# GOLD AND BISMUTH NANOPARTICLES MODIFIED ELECTRODES FOR SIMULTANEOUS DETECTION OF Pb(II) IONS, Cd(II) IONS AND Cu(II) IONS

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# DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled 'Gold and Bismuth Nanoparticles Modified Electrodes for Simultaneous Detection of Pb(II) Ions, Cd(II) Ions and Cu(II) Ions'. I also declare that it has not been previously submitted for the award of any degree and diploma or other similar title of this for any other examining body or University.

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# LIST OF SYMBOLS

| | |
|---|---|
| A | Ampere |
| C | Celsius |
| c | Centi |
| E | Standard Reduction Potential |
| G | Gibbs Free Energy |
| g | Gram |
| Hz | Hertz |
| L | Liter |
| M | Molarity |
| m | Meter |
| n | Nano |
| s | Second |
| V | Volt |
| $\Delta$ | Delta |
| $\theta$ | Theta |
| $\mu$ | Micro |
| ° | Degree |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AAS | Atomic absorption spectrometry |
| ABS | Acetate Buffer Solution |
| AdASV | Adsorptive Anodic Stripping Voltammetry |
| AdCSV | Adsorptive Cathodic Stripping Voltammetry |
| AdSV | Adsorptive Stripping Voltammetry |
| AFM | Atomic Force Microscopy |
| AFS | Atomic Fluorescence Spectrometry |
| AgNP | Silver Nanoparticles |
| ASV | Anodic Stripping Voltammetry |
| AuNPs | Gold Nanoparticles |
| AuNSs | Gold Nanostars |
| CB | Carbon Black |
| CE | Counter Electrode |
| CNTs | Carbon Nanotubes |
| CPE | Carbon Paste Electrode |
| CV | Cyclic Voltammetry |
| CVASV | Cyclic Voltammetry Anodic Stripping Voltammetry |
| DBDR | Dielectric Barrier Discharge Reactor |
| DNA | Deoxyribonucleic Acid |
| DPASV | Differential Pulse Anodic Stripping Voltammetry |
| EMF | Electromotive Force |
| $E_{pa}$ | Anodic Peak Potential |
| FAO | Joint Food and Agricultural Organization |
| FETs | Field-Effect Transistors |
| GCE | Glassy Carbon Electrode |
| GE | Graphite Electrode |
| Gr | Graphene |
| AuNPs | Gold Nanoparticles |
| AuNSs | Gold Nanostars |
| HMIs | Heavy Metal Ions |
| HMs | Heavy Metals |

| | |
|---|---|
| IARC | International Agency for Research on Cancer |
| IC | Ion Chromatography |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| $I_{fwd}$ | Forward Voltage Pulse |
| ILGPE | Ionic Liquid-Graphite-Based Paste Electrode |
| $I_{pa}$ | Anodic Peak Current |
| $I_{pc}$ | Cathodic Peak Current |
| $I_{rev}$ | Reverse Voltage Pulse |
| ISEs | Ion-Selective Electrodes |
| ISFET | Ion Selective Field-Effect Transistor |
| ITO | Indium-Tin Oxide |
| LIBS | Laser Induced Breakdown Spectroscopy |
| LOD | Limit of Detection |
| LSASV | Linear Sweep Anodic Stripping Voltammetry |
| LSV | Linear Sweep Voltammetry |
| MW | Molecular Weight |
| NPs | Nanoparticles |
| NPV | Normal Pulse Voltammetry |
| ppb | Parts Per Billion |
| ppm | Parts Per Million |
| RE | Reference Electrode |
| rGO | Reduced Graphene Oxide |
| RSD | Relative Standard Deviation |
| SPCE | Screen Printed Carbon Electrode |
| SPR | Surface Plasmon Resonance |
| SWadCSV | Square Wave Anodic Stripping Adsorptive Cathodic Stripping Voltammetry |
| SWCNT | Single-Walled Carbon Nanotube |
| SWV | Square Wave Voltammetry |
| UV-Vis | UV-Visible Spectroscopy |
| WE | Working Electrode |
| WEPA | Water Environment Partnership in Asia |
| WHO | World Health Organization |
| XRD | X-Ray Diffraction |

# LIST OF APPENDICES

Appendix A Crystallographic Data

# PARTIKEL NANO EMAS DAN BISMUT SEBAGAI PEGESAN SERENTAK ION PB(II), CD(II) DAN CU(II) SECARA

## ABSTRAK

Pencemaran logam berat di dalam air alam sekitar telah membahayakan kesihatan manusia dan ekosistem. Beberapa kaedah spektroskopi telah digunakan untuk pengesanan logam berat. Walau bagaimanapun kaedah ini memerlukan alatan yang mahal, pengendali berpengalaman dan tidak sesuai untuk analisis di tapak. Pengesan elektrokimia boleh mengatasi kekangan ini tetapi memberikan sensitiviti rendah terhadap pengesanan logam berat. Oleh itu, sensitiviti elektrod pengesan boleh dipertingkatkan dengan mengubahsuai elektrod dengan partikel nano. Dalam kajian ini, partikel nano emas (AuNPs) dienap di atas elektrod indium timah oksida (ITO) melalui kaedah elektroenapan. Sifat elektrokimia elektrod yang diubah dikaji menggunakan analisa voltammetri berkitar (CV) dan voltammetri pelucutan anodik gelombang persegi (SWASV) untuk mengkaji pengesanan logam berat. Elektrod AuNPs/ITO yang disediakan dalam 0.1 M $HNO_3$ dengan keadaan yang tidak dirawat panas dan tidak dibersihkan dipilih sebagai optimum kerana mempamerkan prestasi elektrokimia yang baik dan sensitiviti tinggi terhadap pengesanan logam berat. Julat linear Pb(II), Cd(II) dan Cu(II) adalah 10 ppb hingga 50 ppb dengan sensitiviti 0.121 $\mu A\ ppb^{-1}\ cm^{-2}$, 0.109 $\mu A\ ppb^{-1}\ cm^{-2}$ dan 0.039 $\mu A\ ppb^{-1}\ cm^{-2}$ mengikuti had pengesanan (LOD) 26.16 ppb, 21.17 ppb dan 50.68 ppb. Platform pengesan yang ditunjukkan oleh elektrod yang diubahsuai bersama dengan nanopartikel bismut (BiNPs) dan AuNPs adalah baik. Berbanding dengan elektrod AuNPs/ITO, kesan sinergi Bi-AuNPs pada elektrod ITO menunjukkan prestasi elektrokimia dan tindak balas pelucutan yang lebih baik dalam pengesanan logam berat.

