HEAVY METAL PROFILING OF HAIR AND NAIL SAMPLES AMONG THE TRAFFIC POLICE PERSONNEL

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HEAVY METAL PROFILING OF HAIR AND NAIL SAMPLES AMONG THE TRAFFIC POLICE PERSONNEL

By

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that is not has been previously on concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
As	Arsenic
ACGIH	American Conference of Governmental Hygienists
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BEIs	Biological Exposure Indices
Cd	Cadmium
Cps	Count per second
Co	Cobalt
Cr	Chromium
Cu	Cooper
DNA	Deoxyribonucleic Acid
EPA	United State Environmental Protection Agency
Fe	Iron
FVC	Forced Vital Capacity
HEALS	Health Effects of Arsenic Exposure Longitudinal Study
IARC	International Agency for Research on Cancer
IAEA	International Atomic Energy Agency
ICP-MS	Inductive Coupled Plasma Mass Spectrometry
Mn	Manganese
Ni	Nickel
NIOSH	National Institute of Occupational Health

OSHA	Occupational Safety Health Association
PEFR	Peak Expiratory Flow Rate
PEL	Permissible Exposure Limit
Pb	Lead
Pt	Platinum
PPE	Personal Protective Equipment
REL	Recommended Exposure Limit
REL	Recommended Index Level
Sb	Antimony
Se	Selenium
WHO	World Health Organization
Zn	Zinc

LIST OF SYMBOLS

$=$	Equal
$>$	Greater than
$<$	Less than
Σ	Standard Deviation
$\pm$	Statistical Margin of Error

PEMPROFILAN LOGAM BERAT DARIPADA SAMPEL RAMBUT DAN KUKU DALAM KALANGAN POLIS TRAFIK

ABSTRAK

Logam-logam berat terbentuk dan berkumpul di bumi akibat aktiviti manusia. Secara umumnya, sumber logam-logam berat adalah dari perlombongan, bahan buangan industri, baja, cat dan asap kenderaan. Pelepasan asap kenderaan adalah penyumbang utama bahan percemaran di udara merangkumi arsenik, kadmium, kobalt, nikel, plumbum, antimoni, zink, platinum dan banyak lagi. Logam-logam berat meresap ke dalam tumbuhan, haiwan, dan tisu manusia melalui pernafasan, diet, penyerapan melalui kulit dan pengendalian manusia. Logam-logam berat boleh terkumpul dan dikesan di dalam matriks biologi seperti rambut dan kuku kerana ia sukar untuk dimetabolismakan dan secara langsung menyebabkan kesan keracunan. Penyelidikan ini merupakan satu kajian perbandingan yang memberi focus terhadap penilaian tahap pendedahan logam-logam berat di kalangan anggota polis trafik lapangan berbanding dengan anggota polis trafik dalaman Kuala Lumpur. Kajian ini melibatkan 86 orang responden daripada angota polis trafik Balai Polis Trafik, Jalan Tun H. S. Lee. Soal selidik mengenai faktor-faktor mempengaruhi pendedahan diedarkan untuk mengumpul data serta fakta demografi. Sampel rambut dan kuku dari responden telah diambil. Sampel rambut dan kuku telah dirawat dengan menggunakan kaedah pencernaan asid dibantu gelombang mikro. Sampel dianalisis menggunakan Spektroskopi Jisim Plasma Gabungan Teraruh (ICP-MS) untuk mengukur tahap logam-logam berat seperti arsenik (As), kadmium (Cd), plumbum (Pb), nikel (Ni) dan zink (Zn). Tahap purata logam-logam terpilih dalam rambut dan kuku dari anggota polis trafik lapangan dengan bacaan anggota polis trafik dalaman sebagai rujukan telah ditentukan dan dibandingkan. Hasil kajian menunjukkan bahawa tiada perbezaan yang ketara antara tahap purata logam berat di dalam sampel rambut dan kuku angota polis trafik luaran dan dalaman kecuali As dan Zn dalam rambut serta Cd dan Ni dalam kuku ($p < 0.05$). Walau bagaimanapun, dapatan kajian menunjukkan bahawa tidak ada kaitan antara pendedahan pekerjaan dengan tahap purata logam berat yang dikaji. Ujian sampel *t* berpasangan telah menunjukkan bahawa tahap purata Zn bagi sampel rambut adalah jauh lebih tinggi daripada paras dalam sampel kuku ($p < 0.05$). Ujian ANOVA pelbagai faktor menunjukkan bahawa risiko seperti jenis kumpulan, umur, pengalaman bekerja, jumlah jam bekerja seminggu dan cara kehidupan seperti pendedahan kepada asap elektronik mempunyai impak yang besar ke arah tahap purata As. Manakala jumlah tahun bekerja, pendedahan kepada shisha dan bahan kimia mempunyai pengaruh yang besar ke atas tahap purata Pb. Tahap purata untuk Ni pula dipengaruhi oleh jantina, jumlah tahun bekerja dan pendedahan kepada petroleum semasa bekerja. Kumpulan, jantina dan jumlah jam bekerja seminggu mempunyai pengaruh yang besar terhadap tahap purata Zn. Keputusan daripada kajian ini boleh digunakan sebagai data asas bagi pihak berkuasa yang berkenaan untuk membangunkan dasar dan mengambil langkah yang perlu bagi menangani risiko pendedahan logam-logam berat dan juga memupuk kesedaran mengenai isu-isu ini demi kesihatan awam.

