CHAPTER ONE

INTRODUCTION, PRELUDE, LITERATURE REVIEV, JUSTIFICATION, OBJECTIVES AND LAYOUT OF THE THESIS

CHAPTER ONE

INTRODUCTION

1.1. Prelude

Several metal ions such as sodium, potassium, magnesium, and calcium are essential to sustain biological life. At least six additional metals, chiefly transition metals are also essential for optimal growth, development, and reproduction, i.e. manganese, iron, cobalt, copper, zinc, and molybdenum.

An element, which is required in amounts smaller than 0.01% of the mass of the organism, is called a trace element. Trace metals function mostly as catalysts for enzymatic activity in human bodies. However, all essential trace metals become toxic when their concentration becomes excessive. Usually this happens when the levels exceed by 40-200 fold those required for correct nutritional response. In addition to the metals essential for human life, our diet including the water we drink and the air we breathe may contain toxic metals like mercury, lead, cadmium, chromium, silver, selenium, aluminium, arsenic, and barium. These metals can cause chronic or acute poisoning and should be eliminated as much as possible from the living environment (Mahajan et al., 2005).

Interest in trace element research in clinical medicine, biology, environmental studies, toxicology, and nutrition has become an exciting frontier, and during the last two decades the number of publications on this subject has progressively increased. Recent developments in instrumentation have lowered the limits for determining many trace elements to the low ranges, thus enabling determination of parts per billion (ng/g) and, in some cases, even less (Amos et al., 1971).

The degree of contamination of the organism by metals depends on their levels in the environment as well as on the effectiveness of all the ways in which they are transported and absorbed by the organism. A number of tissues in human body such as the kidney and liver can be used for metal analysis, but these are not easily accessible in living individual. Exposure of an organism to metals can be monitored by determining their concentrations in urine, blood, nails and hair. The hair is a good biological indicator because of the simplicity of sampling and storage. Hair analysis usually leads to higher concentrations of analytical solutions than those in biological fluids (Naginiene et al., 2002; Yoo et al., 2002).

Moreover, there is a correlation between blood or other tissue levels of some metals and those seen in hair. However, hair reflects to a certain extent the total uptake rather than a current level of the metal in the organism. Accumulation of metals in the hair can be endogenous (from circulating blood), or exogenous (from ambient air, soil, dust, shampoo, etc.).A clear distinction between both ways cannot always be made (Vahter et al., 2002).

1.2. Literature review

Age, sex, site of growth and colour of hair are factors affecting the level of endogenous metals. Younger subjects usually have higher metal levels in hair than older ones. Females usually have higher levels than males. Levels of iron, copper, nickel and zinc were higher in red hair compared with brown; whereas mercury was lower. Site of growth (scalp or pubis) significantly influenced the concentration of several elements (Merzenich et al., 2001).

Exogenous metals can be adsorbed and/or bounded to hair. Adsorbed metals can be removed to some extent by several washing procedures depending on the metal and the procedure applied (Case et al., 2001; Maurice et al., 3002). A relationship

has been established between the mean levels of metals in scalp hair and environmental exposure (Seifert et al., 2000) and health status (Naginiene et al., 2002; Miekeley et al., 2001).

The chemical analysis of human hair has some practical values today. However, this remains extremely limited with respect to the assessment of the mineral status of the individual subject (Hambidge, 1982).

Micronutrients form an intricate yet integral part of human body and analysis of hair, in terms of their properties, gives us volumes of information on diseases, metabolic disorders, environmental exposures, and nutritional status (Maurice et al., 3002; Takagi et al., 1988).

In most instances, the demonstrated usefulness of hair analysis has been confirmed to the comparison of population groups and, even then, the physiological or pathological significance of statistically significant differences is frequently obscure (Hambidge, 1982).

Evaluation of trace metals in human tiussues such as hair and nails has proven useful in the studies pertaining to chronic body exposure. These have also been suggested as indexes to evaluate environmental exposure by toxic trace metals. Trace element profile of human hair has been uniquely linked to an individual's identity much the same way as finger prints. Since this tissue after a rapid growth remain somewhat isolated from the metabolic activities of the human body, trace elements accumulated in it reflect largely chronic exposure (Takagi et al., 1988).

Since ancient times, the nails growth is used as an indicator of health and physiological imbalances of human. The fingernails and toenails are used as indication of aging, in addition of various properties, such as thickening, thinning, discoloration, splitting, grooves concave and convex shape so that flatness can be used as indicate disease in the body, nutrient deficiencies, drug reaction and poisoning or nail injury. The nails thickness change or loosened and infected with bacteria are illness signed of certain disease (Suhonen et al., 1999). Human nail is permeable than skin and the composition consists of 7% - 12% of water, so that it is a solid part in body. Mechanical pressure on nails can cause harmful pain; also the nails are affected with stretched, tight and cosmetics. Nails after growth is remain isolated from other metabolic activities in the human body, which is considered as a good reflection of long-term exposure (Takagi et al., 1988). The advantages of nails in elements evaluation are preferable biologic medium because of ease of collection, storage convenience, ease of handling and reproducibility of later analysis results. The nails from various fingers in feet and hands are growth in several weeks of time between formation and clipping and that indicates exposure to elevated concentration contamination integrated over a 2 - 12 month period (Hunter, 1990). The trace elements levels in nails are subject of interest in the biomedical and environmental sciences since recent years. Nails measurement remains the subject of interest as indices for assessing nutritional status, diagnosing diseases, identifying systemic intoxication and environmental exposures. The determination of elements contents in the nails can be considered as an indicator of level in other tissues and that reflect mineral metabolism in the body (Kanabrocki et al., 1979; Abdulrahman et al., 2012). The measurement of elements in nails is used as biomarker for exposure, so that the appropriate selection of these elements is of critical importance for health care management purposes, public health decision making, and primary prevention activities (Garland et al., 1993).

1.3. Justifications

Several epidemiological studies have demonstrated that exposure to metals has so much toxic and carcinogenic effects on humans.

The concentration of trace elements and heavy metals has not been fully investigated among our local population, therefore this study aimed to determine the level trace elements and heavy metals using available resources to achieve the cost effectiveness.

1.4. Objectives

1.4.1. Hypothesis

Can we determine by physics whether there is environmental pollution or not?

The answer is yes, we can

1.4.2. General objective

To find out the degree of environmental pollution by physical techniques.

1.4.3. Specific objectives

1. To assess our method in determining the concentration of metals in hair and nails.
2. To determine whether there is a difference or no in the degree of environmental pollution between different places within western Kordufan state.
3. To determine the effect of environmental pollution with respect to sex, age and the hair colour.
4. To find out is there any discrepancy between hair and nails in the degree of metal absorption.

1.5. Layout of the thesis

- The first chapter provides a short introduction to elemental concentration in hair and finger nails as well as their measurements with different techniques. The justifications of the research and its objectives are explained.
- **In chapter 2** we review literature and provide historical background of spectroscopic measurements and the information needed throughout this work which is put into perspective.
- **Chapter 3** describes the general structure, study design, data collection, sample process and data analysis.
- **Chapter 4** explores the results of data collected.
- **In chapter 5 we** discusses the results that were obtained, gives some conclusion remarks and recommendations for future work.
- The thesis ended with a lengthy list of relevant reference written in Harvard's style of referencing.

CHAPTER TWO

Theoretical background
and
previous studies

CHAPTER TWO

2. Theoretical background and previous studies

2.1. Historical background

The concept of biological monitoring has been practiced by the medical profession for decades; however, the formal application of this technique on a broad scale to industrial hygiene is attributed to Elkins (1954). Elkins subsequently attempted to correlate exposure level and urinary level with toxicity of chemicals. Since then, the concept of biological monitoring for trace elements and xenobiotics has been extended to include monitoring of the bile and feces, exhaled air, blood, perspiration, tears, nails of fingers and toes, hair, milk, teeth, and saliva (Gude, 2011).

2.2. Advantages and disadvantages of hair and nails analysis

In comparison to other biological samples, there are many advantages in utilizing nails as a biomarker of toxic element exposure and nutritional mineral status. These include (1) the ease with which nails can be sampled, stored, transported, and handled, (2) standardized methods are available for collection, washing, preparation, sophisticated instrumental analysis with use of quality control samples for precision and accuracy; (3) storage of aliquots of sample is simple for reanalysis and toenail measure of arsenic (As) is reproducible over a period of several years; (4) once elements are incorporated into keratin of nails, the levels remain isolated from other metabolic activities in the body with no

fluctuation in element levels sue to changing body metabolic activities, unlike the blood; (5) nails, which conserve the pattern of trace element composition beyond the life span of the cells and because of their resistance to decay, are exceptionally well suited for long- distance transport; also, a large number of specimens can be procured and stored, a requirement for population studies; (6) toenails take several months to year to grow out and are used for the measure of past exposure; (7) a number of trace elements are considerably more concentrated in nails than in urine or blood; and (8) the ability to analyze nail content of several elements (Gude, 2011).

Hair is formed from a cluster of matrix cells that make up the follicle. During the growth phase of the hair, metabolic activity is greatly increased, exposing the hair to the internal metabolic environment; extracellular fluids, circulating blood and lymph. As the hair reaches the surface, its outer layers harden, locking in the metabolic products accumulated during this period of hair formation, providing a permanent record of metabolic activity. Determining the levels of the elements in the hair is a highly sophisticated analytical technique, when performed to exacting standards and interpreted correctly (Ahmad, 2013).

Hair analysis has several potential advantages. Sample collection is simple and physically atraumatic. The ease of storage and transportation compares favorably with most other sample materials. The relatively high concentrations of several trace elements in hair facilitate analyses, including simultaneous multi-element analyses. Thus, there are a number of reasons why the value of hair analysis merits careful investigation. However, none of these potential advantages is of any value in practice unless the results can be interpreted in a manner that is relevant to the health of the individual subject (Sukumar, 2006).

2.3. Available analysis methods

Techniques for the analysis of trace elements have developed rapidly in response to the increasing need for accurate measurements at extremely low amount of contents in diverse matrices (Brown et al., 2005). Many elements occur in a variety of matrices at sufficiently low levels that, when instrumental analytical methods were first developed in the nineteenth century, they were undetectable (Mc Naught and Wilkinson, 1997). As analytical technology improved, and it became known that elements were present at these very low levels, the term ‘‘trace’’ was coined to describe them. Although modern analytical methods allow accurate, repeatable determination of elements at such low levels, the generic terms ‘‘trace’’ and ‘‘trace element’’ are still in use. The boundaries of trace analysis are described by the definition of ‘‘trace element’’ in the Compendium of Chemical Terminology, Second Edition (Mc Naught and Wilkinson, 1997): ‘‘any element having an average concentration of less than about 100 parts per million atoms or less than 100 μg/g’’. As analytical techniques have become more sophisticated, detection capabilities have improved and, in several fields, this upper boundary of the definition of ‘‘trace’’ is now too far away from the capabilities of analysis that new terms such as ‘‘ultra trace analysis’’ are in common use. There is no agreement about the range of ultra trace analysis and no rigorous definition. Within the literature, the term is used for the definition of elements at mass fractions less than 10 to-6 and 10 to -8 g/g (1 ppm and 10 ppb) (Ahuja et al., 2015).

There are very few methods for determining trace elements in hair and nails. The reliability of any analyte determination is affected by the extent of contamination during collection, containment, processing, and analysis of the specimen. It is also influenced to a considerable extent by the accuracy with which a value may be

assigned to the standards and (or) controls and the stability of the specimens, standards, and controls during containment (Anand et al., 1975).

The development of analytical instrumentation over the past 30–40 years has allowed us not only to detect trace metals level, but also to know its valency state, biomolecular form, elemental species, and isotopic structure. Lead was the most commonly studied of all the trace elements and the techniques that developed early in time are mostly described on the basis of their lead estimation capacities (Thomas, 2002).

2.3.1. X-Ray Fluorescence (XRF) Technique

X-ray techniques have been used widely for trace element analysis. These have found useful applications in analysis of geological materials, steels, cements, archeological samples, forensic samples and environmental samples. A widely used technique in this domain is X-Ray fluorescence (XRF), which is non-destructive and can be used to analyze almost any element in the periodic tablefrom Flourine (Z=9) upwards (Birks, 1976).

The sensitivity of XRF depends upon the energy of incident radiation, geometry of the instrument used and the efficiency of the detector. The overall precision of the XRF measurement is usually limited by the statistics of the detected photons. The limit of detection depends upon sensitivity of the instrument and background level of sample matrix being analyzed (Dean, 2003). The lower limit of detection is expressed by the relation (Jenkins and Vries, 1970):

$$\textit{Lower limit of detection (LLD)} = \frac{3}{M}\sqrt{Rb/Tb} \qquad \text{eqn.} \rightarrow \mathbf{1}$$

where, Rb is count rate at the background, Tb is time spent counting background and M is sensitivity of the spectrometer.

Samples to be analyzed by XRF can be coal fly ash, vegetation sample like plant samples, sand, water etc. As an illustration, in vegetation sample, XRF technique complies with desired features like the possibility to perform analysis directly on solid sample. It can be applied to Chips/Films of Nail Polishes (Misra et al., 1992). It is less time consuming, requiring less amount of test sample, having multi element capability with wide dynamic range (Margui et al., 2005). The main drawback of XRF instrumentation, restricting more frequent use of the technique for environmental purposes is its limited sensitivity for some pollutants like Pb and Cd and poorer accuracy and precision as compared to atomic absorption spectroscopic techniques. However with the advent of some digital signal processing techniques, precision and accuracy especially in environmental samples are improved drastically (Margui et al., 2007).

Two configurations of XRF spectrometers are widely used viz. energy dispersive X-ray fluorescence (EDXRF) and wavelength dispersive X-ray fluorescence (WDXRF). EDXRF as analytical tool was used for monitoring of urban air pollution in Chandigarh. Aerosol samples were collected on 47mm diameter, 0.8μm pore size cellulose nitrate filter papers. The elemental concentrations were determined using fundamental parameter approach (Bandhu et al., 1998). Pb in aerosol samples from Mexico valley was determined using EDXRF. This technique being quick, reliable and non-destructive was used and compared with atomic absorption spectrometric technique and a good correlation was obtained (Chibowsski, 2000). Heavy metal contents in fruits and vegetables from Lublin, Poland were analysed using EDXRF. The XRF spectrometer by Canberra was equipped with Si (Li) detector and A/D conversion Canberra 1510 detector. X ray radiation was induced by 109Cd, 55Fe and 241 Am. Quantitative analysis was done with AXIL software (Richard, 2001).

Sensitivity of XRF instrument is defined as being the net intensity obtained per unit of concentration. For calculating it, the peak intensities of the analyte have to be measured on certified reference materials (erroneously known in popular language as standards) of composition similar to that of the unknown samples to be analyzed. To calculate the sensitivity, the measured intensities must not be corrected for matrix effects and one must assume a linear relation between intensity and concentration. The sensitivity for each analyte i is calculated from the slope mi of the calibration line as follows. The general form of the equation for a straight line is

$$Y = mX + b \qquad \text{eqn.} \rightarrow \quad 2$$

If the calibration line is a plot of the peak intensity I_p of an analyte i as a function of the concentration C_i, the equation (**2**) becomes:

$$I_p = m_i C_i + I_b \qquad \text{eqn.} \rightarrow \quad 3$$

where the true background intensity Ib is given by the intercept of the calibration line on the Y axis. The slope mi is then given by;

$$m_i = \frac{I_p - I_b}{C_i} \qquad \text{eqn.} \rightarrow \quad 4$$

where C is the concentration of the analyte i in % or ppm. It is the slope of the calibration line that enables to convert measured net intensities into concentrations (Lartigue et al., 2001).

The elemental concentration of Uranium in the samples collected from ground and canal water in Bathinda district of Punjab state, India have been investigated using EDXRF technique. The residue obtained after drying water sample were analyzed using EDXRF spectrometer consisting of Mo anode X- ray tube equipped with selective absorber as an excitation source and Si (Li) detector (Alrakabi et al., 2012).

Maha Bashir Omer (2015) in local study of M.Sc. in Nuclear Sciences & Technology thesis entitled "Assessment of Some Heavy Metals in Fruit from Local Market in Khartoum State" used XRF spectrometer system. She concluded that high concentrations obtained may probably be attributed to pollutants in the high ways traffic or stocking processes, and recommended future conduction of a national survey.

2.3.2. Atomic spectroscopic (AS) techniques

Atomic spectroscopy refers to several methods which are used for spectroscopic determination of elements:

1. Atomic absorption spectroscopy (**AAS,** flame and graphite furnace)
2. Atomic emission spectroscopy (**AES**, flame)
3. Optical emission spectroscopy with inductively coupled plasma **(ICP-OES**)
4. Mass spectrometry with inductively coupled plasma (**ICP-MS**)

These methods are used for quantitative and qualitative determination of many of the elements in the periodic table. Atomic spectroscopy is used in particular for the detection of trace metals in foods, in dyes and pigments, or in drinking water and river water (Bings et al., 2013).

As recently as the early 1960s, trace elemental determinations were predominantly carried out by traditional wet chemical methods such as volumetric, gravimetric, or colorimetric assays. It wasn't until the development of atomic spectroscopic (AS) techniques, in the early to mid-1960s that the clinical community realized that they had a highly sensitive and diverse trace element technique that could be automated. Every time there was a major development in AS, trace element detection capability, sample throughput, and automation dramatically improved (Thomas, 2002). The developments and recent breakthroughs in atomic spectroscopy have directly affected our understanding of the way trace metals interact with the human body. The major element studied in humans was lead for a long time and various methods were devised for this purpose (Mahajan et al., 2005).

2.3.3. Dithizone colorimetric

Lead assays were initially carried out using the dithizone colorimetric method, which was sufficient for the time (late 1950s, early 1960s), but was very slow and labor-intensive. It became more automated with the development of anodic stripping voltammetry (Thomas ,2002), but blood lead analysis was not considered a truly routine method until AS techniques became available (Mahajan et al., 2005).

2.3.4. Flame atomic absorption (FAA)

Flame atomic absorption spectrometry works by introducing the sample into a flame where it is dissociated into its constituent atoms. Electromagnetic radiation in the UV/Visible part of the spectrum is directed through the flame and is partially absorbed in a manner characteristic of the atoms present. FAAS is relatively

inexpensive and simple to operate. It encounters little interference. However, some refractory elements cannot be determined with good sensitivity because flame temperatures are often not hot enough to induce complete atomisation. At its worst, this problem means that trace levels of B, W, Ta, Zr, As and Sn may not be determined by FAAS. FAAS can be used for the analysis of liquid samples only and relatively large sample volumes are required.