# GOLD AND BISMUTH NANOPARTICLES MODIFIED ELECTRODES FOR SIMULTANEOUS DETECTION OF PB(II) IONS, CD(II) IONS AND CU(II) IONS

## ABSTRACT

Recently, heavy metal pollution in the environmental water has put human health and ecosystem at risk. Several spectroscopic techniques have been used for heavy metal detection. However, these techniques are costly, require experienced operators and are inappropriate for on-site analysis. Electrochemical sensors can overcome these constraints but they have low sensitivity towards heavy metal detection. Therefore, by modifying working electrode with nanoparticles, the sensitivity of the electrode can be improved. In this work, gold nanoparticles (AuNPs) were fabricated on indium-tin oxide (ITO) working electrode by electrodeposition method. The electrochemical properties of modified electrode were studied by cyclic voltammetry (CV) and square wave anodic stripping voltammetry (SWASV) was performed for heavy metal detection. AuNPs/ITO electrode prepared in 0.1 M $HNO_3$ with unheat-treated and uncleaned conditions was selected as the optimum as the electrode exhibited better electrochemical performance and high sensitivity towards heavy metal detection. The linear range of Pb(II), Cd(II) and Cu(II) was 10 ppb to 50 ppb with sensitivities of 0.121 $\mu A\ ppb^{-1}\ cm^{-2}$, 0.109 $\mu A\ ppb^{-1}\ cm^{-2}$ and 0.039 $\mu A\ ppb^{-1}\ cm^{-2}$ and limits of detection (LOD) of 26.16 ppb, 21.17 ppb and 50.68 ppb, respectively. A promising sensing platform was demonstrated by electrode co-modified with bismuth nanoparticles (BiNPs) and AuNPs. Comparing with AuNPs/ITO electrode, the synergistic effect of Bi-AuNPs on ITO electrode showed improved electrochemical performance and better stripping response in heavy metal detection.

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Metals are considered as natural elements that presence in the environment. Metals have high electrical conductivity as cations can be produced by readily losing their electrons. Metals exist in many different places on the Earth which include atmosphere, water systems as well as earth crust and can bioaccumulate in humans, animals and plants. The abundance and distribution of metals are strongly affected by the natural earth processes (Jyothi, 2020). There are total of 35 existing natural metals presence in the environment, 23 of them have elemental density greater than 5 g/cm$^3$ and are known as heavy metals (Csuros & Csuros, 2016). Several examples of heavy metal elements are cadmium (Cd), copper (Cu), lead (Pb) and more. In very trace amount, some of these heavy metals are essential elements for different physiological and biochemical behaviors toward human body, but they may cause potential acute or chronic toxicity when presence in high doses (Jyothi, 2020).

Over the past years, industrialization and urbanization have progressing at a very rapid pace, thereby over-exploration of the earth's natural resources has progressively occurred, resulting in the global issue of environmental pollution. In this regard, the increasing anthropogenic activities in both terrestrial and aquatic ecosystems has led to serious pollution of heavy metal in the environment (Raymond et al., 2011). Heavy metals cannot be easily broken down and tend to bioaccumulate in human body through several routes such as inhalation, digestion and also skin contact (Briffa et al., 2020). As such, high level of toxicity and carcinogenic of heavy metals can cause adverse health effects in the human body. Many studies have proven that long-term exposure of heavy metals can result in numerous diseases and disorders in cardiovascular, gastrointestinal, hemopoietic, neurological, respiratory, reproductive and renal systems. Besides, molecules of DNA, proteins and lipids may be damaged by these toxic heavy metals, leading to oxidative stress to occur and support the progression of carcinogenesis in the body (Witkowska et al., 2021). Heavy metals are well-known as one of the major threatening environmental contaminants, tremendously disastrous to environment and also detrimental to human health. Thus, the determination and quantification of these heavy metal in environmental samples is of great significance.

Heavy metals have been established as the precedence elements to be monitored and controlled by different international agencies and organizations including Environmental Protection Agency (EPA), Joint Food and Agricultural Organization (FAO) and World Health Organization (WHO). The regulatory limits of these heavy metals have been strictly set for their concentrations in water. Therefore, high sensitivity and selectivity analytical techniques must be developed to detect the subparts per billion (ppb) level of heavy metals that exist in a wide variety of environmental matrices such as soil, natural and waste water (Bansod et al., 2017).

Several conventional analytical methods including atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS), laser induced breakdown spectroscopy (LIBS) and UV-Visible spectroscopy (UV-VIS) have been used for heavy metal ions (HMIs) detection (Lu et al., 2017; Eddaif et al., 2019; Mohamad Nor et al., 2021). These methods are capable for heavy metal ions detection in a wide selection of elements and giving very low limit of detection in femtomolar concentration range. However, these techniques require expensive instrumentation, complex sampling preparation and the need of skilled operator in handling the complex equipment. Also, the spectroscopic techniques are only applicable for quantitative detection and some techniques require coupling with additional chromatographic techniques such as ion chromatography (IC) for executing speciation of heavy metal ions (Eddaif et al., 2019). In addition, there are many sensors can be employed in determination of heavy metal ions including chemical sensor, electrochemical sensor, biosensor, optical sensor, gravimetric sensor, electronic tongue, chemometric and flow sensing of heavy metal. However, a number of limitations for some of these sensors such as inhomogeneity and unstable enzyme action of biosensor, limited long-run stability of optical sensor, destructive nature and time-intensive of gravimetric sensor, easily affected by environmental conditions of electronic tongue, low reversibility and restricted data availability of chemometric sensing together with high power requirement and error-prone in flow sensing of heavy metal (Vigneshvar et al., 2016; Enciso et al., 2007; Lobnik et al., 2012; Baldwin et al., 2011; Wong & Khor, 2019; Pandey & Mishra, 2018).

