

**STUDY OF TRACE ELEMENTS IN HAIR FROM A
SELECTED MALE POPULATION IN PENANG AND
FALLUJAH USING XRF**

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by

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LIST OF SYMBOLS

α	Alpha
β	Beta
ϕ	Phi
γ	Gamma
λ	Decay constant
μ	micro
μ_l	Linear attenuation coefficient
μ_m	Mass attenuation coefficient
θ	Theta
s	Standard deviation
ϵ_{ip}	Intrinsic peak efficiency
A	Mass number
Ag	Silver
Al	Aluminium
As	Arsenic
As_2O_3	Arsenic trioxide
Br	Bromine
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium

Cu	Copper
E_s	Scattered photon energy
Fe	Iron
Hf	Hafnium
Hg	Mercury
KBr	bromide potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Nb	Niobium
Ni	Nickel
Pb	Lead
Pd	Palladium
Rb	Rubidium
Se	Selenium
Sn	Tin
Sr	Strontium
Ta	Tantalum
V	Vanadium
W	Tungsten
Z	Atomic number
Zn	Zinc
Zr	Zirconium

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
Bq	Becquerel
Ci	Curie
CPS	Counts per second
ED- XRF	Energy dispersive x-ray fluorescence
EPA	Environmental protection agency
eV	Electron volt
FAAS	Flame atomic absorption spectrophotometry
FWHM	Full width at half maximum
Ge(Li)	Lithium drifted germanium
GHz	Gigahertz
Gy	Gray
HPGe	High purity germanium
HVL	Half value layer
IAEA	International atomic energy agency
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ITS	Integrated tiger system
keV	Kilovolt electron volt
LEGe	The low energy germanium detector
MCA	Multichannel analyzers
MCNP	Monte carlo n-particle

MCNP5	Monte Carlo N-Particle version 5
MDL	Minimum detectable limit
MeV	Mega electron volt
NAA	Neutron activation analysis
NaI(Tl)	Sodium iodide thallium
ppm	Parts-per-million
RAM	Random access memory
SDD	Source-to-detector distance
Si(Li)	Lithium drifted silicon
sr	Steradian
SSD	Source-to-sample distance
Sv	Sievert
TXRF	Total reflection x-ray fluorescence
WHO	World Health Organization
XRF	X-ray fluorescence

KAJIAN UNSUR SURIH DALAM RAMBUT DALAM KALANGAN POPULASI LELAKI DI PULAU PINANG DAN FALLUJAH MENGGUNAKAN XRF

ABSTRAK

Negeri Pulau Pinang merangkumi kawasan industri yang berpotensi mempunyai pelbagai jenis bahan pencemar industri yang akan meninggalkan kesan sampingan kepada penduduknya dan alam sekitar. Sama juga dengan bandar Fallujah di Iraq yang terdedah kepada banyak bahan toksik dalam bentuk senjata kimia dalam peperangan pada tahun 2004. Pendedahan begitu mampu memberi kesan buruk kepada alam sekitar dan penduduk kedua-dua buah bandar. Walaupun ada yang menghujahkan bahawa pengetahuan tentang aras semulajadi unsur surih dalam rambut sangat penting dalam membuat penilaian ke atas tahap pencemaran manusia di kawasan-kawasan di mana unsur-unsur ini dijangka akan menunjukkan kepekatan yang agak berbeza, data sebegini tidak sampel rambut dari terdapat bagi Pulau Pinang dan Fallujah. Dalam kajian ini, Arsenik, Merkuri, Plumbum, Besi, Kuprum dan Zink dalam sampel rambut dari Pulau Pinang dan Fallujah telah dianalisis untuk menilai pengambilan unsur-unsur tersebut dari persekitaran. Aras semulajadi elemen-elemen surih dalam rambut telah dipantau sebagai nilai rujukan untuk penilaian aras pencemaran kepada manusia. Dalam kajian ini, kepekatan Hg, As, Cu, Zn, Fe and Pb dalam rambut di kulit kepala 100 orang penduduk Pulau Pinang dan 28 orang lagi di Fallujah telah ditentukan dengan menggunakan XRF.

Satu sistem pendarfluor sinar-X termasuk sumber radio-isotop dan sebuah sistem pengesanan telah dibangunkan untuk tujuan mengukur unsur surih. Untuk memastikan penggunaan yang terbaik, jarak sumber-kepada-pengesanan (SDD) dan jarak sumber-kepada-sampel (SSD) telah ditetapkan berada pada jarak yang terhampir. Sistem itu terdiri daripada satu radio-isotop sebagai sumber foton, satu alat pengesan, pengkolimat, sampel, perisai dan instrumen pemerolehan data. Perisian padanan PeakFit telah digunakan dalam analisis spektrum untuk menentukan data pengujian dan sinar-X pendarfluor. Penentuan untuk arsenik, merkuri, besi, kuprum, plumbum dan zink telah dilaksanakan untuk menentukan aras minima keboleh-kesan melalui kaedah eksperimentasi. Kod Monte Carlo MCNP5 digunakan untuk menguji dan mengesahkan aras keboleh-kesan minimum (MDL) dan membuktikan kebolehan sistem tersebut mengesan unsur-unsur surih yang paling kecil.