FAAS is also a relatively slow technique and is best used when only single elements or a few elements are to be determined within a sample. When a large number of elements require measurement, other techniques may be substantially quicker.

The presence of interferences, such as overlapping peaks from interfering species or incomplete atomisation of non-analyte species, can confer positive bias on measurement results. Incomplete atomisation of the target element itself may result in a negative bias when using these techniques. This is exacerbated when there are present matrix effects that often suppress the signal from a known quantity of element within the sample, as compared to the same amount of element in a calibration standard (Vandecasteele and Block, 1993).

Characteristic concentration in atomic absorption (sensitivity) is defined as the concentration of an element (expressed in mg/l) required to produce a signal of 1% absorption (0.0044 absorbance units). As long as measurements are made in the linear working range, characteristic concentration can be determined by reading the absorbance produced by a known concentration of the element, and solving the following equation:

$$\textit{Characteristic Concentration} = \frac{(Conc.\ of\ Std.\ \times 0.0044)}{Measured\ Abs.} \qquad \text{eqn.} \rightarrow 5$$

The characteristic concentration values for each element at different primary wavelengths are listed in the Standard Conditions section. Knowing the expected characteristic concentration allows the operator to predict the absorbance range which will be observed for a known concentration range of the element of interest. The characteristic concentration check value is the concentration of element (in mg/l) that will produce a signal of approximately 0.2 absorbance units under optimum conditions at the wavelength listed.

Using the characteristic concentration check, the operator can determine whether instrumental parameters are optimized and whether the instrument is performing up to specifications.

The detection limit is defined as the concentration of the element which will produce a signal/noise ratio of 3. Thus, the detection limit considers both the signal amplitude and the baseline noise and is the lowest concentration which can be clearly differentiated from zero. The standard procedure for establishing detection limits by flame atomic absorption is as follows: Two concentrations of the element are prepared, with entirely separate volumetric glassware used for each to reduce the possibility of contamination to a minimum. The absorbance means of the two are established as explained below. The lower concentration standard is made approximately 5× the expected detection limit, and the second standard is made twice this concentration. After establishing what are considered to be optimum conditions, take a reading for each standard alternately, ten or more times. A blank reading (solvent only) is made between each standard reading. The sequence is: blank, low-concentration standard, blank, high concentration standard; repeat the sequence.

Having obtained the data, make the calculation as follows:

1. Average the two blank readings taken immediately before and after each standard and subtract from the standard reading.
2. Calculate the mean and standard deviation for the set of corrected high-standard readings. Do the same for the set of corrected low standard readings.
3. If the ratio of the means does not correspond to the ratio of the concentration prepared to within statistical error, reject the data.
4. If the data pass the ratio-of-the-means test, calculate the concentration detection limit as follows:

$$\text{Detection Limit} : \frac{Standard\ Conc. \times 3\ Std.\ Dev.}{Mean} \qquad \text{eqn.} \rightarrow 6$$

The calculation is made independently for each standard concentration, and the detection limit is the average of the two results.

Routine analytical measurements at the detection limit are difficult because, by definition, noise makes up a significant percentage of the total measurable signal. By definition, the precision obtained at detection limit levels is ±33% when a 3-standard-deviation criterion is used. Therefore, while it is possible to distinguish analyte concentrations at the detection limit from zero, for good precision it is necessary to limit routine analytical work to concentrations higher than the detection limit. It is important to remember that characteristic concentration expresses the size of the absorption signal, the detection limit considers both the signal amplitude and the baseline noise. It is possible to have the same characteristic concentration, but different detection limits (Ahuja et al., 2015).

Flame atomic absorption spectrometry (FAAS) is a mature analytical method, which is present in almost any analytical laboratory as a working horse for elemental determinations of elemental metals (Bings et al., 2013).
Flame atomic absorption spectrometry (FAAS) with its relative low cost and good analytical performance, is the main instrument in the research laboratories for determining a variety of heavy metals. Accurate determination of traces heavy metals by flame atomic absorption spectroscopy is the one of the important problem for the analytical chemist because of their low concentrations. Also other important problem in FAAS determinations of heavy metals is influences of the matrix of the analyzed samples (Divrikli et al., 2007).

When flame atomic absorption (FAA) was first developed, an elevated blood lead level was considered to be 60 μg/dL (600 ppb), well above the FAA detection limit of 2 μg/dL (20 ppb) at the time. But when preparation of the blood samples was taken into consideration, FAA struggled to meet this level. Preparation typically involved either dilution with a weak acid followed by centrifuging and filtering, acid digestion followed by dilution and centrifuging and filtering, or, more recently, dilution with a strong base such as tetramethylammonium hydroxide (TMAH). When sample preparation was factored into the equation, the elevated blood lead concentration of 60 μg/dL was reduced to 2–5 μg/dL (20–50 ppb)—virtually identical to the FAA detection limit (Thomas, 2002).

Aqueous samples can be generally introduced for analysis directly and without any prior treatment. The only major problem associated with working with solutions is their collection and storage. Concerning atomic spectroscopic analysis itself, no particular precautions have to be taken. Non-aqueous samples can sometimes be run directly, but this depends significantly on their viscosity. In FAAS analysis, the

viscosity should be similar to that of water for which most nebulizers are designed. Only some organic solvents, such as ethanol or methyl isobutyl ketone, are often used for dilution of organic liquids, the major drawback, encountered with these techniques is the dilution factor, which reduces the metal content per unit volume (Bader, 2011).Standards can be prepared in the pure solvent. Elements in organic solvents usually give an FAAS analysis response similar to that given by the same element in aqueous solution (Bader, 2011; Mahajan et al., 2005;Thomas, 2002).

Solid samples are usually analyzed by dissolving the sample to form a liquid solution that can be introduced into the flame or furnace. Dissolution can be accomplished by mineral acid digestion (wet ashing), fusion of solids with molten salts and dissolution of the fusion bead, dry ashing of organic solids with acid dissolution of the residue, combustion in oxygen bombs, and other procedures too numerous to mention. The main disadvantage of acid dissolution is the risk of loss and contamination particularly at ppm and ppb levels of analyte.
Dissolution is still the most common approach to atomic absorption spectrometry (AAS) analysis of solids. Solid particulates in air, gas and fluid streams can be collected by filtration and analyzed by digesting the filter and the collected particulates (Anwar et al., 2004).

There are many different techniques which could be used in order to separate and pre-concentrate the metal ions such as chromatography (Bader, 2010; Rathore et al., 2006), solid phase extraction (Gama et al., 2006; Ganjali et al., 2004; Mashhadizadehet al., 2004; Mashhadizadeh et al., 2008; Shemirani et al., 2004,), cloud point extraction (Ghaedi et al., 2009; Giné et al., 2008; Matoset al., 2009; Ojeda et al., 2010), and Co-precipitation (Bader, 2011; Sato and Ueda, 2001).

2.3.5. Delves Cup

An accessory called the Delves Cup was developed in the late 1960s to improve the detection limit of FAA (Thomas 2002). The Delves Cup approach used a metal crucible or boat usually made from nickel or tantalum, which was positioned over the flame. The 10-100μL sample was pipetted into the cup and the heated sample vapor passed into a quartz tube, which was also heated by the flame. The ground state atoms generated from the heated vapor were concentrated in the tube and therefore remained in the optical path for a longer period of time (Mahajan et al., 2005).

This resulted in much higher sensitivity and lower detection capability, which meant that the elevated blood lead level of 60 μg/dL could be detected with comparative ease. Because of its relative simplicity and low cost of operation, the Delves Cup became the standard method for carrying out blood lead determinations for many years. Unfortunately, the Delves Cup approach was found to be very operator-dependent and not very reproducible; sometimes, it involved complicated sample preparation and required calibration with blood matrix standards (Mahajan et al., 2005; Thomas, 2002).

2.3.6. Electro Thermal Atomization (ETA)

Delves Cup approach became less attractive after the commercialization of Electro Thermal Atomization (ETA) in the early 1970s. This approach offered detection capability for lead of ~0.1 ppb — 200 fold better than FAA.However, its major benefit for the analysis of blood samples was the ability to dilute and inject the sample automatically into the graphite tube with very little off-line sample preparation. In addition, because the majority of the matrix components were

"driven-off" before atomization at 2700°C, interferences were generally less than with the Delves Cup, which used a much cooler acetylene flame to generate the atoms. This breakthrough meant that blood lead determinations, even at extremely low levels, could now be carried out routinely in an automated fashion (Mahajan et al., 2005).

2.3.7. Zeeman background correction (ZBGC)

The next major milestone in AS was the development of Zeeman background correction (ZBGC) in 1981, which compensated for nonspecific absorption and structured background produced by complex biological matrices like blood and urine (Mahajan et al., 2005).

2.3.8. Spectrophotometer

Although measurement of trace elements in hair has been done for many years ago, interest in the utility of this procedure has accelerated lately. Reference to hair as a biopsy material began in 1970. Spectroscopic methods of hair analysis have become common analytic services are available nowadays (Klevay et al., 1987).
Despite its usage in hair analysis it is role in measurement of trace elements in nails sample not fully evaluated yet (Ahmad et al, 2013).

2.3.8.1. Principles of Spectrophotometry

A spectrophotometer consists of two instruments, namely a *spectrometer* for producing light of any selected color (wavelength), and a *photometer* for

measuring the intensity of light. The instruments are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device, normally a galvanometer. The signal changes as the amount of light absorbed by the liquid changes.

If development of color is linked to the concentration of a substance in solution then that concentration can be measured by determining the extent of absorption of light at the appropriate wavelength (Skoog and Holler, 2007).

Spectroscopy is based, principally, on the study of the interaction between radiation and matter. This interaction causes in the atom an electronic transition from a lower energetic level, m, to a higher level, l, occurring energy absorption from the atom equal to the energy difference between both levels, El - Em.

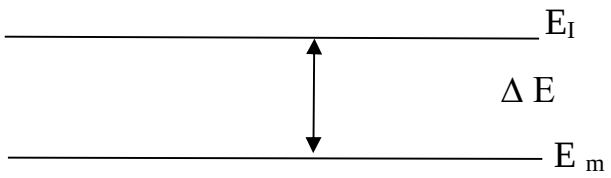

Fig. → 1

$$\Delta E = h\vartheta = \frac{hc}{\lambda}$$ eqn. → **7**

Where $\boldsymbol{h}$ = plank constant, $\boldsymbol{\vartheta}$= frequency, $\boldsymbol{c}$ = light speed in space, and $\boldsymbol{\lambda}$ = wavelength.

A plot of these latter processes as a function of radiation wavelength is known as spectrum that offers information about the difference of energy involved in each electronic transition. Different types of spectroscopy can be found depending on the wavelength of the incident radiation as can be seen in table (1) below;

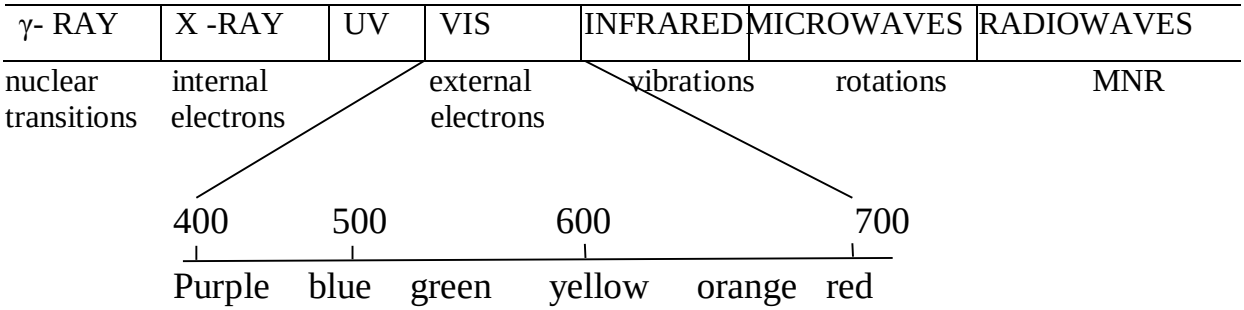

Table 1: Electromagnetic spectrum as a function of the wavelength radiation.

UV-Visible spectroscopy (radiations with wavelengths between 10 and 1000 nm) offers information about the transition of the most external electrons of the atoms. Since atoms or molecules absorb UV-visible radiation at different wavelength, spectroscopy/spectrometry is often used in physical and analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them. This technique is also used to assess the concentration or amount of a given species using the Lambert-Beer- law (Skoog and Holler, 2007; Yao et al., 2001).

When monochromatic light (light of a specific wavelength) passes through a solution there is usually a quantitative relationship (Beer's law) between the solute concentration and the intensity of the transmitted light that is (**eqn. 8**),

$$I = I_0 \times 10^{-kc1} \qquad \text{eqn.} \rightarrow 8$$

where I sub 0 is the intensity of transmitted light using the pure solvent, I is the intensity of the transmitted light when the colored compound is added, c is

concentration of the colored compound, l is the distance the light passes through the solution, and k is a constant. If the light path l is a constant, as is the case with a spectrophotometer, Beer's law may be written (**eqn 9**),

$$I \div I_0 = 10^{-kc} = T \qquad \text{eqn.} \rightarrow 9$$

where k is a new constant and T is the transmittance of the solution. There is a logarithmic relationship between transmittance and the concentration of the colored compound. Thus (**eqn 10**),

$$-\log T = 1/T = Kc = optical\ density\ (O.D) \qquad \text{eqn.} \rightarrow 10$$

The O.D. is directly proportional to the concentration of the colored compound. Most spectrophotometers have a scale that reads both in O.D. (absorbance) units, which is a logarithmic scale, and in % transmittance, which is an arithmetic scale. As suggested by the above relationships, the absorbance scale is the most useful for colorimetric assays.

At the spectrophotometer, one should have two cuvettes in a plastic rack. Solutions which are to be read are poured into cuvettes which are inserted into the machine. One should be marked "B" for the blank and one "S" for sample. A wipette should be available to polish them before insertion into the cuvette chamber. Cuvettes are carefully manufactured for their optical uniformity and are quite expensive. They should be handled with care so that they do not get scratched, and stored separate from standard test tubes. One should not to try to touch them except at the top of the tube to prevent finger smudges which alter the reading. For experiments in which minor in precision is acceptable, clean, unscratched 13 x 100 mm test tubes may be used (Skoog and Holler, 2007; Yao et al., 2001). Figure 2 shows the light path for spectrophotometer.

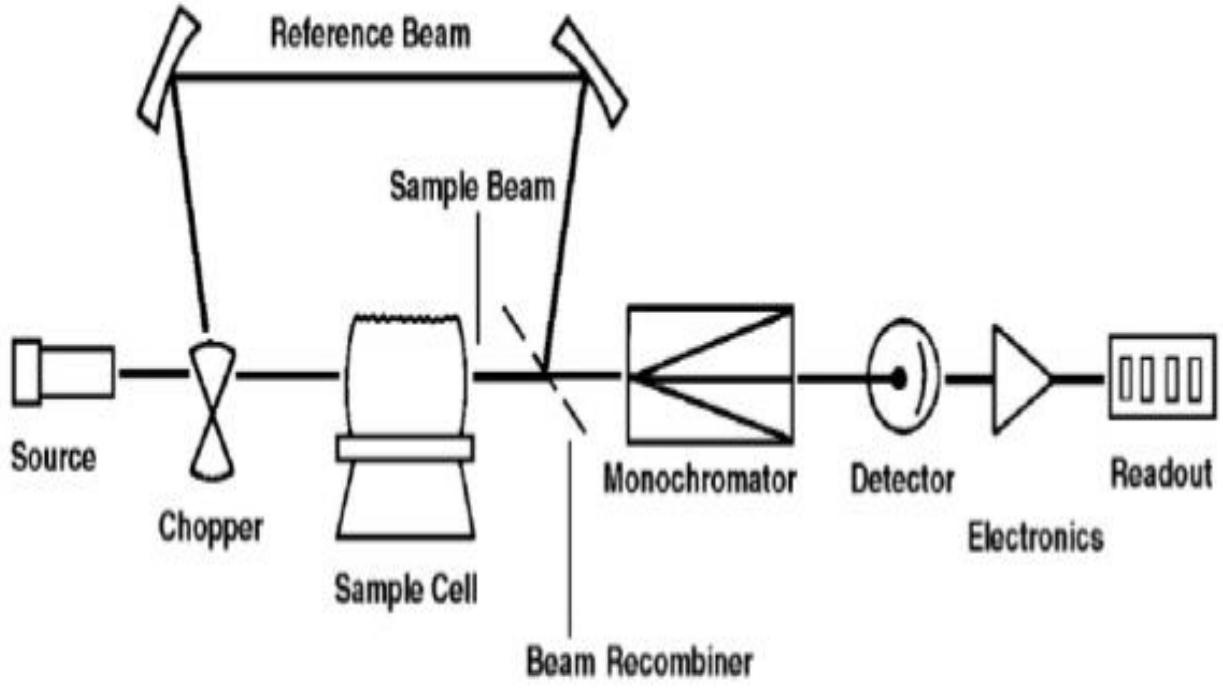

Figure 2: Light Path for a double beam Spectrophotometer

2.3.8.2. Atomic absorption spectroscopy (Spectrometer)

Guystav Kirchoff and Robert Bunsen first used atomic absorption spectroscopy—along with atomic emission—in 1859 and 1860 as a means for identify atoms in flames and hot gases. Although atomic emission continued to develop as an analytical technique, progress in atomic absorption languished for almost a century. Modern atomic absorption spectroscopy has its beginnings in 1955 as a result of the independent work of Walsh (1991). Commercial instruments were in place by the early 1960s, and the importance of atomic absorption as an analytical technique was soon evident.

2.3.8.2.1. Instrumentation of atomic absorption spectrometer

Atomic absorption spectrophotometers use the same single-beam or double-beam optics described earlier for molecular absorption spectrophotometers. There is, however, an important additional need in atomic absorption spectroscopy—we must covert the analyte into free atoms. In most cases our analyte is in solution form. If our sample is a solid, then we must bring it into solution before the analysis. When analyzing a lake sediment for Cu, Zn, and Fe, for example, we bring the analytes into solution as Cu^{2+}, Zn^{2+}, and Fe^{3+} by extracting them with a suitable reagent.

