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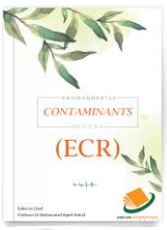

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REVIEW ARTICLE

A REVIEW ON THE IDENTIFICATION AND ANALYSIS OF HEAVY METALS IN HERBS USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICPMS)

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ABSTRACT

Pollution becomes a severe issue in Malaysia due to the economic development and increased industrialisation activities, with the most recent case of chemical residues that polluted the Kim Kim River, at Pasir Gudang, Johor in March this year. This serious situation may cause by various contaminants such as pesticides or chemical fertiliser for water pollution, smoke and emission from automotive for air pollution and solid waste for land pollution. The application of pesticides and chemical fertilisers in agriculture industry lead to heavy metal contamination in herbs products, thus reduces health quality. The government authority involves in monitoring and preventing the incidences uses suitable method in detecting the heavy metal contaminant such as Atomic Absorption Spectrophotometry (AAS), Inductively Coupled Plasma (ICP), and Neutron Activation Analysis (NAA). Other than that, Flame Atomic Absorption Spectrometry (FAAS), Energy Dispersive X-ray Fluorescence (EDXRF), and Electrothermal Atomic Absorption Spectrometry (ETAAS) were also used. Among the methods, ICPMS has been the most chosen and a convenience method to detect heavy metal contamination.

KEYWORDS

Heavy metal, Herbs, ICPMS, robust technique.

1. INTRODUCTION

Heavy metals are known as naturally occur components on the Earth. They are freely distributed throughout the environment or nature, especially in soil and water and can neither be degraded nor destroyed. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at high concentrations, they can lead to poisoning. To a small extent, they enter our bodies via food, drinking water and through the air.

Heavy metals are dangerous because they tend to bioaccumulate in body organs that lead to various health problems [1]. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds will accumulate in living things any time they are taken up and stored faster than they are broken down. These metals known as the significant chemical contaminants in plants that are intended for consumption [2]. The practise of using plants protection agents (e.g. pesticides) is one of the factors that cause the contamination of heavy metal [3].

Herbs or medicinal plants have been universally used as a treatment to cure various diseases. The beneficial compounds including alkaloids, tannins, flavonoids and phenolic compounds in this type of plants make them more desirable to be consumed and obtained people's satisfaction as remedies [4,5]. They are subjected to be contaminated by heavy metals through the soil, water and air [4]. As plants took up elements through the root, there is a possibility for the heavy metals to be assimilated as well. The heavy metals will then accumulate throughout plant tissue and consumed by man and lead to various chronic diseases. Therefore, the analytical method is necessary in order to ensure herbs are free or contain a safe amount of heavy metal to be consumed by human. Inductively coupled plasma mass spectrometry has been chosen as the primary method to analyse multi-elements in an aqueous or liquid solution. Also, some steps need to be included before the analytical method to ensure the accuracy of the result.

1.1 Heavy Metals

Generally, the term heavy metal refers to any metallic chemical elements with relatively high density, which is greater than 4.5 g/cm^3. Other than that, heavy metals are known for their toxic or poisonous characteristic, even at low concentrations [4,6]. These metallic elements are known as essential pollutants to the environment as well as the food supply. Metals which are classified as heavy metals include copper (Cu), cobalt (Co), iron (Fe), zinc (Zn), nickel (Ni), mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). These metals may further divide into essential and non-essential elements.

The essential elements or microelements which consists of Cu, Se, Zn, Fe, Cr and Co are crucial for various metabolic processes and are closely linked to human growth and general health for instance, copper and zinc are needed by the body for normal physiological functions [7,8]. These essential metals usually occur in trace amount. Hence, their deficiency as well as excess, will significantly affect the living organisms, which could cause physiological disorders [9].

While, for the nonessential elements are elements with unknown function towards biological system [9]. Mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) are considered as non-essential elements with known adverse

effects. A group researcher mentioned that those stated heavy metals are known as the "Big Four" heavy metals and considered as the first category of element which are considered as highly toxic elements as well as have mutagenic effects even at low concentration [4,10]. Moreover, some researchers also claimed that cadmium, mercury and lead as the most important contaminants of the food chain and problems to the environment [11].

1.2 Heavy Metals Sources

The presence of heavy metals entering the environment is due to natural processes and through human activities. The migration of heavy metals in the environment are mainly related to volcanic activity and weathering, dissolution, vaporisation; soil erosion, natural weathering of the Earth's crust; rock decay, volcano eruptions, evaporation of oceans, forest fires and soil formation processes [6,9,12].