Untuk sampel-sampel rambut yang diperolehi dari subjek di Pulau Pinang, isi kandungan As (julat 0.14 - 2.98 mg/kg); Hg (julat 1.12- 11.65 mg/kg); Cu (julat 5.8- 35 mg/kg); Fe (julat 4.6- 140 mg/kg); Zn (julat 16.5- 377 mg/kg) dan Pb (julat 0.75- 40.1 mg/kg) telah diperolehi. Sebaliknya, untuk rambut penduduk Fallujah nilai yang telah diperolehi ialah As (julat 0.66- 5.2 mg/kg); Hg (julat 0.9- 13 mg/kg); Cu (julat 2.5- 24 mg/kg); Fe (julat 12.7- 80.5 mg/kg); Zn (julat 20.2 - 379 mg/kg) dan Pb (julat 1- 18.9 mg/kg). Apabila data dan keputusan-keputusan yang diperolehi dalam kajian ini dibandingkan dengan penduduk bandar-bandar lain, didapati bahawa nilai-nilai julat berada dalam tertib magnitud yang sama.

**STUDY OF TRACE ELEMENTS IN HAIR FROM A SELECTED MALE
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ABSTRACT

The state of Penang encompasses an industrial region with a potential for the existence of a variety of industrial pollutants which would certainly have a possible impact on the environment and the people. Similarly, the city of Fallujah in Iraq has been exposed to a lot of toxic materials in the form of chemical weapons during the war in 2004. Such exposure would certainly have a detrimental impact on the environment and the people in these two cities. Although it has been argued that knowledge of the natural levels of trace elements in hair is extremely essential for assessing the degree of human contamination in areas where these elements are expected to show anomalous concentrations, these data from hair samples from Penang and Fallujah are not available. In this study, arsenic, mercury, lead, iron, copper and zinc in hair samples from Penang and Fallujah were analysed to assess the environmental uptake of these elements. The natural levels of trace elements in hair were hence monitored as reference values for the assessment of the possible human contamination levels. In this work, the concentrations of Hg, As, Cu, Zn, Fe and Pb in human scalp hair of 100 residents of Penang and 28 in Fallujah were determined using XRF.

An X-ray fluorescence system including a radioisotopic source and a detection system has been developed for the purpose of measuring trace elements. For the best use, the source -to-detector distance (SDD) and the source-to-sample distance (SSD)

were set at the closest distance. The system comprises of a radioisotope as the source of photons, a detector, collimators, sample, shielding, and data acquisition instruments. The PeakFit fitting software was used for spectral analysis to determine the data of the excitation samples and the fluorescent X-rays. Calibration for arsenic, mercury, iron, copper, lead, and zinc was performed to determine the minimum detectable level (MDL) by experimentation. The Monte Carlo code MCNP5 was used to test and confirm the MDL and verify the ability of the system to detect the smallest amount of trace elements.

For samples of hair obtained from people in Penang, the content of As (range 0.14 - 2.98mg/kg); Hg (range 1.12- 11.65 mg/kg); Cu (range 5.8- 35 mg/kg); Fe (range 4.6- 140 mg/kg); Zn (range 16.5- 377 mg/kg) and Pb (range 0.75- 40.1 mg/kg) were determined. On the other hand, for Fallujah hair, the contents of As (range 0.66- 5.2 mg/kg); Hg (range 0.9- 13 mg/kg); Cu (range 2.5- 24 mg/kg); Fe (range 12.7- 80.5 mg/kg); Zn (range 20.2 - 379 mg/kg) and Pb (range 1- 18.9 mg/kg) were determined. When the data and results obtained in this study were compared with those of other cities, it has been found that range values are within the same order of magnitude.

CHAPTER ONE: INTRODUCTION

1.1 Background

X-ray fluorescence (XRF) is an analytical technique which uses X-rays interacting with a target material to determine the composition (set of elements and proportions) of the material. XRF is a completely safe and non-destructive method because samples to be tested can be analyzed under normal atmospheric pressure and room temperature without the risk of damage to the sample (Lab, 2013). XRF which is one of the most functional and flexible techniques available for the analysis and characterization of objects nowadays has gone through many developments in the last few decades. Over the years XRF has become a significant technique for elemental investigation of many different types of materials.

XRF technique has many important applications in different areas such as geology and mineralogy. In addition to this, the XRF technique has found applications in the analysis of minerals, ecology, environmental management, archaeology, and forensics and in industry, clinical and biological settings. In this research XRF has been used to study the content of trace elements in particular some heavy metals in human hair scalp.

It is clear that the benefits of using the XRF instead of conventional analysis methods is that they are fast and comprehensive and multiple tests are not required .

The XRF setup is actually fairly straightforward being made up of four necessary components: a detector, an excitation source, the sample to be interrogated, the data collection and analyzing system.

An important consideration in the design of an XRF system for studies on humans is the amount of radiation dose endured by the person during the exposure to X-rays.