Electrochemical sensors which are inexpensive can overcome the constraints of other sensors by providing real-time continuous measurements, improving the portability and promoting a fast response (Eddaif et al., 2019). Electrochemical sensors are typically classified under the category of chemical sensor in which an electrode is employed as a transducer component in the presence of complex matrix. Thus, the sensing mechanism of electrochemical sensors is dependent on the signal of output transducer in which a potentiostat is used to measure the potential difference. Electrochemical sensor is allowed to be packed into a compact system when the sensing signal of these sensors are collected using conducting wires. Furthermore, the sensing of heavy metals ions by bare electrodes is allowable due to their defined redox potential and subsequently eliminates the requirement of a molecular recognition probe in the use of optical sensors (Waheed et al., 2018).

There are several techniques in the electrochemical sensing including electrochemical potentiometry, voltammetry, amperometry, conductometry as well as impedimetry. In general, electrochemical techniques such as ion-selective electrodes (ISEs), field-effect transistors (FETs), linear sweep voltammetry (LSV), cyclic voltammetry (CV), square wave anodic stripping voltammetry (SWASV) as well as differential pulse anodic stripping voltammetry (DPASV) have been developed in order to overcome the limitations of the conventional analytical techniques. These electrochemical techniques permit easy operation and are convenient to assemble on tiny circuits that turn into portable devices for the purpose of *in-situ* monitoring of polluted samples. Besides, electrochemical techniques also offer short-time analysis when compared to conventional analytical techniques and allow for on-line tracking of environmental samples (Bansod et al., 2017). Nonetheless, these electrochemical techniques have limitations in providing low sensitivity and detection limits as contrasted to other optical and spectroscopic techniques. Thereby new development is required to enhance the electrochemical performance in heavy metal ions detection. Hence, by modifying the electrode with various interface materials, the selectivity and sensitivity of electrochemical sensor can be significantly improved in sensing of HMIs (Malik et al., 2019).

Nanoparticles such as gold nanoparticles (AuNPs) and bismuth nanoparticles (BiNPs) have been widely used for the modification of electrode surfaces in the advancement of novel electrochemical sensors. Nanoparticles which are in the size range of 1-100 nm, can be used to improve sensing devices owing to their large active surface area, high conductivity, improved mass-transport rate and rapid electron transfer (Tukur et al., 2014). Electrodeposition is a useful electrochemical approach to deposit stable and robust nanoparticles onto electrode surface for electrode modification, in which CV provides potential advantages over other electrochemical methods. Various experimental parameters during electrodeposition play significant role in the formation of nanoparticles film on modified electrode surface. Also, several analytical properties in HMIs detection which include sensitivity, limit of detection, stability of response and capability of simultaneous detection are also greatly affected.

Furthermore, the catalytic characteristics of nanoparticles rely on the particle size wherein the problem of agglomeration of nanoparticles can be reduced via functionalization which also allows stabilization of nanoparticles dispersion. Additionally, hybridization and cooperation of different nanoparticles is capable to promote high sensitivity (ability to detect very low concentration of targeted HMIs in analyte) and selectivity (ability to detect targeted HMIs in analyte without interference from foreign ions) in the design of electrochemical sensing platform (Waheed et al., 2018).

## 1.2 Problem Statement

The presence of heavy metals in the environmental waters due to increasing anthropogenic activity has put human health and ecosystem at risk. The negative effects of this water pollution have demanded many researchers to develop appropriate detection techniques for monitoring and controlling these toxic heavy metals in water samples. Thus, electrochemical techniques are selected as a strong sensing platform for heavy metal ions (HMIs) detection due to its accurateness, highly sensitivity, cost-effective, user-friendly, time economic and suitability for on-site detection (Malik et al., 2019). Nonetheless, bare electrodes employed in the electrochemical sensing tool has very low active surface area which may cause limitation in detecting HMIs which include low sensitivity, selectivity as well as limit of detection (LOD). Therefore, the electrochemical performance of these sensors can be significantly enhanced by chemically modify bare electrodes with effective electron mediators. Electron mediators are defined as redox active constituents that allow mediating electron transfer from donors to acceptors. The large active surface area and remarkable quantum mechanical characteristics of nanoparticles allow them to become excellent electron mediators and promising interface materials for electrode modification (Waheed et al., 2018).

In recent years, gold nanoparticles (AuNPs) have been widely used in electrochemical sensors as they can be easily deposited onto various electrode surfaces and able to reduce the overpotentials of the electroanalytical reaction, inducing less energy than thermodynamically expected to drive a reaction. AuNPs possess large specific surface area, high stability, electrical conductivity and catalytic activities. For example, AuNPs were successfully electrodeposited onto screen printed carbon electrode (SPCE) arrays and showed excellent electrocatalytic activity towards Pb(II) and Cu(II) detection with detection limits of 2.1 ng/L and 1.4 ng/L, respectively (Kanyong et al., 2016). Nonetheless, various experimental parameters including electrolyte composition, heat treatment process and cleaning step during electrode modification by electrodeposition method influence the nanoparticles' nucleation and growth that relates to particle sizes, nanostructure morphologies (shape, texture and distribution) and topographies (quantitative dimensional measurement) of deposited AuNPs film on electrode surface (Zakaria et al., 2021). To the best of our knowledge, no studies have been reported on the effect of heat treatment and cleaning process on the AuNPs modified electrode.

