STUDY OF TRACE ELEMENTS IN HUMAN HAIR FROM AIR POLLUTION BY INSTRUMENT NEUTRON ACTIVATION ANALYSIS

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STUDY OF TRACE ELEMENTS IN HUMAN HAIR FROM AIR POLLUTION BY INSTRUMENT NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Environmental pollution has been remarkably concerned as a major problem in Thailand. In Bangkok, sources of air pollution are from pollutants emitted from vehicles in the congested traffic areas. In this study, elemental concentrations in human scalp hair samples from female students living in two Bangkok areas with different degrees of air pollution were determined by Instrument neutron activation analysis (INAA).

Hair samples were collected from 30 female students, 13 to 18 years old, living in Talingchan and Bangkoknoi-Bangplad districts. From the report of the National Pollution Control Department, the first district is less air-polluted than the second one. After appropriate treatments, the hair samples along with standard reference material and in-house standard solutions were irradiated under neutron beam at optimum experiment conditions for determining short and long lived radioisotopes. Qualitative and quantitative analysis were achieved by HPGe gamma spectrometry system.

Ten elements, Mg, Ca, Cl, Al, I, Mn, Na, Br, Hg and Zn were found, six of them are trace elements (Al, I, Br, Mn, Hg and Zn). The contents of Ca, Cl, I, Mn, Na, Br and Zn in both groups of subjects are similar and found to be within the average values reported by several published data. Statistical analysis showed significance higher Al and Hg concentrations in Talingchan group at $p < 0.05$. However, the high concentrations of Al and Hg in the lower air-polluted area (Talingchan) cannot be explained by the degree of air pollution but could probably attributed from other factors relating to the state of health, disease and lifestyle.

Accuracy and precision of method of 33% and 8.8% are well accepted for trace element analysis by INAA technique. Variations in the concentrations presented indicate dependence on other environment factors. On-going research with increasing numbers of samples and sampling sites should be designed to obtained more database of elemental composition of human hair. To ensure comparable data, standardization of hair sample collection and treatments are also needed to reduce inconsistencies due to different methodologies.

KEY WORDS: HAIR ANALYSIS / TRACE ELEMENTS / NEUTRON ACTIVATION ANALYSIS / AIR POLLUTION / BANGKOK

64 P.

ศึกษาธาตุปริมาณน้อยในเส้นผมจากมลพิษในอากาศโดยการอาบนิวตรอน (STUDY OF TRACE ELEMENTS IN HUMAN HAIR FROM AIR POLLUTION BY INSTRUMENT NEUTRON ACTIVATION ANALYSIS)

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บทคัดย่อ

มลพิษในสิ่งแวดล้อมก่อให้เกิดปัญหามากมายในปัจจุบัน โดยเฉพาะมลพิษทางอากาศในเขตกรุงเทพมหานคร เนื่องจากปัญหาจราจรที่แออัดในเมือง การวิจัยนี้เป็นการศึกษาธาตุปริมาณน้อยในเส้นผมของกลุ่มตัวอย่างนักเรียนหญิง ที่อาศัยอยู่ในพื้นที่ที่มีระดับของมลพิษในอากาศต่างกันโดยเทคนิคการอาบนิวตรอน

ทำการเก็บตัวอย่างเส้นผมจากนักเรียนหญิง 2 กลุ่ม อายุระหว่าง 13-18 ปี รวม 30 ตัวอย่างที่อาศัยอยู่ในเขตตลิ่งชัน และเขตบางกอกน้อย-บางพลัด ภายใต้เงื่อนไขการทดลองสำหรับการวิเคราะห์ไอโซโทปรังสีที่มีครึ่งชีวิตสั้นและยาวเพื่อวิเคราะห์ตัวอย่างเชิงคุณภาพและปริมาณด้วยเครื่องวัดรังสีแกมมาชนิดหัววัดแบบกึ่งตัวนำ

ผลการศึกษาพบธาตุทั้งหมด 10 ธาตุคือ แมกนีเซียม แคลเซียม คลอรีน อลูมิเนียม ไอโอดีน แมงกานีส โซเดียม โบรมีน ปรอท และสังกะสี ประกอบด้วยธาตุปริมาณน้อย 6 ธาตุได้แก่ อลูมิเนียม ไอโอดีน แมงกานีส โบรมีน ปรอท และสังกะสี ปริมาณของแคลเซียม คลอรีน ไอโอดีน แมงกานีส โซเดียม โบรมีน และสังกะสีในตัวอย่างทั้งสองกลุ่มไม่แตกต่างกันและ มีค่าใกล้เคียงกับผลการศึกษาอื่นที่เคยมีการรายงานไว้ ปริมาณเฉลี่ยของอลูมิเนียมและปรอทในเส้นผมนักเรียนที่อาศัยในต่างเขตมีความแตกต่างที่ระดับนัยสำคัญ 0.05 ปริมาณเฉลี่ยของอลูมิเนียมและปรอทในเส้นผมของนักเรียนกลุ่มที่อาศัยอยู่ในเขตมลพิษต่ำ มีค่าสูงกว่ากลุ่มที่อยู่ในเขตที่มีระดับมลพิษสูงกว่า อาจเป็นผลมาจากปัจจัยอื่น ที่ไม่ใช่มลพิษทางอากาศ เช่น ความแตกต่างทางสุขภาพ และการใช้ชีวิตในสภาพแวดล้อมที่แตกต่างกันของแต่ละบุคคล

เทคนิคการอาบนิวตรอนสามารถใช้วิเคราะห์ธาตุปริมาณน้อยในเส้นผมได้ จากผลการวิเคราะห์ธาตุส่วนใหญ่ในกลุ่มทั้งสองไม่มีความแตกต่างและอยู่ในเกณฑ์ปกติ อาจเนื่องจากระดับความรุนแรงของมลพิษไม่สูงพอที่จะแสดงความแตกต่างของการสะสม ความถูกต้องและความแม่นยำของการวิเคราะห์เท่ากับ 33% และ 8.80% อยู่ในระดับที่ยอมรับได้สำหรับการวิเคราะห์ธาตุปริมาณน้อย เพื่อให้ผลวิเคราะห์ที่มีความแปรปรวนน้อยลงควรเพิ่มจำนวนพื้นที่ทดสอบและจำนวนตัวอย่างที่นำมาวิเคราะห์และควบคุมวิธีการให้ได้มาตรฐานเพื่อสามารถนำไปเปรียบเทียบกับรายงานอื่นๆ ได้

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CHAPTER I

INTRODUCTION

1.1 Background and rationale

Environmental pollution has been remarkably concerned as a world's problem. Many studies have demonstrated an association between environmental exposure and health problems. In Thailand, studies have shown that air pollution has harmfully affected the health of Bangkok residents.

The data from United Nations Environment Program (UNEP) between 1992-2002 (1), reported that in 1992 the Thai capital was one of the most air-polluted cities in the world due to the city's traffic problems. Approximately one million Bangkok residents suffered from allergies and respiratory problems from air pollution. In 2001, airborne particulate matter was resulted in estimated causes of 3,300 premature deaths and almost 17,000 hospital admissions, at a total health care cost of up to 220.5 billion Baht. In 2002, World Bank reported that while the overall air quality in Thailand has improved in the last years, it is still a problem in areas with crowded traffic density like Bangkok.

Study of trace elements in human body is important in searching for relationship between human health and exposure to elements through air environment. In recent years, the determination of trace element levels in human hair has become popular for monitoring environmental exposures.

The analytical methods currently used to measure trace elements in hair include Atomic absorption spectrometry, Neutron activation analysis, x- ray fluorescence, Mass Spectrometry, Inductive Coupled Plasma Atomic Emission Spectrometry (ICP) and other chemical method etc.

The purpose of this study was to determine the elemental concentrations in human scalp hair samples by instrument neutron activation analysis (INAA). In this study, two areas with different degree of environmental pollution due to traffic congestion were chosen for the observation.

1.2 Objectives

1.2.1 To determine trace element compositions in human hair.

1.2.2 To analyze trace element concentrations in human hair by nuclear technique.

1.2.3 To compare trace element levels in hair from two different air pollution areas.

1.3 Expected outcome and benefits

1.3.1 Trace element contents in human scalp hair detected with neutron activation analysis technique may be a useful indicator of environmental exposure to air pollution.

1.3.2 The method is applicable as a tool for monitoring pollution level of groups of individual living in areas with different degrees of air pollution.

1.3.3 The results of this study will provide useful information for further study which would help actions being taken to reduce air pollution over the city.

CHAPTER II

LITERATURE REVIEW

2.1 The need for elements in human body

Microelements are very important for the functioning of all living organisms. However, human body cannot manufacture elements itself. Elements play an important role in many bodily functions and are contained in body tissues and fluids. Many elements are assisting in the production of energy and other important biochemical processes, particularly enzyme reactions. Deficiencies or imbalances among elements lead to health problems (2).

Most of the human body is made up of water (H_2O) with cells consisting of 65-90% water by weight and the major six elements are oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorus. All elements in human body are listed as follows; Oxygen (65%), Carbon (18%), Hydrogen (10%), Nitrogen (3%), Calcium (1.5%) , Phosphorus (1.0%), Potassium (0.35%), Sulfur (0.25%), Sodium (0.15%), Magnesium (0.05%), Copper, Zinc, Selenium, Molybdenum, Fluorine, Chlorine, Iodine, Manganese, Cobalt, Iron (all in 0.70%), and trace amounts of Lithium, Strontium, Aluminum, Silicon, Lead, Vanadium, Arsenic, Bromine (3).

More than 50 elements are found in the human body. About 25 elements have been found to be essential as depletion in the concentration may cause various metabolic instabilities due to enzyme dysfunction (4). The main elements in human body which have the important functions in body metabolism are listed in Table 1 (5).

Table 1. The functions of main elements in human body.

Elements	Functions
Boron (B)	- Assist and improve retention of calcium, magnesium, and phosphorus. - Necessary for brain function, memory and alertness as well as for the activation of vitamin D.
Calcium (Ca)	- Found mainly in bones and teeth. - Important for membrane function, nerve impulses, muscle contractions, and blood clotting.
Carbon (C)	- Found in all organic molecules.
Chlorine (Cl)	- Important for membrane function and water absorption. - Chloride is the major anion in body fluids and part of hydrochloric acid (HCl) in gastric juices.
Chromium (Cr)	- Master regulator of insulin. - Potent metabolic hormone in the metabolism of proteins, carbohydrates, and fats. - Assist neurotransmitters. - Help with the function of the brain, thyroid, and hormonal balance.
Cobalt (Co)	- A vital part of vitamin B12. - Stimulate numerous enzymes. - Help build red blood cells and with iron absorption.
Copper (Cu)	- Involved in the synthesis of hemoglobin, melanin, and elastin. - An enzyme cofactor. - Part of some cytochromes in cell respiration. - Assist in phospholipid synthesis, protein metabolism, vitamin C oxidation, and the formation of RNA.
Germanium (Ge)	- Helps activate various organs to attract more oxygen. - Expel harmful pollutants and pathogens from the body. - Help maintain a strong immune system by assisting in the production of killer cells and T-suppresser cells. - Assist in electron transmissions.

Table 1. The functions of main elements in human body. (continued)

Elements	Functions
Hydrogen (H)	- A component of water and most other compounds in the body.
Iodine (I)	- A major component of thyroid hormones (thyroxine and triiodothyronine). - Necessary for the metabolism of fats and such minerals as calcium, silica, and phosphorus. - Essential for spleen, liver, and brain function. - Neutralizes albumin.
Iron (Fe)	- Essential for oxygen transport and energy capture. - Component of hemoglobin, myoglobin, and cytochromes in cell respiration.
Magnesium (Mg)	- Required for activation of several enzymes. - Vital for strong bones and teeth. - Essential for brain and liver function. - Calm nerves. - Promote cell growth. - Increase tissue elasticity. - Necessary for metabolism of ATP-ADP.
Manganese (Mn)	- Cofactor for some enzymes. - Found with lecithin and involved in the synthesis of fatty acids and cholesterol. - Strengthens nerves and thought processes. - Element in body linings and connective tissues. - Help with eyesight. - Enhance body's recuperative abilities and resistance to disease.
Nitrogen (N)	- Found in proteins, nucleic acids, and other organic compounds - 78% of the air we breathe is nitrogen.
Oxygen (O)	- A component of water and other compounds. - Oxygen gas is essential for respiration.

Table 1. The functions of main elements in human body. (continued)

Elements	Functions
Phosphorus (P)	- Found in the nucleus of every cell in the body (including white blood cells), nucleic acids, high-energy compounds, and phosphate buffer system. - A major component of outer bone. - Combine and calcium to build and maintain bone. - Necessary for the reproductive system and sexual function. - Necessary for muscle tissue and growth. - An essential nutrient for the nerves.
Potassium (K)	- Important for proper membrane function, nerve impulses, and muscle contractions. - Major cation in cytoplasm. - A primary electrolyte and alkalizer. - Attract oxygen to tissues. - Helps eliminate toxins from the body.
Selenium (Se)	- A powerful antioxidant; vital to the immune system; major part of apoptosis (normal cell death in the body). - Help maintain cell integrity. - Support heart function. - Help slow the aging process. - Delay oxidation of polyunsaturated fatty acids.
Sodium (Na)	- Stored in stomach walls, joints, and gallbladder. - Help prevent blood clotting. - Important for membrane function, nerve impulses, and muscle contractions. - Major cation in body fluids. - Contribute to the alkalinity of the lymph and blood. - Work with the bicarbonate buffer system in the digestive tract to prevent hydrochloric acid from burning stomach walls. - Help retain calcium and cholesterol liquid in the body. - Help with excretion of carbon dioxide (CO_2).

Table 1. The functions of main elements in human body. (continued)

Elements	Functions
Sulphur (S)	- Found in many amino acids as well as thiamine and biotin. - Necessary for developmental and neurological processes and for synthesis of collagen. - Detoxify. - Increase blood circulation. - Reduce muscle cramping and back pain. - Remove inflammation. - Assist in the healing of muscles. - Help the liver produce chorine. - An important element in nerves and the myelin sheath. - Stimulate flow of bile. - Regulate heart and brain function. - Promote healthy skin, nails, and hair. - Help lubricate joints.
Zinc (Zn)	- Found in all body fluids, including urine as well as the moisture found in the eyes, mouth, lungs, and nose. - A cofactor for enzyme function, especially carbonic anhydrate needed for carbon dioxide transport. - Part of peptidases needed for protein digestion. - Necessary for normal taste sensation. - Important in wound healing. - A necessary part of DNA and for cell division and synthesis. - Necessary for hormone production and for the prostate gland. - A vital part of the immune system.

2.2 Trace elements

There are three groups of element that are classified by concentration levels in human body; *Major, Minor and Trace elements*. Major elements are those with concentrations exceeding 1% by mass which include the basic elements of organic compounds as carbon, hydrogen, oxygen, nitrogen and the other elements present in high quantities as calcium and phosphorus. Minor elements are those present at medium concentration levels, in the range 0.01% to 1% by mass that are mostly assemble in specific organs such as potassium, sodium, chlorine, sulfur, and magnesium.