In addition to the effects on the human body from X-ray sources, and the effect of the thickness of the sample, which is made up of different materials, the resulting radiation to be measured is generally weak compared to the inherent noise and a large background radiation, which leads to a weakening of the ability of detection of radiation within the XRF system. Therefore, there is a need for an approach to improve the system and increase the XRF analytical capacity and access to the ability to detect the lowest possible concentrations of elements within the sample.

The work focussed on using a source of excitation that maximizes signal and increases the interaction of the photoelectric process, while at the same time minimizing the background radiation using proper shield design and appropriate positioning of the source of radiation, the sample and the detector. The background can be minimized by shifting the characteristic X-ray peaks far from the peak of Compton scatter, or with the polarization of the incident radiation. There are of course three necessary requirements for the detection system : (1) good counting

efficiency, (2) high energy resolution and (3) compactness of the set up to satisfy space requirements (Gardner R.P et al., 1999).

Si (Li) (Silicon lithium) detectors or pure germanium (Ge) detectors are generally the detectors of choice to record the characteristic lines measured. Both detectors traditionally work at liquid nitrogen temperatures. Now a new generation detectors are available working at room temperature, and with accepted energy resolution (Gardner R.P et al., 1999).

There are three core analytical components of an XRF system: The first one is the determination of intensity of the emitted characteristic lines in the X-ray spectrum. The second step is the determination of the concentrations of elements from intensity of the characteristic lines that were measured. Third is processing of error analysis (Gardner R.P et al., 1999).

Systems of calibration for the XRF setup requires special care due to the fact that the trace element distribution is typically uniform in the human body and there is the possibility of high self-attenuation of the incident radiation.

1.2 Scope of Work

The present study covers the construction, improvement and evaluation of an XRF system which can support and to some extent cater to requests for measurements. The focus is on a radioisotopic XRF system that is cost-effective in the long term. The

Monte Carlo simulations program utilized in this work is the Monte Carlo N-Particle version 5 (MCNP5). This code modifies specific parameters in the simulation and enables the researcher to determine the effect of changes in the various parameters on system performance of the XRF system. Calibration of concentration for selected elements was also carried out using MCNP5. The optimal system calibration was chosen using computer simulation and the experimental measurements carried out at the same time.

Once the XRF setup was developed and optimized, for actual measurements human hair was chosen as the sample for study. This is a good option since the determination of levels of trace elements in hair has developed into a technique of option for reviewing nutritional status, monitoring exposure to environmental factors, heavy metal poisoning estimation, and disease diagnosis (Tavakkoli et al., 2000, Bencko, 1995, Moo and Pillay, 1983).

The selection of hair as the major bioplate in the multi-element analysis is defensible as follows: (1) As a sampling tissue hair is useful since it is a tissue of low metabolic activity. It therefore can become a useful tool to document metabolic processes occurring in an organism over an extended period (Vazina et al., 1998). (2) Hair is considered to be stable for a long time period of time (Foo and Tan, 1998). (3) Hair is also known to contain higher concentrations of metals compared with those found in blood, urine or other usual body components. This makes such analysis more appropriate (Foo and Tan, 1998, Airey, 1983, Maugh, 1978, Laker, 1982). (4) The elemental content in hair reflects the unique features and characteristic peculiarities of a human being, such as sex, age, dietary and

environmental conditions, pharmacological effects, etc (Vazina et al., 1998). (5) Samples of hair can be conveniently collected in a non-invasive manner. (6) They can be kept for a long time without specialised storage space. (7) They can be utilized repeatedly for recurrent analyses without deterioration, and they are easily transportable and can be mailed (Vazina et al., 1998). (8) Hair of regular, healthy individuals in general contains all trace elements within a well defined concentration range.

Human hair is estimated to have a growth rate of 1 to 1.5 cm per month. There are three international organizations who recommend the use of hair as a significant biological material for universal environmental screening. These three organizations are the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the Environmental Protection Agency (EPA) (Sthiannopkao et al., 2010). In this study, trace element levels in hair are monitored.

Recently, for a common toxic trace metal in hair this method was shown to be a suitable as a biological marker of work-related and environmental exposure of man for most toxic trace metals (Bencko, 1995). Hair samples are appropriate pointers for exact toxic element determination to which subjects are exposed to throughout the previous 2–18 months (Samanta et al., 2004).

In this work, the limits to what can be accomplished can be clarified into three components: First, the detector used in the experiment cannot read energies less than 3 keV, so all the elements which provide emission energies less than 3 keV cannot be

studied, such as silicon, chlorine, sodium, aluminium and fluorine, carbon, oxygen and hydrogen, and several other elements. Second, the photon γ -ray source used, which is the Am-241, has a maximum energy of 59.9 keV, therefore the elements that have energies greater than 59 keV, cannot be studied using this source.

The third limitation is because of the intensity of the radiation from the Am-241 source, the dry samples can only be used in this study because of the ease of being installed in the system without needing a holder or a vial. This is because the vial will absorb a large part of the incoming rays to scan the sample and it will absorb a large part of the radiation emerging from the sample, therefore the detector cannot detect this ray.