HEAVY METAL PROFILING OF HAIR AND NAIL SAMPLES AMONG THE TRAFFIC POLICE PERSONNEL

ABSTRACT

Heavy metals are formed in the earth and became concentrated as a result of human caused activities. Sources of heavy metals are generally from mining, industrial wastes, fertilisers, paint, and vehicle emissions. Vehicle emissions are major source of airborne contaminants including arsenic, cadmium, cobalt, nickels, lead, antimony, zinc, platinum and others. Heavy metals come into plant, animal, and human tissues through inhalation, diet, absorption via skin contact and human handling. Heavy metals can accumulate and traced in biological matrices such as hair and nail as they are hard to metabolise and directly produce the toxicity effects. This research was a comparison study which focused on the evaluation of the heavy metals exposure concentrations among outdoor traffic police personnel as compared to the indoor traffic personnel. This study involved 86 respondents from traffic police personnel of Jalan Tun H. S. Lee, Police Traffic Station. Questionnaires regarding the underlying factors of exposure were distributed to collect data as well as demographics facts. The samples of hair and nail from respondents were collected. The hair and nail samples were pre-treated using microwave assisted acid digestion method. Samples then were analysed using Inductively Coupled Plasma-mass Spectrometry (ICP-MS) to measure the concentrations of heavy metals namely arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni) and zinc (Zn). The mean concentrations of selected metals in hair and nail samples from outdoor with regards indoor traffic police personnel were determined and compared. The results indicated that there was no significant difference between the mean concentrations of heavy metals in hair and nail samples of outdoor and indoor traffic police personnel. This was true except for As and Zn concentration in hair as well as Cd and Ni in nails ($p < 0.05$). However, it was demonstrated that there was no correlation between occupational exposure with the mean concentrations of heavy metals of interest. A paired sample *t*-test showed that the Zn concentration was significantly higher in hair than in the nail sample ($p < 0.05$). Multifactorial ANOVA test demonstrated that the risk or underlying factors such as group, age, working experience, working hours per shift and lifestyle factors such as electronic smoke exposure have a significant impact on the mean concentration of As. Meanwhile, years of working experience, shisha and chemical exposure have a significant influence on the mean concentration of Pb. The mean of Ni was influenced by gender, working experience and exposure of petroleum. Group, gender and working hours per week have a significant influence on the mean concentration of Zn. Results from this study can be used as a baseline data for relevant authorities to develop policies and taking necessary intervention to address the risks of heavy metals exposure as well as to create awareness regarding these issues for public health.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Human activities results in the formation of heavy metals in the earth. Industrial wastes, fertiliser, mining, vehicle emissions and many more are the common sources of heavy metals. These heavy metals are absorbed by plants, animals and humans through breathing, eating or drinking and dermal uptake (Al-Awadeen *et al.*, 2014). Toxicity effects can be due to the accumulation of heavy metals in an organism since they are not easily metabolised and excreted (Khalique, 2008).

Heavy metals are distributed and presented in the environment due to urban construction, industrialisation and traffic activities. The decay of metal or building material and transferring of contaminated soil by automobile cause heavy metal to accumulate in the environment in a form of road dust especially in the urban area (Tchounwou *et al.*, 2012). A study on road dust composition of selected metals in Kuala Lumpur City Centre showed the elevated concentration of heavy metals including Fe, Cu, Mn, Zn, Pb, Ni, Cr and Cd (Han et al., 2014). A similar study was conducted by Ramlan & Badri with regards to heavy metals pollution in the city of Kuala Lumpur by analysing soil and street dust samples. The result showed that, there was a high concentration of metals pollution contributed by Mn, Zn and Pb in the air (Yuen *et al.*, 2012). Another study was carried out in Malaysia which involved heavy metal distribution in ambient air in the industrialisation area indicated there was a high concentration of Cr, Cu, Cd, Pb, Ni and Zn (Saphira *et al.*, 2014).

Highly contaminated environment with heavy metals may pose an occupational exposure treat especially to those who work in high traffic volume and urban settings due to constant exposure towards metal polluted environment. Surif and Chai have carried out a study on screening of lead exposure among workers in Selangor and the Federal territory in Malaysia, involving workers at petrol kiosk, vehicle shop, bus driver and traffic police personnel have proven that there was a positive correlation between lead burden and lead exposure (Hashim *et al.,* 2005). Data indicated that traffic police personnel have a high risk of heavy metal exposure due to frequent exposure towards highly contaminated working atmosphere (Sebastiampillai *et al.,* 2014). Another study was carried out in Pakistan which involved traffic police personnel indicated that occupational exposure of lead was associated with blood lead concentration (Agha *et al.*, 2005).

Occupational Safety and Health Administration (OSHA) has reported the importance of biological monitoring on heavy metal exposure among high risk worker due to its harmful effect towards health (OSHA, 2005). Biological monitoring usually involves analysis of biological samples such as blood and urine. New evidence suggested that hair and nail analysis can provide useful information on occupational and environmental exposure to toxic elements (Daniel *et al.*, 2004). Heavy metals that enter into a human biological system can be deposited in keratin cell of hair and nail. Analysis of hair and nail can act as useful aid in the diagnosis of certain diseases relating to trace elements and provide information about the use of certain drugs. It is clear from the literature that in order to make hair and nail analysis a reliable and reproducible technique, certain aspects need to be investigated. Most importantly, exogenous contamination and washing procedures need to be addressed in order to put the final results into perspective (Mehra & Juneja, 2005a; Saat *et al.*, 2013a). World Health Organization (WHO) has also indicated benefits of hair testing in some cases and the International Atomic Energy Agency (IAEA) uses hair and nail to monitor global trends of elements (Samantaa *et al.*, 2003). Hence, hair and nail are recognised as asset to monitor environmental and occupational exposure due to the fact that values of elements are correlated to values in other body tissues which set the non-toxic and toxic limits (Nyambura, 2012).