Furthermore, electrochemical detection by a single nanomaterial modified electrode may lead to certain limitations, and thus synergetic binary nanoparticles are introduced to promote significant performance improvement for electrochemically sensing towards HMIs (Lu et al., 2019). Bismuth nanoparticles (BiNPs) are extraordinary to be used for the electrode modification because they may improve catalytic activity, electroanalytical ability and higher signal-to-noise ratio (Ariño et al., 2017). One literature has been reported by Lu et al. (2017) on the fabrication of Bi/AuNPs/SPCE for reliable and sensitive HMIs detection using DPASV technique. The intergration of AuNPs and BiNPs greatly enhanced the electrochemical performance of modified electrode. To our knowledge, limited research is found on HMIs detection based on electrode modified with AuNPs and BiNPs.

In the current work, to fabricate a good and uniform electrodeposited AuNPs film on electrode by CV technique, the effect of different background electrolyte solutions utilized in electrodeposition of AuNPs was studied. This is because different nanostructure morphologies of AuNPs produced can have different impact on electrochemical properties of modified electrodes. In addition, the effect of heat treatment and cleaning process on the AuNPs modified electrode was also studied to evaluate the relationship between heat treatment, cleaning process and electrochemical performance of AuNPs modified electrode. Besides, the electrocatalytic performance of single nanoparticles (AuNPs) and binary nanoparticles (Bi-AuNPs) modified electrodes was also investigated by CV and SWASV analyses for HMIs detection.

## 1.3 Objectives

The objectives of this project are:

i. To optimize the fabrication process of AuNPs/ITO modified working electrode

ii. To determine the sensitivity, limit of detection (LOD), selectivity and performance in real water sample of AuNPs/ITO modified working electrode for heavy metal detection

iii. To investigate feasibility of electrodeposited Bi-AuNPs binary modified electrode as the working electrode for heavy metal detection

## 1.4 Scope of Research

In this work, AuNPs/ITO modified electrodes with different background electrolyte solutions were fabricated using electrodeposition technique by cyclic voltammetry (CV) mode. The properties of the as-deposited AuNPs film on ITO electrode were studied using X-ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM) and Atomic Force Microscopy (AFM). The effect of heat treatment and cleaning process on conductivity and electrochemical performance of AuNPs/ITO electrode was investigated using CV analysis and square wave anodic stripping voltammetry (SWASV). After optimizing the fabrication of AuNPs/ITO electrode, the sensitivity of the modified electrode for simultaneous detection of Pb(II), Cd(II) and Cu(II) was determined using SWASV analysis. The obtained stripping response of AuNPs/ITO modified electrode was then analyzed to determine the limit of detection (LOD) for each heavy metal ions. Next, interference study of foreign metal ions, reproducibility study as well as the application of AuNPs/ITO modified electrode in the real seawater sample were also conducted. In addition, Bi-AuNPs/ITO modified electrode was fabricated through electrodeposition in order to compare with AuNPs/ITO modified electrode for their electrochemical performances using CV and SWASV analyses.

## 1.5 Dissertation Outline

This thesis is organized into five chapters. The first chapter covers the research background, problem statement, objectives and scope of research. The second chapter discusses the literature reviews on heavy metal, conventional technique and electrochemical technique for heavy metal detection as well as nanomaterials modified working electrode. In the third chapter, the materials, apparatus and equipment used, as well as experimental procedure, parameters and characterization methods are discussed in details. Chapter 4 focuses on results and discussion. Finally, Chapter 5 concludes the research and suggests for future work.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Heavy metals contributed to environmental contamination which has drew serious attention all over the world. Heavy metals can enter the food chain and appear in the daily consuming food and beverages, developing significant risks and hazards towards human health, environmental persistence and bioaccumulative potential due to the high toxicity of heavy metals. In this chapter, the sources and exposure of heavy metals together with their toxicological effects on humans are discussed. Besides, various techniques for heavy metal ions detection that are currently employed including conventional techniques, sensors and electrochemical techniques are elaborated. Finally, the use of nanomaterials in the modification of electrodes for the electrochemical heavy metal sensing are also explained in detail.

## 2.2 Heavy Metals

Metals are normally known as elements that are ductile, malleable and with a metallic lustre. In addition, metals are common conductors of electricity, possess basic oxides and able to produce cations. Metals are widely used in various applications and importance for industries. Part of metals and semimetals (metalloids) are biochemically and physiologically significant towards biological systems and are vital for biological life (Norbert, 2001). According to Csuros & Csuros (2016), heavy metals (HMs) can be described as metals with elemental density greater than 5 g/cm$^3$ while Duffus (2002) defined “HMs” as classification of metals and metalloids that are associated with pollution and contamination due to potential toxicity. Heavy metals can be categorized into essential and non-essential HMs according to their functions in biological systems.

Essential HMs such as iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are crucial for biological life and needed in the body in very trace amount whereas non-essential HMs including mercury (Hg), lead (Pb) and cadmium (Cd) which are toxic, possess no significant biological function in living creatures (Türkmen et al., 2009). Some of the HMs like Cu, Mn and Zn are known as micronutrients which are essential for the growth of plants and stress resistance (ability of plants to survive under stress conditions). These micronutrients are also essential for biosynthesis in plants as well as function of diverse biological molecules for instance carbohydrates, nucleic acids, chlorophyll and so on. Either excess or deficiency of these essential HMs can result in anomalous conditions or even various diseases. Nevertheless, different categories of organisms including microorganisms, animals and plants require different types of essential HMs (Appenroth, 2010).