1.3 Research Objectives

In the course of this work, experiments have been made to achieve the following research objectives:

1. To develop and optimize a radioisotopic based XRF system for determination of trace elements.
2. To verify the feasibility of determining concentrations of trace elements in multi elemental combinations simultaneously.
3. To use the XRF system developed in this work to measure elemental concentration in hair samples of human male populations in Penang
4. To use the XRF system developed in this work to measure elemental

concentration in hair samples of human male populations in Fallujah and to compare them with existing data from other locations.

1.4 Thesis Organization

This thesis consists of six chapters. Inside this thesis, explanations about an XRF setup and development with the ability for work is discussed.

Chapter One provides the introduction, research objectives, work limitations and work organization.

Chapter Two explains the historical evolution of XRF technology since the discovery of X-rays, and the XRF is currently known, as well as important uses of this technology in the field of early detection of certain types of cancers and their use in the detection of environmental impacts on humans. Using this technique can help in tracing mineral elements in different tissues of humans, including human hair.

Chapter Three focuses on the theoretical background for this study and the classification of parameters which will significantly affect the XRF system. The explanations about the Monte Carlo code MCNP5 utilized in developing the system are also incorporated.

Chapter Four provides a detailed explanation of the features of each part of the XRF setup and includes an explanation of how these factors were optimized via computer simulation using MCNP5 and also via experimentation including the calibration of the XRF system for the elements lead, iron, copper, zinc, mercury and arsenic.

Chapter Five presents the results of human hair samples that have been collected from the barber shop in Penang and then the results analyzed using the XRF system, with the study focussing on six important mineral elements, which are arsenic, mercury, lead, iron, copper and zinc. The results were then compared with previous studies for cities in Malaysia and other cities around the world.

The second part presents of chapter Five presents the results of human hair samples which were obtained from the Iraqi city of Fallujah, and compared with previous studies of other cities from around the world. Chapter six contains conclusions and suggestions for future studies.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The focus of this chapter is on the chronological background of XRF and explains the uses of XRF in human tissue studies. Following that the various studies involving measurements of trace elements in human hair for environmental and diseases studies is illustrated historically, and a review made of the used of XRF for detecting trace elements in hair. Finally, this chapter summarises studies about detection of trace elements in human hair in Malaysia.

2.2 Using XRF to Detect Trace Elements in Human Tissue

Trace elements take a significant function in biological progressions. Indeed there is a connection between the levels of trace elements and the existence of diseases (Theodorakou and Farquharson, 2008). Trace elements here indicate chemical elements found in minute amounts (0.01% by mass of the human body composition) (Theodorakou and Farquharson, 2008). Trace elements are useful for the human body and the main tasks of trace elements are as stabilizers, components of construction, necessary elements for hormonal roles and as enzymes co-factors (Yaman, 2006).

As an example of trace elements, Zn is an antioxidant trace element. There is actually an inverse association between Zn and cancer risk (Silvera and Rohan, 2007).

Copper is also an antioxidant trace element. Fe is also known to be related with cancer; it is the major regulator of angiogenesis. Se avoids oxidative stress and antagonises the toxic consequences of heavy metals such as Ag, Cd and Hg and concurrently the state of Se changes after exposure to these heavy metals (Kucharzewski et al., 2003).

The detection and quantification of trace elements in biological samples are completely achievable by a number of methods such as inductively coupled plasma-atomic emission spectrometry (ICP-AES), X-ray fluorescence (XRF), neutron activation analysis (NAA), total reflection X-ray fluorescence (TRXRF), flame atomic absorption spectrophotometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS) (Yaman, 2006).

Theodorakou and Farquharson (2008) summarized that the concentrations of K, Zn, Cu, Ca and Fe in breast cancer tissue samples were found to be statistically higher than those found in breast tissue samples which are considered normal or non-diseased while the elemental concentrations of the elements Zn, Ca, Mn, S, K, Fe, Se, Rb and P in breast cancer tissue samples were higher than those found in benign breast tissues, at the same time as the Cu levels were low in tumour samples. They also summarized that the concentration of Cd, Zn, Pb, Cu, Se, Ti and Rb are found to be reduced in kidney cancerous tissue samples if compared to healthy tissue while Fe and Zr were found to be higher in cancerous tissues. The concentrations of, Mn Ca, Cr and Fe are raised in cancerous prostate tissues while Cu, Zn and V are suppressed in such tumours (Theodorakou and Farquharson, 2008). Regarding thyroid diseases, they said that the highest concentrations of Cu and Se were found in patients with nodular goitre and lowest in patients with Graves' disease (Cu) and with thyroid cancer (Se). The highest

Zn concentrations were found in patients with Graves' disease while the lowest was found in patients with thyroid cancer. In the end, they said the concentrations of P, Ti and Pb were found to be higher in the lung cancer tissue compared with those in benign tissues and Ca, Fe, Cu and Zn were significantly lower in the benign tissue samples (Theodorakou and Farquharson, 2008).