Till date there is no conclusive and comprehensive studies on environmental and occupational exposure among high risk population such as traffic police personnel have been conducted in Malaysia. This study was carried out to determine the presence of heavy metals and their concentrations in hair and nail samples of traffic police personnel who work in Kuala Lumpur, Malaysia. The main benefits of this study is to provide the safety data which indirectly could assist in the policy development as well as creating public awareness with regards to heavy metals associated with occupational and environmental exposures.

1.2 Objectives of the Study

The general and specific objectives of this study are as follows:

1.2.1 General Objective

To evaluate the heavy metals exposure concentrations (As, Cd, Pb, Ni and Zn) among outdoor and indoor traffic police personnel in Kuala Lumpur

1.2.2 Specific Objectives

1. To determine the presence of heavy metals in hair and nail samples among outdoor and indoor traffic police personnel who work in Kuala Lumpur.
2. To measure the concentration of heavy metals in hair and nail samples among outdoor and indoor traffic police personnel who work in Kuala Lumpur.
3. To determine the underlying factors which influence the heavy metals exposure among outdoor and indoor traffic police personnel who work in Kuala Lumpur.

1.3 Research Hypothesis

The Null Hypotheses of this study are:

1. There is no difference in the concentration of heavy metals in hair and nail samples between outdoor and indoor traffic police personnel.
2. There is no underlying factor which influences the heavy metals exposure among outdoor and indoor traffic police personnel in Kuala Lumpur.

1.4 Problem Statement

There is no established data on heavy metals exposure among police traffic personnel in Malaysia and its association with health in Malaysia. According to the World Health Organisation (WHO), air pollution exposure and its side effects can be considered as a public health concern due to rapid urbanisation and industrialisation particularly in developing countries like Malaysia. Air contamination mostly due to hazardous heavy metals like As, Cd, Pb, Ni and Zn. These heavy metals elements are introduced through various human activities particularly from foods and the use of vehicles and transportations (Aminah *et al.*, 2007; Al Fatawi & Al-Awani, 2012; Han *et al.*, 2014).

Various studies have reported the short and long term exposures of heavy metals are related to toxicity effects on human tissues and organs as well as allergic reactions and cancer development (Morais *et al.*, 2012; Onuwa *et al.*, 2012). Hence, heavy metals exposure proves to be a concern for those who work in a polluted environment such as traffic police personnel due to its negative effects towards health. An assessment of blood Pb concentration and its correlation with parameter of DNA damage and other biochemical parameters such creatine and uric acid done in Pakistan showed that traffic police personnel who work in fields were found to have higher blood Pb concentration and DNA damage parameters as compared to control subjects and the study has also indicated a positive correlation between Pb concentration and biochemical parameters (Pervez *et al.*, 2015).

A recent study done in Malaysia has demonstrated elevated concentration of DNA damage among traffic police personnel as opposed to control respondents (Nur & Zurahanim, 2014). Both of these studies emphasised on the long-term health effect of DNA damage which can lead to DNA mutation and cancer development as well as other related diseases such as renal dysfunction disorders. Thus, this present study is essential to provide the baseline quantitative data on the heavy metals exposure particularly on traffic police personnel.

1.5 Potential Benefit

Quantitative data and information on personal heavy metal exposure among police traffic personnel will be used as a baseline data for preventive and control measures in order to minimise the additional health hazards toward workers at the workplace. The research findings will be shared with the Royal Police Malaysia (PDRM) as part of a memorandum of agreement (MOA) partnership between PDRM and USM and can be used for monitoring and improvement of safety precautions especially for the benefit of traffic police personnel and related to occupational health.

1.6 Thesis Outline

The outline of the study is as follows: The first chapter gives the overview of heavy metals exposure and their determination by using hair and nail samples analysis. The objectives and the problem statement of research are also covered in this chapter.

The second chapter is the summary on literature from related studies. Previous research on heavy metals' adverse health effect, the relationship of heavy metals exposure and the use of hair and nail samples as a heavy metals exposure indicator are addressed in this chapter.

The third chapter describes the comprehensive explanation on the research methodologies which includes statistical method, sample collection, sample preparation and sample analyses.

Fourth chapter provides the research findings and discussion of the entire study. Summary on the research findings, the limitation of the study as well as recommendations for future work are presented in the last chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Heavy metals are defined as a specific metals or mixture of metals which can cause harmful effects to human. There are many types of heavy metals, some are toxic in nature by a small amount exposure and some are essential in sustaining life (Jaishankar *et al.*, 2014). In a large amount of exposure, these heavy metals can be considered as a health hazard. OSHA (2010a) has provided information and regulation regarding the safe range of heavy metals exposure. Hence, occupational and environmental heavy metals exposure should be assessed and monitor in order to maintain a healthy population (Perry & Potter, 2008; Oregon Public Health Division (OPHD), 2011).

A long-term exposure may cause an accumulation of heavy metals in a soft tissue and resulting to heavy metals poisoning (Onuwa *et al.*, 2012). There are a few heavy metals that generally associated with heavy metal intoxication such as As, Cd, Pb, Ni and Zn which are also considered as 'toxic metal' (Grabenklis *et al.*, 2010). Most of these heavy metals element are mutagenic and carcinogenic which may pose such a threat to human life. Studies have shown that heavy metals may cause serious health problems like cardio, respiratory, neurological to cancer diseases (Duruibe & Ogwegbhu, 2007; Saat *et al.*, 2013b).

Heavy metals can be traced in human biological features such as blood, urine, hair and even nails. However, a few studies have suggested and recommended the use of hair and nail for measuring the concentration of heavy metals exposure and indirectly act as biological indicators for heavy metals risk assessment (Mehra & Juneja, 2005b; Akan *et al.*, 2012).