Trace elements (6), also known as micronutrients. They are required in amounts smaller than 0.01% by mass of the organism. Its very low quantity may be expressed in parts per million (ppm: μg/g) or parts per billion (ppb: ng/g) level. Trace elements can be categorized into those which are essential for human life (often referred to as minerals) such as iron, manganese, zinc, copper, iodine, cobalt, molybdenum, selenium, chromium and silicon, and others, and those which are potentially toxic such as aluminium, arsenic, cadmium, lead, mercury, nickel and etc. Some essential trace elements (Co, Cr, Fe, Mn, and Se) may also be toxic when concentrations are raised above specific cut-off levels, above the safe range of population mean intakes (7).

During the last few decades, clinical interesting in the trace elements has been expanding with steadily growing momentum. Major reasons for this development include (8):

- the elucidation of the physiological roles of several of the ‘essential’ trace elements at a molecular level in many vital metabolic processes in mammalian;
- the identification of the roles for an increasing number of the trace elements in mammalian nutrition;
- the documentation of clinical problems resulting from ‘new’ trace deficiency states;
- the rapidly increasing use of chemically defined ‘elemental’ diets in medical practice. Many formulated foods are now enriched with essential trace elements such as copper, iodine, iron or zinc;
- the identification of a growing number of inherited disorders of human trace metal metabolism;

- the realization that environmental pollution may be an important contributory factor to some major chronic diseases.

Imbalance elemental levels, as well as the trace elements, are associated with many metabolic disorder in man (9,10,11,12). For example, Inadequate calcium causes bone maintenance calcium deficiency, muscle contraction and abnormal heartbeat. Sodium and potassium deficiency also frequently results in diuretics including effect of nerve and muscle functions.

Trace minerals are essential to metabolic functions in all phases of the life process. They not only provide the building blocks to life itself, but are also necessary in the production of hormones and enzyme activity. For example, iodine deficiency leads to goiter and cretinism. Severe iron deficiency results in anemia and has a low hemoglobin concentration in red blood cells. Deficiency of chromium may cause diabetes. Excess cobalt causes reduced growth and congestive heart failure and etc. Zinc is involved in the production, storage and secretion of insulin, and is necessary for growth hormones. Magnesium is required for normal muscular function, especially the heart. A deficiency has been associated with an increased incidence of heart attacks, anxiety and nervousness. Potassium is critical for normal nutrient transport into the cell. A deficiency can result in muscular weakness, depression and lethargy. Excess sodium is associated with hypertension. Elevated lead, particularly in children, can contribute to learning disabilities and hyperactivity.

Furthermore, imbalance trace element can indicate from the change of external organs. For examples, white spots on the fingernails are often a sign of zinc deficiency; while vertical lines on nail can indicate poor nutrition or iron deficiency, and a variety of copper deficiency, symptom have been associated with hypopigmentation of hair and skin, and crimpy or steely hair (13).

2.3 Hair structure

Hair is fine strand of tissue, which appears above every part of the skin surface. They cover most of the body, with the exception of the eyelids, the palms of the hands and the soles of the feet. Hair varies greatly in color, length, and thickness in different parts of the body and in different races of humans. On average, the total number of

hair follicles for an adult human is estimated at 5 million, of which 100,000 alone cover the scalp (14).

The hair is divided into two parts; the hair root and the hair shaft. The hair root is the structure beneath the dermis layer while the hair shaft travels through the epidermis layer to appear on the skin surface. Below the surface of the skin is the hair root, which is enclosed within a hair follicle. The root is nourished by a network of delicate blood vessels, which deliver vitamins, minerals and trace elements to the outer layers of the hair shaft. At the base of the hair follicle is the dermal papilla. The dermal papilla is feed by the bloodstream, which carries nourishment to produce new hair. The dermal papilla is a structure very important to hair growth because it contains receptors for hormones. The hair structure is illustrated in Figure 1.

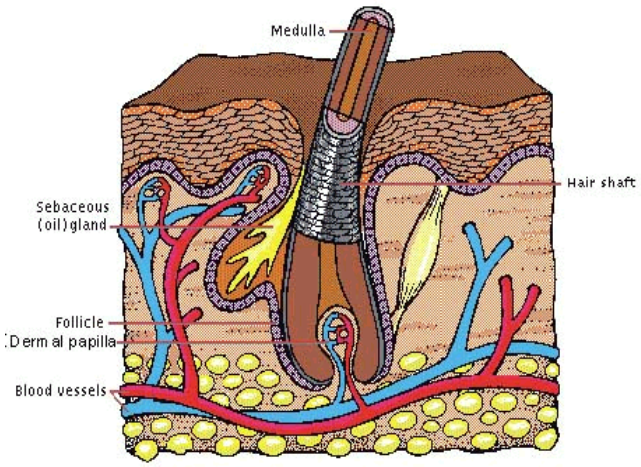

Figure 1. Hair structure.

Hair is composed of strong structural protein called keratin. This is the same kind of protein that makes up the nails and the outer layer of skin. Each strand of hair consists of three layers. An innermost layer or *medulla*, is only present in large thick hairs, the middle layer known as *the cortex* that provides strength and both the color and the texture of hair, and the outermost layer is known as *the cuticle* which is thin and colorless and serves as a protector of the cortex (15). The bundles of fibers found in the cortex are made from molecules of Amino Acids. There are about twenty-two

amino acids in the hair, and the molecules of each contain atoms of elements in different proportions.

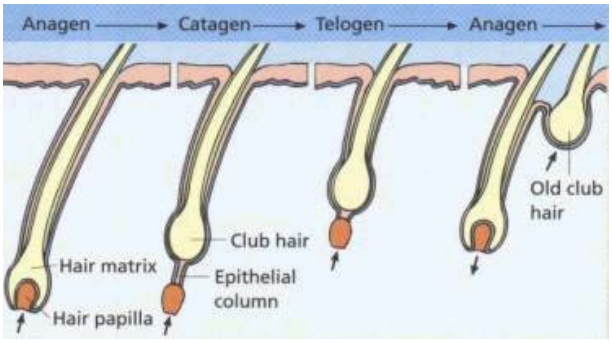

Figure 2. The hair growth cycle (16).

Hair is constantly growing. Over a period of between one and six years an individual hair actively grows, then stops, rests and degenerates, and finally falls out. The rate of growth is approximately 1.25 centimeters, or about 0.5 inches, per month (17). Before the hair leaves the follicle the new hair is normally ready to replace it. At any one time we only have around 85% of our hair on our head at a time, the rest being in the resting stages. The hair growth cycle has three distinctive phases; *Anagen* is the period of active growth, *Catagen* is the period of breakdown and change, and *Telogen* is the resting stage before resumption of growth (Figure 2). The regeneration of hair is influenced by many factors such as health, hereditary factors, diet, hormone balance, age, physical condition, climate, chemical effects, sex and effects of disease (18).

2.4 Trace elements in human hair and the effects to health

Usually blood, urine, liver biopsy, hair, or nails are the most commonly objects used for elemental analysis. Most of the metabolic processes taking place in the tissues are at the cellular or mitochondrial level. For example, blood levels of many substances depend on the level of hydration, composition of recent meals, activity level, and even the time of day. Urine shows only what is excreted from the body by the kidneys (19). Blood serves mostly as a transporter of nutrients and is not representative of tissue levels. Also, the elements in the blood are in a much more

diluted form with lower concentrations, causing analytical difficulties. Certain elements like magnesium and potassium have a much higher concentration in the cells than in the extra cellular fluids like blood (20).

Compared to other types of clinical specimens, hair has different uses and even advantages over blood or urine. While urine and blood tend to show current or recent body status, hair represents a longer time frame, potentially years. Elements also occur in hair at higher levels (as shown in Table 2.) (21) , allowing for more sensitive and, because of the higher levels, more analytically accurate results, and this led to the use of hair in clinical status studies and it has been recognized as an indicator of exposure to many metals (22).

Scalp hair is considered a suitable biological sample for estimating the intake of environmental exposure and some trace elements. Hair is formed in the matrix cell, where it incorporates various elements from the blood at a relatively constant rate. After formation, the hair is separated from the body's internal metabolism; therefore, its composition reflects the concentration of elements in blood at the time of formation (4).

Trace element analysis of human hair has become popular due to the hair property of retaining trace elements, the ease of sample collection, transport and storage (23). Human hair contains minute traces of more than 30 elements such as Al, As, Br, Ca, Cd, Cl, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Sb, Sc, Se, V, Zn, etc. Their concentrations can reveal interesting facts about health, diets, occupation and impact of environmental pollution.

In 1997, Georgescu et el studied hair analysis of metallurgical worker by instrument neutron activation analysis (INAA). They concluded that high concentration levels of Al, Co, Cu, Fe, Mn, Sb, V and Zn in the hair of metallurgical worker. They also compared hair concentration levels of healthy people from the control group with some published results of healthy people from different countries in which the hair samples have been washed according to the procedure described by the International Atomic Energy Agency (IAEA) in Table 3. Ranges compiled for a normal population from different nationalities presented in the report of the IAEA coordinated research program which were also shown in Table 3 (24,25).

Table 2. The normal levels of some essential and trace elements in blood, urine and hair.

Element	Blood serum (mg/L)	Urine (mg/L)	Hair (mg/kg)
Copper	0.8 - 1.8	0.03 - 0.06	7 - 40
Iron	0.7 - 1.5	0.10 - 0.15	15 - 175
Zinc	0.8 - 1.1	0.40 - 0.60	150 - 250
Cadmium	0.001 - 0.007*	0.001 - 0.005	0.4 - 2.4
Mercury	0.002 - 0.006	0.001 - 0.02	0.5 - 10
Lead	0.002 - 0.2*	0.006 - 0.012	5 - 50

* whole blood

Table 3. Elemental concentrations of scalp hair of healthy people of different nationalities and the normal range published by IAEA.

Element	Georgescu et el. (Romania)	Tomza et al. (Poland)	Wiesner (Germany)	Vance et el. (USA)	Takeuchi et el. (Japan)	Normal range IAEA comp.
Al, ppm	14.7 ± 1.70	15 ± 2.2	17.7 ± 1.80	-	10.3 ± 1.90	3.7 – 10.3
Co, ppb	20 ± 2.21	190 ± 3.5	300 ± 1.82	29.4 ± 1.14	41 ± 2.5	28 – 170
Cu, ppm	11.5 ± 1.43	11.0 ± 1.5	11.7 ± 1.48	-	11.1 ± 1.59	9.6 – 20.6
Fe, ppm	24 ± 1.80	108 ± 2.2	45.3 ± 1.71	9.16 ± 1.10	28 ± 1.73	27 – 106
Mg, ppm	123 ± 2.10	52 ± 1.8	-	-	78 ± 2.4	48 – 115
Mn, ppb	750 ± 2.25	1110 ± 2.3	1340	-	480 ± 2.6	140 – 8800
Sb, ppb	46 ± 1.96	170 ± 2.5	-	26 ± 1.11	65 ± 2.3	28 – 858
Se, ppb	460 ± 1.47	800 ± 1.70	310 ± 1.31	544 ± 1.04	700 ± 1.89	332 – 3740
V, ppb	31 ± 1.94	80 ± 3.3	-	-	30 ± 2.0	21 – 57
Zn, ppm	170 ± 1.19	166 ± 1.4	151	150 ± 5.3	178 ± 1.30	128 – 261

A number of studies have reported an association of scalp hair trace elements concentration with health and diseases. For example, the comparative study of the copper content of the liver and hair of African children with Kwashiorkor disease suggested a direct correlation between the reduced levels of copper in the hair and the liver among these subjects (26). In 1998, Lin and Huang report that consumption of

drinking water with elevated arsenic concentrations showed a correlation with hair arsenic which was elevated in patient with blackfoot disease (27). Determination of 17 elements in the hair and nails of Alzheimer's disease revealed significant imbalances in the concentrations of six elements (Br, Ca, Co, Hg, K, and Zn) (28).

Many parameters have been shown to influence elemental concentrations of the hair such as gender, ethnicity, diet, age, geographic location, and season. Sky-Peck (29) demonstrated variation in elemental concentrations among a healthy Midwestern American population, as follow:

1. Gender – females had higher Ca and Ni and lower Pb, Br and Se compared to males;
2. Hair color - blondes had less Fe than brunettes, red-heads had more Fe and Cu;
3. Ethnicity/race - Blacks had increased Ca, Fe, Ni, Cr, Mn, As, and Pb, and decreased Hg, compared to Caucasians; Orientals had decreased Ca, Fe, Cu, Mn and Pb;
4. Age – a decrease in S, Ca and Sr, and an increase in Pb with age;
5. Geography – increased hair strontium in areas with elevated strontium in drinking water, and increased hair lead in industrial/older residential areas.

Sky-Peck also noted that some of these differences may due to differences in natural living habits, for example hair treatment and/or environmental exposure (30). It is known that various chemicals including metals will bind to melanin (31).

Sukumar and Subramanian (32) reported that hair composition may be changed by exogenous means such as smoking habit, years of occupation and income of family. The Cu level was found to be higher in the middle age group (31–45 years) than in the older age group (46–60 years) of fishermen. But the Cu and Cd levels were lower in the younger age group (16–30 years) than in the middle age group of businessmen and the older age group of non-mining workers. Thus, both the higher and lower concentrations of elements were observed in the younger age group of subjects when compared to the element concentrations found in the older age groups. Likewise, Petering et al.(33) found lower levels of Pb in the hair of males and higher levels in the females up to the age of 35 years and then a sharp decrease.

For hair colors, Sky-Peck found no differences in elemental concentrations between gray hair and natural hair while other investigators have noted pigmentation effects (30). Data collected from Hair Tissue Mineral Analyses (HTMA) of individuals having a variety of hair colors, suggests that trace mineral levels in human hair are relatively independent of hair color (34). For people who have gray or white hair, there is a general decrease of hair mineral concentration with age (35). Further studies are needed to clarify whether the older individuals belonging to this age group lose their hair pigments because of metabolic difficulties caused by impaired digestion and/or absorption which in turn may result in deficiencies of different nutrients.

An international database of the baseline concentration for elements in clinical specimens including hair has been set up (36,37). International differences in the concentrations of Zn, Cd, Cu, Mn and Pb in hair are identified. Some of these geographical differences may be due to differences in environmental metal concentrations, industrialization, and etc. Seasonal differences in hair element concentrations, e.g. cadmium, may be due to time spent outdoors and contact with soil, dust, and etc.

2.5 Hair trace elements from environmental pollution

Because of growing public concern about the environmental contamination, it is becoming increasingly important to better understand both the natural and human processes. Yukawa et al. studied the distribution of trace elements in infant hair (2-7 years of age) using neutron activation analysis (38). Increased concentrations of I, Mg, Ca and Cu was found from the scalp end to the tip while Cl and Br decreased inversely. Different profiles of the concentrations of Hg, Se, Ca and Mn were seen in each sample. These results were discussed with reference to the indication of environmental pollution.