Heavy metal pollution caused by human activities is mostly related to agriculture and industrial waste. The source of heavy metals may naturally originate from the soil or water, which are the result of the mineral content of the soil or source of the water. However, they also may be artificially introduced by the application of plant protection agents such as fertilisers, herbicides, and pesticides which are commonly used by farmers. According to a study, the soil is commonly subjected to this contamination through the discharge of heavy metals, mainly from industrial activities [4]. Furthermore, heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. The contamination may cause by different branches of industry like power industry, transportations, municipal waste management, waste dumping sites, fertilisers and waste used to fertilise soil [6].

1.3 Heavy Metals and Human Health

Toxic heavy metals have proven to be a significant threat to human and have been known to cause various serious diseases. They tend to accumulate in the food chain. High concentration or overconsumption of heavy metals is the main reason for the association of many diseases and health problems. Plus, kidney is the critical target organ because heavy metals excretion rate is very slow that promotes their accumulation for a relatively long time and leads to the damage effects on human even at very low concentrations [13]. Their toxicity depends on several factors including the dose, route of exposure as well as the age, gender, genetics and nutritional status of the consumers [2].

Some group researcher mentioned on the harmful effect of heavy metals after entering the human body that could result to malfunction and may interrupt with the normal function of central nervous system, liver, lungs, heart, kidney, and brain and lead to hypertension, abdominal pain, skin eruptions, intestinal ulcer, and different type of cancers [6]. Other than that, it can also promote to the permanent intellectual and developmental disabilities as well as reading and learning disabilities, behavioural problems, loss sense of hearing, a problem in paying attention and interruption in the development of a visual and motor function.

As lead (Pb) and cadmium (Cd) are known as the significant contaminants in the environment, their accumulation is of most dangerous to human health. Some researchers in their study mentioned that, lead especially to attack the function of the brain or the central nervous system [8]. The exposure to lead can also leads to anaemia and cause slow growth, learning disabilities, as well as antisocial behaviour. While, the contamination related with cadmium usually accumulate in the kidneys and liver and thus will severely damage the organs that can be indicated by formation of high levels of urea in blood, as well as it affects the bone by disturbance with calcium and phosphate also has the carcinogenic effects [14].

2. HERBS

The word herbs derived from the Old French word "herbe" and the Latin word "herba", has already been used over 4000 years by European and Mediterranean cultures as a medicine to treat diseases. Initially, herbs were categorised as the non-woody plants; however, nowadays, this traditional medicine refers to any part of any plants that it could be the flower, leaf, bark, root or even its fruit. Other than their exclusive benefits as medicine, herbs have widely been used in cooking to enhance the aroma and flavour of the dishes, for example, black pepper, cinnamon, ginger, and many more.

Herbs contain nutritional value(s) which are essential for the human body. The presence of essential phytochemical content like alkaloids, tannins, flavonoids and phenolic compounds make herbs the valuable plants [4]. Moreover, the herbs' content of active organic compounds of varying structures have the remedial powers to use as a medicine [7]. Thus, these plants have been used widely in the prevention and treatment of several health diseases such as cardiovascular diseases, inflammatory diseases, arthritis, diabetes and others [15].

2.1 Herbs and Heavy Metal Contamination

All plants are subjected to expose with various kinds of pollutants, especially with pesticides, microbial contaminants, heavy metals, chemical toxins and adulterated with conventional drugs during growth and processing [7]. Herbs have been associated with the transfer of trace elements from soil to man. The heavy metals contamination in herbs or medicinal plants are at different concentrations for different places, and this is because their contents are depending on the geographical environment of soil. The concentration of heavy metals throughout the plants is also not uniformly distributed with roots contained the highest level of heavy metals followed by vegetative tissue.

Plants uptake or accumulate minerals which are essential for growth (Fe, Mn, Zn, Cu, Mg, Mo, and Ni) from the environment [16]. This bioaccumulation feature of plants results in the accumulation of some unwanted or unknown biological function elements includes lead, chromium, cadmium, mercury and many more. A researcher in his work stated that heavy metals accumulation in plants may cause by several factors including the type of plant, nature of soil, climate and agriculture practices [17]. Also, in other study they had emphasize that herbal drugs might be contaminated throughout growing and processing [18].

As the heavy metals impact the quality of human life as well as other biological creatures, their content in edible plants and medicinal purposes must be limited. Health authorities has started listed the recommendation for limits of heavy metals in consumable plants since 1991 by German Ministry of Health with lead 5 mg/kg, cadmium 0.2 mg/kg, mercury 0.1 mg/kg, with certain exemptions for cadmium in linseed, hawthorn and yarrow 0.3 mg/kg, and 0.5 mg/kg for birch leaf, St. John's wort, willow bark and mate [19]. Meanwhile, the World Health Organisation (WHO) recommends limits for various medicinal plants with no more than 10 mg/kg for lead and 0.3 mg/kg for cadmium.