2.2 Heavy Metals Exposure

Heavy metals are usually present at a low concentration of parts per billion (ppb) range in the environment and it may cause harm at a higher concentration (Akan *et al.*, 2012; Tchounwou *et al.*, 2012). Both heavy metals that serve biological and non-biological functions can be considered as dangerous if exposed to human at a higher concentration (OSHA, 2005). Heavy metals such as Cu, Ca and Fe are useful to human physiology such as for building bone, transportation of oxygen in the body and useful for brain growth (Morais *et al.*, 2012). Some toxic metals such as Pb, Cd, As and many more are considered as xenobiotic or as a foreign substance and serve no purpose but harm as it enters into human body (Eibensteiner *et al.*, 2005).

Repeated exposure to heavy metals whether occupational exposure or environmental exposure in a long period of time may cause serious health effects to a person and even a population (Duruibe & Ogwegbhu, 2007). Heavy metals may cause various health symptoms ranging from cardio diseases, respiratory diseases, and neurological diseases to cancer diseases (OSHA, 2010a; OPHD, 2011).

Heavy metal presents in the form of soil, road dust and even ambient air especially in a highly polluted area of urban cities. Environment such as urban city setting with building surroundings can limit the air circulation which contributes in the gathering of street dust (Agha *et al.*, 2005; Nyambura, 2012). A study indicated that there were a traceable amount of elements such as Al, Fe, Cu, Co, Cd, Cr, Pb and Zn from soil collected in the urban area of Klang (Yuswir *et al.*, 2015). Heavy metals can also be found in the air as the result of the formation of street dust and industrial activities. A study in an industrial area of Batu Pahat, Johor demonstrated that Cd, Cr and Ni concentration in air surpass the standard concentration which were 0.0050 $\mu g/m^3$, 0.0100 $\mu g/m^3$, and 0.0150 $\mu g/m^3$ correspondingly (Saphira *et al.*, 2014).

National Institute of Occupational Health (NIOSH) recognised the guideline given and suggested Recommended Exposure Limits (REL) that can be used to monitor heavy metals in milligrams per cubic meter (mg/m^3) and micrograms per cubic meter ($\mu g/m^3$). REL can be used to measure present of element in a liquid or air (NIOSH, 2007). A nonprofits organisation called ACGIH developed a guideline parameter in assessing hazards such as chemicals in a workplace (OSHA, 2012). ACGIH has introduced Biological Exposure Indices (BEIs) which is a measurement of concentration from biological samples such as blood, urine, hair, and nails. BEIs represent the bioaccumulation of chemicals from the sample collected and is measured in milligrams per kilogram (mg/kg) or milligrams per gram (mg/g) (OSHA, 2010a). BEIs can be used as a guideline in measuring solid biological substances such as hair and nail with reference to blood and urine concentrations. BEIs guide for monitoring biological samples is presented in Table 2.1.

Table 2.1: Biological Exposure Indices (BEIs)

Element	Type of Sample	Concentration	Reference
As	Urine	35 µg/L	(OSHA, 2012)
	Blood	1 µg/L	(OSHA, 2012)
Cd	Urine	5 µg/L	(OSHA, 2012)
	Blood	5 µg/L	(OSHA, 2012)
Pb	Urine	0.0677 µg/L	(Agency for Toxic Substance and Registry (ATSDR), 2005b)
	Blood	30 µg/L	(OSHA, 2012)
Ni	Urine	3 µg/L	(ATSDR,2005c)
	Blood	0.2 µg/L	(ATSDR,2005c)
Zn	Urine	1000 µg/L	(OSHA,2012)
	Blood	5800 µg/L	(Australian Laboratory Services (ALS), 2015)

Heavy metals can be inhaled as part of small particulate matter through the lung or even ingested through food and water. A study stated that major outdoor pollutant was formed by particulate matter (Khalique, 2008). Inhalation of air pollutant is associated with health problems, especially respiratory problem. In Malaysia, study was conducted on work stress, air pollution, and asthma (Irniza, 2011). The research involved traffic police personnel using Police Stress Questionnaire (PSQ) to assess psychological factor, Dustrak aerosol monitor was used to measure the concentration of particulate matter (PM_{10}) exposed and the General Health Questionnaire (CHQ) was used to study subject health status. The study indicated that 10% of respondents were diagnosed with asthma disease and the mean concentration of exposed particulate matter was 273 $\mu g/m^3$ with urban areas recorded to have a higher reading (Irniza, 2011). Thus, heavy metals exposure in the form of particulate matter may lead to respiratory health problems.

The urban environment itself is not a healthy place for working setting especially for outdoor workers such as traffic police personnel due to major occupational hazard of heavy metals exposure (Mormontoy *et al.*, 2006). Studies indicated that the risk of heavy metals exposure among traffic police personnel were due to the duties itself that required them to be deployed in streets, road intersections and public place regularly during their working hours (Eibensteiner *et al.*, 2005; Khalique, 2008). A study in Islamabad reported that, police traffic usually spent about an average of 8 hours per day in 6 days per week basis (Agha *et al.*, 2005).

The results of the mean blood Pb, Cu and Mn concentration traffic police personnel in Islamabad was found to be higher than the control group (Agha *et al.*, 2005). The study indicated that heavy metals can be traced from blood sample of traffic police personnel and the exposure concentrations were elevated as compared to control group. Another study carried out in the city of Bursa, Turkey has demonstrated that there were difference blood Pb concentrations between traffic police personnel working outdoor and those working on indoor basis with average blood Pb concentrations of 9.4 µg/L and 8.7 µg/L (Pala *et al.*, 2002). Hence, it was evident that traffic police personnel are highly vulnerable to heavy metals exposure due to their working condition.