Heavy metal elements constitute an ill-defined group of inorganic chemical hazards which give rise to the most investigated environmental contaminants. Depending on the exposure doses and durations, nearly all HMs exert potential toxic to living organisms. The most common hazardous HMs exist in the environment as reported by Briffa et al. (2020) are arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg) and zinc (Zn). Sources of these toxic HMs in the environment can be found from natural process or anthropogenic activities. Volcanic activity, soil erosion as well as weathering of rocks and minerals have become the geological or natural sources of HMs in the environment. Also, the rising in the anthropogenic share of HMs in the environment as a consequence of global patterns of economic growth and urbanization as well as industrialization (Nagajyoti et al., 2010). Due to the reason of the astounding increase of anthropogenic activities in both terrestrial and aquatic ecosystems, heavy metal contamination has occurred and turned into the dominant cause of pollution in the environment. Few economic sectors such as industry, agriculture, manufacturing and mining tend to release high toxicity and carcinogenic heavy metal elements into the atmosphere. Anthropogenic activities such as rapid growing of industrial area, mine tailing, land application of fertilizers, pesticides, wastewater irrigation, combustion of fossil fuels and also atmospheric deposition have polluted HMs into the environment (Raymond et al., 2011).

## 2.3 Sources, Exposure and Effect of Heavy Metals

Although some HMs are physiologically and biochemically required in the body, exceeding HMs threshold concentration, HMs can act as carcinogenic agents and exert chronic toxicities, profoundly affecting the ecosystem and human health. These HMs can be dissolved and released into the environment in various forms as water pollutants, air pollutants and also soil pollutants (Jyothi, 2020). HMs are non-biodegradable as they enter and bioaccumulate in human body and living creatures through several routes by drinking polluted water, consuming contaminated food, inhaling from atmosphere and skin contact (Briffa et al., 2020). Distribution of most HMs in cells and tissues in human body is via blood. Taking Pb as an example, Pb is distributed in kidney and liver by red blood cells and consequently transported to bone, teeth and hair mainly as phosphate salt. As for Mn, it is carried by blood to lung by diffusing through the lung membrane to the central nervous system which contains brain and spinal cord. Inorganic salt of Mn which is water soluble is distributed in kidney and plasma to eliminate renal whereas organic Mn salt which is water insoluble (lipid soluble) is distributed in the intestine to eliminate fecal. Besides, for Cd, it firstly binds itself to albumin and blood cells, and consequently connects with metallothionein tissue in liver and kidney. As is carried by blood to the vital organs such as liver, kidneys, lungs and heart, muscle and nervous tissue and also distributed in the hair, skin and nails (Engwa et al., 2019). Table 2.1 shows the regulatory limit of some common HMs approved by few administrations and agencies.

Table 2.1: Regulatory limit of heavy metals (Engwa et al., 2019)

| **Heavy metal** | **Limit of EPA (Environmental Protection Agency) in drinking water (ppm)** | **Limit of OSHA (Occupational Safety and Health Administration) in workplace air (ppm)** | **Limit of FDA (Food and Drug Administration) in bottled water or food (ppm)** |
|---|---|---|---|
| Arsenic (As) | 0.01 | 10 | - |
| Cadmium (Cd) | 0.005 | 5 | 0.005 |
| Chromium (Cr) | 0.1 | 1 | 1 |
| Lead (Pb) | 0.015 | 0.15 | - |
| Mercury (Hg) | 0.002 | 0.1 | 1 |
| Zinc (Zn) | 5 | 5 | - |

Transportation of HMs into the body cells, tissues, organs and systems chemically binds to proteins, causing the macromolecules to be destructed by nuclei acids and their cellular systems are severely damaged and disrupted. Thereby, high level of toxicity and carcinogenic of HMs possesses hazardous effects on human health including mental disorder caused by the disrupted central nervous systems, damaged organs such as kidney, liver and lung as well as destroyed blood components, leading to different diseases and expose towards cancer. Moreover, prolonged exposure and accumulation of HMs in the body is possible to progressively retard the development of physical, muscular and neurodegenerative activities that imitate several disorders for instance multiple sclerosis, muscular dystrophy, Alzheimer’s disease as well as Parkinson's disease. Apart from that, consistent direct contacting with HMs may destroy nuclei acids and promote cell mutation, leading to the imitation of hormones thereby interfering with endocrine glands in the reproductive system and sooner or later may develop cancer (Engwa et al., 2019). According to the reports of International Agency for Research on Cancer (IARC), several toxic HMs such as As, Cd and Cr exert considerable causing agents towards cancer (Kim et al., 2019). The negative impacts of various HMs (Ar, Cd, Cr, Cu, Hg, and Pb) on human health are discussed in the following sections and the lists of health effect caused by different HMs is presented in Table 2.2.

Table 2.2: Health effect of different heavy metals towards human health (Briffa et al., 2020)

| Heavy metal | Effect |
|---|---|
| Arsenic (As) | • Lung irritation<br>• Increased risk of cancer<br>• Heart problem<br>• Brain damage<br>• Infertility and miscarriages<br>• DNA damage |
| Cadmium (Cd) | • Infertility<br>• Psychological disorders<br>• Gastrointestinal disorders<br>• DNA impairment<br>• Immune system deficiencies<br>• Cancer |
| Chromium (Cr) | • Liver damage<br>• Bronchitis<br>• Coma<br>• Lung cancer<br>• Liver and kidney disease<br>• Death (usually at 1-3 g) |
| Copper (Cu) | • Cytotoxic to erythrocytes leading to hemolysis<br>• Hepatic and kidney disease<br>• Hepatocellular degeneration<br>• Wilson’s disease<br>• Death |
| Lead (Pb) | • Renal impairment<br>• Premature and low birth<br>• Miscarriages<br>• Pica<br>• Cognitive impairment<br>• Brain injury |
| Mercury (Hg) | • DNA and chromosomes damage<br>• Mongolism<br>• Minamata disease<br>• Teratogenic<br>• Lung cancer<br>• Colorectal cancer |