Analysis of human hair trace elements has become popular for its utilization as a tool for monitoring environmental pollution or intake of toxic elements. Georgescu et al. (24) demonstrated that occupational exposure to the metallic elements reflected in significant higher concentration in hair.

Environment toxic elements may inhibit enzymes in the body, weaken cell membranes, or impair nutrient delivery, which can lead to illness. For example (39,40), excess lead is associated with fatigue, constipation, insomnia, emotional disturbances, hyperactivity, and learning disabilities in children. Excess aluminum is associated with Alzheimer's disease and may also lead to the depletion of phosphorus in the body, which is a disadvantage for bone. Copper is an essential substance to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. Low-level chromium exposure can irritate the skin and cause ulceration, and long-term exposure can cause kidney and liver damage, and damage to circulatory and nerve tissue. Excess arsenic has impact on respiratory system, skin problems, and tingling in the extremities. Mercury causes damage to the brain and the central nervous system, while foetal and postnatal exposures have given rise to abortion, congenital malformation and development changes in young children. Excess cadmium is related with tissue aging, musculoskeletal pain, anemia, renal dysfunction and hypertension. High exposure can lead to obstructive lung disease and has been linked to lung cancer. Selenium accumulation causes hair and fingernail loss, damage to kidney and liver tissue, damage to circulatory tissue, and more severe damage to the nervous system.

There are many analytical results of trace elements in human hair from different environmental or pollution areas. Cho et al. (41) reported hair elemental concentration of Korean populations, the concentrations of Ca, Mg, Zn, Cu, Na, Br, Mn, I, and S in hair samples from metropolitan residents were higher than the rurals. Sarmani (42) demonstrated high concentrations of As, Br, Cr, Hg, Sb and Se in urban population of Malaysia while the concentrations of As and Hg in the rurals are within the normal range (Table 4). The concentrations of As and Hg in healthy adults are in the range of 0.13 – 0.71 ppm and 1.25 – 7.6 ppm respectively (43). Table 4 compared elemental concentrations in hair samples between metropolitan and rural residents of Malaysia and Korea.

In 2002, an air pollution study of dust sample in Bangkok has been monitored by the INAA technique. The results showed that heavy metals from vehicle exhaust and other source in the air have been detected. The elements found are As, Sb, Zn, Cr, Au, Br and others (44).

Table 4. Trace element concentrations (ppm) in hair sample of metropolitan and rural residents of Malaysia and Korea.

Malaysia			Korea		
Element	Metropolitan (n = 25)	Rural (n = 40)	Element	Metropolitan (n = 32)	Rural (n = 10)
As	0.83 ± 0.56	0.29 ± 0.17	Al	15.7 ± 6.12	16.6 ± 10.3
Br	9.1 ± 7.0	1.29 ± 0.61	As	0.15 ± 0.12	0.18 ± 0.05
Co	0.06 ± 0.02	0.05 ± 0.03	Br	9.68 ± 5.24	7.43 ± 8.76
Cr	1.63 ± 1.03	1.07 ± 0.48	Ca	1499 ± 1052	761 ± 567
Fe	52 ± 23	73 ± 22	Cu	31.5 ± 11.7	22.4 ± 15.6
Hg	6.62 ± 3.73	0.82 ± 0.31	I	0.64 ± 0.45	0.37 ± 0.13
Sb	0.27 ± 0.21	-	Mg	132 ± 80.1	91.0 ± 42.0
Se	0.58 ± 0.18	0.44 ± 0.25	Mn	2.29 ± 1.78	0.97 ± 0.77
Zn	187 ± 83	136 ± 79	Na	8.27 ± 4.53	7.39 ± 2.56
			S	48052 ± 3040	44875 ± 4299
			Zn	307 ± 84	315 ± 68

2.6 Nuclear reactor

Most nuclear reactors consist of a core that contains the reactor fuel as well as neutron moderating and cooling materials. Uranium-235 is the most commonly used fuel. The major source of neutrons is the fissioning of U-235. When U-235 fissions, neutrons are produced that have energies ranging from 0.1 to above 20 MeV. The energetic neutrons resulting from fission collide with the nuclei of the moderator and slow down. This result in a more efficient fission process since the fission cross section of U-235 is larger for moderated neutrons than for immoderate neutrons. The energy distributions of neutrons in a nuclear reactor can be classified into three principal components as shown in Figure 3

(1) Thermal or slow neutrons have an average energy of about 0.025 eV

(2) Epithermal neutrons have energy extending from 0.5 eV- 1 MeV

(3) Fast neutrons have energy above 1 MeV

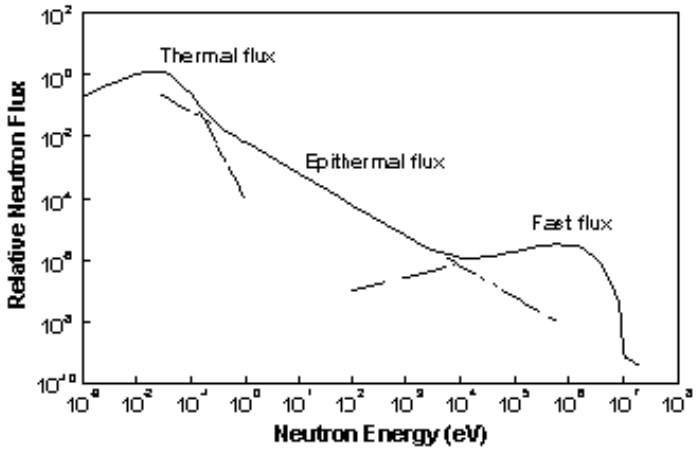

Figure 3. A typical neutron energy spectrum from a nuclear reactor showing the various components used to describe the neutron energy regions.

2.7 Neutron reaction

Several nuclear reactions of neutron are possible depending on the target nucleus and the neutron energy. Nuclear reactions of interest in neutron activation analysis are given below (45).

2.7.1 Neutrons capture

Neutrons capture, (n,γ) reaction is the most common reaction from thermal neutrons (0.025 eV) absorbed by a nucleus with the prompt emission of a gamma ray. The target and isotope are of the same atomic number but the mass numbers differ by one unit; for example

$$^{59}_{27}\mathrm{Co}\,(\mathrm{n},\gamma)\,^{60}_{27}\mathrm{Co}$$

2.7.2 Transmutation

A nucleus may absorb a neutron forming a compound nucleus, which then de-energizes by emitting a charged particle, either a proton or an alpha particle. This produces a nucleus of a different element. Such a reaction is called a transmutation as neutron-proton (n, p) and neutron-alpha (n,α) reactions.

$$^{16}_{8}\mathrm{O}\,(\mathrm{n},\mathrm{p})\,^{16}_{7}\mathrm{N}$$

$$^{10}_{5}\mathrm{B}\,(\mathrm{n},\alpha)\,^{7}_{3}\mathrm{Li}$$

2.7.3 Inelastic scattering

The neutron transfer only part of its kinetic energy to the target nucleus and escaping with only degradation in its energy. The processes of inelastic scattering for the production of radioactive isotopes are two reactions as given.

(a) The (n, n′) reaction

This reaction takes place when the scatter neutron imparts sufficient energy to the target nucleus to raise its energy to a metastable state, for example

$$^{103}_{45}\mathrm{Rh}(\mathrm{n},\mathrm{n}')^{103\mathrm{m}}_{45}\mathrm{Rh}$$

(b) The (n,2n) reaction

This reaction may occur when the scattered neutron imparts enough energy to exceed the binding energy of the least bound neutron in the target nucleus. The net result is the removal of one neutron from the target nucleus.

$$^{23}_{11}\mathrm{Na}\ (\mathrm{n},2\mathrm{n})^{24}_{11}\mathrm{Na}$$

2.7.4 Fission

Nuclear fission is the process of splitting nucleus; the process involves the absorption of a neutron into the very heaviest ($Z \geq 90$) and results in the two fragments nucleus which are called fission product or fission fragment, with the concomitant release of two to three neutrons.

2.8 The rate of radionuclides production and radioactive decay

When a sample is bombarded with neutron, charge particles or photons radioactive nuclei are often produced. The rate of radionuclides production is dependent on the number of target nuclei, the neutron flux and neutron cross-section, as given

$$P = N\sigma\phi \qquad \text{…….. (1)}$$

Where P = The rate of radionuclides production

N = The number of atoms of target nuclei

σ = Cross-section of the investigation reaction, in barn (10^{-24} cm^2)

ϕ = The neutron flux, in neutron/cm^2.sec

Since the radionuclide decay during the irradiation with its characteristic half-life at a rate proportional to the amount present at any specific time, the rate of change of the product nuclei during irradiation is thus given by the difference between the production rate and the decay rate (λN), or

$$\frac{dN(t)}{dt} = P - \lambda N(t) \qquad \text{..... (2)}$$

where N(t) = number of atom of radionuclide at any time t

λ = the decay constant, $\lambda = 0.693 / T_{1/2}$

$T_{1/2}$ = half-life of radionuclide

The solution of equation 2 is given by

$$N(t) = \frac{P}{\lambda}(1 - e^{-\lambda t}) \qquad \text{..... (3)}$$

Where at time t = 0, N(0) = 0 and the activity of the radionuclide at the end of the irradiation period t_i is

$$A(t_i) = \lambda N(t_i) = P(1 - e^{-\lambda t_i})$$

$$A(t_i) = N\sigma\phi(1 - e^{-\lambda t_i}) \qquad \text{..... (4)}$$

After irradiation, the activity at any decay time (t_d) can be determined from

$$A(t_d) = N\sigma\phi(1 - e^{-\lambda t_i})e^{-\lambda t_d} \qquad \text{..... (5)}$$

Equation 5 may be also expressed in terms of the measurement parameter, for example, count rate (R(t)) and weight of element (w), since

$$R(t) = A(t)f(E)\varepsilon(E) \qquad \text{..... (6)}$$

where $\varepsilon(E)$ = absolute photo peak efficiency at energy E

f(E) = intensity of gamma ray at energy E

And the number of target nuclide (N) in the sample can be calculated from

$$N = \frac{N_A w \theta}{M} \qquad \text{..... (7)}$$

where N_A = Avogadro's number (6.022×10^{23} atoms/gram-atom)

θ = The isotopic abundance of target nuclide

w = The weight of target nuclide in the specimen

M = The atomic weight of the target

By substitute equation 6 and 7 into equation 5, the equation can be written as

$$R(t_d) = f(E)\varepsilon(E)\frac{N_A w \theta}{M}\sigma\phi(1 - e^{-\lambda t_i})e^{-\lambda t_d} \qquad \text{..... (8)}$$

2.9 Radiation measurement

2.9.1 Interaction of gamma ray with matter

When gamma ray penetrates matter, it can interact with the atoms in various ways. The three main interaction processes are:

(a) Photoelectric Effect

In this process, the gamma ray interacts with a bound electron, and all of its energy is absorbed. The electron is ejected from the atom with kinetic energy E_e approximately equal to

$$E_e = E_\gamma - E_b \qquad \text{..... (9)}$$

where E_γ = the energy of incident gamma ray

E_b = the electron binding energy

Since the ejected electron leaves a hole in a shell of the atom, this atom will deexcite with the emission of one or more x-rays. This effect is the dominant mode of interaction at low energies in the material with high atomic number.

(b) Compton Scattering

In Compton scattering, the incoming gamma ray photon is deflected through an angle θ with respect to its original direction. The photon transfers a portion of its energy to the electron, which is then known as a recoil electron, or a compton electron.

The photon is scattered through angle θ with degrade energy hν′. The recoil electron is ejected at angle ϕ with energy E_e.

$$E_e = h\nu - h\nu' \qquad \text{……..(10)}$$

The equation of the degraded gamma ray energy is

$$h\nu' = \frac{h\nu}{1+(1-\cos\theta)h\nu / m_e c^2} \qquad \text{……. (11)}$$

where θ = angle of the scattered gamma ray with respect to the incident gamma ray

hν′ = the energy of the degraded gamma ray

hν = the energy of the incident gamma ray.

The Compton effect is more important for intermediate gamma ray energies.

(c) Pair Production

If a photon enters to matter with energy in excess than twice the rest mass of the electron ($2m_ec^2$ = 1.022 MeV), it may interact with the electric field of the nucleus. The photon is absorbed and reappears as a positron-electron pair. These particles share the kinetic energy that is equal to the difference between the incident gamma ray energy and the two electron rest mass energies, then an energy balance yields.

$$E^+ + E^- = h\nu - 2m_ec^2 \qquad \text{……. (12)}$$

Where E^+ = the kinetic energy of the positron

E^- = the kinetic energy of the negatron

$2m_ec^2$ = the rest mass equivalent energy of the two particles.

The positron will finally react with an electron and annihilate. If this process occurs, two photons with energies of about 0.511 MeV are generated. Each 0.511 MeV photon will interact further by Compton scattering or Photoelectric effect.

2.9.2 Gamma-ray spectrometry

Radioactive nuclides are determined qualitative and quantitative by the interactions of their emitted radiation with matters, described above. Gamma rays are detected by the ionization interactions of secondary charge particles. Gamma- ray spectrometry is the detection system that measures gamma ray energy and intensity. The instrumentation used to measure gamma rays from radioactive samples generally consists of a detector, associated electronics and a computer-based as shown in Figure 4.

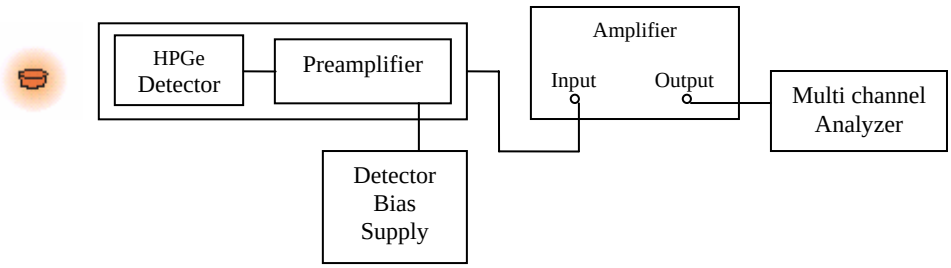

Figure 4 . The schematic of gamma ray spectrometry.

(a) Semiconductor detector

Semiconductor is defined as a material that is immediately between insulator and conductor, has been useful in activation analysis. Silicon and germanium detectors, cooled to temperatures slightly above that of liquid nitrogen (77 K), are used for precise measurements of x- ray and gamma- ray energies and intensities. Germanium detectors can be used for the detection of high- energy gamma radiation over the range of 3 keV to 10 MeV. The used of semiconductor detector is based on the production of electron-hole pairs, which are free- charge carrier and the collection of the liberated charge by application of an electric field.