What reagent we choose to use depends on our research goals. If we need to know the total amount of metal in the sediment, then we might use a microwave digestion using a mixture of concentrated acids, such as HNO_3, HCl, and HF. This destroys the sediment's matrix and brings everything into solution. On the other hand, if our interest is biologically available metals, we might extract the sample

under milder conditions, such as a dilute solution of HCl or CH_3COOH at room temperature (Koirtyohann, 1991).

2.3.8.2.2. Atomization

The process of converting an analyte to a free gaseous atom is called atomization. Converting an aqueous analyte into a free atom requires that we strip away the solvent, volatilize the analytes, and, if necessary, dissociate the analyte into free atoms. Desolvating an aqueous solution of $CuCl_2$, for example, leaves us with solid particulates of $CuCl_2$. Converting the particulate $CuCl_2$ to gas phases atoms of Cu and Cl requires thermal energy.

$$CuCl_2\ (aq) \rightarrow CuCl_2\ (s) \rightarrow Cu\ (g) + 2Cl\ (g) \qquad \text{eqn.} \rightarrow 11$$

There are two common atomization methods: flame atomization and electro-thermal atomization, although a few elements are atomized using other methods (Salvin, 1991).

2.3.8.2.2.1. Flame atomizer

The aqueous sample is drawn into the assembly by passing a high-pressure stream of compressed air past the end of a capillary tube immersed in the sample. When the sample exits the nebulizer it strikes a glass impact bead, converting it into a fine aerosol mist within the spray chamber. The aerosol mist is swept through the spray chamber by the combustion gases—compressed air and acetylene in this case—to the burner head where the flame's thermal energy desolvates the aerosol mist to a dry aerosol of small, solid particles. The flame's thermal energy then volatilizes the particles, producing a vapor consisting of molecular species, ionic species, and free atoms (Parsons et al., 1983; Salvin, 1991).

2.3.8.2.2.1.1. Burner

The slot burner provides a long optical path- length and a stable flame. Because absorbance increases linearly with the path length, a long path length provides greater sensitivity. A stable flame minimizes uncertainty due to fluctuations in the flame. The burner is mounted on an adjustable stage that allows the entire assembly to move horizontally and vertically. Horizontal adjustments ensure that the flame is aligned with the instrument's optical path. Vertical adjustments adjust the height within the flame from which absorbance is monitored. This is important because two competing processes affect the concentration of free atoms in the flame. The more time the analyte spends in the flame the greater the atomization efficiency; thus, the production of free atoms increases with height. On the other hand, a longer residence time allows more opportunity for the free atoms to combine with oxygen to form a molecular oxide. For an easily oxidized metal, the concentration of free atoms is greatest just above the burner head. For metals, which are difficult to oxidize, the concentration of free atoms increases steadily with height. Other atoms show concentration profiles that maximize at a characteristic height (1983; Salvin, 1991; koirtyohann, 1991).

2.3.8.2.2.1.2. Flame

The flame's temperature, which affects the efficiency of atomization, depends on the fuel–oxidant mixture. The air–acetylene and the nitrous oxide–acetylene flames are the most popular. Normally the fuel and oxidant are mixed in an approximately stoichiometric ratio; however, a fuel-rich mixture may be necessary for easily oxidized analytes.

The primary combustion zone is usually rich in gas combustion products that emit radiation, limiting is usefulness for atomic absorption. The interzonal region

generally is rich in free atoms and provides the best location for measuring atomic absorption. The hottest part of the flame is typically 2–3 cm above the primary combustion zone. As atoms approach the flame's secondary combustion zone, the decrease in temperature allows for formation of stable molecular species (Salvin, 1991; Walsh, 1991).

2.3.8.2.2.1.3. Sample introduction

The most common means for introducing samples into a flame atomizer is a continuous aspiration in which the sample flows through the burner while we monitor the absorbance. Continuous aspiration is sample intensive, typically requiring from 2–5 mL of sample. Flame micro sampling allows introduction of a discrete sample of fixed volume, and is useful when we have a limited amount of sample or when the sample's matrix is incompatible with the flame atomizer.

Flame micro sampling is accomplished using a micro pipet to place 50–250 μL of sample in a Teflon funnel connected to the nebulizer, or by dipping the nebulizer tubing into the sample for a short time. Dip sampling is usually accomplished with an automatic sampler. The signal for flame micro sampling is a transitory peak whose height or area is proportional to the amount of analyte that is injected.

2.3.8.2.2.1.4. Advantages and disadvantages of flame atomization

The principal advantage of flame atomization is the reproducibility with which the sample is introduced into the spectrophotometer. A significant disadvantage to flame atomizers is that the efficiency of atomization may be quite poor. There are two reasons for poor atomization efficiency. First, the majority of the aerosol

droplets produced during nebulization are too large to be carried to the flame by the combustion gases. Consequently, as much as 95% of the sample never reaches the flame. A second reason for poor atomization efficiency is that the large volume of combustion gases significantly dilutes the sample. Together, these contributions to the efficiency of atomization reduce sensitivity because the analyte's concentration in the flame may be a factor of 2.5 × 10–6 less than that in solution (Walsh, 1991; koirtyohann, 1991).

2.3.8.2.2.2. Electrothermal atomizers

A significant improvement in sensitivity is achieved by using the resistive heating of a graphite tube in place of a flame. A typical electrothermal atomizer, also known as a graphite furnace, consists of a cylindrical graphite tube approximately 1–3 cm in length and 3–8 mm in diameter.

The graphite tube is housed in a sealed assembly that has optically transparent windows at each end. A continuous stream of an inert gas is passed through the furnace, protecting the graphite tube from oxidation and removing the gaseous products produced during atomization. A power supply is used to pass a current through the graphite tube, resulting in resistive heating. Samples of between 5–50 μL are injected into the graphite tube through a small hole at the top of the tube. Atomization is achieved in three stages.

In the first stage the sample is dried to a solid residue using a current that raises the temperature of the graphite tube to about 110 ^{o}C.

In the second stage, which is called ashing, the temperature is increased to between 350–1200 ^{o}C. At these temperatures any organic material in the sample is

converted to CO_2 and H_2O, and volatile inorganic materials are vaporized. These gases are removed by the inert gas flow.

In the final stage the sample is atomized by rapidly increasing the temperature to between 2000–3000 ºC.

The result is a transient absorbance peak whose height or area is proportional to the absolute amount of analyte injected into the graphite tube. Together, the three stages take approximately 45–90 s, with most of this time used for drying and ashing the sample. Electrothermal atomization provides a significant improvement in sensitivity by trapping the gaseous analyte in the small volume within the graphite tube. The analyte's concentration in the resulting vapor phase may be as much as 1000× greater than in a flame atomization. This improvement in sensitivity and the resulting improvement in detection limits are offset by a significant decrease in precision. Atomization efficiency is strongly influenced by the sample's contact with the graphite tube, which is difficult to control reproducibly (Parsons et al., 1983).

2.3.8.2.3. Developing a quantitative method

Flame or Electrothermal Atomization? The most important factor in choosing a method of atomization is the analyte's concentration. Because of its greater sensitivity, it takes less analyte to achieve a given absorbance when using electrothermal atomization. Table 2 which compares the amount of analyte needed to achieve an absorbance of 0.20 when using flame atomization and electrothermal atomization, is useful when selecting an atomization method. For example, flame atomization is the method of choice if our samples contain 1–10 mg Zn^{2+}/L, but

electrothermal atomization is the best choice for samples containing 1–10 μg Zn^{2+}/L (Parsons et al., 1983; Walsh, 1991; koirtyohann, 1991).

Table 2: Concentration of analyte yielding an absorbance of 0.20

Element	Concentration (mg/L)	
	Flame atomization	Electrothermal atomization
Ag	1.5	0.0035
Al	40	0.015
As	40	0.050
Ca	0.8	0.003
Cd	0.6	0.001
Co	2.5	0.021
Cr	2.5	0.0075
Cu	1.5	0.012
Fe	2.5	0.006
Hg	70	0.52
Mg	0.15	0.00075
Mn	1	0.003
Na	0.3	0.00023
Ni	2	0.024
Pb	5	0.080
Pt	70	0.29
Sn	50	0.023
Zn	0.3	0.00071

2.3.8.2.4. Selecting the Wavelength and Slit Width

The source for atomic absorption is a hollow cathode lamp consisting of a cathode and anode enclosed within a glass tube filled with a low pressure of Ne or Ar. Applying a potential across the electrodes ionizes the filler gas. The positively charged gas ions collide with the negatively charged cathode, sputtering atoms from the cathode's surface. Some of the sputtered atoms are in the excited state and emit radiation characteristic of the metal(s) from which the cathode was manufactured. By fashioning the cathode from the metallic analyte, a hollow cathode lamp provides emission lines that correspond to the analyte's absorption spectrum.

Each element in a hollow cathode lamp provides several atomic emission lines that we can use for atomic absorption. Usually the wavelength that provides the best sensitivity is the one we choose to use, although a less sensitive wavelength may be more appropriate for a larger concentration of analyte. For the Cr hollow cathode lamp in table 3, for example, the best sensitivity is obtained using a wavelength of 357.9 nm. Another consideration is the intensity of the emission line. If several emission lines meet our need for sensitivity, we may wish to use the emission line with the largest relative P0 because there is less uncertainty in measuring P0 and PT. When analyzing samples containing ≈10 mg Cr/L, for example, the first three wavelengths in table 3 provide an appropriate sensitivity. The wavelengths of 425.5 nm and 429.0 nm, however, have a greater P0 and will provide less uncertainty in the measured absorbance. The emission spectrum from a hollow cathode lamp includes, besides emission lines for the analyte, additional emission lines for impurities present in the metallic cathode and from the filler gas. These additional lines are a source of stray radiation that leads to an instrumental deviation from Beer's law. The monochromator's slit width is set as wide as

possible, improving the throughput of radiation, while, at the same time, being narrow enough to eliminate the stray radiation (Parsons et al., 1983; koirtyohann, 1991).

Table 3: Atomic Emission Lines for a Cr Hollow Cathode Lamp

wavelength (nm)	slit width (nm)	mg Cr/L giving A = 0.20	P0 (relative)
357.9	**0.2**	**2.5**	**40**
425.4	**0.2**	**12**	**85**
429.0	**0.5**	**20**	**100**
520.5	**0.2**	**1500**	**15**
520.8	**0.2**	**500**	**20**

2.3.8.2.5. Preparing the sample

Flame and electrothermal atomization require that the sample be in solution. Solid samples are brought into solution by dissolving in an appropriate solvent. If the sample is not soluble it may be digested, either on a hot-plate or by microwave, using HNO_3, H_2SO_4, or $HClO_4$. Alternatively, we can extract the analyte using a Soxhlet extractor. Liquid samples may be analyzed directly or extracted if the matrix is incompatible with the method of atomization. A serum sample, for instance, is difficult to aspirate when using flame atomization and may produce an unacceptably high background absorbance when using electrothermal atomization. A liquid–liquid extraction using an organic solvent and a chelating agent is frequently used to concentrate analytes. Dilute solutions of Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}, for example, can be concentrated by extracting with a solution of ammonium pyrrolidine dithiocarbamate in methyl isobutyl ketone (Walsh, 1991).

2.3.8.2.6. Minimizing spectral interference

A spectral interference occurs when an analyte's absorption line overlaps with an interferent's absorption line or band. Because they are so narrow, the overlap of two atomic absorption lines is seldom a problem. On the other hand, a molecule's broad absorption band or the scattering of source radiation is a potentially serious spectral interference. An important consideration when using a flame as an atomization source is its effect on the measured absorbance. Among the products of combustion are molecular species that exhibit broad absorption bands and particulates that scatter radiation from the source. If we fail to compensate for these spectral interference, then the intensity of transmitted radiation decreases. The result is an apparent increase in the sample's absorbance. Fortunately, absorption and scattering of radiation by the flame are corrected by analyzing a blank. Spectral interferences also occur when components of the sample's matrix other than the analyte react to form molecular species, such as oxides and hydroxides. The resulting absorption and scattering constitutes the sample's background and may present a significant problem, particularly at wavelengths below 300 nm where the scattering of radiation becomes more important. If we know the composition of the sample's matrix, then we can prepare our samples using an identical matrix. In this case the background absorption is the same for both the samples and standards. Alternatively, if the background is due to a known matrix component, then we can add that component in excess to all samples and standards so that the contribution of the naturally occurring interferent is insignificant. Finally, much interference due to the sample's matrix can be eliminated by increasing the atomization temperature. For example, by switching to a higher temperature flame it may be possible to prevent the formation of interfering oxides and hydroxides. If the identity of the matrix interference is

unknown, or if it is not possible to adjust the flame or furnace conditions to eliminate the interference, then we must find another method to compensate for the background interference. Several methods have been developed to compensate for matrix interferences, and most atomic absorption spectrophotometers include one or more of these methods. One of the most common methods for background correction is to use a continuum source, such as a D2 lamp. Because a D2 lamp is a continuum source, absorbance of its radiation by the analyte's narrow absorption line is negligible. Only the background, therefore, absorbs radiation from the D2 lamp. Both the analyte and the background, on the other hand, absorb the hollow cathode's radiation. Subtracting the absorbance for the D2 lamp from that for the hollow cathode lamp gives a corrected absorbance that compensates for the background interference. Although this method of background correction may be quite effective, it does assume that the background absorbance is constant over the range of wavelengths passed by the monochromator. If this is not true, subtracting the two absorbances may underestimate or overestimate the background (Walsh, 1991; koirtyohann, 1991).

2.3.8.2.7. Minimizing chemical interferences

The quantitative analysis of some elements is complicated by chemical interferences occurring during atomization. The two most common chemical interferences are the formation of nonvolatile compounds containing the analyte and ionization of the analyte. One example of the formation of nonvolatile compounds is the effect of PO_4^{3-} or Al^{3+} on the flame atomic absorption analysis of Ca^{2+}. In one study, for example, adding 100 ppm Al^{3+} to a solution of 5 ppm Ca^{2+} decreased the calcium ion's absorbance from 0.50 to 0.14, while adding 500 ppm

PO_4^{3-} to a similar solution of Ca^{2+} decreased the absorbance from 0.50 to 0.38. These interferences were attributed to the formation of nonvolatile particles of $Ca_3(PO_4)_2$ and an Al–Ca–O oxide (Hosking et al., 1977). When using flame atomization, we can minimize the formation of nonvolatile compounds by increasing the flame's temperature, either by changing the fuel-to-oxidant ratio or by switching to a different combination of fuel and oxidant. Another approach is to add a releasing agent or a protecting agent to the samples. A releasing agent is a species that reacts with the interferent, releasing the analyte during atomization. Adding Sr^{2+} or La^{3+} to solutions of Ca^{2+}, for example, minimizes the effect of PO_4^{3-} and Al^{3+} by reacting in place of the analyte. Thus, adding 2000 ppm $SrCl_2$ to the Ca^{2+}/PO_4^{3-} and Ca^{2+}/Al^{3+} mixtures described in the previous paragraph increased the absorbance to 0.48. A protecting agent reacts with the analyte to form a stable volatile complex. Adding 1% w/w EDTA to the Ca^{2+}/PO_4^{3-} solution described in the previous paragraph increased the absorbance to 0.52. Ionization interferences occur when thermal energy from the flame or the electrothermal atomizer is sufficient to ionize the analyte

$$M(g) \rightleftarrows M^+(g) + e^- \qquad \text{reaction} \rightarrow 1$$

where M is the analyte

Because the absorption spectra for M and M+ are different, the position of the equilibrium in reaction 1 affects absorbance at wavelengths where M absorbs. To limit ionization we add a high concentration of an ionization suppressor, which is simply a species that ionizes more easily than the analyte. If the concentration of the ionization suppressor is sufficient, then the increased concentration of electrons in the flame pushes reaction 1 to the left, preventing the analyte's ionization.

Potassium and cesium are frequently used as an ionization suppressor because of their low ionization energy (Hosking et al., 1977; Parsons et al., 1983).

2.3.8.2.8. Standardizing the method

Because Beer's law also applies to atomic absorption, we might expect atomic absorption calibration curves to be linear. In practice, however, most atomic absorption calibration curves are non- linear, or linear for only a limited range of concentrations. Nonlinearity in atomic absorption is a consequence of instrumental limitations, including stray radiation from the hollow cathode lamp and the variation in molar absorptivity across the absorption line. Accurate quantitative work, therefore, often requires a suitable means for computing the calibration curve from a set of standards. When possible, a quantitative analysis is best conducted using external standards. Unfortunately, matrix interferences are a frequent problem, particularly when using electrothermal atomization. For this reason the method of standard additions is often used. One limitation to this method of standardization, however, is the requirement that there be a linear relationship between absorbance and concentration (Hosking et al., 1977).

2.3.8.3. Evaluation of atomic absorption spectroscopy

2.3.8.3.1. Scale of operation

Atomic absorption spectroscopy is ideally suited for the analysis of trace and ultratrace analytes, particularly when using electrothermal atomization. For minor and major analyte, sample can be diluted before the analysis. Most analyses use a macro or a meso sample. The small volume requirement for electrothermal

atomization or flame microsampling, however, makes practical the analysis micro and ultramicro samples (Crawford et a, 1985).

2.3.8.3.2. Accuracy

If spectral and chemical interferences are minimized, an accuracy of 0.5–5% is routinely attainable. When the calibration curve is nonlinear, accuracy may be improved by using a pair of standards whose absorbances closely bracket the sample's absorbance and assuming that the change in absorbance is linear over this limited concentration range. Determinate errors for electrothermal atomization are often greater than that obtained with flame atomization due to more serious matrix interferences.

2.3.8.3.3. Precision

For absorbance values greater than 0.1–0.2, the relative standard deviation for atomic absorption is 0.3–1% for flame atomization and 1–5% for electrothermal atomization. The principle limitation is the variation in the concentration of free analyte atoms resulting from variations in the rate of aspiration, nebulization, and atomization when using a flame atomizer, and the consistency of injecting samples when using electrothermal atomization.