### 2.3.1 Arsenic

Although As is a semi-metal, but it is discussed under this section due to the reason that it is toxic and carcinogenic in nature. According to the research about the adverse effects of As exposure to human which was studied by Abdul et al. (2015), most of the people exposed to As via various means such as underground water, air in atmosphere and food stuffs. Systems of cardiovascular, reproductive, renal and endocrine may be destroyed by this hazardous HMs. Groundwater was reported as the main exposure route in the countries in South Asia including Bangladesh, Pakistan and India. A study from Shahid et al. (2018) reviewed that almost 47 millions of Pakistan's citizens were in jeopardy because of As pollution in the groundwater and the authorities discovered that nearly 50 % of groundwater contained excessively high level of As compared to the WHO regulated limit of As in drinking water. A recent study by Huq et al. (2020) declared that the severe pollution of As in groundwater of Bangladesh was due to the excessive land usage for the purpose of agrochemicals and agriculture. As a consequence, the population who had been exposed to As showed 40 % higher in risk of having cancer than non-exposer. As is able to bind to DNA-binding proteins and causes DNA (Deoxyribonucleic acid) repair process to be damaged thereby accelerating the possibilities of carcinogenesis. Also, As has harmful carcinogenic impact on prostate glands, leading to the diagnosis of leukemia, prostate cancer and liver cancer caused by the occurrence of liver lesions in hepatic areas. Furthermore, As poisoning may also cause skin and Kupffer cell cancers in human body (Engwa et al., 2019).

### 2.3.2 Cadmium

Contaminated food and drinking water are the main exposure route of Cd towards human beings. Besides, inhaling cigarette smoke can also cause Cd exposure. Genchi et al. (2020) recommended an effective way to eliminate Cd from food stuffs through microbial fermentation. Incineration process of waste and phosphate fertilizers lead to another primary Cd source in the environment. As a result, cigarette smokers and non-smokers are showing a great discrepancy of Cd levels in their blood. The existence of Cd in human body tends to attack brain and results in Alzheimer's disease (Bakulski et al., 2020). Since Cd cannot be broken down by body system, it thereby accumulates at the kidney region after absorption and causes high concentration of urinary Cd in human. People who are working in pigment industries, electroplating processes and battery manufacturing have higher risk of exposure to Cd. Although there is only low amount of Cd exposure, it is considered as toxic due to long term accumulation of Cd in human body (Zhang et al., 2019). Cd is well known as potent carcinogenic as it leaves dangerous effects towards human's organs particularly in lung, prostate, pancreas and kidney. In addition, Cd is also hazardous towards gastric system and may cause gastric cancer, lung cancer, breast cancer and renal cancer due to the damaged excretory system. Several adverse health effects are also implicated by Cd such as oxidative stress, apoptosis, DNA damage and DNA methylation. The toxicity promoted by Cd may lead to decrease in density of bone mineral, initiating bone fracture (Briffa et al., 2020).

### 2.3.3 Chromium

Cr possesses several oxidation states ranging from $Cr^{2+}$ to $Cr^{6+}$. $Cr^{3+}$ has less toxicity and it is needed by human body in trace concentration for protein and lipid metabolism and acting as cofactor for insulin mechanism of action (Vincent, 2019). As opposed to that, hexavalent form of Cr such as $Cr^{6+}$ is the most common toxic forms and tends to be corrosive, causing allergic reactions, various pathologies and diseases to human body. Thus, inhaling excessive $Cr^{6+}$ may induce irritation of nose lining and lead to nose cancers. $Cr^{6+}$ can also lead to anemia, sperm damage and malfunction male reproductive system as well as irritations and ulcers in stomach and small intestine

(Engwa et al., 2019). Human exposes to high concentration of $Cr^{6+}$ can have severe respiratory, cardiovascular, hematological, hepatic, renal, neurological and gastrointestinal effects in body as well as possibly lead to death (Shekhawat et al., 2015). According to the reports of IARC on 2018, hexavalent Cr(VI) was categorized into Group I occupational carcinogen as they disclosed that out of 973,697 workers, approximately 11,000 of them were diagnosed with cancer (Deng et al., 2019). The major source for non-occupational exposure is through consuming contaminated food and water by Cr and also skin contact with Cr containing products. In addition, huge amount of Cr is released into environment by chemical, refractory and metallurgical industries which results in adverse health effect on human beings, animals and plants. This is because Cr has a tendency to bioaccumulate in human body and causes different diseases and disorders (Fang et al., 2014). Ulcer formation for example nasal septum ulcer is frequently happened in chromate workers. Moreover, exposure to high doses of Cr may cause suppression of erythrocyte glutathione reductase, which subsequently reduces the ability to convert methemoglobin to hemoglobin. DNA damage can also be induced in various methods in human body due to the high concentration of chromate compound, resulting in chromosomal aberrations, DNA adducts forms, conversion in exchange of replication sister chromatid and DNA transcription (Matsumoto et al., 2006). It is also proven that Cr exerts considerably toxic and carcinogenic by promoting stomach tumors in both humans and animals who were ingesting Cr containing water (Engwa et al., 2019).

### 2.3.4 Copper

Cu is normally required by many enzymes to perform their roles regularly and hence it is known as essential heavy metal elements. Cupronzymes promote changing states of $Cu^{+}$ from $Cu^{2+}$ via redox reaction. $Cu^{+}$ compound is toxic as it presents as superoxide and initiates the formations of hydroxyl radicals. The radicals formed can react with some biomolecules and trigger allergic reactions in human body (Briffa et al., 2020). Several studies also confirmed that Cu is capable of promoting breakage of DNA strands, causing bases to oxidize by hydroxyl radicals and oxygen free radicals. Besides, low-density lipoprotein (LDL) oxidation can also be induced by Cu as claimed by some *in* vitro studies (Engwa et al., 2019). Short term exposure of Cu causes gastrointestinal manifestations. Nearly all reports of acute Cu toxicity are from ingestion of contaminated food and drink. In the majority of cases, Cu may cause severe nausea, vomiting, diarrhea, failure of various organs, shock and possibly death have been reported in persons that were exposed to very high concentration. Effects of chronic Cu exposure are shown in serious disease condition which can be best described by Wilson disease which is an autosomal recessive genetic disorder owing to the Wilson protein's mutation. Accumulation of Cu in liver causes expression of the gene as Cu cannot be absorbed to ceruloplasmin and transported to bile system (Araya et al., 2007).