The total number of pairs produced within the germanium is E/ε, where E is the total energy dissipated, and ε is the average energy required for the production of an electron-hole pair. Typical values of ε for germanium range from 2.94 to 2.98 eV. The charge collected at the electrodes is proportional to the energy lost by the incident radiation.

(b) Preamplifier

Preamplifier generally comes as an integral part of the detector assembly. In order to provide one or more of the following functions: i) minimize the electronic noise and amplify the detector output pulse; ii) preliminary pulse shaping; iii) matching of the detector output impedance with that of the output signal cable to the main amplifier; iv) charge- to –voltage conversion of the detector output pulse.

(c) Amplifiers

The important characteristic of amplifier is the linearity; complete the amplification of the detector signals from the preamplifier to amplitudes appropriate for subsequent pulse analysis. The other functions are shaping the output pulse, the gain stability and the noise level.

(e) Multichannel pulse height analyzer

Multichannel pulse height analyzer (MCA) is an appropriate memory device and supporting data presentation equipment, which can collect the entire spectrum at once. MCA is generally used to perform analysis of complex mixtures of gamma ray emitting radionuclides. It is operated in conjunction with analog- to- digital converter (ACD), memory and a method of display (either an oscilloscope or a computer monitor). The output of the ACD is stored in a computer- type memory. It has many addressable locations as the maximum number of channel, which the recorded spectrum can be subdivided.

2.10 Neutron activation analysis

Neutron activation analysis (NAA) (46) is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analyses. NAA is a method of high efficiency for the precise determination of a number of main-components and trace elements in different types of samples. It is a useful method for the simultaneous determination of about 25-30 major, minor and trace elements of geological, environmental and biological samples in parts per million or better (ppb-ppm) range without or with chemical separation.

In NAA, neutrons are used to activate the nuclides in the sample. As a result of the irradiation with neutrons, a mixture of radionuclides is included in the sample. These radionuclides disintegrate and mostly emit photons or gamma ray, which are

measured by a Germanium semiconductor detector, resulting in a gamma ray spectrum. The type of radionuclide can be identified from the gamma ray energies and its concentration from the associated peak area or count rate. The value of detection limit (μg) for each element that can be detected by NAA technique is shown in Figure 5.

The form of neutron activation analysis applied mostly is nondestructive or Instrumental Neutron Activation Analysis (INAA), in which the sample does not undergo any chemical processing prior to analysis. If chemical separations are used to samples either before or after irradiation to remove interference or to concentrate the radioisotope of interest, the technique is called radiochemical neutron activation analysis (RNAA).

Furthermore, INAA technique can be categorized according to the time of measurement. Prompt gamma ray NAA (PGNAA), where gamma rays are measured during neutron irradiation and Delayed gamma ray NAA (DGNAA), when the measurement of radioactive decay after the end of the irradiation. The process of neutron capture or (n, gamma) reaction is illustrated in Figure 6. Only DGNAA technique was chosen in this research, due to limitation of facility to measure gamma ray while irradiation.

Delayed gamma ray NAA (DGNAA), sometimes called conventional NAA, is useful for the vast majority of elements that produce radioactive nuclides. The technique is flexible with respect to time, such that the sensitivity for a long-lived radionuclide, which is interfered by some shorter-lived radionuclide, can be improved by waiting for the short-lived radionuclide to decay. This selectivity is a key advantage of DGNAA over other analytical methods.

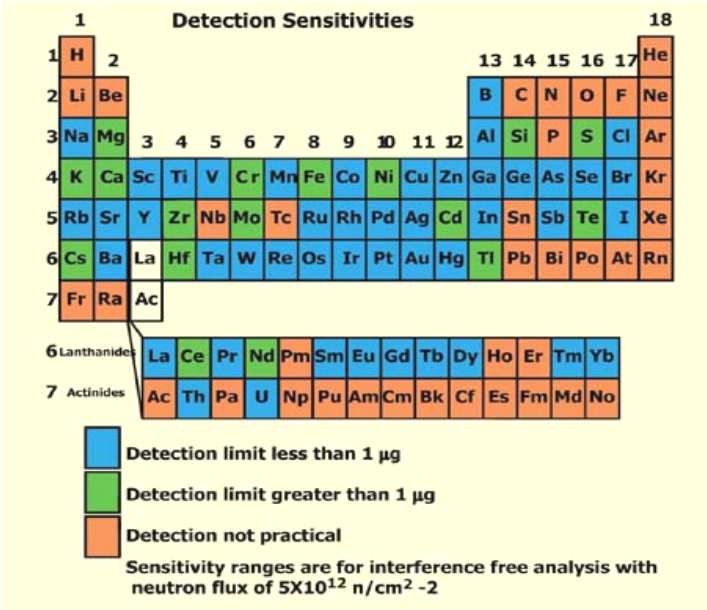

Figure 5. Periodic table showing elements that can be detected with NAA technique and value of detection limit (µg) for each element (47).

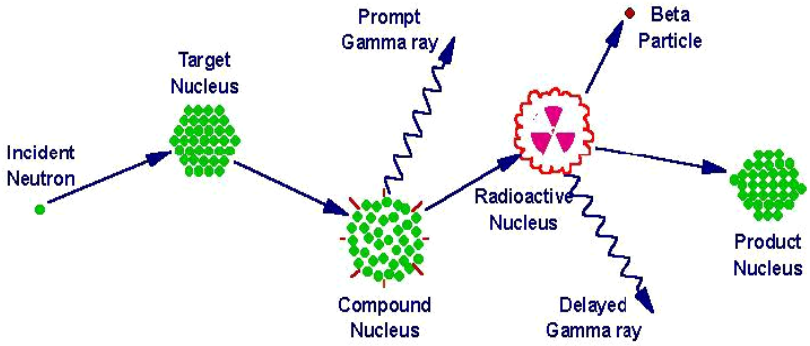

Figure 6. The process of neutron capture or (n,γ) reaction followed by the emission of gamma rays.

2.11 Qualitative analysis

Qualitative means identification of element compositions, which present in the sample at detectable level from the nuclear decay data of radionuclides. The qualitative analysis can be readily accomplished by measuring the gamma ray energies and maybe half-lives, and compare those values with the standard decay data library.

2.12 Quantitative analysis

The quantitative analysis determines the quantity of each element in the sample. It can be accomplished by using the absolute method or the comparative method.

(a) Absolute method

From equation 8, the amount of the element present in the sample can be calculated from

$$w = \frac{R(t_d)M}{f(E)\,\varepsilon(E)\,\theta\sigma\phi(1-e^{-\lambda t_i})e^{-\lambda t_d}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

where the values of $R(t_d)$, $\varepsilon(E)$, t_i and t_d are obtained from the experiment and the rest can be taken from nuclear data library. However due to the uncertainty of nuclear data value, the accuracy of the result is quite low.

(b) Comparative method

In this method, a standard which known composition of the sought elements and the unknown sample are irradiated simultaneously for the same time and neutron flux. The sample and standard will be measured with identical conditions at different decay time. From equation 8, the count rate of sought element in sample and standard are:

$$w_{sam} = \frac{R_{sam}(t_{d1})M}{f(E)\,\varepsilon(E)\,\theta\sigma\phi(1-e^{-\lambda t_i})e^{-\lambda t_{d1}}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (14)$$

$$w_{std} = \frac{R_{std}(t_{d2})M}{f(E)\,\varepsilon(E)\,\theta\sigma\phi(1-e^{-\lambda t_i})e^{-\lambda t_{d2}}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (15)$$

Where subscript sam and std indicate the value that obtain from sample and standard respectively. From equation 14 and 15, the weight of sought element in the sample can be determined from the following equation.

$$w_{sam} = \frac{R_{sam}(t_{d1})e^{\lambda t_{d1}}}{R_{std}(t_{d2})e^{\lambda t_{d2}}} \cdot w_{std} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (16)$$

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and equipments

3.1.1 Non- ionic detergent

3.1.2 Deionized water

3.1.3 Polyethylene sheet

3.1.4 Whatman No.41 filter paper

3.1.5 Reference material from National Institute of Standard and Technology (NIST)

- Standard reference material 1547: Peach leaves
- Standard reference material 2709: San Joaquin Soil
- Reference material 8435: Whole milk powder

3.1.6 Atomic absorption standard Zinc solution 1001 ug/ml from SCP Science

3.1.7 ICP spectroscopy standard Hg solution 1000 ug/ml from Fisher Scientific

3.1.8 Potassium iodide (KI) AnalaR from BDH Limited Poole England

3.1.9 Heating lamp and heat sealer

3.1.10 Balance

3.1.11 Other accessories such as beakers, tissue paper, forceps, spoon, scissors, micropipette, magic ink pen and tray

3.2 Sampling areas

Hair samples were collected from female students living in two different areas in Bangkok, Thailand. Areas selection was based on the data from Pollution Control Department, Ministry of Natural Resources and Environment published in 2003. The group A area, Talingchan district is in the suburb with relatively low air pollution and far from the main road or any large visible pollution source. On the contrary, the group B area is in Bangkoknoi-Bangplad district which locates near main roads and at risk to vehicular pollutants.

3.3 Sampling procedure

Fifteen hair samples of female students from each area were collected by the following inclusion and exclusion criteria.

- Age range from 13 to 18 years old.
- Have been lived in sampling area more than six months.
- Apparently healthy.
- Have never treated hair with dyed, colored, bleached, permed or otherwise chemically treated within six months, normal scalp; without scratch or lesion.

The questionnaire was used to collect individual data that will be relevant to the interpretation of the result. Approximately 500 mg of hair was taken, approximately 1 cm from the scalp, from different part around the head with stainless steel scissors. The samples were kept in the polyethylene bag. Before analysis, the samples were washed for 5 minutes in non- ionic detergent, rinsed 2 times with deionized water and air-dried in a dust free area for 24 hours. Next, weighted 200 mg of sample and put into polyethylene bag (2x2 cm^2) for analysis.

3.4 Preparation of standards

Because standard reference material for trace element analysis of human hair is not available, other standard reference materials and standard solutions were used in the validation of analytical procedures.

b) Standard reference material from National Institute of Standard and Technology, USA; SRM 1547: Peach leaves, SRM 2709: San Joaquin Soil and RM 8435: Whole milk powder.

The selecting of standard material was considered from the component and amount of elements in the selected reference materials. The SRM 1547 (Peach leaves) is the appropriate standard material. About 100 mg of standard was weighted and put into polyethylene bag (2x2 cm^2). The certificate of SRM 1547 for certified values and non–certified values are tabulated in Table 5 and Table 6 respectively.

This standard not only uses for the determination of concentration but also use together with SRM 2709 and RM 8435 as quality control materials.

c) Single elemental in-house standard for zinc, mercury and iodine were use as standard due to the limitation of these element analyses from NIST reference materials. The stock solution of 50 ppm zinc and 25 ppm mercury were prepared from standard solution of zinc and mercury, respectively. For iodine, the stock solution of 4 ppm iodine was prepared by dissolved KI and diluted with distilled water. The stock standard solutions were pipetted onto a sheet (2x2 cm^2) of Whatman No.41 filter paper, dried by heating lamp and placed immediately into clean polyethylene bags. The preparation condition is shown in Table 7.

Table 5. Certified values of Standard reference material 1547 (Constituent Elements in Peach Leaves).

Element	Wt %
Calcium	1.56 ± 0.02
Magnesium	0.432 ± 0.008
Nitrogen (total)	2.94 ± 0.12
Phosphorus	0.137 ± 0.007
Potassium	2.43 ± 0.03

Element	μg/g
Aluminium	249 ± 8
Arsenic	0.060 ± 0.018
Barium	124 ± 4
Boron	29 ± 2
Cadmium	0.026 ± 0.003
Chlorine	360 ± 19
Copper	3.7 ± 0.4
Iron	218 ±14
Lead	0.87 ±0.03
Manganese	98 ± 3

Element	μg/g
Mercury	0.031 ± 0.007
Molybdenum	0.060 ± 0.008
Nickel	0.69 ± 0.09
Rubidium	19.7 ± 1.2
Selenium	0.120 ± 0.009
Sodium	24 ± 2
Strontium	53 ± 4
Vanadium	0.37 ± 0.03
Zinc	17.9 ± 0.4

Table 6. Noncertified values of Standard reference material 1547 (Constituent Elements in Peach Leaves).

Element	Wt %
Nitrogen (Kjeldahl)	2.96
Sulfur	0.2

Element	μg/g
Antimony	0.02
Bromine	11
Cerium	10
Chromium	1
Cobalt	0.07
Europium	0.17
Gadolinium	1
Iodine	0.3
Lanthanum	9

Element	μg/g
Neodymium	7
Samarium	1
Scandium	0.04
Terbium	0.1
Thorium	0.05
Tin	< 0.2
Uranium	0.015
Ytterbium	0.2

Table 7. Preparation of standard solutions.

Element	Stock solution concentration (ppm)	Pipette volume (ml)	Weight of element (μg)
Zn	50	0.25	12.5
Hg	25	0.25	6.25
I	4	0.25	1.0

3.5 Neutron source

Thai Research Reactor - 1 / Modification 1 (TRR-1/M1) (48) at the Office of Atoms for Peace (OAP), Thailand was used for the neutron irradiation. It is an open pool type, TRIGA Mark III Reactor with maximum power of 2 MW. The reactor is operated 12 hours per day from Tuesday to Friday at 1.2 MW. The diagram of core configuration including irradiation facilities is shown in Figure 7. In this study, the pneumatic system and Lazy Susan rotating rack (LS) are used for the determination of short-lived radioisotopes and long-lived radioisotopes respectively. The neutron flux in these irradiation facilities is shown in Table 8.

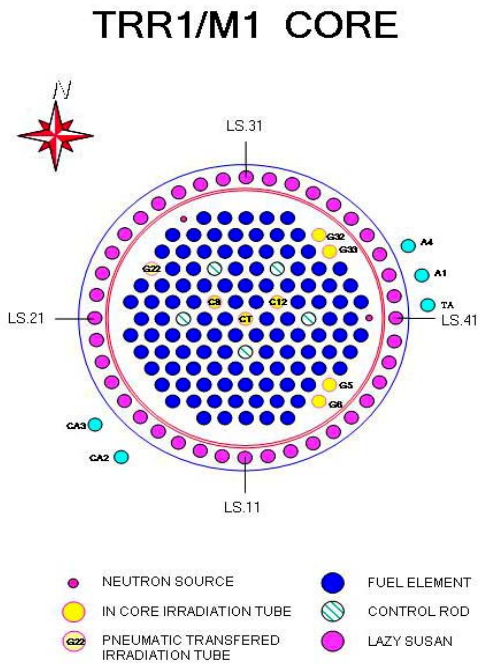

Figure 7. The core of Thai research reactor.

Table 8. Neutron flux in irradiation facilities.