3. HEAVY METAL DETECTION

The safety and quality of herbs to be consumed need to take into consideration in order to protect the consumers. Therefore, heavy metal analysis is an important parameter to ensure the consumable plants are safe to be ingested. There are many studies related to the analysis or detection of heavy metals in herbs such as analysis of toxic elements in herbal tablets, analysis of "Big Four" heavy metals in Seabuckthorn leaf extract [2,10]. Besides that, a group researchers determine the concentration of heavy metals and compare the result with the international permitted levels [8]. Moreover, a study on determination of wholesome elements (Al, Ca, Co, Cr, Fe, Mg, Mn, Mo, Ni, P, Se, Sr, V and Zn) as well as heavy metals in safflower (*Carthamus tinctorius L.*) which is a traditional Chinese medicinal herb from Xinjiang and Henan province of China [20].

3.1 Analytical techniques

Quantitative analysis of heavy metals has been established for determining the quality of medicinal plants as well as herbs. World Health Organization (WHO) has developed guidelines for the preparation of herbs, including procedures for the standardisation of herbal medicine

and also for assessing the quality of herbal medicines [21]. Therefore, the existence of available methods or techniques to analyse the presence and quantity or level (concentration) of heavy metals is vital in order to ensure the safety of the herbs. For example, the primary methods commonly used for quantitative analysis of toxic heavy metals are Atomic Absorption Spectrophotometry (AAS), Inductively Coupled Plasma (ICP), and Neutron Activation Analysis (NAA) [2]. Other than that, Flame Atomic Absorption Spectrometry (FAAS), Energy Dispersive X-ray Fluorescence (EDXRF), and Electrothermal Atomic Absorption Spectrometry (ETAAS) are also used [17].

3.2 Inductively Coupled Plasma (ICP)

Generally, ICP has been commercially available for almost 50 years and is widely used for the measurement of trace metals in variety kinds of solutions. ICP is chosen as the instruments for heavy metal detection and can be divided into two techniques which are inductively coupled plasma – optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS). ICP has been chosen over other techniques abovementioned.

3.3 Inductively Coupled Plasma- Mass Spectrometry

Between the two mentioned ICP techniques, inductively Coupled Plasma Mass Spectrometry (ICPMS) has been widely used. The ICPMS has been the most targeted method due to its high sensitivity and specificity and it is the most reliable and an effective method for multi-element determination [2,17,20]. ICPMS is relatively a new method for the identification and analysis of trace multi-elemental and isotopic concentrations in liquid, solid, or gaseous samples. Besides that, there are two different types of ICPMS, which are quadrupole ICPMS and high-resolution ICPMS [22]. This machine can be used to analyse many kinds of the sample, including biological sample (e.g. blood, serum and urine), environmental sample (e.g. soil, sewage and industrial effluents), food sample (e.g. seafood, vegetables and wine) as well as chemical reagent [23]. Moreover, the sample used must be appropriately treated before introducing it into the machine.

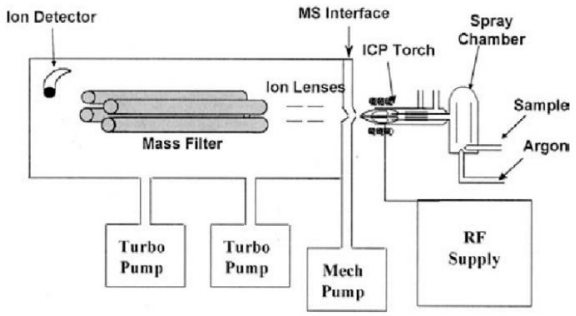

Figure 1: Basic instrumental components of ICPMS (Thomas,2004)

The necessary components of this instrument are sampling interface, a peristaltic pump leading to a nebuliser, a spray chamber, a plasma or ICP torch, a detector, an interface, and ion- focusing system. The basic operation of ICPMS starts with a sample pump into the nebuliser, which is to convert the sample into a spray. Then, the internal standard such as germanium pump into a mixer along with the sample before nebulization to compensate for matrix effects. Large droplets are filtered out, and small droplets will continue into the plasma torch where ionisation occurs. The mass separation device will then separate the ions according to their mass-to-charge ratio. Finally, an ion detector converts the ions into an electrical signal, which is multiplied and read by computer software [24].