2.2.1 Arsenic (As)

As is an inorganic soluble substance which spreads throughout earth's crust, commonly as metal arsenate or arsenic sulphide (ATSDR, 2007). As can be widely spread to the atmosphere through human activities such as agricultural pesticide usage, fossil fuel usage, and mining as well as natural activities such as the dissolution of mineral and volcanic eruption. It can be deposited into the atmosphere and can be present in land or water (WHO, 2010). As a result, As will be inspirited by a human as a particle or absorbed as drinking water as a result of water contamination. NIOSH has identified the exposure risk of As and indicated that As's REL for health monitoring should be at 2 $\mu g/m^3$ (NIOSH, 2007).

There is evidence suggested the relationship between As exposure and health issues. As is also known to have a non-metabolic biological function to human and view as an awful cancer agent (Lindberga *et al.*, 2008). It is a toxic metal with a reputation to cause health harm even with a low dose of exposure. It is found that if the dose is as low as 0.1 to 1.82 µg/L in a drinking water it can still exhibit increased risk of vascular and cardio disease (ATSDR, 2010). In addition, As can also cause a decrease in red blood cells and white blood cells count (Akan *et al.*, 2012; Shukri *et al.*, 2013). Long-term intake of this inorganic substance may cause the development of health diseases such as skin, neuropathy, endocrine, cardiovascular, respiratory and renal disease (WHO, 2010).

According to a cohort study that was conducted in Ariahazar, Bangladesh which was based on data from the Health Effects of As Exposure Longitudinal Study (HEALS), it was demonstrated that there was a strong relationship between As exposure and respiratory symptoms such as coughing problem and breathing problem as well as the present of blood in sputum (Parvez *et al.*, 2010). A similar study was carried out in the United States involving subjects with 20 years of inorganic As exposure whom reportedly have more than 2 μg/L As in blood were likely to have a medical history of heart problems, depression, and even diabetics (Zierold *et al.*, 2003).

2.2.2 Cadmium (Cd)

Cd is a product from industrial and agricultural activities and is considered as a potent toxic metal. Cd is often used in the production of alkaline batteries and application of fertiliser and can be found in our daily intake such as crops and green leafy vegetable as well as from tobacco smoke (Benard, 2008). Previous studies have demonstrated that, the average intake of Cd from food may vary from 8 to 25 μg per day and it was estimated that an active smoker inhaled about 1 μg of Cd daily (Lars & Åkesson, 2009). Like other toxic metals, Cd serves no essential biological role. Cd can cause impairment of kidney and lung function in a short term exposure and subsequently increase the risk for both organs to develop cancers in a long term exposure (OPHD, 2011). According to OSHA (2010b), Cd exposure concentration in the environment should be monitored and suggested that Permissible Exposure Level (PELs) should be at 5 $\mu g/m^3$. Cd is also a carcinogenic and classified by International Agency for Research on Cancer (IARC) as Group 1 carcinogen to human and induce free radical release in human physiology as it reacted to the enzyme and caused the development of cancer cell (WHO, 2010).

An investigation which was conducted on 103 welder workers in Cd contains working environment, 17% of respondents' sample were exceeded the threshold limit and 6 respondents have developed tubular dysfunction disease when samples of urine were collected and analysed (Ding *et al.*, 2011). Similar study in southern part of Sweden involving 1021 population of battery plant worker group and reference group showed that 50% of the exposed group developed tubular proteinuria as compared to 5% of control group (Lars *et al.*, 2000).

2.2.3 Lead (Pb)

The most common heavy metal which present in our environment is Pb. Pb contaminant in the air is a result of the burning of fossil fuel, mining, and even manufacturing activities. It is also widely used in oil and gas industry and production of batteries. Pb is a highly toxic metal that serves no biological purpose in the human body and exposure may lead to health concern (OPHD, 2011).

Pb exposure is associated with health diseases ranging from cardiovascular, reproductive and systemic system and even can cause cognitive impairment (Chen *et al.*, 2015). Short-term exposure may cause mild health symptoms such as a headache, nausea fatigue and many more. A long-term exposure may cause neurological damage, increase blood pressure and development of cancer (OSHA, 2005). A study conducted in Peru on Pb exposure has shown that sperm motility and viability was significantly declined with elevated concentration of blood Pb (Eibensteiner *et al.*, 2005).

According to NIOSH, REL for Pb exposure monitoring in a workplace is 5 μg/m³ (NIOSH, 2007). The Commission of Human Biological Monitoring was set up by the United State of America in 1983, in order to study and develop standards or recommendation value for monitoring heavy metals in the environment. The organisation has indicated that reference value for Pb should be 60 μg/L, 90 μg /L and 120 μg/L, respectively for children, adult female and male based on blood Pb concentration measurement (Wilhelm *et al.*, 1999).

2.2.4 Nickel (Ni)

Ni is a widespread metal which can be traced from plant and another living organism. Ni can exist in a natural state of mineral forms such as volcanic ashes or result of combustion of coal, fossil fuel, and diesel oil. Ni is an essential heavy metal which is used in the production of alloys, jewellery and battery (Beukers *et al.*, 2015). Ni can be deposited into the environment as a result of a metal plate of alloys manufacturing and chemical reaction which induced by a Ni-Cd battery of motor vehicles. Like any other heavy metals, Ni also can accumulate in street dust and can be inhaled in gaseous form. Ni is water soluble in nature and can be dissolved in water (ATSDR, 2005a; Das *et al.*, 2008). It has a potential to produce toxicity effects and can be carcinogenic, haemotoxic, immunotoxic, pulmonary toxic and even nephrotic agent. Ni can be exposed through inhalation, indigestion, and dermal absorption and serves no purpose in human physiology as it disturbs the physiology of Mg, Zn and Ca that eventually results in brain injury and cardiac problem (C. J. Martin *et al.*, 1999).