Irradiation facility	Neutron flux ($n/cm^2.sec$)
Pneumatic system	2×10^{12}
Lazy Susan (LS 1 – LS 40)	$2.8 \times 10^{11} – 4.1 \times 10^{11}$

3.6 Counting system

Two high resolution gamma ray spectrometers are used in this study.

3.6.1 Spectrometer I locates in the third floor of reactor building, was used for the analysis of short-lived radioisotopes. It consisted of: Hyper Pure Germanium detector or HPGe model GCW 2018 with a resolution of 2 keV at 1332.5 keV of Co-60, Biased supply model 3102D, Spectroscopy amplifier model 2024D, S-100 Multichannel analyzer card and computer and GENIE 2000: Basic spectroscopy software for controlling multichannel

analyzer function and spectrum analysis. All equipments are from Canberra Industry, USA.

3.6.2 Spectrometer II locates in Physics Project laboratory, was used for the analysis of long-lived radioisotopes. It consisted of: HPGe detector model GCW 1522 with a resolution of 2 keV at 1332.5 keV of Co-60, Biased supply model 3106D, Spectroscopy amplifier model 672, Accuspec A: Acquisition interface board with computer and GENIE 2000: Basic spectroscopy software. All equipments are from Canberra Industry, USA except spectroscopy amplifier which manufacturer by EG & ORTEC, USA.

3.7 Qualitative Analysis

3.7.1 Short -lived radioisotopes

A specimen was put into a polyethylene rabbit, irradiated in pneumatic system and measured with Spectrometer I. The experiments were performed with selected samples and standards (SRM 1547: Peach leaves and I standard solution) one at a time by varying irradiation time, decay time and counting time as shown in Table 9. The results were evaluated to determine the optimum condition for quantitative analysis.

3.7.2 Long -lived radioisotopes

The samples and standards (SRM 1547: Peach leaves and Hg-Zn standard solution) were packed together and wrapped with aluminium foil, put into aluminium container and irradiated in Lazy Susan. After decaying for 3 days, the specimens were measured with Spectrometer II. The experiment was performed by varying irradiation time, decay time, counting time and counting geometry to optimize the experimental condition for quantitative analysis.

Table 9. The experimental conditions for qualitative activation analysis.

Facility	Irradiated time	Decay time	Counting time	distance
Pneumatic	15, 20, 25 sec	3, 6, 9 min	3 min	fixed at 5 cm.
		5, 10, 15 min	5 min	
Lazy Susan	12, 24 hours	3, 5, 10, 14, 21 days	10, 15, 30 min	0, 1,2,5 cm.

3.8 Quantitative analysis

For short-lived radioisotope, the samples and standards were performed by using the experiment conditions from 3.7.1 and the spectrum data of each specimen were kept for further analysis.

For long-lived radioisotope, the specimens were left for two weeks after the completion of short lived radioisotope analysis. The experiments were performed by using the conditions from 3.7.2.

Those spectrum data were analyzed for peak area through the Genie 2000 software which is available from Canberra (49), and the concentration of elements were calculated with equation 16.

3.9 Accuracy and reproducibility test

The accuracy of the experiments was checked by analyzing the concentration of some elements in SRM 2709 and RM 8435, by comparing with SRM 1547.

The reproducibility of repeated trial or the precision under the same condition was determined in SRM 1547 and standard solutions of zinc-mercury and iodine. The limit of acceptability for the reproducibility test is within ±10%.

3.10 Statistical analysis

Ordinary statistical methods were used to calculate means and standard deviations (SD). Comparison of concentrations for any given pair of element was made by Student’s t- test. Levene's test (F-test) is used to test for equality of variances. P- values < 0.05 was considered significant.

CHAPTER IV

RESULTS

4.1 Qualitative analysis of hair samples by Instrument Neutron Activation Analysis

Ten elements were found from hair samples; Magnesium, Chlorine, Aluminium, Calcium, Iodine, Manganese, Sodium, Bromine, Mercury and Zinc. The nuclear properties of these radioisotopes are shown in Table 10.

The optimum experimental conditions for quantitative analysis could be considered from varying irradiation time, decay time and counting time through the qualitative analysis. Varying of decay time can identify the elemental components in sample by checking on half life of radioisotope. Because at initial decay time, high activity of radioisotope results to high percentage of the dead time and the reliable of counting statistics will be lose. Furthermore, long irradiation time is practical to determine the radioisotope which has long half –life, in order to obtain high activity and reduce on the decay time and/or counting time.

For short lived radioisotopes, the optimum condition for the experiment is 20 seconds irradiation time, 6 minutes decay time and 5 minutes counting time. The peaks found in the spectrum had which are suitable for quantitative analysis.

For long lived radioisotopes, the optimum decay period before counting will depend on the background activity and interference. Irradiation time at 24 hours, the spectrum showed the peaks which are suitable for quantitative analysis. At first period of decay time (3 days), high intensity peak of Na and Br interfered to peak of Zn and Hg therefore the specimen were left for 3 week before counting of Zn and Hg. The results of qualitative analysis and optimum conditions are shown in Table 11.

Blank samples (plastic bag and plastic bag + filter paper) were irradiated at the same time of samples and standards. The comparison of peak area between blanks and specimens spectrum illustrated less than 5% of specimen peak areas in blanks (Table 12). There were not found peak of I, Hg and Zn in blank of plastic+ filter paper.

Table10. Nuclear Properties of radioisotopes for qualitative activation analysis.

Product Isotope	Cross section (barn)	Product Half-life	Photon energy (keV)
Mg-27	0.037	9.46 min	1014.43
Al-28	0.226	2.24 min	1778.99
Na-24	0.513	14.96 hour	1368.60
Cl-38	0.423	37.24 min	1642.69
Ca-49	1.120	8.72 min	3084.54
Mn-56	13.20	2.58 hour	846.76
Zn-65	0.726	243.9 day	1115.55
Br-82	2.580	35.3 hour	776.52
I-128	4.040	24.99 min	442.90
Hg-203	4.350	46.61 day	279.20

Table 11. The optimum experimental conditions for quantitative activation analysis.

Irradiation facility	Irradiated time	Decay time	Counting time	Target Isotope
Pneumatic	20 sec	6 min	5 min	Mg-27, Cl-38, Al-28, Ca-49, I-128, Mn-56
Lazy Susan	24 hours	3 days	10 min	Na-24, Br-82
		3 weeks	10 min	Hg-203, Zn-65

Table 12. The analytical result of blank samples.

Isotope	Energy (keV)	Peak Area	
		Blank1 (plastic)	Blank2 (plastic+ filter paper)
short lived radioisotope			
Mg-27	1014.43	8.39	10.05
Cl-38	1642.69	14.13	20.40
Al-28	1778.99	47.79	85.56
long lived radioisotope			
Na-24	1368.60	81.75	90.56

4.2 Quantitative analysis of hair samples by Instrument Neutron Activation Analysis

The gamma ray spectrum of samples and standards were analyzed by Genie 2000 for the peak area. The quantitative determination was determined by comparative method. The concentration of each element was calculated from equation 16 by using peak area of the selected gamma ray energy of each radioisotope as shown in Table 10. The spectrum of samples and standards at appropriate experimental conditions for short and long lived radioisotope are shown in the appendix (Figure A1 through A7).

Considering from concentration level of 10 elements, one of them (Ca) is major element, three (Mg, Na, Cl) are minor elements and six (Al, I, Br, Mn, Hg and Zn) are trace elements. The concentration of the analyzed elements in hair samples are presented in Table 13a and Table 13b, and the analytical results for 10 elements are summarized in Table 14a and Table 14b for group A and B respectively. Concentration range, mean, standard deviation (SD) and median are reported in ppm for each element.

4.3 Accuracy and reproducibility

The SRM 2709 and RM 8435 were analyzed and compared with SRM 1547 for quality assurance. The percent accuracy of 4 selected elements ranged from 5.69% to 33.01%, Mn and Br were presented to be low at 5.69% and 13.96% while Mg and Zn were at 26.65% and 33.01% respectively (Table 15).

The values of gamma peak area divided by material weight and coefficient of variation were calculated for precision and presented in Table 16. After the repetitive analysis of SRM 1547 and standard solutions (Zn-Hg and I standard solutions), the percent coefficient of variation ranged from the lowest 1.24% to the highest 8.8%. The %CV of 7 elements (Mg, Cl, Al, Ca, Mn, Na and Zn) were found to be lower than 6% and 3 elements were above 6% but still within an acceptable limit of 10%.

4.4 Comparison of trace element concentrations in hair between group A and B

Difference in the mean concentration of each element between groups was determined by Student's *t*-test and equality of the variance by Levene's test. Only iodine showed inequality of the variance ($p=0.01$). Group A demonstrated significantly higher concentrations of Al and Hg than the group B ($p<0.05$). The mean concentrations of Cl and I were higher in the group B but statistically not significant. The other elemental concentrations were found to be within the same magnitude (Table 17).

Table 13a. The elemental concentrations of hair samples (mean and standard deviation) in ppm for group A.

Hair	Mg	Ca	Cl	Al	I	Mn	Na	Br	Hg	Zn
A1	306 ± 31	1209 ± 22	118 ± 22	38.0 ± 3.0	0.8 ± 0.3	0.90 ± 0.16	38 ± 2	7.7 ± 0.2	39.3 ± 3.1	154.2 ± 25.0
A2	380 ± 20	2191 ± 48	151 ± 19	40.7 ± 1.6	0.8 ± 0.3	0.62 ± 0.08	141 ± 4	26.9 ± 3.4	157.0 ±12.8	399.9 ± 57.0
A3	295 ± 0	1261 ± 115	161 ± 15	31.4 ± 0.1	0.9 ± 0.2	0.26 ± 0.02	46 ± 0	5.2 ± 0.5	66.1 ± 5.6	182.3 ± 32.9
A4	262 ± 71	1057 ± 98	275 ± 33	30.7 ± 0.5	0.6 ± 0.3	0.27 ± 0.02	50 ± 6	3.4 ± 0.4	57.0 ±17.2	374.2 ± 70.0
A5	314 ± 33	3433 ± 29	97 ± 10	35.3 ± 1.8	2.9 ± 0.1	0.79 ± 0.19	133 ± 1	73.1 ± 3.9	79.4 ± 9.9	178.3 ± 9.6
A6	253 ± 31	1758 ± 8	286 ± 35	42.9 ± 1.1	1.0 ± 0.4	2.43 ± 0.06	86 ± 8	19.9 ± 0.1	104.9 ±36.0	379.5 ± 40.5
A7	279 ± 26	833 ± 37	718 ± 86	33.5 ± 0.8	1.1 ± 0.3	0.71 ± 0.05	80 ± 25	4.7 ± 0.1	48.3 ±10.5	180.9 ± 15.0
A8	243 ± 35	1394 ± 17	209 ± 12	29.1 ± 0.8	1.7 ± 0.3	0.35 ± 0.00	60 ± 3	4.6 ± 0.1	67.3 ± 2.8	173.7 ± 27.2
A9	266 ± 46	1345 ± 86	82 ± 7	27.4 ± 2.6	0.8 ± 0.2	0.24 ± 0.00	59 ± 1	5.8 ± 0.1	98.1 ±10.5	216.1 ± 44.8
A10	299 ± 31	1956 ± 142	85 ± 6	31.2 ± 3.7	1.1 ± 0.5	0.68 ± 0.08	191 ± 22	16.7 ± 0.4	48.0 ±11.0	186.9 ± 4.0
A11	300 ± 94	1980 ± 78	115 ± 4	33.2 ± 1.1	0.8 ± 0.5	0.79 ± 0.05	72 ± 21	6.7 ± 1.6	65.6 ±12.6	140.6 ± 11.5
A12	268 ± 91	1424 ± 170	163 ± 19	38.9 ± 1.1	2.2 ± 0.0	0.87 ± 0.03	55 ± 19	3.8 ± 0.1	72.5 ±10.0	210.6 ± 18.9
A13	288 ± 14	853 ± 35	323 ± 41	24.6 ± 1.3	3.1 ± 0.2	2.31 ± 0.09	74 ± 14	5.7 ± 0.1	39.1 ± 2.0	157.9 ± 21.0
A14	218 ± 95	996 ± 86	512 ± 4	27.1 ± 1.4	0.9 ± 0.3	0.63 ± 0.11	64 ± 4	5.1 ± 0.1	50.4 ± 5.7	550.0 ± 29.7
A15	266 ± 72	941 ± 158	144 ± 21	49.8 ± 1.2	2.2 ± 0.5	0.82 ± 0.11	40 ± 31	8.3 ± 3.7	85.0 ±21.8	161.6 ± 27.5

Table 13b. The elemental concentrations of hair samples (mean and standard deviation) in ppm for group B.

Hair	Mg	Ca	Cl	Al	I	Mn	Na	Br	Hg	Zn
B1	276 ± 48	2194 ± 134	132 ± 14	21.7 ± 1.2	3.7 ± 0.4	0.86 ± 0.07	114 ± 14	66.7 ± 4.5	33.6 ± 3.1	177.8 ± 12.1
B2	391 ± 82	2620 ± 303	265 ± 13	25.9 ± 6.2	3.6 ± 0.4	0.91 ± 0.01	200 ± 30	19.5 ± 2.7	44.7 ± 10.3	237.7 ± 30.0
B3	192 ± 65	1032 ± 147	188 ± 19	24.6 ± 3.0	0.7 ± 0.0	0.42 ± 0.00	11 ± 3	4.1 ± 0.5	60.9 ± 7.6	189.4 ± 33.5
B4	269 ± 0	2000 ± 111	149 ± 6	30.6 ± 0.1	5.0 ± 0.4	1.20 ± 0.04	19 ± 2	3.3 ± 0.3	44.2 ± 22.6	265.3 ± 36.4
B5	206 ± 11	1175 ± 150	273 ± 3	25.9 ± 1.3	0.8 ± 0.2	0.71 ± 0.05	15 ± 1	3.9 ± 0.5	41.1 ± 13.0	185.6 ± 26.9
B6	189 ± 22	825 ± 162	232 ± 13	22.5 ± 0.7	0.7 ± 0.0	0.74 ± 0.15	13 ± 1	3.5 ± 0.5	43.6 ± 2.7	200.3 ± 54.0
B7	331 ± 79	1099 ± 76	134 ± 29	26.5 ± 4.7	1.3 ± 0.4	1.06 ± 0.06	24 ± 6	2.0 ± 0.3	84.9 ± 35.5	190.7 ± 24.3
B8	235 ± 90	581 ± 25	885 ± 5	33.4 ±12.3	6.7 ± 0.0	0.60 ± 0.10	46 ± 9	13.7 ± 2.2	43.0 ± 1.1	449.8 ± 7.9
B9	214 ± 54	630 ± 93	519 ± 54	22.3 ± 1.4	2.4 ± 0.3	0.42 ± 0.06	12 ± 2	5.4 ± 0.5	66.6 ± 4.3	211.5 ± 14.7
B10	210 ± 40	582 ± 68	431 ± 38	26.8 ± 1.4	1.5 ± 0.3	0.29 ± 0.04	56 ± 8	9.6 ± 0.7	25.1 ± 2.6	136.5 ± 10.1
B11	231 ± 38	1039 ± 81	234 ± 22	19.2 ± 1.0	0.8 ± 0.2	0.25 ± 0.03	72 ± 10	5.7 ± 0.5	31.7 ± 2.4	133.3 ± 9.6
B12	264 ± 142	715 ± 30	502 ± 4	25.1 ± 6.1	0.8 ± 0.3	0.52 ± 0.04	63 ± 11	5.4 ± 0.5	40.0 ± 5.4	186.4 ± 15.3
B13	226 ± 0	802 ± 79	569 ± 40	20.3 ± 1.6	0.8 ± 0.5	1.18 ± 0.08	109 ± 3	4.4 ± 0.1	34.9 ± 5.1	427.9 ± 77.7
B14	260 ± 7	1849 ± 458	205 ± 32	30.3 ± 7.1	1.0 ± 0.2	0.25 ± 0.02	77 ± 26	4.5 ± 1.2	32.7 ± 7.0	164.8 ± 28.7
B15	260 ± 46	857 ± 85	201 ± 23	22.5 ± 1.3	1.1 ± 0.2	0.31 ± 0.05	54 ± 7	2.7 ± 0.3	46.4 ± 3.2	130.9 ± 9.5

Table 14a. Analytical results of elements in hair of group A in ppm.