2.3.8.3.4. Sensitivity

The sensitivity of a flame atomic absorption analysis is influenced strongly by the flame's composition and by the position in the flame from which we monitor the absorbance. Normally the sensitivity of an analysis is optimized by aspirating a standard solution of the analyte and adjusting operating conditions, such as the fuel-to-oxidant ratio, the nebulizer flow rate, and the height of the burner, to give

the greatest absorbance. With electrothermal atomization, sensitivity is influenced by the drying and ashing stages that precede atomization. The temperature and time used for each stage must be optimized for each type of sample. Sensitivity is also influenced by the sample's matrix. We have already noted, for example, that sensitivity can be decreased by chemical interferences. An increase in sensitivity may be realized by adding a low molecular weight alcohol, ester, or ketone to the solution, or by using an organic solvent.

2.3.8.3.5. Selectivity

Due to the narrow width of absorption lines, atomic absorption provides excellent selectivity. Atomic absorption can be used for the analysis of over 60 elements at concentrations at or below the level of μg/L.

2.3.9. Neutron activation analysis (NAA)

The term activation analysis refers to identification and quantitative determination by use of radio nuclides produced from a target element. NAA is the most common variant in which neutrons are used to irradiate and to activate the sample. When the measurement is carried out without prior chemical separation, the method is called instrumental NAA (INAA). As a result of a nuclear reaction between the neutron and the isotope of the element of interest, radio nuclides with characteristic half-lives may be produced, emitting radiation of varying energies that may be measured by a suitable detector and are characteristic of the element from which they were produced. Applications of the NAA technique have been in the analysis of very pure silicon (where LODs for some elements may be mass fractions of 10 to-15 or below), the trace determination of elements in biological samples, and the

multi-element analysis of airborne particulate matter (Ali and Tarafdar 2003). It can be applied to Analyse the cancerous tissues, trace elements in human hair and drinking water (Lal et al., 1987; Bhandari et al., 1987).

Activation analysis is suited to the analysis of solid samples where these may be irradiated with no dissolution step. Liquid samples may be analysed but pre concentration is often required prior to analysis. Mass fractions down to the 10 to -15 levels can be detected.

The detection limit represents the ability of a given NAA procedure to determine the minimum amounts of an element reliably. The detection limit depends on the irradiation, the decay and the counting conditions. It also depends on the interference situation including such things as the ambient background, the Compton continuum from higher energy-rays, as well as any-ray spectrum interferences from such factors as the blank from pre-irradiation treatment and from packing materials. The detection limit is often calculated using Currie's formula:

$$\boldsymbol{DL = 2.71 + 4.65B} \qquad \textit{eqn.} \rightarrow 12$$

Where: DL is the detection limit and B is the background under a gamma-ray peak. This relation is valid only when the gamma-ray background (counting statistical error) is the major interference (Hamidatou et al., 2013).

Neutron Activation Analysis sensitivities and accuracy are dependent on the concentration of particular element and radionuclide parameters (i.e. parent isotope abundance, neutron cross section, half-life, and gamma ray abundance). Element

sensitivities vary from 10-3 to 10-10 grams per gram of sample. To Calculate Element Concentration using gamma ray counts:

The procedure used to calculate concentration (i.e., ppm of element) in the unknown sample is to irradiate the unknown sample and a comparator standard containing a known amount of the element of interest together in the reactor. If the unknown sample and the comparator standard are both measured on the same detector, then one needs to correct the difference in decay between the two. One usually decay corrects the measured counts (or activity) for both samples back to the end of irradiation using the half-life of the measured isotope. The equation used to calculate the mass of an element in the unknown sample relative to the comparator standard is:

$$\frac{A_{sam}}{A_{std}} = \frac{A_{sam}}{A_{std}} \frac{\left(e^{-\lambda T_d}\right)_{sam}}{\left(e^{-\lambda T_d}\right)_{std}} \qquad \text{eqn.} \rightarrow 13$$

where *A*=activity of sample (*sam*) and standard (*std*), *m*=mass of the element, λ=decay constant for the isotope, and *Td*=decay time. When performing short irradiations, the irradiation, decay and counting times are normally fixed the same for all samples and standards such that the time-dependent factors cancel. Thus the above equation simplifies into:

$$C_{sam} = C_{std} \frac{W_{std}}{W_{sam}} \frac{A_{sam}}{A_{std}} \qquad \text{eqn.} \rightarrow 14$$

where *C*=concentration of the element and *W*=weight of the sample and standard (Ahuja et al., 2015).

Sensitivities and LODs can vary widely with some elements being difficult to detect at all. In principle, NAA offers a robust analysis technique providing very accurate results down to ultra-low concentrations. Most sources of systematic and random error (e.g., interfering nuclear reactions, overlap of spectral lines and dead-time losses) are identifiable, since the physical principles of NAA are well understood and described (Marques et al., 2000).

Non-destructive neutron activation analysis has proved to be an effective method of analysing trace elements in Soils. More importantly is the fact that the basic major elements which is universally present in soil samples are clearly identified in percentage concentrations and ppm; as well as the key elements needed to enhance the elemental properties of soils such as Al, Fe, Zn and Mn are also found in moderate quantities (Kogo et al., 2009).

Additionally, since NAA is based on principles fundamentally different from the other analytical techniques, it is prone to completely different systematic biases and is therefore extremely useful in the analysis of reference materials or in assessing the comparability of measurement results. However, the throughput of NAA techniques is low, and the technique is expensive.

An important feature of the Instrumental Neutron Activation Analysis (INAA) technique is its methods of standardization. A widely used standard method for determining concentration of elements in an unknown sample is the relative standardization where a standard and a sample of unknown elements (with unknown concentrations) are co-irradiated and measured separately on the same detector, in the same geometry, for the same time (Nuviadenu et al., 2012).

This method is very accurate with easy error propagation. Its disadvantage is that multi-element analysis is only possible with multi-element standards. It is also quite sensitive to unrecognized interferences (Nuviadenu et al., 2011).

Each collected sample washed three times with 10 mL of buffer solution, air-dried and immediately weighed and packaged for irradiation. The prepared samples will be irradiated for 1 h at a neutron flux of $5x10^{11}$ neutrons/cm^2/s in the inner channel of a 30 kW Miniature Neutron Source Reactor. After irradiation, the samples will be allowed to decay for 24 h in order to reduce the activity to safety levels and to minimise the background effects. Each sample will be counted for 30 min and the peak areasof interest obtained from the spectra will be used in concentration calculations. Nuviadenu, et al. in Ghana in thier study of Trace-elements Exposure Levels in Artisanal "Galamsey" Mines in Wassa-West District, Ghana, used this technique of sample analysis and found that the four elements (Hg, Au, As and Sb) detected in fingernails of the *galamsey* miners, in the Wassa-West District of Ghana, were on the average at concentrations that depict high exposure levels. Some individual concentrations were also critically high, raising health concerns. They concluded routine health screening for populations and frequent monitoring programs be carried out to ensure best practices of safety and standard procedures (Nuviadenu et al., 2012).

Similarly Vance, et al. in USA used the similar method in their study; Trace element content in fingernails and hair of a non-industrialized versus control population (Vance et al., 1988).

Again Nuviadenu, et al. (2011) in another study in Ghana, used *k0* standardization method of INAA and concluded that there is a good agreement between the two methods was obtained. Owing to the fact that *k0*-method is carried out without the

need to prepare standards, it may be prudent to adopt this method for elemental analysis, especially when studying samples with very small mass like fingernails.

2.3.10. Proton induced X-Ray emission technique (PIXE)

X-ray emission may also be induced by heavy charged particles (particle-induced X-ray emission, PIXE). Calibration is often by means of thin-film standards, or by using fundamental physical parameters in conjunction with an experimentally determined efficiency curve. Using this technique, LODs of a few ng/cm^2 have been claimed for particulate material on ambient air filters for a range of elements, with repeatabilities of 1% and an accuracy of 5% (Ahuja et al., 2015).

For a given trace element A with atomic number Z in a given matrix B, the detection limit is determined by statistical fluctuation of background and therefore it is defined by:

$$\boldsymbol{N_A \geq 3 \times \sqrt{N_B}} \qquad \text{eqn.} \rightarrow 15$$

where N_A is the total number of counts of a characteristic X-ray peak for a given line i of *A*, and N_B is the number of background counts included in the full width of half maximum (FWHM) of the characteristic X-ray peak or the detection limit of *A* in the matrix *B* is given in units of parts per million by the following Formula:

$$\frac{\boldsymbol{n_A}}{\boldsymbol{n_B}} = \frac{\boldsymbol{3 \times \sqrt{N_B} \times 10^5}}{\boldsymbol{n_B \times N_P \times \frac{\Omega}{4\times\pi} \times \varepsilon_f(Z) \times ab(Z) \times \sigma_z^i}} \qquad \text{eqn.} \rightarrow 16$$

Where n_B is the atomic concentration of the matrix element, n_A is that of the trace element *A*, N_p is the number of projectiles, Ω is the solid angle subtended by a detector, and σ^i_Z , $ab(Z)$,ε_f (Z) are, respectively, the production cross section of K X-rays for the trace element, absorption of X-rays by windows and others (air, target) and the detection efficiency (Koumeir et al., 2010).

The trace elements in medicinal plants had been largely detected by PIXE technique as it is one of the most powerful techniques for its quick multi elemental trace analysis capability and high sensitivity. Some of the common trace elements determined by this technique are K, Ca, Fe, Zn, Sr. etc. (Singh et al., 2009).

CHAPTER THREE

Materials
and
method

CHAPTER THREE

3. Materials and method

3.1. Study design

Prospective cross sectional study from November 2011 to 2015

3.2. Study setting

A community based study was carried out in rural area of Western Kordufan state. These samples were analyzed by using (FAA). Additional samples were also collected from Khartoum at Haj Yousif area (Mansura area) and analyzed by XRF.

3.3. Study population

Randomly selected candidates from general population from different strata of rural area were enrolled into our study.

3.4. Sample size

Non probability total coverage study

3.5. Inclusion criteria

- All candidates from both genders irrespect to their age.
- Candidates who accept the informed consent
- Candidates who did not trim their nails for a couple of weeks or longer.
- Candidates who did not bleached their hair or treated with permedication during the last 3 months.

3.6. Exclusion criteria

1. Candidates who did not accept the informed consent
2. Candidates who trimmed their nails within the last couple of weeks.

3. Candidates who bleached their hair or treated with permedication during the last 3 months.
4. Samples that was not collected adequately or stored appropriately.

3.7. Procedures

The concentrations of copper (Cu), iron (Fe), calcium (Ca), magnesium (Mg), lead (Pb), and zinc (Zn) were determined by atomic absorption spectrometry, and XRF Technique.

3.7.1. Hair samples

3.7.1.1. Collection of hair sample

The scalp hair samples were cut close to the skin at the nape area with stainless steel scissors that was routinely immersed in sprit in between participants to make sure it is cleanliness before usage among each participant. As a minimum of about 0.5 gm of hair sample was targeted this is about one heaped tablespoon full. There after the collected hair samples were immediately placed in clean blood collection plain plastic tubes, and appropriately labeled.

3.7.1.2. Preparation of hair sample

3.7.1.2.1. For Atomic Absorption (FAA)

The samples were washed according to the procedure described by the International Atomic Energy Agency (IAEA) to remove external contaminants (Ryabukhin, 1978).

Wash the hair sample in a 500-mL polyethylene bottle containing 150 mL of a 1% solution of 7X-o-Matic (non-ionic detergent, Linbro Chemical, New Haven, CT.), by agitating on a mechanical mixer for 30 minutes at room temperature. Thereafter, we transfer the sample to a polyethylene filter crucible and rinse with a total of one liter of deionized water. Then after, dry overnight at 110 °C, weigh and transfer to a 50-mL Erlenmeyer flask. Dry weight adjusted to be about 0.5 g. we added 6 mL of HNO3 and allowed to react at room temperature.

This is followed by warming the digest and adding 1 mL of HClO4 and heated at 200 °C until dense white fumes are evolved. Then we ensured that the solution is kept water clear. Lastly, we transferred it to a 5-mL volumetric flask and diluted to volume with deionized water. This solution used for the determination of Cu, Fe and Mg. A further dilution was required for Zn. A blank sample was prepared by following exactly the same procedure, but without the hair.

3.7.1.2.2. For X-Ray Fluorescence Spectrometer (XRF)

The samples were directly exposed to XRF radiation. The exposure time and orientation of the device was made the same for all samples.

3.7.2. Nail sample

3.7.2.1. Collection of nail sample

To obtain more nail masses, participants had not trimmed their nails for a couple of weeks or longer. Nails were collected by clipping with a stainless steel clipper from all ten fingers. Clippers were routinely immersed in sprit in between participants to make sure their cleanliness before usage among each participant. The aim was to collect nails as much as possible.

These nail samples were placed in an appropriately labeled clean plastic blood collection containers and stored at room temperature in the driest condition before it is analysis.

3.7.2.2. Preparation of nail sample

3.7.2.2.1. For Flame Atomic Absorption (FAA)

The nail samples were scraped with Teflon-coated forceps to remove surface dirt. The sample is placed in a polyethylene bottle containing 25 mL of a 1% solution of 7X-o-Matic (non-ionic detergent, Linbro Chemical, New Haven, CT.) and shaken on a mechanical shaker for 30 minutes. The sample is rinsed vigorously with four 250-mL portions of deionized water, dried overnight at 150 °C, weighed and digested in a 10-mL Erlenmeyer flask with 1 mL of HNO3 and 0.5 mL of HClO4. The sample is transferred to a 5-mL volumetric flask and diluted to volume with deionized water. This solution is suitable for the determination of Cu and Fe. For the determination of Ca, Zn and Mg, a 1-mL aliquot of this solution is diluted to 5 mL with deionized water. A blank sample was prepared by following exactly the same procedure, but without the fingernail (Harrison and Tyree, 1971).

3.7.2.2.2. For X-Ray Fluorescence Spectrometer (XRF) technique

The samples were directly exposed to XRF radiation. The orientation and exposure time were made the same for all samples.

3.7.3. Experiments setup

In the present study, measurement of Cu, Fe, Ca, Mg, Pb, and Zn were carried out using the Atomic absorption spectrometer (Buck Model 210 VGP Atomic Absorption Spectrophotometer). Appendix 1 shows the schematic diagram of the atomic absorption spectrometer used. Appendix 2, 3 and 4 show Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer.

3.7.3.1. General specifications (Buck Model 210 VGP AAS)

Electrical:

110V AC nominal (+10%), 50/60 Hz 220, 240V AC, 50/60 Hz Power Consumption: 50W

Optics:

Detector: model 928; wide range general purpose, 190-930nm

Optional Detectors: model 955; UV enhanced, wide-range, 190-930nm

model EMI9783B; narrow range furnace/hydride application, 165-600nm

Lenses: Supracil - amorphous silica

Monochromator: 0.25m Ebert mount

Grating: 32nm x 27nm; 600 grooves/mm

Wavelength adjustment: 3 digit mechanical, 0 to 1000nm +1 nm

Reproducibility: +0.2 nm Resolution: variable slit - 2Å, 7Å, and 20Å

Operating Modes:

Absorbance/Emission: -0.0820 to 3.2000

Concentration: to 5 significant digits

Integration Period: 0.5 to 10 seconds

Screen Refresh: 0.5 to 1.5 seconds

Recorder Output: 1V/ABS (-0.08 to 3.2V)

Background Correction: In-line Deuterium Arc Giant Pulse (Self-reversal)

Hollow Cathode Lamps:

Dimension: 1.5" OD

Striking Voltage: 500V

Lamp Current: 0 to 18 mA average current

Duty Cycle: 25%

Modulation Frequency: variable; 33 to 200 Hz (142 Hz Norm.)

Burner Assembly:

Design: Polyethylene Pre-mix chamber, glass impact bead dispersion

Burner Head: Titanium; air-acetylene head - 4" x 0.026" single slot (nitrous oxide head - 2" x 0.019" single slot)

Adjustments: Horizontal g

Performance:

Average Noise (at 3s): 0.0018 ABS (Cu at 324.7nm, 7Å slit, 5 sec. int.)

Reproducibility: <+5%

3.7.3.2. Specification of X-Ray Fluorescence spectrometer (XRF)

The X-MET5100 is a hand-held elemental analyzer with an integrated Personal Digital Assistant computer (PDA) intended for various different applications (Appendix 5 and 6). It provides a method for chemical analysis or sample identification (sorting) directly from samples in a various forms.

Within the X-MET analysis program, we can select analytical methods, view spectra and save data.

Components of X-MET:

– **Analyzer**	There are two indicator lights on the rear of the analyzer. The green light is always on when the power is on. The red light is on when X-rays are being generated.
– **Battery (x2)**	Batteries are situated inside the handle. Each fully charged battery will operate for approximately 4 to 7 hours depending on use.
– **PDA computer**	Installed in the cradle of the instrument. The display is a color touch screen, which can be operated either with a fingertip or the stylus.

Environmental operating conditions for the X-MET:

– **Temperature**	• Analyzer: -10 to 50 ^{0}C • Charger: 0 to 40 ^{0}C, operating -20 to 70 ^{0}C, non-operating
– **Humidity**	Continuous operation at 20 to 95% RH, non considering. The charger is designed for indoor use only.
– **Weather proofing**	With PDA rain cover installed instrument can be used in rainy and dusty environment (IP54 compatible).
– **Shock resistance**	In transport and operation the instrument must not be dropped or left in exceptional conditions, which might damage its sensitive components.
– **Line Voltage**	Analyzer (Charger): 100-240b V, 46-64 Hz, 0.6A. PDA: 100-240V, 50-60 Hz

3.7.4. Standard preparation for FAA

The standard stock solutions of each element were prepared as follow:

25 ml was taken from the stock standard solution (l000 ppm) in 250 ml volumetric flask and made up to the mark with 0.5 M Nitric acid, to prepare (l0 ppm) working solution.

From the working solution, a series of dilution for each element were prepared according to the concentration expected in the sample. Solutions were prepared to be suitable to the concentration of element in the sample solution by the following:

Cu: a series of 10, 40 and 50 µl were taken from the intermediate standard solution (10mg/l), in a 10ml volumetric flask with a micro pipette and were made up to the marks with HNO_3 0.5 M solution to be similar to the solvent of the sample corresponding to 10, 40 and 50 ppb.

Fe: a series of 1, 2 and 4 ml were taken from the working standard solution (10mg/l), in a 10 ml volumetric flask with a micro pipette and were made up to the marks with HNO_3 0.5 M solution to be similar to the solvent of the simple (to avoid the physical interference). The resulted iron solutions are l, 2 and 4 ppm respectively.