3.3.1 Sensitivity

This technique has advantages over inductively coupled plasma optical emission spectrometry (ICP-OES) due to its ultra-high sensitivity and specificity. ICPOES has been introduced in the mid of 1970s and also known as inductively coupled plasma atomic emission spectrometry [22]. The advantages of this technique also mentioned, where the ICPMS offers high sensitivity as well as simultaneous multi-element analysis capability [20]. They used both ICPMS and ICPAES to determine the presence of heavy metals as well as the low level of elements and to measure the higher concentration of elements respectively. Both ICPMS and ICPAES methods are high-speed and high-throughput multi-element analysis, however, the difference in ion formation and detection methods have a significant impact on the sensitivities of the two techniques. ICPMS is relatively more sensitive than ICPAES as the detection of 1-10 ppt as compared to ICPAES with 1-10 ppb of the detection limit. Their results showed that the concentration of all studied heavy metals in safflower samples in both regions were at low and met the national hygiene standards except for Pb in Xinjiang sample.

3.3.2 Advantages and Disadvantages

Inductively coupled plasma mass spectrometer known to be the best method for the analytical study of various trace elements, including heavy metals. It is the most reliable method over the other methods despite its expensive operation cost. Table 1 summarises the important features of several methods including ICPMS, ICPOES, AAS and NAA. As refer to the table, the advantages of ICPMS over the other methods are low detection limit up to parts per trillion (ppt) in the range of 0.01 to 0.1 mg/L for many elements which is a favourable detection limit, require simple sample preparation, high throughput (can load about 40 samples per hour) as well as can measure more than one element simultaneously or multi-elemental analysis [23].

A major limitation of ICPMS is that it is an expensive, complicated and high maintenance machine [22]. Other than that, spectroscopic (molecular ions interferences from other matrix constituents) and matrix (large amount of other matrix constituents that affect the ionising conditions in the plasma) interferences are problems that could arise during the operation [24]. Heavier elements such as lead are more suitable for ICPMS analysis as compared to lighter elements which are prone to interferences. Chromium and iron are the examples of elements which are not amenable to assay by ICPMS. Moreover, the intensity of the signal varies with each isotope, and there is a large group of elements that cannot be detected by ICPMS.

The elements consist of hydrogen (H), helium (He) and most gaseous elements, carbon (C) and elements without naturally occurring isotopes, including most actinides. Other kinds of interferences that may occur with ICPMS is when plasma-formed species have the same mass as the ionised analyte species [25]. However, these interferences are predictable and can be corrected with element correction equations or by evaluating isotopes with lower natural abundances. Using a mixed gas with the argon source can also alleviate the interference. Besides that, the accuracy of ICPMS is highly dependent on proper skill and technique. Last but not least, preparation of the sample and standard need the utmost care to avoid conflicting species that can be formed in the plasma and produce false positives. Table 1 summaries each method advantages and disadvantages.

Table 1: Comparison of important features among different methods (ICPMS, ICPOES, AAS, & NAA)

Types of Method / Specification	Inductively coupled plasma mass spectrometry (ICPMS)	Inductively coupled plasma optical emission spectrometry (ICPOES)	Atomic Absorption Spectrophotometry (AAS)	Neutron Activation Analysis (NAA)
Detection limit	Parts per trillion (ppt)	Parts per billion (ppb)	Parts per billion (ppb)	A few parts per million (ppm) to a few parts per billion

Sample throughput	High	High	High	High
Sensitivity	Highly sensitive	Sensitive	Sensitive	Very sensitive
Numbers of detected elements	82	73	70	74
Multi-element detection	Yes	Yes	No	Yes
Cost	Expensive	Less expensive	Less expensive	Less expensive
Form of sample	Liquid, solid and gaseous (with correct sample preparation)	Solid sample (e.g. metal, trace minerals, food substances)	Liquid only (solid sample need to undergo appropriate sample preparation)	Solid, liquid, suspension, slurry and gases with minimal preparation
Volume of sample required	Very small	Small	Small	Small
Time of detection	Rapid	Fast	Fast	Fast
Method of development	Difficult to handle (should be monitored by a specialist)	Simple and easy to handle	Simple to handle	Simple to handle

4. CONCLUSION

High content of essential phytochemical compounds (alkaloids, tannins, flavonoids and phenolic compounds) make herbs the most interesting remedies for many diseases. As people widely consume herbs as medicine, the studies on the safety of herbs intake are of importance. Health authorities of every region have listed the recommendation limit of toxic metals in consumable plants. Meanwhile, the standard limit listed by WHO are 10 mg/kg for lead while, 0.3 mg/kg for cadmium. The presence of various analytical techniques makes the detection of heavy metal toxicity in medicinal plants easier. The analytical methods to determine the concentration of heavy metals in food chain are one of the effective ways to ensure the quality of consumable herbs or traditional herbs, even medicinal plants. Consequently, essential trace elements can also be detected which are needed for human body function. Inductively coupled plasma mass spectrometry (ICPMS) is a commonly used method due to its high sensitivity and specificity.

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