Element	Range	Mean ± SD	Median
Mg	218.3 – 379.5	282.3 ± 37.3	280.0
Ca	832.9 – 3432.5	1508.7± 682.7	1345.1
Cl	82 - 718	229 ± 177	161
Al	24.6 - 49.8	34.3 ± 6.8	33.2
I	0.6 - 3.1	1.4 ± 0.8	1.0
Mn	0.24 - 2.43	0.85 ± 0.65	0.71
Na	38 - 191	79 ± 43	63.6
Br	3.4 - 73.1	13.2 ± 17.9	5.8
Hg	39.1 - 157.0	71.9 ± 30.8	66.1
Zn	140.6 - 550.0	243.1 ± 122.0	182.3

Table 14b. Analytical results of elements in hair of group B in ppm.

Element	Range	Mean ± SD	Median
Mg	189.4 – 391.2	250.3 ± 54.1	235.4
Ca	581.0- 2620.1	1200± 647.7	1032.1
Cl	132 - 885	328 ± 212	234
Al	19.2 - 33.4	25.2 ± 3.99	25.1
I	0.7 - 6.7	2.1 ± 1.9	1.1
Mn	0.25 - 1.20	0.65 ± 0.33	0.60
Na	11 - 200	59 ± 51.8	53.8
Br	2.0 - 66.7	10.3 ± 16.3	4.53
Hg	25.1 - 84.9	44.9 ± 15.4	43.0
Zn	130.9 - 449.8	219.2 ± 96.4	189.4

Table 15. Results of accuracy test.

Reference material	Element	Mean ± SD		% accuracy
		Certified values	Results	
SRM 2709	Mg	1.51 ± 0.05 %	1.108 ± 0.061 %	26.65
	Mn	538 ± 17ppm	507.4 ± 21.3 ppm	5.69
RM 8435	Br	20 ± 10 ppm	22.792 ± 3.99 ppm	13.96
	Zn	28.0 ± 3.1 ppm	18.753 ± 3.84 ppm	33.01

Table 16. Results of reproducibility test.

Isotope	W(μg)	Area	Area/W	Mean ± SD (Area/W)	%CV
Mg-27	464.83	1349.22	2.903	3.14 ± 0.18	5.71
	460.08	1471.72	3.199		
	459.99	1532.75	3.332		
	462.97	1454.73	3.142		
Cl-38	38.74	662.42	17.10	17.69 ± 0.43	2.46
	38.34	695.63	18.14		
	38.33	682.40	17.80		
	38.58	683.64	17.72		
Al-28	26.79	5752.35	214.70	211.20 ± 3.11	1.47
	26.52	5644.06	212.83		
	26.51	5512.87	207.93		
	26.69	5586.61	209.35		
Ca-49	1678.56	1731.05	1.03	1.02 ± 0.02	2.39
	1661.40	1736.25	1.05		
	1661.09	1664.44	1.00		
	1671.85	1660.50	0.99		
Mn-56	10.54	19045.20	1806.12	1810.90 ± 22.45	1.24
	10.44	18857.93	1806.83		
	10.44	19222.40	1842.10		
	10.50	18784.56	1788.55		
I-128	1.00	967.64	967.64	881.20 ± 77.44	8.79
	1.00	921.64	921.64		
	1.00	797.32	797.32		
	1.00	838.21	838.21		
Na-24	2.54	714.36	281.04	260.13 ± 14.93	5.74
	2.56	665.77	260.47		
	2.56	633.52	247.90		
	2.57	645.82	251.09		
Br-82	1.17	752.88	646.24	603.41 ± 53.09	8.80
	1.17	753.34	643.06		
	1.17	692.08	590.87		
	1.18	628.90	533.48		
Hg-203	6.25	2078.15	332.50	312.41 ± 24.89	7.97
	6.25	1982.13	317.14		
	6.25	1726.83	276.29		
	6.25	2023.26	323.72		
Zn-65	12.50	772.87	61.830	60.11 ± 3.12	5.19
	12.50	791.33	63.306		
	12.50	737.75	59.020		
	12.50	703.32	56.266		

Table 17. Statistical comparison of trace element in hair samples between group A and B.

Element	Mean ± SD (ppm)		Level of significance (p)	
	Group A	Group B	F-test	t-test
Mg	282.3 ± 37.3	250.3 ± 54.1	NS	NS
Ca	1508.7± 682.7	1200± 647.7	NS	NS
Cl	229 ± 177	328 ± 212	NS	NS
Al	34.3 ± 6.8	25.2 ± 3.99	NS	<0.05
I	1.4 ± 0.8	2.1 ± 1.9	<0.05	NS
Mn	0.85 ± 0.65	0.65 ± 0.33	NS	NS
Na	79 ± 43	59 ± 51.8	NS	NS
Br	13.2 ± 17.9	10.3 ± 16.3	NS	NS
Hg	71.9 ± 30.8	44.9 ± 15.4	NS	<0.05
Zn	243.1 ± 122.0	219.2 ± 96.4	NS	NS

NS = Not significant difference

CHAPTER V

DISCUSSION

The elemental composition analysis of 30 female student's hair samples from Talingchan district (group A) and Bangkoknoi-Bangplad district (group B) were performed by instrumental neutron activation analysis technique. Ten elements were found as shown in Table 11. Six elements Mg, Cl, Al, Ca, I and Mn were determined from short-lived radioisotope and the rest, Na, Br, Hg and Zn were determined from long- lived radioisotope.

The elements in hair sample can be classified by concentration levels into 3 groups, one major element (Ca), 3 minor elements (Mg, Na and Cl) and the other with 6 trace elements (Al, I, Br, Mn, Hg and Zn).

Table 18 shows the ranges of hair levels of the two studied for Ca, Cl, I, Mn, Na, Br and Zn of two groups which agreed with some published results of healthy people in Thailand (23) and in some other countries (England, New Zealand) except for Mg Al and Hg which are higher (25,50,51).

The mean values of Mg, Ca, Al, Mn, Na, Br, Hg and Zn concentration in group A are higher than group B. Most of the median values of both groups show similar results to the mean values except for Zn, however no significant difference is observed. The mean concentrations of the elements Ca, Cl, I, Mn, Br and Zn in the hair of subjects from 2 different areas presented in this study agree with some published results of healthy people from several countries (23,25,50,51) except for Mg, Al and Hg which are higher whereas Na is lower (Table 19).

When compared with the Malaysian and Korean residents, the concentration levels of Mg, Al and Hg are much higher (41,42). In the hair of Thai students, the average Mg and Al levels of the two studied groups is 1.8 to 2 times higher than the average levels of the Korean residents in the rural and the metropolitan (226 vs 112 ppm for Mg and 30 vs 16 ppm for Al) (Table 20). The mean level of Hg is dramatically higher, 8.8 times when compared with the Malaysian residents in the

metropolitan area and more than 70 times when compared with the rural residents. In the Malaysian residents, the high Hg level in the metropolitan area residents can be explained by the high degree of air-polluted environment (6.62 vs 0.82 ppm), but in our study we could not find any other environmental factors to clarified these findings.

For Br, Cl and I, the relative errors of measurements are quite high corresponding to the study of Cho et al. (41) and Tavakkoli et al. (52), a high variable for these elements analysis is shown in Table 21.

Table 18. Comparison of ranges of the hair element contents between the present study and some other published results of the normal (in ppm).

Element	This study		Thailand (n=44) (2002)	England (1987)	New Zealand (1984)	Normal range IAEA comp.
	Group A	Group B				
Mg	218.3 -379.5	189.4 –391.2	-	30.37-81.65	73.45-149.31	48-115
Ca	832.9 -34325	581.0- 2620.1	821-3921	150-1620	250-1380	-
Cl	82 -718	132 -885	25.7- 365.7	110-920	250-1380	-
Al	24.6 -49.8	19.2 -33.4	23.7-100.2	1.79-9.43	6.157-10.833	3.7-10.3
I	0.6 -3.1	0.7 -6.7	0.3-10.8	0.176-4.975	1.110-3.474	-
Mn	0.24 -2.43	0.25 -1.20	0.0005-0.0162	0.208-3.976	0.573-1.679	0.14 -8.80
Na	38 -191	11 -200	24-707	55-445	30-80	-
Br	3.4 -73.1	2.0 -66.7	1.1-1.6	0.55-8.11	6.00-16.69	-
Hg	39.1-157.0	25.1-84.9	0.5-38.9	0.530-4.720	1.498-2.809	-
Zn	140.6 -550.0	130.9 -449.8	134-630	141.9-259.6	157.6-293.2	128-261

Table 19. Comparison of mean hair element contents between the present study and some other published results of the normal (in ppm).

Element	This study		Thailand (n=44) (2002)	England (1987)	New Zealand (1984)	Normal range IAEA comp.
	Group A	Group B				
Mg	282.3 ± 37.3	250.3 ± 54.1	-	42.47 ± 9.56	109.51 ± 23.7	48-115
Ca	1508.7 ± 682.7	1200 ± 647.7	1840 ± 362	380 ± 275	710 ± 280	-
Cl	229 ± 177	328 ± 212	96.6 ± 23.3	290 ± 145	680 ± 280	-
Al	34.3 ± 6.8	25.2 ± 3.99	59.4 ± 13.6	4.77 ± 1.828	7.491 ± 2.285	3.7-10.3
I	1.4 ± 0.8	2.1 ± 1.9	1.6 ± 0.6	1.453 ± 0.466	1.842 ± 0.705	-
Mn	0.85 ± 0.65	0.65 ± 0.33	0.004 ± 0.0005	1.474 ± 0.464	0.964 ± 23.72	0.14 -8.80
Na	79 ± 43	59 ± 51.8	186 ± 71	145 ± 80	110 ± 25	-
Br	13.2 ± 17.9	10.3 ± 16.3	3.0 ± 0.5	3.30 ± 1.29	10.85 ± 3.39	-
Hg	71.9 ± 30.8	44.9 ± 15.4	2.9 ± 1.2	1.752 ± 0.898	2.117 ± 0.368	-
Zn	243.1 ± 122.0	219.2 ± 96.4	331 ± 82	196.0 ± 35.9	202.3 ± 45.4	128-261

Table 20. Comparison of mean hair element contents to the Malaysian and Korean residents living in metropolitan and rural areas.

Element	This study		Malaysia		Korea	
	Group A	Group B	Rural	Metropolitan	Rural	Metropolitan
Mg	282.3 ± 37.3	250.3 ± 54.1	-	-	91.0 ± 42.0	132 ± 80.1
Ca	1508.7 ± 682.7	1200 ± 647.7	-	-	761 ± 567	1499 ± 1052
Cl	229 ± 177	328 ± 212	-	-	-	-
Al	34.3 ± 6.8	25.2 ± 3.99	-	-	16.6 ± 10.3	15.7 ± 6.12
I	1.4 ± 0.8	2.1 ± 1.9	-	-	0.37 ± 0.13	0.64 ± 0.45
Mn	0.85 ± 0.65	0.65 ± 0.33	-	-	0.97 ± 0.77	2.29 ± 1.78
Na	79 ± 43	59 ± 51.8	-	-	7.39 ± 2.56	8.27 ± 4.53
Br	13.2 ± 17.9	10.3 ± 16.3	1.29 ± 0.61	9.1 ± 7.0	7.43 ± 8.76	9.68 ± 5.24
Hg	71.9 ± 30.8	44.9 ± 15.4	0.82 ± 0.31	6.62 ± 3.73	-	-
Zn	243.1 ± 122.0	219.2 ± 96.4	136 ± 79	187 ± 83	315 ± 68	307 ± 84

Table 21. Percentage coefficient of variations in the measurement of hair elements (Cl, I and Br) (41,52).

Element	This study		Cho et el. (Korea)		Tavakkoli et al. (Iran)	
	Group A	%cv	Rural	%cv	-	%cv
Cl	229 ± 177	77.29	-	-	1231 ± 1081	87.81
I	1.4 ± 0.8	57.14	0.37 ± 0.13	35.14	0.57 ± 0.44	77.19
Br	13.2 ± 17.9	135.61	7.43 ± 8.76	117.9	3.27 ± 1.85	56.57

Different normal levels of some elements in hair compared to other countries may be affected from the criteria of sampling and wide variation of the elemental concentrations in normal populations living in various parts of the world. Furthermore, some elements levels like Al, Mg and Zn have been reported to be higher in the hair of younger persons (24).

The mean differences of hair element contents between two groups of subjects from two sampling areas were tested by Student's t-test at $p < 0.05$ level. No significant difference is observed in the contents of Mg, Ca, Na, Cl, I, Br, Mn, and Zn between 2 groups. Talingchan subjects (group A) demonstrated significantly high concentrations of Al and Hg when compared with the Bangkoknoi-Bangplad (group B) students ($p<0.05$). The mean concentrations of Al and Hg are 34.3 ± 6.8 and 71.9 ± 30.8 ppm in group A, and, 25.2 ± 3.99 and 44.9 ± 15.4 ppm in group B respectively.

High hair concentration for Al is probably related to the many other sources of pollution, e.g. contamination originated from washing process or aluminium in cookware (53,54). The concentrations of Hg in both groups in this study are markedly high when compared with the previously reported values for the population in Thailand (2.6 ppm), People's Republic of China (8 ppm) and Malaysia (8 ppm) (21).

High values of Al and Hg in the hair sample from low pollution area may possibly be linked to the use of hair care, hair shampoo, daily intake food, supplementary food, water and other sources of pollution which are not associated with the exposure to traffic pollution.