2.3 Trace Elements in Human Hair for Environmental and Disease Studies

As emphasised before X-ray fluorescence (XRF) is a useful analytical tool for the determination of many elements in biological samples, like body fluids and other human tissues (Khuder et al., 2007). Human hair is a hard tissue like bone (Carvalho et al., 1998, Dietz et al., 2006). Human hair consists of proteins, lipids, water, trace elements and pigments (Robbins, 2012)

It is fairly well-known that hair has unique capabilities to reveal and detect information concerning the effects of nutritional status and the results of exposure to influences from the surrounding environment (Gellein et al., 2008). Hair grows at a rate of roughly 1 cm per month. Trace elements are incorporated into hair for the duration of the growth progression and can be an indication of the composition of trace elemental components in blood plasma at the moment of configuration (Benner Jr and Levin, 2005).

Additionally to the possibility of being utilized as biological markers hair sample analysis has a number of advantages. The main constituent of hair is keratin, which

makes it steady and strong. Hair can also be simply collected and does not need particular storage space for keeping. Furthermore, a lot of trace elements accumulate in hair at concentrations of at least ten times greater than in urine or blood serum (Maugh, 1978).

Scalp hair has been effectively applied in medical forensics, screening populations for heavy metal poisoning, and for environmental contaminant monitoring (Sky-Peck, 1990). Hair samples from the scalp of the human head were used in an initial evaluation of heavy metals exposure (Cr, Cu, Zn, Cd, Mn and As) of the human population (Pereira et al., 2004).

It may be worth noting that since 1965 several research programmes have been developed and implemented by the International Atomic Energy Agency (IAEA) to study and evaluate the concentrations of trace element concentrations in human hair. Such programmes were organized in the framework of Nuclear Based Methods for the Analysis of Pollutants in Human Hair. It was following such studies that the decision was made of using hair as a marker of biological samples and development of analytical techniques for hair trace elemental evaluation (Watts, 2000).

Bate (1966) mentioned that in 1962 he started studying the content of trace elements in human hair as a means to compare the hair samples for physical evidence (Bate, 1966). Bate had also predicted that hair is to be of significant value in many applications, because the content of the trace elements does not change rapidly with time. Changes however do occur over relatively extended periods and predicted these changes can be due to natural growth processes and/or contamination from the

environment, or because of adsorption of environmental trace elements by hair (Bate, 1966).

Other trace elemental studies of human hair have included work by Harrison who studied trace elements for hair using atomic absorption spectroscopy (Harrison et al., 1969). Pearson and Pounds studied arsenic in human hair in 1971 using NAA (Pearson and Pounds, 1971). In 1973, Gordu studied the factors that affects trace elements of human hair like environment and personal features as age, sex, height and weight (Gordus, 1973). Fe, Co, Zn, Cr, Ag, Cs, Hg, Rb, Sb and Se were studied in human hair for Italian population by Clemente using NAA (Clemente et al., 1977). Ryabukhin used XRF and atomic absorption spectroscopy to analyse hair as an indicator of contamination by trace elemental pollutants from the environment (Ryabukhin, 1978). In 1979 Imahor carried out multi-element NAA measurements of human scalp hair in the local population of Tokyo (Imahori et al., 1979). Moo (1983) measured trace elemental profiles of the scalp hair of patients suffering from cancer using NAA, and he compared them with results from healthy individuals. He observed the significant differences for a number of elements, notably for Se, I and Au (Moo and Pillay, 1983). Muramatsu in 1988 studied the relationship between human scalp hair and internal organs: kidney and liver from 30 Caucasian Swedish subjects and carried out a NAA analysis to monitor Ag, Co, Cr, Hg, Sb, Se and Zn by neutron activation analysis (NAA) (Muramatsu and Parr, 1988) . In 1992, Rauf used INNA to study human scalp hair as an environmental monitor for Indonesian and Canadian population groups (Rauf and Jervis, 1992). Bencko studied human hair as a biomarker for exposure assessment to certain pollutants with focus on occupational and environmental settings (Bencko,

1995). In 1996, Bertazzo used atomic absorption to determine quantities of Cu and Zn in human hair and determined its relationship with age, sex and hair pigmentation (Bertazzo et al., 1996). In 1996, Man and Zheng found significantly lower values of strontium and significantly higher values of arsenic, iron and vanadium in the hair of Nasopharyngeal Carcinoma patients (Man et al., 1996). Kehrig and Malm in 1997, using ECD-gas chromatographer studied methylmercury in hair samples from different riverine groups in fishing villages and gold milling areas in the Amazon region, Brazil (Kehrig et al., 1997). Carvalho and Marques in 1998 explained how to use XRF spectrometry in trace elemental research in human tissues, and they studied Cu, Mn, Fe, Zn, Se, Br, Sr and Rb from patient's tissue with cirrhosis (Carvalho and Marques, 2001). Foo and Tan in the same year studied trace elements in the hair of the people from South-east Asian islanders. They studied Hg, Cd, Pb, and Cu by taking hair samples from Singapore (85 samples) and from two islands of Batam, Indonesia (68 samples). Their study was focused on measuring the environmental uptake of elements. (Foo and Tan, 1998). In 1999 Krejdo, Olejnik studied the relationships between Zn, Pb, Cd and Cu content in children's scalp hair, and the level of environmental exposure. The population that was looked at was a group of 93 children (62 boys and 31 girls), aged between 5 and 16 years, living in contaminated regions of the copper mines in Poland, and they compared their results with healthy people in unexposed areas (Krejdo et al., 1999). In 2000 Tavakkoli and Ahmadiniar used NAA and measured of trace elements (Al-Mn, Al-V, Mg-Ca, Mn-V) in hair content from 100 healthy people staying in Esfahan, Iran and the results of their study were compared with the results of measurements from England and New Zealand (Tavakkoli et al., 2000). Again, another researcher studied Amazon area in Brazil, Vasconcellos and Bode in 2000 used INNA to