Ca: A series of 20, 60, 200, and 800 µl from calcium stock solution (100ppm) were taken in a series of 10 ml volumetric flask and were made up to the marks with 0.5 HNO_3 solution these corresponding to 2, 6, 20, 60, and 80 ppm. Then 0.4% strontium (Sr^{+2}) was prepared from strontium chloride (as matrix modifier) after reform each standard was diluted with this solution to get 0.08% strontium (Sr^{+2}) corresponding to 1, 3, 10, 30, and 40 ppm.

Mg: A series of 500, 1000, 2000, 2600 µl were taken from the intermediate standard solution (10 ml/L), in volumetric flasks 10 ml with a micro pipette and

were made up to the marks with HNO_3 0.5 N solution. These corresponding to 0.5, 1, 2 and 2.6 ppm and same as the calcium these solution was fold by strontium to prevent interference and the final concentration of standards were 0.25, 0.5, 1, and 1.3 ppm respectively.

Pb: A series of 10, 100, 200, 300, 500, 100 µl were taken from the intermediate standard solution (10 ml/L), in volumetric flasks 10 ml with a micro pipette and were made up to the marks with HNO_3 0.5 N solution. These corresponding to 0, 0.01, 0.05, 0.1, 0.25 and 0.5 ppm.

Zn: A series of 400, 700 and 1000 µl were taken from the working standard solution (10mg/l), in a 10 ml volumetric flask with a micro pipette and were made up to the marks with HNO_3 0.5 M solution to be similar to the solvent of the sample corresponding to 0.4, 0.7 and 1 ppm of zinc respectively.

3.7.5. Measurements

3.7.5.1. For Flame Atomic Absorption (FAA)

The measurement is as follow;

After preparation the samples and standard solution the main power supplier of the AAS was turned on the valves of acetylene, and air compressor was operated at 0.09 and 0.35 trip respectively.

The personal computer was open and the method parameters were setup on the control program. Hallow cathode lamp of elements under study were set. The operating conditions of the lamps used were showed in Table 4.

It serves as a quick reference guide to the sensitivity and performance using flame techniques. The detection limits are determined as the lowest concentration given

an absorbance detectable above the noise range. These values were determined empirically under Buck Scientific standard test conditions.

Sensitivity is a measure of the instrument response to the analyte, and by convention, shows the concentration of each element required to absorb 1% of the incident light energy. This corresponds to an absorbance value of 0.0044. Elements with greater sensitivity will have the lowest concentration values in that category. The values for "sens. check" in table and 4 are the amounts in mg/l required to give an absorbance reading of 0.200 abs.

The "linear range" is the amount of analyte in mg/l which will produce an absorbance of approximately 0.300 and safely keep the analysis in the linear part of the calibration curve. This area of the curve requires only one standard to be run but an additional standard run as a check is good practice. Above this area a multi-point calibration must be used.

Table 4: Atomic absorption concentration ranges

Element	Wl (Nm)	Silt (Nm)	Detec Limit (mg/L)	Sens Check (mg/L)	Linear Range (mg/L)
Cu	**324.8**	**0.7**	**0.005**	**2**	**5**
Fe	**248.3**	**0.2**	**0.05**	**2.5**	**5**
Ca	**422.7**	**0.7**	**0.05**	**2**	**5**
Mg	**285.2**	**0.7**	**0.005**	**0.015**	**1.5**
Pb	**283.3**	**0.7**	**0.08**	**10**	**20**
Zn	**213.9**	**0.7**	**0.005**	**0.5**	**2.5**

NB: Wl; Wavelength, Detec Limit; Detection Limit, Sens Check; Sensitivity Check.

After line search/beam balance was finished successfully flame was ignited and deionizer water was sucked for 5 minutes then auto zero command was executed. The electromagnetic radiation from hollow cathode is passed through the flame. The sample solution was introduced to the instrument through the sample capillary tube.

The nebulizer capillary tube was transferred to the standard one solution flask and the reading of the absorption was taken, then the tube was returned to the denionized water cup and waited till the digital reading reach zero value the continued on the same way for other standard solution.

When the sample reached the flame it is changed into gaseous state and then brought to its atomic state (free atom form) in the flame. Elements found in the ground state were absorbed radiation predominantly of wave length corresponds to transitions from ground state to upper excited states. Theses free atoms absorb a part of the radiation. By knowing the intensity of electromagnetic radiation from the light source and the intensity of the transmitted light, the concentration of the element under investigation can be calculated.

Before the reading of the standard or samples solution, the absorption value of the blank solution was taken. The absorbance values were taken from samples solution and the blank value from the sample value.

3.7.5.2. For X-Ray Fluorescence Spectrometer (XRF)

The samples were put in small plastic containers having the same size. The orientation and exposure time are the same for all samples.

3.7.6. Calculation

3.7.6.1. For Flame Atomic Absorption (FAA)

The calibration curve for each element was made using prepared standard solutions mentioned previously. The concentration of the element was calculated using Beer's law.

$$\text{Element } (\mu g / g) = \frac{(C)(V)(d.f)}{(W)} \qquad \text{eqn.} \rightarrow 17$$

where C is the concentration of the element in the sample solution in mg/l; V is the volume of the undiluted sample solution in ml; W is the sample weight in grams; and d.f. is the dilution factor, if used, as described below:

$$\text{d. f .} = \frac{(volume\ of\ diluted\ sample\ solution\ in\ ml)}{(volume\ of\ aliquot\ taken\ for\ dilution\ in\ ml)} \qquad \text{eqn.} \rightarrow 18.$$

3.7.6.2. For X-Ray Fluorescence Spectrometer (XRF)

To convert the reading of XRF to micro gram (x) per gram ($x_\circ$= 1) from concentration (%) one use the following formula (egn. 19):

$$\frac{\boldsymbol{x \times 10^{-6}}}{\boldsymbol{x_\circ}} \times \mathbf{100} = C$$

$$\boldsymbol{x = x_\circ C \times 10^4 = 1 \times C \times 10^4 C} \qquad \text{eqn.} \rightarrow 19$$

3.8. Data collection

Data collected using a predesigned questionnaire containing personal data such as gender, age, residence, tribe and occupation, as well social habits, resources of water, use of make-up, chronic disease and use of chronic medication (Appendix 7 and 8).

3.9. Data analysis

Collected data were spread on master sheet, thereafter, entered computer and analysis was performed using SPSS version 21.0 for Windows. All quantitative data were presented as mean values ± standard deviation (SD). We used the Chi-square test (χ 2) to compare the differences between groups with confidence level 95%. Student's t test was used to compare a result of a single group. P value <0.05 considered to be significant.

To provide an index of normality, each set of hair and nail element results compared with reference intervals which have been derived from Biolab's database, which validated and certified by the both National Institute for Environment Studies, Japan and from the Community Bureau of Reference of the Commission of the European Communities (Ibaraki, 2010) shown in table 5.

To test the accuracy and strength of the machine used in trace elements measurement we calculated the sensitivity, specificity, positive predictive and negative predictive values. The results were expressed as figures or tables as appropriate.

* **Positive predictive value:** Probability that increase of trace element concentration in the sample is present when the test is positive.

* **Negative predictive value:** Probability that increase of trace element concentration in the sample is not present when the test is negative.
* **Sensitivity:** Probability that there is increase of trace element concentration measurement in the sample when actually there is increase of trace element concentration (True positive test).
* **Specificity:** Probability that the test result will be negative when actually there is no increase of trace element concentration (True negative test).

3.10. Ethical considerations

1- The acceptance of the proposal by faculty board – Faculty of higher education, Sudan University.
2- Pre-investigation informed consent for both sample providence and research purposes was obtained from study candidates, during whom participants informed also about the confidentiality of the results and if he wish to withdraw from study has rights to withdraw at any step.

CHAPTER FOUR

Results and discussion

CHAPTER FOUR

4.1. Results

4.1.1. Demography

The whole sample size was 72 participants. In Five candidates the sample was inappropriate, so were excluded from the study. A total 67 participants were enrolled into this cross sectional study after acceptance of the pre-given informed consent. There were 55.2% child, and 44.8% adults (Figure 3 and Table 6).

Their age distribution ranged from 6 to 65 years, and mean ± standard deviation (SD) being 21.6±14.2 years. They descent were from different community strata of the rural area around Elnihoud city (Figure 4 and Table 7). The tribe compositions of study group were variable; 49.3% Hamar, 10.4% Bidairya and 6% Gawama, as shown in Table 8 and Figure 5.

Most of the participants are students and farmers where they make up the social component of the research population as observed in 70.1% and 25.4% respectively (Figure 6).

4.1.2. Sociology

Since this study was conducted in a rural area where the population depends on farming and herding career. The research sample, all share the same food quality and sources of their own local products.

They also use drinking water from the available water resources they have, which is composed of excavations and wells or both as seen is 44.8%, 14.9%, and 40.3% respectively (Figure 7).

The inhabitants of this region can be consider are from conservative societies where the number of smokers and tobacco snuffers accounts for only about 4.5% of the studied sample, and of great note, none of them has a history of alcohol intake as shown in Table 9.

Given to the use of make-up, it is noticeable use of Henna, hair dye and manicure (Nail varnish) by some participants as used in 26.9%, 12% and 10.4% respectively (Table 10 and figure 8).

It should be noted that participants in the research does not have a satisfactory history of chronic diseases as well as use of medical drugs in a sustainable manner, except in one of them (1.5%) who suffered from hypertension and has to use of the necessary antihypertensive medicine.

It is further noted that there is no one of them has a previous history of poisoning or chronic skin allergy.

4.1.3. Trace elements concentration analysis

The populations were subdivided into two categories, the children (majority) and adults (males and females).

4.1.3.1. Hair

The mean hair weight collected from participants was 39.7 ± 74.7 mg. (Range, 10.1 to 449 mg).

The results of element concentration were obtained in milligram per kilogram.

4.1.3.1.1. Overall trace element concentration in hair samples

The overall mean, maximum, and minimum of each trace element concentrations in hair samples are shown in Table 11. The trace of the element concentrations varied widely in the hair samples as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the hair sample is Ca> Mg > Fe > Pb > Zn > Cu. Overall mean concentrations of Ca (606.06 μg/g), Mg (562.07 μg/g), Fe (314.7 μg/g), Pb (187.6μg/g), Zn (118.9 μg/g), and Cu (19.14 μg/g) were found in the hair samples.

Collectively, the study showed that there is increase that exceeds the reference range of the measured element concentration in the participants hair; Fe in 9%, Mg in 9%, Pb in 7.5%, Cu in 6%, Ca in 6%, and Zn in 4.5% (Table 12). This increment was seen in 17 candidates (25.4%).

To further gain a better insight and understanding of the inter-element association of trace elements in the scalp hair samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 13 presents the summary of the correlation of the inter-element associations in the hair samples.

Generally, the levels of the trace elements in hair samples were strongly correlated. High significant correlations ($p<0.01$) were found between almost all trace elements except between Ca and Cu it was less powerful ($r = 0.259$, $p = 0.017$). However, weak poor correlation was found between the levels of Ca and Fe ($r = 0.194$, $p = 0.057$).

4.1.3.1.2. Trace element concentration in children's hair samples (n=37)

The children's mean, maximum, and minimum of each trace element concentrations in their hair samples are shown in table 14. The trace of the element concentrations varied widely as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the hair sample is Ca> Mg > Fe >Pb> Zn > Cu. Overall mean concentrations of Ca (560.1 μg/g), Mg (483.1 μg/g), Fe (372.5 μg/g), Pb (164.9 μg/g), Zn (103.6 μg/g), and Cu (18.1μg/g) were found in their hair samples.

To further gain a better insight and understanding of the inter-element association of trace elements in the children's scalp hair samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 15 presents the summary of the correlation (r) of the inter-element associations in the hair samples.

Generally, the levels of the trace elements in their hair samples were correlated ($p<0.05$), except between Ca& Cu, Ca& Fe and Ca& Zn where there is poor correlation ($p>0.05$).

Among elements correlated this correlation was high ($p<0.01$) between almost all elements, whereas, it was less between Ca and Pb ($r = 0.317$, $p = 0.028$).

4.1.3.1.3. Trace element concentration in female's hair samples (n=23)

The female's mean, maximum, and minimum of each trace element concentrations in their hair samples are shown in table 16. The trace of the element concentrations varied widely as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the hair sample is Mg >Ca> Fe >Pb> Zn > Cu. Overall mean concentrations of Mg (680.04 μg/g), Ca (661.56 μg/g), Fe (232.97 μg/g), Pb (216.13 μg/g), Zn (137.14 μg/g), and Cu (21.68 μg/g) were found in their hair samples.

To further gain a better insight and understanding of the inter-element association of trace elements in the female's hair samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 17 presents the summary of the correlation (r) of the inter-element associations in the hair samples.

Generally, the levels of the trace elements in their hair samples were correlated ($p<0.05$), except between Cu & Fe, Cu & Mg, Fe &Ca, Mg & Fe, Mg &Ca, Mg &Pb and Mg & Zn where there is poor correlation ($p>0.05$).

Among elements correlated this correlation was less strong between Cu and Pb ($r= 0.359$, $p = 0.046$), whereas, it was highly strong between the remainder ($p<0.01$).

4.1.3.1.4. Trace element concentration in male's hair samples (n=7)

The male's mean, maximum, and minimum of each trace element concentrations in their hair samples are shown in table 18. The trace of the element concentrations varied widely as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the hair sample is Ca> Mg > Fe >Pb> Zn > Cu. Overall mean concentrations of Ca (666.68 μg/g), Mg (591.92 μg/g), Fe (277.88 μg/g), Pb (213.37 μg/g), Zn (139.29 μg/g), and Cu (16.10 μg/g) were found in their hair samples.

To further gain a better insight and understanding of the inter-element association of trace elements in the male's hair samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 19 presents the summary of the correlation (r) of the inter-element associations in the hair samples.

Generally, the levels of the trace elements in their hair samples were almost poorly correlated ($p>0.05$). Only there is high strong correlation between Fe & Mg and Fe &Pb ($p<0.01$) and less high correlation between Pb and Mg ($p<0.05$ and >0.01).

4.1.3.1.5. Trace element concentration in the hair samples among genders (n=67)

Accumulation of trace elements in hair and it is variations was not influenced by gender as the difference was statistically not significant between groups in study participants ($p>0.05$) as shown in table 20.

4.1.3.1.6. Trace element concentration in the hair samples between age groups (n=67)

Accumulation of Mg in hair was significantly influenced by age (p=0.000). The study indicated higher Mg concentration (μg/g) with increased age, whereas, the concentration of the remainder elements in hair was not significantly influenced age (p>0.05) as explored in table 21.

4.1.3.1.7. Trace element concentration in the hair samples depends on geographical location (n=67)

The variation of elements in hair between subjects was not affected by geographical residence as there were no significant differences in trace element concentration in the hair between the locations (p>0.05) as shown in table 22.

4.1.3.1.8. Trace element concentration in the hair sample depends on tribe (n=67)

The variation of elements in hair between subjects was not affected by their tribe. This observation was obviously seen in the current study as it revealed that there were no significant differences in trace element concentration in the hair between the tribes of study sample (p>0.05) as shown in table 23.

4.1.3.1.9. Trace element concentration in the hair sample depends on work (n=67)

In order to evaluate the relations between the work nature of study group and trace element concentrations in their hair, the result was statistically significant for Mg that showed increased concentration among farmers (p=0.003) as showed in table 24.

In the same way, when comparing the groups regarding working hours the study found that as daily working hours increase there is significant increase in Ca and Mg concentration in their hair ($p=0.02$) as presented in table 25.

4.1.3.1.10. Trace element concentration in the hair sample depends on water resources (n=67)

Considering water is important factor supplies human with trace elements, the study found that there is no considerable variation changes of measured trace elements deposition in the hair of participants irrespective to the water that using excavations versus wells as a main water resources as the difference was statistically not significant ($p>0.05$) as illustrated in table 26.

4.1.3.1.11. Effect of social habit on trace element concentration in the hair (n=67)

Social habits in form of cigarette smoking and tobacco snuffing by participants were studied also paid attention in the current study.

The study showed there is high concentration of Fe, Pb and Zn measured in the hair of cigarette smokers and tobacco snuffers. These high variations in their concentration were statistically significant ($p<0.05$), whereas, the variation in the remainder measured elements was statistically not significant ($p>0.05$) as demonstrated in table 27.

4.1.3.1.12. Effect of make up on trace element concentration in the hair (n=67)

Using make up by study group was studied extensively as external parameter providing trace elements to the hair when became in contact with it.

The variation in Mg in hair between subjects was higher for Henna and Hair dye users than the other groups in the current study. There were significant differences in Mg concentrations in their hair ($p=0.042$), whereas the difference in the other trace elements was statistically not significant ($p>0.05$) as demonstrated in table 28.

4.1.3.2. Fingernails

The mean fingernail weight collected from participants was 36.9 ± 51.3 mg. (Range, 32.1 to 305.5 mg).

The findings regarding the content of trace elements in fingernails of the investigated participant's sample together with the basic statistical description were presented separately.

4.1.3.2.1. Overall trace element concentration in fingernail samples (n=67)

The overall mean, maximum, and minimum of each trace element concentrations in fingernail samples are shown in table 29. The trace of the element concentrations varied widely in the fingernail samples as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the fingernail sample is Ca> Mg > Fe >Pb> Zn > Cu. Overall

mean concentrations of Ca (792.5 μg/g), Mg (598.8 μg/g), Fe (344.9 μg/g), Pb (250.6 μg/g), Zn (104.9 μg/g), and Cu (37.5 μg/g) were found in the fingernail samples.

Collectively, the study showed that there is increase exceeding the reference range of the measured element concentration in the participants fingernails; Cu in 29.9%, Mg in 26.9%, Fe in 14.9%, Pb in 7.5%, and Ca in 4.5% (Table 30). This increment was seen in 20 candidates (29.8%).

To further gain a better insight and understanding of the inter-element association of trace elements in the fingernail samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 31 presents the summary of the correlation of the inter-element associations in the fingernail samples. Generally, the levels of the trace elements in fingernail samples were strongly correlated. High significant correlations ($p<0.01$) were found between almost all trace elements except between Pb and Cu it was less powerful ($r = 0.253$, $p = 0.020$).