### 2.3.5 Mercury

Hg has been extensively studied and received attention worldwide because of its nature of toxicity and effortlessly enter into food chains. Hg normally presence in three forms which are metallic or elemental mercury ($Hg^0$), organic mercury including ethyl mercury (Et-Hg) and methyl mercury (Me-Hg) as well as inorganic mercury ($Hg^+$ and $Hg^{2+}$) (Li et al., 2017). Compounds of organic Hg is more toxic compared to inorganic compounds. Hg compounds have been widely used in mining industry for gold extraction and several industrial processes as well as in lamp manufacturing factory for producing fluorescent light bulbs. In addition, Hg pollution has also occurred in soaps and skin brightening creams. Mercury chloride ($HgCl_2$) is one of the essential constituents in skin lightning creams to clear away spots and freckles of the skin by inhibiting the tyrosinase action permanently which tends to induce melanin (Chen et al., 2020). In aquatic environment, microorganisms carry out biomethylation reactions naturally to form Me-Hg which enters the food cycle of marine living creatures and finally enters the human body by consuming the fish. An incident of organic Hg exposure due to polluted fish has happened in Minamata Bay, Japan and the illness is called as Minamata disease (Moriarity et al., 2020). Acute Hg toxicity can lead to neurological damage such as disturbance in chewing, swallowing and speech, muscle weakness, ataxia, numb limbs and increasing of tendon reflex for individuals that have been exposed to high doses of Me-Hg. Poisoned pregnant women have the possibility to give birth to infants with chronic developmental disabilities. Research from neural electromyography declared that occupational exposure to Hg could result in chronic injuries and peripheral nerves (Chen et al., 2019). Moreover, Hg prefers to accumulate in the kidney and leave detrimental effects exceptionally in the proximal tubule. There is a deleterious relationship between high Hg concentration in blood and regular alcohol drinking. Hg exposure causes impairment of cellular metabolism, oxidation stress and cell death due to damage of liver, increased liver enzymes in GGT (Gamma-glutamyl Transferase Test) level (Choi et al., 2017). Hg compounds are able to induce renal effects and causes impairment of renal function, especially in females and elders as reviewed by the study carried out by Lie et al. (2015). Cancers including lung cancer, skin cancer, brain cancer and colorectal cancer promoted by Hg is via the free radicals generation which induces oxidative stress and thus causes biomolecules damages, disruption of DNA molecules, DNA repair and maintenance system (Engwa et al., 2019).

### 2.3.6 Lead

Pb is a hazardous environmental contaminant which exert massive adverse effects on human health, especially to multiple body organs. Even though dermal contact is one of the exposure routes for Pb, it is primarily absorbed from digestive and respiratory system. An incident of elevated Pb level in blood occurred in pregnant women in Bangladesh with range of above 5 µg/dL as evaluated by Forsyth et al. (2018). They discovered that the main Pb exposure source was from Pb-soldered food storage cans. A recent study from Australia found the high Pb concentrations in children was due to the high Pb level in soil and petri-dish dust at their houses (Dong et al., 2020). Pb poisoning is toxic due to Pb exposure and it is generally correlated to the central nervous system and gastrointestinal tract in human. The international Level of Concern of lead poisoning is around 10 µg/dL in the blood. Pb poisoning can be in both acute and chronic situation. Acute Pb exposure may cause loss of appetite, fatigue, headache, hypertension, renal dysfunction and arthritis whereas chronic Pb exposure can cause allergies, autism, mental retardation, weight loss, birth defects, muscular weakness, brain damage, kidney damage, coma and possibly death (Martin & Griswold, 2009). Although lead poisoning can be avoided, it still exerts deleterious health effects by interrupting the function of oxidant-antioxidant system and promoting inflammatory actions on most organs in the body. Exposure of high Pb doses may result in moving of plasma membrane of the blood brain barrier into the interstitial spaces, causing edema. Besides, intracellular second messenger systems can also be disturbed by Pb exposure, retarding the performance of the central nervous system. According to a study conducted by Brown & Margolis (2012), disabilities in intellectual development have been manifested in children who was exposed to trace amount of Pb (5-25 µg/dL in blood), showing lower intelligence quotient (IQ) point.

## 2.4 Heavy Metal Determination Techniques

Environmental contamination by heavy metals has become a worldwide issue as it damages public health. Growing in anthropogenic activities including industrialization and urbanization which releases metal pollutants into the environment, leading to the continuous increment of heavy metal levels in soil, ground water, drinking water and food. Therefore, simple, rapid and highly sensitive analytical techniques to detect and efficiently remove heavy metals are exceptionally required for monitoring and controlling the environment (Timothy & William, 2019). Quantitative analysis of trace amount of heavy metals in the environment is required to actively monitor and control the external distribution, accumulation and exposure limit of heavy metals to the living organisms.

Various analytical methods have been used for heavy metal ions (HMIs) detection for their trace concentrations in the water environment such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS), laser induced breakdown spectroscopy (LIBS), X-ray fluorescence (XRF), ion chromatography (IC) together with UV-Vis spectrometry (UV-Vis) (Lu et al., 2017; Eddaif et al., 2019; Mohamad Nor et al., 2021). These conventional techniques offer several benefits such as sensitive, accurate, versatile which make them suitable for many elements detection as well as their good limit of detection (LOD). Although these spectroscopy techniques have their advantageous characteristics, they are costly instrumentation, complex sampling procedures, requires specialized operators, complex analytical processing, long analysis time and are inappropriate for on-site measurement (Eddaif et al., 2019). Different sorts of analytical methods to determine HMIs are briefly exemplified in the following sections.