determine mercury and selenium contamination in hair samples of Brazilian Indian populations existing in the Amazonic region. Elevated levels of mercury were found in hair samples as compared to groups of control. They found correlations between selenium and mercury concentrations in Brazilian controls and in the Indian people groups (Vasconcellos et al., 2000). Chai in 2004 performed measurements in Beijing, China, and determined the occupational health impact of atmospheric pollution on exposed workers at an iron and steel complex. He used NAA to measure Fe, Co, Zn and Ca in scalp hair (Chai et al., 2004). In 2005 Mosaferi focused on the relationship between arsenic concentration in water of drink and human hair in three villages of Bijar city in the Kurdistan province of Iran using Neutron Activation Analysis method (Mosaferi et al., 2005). In 2006 Sidney Katz and Roya Katz (2006) studied the correlation between mercury concentrations in human scalp hair and environmental or food mercury exposures (Katz and Katz, 2006). Trojanowski determined the levels of lead and cadmium by atomic absorption spectrophotometry in central Pomerania (northern Poland) (Trojanowski et al., 2010). In 2012 Onuwa and Nnamonu determined levels of Cr, Mn, Ni, Cu, Zn and Mo in human scalp hair using energy dispersive x-ray fluorescence (ED- XRF). They investigated the relationships between ages; body mass, height, and heavy elements concentrations. The results indicated that there is no relationship between the body mass and height in heavy elements concentration in hair (Onuwa et al., 2012).

Wołowiec and Michalak (2013) have articles on hair analysis in health evolution because Wołowiec and Michalak reviewed studies published in 1997-2013 described the correlations between trace elements of hair and the physical or mental disorders and

occurrence of some diseases. They concluded that most of the authors of these research articles found out that the profile of hair trace elements imbalance might be functional as a diagnostic tool used for the early diagnosis of a lot of diseases. On the other hand, it seems that there is a necessity to make standard sample preparation procedures, in especially washing and mineralization methods (Wołowiec et al., 2013).

2.4 Using XRF for Detection of Trace Elements in Hair

X-ray fluorescence (XRF) technique, which can be based on either by radioactive sources or by X-ray tubes, has found many applications in the area of medicine, particularly in the determination of trace elements. It is a non-destructive physical process employed for trace element analysis of human tissue in the solid or liquid state; it is a simple method that has been expanded to be used for the determination of trace element concentrations in hair. (Toribara et al., 1982, Vazina et al., 1998).

The determination of trace elements in organic samples like hair, is actually extremely significant when the correlation between an irregular amount of a given element and a pathological situation is taken into account (Cesareo, 1976). Pre-concentration method and XRF determination of heavy metals were applied for the analysis of hair from an exposed group of workers from Sudan for the elements Fe, Cu, Ni, Zn and Pb (Eltayeb and Van Grieken, 1989a).

Toribara and Jackson (1982) used an X-ray fluorescence spectrometer to measure, non-destructively, the concentrations Fe, Ca, Ti, Cu, V, Cr, Mn, Zn, Ni, As, Se,

Br, Rb, Sr, Hg, and Pb in a 1 mm length from one single strand of hair (Toribara and Jackson, 1982).

Havranek et., al (1989) described a method to deal with samples in XRF analysis of hair in view of the effect on investigative results. Havranek described a method which depends on the partial ashing of hair followed by pellet preparation. The suitability of this method is evaluated on the XRF analysis of real hair samples gathered from four healthy adults (Havranek et al., 1989). In 1988 Muramatsu and Parr studied the relationship between hair and internal organs like liver and kidney. They observed an extremely important positive relationship between the Hg content in hair and the kidney cortex. On the other hand a very weak relationship was found among Hg in kidney cortex and liver and Se in hair and kidney cortex. The concentration of cobalt in liver is related with that in the kidney cortex (Muramatsu and Parr, 1988).

In another study, human tissues (i.e., bone, hair, liver and kidney) from 61 persons were collected and they were analyzed by energy dispersive x-ray fluorescence (EDXRF) spectrometry. Every sample was studied for its elements of Fe, Cu, Mn, Zn, Se, As, Sr, Rb and Pb to find out about the probable relationships among these elements in the various tissues. A noteworthy difference between male and female was found just for Zn. No important connection exists amongst the studied elements in hair, except for Fe and Cu (Carvalho et al., 1998).