4.1.3.2.2. Trace element concentration in children's fingernails samples (n=37)

The children's mean, maximum, and minimum of each trace element concentrations in their fingernail samples are shown in table 32. The trace of the element concentrations varied widely as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the fingernails sample is Ca> Mg > Fe >Pb> Zn > Cu. Overall mean concentrations of Ca (821 μg/g), Mg (629.5 μg/g), Fe (343.2 μg/g), Pb (253.2 μg/g), Zn (96.4 μg/g), and Cu (33.8 μg/g) were found in their fingernail samples.

To further gain a better insight and understanding of the inter-element association of trace elements in the children's fingernail samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 33 presents the summary of the correlation (r) of the inter-element associations in the fingernail samples.

Generally, the levels of the trace elements in their fingernail samples were correlated ($p<0.05$), except between Pb& Cu, Pb& Mg and Mg & Zn where there is poor correlation ($p>0.05$).

Among elements correlated this correlation was less strong between Cu & Fe, Cu &Ca, Cu & Mg, Cu & Zn, Ca& Mg , Ca& Zn and Pb& Zn ($p<0.05$ and > 0.01), whereas, it was highly strong between the remainder ($p<0.01$).

4.1.3.2.3. Trace element concentration in female's fingernails samples (n=23)

The female's mean, maximum, and minimum of each trace element concentrations in their fingernail samples are shown in Table 34. The trace of the element concentrations varied widely as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the fingernails sample is Ca> Mg > Fe >Pb> Zn > Cu. Overall mean concentrations of Ca (838.9 μg/g), Mg (600.9 μg/g), Fe (376.6 μg/g), Pb (265.1 μg/g), Zn (113.9 μg/g), and Cu (43.8 μg/g) were found in their fingernail samples.

To further gain a better insight and understanding of the inter-element association of trace elements in the female's fingernail samples, the levels of trace elements

were subjected to Pearson Bivariate correlation (r). Table 35 presents the summary of the correlation (r) of the inter-element associations in the fingernail samples.

Generally, the levels of the trace elements in their fingernail samples were correlated ($p<0.05$), except between Ca& Zn and Ca& Mg where there is poor correlation ($p>0.05$).

Among elements correlated this correlation was highly strong between almost all elements ($p< 0.01$), whereas, it was less strong between Cu & Mg and Pb& Zn ($p<0.05$ and > 0.01).

4.1.3.2.4. Trace element concentration in male's fingernail samples (n=7)

The male's mean, maximum, and minimum of each trace element concentrations in their fingernail samples are shown in table 36. The trace of the element concentrations varied widely as demonstrated by the large range of trace element concentrations. The order of the mean trace element concentrations in the fingernails sample is Ca> Mg > Fe >Pb> Zn > Cu. Overall mean concentrations of Ca (489.7 μg/g), Mg (429.7 μg/g), Fe (249.7 μg/g), Pb (189.2 μg/g), Zn (120.2 μg/g), and Cu (37.1 μg/g) were found in their fingernail samples.

To further gain a better insight and understanding of the inter-element association of trace elements in the male's fingernail samples, the levels of trace elements were subjected to Pearson Bivariate correlation (r). Table 37 presents the summary of the correlation (r) of the inter-element associations in the fingernail samples.

Generally, the levels of the trace elements in their fingernail samples were poorly correlated ($p>0.05$). However, strong significant correlations were found between Ca and Zn ($r = 0.862$, $p = 0.006$) and less strong significant correlation between Fe and Mg ($r = 0.809$, $p = 0.014$).

4.1.3.2.5. Trace element concentration in the fingernail samples among genders (n=67)

Accumulation of trace elements in the fingernails and it is variations was not influenced by gender as the difference was statistically not significant between groups in study participants ($p>0.05$) as shown in table 38.

4.1.3.2.6. Trace element concentration in the fingernail samples between age groups (n=67)

In order to evaluate the relations between age and trace element concentrations in the fingernails, the result was non-statistically significant where $p>0.05$ in all elemental correlation as shown in table 39.

4.1.3.2.7. Trace element concentration in the fingernail samples depends on geographical location (n=67)

The relation between residence and Fe concentration in fingernails was higher in Bayadh and Abumarigah villages when compared to the other places and this difference was statistically significant ($p<0.05$).

On other hand, the relation between residence and Mg concentration was higher in Bayadh and Salami villages when compared to the other places and this difference was statistically significant ($p<0.05$), whereas correlations between residence and Cu, Ca, Pb, and Zn were negative as the difference was non-statistically significant ($p>0.05$) as shown in table 40.

4.1.3.2.8. Trace element concentration in the fingernail sample depends on tribe (n=67)

The relation between tribe of participants and Cu, Fe and Pb concentration in fingernails was higher in Hamar tribe when compared to the other tribe in the current study and this difference was statistically significant ($p<0.05$), whereas, no correlations found between tribe and Ca, Mg and Zn as the difference was statistically not significant ($p>0.05$) as shown in table 41.

4.1.3.2.9. Trace element concentration in the fingernail sample depends on work (n=67)

In order to evaluate the relations between the occupation of study group and trace element concentrations in the fingernails, the result was non-statistically significant where $p>0.05$ in all elemental correlation as showed in table 42.

When comparing the groups regarding working hours the study found that as daily working hours increase there is increase in Zn concentration in fingernails among farmers ($p=0.02$) as shown in table 43.

4.1.3.2.10. Trace element concentration in the fingernail sample depends on water resources (n=67)

Considering water is important factor supplies human with trace elements, the study found that there is considerable increase of Cu deposition in the fingernails of participants whom using excavations as a main water resources as the difference was statistically significant ($p=0.01$), whereas, for the rest of trace elements measured the difference was statistically not significant where $p>0.05$ as shown in Table 44.

4.1.3.2.11. Effect of social habit on trace element concentration in the fingernails (n=67)

The variation of trace elements concentration in the fingernails among participants was not affected by the negative social habits in form of cigarette smoking and tobacco snuffing. The difference between groups was statistically not significant ($p>0.05$) as demonstrated in table 45.

4.1.3.2.12. Effect of make up on trace element concentration in the fingernails (n=67)

Using make up by participants was studied extensively in the current study as one of the external parameter that providing fingernails with the trace elements when become in contact with it. The differences in the variations of all trace elements measured in fingernails between subjects were statistically not significant ($p>0.05$) as demonstrated in table 46.

4.1.3.3. Relations of trace element variation between hair and nails

The levels of trace elements in hair samples compared to fingernail samples from study subjects is as presented in table 47.

The variation difference in trace elements concentration in hair versus fingernails is statistically not significant as $p > 0.05$ as explored in table 48.

Broadly speaking, 20 (29.8%) participants showed moderate increase of the trace elements concentration in their fingernails sample, and 17 (25.4%) participants showed moderate increase of the trace elements concentration in their hair sample. Study also found that all participants with increased hair trace elements concentration they had increased of at least the same trace elements in their fingernails sample, whereas, in the remainder 3 participants (4.5%) of them those with increased trace elements in their fingernails they did not have same increase trace element concentration in their hair.

From the point of view, the sensitivity and specificity of the machine found to be acceptably very high. The obtained sensitivity and specificity of the machine used in detection of trace elements concentration and its correlation ability between hair and fingernail samples were calculated and found to be 91.96% and 100% respectively. Similarly, its accuracy obtained by the positive and negative predictive values at 95% confidence interval (95% CI) which was very high, as it was 100% and 94% respectively as shown in table 49.

4.2. Discussion

Hair and nails mineral analysis is being used by health care practitioners and promoted by laboratories as a clinical assessment tool and to identify toxic exposures, despite a 1985 study that found poor reliability for this test. To assess whether the reliability of data from commercial laboratories advertising multi mineral analyses for nutritional or toxicity assessment has improved since the 1985 study. Variations were found in laboratory sample preparation methods and calibration standards. Laboratory designations of normal reference ranges varied greatly, resulting in conflicting classifications (high, normal, or low) of nearly all analyzed minerals. Laboratories also provided conflicting dietary and nutritional supplement recommendations based on their results. Problems with the regulation and certification of these laboratories also should be addressed (Ahmad et al. 2013).

The use of nails in biomonitoring is not as widespread as hair, but the use has increased the recent decades (Oluwole et al., 1994

Hair and nails appeared to be the best indicator tissues for trace element exposure in the current study. The concentrations were within the detection limits for all samples, but the levels were generally higher in nails than hair samples.

The findings of trace elements concentration in hair obtained from participants in the current study in concordance with the other local study performed in Khartoum, Sudan (Osman et al., 2010), where in both studies the mean of trace

elements concentration was within same ranges. Of note the readings were slightly lower.

Whereas, the findings of trace elements concentration in fingernails obtained from participants in the current study when compared to other local study performed in Jabait, eastern Sudan (Ibrahim et al., 2014), the findings of our study showed lower concentration for all elements.

During literature search there is limited knowledge of the relationships between trace elements in hair and nails in published literature, which has mainly focused on correlations between drinking water levels and/or other factors.

The existing knowledge about hair-nail relationships for trace elements is confusing. Most of the correlation analyses in the current study found significant relationship between matched hair and nails samples. The results indicate that incorporation of trace elements is similar for hair and nails. the findings is in concordance with that obtained by Abdulrahman et al. (2012) in Nigeria, whereas, it was in contradicting with the findings from the study by Evjen (2011) in Tanzania as concluded that half of the correlation analyses in his study found no significant relationship between matched hair and nails samples.

The study of Vance et al. (1988) reported that concentrations of non-essential trace elements (Pb) were positively correlated in hair and nail, whereas concentrations of essential elements (Fe, Zn, Cu, Ca, and Mg) were not correlated.

We have analysed hair and fingernails of 67 healthy and occupationally unexposed individuals to investigate the feasibility of this type of study by atomic absorption spectrometer. Their demographic characteristics indicate a relatively young group with about 55.2% having ages below 18 years.

Trace-elements concentrations in the scalp hair and fingernails show great variations between subjects within each residence. These however were not established by a correlation study. A glance at statistical summary of the data reveals mean concentration values for the different trace- elements with large standard deviations. Over the vast majority of the data fall within almost similar standard deviation, making our data hardly normally distributed.

Hair grows at a rate of approximately 1 cm per month, and it grows in phases. It grows in the anagen phase and rests in the telogen phase. About 10% of the scalp hair is in the resting phase at any point in time, and it lasts for approximately 3 months (Gellein et al., 2008). There is a possibility of differences in trace element profiles between strands.

The result of trace elements in this study using atomic absorption spectrometry was performed in a healthy individuals living in a non-industrialized environment it was in concordance with the results from a studies among healthy individuals living in a relatively non-industrial environment, based on analysis of fingernails using Instrumental Neutron Activation Analysis (INAA) method (Hewitt et al., 1995) and scalp hair from local study.

An analytical method for determining essential elements (Zn, Fe, Ca, Mg and Cu) and toxic element (Pb) on scalp hair by atomic absorption spectrometer was assessed. Results obtained directly using of hair was compared with those measured from nails samples and the analytical methods were found to agree well.

The key aspect addressed in this study is whether the results actually can provide information about exposure, intake or nutritional status.

The method of hair and nail analysis appears to be ideally suited for use in this prospective study.

The FAA Technique used to analyze hair samples and nail samples shows some interesting results as tables 11 and 29 indicates. For hair the concentrations in micro gram per gram for Fe is in the range of 22.49 μg/g to 5400 μg/g, for Zn the range is from 10.8 μg/g to 603.5 μg/g, and for Mg the range is from 156.66 μg/g to 2686.83 μg/g.

It is very striking to show that these ranges agree with standard one shown in table (5).

For XRF Technique the analysis of hair samples show that Fe ranges from 400 μg/g to 1400 μg/g, and Zn ranges from 100 μg/g to 200 μg/g as indicated in tables 50, 51 and 52.

4.3. Conclusion

1. Trace elements variation is similarly recorded in hair and nails

2. In such group from conservative community strata the trace elements was not affected by gender and age.

3. The high sensitivity, specificity and accuracy of atomic absorption spectrometer in detection of trace elements in hair and nails.

4. Study performed in healthy individuals, in non industrialized area.

5. The result obtained can be broad casted and validated as a reference of trace element in hair and nails of Sudanese population

4.4. Recommendations

1. Use of atomic absorption spectrometer in detection of trace elements in hair or fingernails is valuable as available resource in such country with economical constrains

2. To determine the health state of individuals or environmental pollution status we can use hair or nail, one of them can suffice as parameter.

3. Validate the obtained result from healthy people from non industrialized environment such as in the current study as a reference in the oncoming studies in the fields.

4. Further similar studies need to be made for other regions of Kordufan and hence to be extended to other Sudanese regions and states.

FIGURES

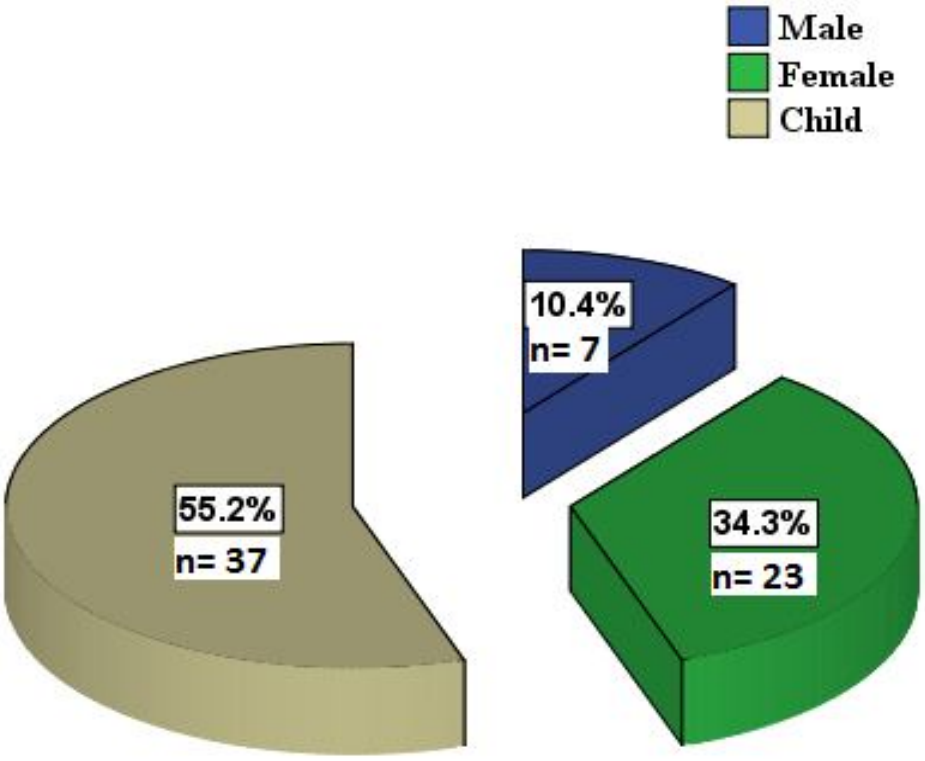

Figure 3: Gender and age strata of study group (n=67)

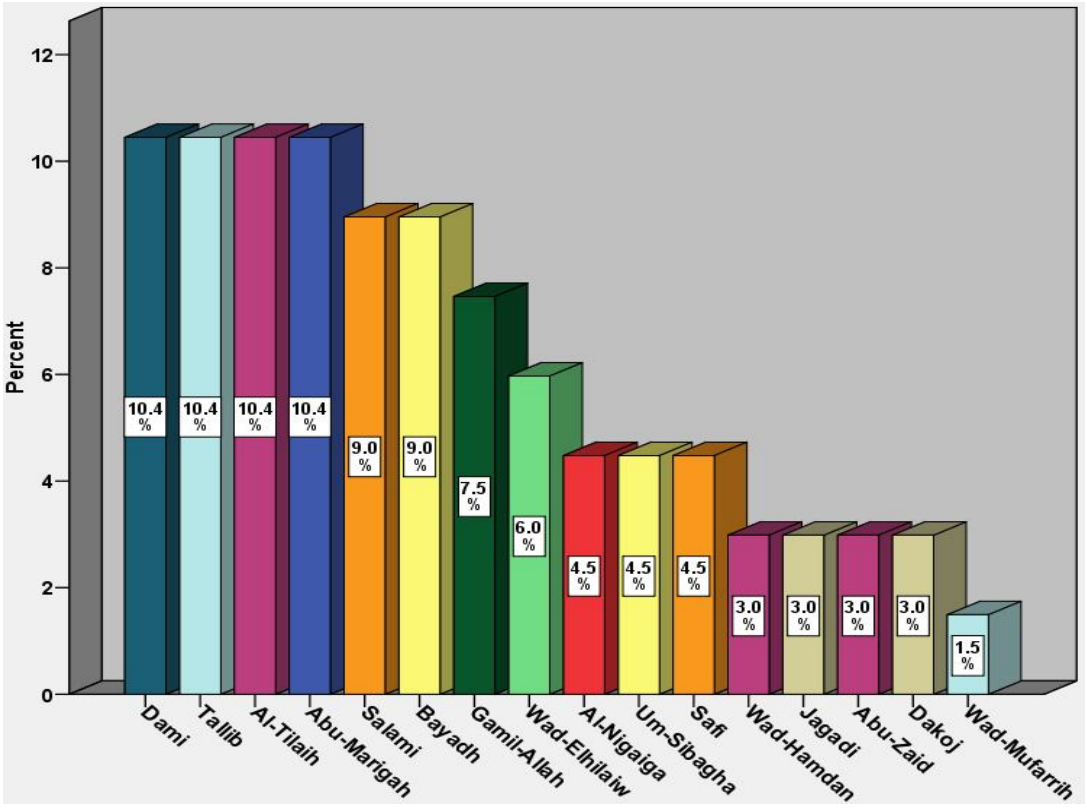

Figure 4: Geographical distribution of the study group (n=67)