Various studies indicated that short-term exposure of Ni in which the exposure lasted for one day may cause mild health harm such as a headache, insomnia, nausea, and vertigo. Short-term exposure may also induce delayed health medical condition such as sweating, tachycardia, and fatigue (Gempel & Nikel, 2006; Sancini *et al.*, 2014). A long-term exposure to Ni which lasted from 10 to more than 100 days may cause serious illness to health (ATSDR, 2005c). According to WHO, there are types of Ni compound which are Ni powder, nickel sulphate ($NiSO_4$), nickel chloride ($NiCl_2$), nickel carbonate ($NiCO_3$) and nickel nitrate ($Ni(NO_3)_2$). Ni powder is described as a chronic toxicant, where else, others are classified as carcinogen class I, reproductive toxicant class II and chronic toxicant (Das *et al.*, 2008).

Studies have reported on the effects of Ni exposure. It was stated that the workers were constantly exposed to 70-1100 $\mu g/m^3$ Ni metals present in fumes in welding industry have developed eye irritation and respiratory problems (Das *et al.*, 2008). Other study shown that 100 days of exposure caused asthma, bronchitis, sinusitis and even development of lung cancer (Beukers *et al.*, 2015). Long-term contact with Ni from jewellery accessories were also reported to cause skin irritations condition, especially for the female (ATSDR, 2005c). According to NIOSH guideline, the Recommended Index Level for nickel's exposure monitoring should be less than 15 $\mu g/m^3$ (NIOSH, 2007).

2.2.5 Zinc (Zn)

Zn is considered as a harmless heavy metal as its play a vital role in human physiology. Zn is essential in human body but excessive Zn deems to be unsafe as it can suppress the absorption of other beneficial elements in the body such as Cu. It may also lead to damaging of protein, lipids, DNA and male reproductive activity (ATSDR, 2005d). Excessive Zn may lead to general health symptoms like abdominal pain, diarrhoea, lethargy and acute respiratory distress syndrome (Plum *et al.*, 2010). Zn is usually exposed to human through inhalation of Zn fume in which Zn oxide is released by Zn welding activity (C. J. Martin *et al.*, 1999). It can also be exposed through inhalation or indigestion of dust particle from street dust or through a daily dietary intake of the high protein food such as beef, pork, and lamb. Short-term exposure of Zn fume could cause mild health problems such as fume fever, nausea, dyspnoea, fatigue, and chills. Long-term exposure of Zn may cause neutropenia, apnea, leukopenia and others abnormal blood pattern (ATSDR, 2005d). Zn can also interact with other elements such as Cu, Pb and Ni and alter human physiological function resulting to disease development after indirectly long term exposure (Jaishankar *et al.*, 2014).

It was reported that, two soldiers were found dead after 25 and 32 days of exposure to zinc chloride containing smoke bomb due to acute respiratory distress (ARDS) (Plum *et al.*, 2010). Conforming to the fact of Zn toxicity, it was also demonstrated that people who take oral tablet of Zn supplement approximately at 100,000-300,000 µg per day may develop abnormal blood pattern such apnea, neutropenia, leucopenia accompanied with mild symptoms of nausea and headache (Nriagu, 2007).

National Academy of Science, Washington, United State of America has recommended that daily intake of Zn for adult male and female should not exceed more than 15 mg per day (U.S. Environmental Protection Agency (EPA), 2003). NIOSH has advised that Recommended Index Level for health monitoring of Zn in a workplace is 2000 $\mu g/m^3$ (NIOSH, 2007).

2.3 Adverse Health Effect of Heavy Metals

Heavy metals are considered as a natural occurring product that can be found in the environment as result of human activities. They are trace elements whereby the element's concentration should be present in the environment at less than 10 $\mu g/m^3$ on types of heavy metals (OSHA, 2010b). Zn and Ni are essential in human bio-metabolism and bodily processes but at a low amount and both of them are considered as a micronutrient (Jaishankar *et al.*, 2014). Whereas As, Cd and Pb are considered as non-beneficial to human biological function (OPHD, 2011).

Most of the heavy metals are toxic elements which can bring harm to human health. Heavy metals are exposed into the biosphere and absorb by human, once absorbed they interact with other ion and enzyme in human biology, modifying enzyme physiology and structure leading to cellular damage and dysfunction that eventually affected organ systems (OPHD, 2011; Jaishankar *et al.*, 2014). Several metals are considered as carcinogenic, nephrotoxic, neurotoxic and cardiotoxic which may cause adverse health effect ranging from respiratory disease, kidney disease, cardio disease and even cancer (Tchounwou *et al.*, 2012).

Heavy metals can be exposed to human as in the form of single metal or combination of metals exposure. There were studies on the combination of a few heavy metals exposure and its adverse effect (Tomei *et al.*, 2006; S. Martin & Grisworld, 2009). A study demonstrated that heavy metals exposure such as Cd, Ni, Pb and Zn contribute health problems such as anaemia, kidney damage, and respiratory problem (Eibensteiner *et al.*, 2005). Cd and Pb were also found to be associated with kidney diseases, high exposure to these heavy metals causes damage in kidney's glomerular filtration function which leads to kidney damage (Duruibe & Ogwegbhu, 2007; Chen *et al.*, 2015). Heavy metals such as Ni and As that present in the air are likely to cause allergic effect as they trigger inflammatory reaction during inhalation of the exposure which leads to asthma and other respiratory problem (Gempel & Nikel, 2006; ATSDR, 2007).