In 1999, the correlations between Zn, Pb, Cd and Cu contents in scalp hair of children, and the stage of environmental exposure in polluted area of the Copper Basin Legnica was studied by Krejpcdo and others. They found the concentration of Cd and Pb

in the hair were considerably higher in the exposed children than reference group (Krejdo et al., 1999). The concentrations of calcium, iron, and zinc in 93 pregnant women from the Tianjin metropolis, China was investigated (Leung et al., 1999). In 2000 Dede and others studied trace element levels in hair of persons living in town and determined by energy dispersive XRF. This study investigated concentrations of Ca, Fe, Cu, Zn and Pb in the scalp hair for two groups, the first group was from a normal environment and the second one was exposed to high levels of contamination as a result of the working environment (Dede et al., 2001).

In 2000, measurements by Kolmogorov and his group showed a significant decrease of the concentrations of Zn and Se, and by the increase of Cr in scalp hair of patients with oncological mammary pathology. The Se deficit was more marked in patients with cancer than in those with hyperplasia (Kolmogorov et al., 2000). In 2001, Toribara used XRF to disclose mercury intake in a single hair (Toribara, 2001).

In samples of hair Baranowska et al. (2004) observed positive correlations between the content of Ca, Fe and P and age. They found differences in Na, K and S content hair between men and women (Baranowska et al., 2004). In 2005 Martin observed the distribution of lead in a hair sample collected from a lead smelter employee using Synchrotron X-ray fluorescence (Martin et al., 2005).

A number of hairs belonging to Napoleon Bonaparte were analyzed by synchrotron induced x-ray fluorescence analysis. This study attempted to find the existence of significant quantities of arsenic, in fact beyond the fatal content, (Chevallier et al., 2006).

Khuder improved the XRF and TXRF methods to apply for multi-element measurement of elements in entire blood and hair samples in non-occupational exposed people existing in Damascus (Khuder et al., 2007). In 2008 A. Khuder studied and determined the concentrations of Ni, Cu, Zn, and Pb in human scalp hair of 281 individuals working in 10 Syrian industrial factories using total reflection X-ray fluorescence (TXRF) analysis (Khuder et al., 2008).

2.5 Trace Elements in Human Hair in Malaysia

In Malaysia, there have been a number of investigations with regards to trace elements of human hair. These studies have revealed that, most of them use the NAA technique. The first part of these investigations is around environmental pollutions. The second part is around toxicity of some elements in water, and the last part around some diseases.

In 1987 Sarmani studied trace element concentrations (Fe, As, Br, Co, Cr, Hg, Sb and Zn) in human hair for various local residents in Malaysia using instrumental neutron activation analysis. In this study, 25 subjects were those residing in Kuala Lumpur, 20 subjects residing in Sepang and 40 people were those living in a small village in Alor Star. The results did not differ considerably from recorded rates for other countries in the world. However, the levels of elements (Hg, As and Sb) are considerably different for a variety of groups. Actually they are related to local environmental features (Sarmani, 1987).

In 1994, Sarmani pursued a very interesting study. In this investigation hair samples were gathered from fishermen and their families living in Kuala Juru Village in

the state of Penang on the West Coast of peninsular Malaysia. Kuala Juru is an industrialized region in Penang. Also hair samples were gathered from Chendering Village in the state of Terengganu on the East Coast of Peninsular Malaysia. Chendering is a non-industrialized region. For evaluation purposes, hair samples were also collected from a group of non-fishermen in Selangor and all of samples analyzed for mercury by using NAA.

Sarmani et al. (1994) conducted their study to estimate the exposure of chosen groups of people to mercury and methyl-mercury, and to estimate potential health hazards in these groups. The hair mercury levels found in the Malaysian study depended on the quantity and the kind of fish consumed. Industrial activities do not influence the mercury level in the hair of the people studied and it was concluded that industries in Malaysia do not release mercury into the environment (Sarmani et al., 1994).

In 2002 Mokhtar studied the concentration of lead in human hair for 107 people working in a Shipyard in Sabah, Malaysia. They argued that the studies of trace elements in human hair for environmental and workers in the mining and factories are still few in Malaysia (Mokhtar et al., 2002). Hair mercury level was measured in four coastal populations in Malaysia with correlation to the fish consumption between gender, age, and country and city region. These regions were in Kedah, Terengganu, Johor, and Selangor (Hajeb et al., 2008).

Hair samples from human residents in different areas of Malaysia, in addition to a sample from Japan, were collected and analyzed. 21 elements were determined using NAA (Abugassa et al., 1999).

2.6 Trace Elements in Human Hair in Fallujah

It is significant to mention that the issue of trace elements in hair in Fallujah has not received adequate attention from researchers. The only study which has focused on trace element is Alaani et al. (2011). However, Alaani et al. has not provided any information or data regarding trace elements in human hair in Fallujah for normal people. Rather, Alaani et al. studied contamination of the parents of the children and of the environment by Uranium and other elements using Inductively Coupled Plasma Mass Spectrometry. Hair samples from 25 fathers and mothers of children diagnosed with congenital anomalies were collected and analysed for uranium and 51 other elements (Alaani et al., 2011).