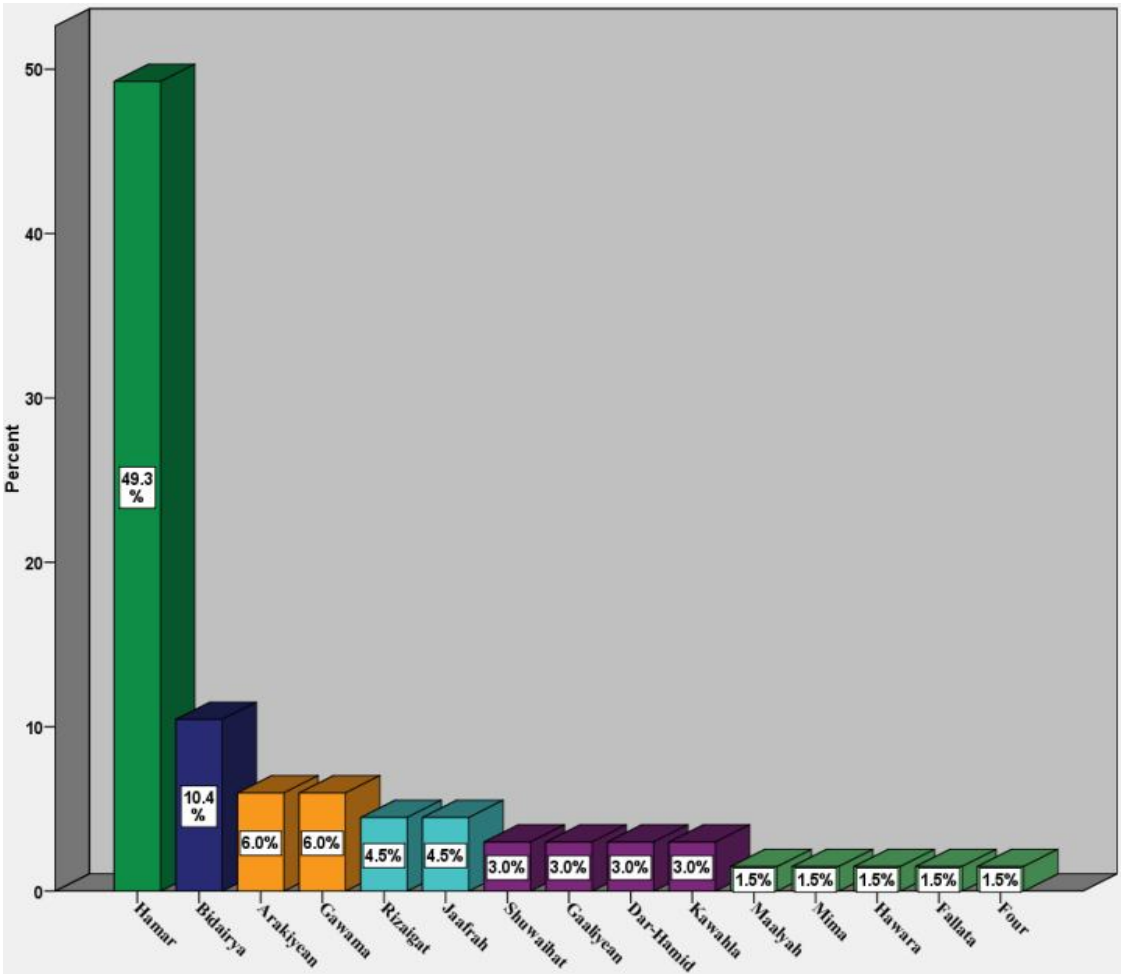

Figure 5: The tribe of study group (n=67)

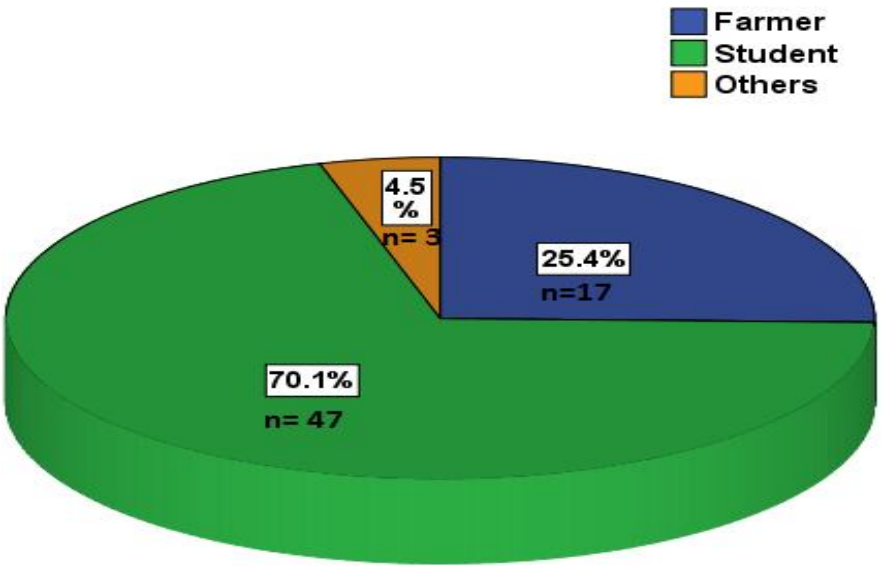

Figure 6: The occupation of study group (n=67)

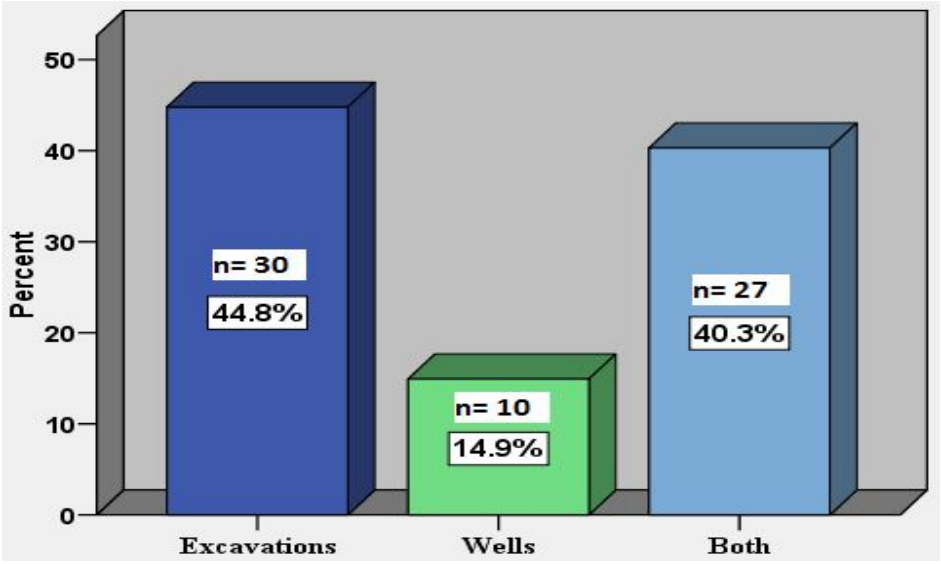

Figure 7: Resources of water used by participants (n=67)

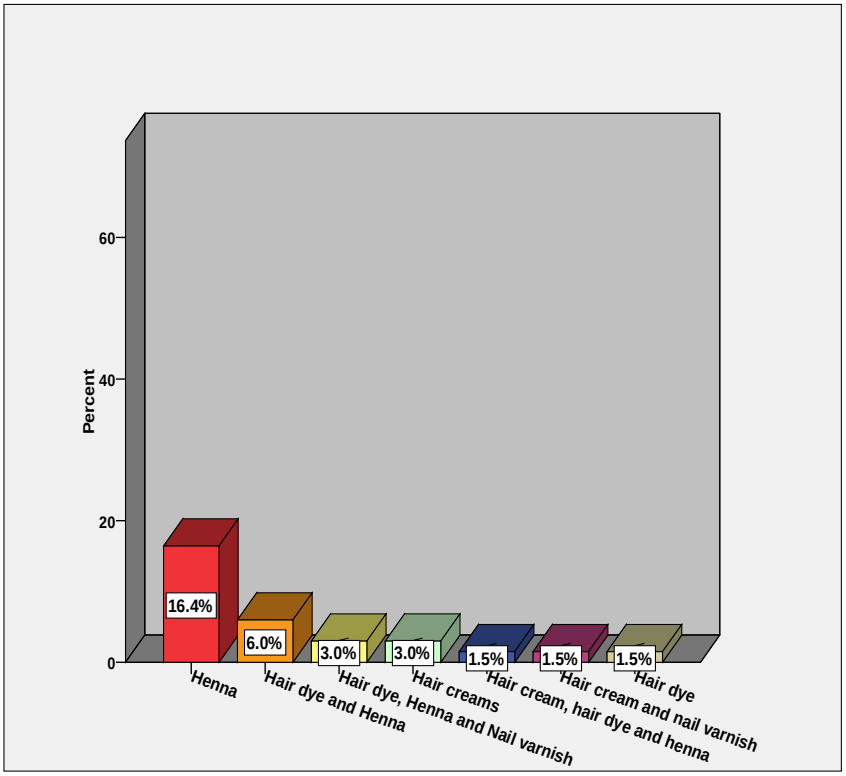

Figure 8: Use of make up by the study group (n=67)

TABLES

Table 5: Reference intervals of trace elements concentration in scalp hair and fingernails validated and certified by the National Institute for Environment Studies, Japan and from the Community Bureau of Reference of the Commission of the European Communities

Element	Reference (µg/g)			
	Hair		Nail	
	Minimum	Maximum	Minimum	Maximum
Cu	11	25	4	51
Fe	20	520	60	555
Ca	130	1280	250	1860
Mg	100	935	90	800
Pb	30	410	50	480
Zn	30	450	40	490

Table 6: Distribution of Age Groups among study group (n=67)

Age Groups	Frequency	Percent
6 – 18	37	55.2
19 – 35	19	28.4
36 – 50	8	11.9
51 – 65	3	4.5
Total	**67**	**100.0**

Table 7: Geographical distribution of the study group (n=67)

Residence	Frequency	Percent
Al-Tilaih	7	10.4
Abu-Marigah	7	10.4
Tallib	7	10.4
Dami	7	10.4
Salami	6	9.0
Bayadh	6	9.0
Gamil-Allah	5	7.5
Wad-Elhilaiw	4	6.0
Safi	3	4.5
Um-Sibagha	3	4.5
Al-Nigaiga	3	4.5
Abu-Zaid	2	3.0
Jagadi	2	3.0
Wad-Hamdan	2	3.0
Dakoj	2	3.0
Wad-Mufarrih	1	1.5
Total	**67**	**100.0**

Table 8: The tribe of study group (n=67)

Tribe	Frequency	Percent
Hamar	33	49.3
Bidairya	7	10.4
Arakiyean	4	6.0
Gawama	4	6.0
Jaafrah	3	4.5
Rizaigat	3	4.5
Gaaliyean	2	3.0
Kawahla	2	3.0
Dar-Hamid	2	3.0
Shuwaihat	2	3.0
Hawara	1	1.5
Maalyah	1	1.5
Mima	1	1.5
Four	1	1.5
Fallata	1	1.5
Total	**67**	**100.0**

Table 9: Social habits of the study group (n=67)

Social habits	Frequency	Percent
No	64	95.5
Smoking	2	3.0
Tobacco Snuffing	1	1.5
Total	**67**	**100.0**

Table 10: Use of make up by the study group (n=67)

	Frequency	Percent
Henna	18	26.9
Hair dye	8	12.0
Nail varnish	7	10.4
Hair creams	4	6.0

Table 11: Overall mean, maximum, and minimum of each trace element concentrations in hair samples of the study group (n=67)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	9.61	200.00	19.1384	24.70688
Fe	22.49	5400.00	314.7216	644.80477
Ca	183.02	1854.83	606.0552	364.22403
Mg	156.66	2686.83	562.0719	411.91479
Pb	47.61	900.00	187.5569	150.72131
Zn	10.80	603.50	118.8539	110.38196

Table 12: Collective element concentration in the hair of study group (n=67)

Element	Reference (µg/g)	< normal		Within range		> range	
		No.	%	No.	%	No.	%
Cu	11- 25	7	10.4	56	83.6	4	6
Fe	20 – 520	—	—	61	91.0	6	9.0
Ca	130 – 1280	—	—	63	94.0	4	6.0
Mg	100 – 935	—	—	61	91.0	6	9.0
Pb	30 – 410	—	—	62	92.5	5	7.5
Zn	30 – 450	2	3.0	62	92.5	3	4.5

Table 13: Overall Pearson correlation of the inter-element associations in the hair samples (n = 67)

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.890(**)	1				
	0.000					
Ca	0.259(*)	0.194	1			
	0.017	0.057				
Mg	0.422(**)	0.441(**)	0.296(**)	1		
	0.000	0.000	0.008			
Pb	0.647(**)	0.677(**)	0.394(**)	0.554(**)	1	
	0.000	0.000	0.000	0.000		
Zn	0.694(**)	0.603(**)	0.319(**)	0.488(**)	0.747(**)	1
	0.000	0.000	0.004	0.000	0.000	

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 14: Trace Element Concentration in Children's Hair Samples (n=37)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	9.61	200.00	18.1308	30.81140
Fe	65.73	5400.00	372.5059	858.73587
Ca^{+2}	187.50	1328.28	560.0819	329.66204
Mg^{+2}	156.71	1800.00	483.0903	287.27155
Pb	50.00	900.00	164.9143	152.76815
Zn	22.92	603.50	103.6224	93.18975

Table 15: Summary of Pearson Correlation (r) of the inter-element associations in the children's hair samples (n=37)

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.983(**)	1				
	0.000					
Ca	0.149	0.260	1			
	0.189	0.060				
Mg	0.763(**)	0.838(**)	0.442(**)	1		
	0.000	0.000	0.003			
Pb	0.807(**)	0.850(**)	0.317(*)	0.866(**)	1	
	0.000	0.000	0.028	0.000		
Zn	0.895(**)	0.909(**)	0.198	0.803(**)	0.833(**)	1
	0.000	0.000	0.121	0.000	0.000	

****** Correlation is significant at the 0.01 level.

***** Correlation is significant at the 0.05 level.

Table 16: Trace Element Concentration in Female's Hair Samples (n=23)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	11.24	86.24	21.6826	16.19958
Fe	22.49	525.80	232.9761	115.30469
Ca	183.02	1854.83	661.5617	413.81706
Mg	255.96	2686.83	680.0465	548.13890
Pb	47.88	551.72	216.1252	146.25301
Zn	10.80	593.10	137.1378	125.35061

Table 17: Summary of Pearson Correlation (r) of the inter-element associations in the female's hair samples (n= 23)

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.075	1				
	0.367					
Ca	0.612(**)	0.311	1			
	0.001	0.075				
Mg	0.140	0.107	0.141	1		
	0.262	0.313	0.261			
Pb	0.359(*)	0.699(**)	0.482(**)	0.252	1	
	0.046	0.000	0.010	0.123		
Zn	0.693(**)	0.560(**)	0.584(**)	0.321	0.711(**)	1
	0.000	0.003	0.002	0.068	0.000	

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 18: Trace Element Concentration in Male's Hair Samples (n=7)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	11.24	21.02	16.1043	3.82501
Fe	69.44	609.09	277.8829	228.91660
Ca	310.43	1266.60	666.6786	387.50466
Mg	156.66	1318.18	591.9157	420.39195
Pb	47.61	427.04	213.3714	156.07696
Zn	57.63	462.63	139.2871	145.28318

Table 19: Summary of Pearson Correlation (r) of the inter-element associations in the male's hair samples (n= 7)

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.461	1				
	0.149					
Ca	0.613	0.481	1			
	0.071	0.137				
Mg	0.418	0.914(**)	0.429	1		
	0.175	0.002	0.168			
Pb	0.421	0.838(**)	0.342	0.783(*)	1	
	0.174	0.009	0.226	0.019		
Zn	0.088	0.528	0.285	0.294	0.572	1
	0.426	0.112	0.268	0.261	0.090	

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 20: Comparison of the variations in trace element concentration in the hair Samples among genders (n=67)

Element	Chi square	P value
Cu	9.060	0.060
Fe	3.935	0.140
Ca	0.770	0.681
Mg	4.026	0.134
Pb	0.749	0.688
Zn	2.183	0.702

Table 21: Comparison of the variations in trace element concentration in the hair Samples between age groups (n=67)

Element	Chi square	P value
Cu	11.955	0.063
Fe	3.171	0.366
Ca	5.836	0.120
Mg	**18.917**	**0.000 ***
Pb	4.165	0.244
Zn	2.315	0.889

*** Statistically significant, p value < 0.05**

Table 22: Comparison of the variation in trace element concentration in the hair Sample depends on geographical location (n=67)

Element	Chi square	P value
Cu	21.396	0.875
Fe	19.692	0.184
Ca	12.711	0.625
Mg	16.480	0.351
Pb	11.284	0.732
Zn	24.615	0.744

Table 23: Comparison of the variations in trace element concentration in the hair Sample depends on the tribe of participants (n=67)

Element	Chi square	P value
Cu	39.999	0.66
Fe	14.860	0.388
Ca	4.383	0.968
Mg	14.967	0.380
Pb	17.854	0.214
Zn	9.693	0.999

Table 24: Trace element concentration in the hair Sample depends on work (n=67)

Element	Chi square	P value
Cu	8.305	0.081
Fe	0.471	0.790
Ca	4.248	0.120
Mg	**11.708**	**0**.003 *
Pb	0.777	0.678
Zn	0.956	0.916

*** Statistically significant, p value < 0.05**

Table 25: Trace element concentration in the hair Sample depends on daily working hours (n=67)

Element	Chi square	P value
Cu	13.271	0.350
Fe	1.503	0.959
Ca	**16.498**	**0.011 ***
Mg	**19.212**	**0.004 ***
Pb	2.083	0.912
Zn	2.315	0.999

*** Statistically significant, p value < 0.05**

Table 26: Trace element concentration in the hair Sample depends on water resources (n=67)

Element	Chi square	P value
Cu	2.871	0.580
Fe	0.133	0.936
Ca	2.871	0.238
Mg	1.632	0.442
Pb	3.017	0.221
Zn	7.248	0.123

Table 27: Effect of social habit on trace element concentration in the hair (n=67)

Element	Chi square	P value
Cu	0.617	0.961
Fe	**14.874**	**0.001 ***
Ca	0.199	0.905
Mg	4.333	0.115
Pb	**12.698**	**0**.002 *
Zn	**21.770**	**0**.000 *

*** Statistically significant, p value < 0.05**

Table 28: Effect of make up on trace element concentration in the hair (n=67)

Element	Chi square	P value
Cu	**19.007**	0.165
Fe	**2.231**	**0**.956
Ca	**3.402**	**0**.845
Mg	**14.589**	**0**.042 *
Pb	**7.732**	**0**.357
Zn	**13.093**	**0**.519