2.4 Methods for Heavy Metals Analysis

An analytical method for heavy metals analysis is very crucial and important for toxicity assessment. The method of analysis may vary and is dependent upon the nature of the sample. There are many methods which can be employed to analyse heavy metals, such as flame Atomic Absorption Spectrometry (AAS), X-ray fluorescence (XRF) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Lawal, 2014).

AAS employed the principle of absorption of specific wavelengths of an atom to be excited from one state to another in order to determine the concentration of a specific analyte (Nyambura, 2012). XRF spectrometry utilised the principle of relaxation of atoms as they are excited by energy source and emit an x-ray photon to allow the determination of analytes

(Guthrie & Jeffry, 2012). ICP-MS applies an electromagnetic principle to separate elements by converting atom of samples into ions and is identified and quantified by mass-spectrometer (Schweitzer, 2008). ICP-MS is found to be a much sensitive method as compared to other methods as its detection limit ranging from pbb up to parts per trillion (ppt). In addition, this method can detect isotope elements and able to analyse multiple elements at a time (Bennan *et al.*, 2015).

2.4.1 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS functions based on electromagnetic principle and the source of the instrument is ICP torch that contains argon plasma which flows and when atomised produce argon ions. The samples are introduced via introducer devices in a liquid aerosol form and converted into an atom and ionised by the plasma (Bazzilio & Weinrich, 2012). The ionised atom continues to travel by lens which electrostatically focused the beam into mass spectrometry device. The ion in the beam positively repels each other and causes electron and unwanted material to leave the beam. Mass spectrometer splits the ion based on mass to charge (m/z) ratio allowing separation of inorganic, organic and even biological matters (Wolf, 2005). The mass analyser causes ion to reach the detector at a different phase based on the mass itself. The detector will convert the ion into electrical signals and the element is detected, quantified and data can be produced (Wisconsin Department of Natural Resources (WDNR), 2004). Figure 2.1 shows the components of ICP-MS

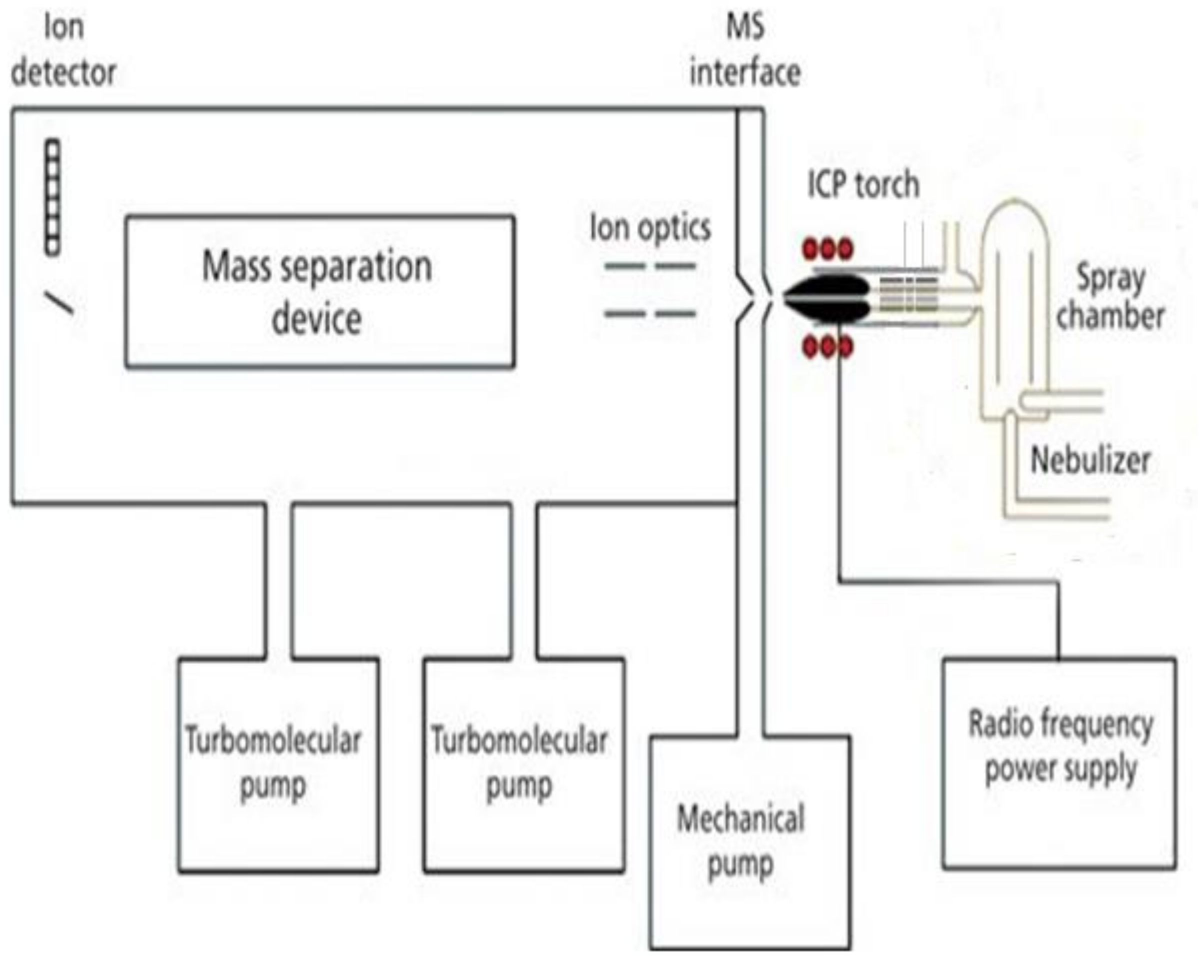

Figure 2.1: Components of ICP-MS (adapted from Bennan *et al.* (2015))

2.5 The Overview of Hair and Nail

The hair consists of hair follicles and hair shaft which are the internal and external parts of scalp respectively. Hair shaft consists of three separate layers which are cuticle, cortex, and medulla (Farlex, 2016). Cuticles, the exterior part of hair shaft which made of extended layers of each cuticle cell designed to protect the inner layer from chemical and mechanical force. A healthy cuticle produces a shiny look of the hair shaft (Leonard *et al*., 1991). The second layer which is cortex consists of keratin cells that synthesised by hair follicles.