Although the concentrations of Br and Cl lie within the values published in the literatures (23,25,50,51). The higher mean concentrations in both studied groups may indicate the exposure to traffic pollution as Br and Cl are known to be associated with vehicular activity. They were found to relate to Pb on motor-vehicle aerosols. Automobile burning leaded gasoline contributes large amounts of Pb, Br and Cl to the atmosphere (55). However we could not demonstrate their relationship to the exposed to vehicular emissions as the used of leaded fuels in our country has been terminated for many years.

Inorganic bromide is naturally present in all foods at low levels. Residues found in rice at or below 20 mg/kg are considered to be naturally occurring. Residues above 20 mg/kg and below 50 mg/kg are considered to arise from the use of methyl bromide as soil fumigation (56). In the study of toxic elements in rice by INAA and RNAA (57), arsenic, cadmium, mercury, bromine, cobalt and zinc have been found in 23 varieties of rice collected from 21 different rice experiment stations throughout Thailand. It is possible that the high concentration of bromine in rice may be caused by the use of fungicide that contains high contents of methyl bromide. In 1982, Ohmori and Hirata (58) have demonstrated high bromide concentrations in the hair of individuals exposed to methyl bromide, as well as individuals ingesting bromide supplements.

The concentration of the elements detected in hair samples were determined by comparison with reference material obtained from the NIST (SRM 1547) and standard solutions (zinc-mercury and iodine solutions). Accuracy and precision of method were 33% and 8.8% which generally accepted for trace element analysis by INAA technique. However, the precision for most of the elements were better than 6% except for Br, I and Hg are very well accepted. The cause for high deviation in I and Hg concentrations (8.79% and 7.97%) may be from the use of in-house standard solution. However the high deviation in Br concentration (8.80 %) of standard material was also observed.

CHAPTER VI

CONCLUSION

Elemental composition of human hair from the study of the two groups of female students comparable in terms of age and levels of education by instrument neutron activation analysis were determined. Ten elements (Magnesium, Chlorine, Aluminium, Calcium, Iodine, Manganese, Sodium, Bromine, Mercury and Zinc) were found which six of them are trace elements (Al, I, Br, Mn, Hg and Zn). The concentrations of Ca, Cl, I, Mn, Na, Br and Zn in both groups are found to be within the average values published in the literatures.

The higher concentration values of Al and Hg found in group of lower traffic pollution may not be an indicator of increase in the levels of these two trace elements in the environment but could probably relate to the state of health and lifestyle. Their high concentrations of Al and Hg may attribute from sources other than air pollution. Changes in environment and dietary habits can reflect changes in elemental concentration of human tissues.

Ranges of elemental concentrations in human scalp hair are too wide so that small variations in concentration value cannot indicate.

It was found that the concentrations of some elements for the student who take supplementary food are quite high when comparing within the group. This problem has to take into consideration for further study. In addition, the sampling site and the number of samples must be increased to reduce inconsistencies in the results.

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APPENDIX

Spectrum of short- lived radioisotopes

Experimental condition

Irradiation time = 20 sec Decay time = 6 min

Counting time = 5 min Distance = 5 cm.

Measurement Gain = 30/0.8

Figure A1, A2 and A3: the spectrum of hair sample, standard (SRM: 1547) and iodine standard solution.

Spectrum of long- lived radioisotopes

Two experimental conditions

- for Na and Br analysis

Irradiation time = 24 hours Decay time = 3 days

Counting time = 10 min Distance = 5 cm.

Measurement Gain = 10/0.8

Figure A4 and A5: the spectrum of hair sample, standard (SRM: 1547)

- for Hg and Zn analysis

Irradiation time = 24 hours Decay time = 3 weeks

Counting time = 10 min Distance = contact

Measurement Gain = 20/0.6

Figure A6 and A7: the spectrum of hair sample and Hg-Zn standard solution.

Spectrum of short- lived radioisotopes

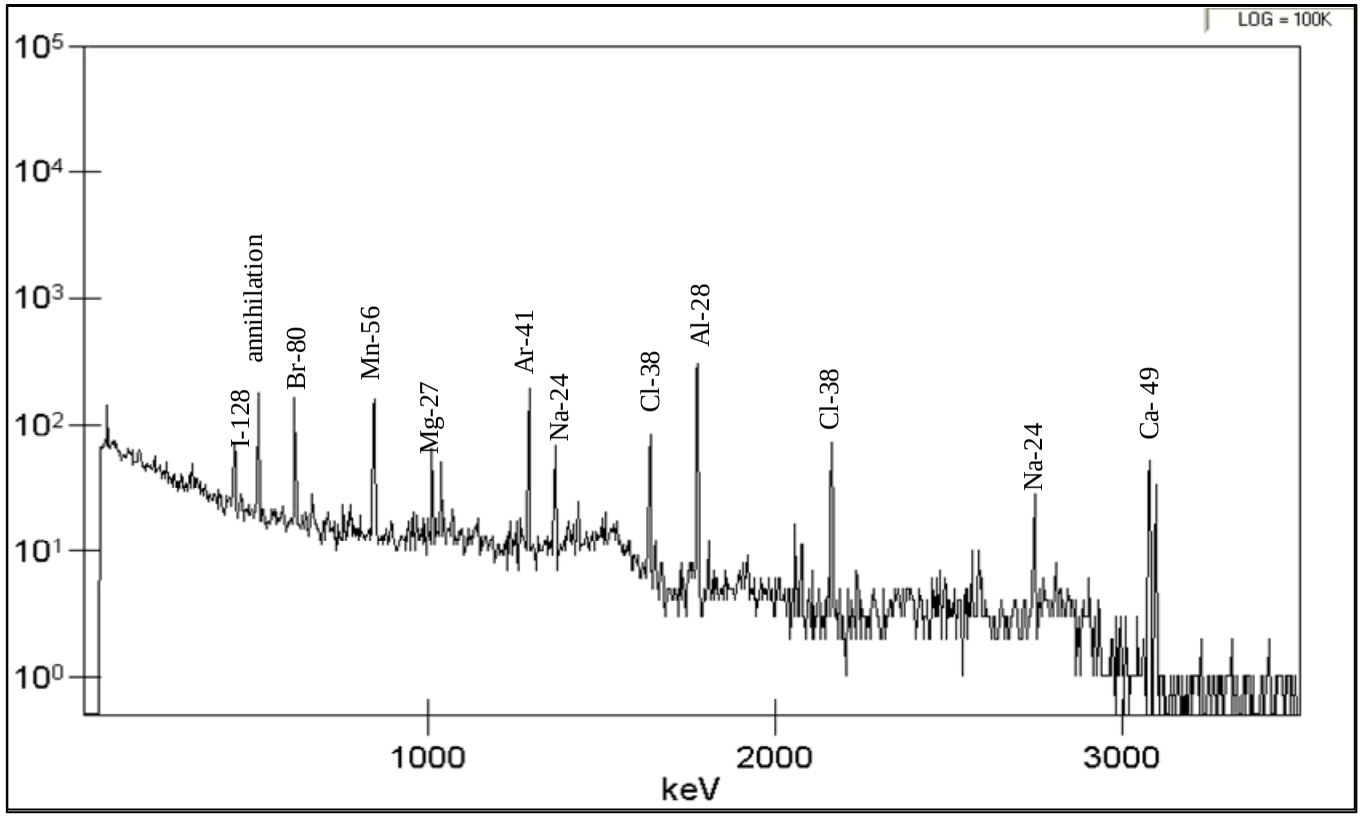

Figure A1. Spectrum of hair sample irradiated in pneumatic system.

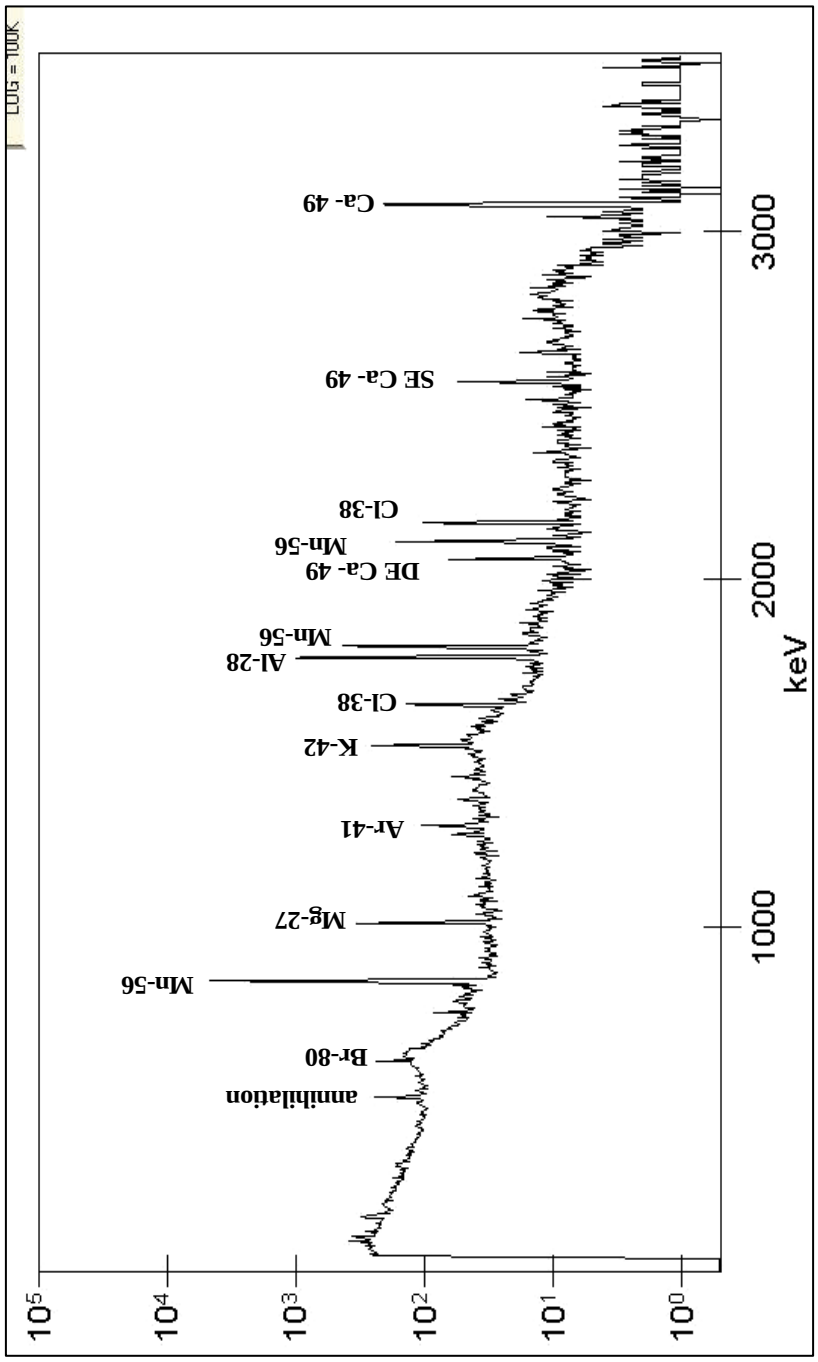

Figure A2. Spectrum of standard (SRM 1547) irradiated in pneumatic system.

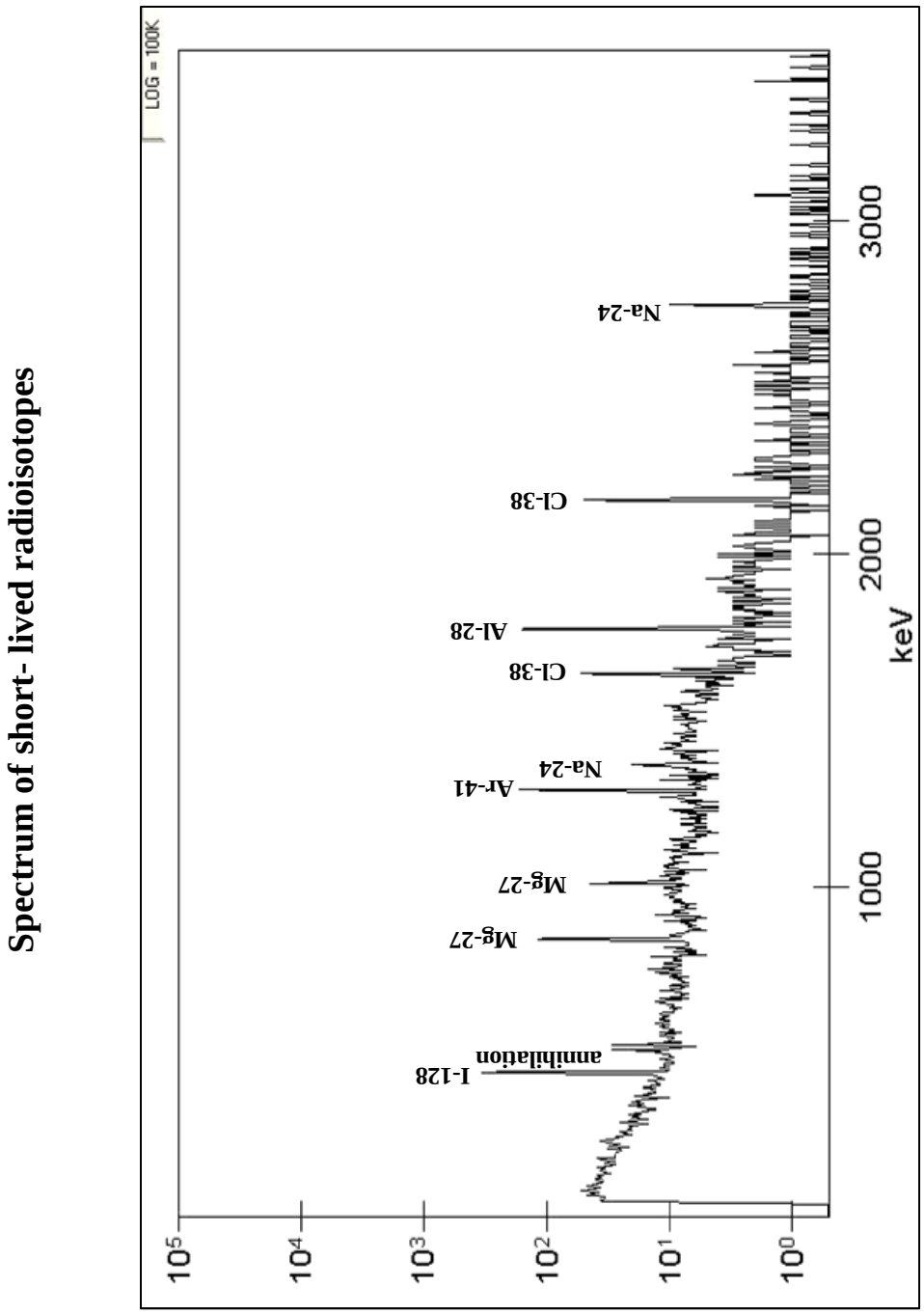

Figure A3. Spectrum of iodine standard solution irradiated in pneumatic system.