*** Statistically significant, p value < 0.05**

Table 29: Overall Trace Element Concentration in Fingernail Samples (n=67)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	3.47	232.00	37.5752	35.40652
Fe	50.91	1037.50	344.9127	199.58788
Ca	132.31	2200.00	792.5218	537.77684
Mg	86.08	2855.26	598.8270	455.06642
Pb	46.88	754.23	250.5748	160.27767
Zn	15.10	306.45	104.9109	61.94968

Table 30: Collective element concentration in the fingernails of study group (n=67)

Element	Reference µg/g	< Normal		Within range		> range	
		No.	%	No.	%	No.	%
Cu	4 -51	2	3.0	45	67.2	20	29.9
Fe	60 – 555	1	1.5	56	83.6	10	14.9
Ca	250 – 1860	4	6.0	60	89.6	3	4.5
Mg	90 – 800	1	1.5	48	71.6	18	26.9
Pb	50 – 480	1	1.5	61	91.0	5	7.5
Zn	40 – 490	5	7.5	62	7.5	—	—

Table 31: Overall Pearson Correlation of the inter-element associations in the fingernail samples (n = 67)

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.397(**)	1				
	0.000					
Ca	0.309(**)	0.536(**)	1			
	0.006	0.000				
Mg	0.323(**)	0.564(**)	0.361(**)	1		
	0.004	0.000	0.001			
Pb	0.253(*)	0.708(**)	0.655(**)	0.300(**)	1	
	0.020	0.000	0.000	0.007		
Zn	0.323(**)	0.471(**)	0.305(**)	0.317(**)	0.329(**)	1
	0.004	0.000	0.006	0.004	0.003	

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 32: Trace Element Concentration in Children's fingernail Samples (n=37)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	3.47	232.00	33.8072	43.78692
Fe	50.91	730.76	343.2424	185.04330
Ca	169.39	1869.96	821.0014	538.17923
Mg	86.08	2855.26	629.5186	515.32434
Pb	46.88	754.23	253.1765	169.45896
Zn	21.24	306.45	96.3886	58.06419

Table 33: Summary of Pearson Correlation (r) of the inter-element associations in the children's fingernail samples (n=37)

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.332(*)	1				
	0.022					
Ca	0.280(*)	0.523(**)	1			
	0.046	0.000				
Mg	0.337(*)	0.595(**)	0.358(*)	1		
	0.021	0.000	0.015			
Pb	0.119	0.602(**)	0.631(**)	0.200	1	
	0.241	0.000	0.000	0.118		
Zn	0.318(*)	0.483(**)	0.359(*)	0.255	0.331(*)	1
	0.028	0.001	0.015	0.064	0.023	

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 34: Trace Element Concentration in Female's fingernail Samples (n=23)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	12.41	92.39	43.7774	21.67548
Fe	67.07	1037.50	376.5774	234.95766
Ca	132.31	2200.00	838.8674	583.69326
Mg	107.65	1662.50	600.9248	383.82932
Pb	55.97	700.00	265.0800	158.31837
Zn	34.90	287.50	113.9587	63.15181

Table 35: Summary of Pearson Correlation (r) of the inter-element associations in the female's fingernail samples (n= 23).

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.674(**)	1				
	0.000					
Ca	0.555(**)	0.553(**)	1			
	0.003	0.003				
Mg	0.352(*)	0.518(**)	0.317	1		
	0.050	0.006	0.070			
Pb	0.708(**)	0.868(**)	0.744(**)	0.495(**)	1	
	0.000	0.000	0.000	0.008		
Zn	0.512(**)	0.609(**)	0.230	0.487(**)	0.463(*)	1
	0.006	0.001	0.146	0.009	0.013	

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 36: Trace Element Concentration in Male's fingernail Samples (n=7)

Element	Minimum µg/g	Maximum µg/g	Mean µg/g	Std. Deviation µg/g
Cu	16.10	56.52	37.1129	18.11412
Fe	142.03	523.80	249.7000	126.04204
Ca	216.37	1035.19	489.7086	271.99779
Mg	105.26	1095.23	429.7071	324.34435
Pb	56.56	350.00	189.1629	114.57755
Zn	15.10	258.49	120.2286	79.54906

Table 37: The summary of the Pearson Correlation (r) of the inter-element associations in the male's fingernail samples (n=7).

Element	Cu	Fe	Ca	Mg	Pb	Zn
Cu	1					
Fe	0.580	1				
	0.086					
Ca	0.271	0.098	1			
	0.279	0.417				
Mg	0.253	0.809(*)	0.576	1		
	0.292	0.014	0.088			
Pb	0.592	0.617	0.045	0.348	1	
	0.081	0.070	0.462	0.222		
Zn	0.023	0.029	0.862(**)	0.535	0.020	1
	0.480	0.476	0.006	0.108	0.483	

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 38: Comparison of the variations in trace element concentration in the fingernails Samples among gender (n=67)

Element	Chi square	P value
Cu	2.875	**0.579**
Fe	2.924	**0.571**
Ca	2.471	**0.650**
Mg	2.184	**0.702**
Pb	2.388	**0.665**
Zn	5.066	**0.079**

Table 39: Comparison of the variations in trace element concentration in the fingernails hair Samples between age groups (n=67)

Element	Chi square	P value
Cu	4.812	0.568
Fe	1.701	**0**.945
Ca	3.215	**0**.781
Mg	7.234	**0**.300
Pb	2.520	**0**.866
Zn	1.021	**0**.796

Table 40: Variation in trace element concentration in the fingernails Sample depends on geographical location (n=67)

Element	Chi square	P value
Cu	35.935	0.210
Fe	**46.394**	**0**.028 *
Ca	32.394	0.349
Mg	**54.519**	**0**.004 *
Pb	43.131	**0**.057
Zn	14.421	**0**.494

*** Statistically significant, p value < 0.05**

Table 41: Variations in trace element concentration in the fingernails Sample depends on the tribe of participants (n=67)

Element	Chi square	P value
Cu	**43.342**	**0.032 ***
Fe	**43.191**	**0.033 ***
Ca	14.212	0.986
Mg	33.051	0.234
Pb	**46.863**	**0**.014 *
Zn	16.412	0.289

*** Statistically significant, p value < 0.05**

Table 42: Trace element concentration in the fingernails Sample depends on work (n=67)

Element	Chi square	P value
Cu	2.422	0.659
Fe	1.457	0.834
Ca	0.447	0.978
Mg	9.392	0.052
Pb	2.804	0.591
Zn	3.495	0.174

Table 43: Trace element concentration in the fingernails Sample depends on daily working hours (n=67)

Element	Chi square	P value
Cu	3.014	0.995
Fe	3.542	0.990
Ca	1.282	1.000
Mg	7.342	0.834
Pb	2.421	0.998
Zn	**15.086**	**0.020 ***

*** Statistically significant, p value < 0.05**

Table 44: Trace element concentration in the fingernails Sample depends on water resources (n=67)

Element	Chi square	P value
Cu	**13.339**	**0.010 ***
Fe	6.796	0.147
Ca	4.471	0.346
Mg	5.975	0.201
Pb	9.295	0.054
Zn	4.626	0.099

*** Statistically significant, p value < 0.05**

Table 45: Effect of social habit on trace element concentration in the fingernails (n=67)

Element	Chi square	P value
Cu	0.913	0.923
Fe	0.617	0.961
Ca	0.366	0.985
Mg	1.243	0.871
Pb	0.309	0.989
Zn	0.253	0.881

Table 46: Effect of make up on trace element concentration in the fingernails (n=67)

Element	Chi square	P value
Cu	10.192	0.748
Fe	11.492	0.647
Ca	3.149	0.999
Mg	14.336	0.425
Pb	3.222	0.990
Zn	1.062	0.994

Table 47: Overall comparison of mean trace element concentration between hair and nails (n=67)

Element	Mean concentration (µg/g)	
	Hair	Fingernails
Cu	19.13840	37.57520
Fe	314.7216	344.9127
Ca	606.0552	792.5218
Mg	562.0719	598.8270
Pb	187.5569	250.5748
Zn	118.8539	104.9109

Table 48: Overall comparison of mean trace element concentration between hair and nails (n=67)

Element	< Reference (µg/g)		Within range (µg/g)		> Reference (µg/g)		P value
	Hair	Fingernail	Hair	Fingernail	Hair	Fingernail	
Cu	7 (10.4%)	2 (3.0%)	56 (83.6%)	45 (67.2%)	4 (6.0%)	20 (29.9%)	0.273
Fe	—	1 (1.5%)	61 (91.0%)	56 (83.6%)	6 (9.0%)	10 (14.9%)	0.523
Ca	—	4 (6.0%)	63 (94.0%)	60 (89.6%)	4 (6.0%)	3 (4.5%)	0.780
Mg	—	1 (1.5%)	61 (91.0%)	48 (71.6%)	6 (9.0%)	18 (26.9%)	0.894
Pb	—	1 (1.5%)	62 (92.5%)	61 (91.0%)	5 (7.5%)	5 (7.5%)	0.524
Zn	2 (3.0%)	5 (7.5%)	62 (92.5%)	62 (92.5%)	3 (4.5%)	—	0.804

Table 49: Validity of Flame Atomic Absorption Spectrophotometer in measurement of trace elements in scalp hair and fingernails (67)

Test	Value	95% CI (Confidence interval)
Sensitivity	91.96%	97.22%
Specificity	100.0%	92.45% — 100%
Positive predictive value	100.0%	83.16% — 100%
Negative predictive value	94.00%	83.45% — 98.75%

- **Positive predictive value:** Probability that increase of trace element concentration in the sample is present when the test is positive.
- **Negative predictive value:** Probability that increase of trace element concentration in the sample is not present when the test is negative.
- **Sensitivity:** Probability that there is increase of trace element concentration measurement in the sample when actually there is increase of trace element concentration (True positive test).
- **Sensitivity:** Probability that the test result will be negative when actually there is no increase of trace element concentration (True negative test).

Table 50: Range of trace elements concentration in Hair samples using XRF Technique

Element	Minimum	Maximum	Mean	Std. Deviation
Fe	400.00	1400.00	657.1429	297.97854
Cr	200.00	400.00	285.7143	66.29935
Zn	100.00	200.00	114.2857	36.31365
Ni	.00	100.00	92.8571	26.72612
Mn	.00	.00	.0000	.00000
Pb	.00	.00	.0000	.00000

Table 51: Range of trace elements concentration in Nail samples using XRF Technique

Element	Minimum	Maximum	Mean	Std. Deviation
Fe	300.00	1000.00	528.5714	201.64161
Cr	200.00	400.00	278.5714	80.17837
Zn	100.00	100.00	100.0000	.00000
Ni	100.00	100.00	100.0000	.00000
Mn	.00	.00	.0000	.00000
Pb	.00	.00	.0000	.00000

Table 52: Correlation of trace elements concentration between Hair and Nails samples using XRF Technique

Element	Chi square	P value
Cr	0.875	0.928
Fe	33.6	0.297
Ni	Constant	—
Mn	Constant	—
Pb	Constant	—
Zn	Constant	—

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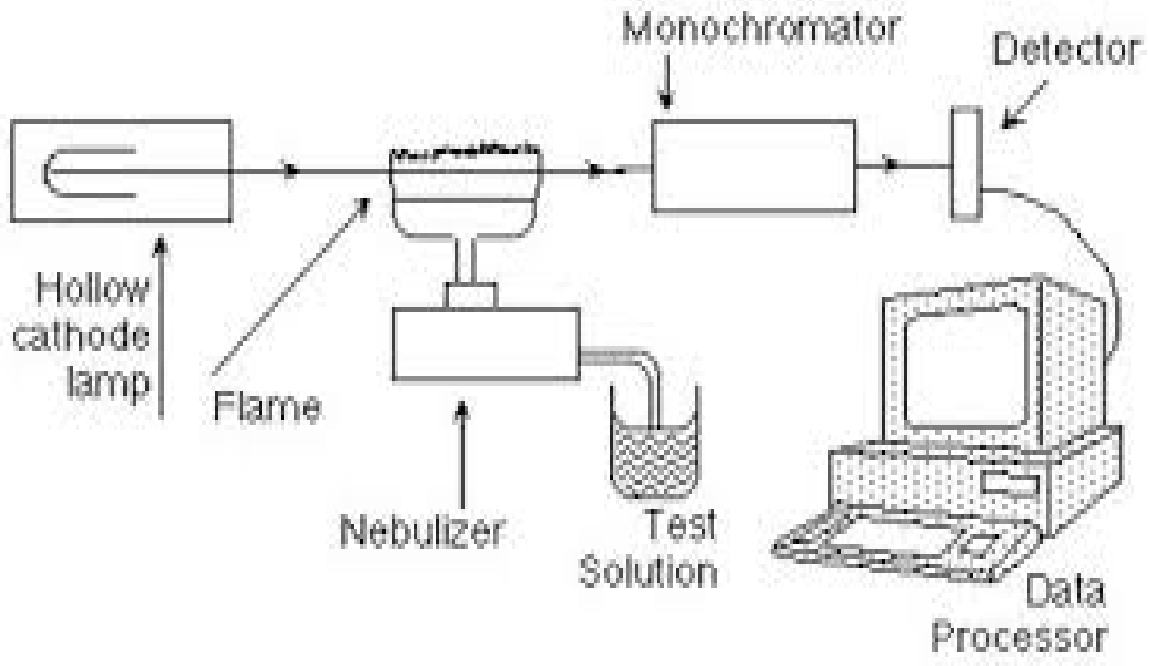
Appendix 1: Schematic diagram of atomic Absorption Spectrometer



Appendix 2: Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer

Appendix 3: Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer; front and rear views

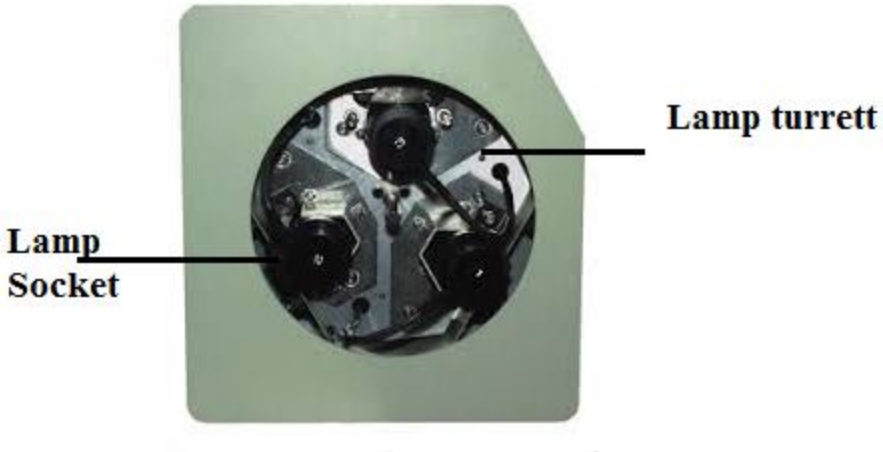
Front view

Rear view

Appendix 4: Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer; right and left

Right side

Left side



Appendix 5: The X-MET5100 rear view

Appendix 6: The X-MET5100 lateral view

Appendix 7: Study's questionnaire (English) Form No ( )

Personal data				
Age	…….			
Sex	Male ○	Female ○		
Residence	……			
	Near to industry ○	Away from industry ○		
Tribe				
Occupation				
Duration of occupation				
Daily hours				
Dietary data				
Type of food	……..			
Sources of food	……..			
Sources of water	……			
Habits				
Cigarette smoking	Yes ○	No ○		
Snuffing tobacco	Yes ○	No ○		
Alcohol intake	Yes ○	No ○		
Chronic use of hair creams	Yes ○	No ○		
	If yes, specify	……		
Chronic use of nail varnish	Yes ○	No ○		
	If yes, duration	……		
Hair dye	Yes ○	No ○		
Hair henna	Yes ○	No ○		
Cut fingernail < 4 wks	Yes ○	No ○		
Use of hair treatment < 12 wks	Yes ○	No ○		
Health data				
Chronic disease	Yes ○	No ○		
	If yes, specify	1. ……	2. ……..	3. ……
Past history of poisoning	Yes ○	No ○		
	If yes, specify	……		
Past history of irradiation	Yes ○	No ○		
Drug history	Yes ○	No ○		
	If yes, specify	……		
Hair				
Weight	……..			
Elements	1.	2.	3.	
	4.	5.	6.	
Nail				
Weight				
Elements	1.	2.	3.	
	4.	5.	6.	

Name and signature of participant ……..

Appendix 8: Study's questionnaire (Arabic)

البيانات الشخصيه				
العمر	.....			
الجنس	ذكر ○	انثى ○		
السكن	.....			
	بالقرب من المصانع ○	بعيد من المصانع ○		
القبيله				
المهنة				
مدة الالتحاق بالمهنة				
عدد ساعات العمل اليومية				
بيانات الأغذية				
طبيعة الاكل	.....	.....		
مصادر الغذاء	.....	.....		
مصادر المياه	.....	.....		
العادات				
التدخين	نعم ○	لا ○		
التمباك	نعم ○	لا ○		
الخمور	نعم ○	لا ○		
الإستخدام المزمن لكريمات الشعر	نعم ○	لا ○		
	اذا كان بنعم، حدد النوع	.....	.....	
الإستخدام المزمن للمناكير	نعم ○	لا ○		
	اذا كان بنعم، حدد المدة	.....		
استخدام صبغة الشعر	نعم ○	لا ○		
استخدام الحناء للشعر او الاطراف	نعم ○	لا ○		
تقليم الاظافر < 4 اسابيع	نعم ○	لا ○		
استخدام علاج واصباغ الشعر<12 اسبوع	نعم ○	لا ○		
البيانات الصحية				
امراض مزمنة	نعم ○	لا ○		
	اذا كان بنعم، حدد	.1	.2	.3
هل حدث لك تسمم في السابق	نعم ○	لا ○		
	اذا كان بنعم، حدد			
هل تعرضت لاي اشعاع علاجي	نعم ○	لا ○		
هل تستخدم علاجات مزمنه	نعم ○	لا ○		
	اذا كان بنعم، حدد	.....	.....	
الشعر				
الوزن	.....			
العناصر	.1	.2	.3	
	.4	.5	.6	
الأظافر				
الوزن	.....			
العناصر	.1	.2	.3	
	.4	.5	.6	

اسم وتوقيع المشترك بالبحث .....