, 2.99 mg/kg-9.50 mg/kg, 1.95 mg/kg-4.32 mg/kg, and 3.34 mg/kg- 7.41 mg/kg respectively. The hazard quotient for As was found to be below 1 while for Pb it was greater than 1. It was also found that 64.3% and 35.7% of the samples had hazard quotient greater than 1 for Ni and Cr respectively. Pb had the highest DDE values than other heavy metals. The mean ADI concentrations of Pb, As, Cr and Ni were found to be 0.85, 0.024, 0.034 and 0.047 respectively. The health risk index was greater than 1 for Pb As and Ni but less than 1 for Cr. The hazard quotient and health risk index that are greater than 1 indicates that the exposure is likely to cause adverse health effects. The concentration of hydroquinone ranged from 0.06- 7.65 % in four creams and in all samples, hydroquinone was not detected. Two of the creams had hydroquinone concentrations above the US FDA threshold limit of 2%. This is very alarming and dangerous to people using these creams hence the need to sensitize the public and also set more stringent measures to monitor the importation of skin lightening products in Botswana.

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