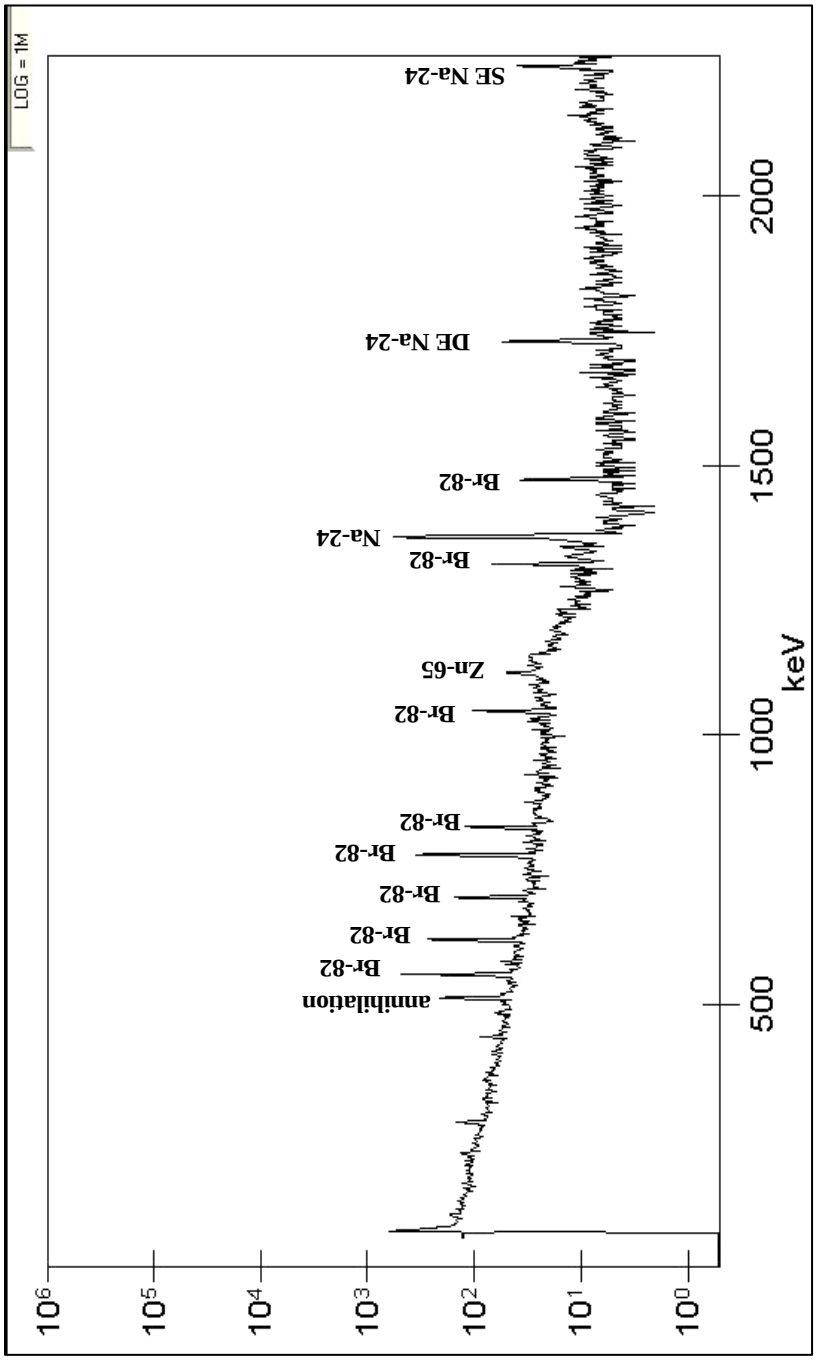

Figure A4. Spectrum of hair sample irradiated in lazy Susan for Na and Br analysis.

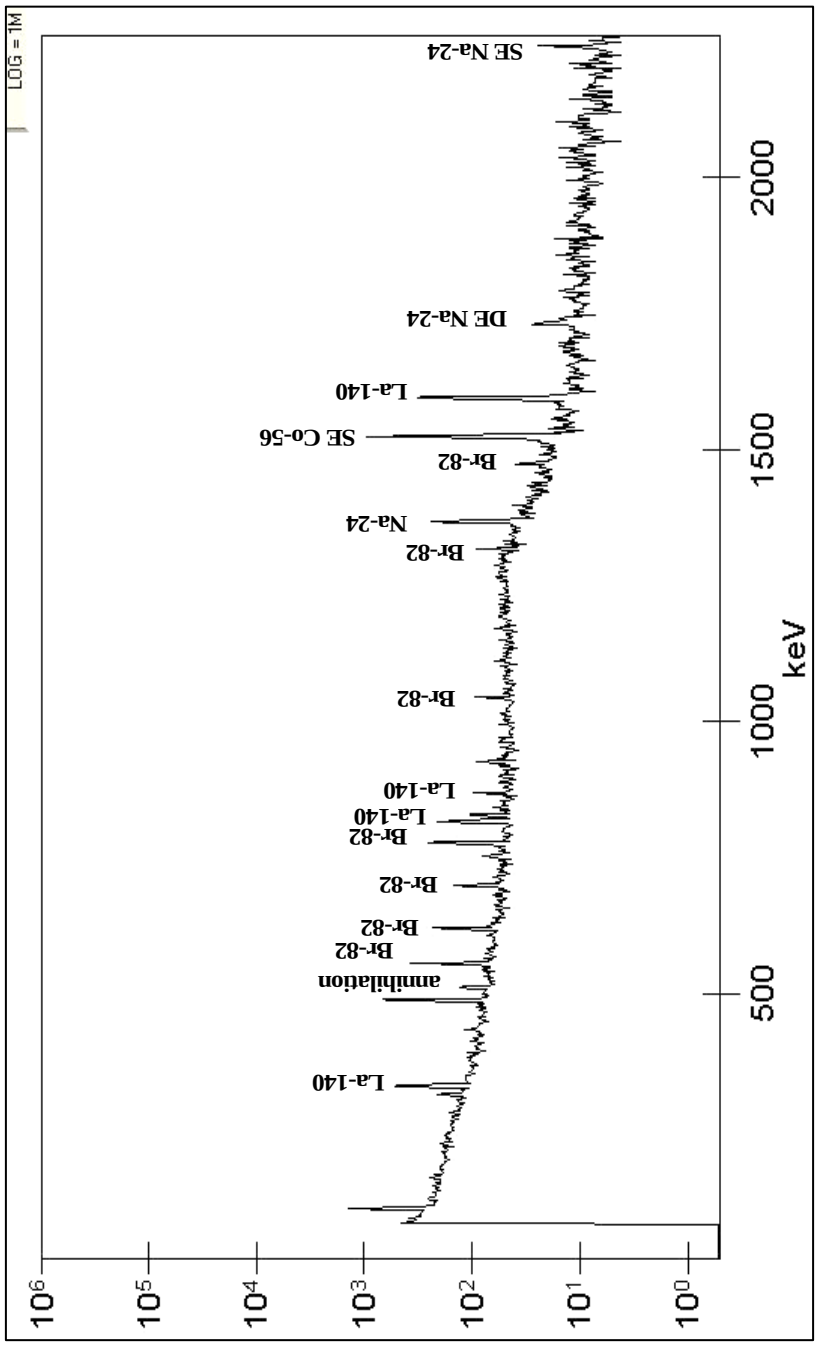

Figure A5. Spectrum standard (SRM 1547) irradiated in lazy Susan for Na and Br analysis.

Spectrum of long- lived radioisotopes

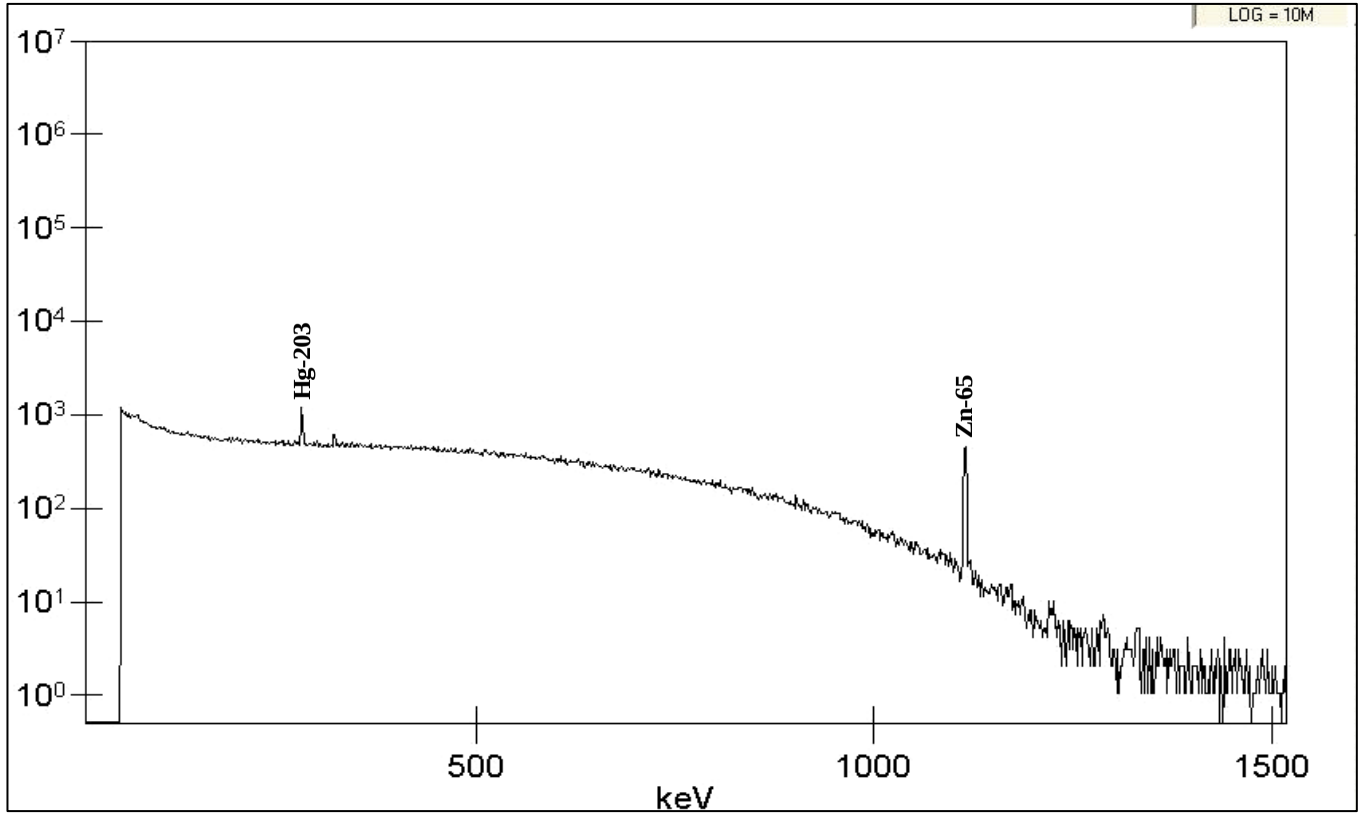

Figure A6. Spectrum of hair sample irradiated in lazy Susan for Hg and Zn analysis.

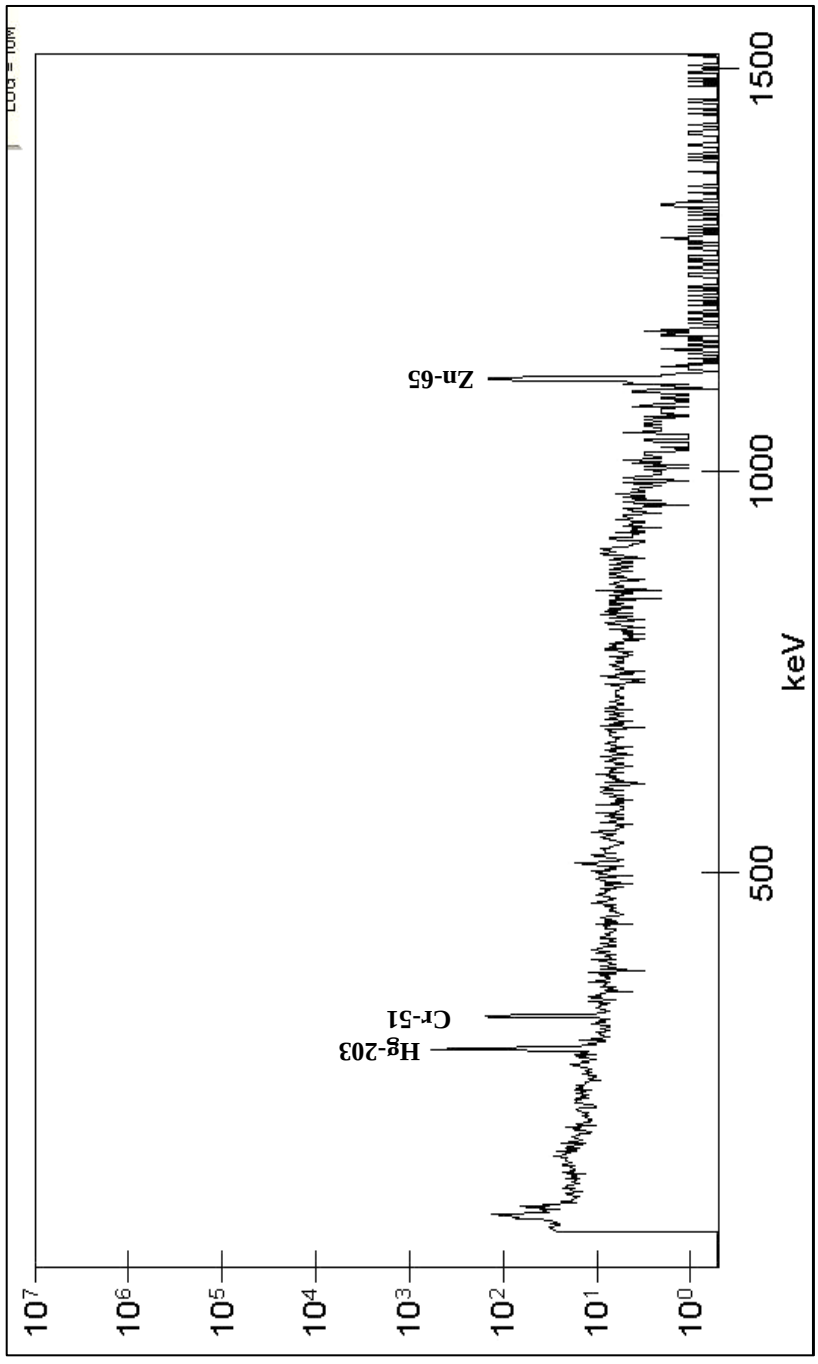

Figure A7. Spectrum of Hg-Zn standard solution irradiated in lazy Susan for Hg and Zn analysis.

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