### 2.4.1 Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrometry (AAS) is an instrumental analysis approach for both quantitative and qualitative detection of trace heavy metal elements in all sample's varieties. Its operation starts by passing a beam of light across the sample, thereby specific quantity of light is absorbed relying on the concentration of element. Eventually, the concentration of element can be calculated by comparing the intensity of the initial beam with the beam after passing the sample. Instrumentation of AAS owns individual light source for each element as each element absorbs specific wavelength of light. Figure 2.1 illustrates the principle and components of AAS which consists of light source, flame, monochromator, photomultiplier and detector. Generally, AAS only allows detection of single element per analysis. Hence, AAS has an advantage of determination of heavy metal element in a very low mass concentration and less labor-intensive due to its fully automated operation. AAS analysis can be conducted by flame (FAAS) or graphite furnace (GFAAS) which is dependent on the expected range of mass concentration and the quantity of sample available. FAAS requires a volume of sample of few mL while GFAAS only needs small volume of sample (10-30 μl). Thus, GFAAS is able to provide lower LOD as compared to FAAS but it takes longer time in analyzing a sample. AAS is normally working in a consecutive mode only for single element. It is useful in detecting certain heavy metal elements including As, Cr, Cd and Pb which exert adverse impacts on human health even in very trace amounts (Zeiner et al., 2007). A study of investigating the quality of drinking water was performed by Elhamili et al. (2016) by measuring the heavy metals level of Cd, Cu, Fe, Pb and Zn using AAS for a possible microbial contamination. The AAS was conducted by comparing the obtained result of tested heavy metal concentrations with the WHO standard. Sisay et al. (2019) declared that AAS was applicable in monitoring the presence of Cd and Pb in roadside soil samples with concentrations around 1.745 mg/kg and 43.42 mg/kg, respectively. The study showed that the high level of Cd and Pb in the examined area was mainly due to goals line effluent and vehicular emission.

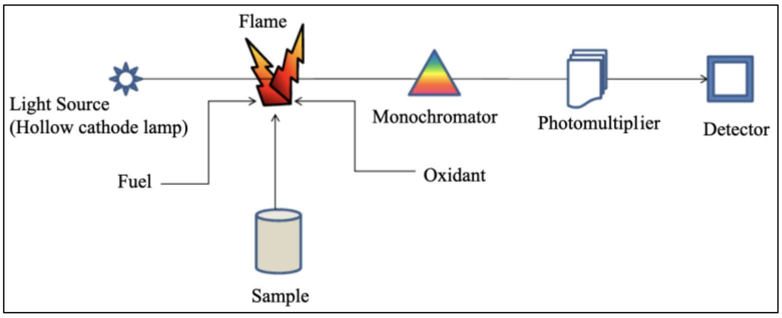

Figure 2.1: Schematic diagram showing the principle and components of AAS (Paul et al., 2017)

### 2.4.2 Atomic Fluorescence Spectrometry (AES)

Atomic fluorescence spectrometry (AFS) is an advanced analytical method to detect heavy metals by analyzing the atoms excitation in the vapor condition of an element with radiation at a specific wavelength and generating atomic fluorescence. The concentration in soil can be measured based on the intensity of fluorescence in which the concentration is directly proportional to the fluorescence intensity. In general, AFS is quite similar to AAS but the source of light is at 90 ° right angle to other components in order to prevent the radiation released from the excitation light source interfering with atomic fluorescence detection signal as shown in Figure 2.2. AFS has the advantage of both atomic absorption and emission. Nevertheless, this technique can only be used on 11 elements such as Hg, Se and As (Li et al., 2019). Thus, utilization of AFS in heavy metal detection is still needed further development as its earliest application field is only for coal, rock, stream sediment, soil and some minerals. AFS can be combined with other techniques and developed into Hg-AFS. Mao et al. (2016) utilized a dielectric barrier discharge reactor (DBDR) to capture As and integrated with Hg-AFS for detection, obtaining a LOD as low as 1.0 ng/L. An instrument of atomic fluorescence heavy metal monitoring was designed for remote monitoring of environmental water samples with great result (Zeiner et al., 2007).

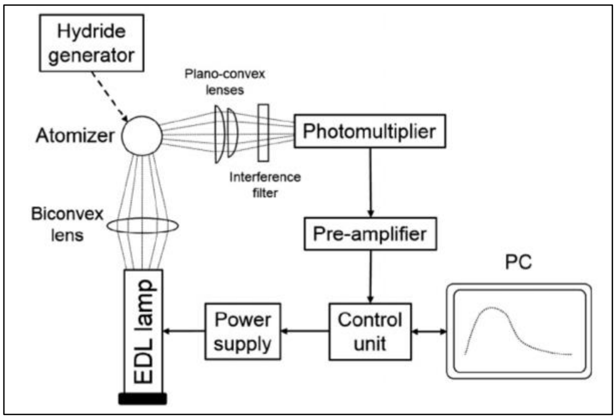

Figure 2.2: Schematic diagram showing the principle and components of AFS (Marschner et al., 2015)

### 2.4.3 Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Inductively coupled plasma mass spectroscopy (ICP-MS) is known as one of the advanced techniques in elemental analysis for detection of ultra-trace amount of heavy metals in broad sample types. Working principle of ICP-MS is by exposing a sample to plasma which is at high temperature, thereby emitting light and ions are formed. The light and ions can then be analyzed by a mass spectrometer (MS) which can separate the ionized atoms or molecules using the difference in mass-to-charge (m/z) ratio. Figure 2.3 displays the component and principle of ICP-MS. Almost all elements in the periodic table can be analyzed by ICP-MS in one single step and give very low LOD (Zeiner et al., 2007). ICP-MS has the advantages of simultaneous multi-element analysis, strong anti-interference, wide linear range, good reproducibility as well as highly sensitive which gives lower detection limit (ppt or ppq-range). However, the drawbacks of ICP-MS are the exceptionally high cost in operation due to the high usage of argon (Jin et al., 2020). ICP-MS is primarily used for the determination of HMIs in wastewater. An automated, remotely extraction and flow injection method of ICP-MS was proposed by Lagerström et al. (2013) in the determination of Co, Cu, Fe, Ni, Mn and Zn, simultaneously. The method of using ICP-MS in determining Co, Mn, Mo, Sb, Tl and V