Medulla is the central layer of the hair shaft that composed of medullar cells that made of hair mass. The base part of the hair is the hair follicle connected to capillaries and sebaceous gland (Farlex, 2016).

Hair is composed mainly of keratin and hair growth is influenced by biological process that varies among race, age, and gender (Yaemsiri *et al.*, 2010). During the development of hair, the blood capillaries supply nutrient and even a bi-product such as heavy metals or drugs around the hair follicle and integrated into the structure of the hair (Lawal, 2014). These products continue to be carried by the hair as the growth continues and therefore they can be analysed for environmental and occupational exposure (Boumba *et al.*, 2006). Figure 2.2 describes the anatomy of hair.

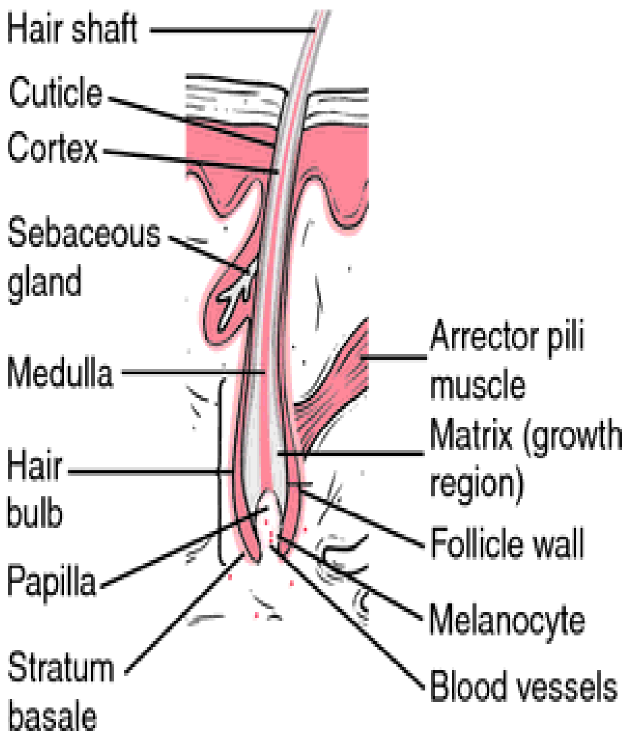

Figure 2.2: The Anatomy of Hair (Farlex, 2016)

Nails are an essential part of human anatomy which covers peripheral part of the body such as fingers and toe. Fingernails and toenails are composed of sturdy protein which also known as keratin and like hair, they also as adjunction of the skin. The anatomy of nail consists of the nail bed, nail matrix, and nail plate (Figure 2.3) (Saylor Academy, 2010). Nail bed is the base of the nails which nail plate is arranged upon it. A nail bed is comprised of network connective tissues and fibbers. Nails matrix is considered as the deepest part of nails bed whereby it connects into the blood vessel and lymph node. It is the fundamental parts of nails which is responsible to produce keratin protein (Fovoro, 2013).

Keratin proteins are produced by amino acids cysteine and many other amino acids and considered as a tough structure with high resistance to chemicals and physical (Sukumar & Subramnian, 2007). It is known that chemical composition such as heavy metals also involves in keratin production. Nails plate is the actual nail made of keratin of nails matrix and the exterior part of nails which separated from the fingers (Daniel *et al.*, 2004). The nails plate is the part of nails which can be analysed to indicate sex, diseases and even exposure to chemicals (Saylor Academy, 2010).

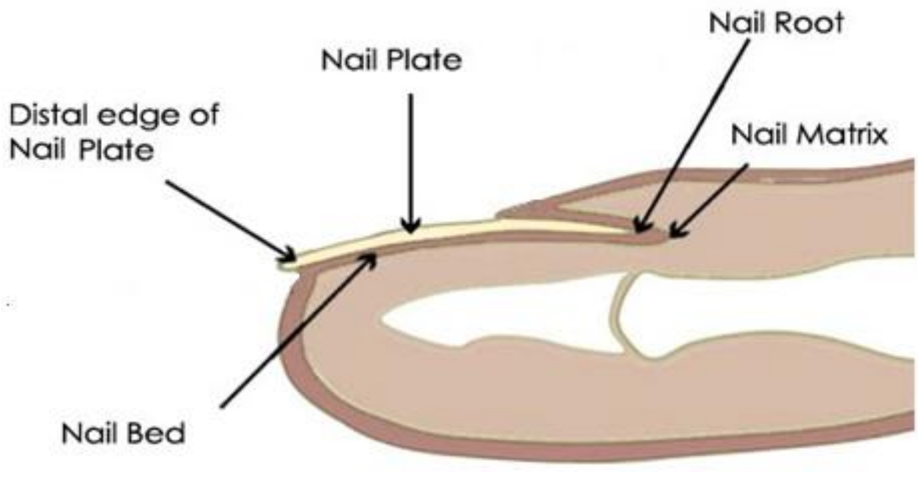

Figure 2.3: The Anatomy of Nail (adapted from Outdoors (2011))