Table 2.1 shows summary of literature review for trace elements in human hair, which indicates the pollutant in environmental and background.

2.7 The Elements Selected

Six elements; arsenic, mercury, iron, copper, zinc and lead were selected and further investigation would focus on the detection of these elements. Elements were selected in the study for several reasons. When the high percentage of mercury, arsenic and lead in the body, they lead to different degrees poisoning(Katz and Katz, 2006), and may lead to death when they arrive to a very high proportions of the body.

Table 2.1: Summary of environmental studies of trace elements in human hair

Reference	Trace Elements	Location / Country	Method	Reason of the existence of trace element
(Pereira et al., 2004)	Cr, Cu, Zn, Cd, Mn and As	Portugal		People living near an abandoned cupric mine.
(Bencko, 1995)	As, Cu, Ni, Co, Mn and Fe	-	AAS	Exposure to pollutants.
(Kehrig et al., 1997)	Hg	Amazon, Brazil	ECD-gas chromatographer.	Fishing villages and gold mining areas.
(Foo and Tan, 1998)	Hg, Cd, Pb, and Cu	Singapore and Indonesia	AAS	Environmental uptake of elements.
(Krejpcdo et al., 1999)	Zn, Pb, Cd and Cu	Poland	FAAS	Level of environmental exposure.
(Tavakkoli et al., 2000)	Al, Mn, Mg, Ca, and V	Esfahan, Iran	NAA	Environment
(Vasconcellos et al., 2000)	Hg and Se	Amazon, Brazil	NAA	Selenium and mercury concentrations.
(Chai et al., 2004)	Fe, Co, Zn and Ca	Beijing, China	NAA	Workers at an iron and steel factory.
(Mosaferi et al., 2005)	As	Kurdistan, Iraq	NAA	Drinking water.
(Trojanowski et al., 2010)	Pb and Cd	Poland	AAS	Environment

Continue Table 2.1

(Onuwa et al., 2012)	Cr, Mn, Ni, Cu, Zn and Mo	-	(ED- XRF)	Relationships between ages; body mass, height, and heavy elements concentrations.
(Leung et al., 1999)	Ca, Fe, and Zn	China	XRF	Pregnant women
(Dede et al., 2001)	Ca, Fe, Cu, Zn and Pb	Ankara, Turkey	ED- XRF	Exposed to high levels of contamination.
(Khuder et al., 2008)	Ni, Cu, Zn, and Pb	Syria	TXRF	Industrial factories
(Sarmani, 1987).	Fe, As, Br, Co, Cr, Hg, Sb and Zn	Malaysia	NAA	Various local residents in Malaysia.
(Sarmani et al., 1994)	Hg	Malaysia	NAA	Fishermen, Kuala Juru Village in the state of Penang.
(Mokhtar et al., 2002)	Pb	Malaysia	FAAS	Shipyard workers, Sabah.
(Hajeb et al., 2008)	Hg	Malaysia,	(ICP-AES)	Fish consumption, in Kedah, Terengganu, Johor, and Selangor.
(Abugassa et al., 1999)	21 elements	Malaysia	NAA	Resident in different areas of Malaysia.
(Alaani et al., 2011)	Ca, Mg, Co, Fe, Mn, V, Zn, Sr, Al, Ba, Bi, Ga, Pb, Hg, Pd and U	Fallujah, Iraq	Inductively Coupled Plasma Mass Spectrometry	25 fathers and mothers of children diagnosed with congenital anomalies.

Iron, copper and zinc are elements in the human body, but rising or decrease concentrations of these elements in the human body may be a sign of a malfunction or organic dysfunction in the body or it may be an indicator of response to some types of cancers. The properties, absorption edge and K X-ray energies of these elements are as shown in Table 2.2 and the role and the functions of the elements selected will be discussed accordingly.

Table 2.2: The properties of the selected elements and its X-ray

Element		Iron	Copper	Zinc	Arsenic	Mercury	Lead
Symbol		Fe	Cu	Zn	As	Hg	Pb
Atomic Number, Z		26	29	30	33	80	82
Atomic weight, A		55.845	63.546	65.38	74.92	200.59	207.2
Density (g/cm³)		7.874	8.96	7.13	5.727	13.546	11.35
K series	$K\alpha_1$	6.403	8.047	8.638	10.543	70.821	74.957
(keV)	$K\beta_1$	7.057	8.904	9.571	11.725	80.258	84.922
	$L\alpha_1$	-	-	-	-	9.987	10.549
	$L\beta_1$	0.717	0.948	1.032	1.317	11.823	12.611

Another point to note is that the elements considered should also be abundant in nature and its physiological behaviour and toxicology are well known. Therefore, it is better to select elements which represent the essential trace elements and toxic elements so that the level of body supply with essential trace elements and the degree of poisoning with toxic elements can both be evaluated. Important trace elements are essential for human in quantities ranging from 18 mg to 50 mg per day and act as catalytic or structural components of larger molecules. They have specific roles to play and are