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RESEARCH ARTICLE

HEAVY METAL CONTAMINATION IN VEGETABLES AND ITS DETECTION: A REVIEWNur Illia Mohd Ruzaidy^a, Azura Amid^{b*}^a Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang Darul Makmur, MALAYSIA^b International Institute for Halal Research and Training (INHART), Level 3, KICT Building, International Islamic University Malaysia, Jalan Gombak, 50728 Kuala Lumpur, MALAYSIA*Corresponding author email: azuraamid@iium.edu.my

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ABSTRACT

The quality of marketed vegetables cannot be guaranteed as many of them has been contaminated with heavy metals such as lead, cadmium and arsenic. These heavy metals can act as either growth supporter or threat depending on their types and amount absorbed by the plants. The consumption of an excessive amount of heavy metals in vegetable may cause several diseases such as renal dysfunction and bone depletion. There are few methods used to detect heavy metal contamination such as Atomic Absorption Analysis (AAS), Neutron Activation Analysis (NAA), X-ray Fluorescence Spectroscopy (XRF) and Inductively Coupled Plasma Spectrometry (ICPMS). This paper discusses all these methods to compare their efficiency, advantages and disadvantages to select the best method for heavy metal detection in vegetables.

KEYWORDS

Contamination, heavy metal, ICPMS, vegetable.

1. INTRODUCTION

Vegetables are a typical food that is consumed by human. It consists of several categories such as allium, fruit, roots and leafy vegetable. Furthermore, it is also essential for human consumption as it contributes many advantages to the consumer such as vitamins, minerals, fibres, antioxidant and antibacterial agent (Ali and Al-qahtani, 2012). Thus, vegetables are widely planted and become one of the most important crops in agricultural industries. In order to produce enough quantity of vegetables, several technologies are used to ensure the growth and development of the vegetables are enhanced and protected. Meanwhile, the community in rural areas are still planting the vegetables by using the traditional method. These vegetables are collected for sale at the market while the vegetables that are available at the supermarket commonly come from bigger planting areas as they need greater product quantity.

However, the quality of these vegetables is highly doubted due to several types of pollution that occurred in many areas. The usage of a chemical substance containing many types of heavy metals affects the health of the soil. These heavy metals are not only disrupting the soil properties and its microbial community but also affecting the vegetables when they take up the elements from the soil into their tissue (Sobolev and Begonia, 2008). Besides, the vegetables can be contaminated by heavy metals through air pollution. The air which is polluted with dust containing harmful metal compounds will directly infect the exposed parts of the plants (Seyyednejad et al., 2011).

Therefore, the heavy metals contaminated vegetables will no longer serve as a nutritional source only but also introduce dangerous pollutants into the human body. The heavy metals are not easily removed from the body. Instead, they will accumulate in the blood and tissue for an extended period (Rc, et al., 2015). The exposure of heavy metals inside the body will lead to many types of disease such as brain damage, kidney failure and infertility (Järup, 2003).

2. HEAVY METALS

Heavy metals are the metallic elements other than alkaline and alkaline earth element that possess contamination and potential toxicity or ecotoxicity (Karshman, 2002). It is high in density and relative atomic weight. Usually, heavy metal has greater than 20 atomic number (Raskin, et al., 1994). Other than that, it has long biological half-lives and undegradable, neither by microbial nor chemical degradation (Kirpichtchikova, et al., 2006; Radwan and Salama, 2006). However, heavy metal can change in terms of its chemical forms and bioavailability. The rate of transport of heavy metal in soil depends on two factors: its chemical form and speciation of metal (Wuana and Okieimen, 2011). It will accumulate once it is introduced into the soil, and the concentration will either remain or increase within an extended period.

Heavy metals usually found at the contaminated area, and most of them are lead, chromium, arsenic, zinc, cadmium, copper and mercury (Washington, 1997). Lead and cadmium are used in batteries manufacturing. Meanwhile, arsenic is found the most in the ashes of coal

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combustion. This compound is adsorbed strongly to soils and transported only over a short distance in groundwater or surface water (Wuana and Okieimen, 2011). None of these metals contributes to plants growth. Nevertheless, they are the toxic agents to the crops. They cannot be removed entirely as some of them initially exist in plant, and some of them are taken up by the plants from the environment (Elbagermi, et al., 2012). Therefore, the crops are frequently exposed to these harmful elements which can affect not only the plants but also the consumers.

2.1 Factors That Lead to Heavy Metal Contamination

2.1.1 Agriculture

Chemical substances are widely used in agriculture to combat any damaging agent that can affect yield production. However, these chemicals are indirectly possessing significant risks to plants and consumers. The fertiliser is added to the soils regularly to ensure that the plants receive an adequate source of nitrogen, potassium and phosphorus but it also supplies harmful trace elements such as cadmium and lead as impurities. Besides, lead and arsenic are the main ingredients used in a mixture of pesticide. Lead arsenate was introduced for fruits planting to control some parasitic insects. Besides, the arsenic-containing compound was also extensively used to control the cattle ticks and pest in banana in New Zealand and Australia with the formulation of copper, chromium and arsenic (CCA) (Wuana and Okieimen, 2011).

These agrochemical sources spread the pollutants, and different types of heavy metal are being released from them (Kayastha, 2007). A study shows that a long exposure of chemical fertiliser and pesticide to the agricultural site in Iran caused the bulk density of the soil increase in a long term due to accumulation of heavy metal (Yarhgoi and Azarneshan, 2014). Cadmium concentration in the soil has exceeded over the standard limit of safe agricultural soil, which marks that the crops produced from the soil are not safe to be consumed. Thus, the pesticide and fertiliser usage in agriculture must be controlled or replaced with safer alternatives for human consumption (Yargholi and Azarneshan, 2014).

2.1.2 Air pollution

The air is polluted by the gas or smoke released from the industrialisation and vehicles (Radwan and Salama, 2006). The metals such as arsenic, cadmium and lead can volatilize during high-temperature processing, which potentially escapes through the air. They will convert into oxides and condense into fine particulates (Wuana and Okieimen, 2011). Through the use of the automobile, the metals containing smoke and combustion of diesel oils and tyres can pollute the vegetable (Seyyednejad, et al., 2011). The dust containing harmful heavy metals penetrates the plants through the plants' exposed area. Most of the vegetables that are planted near to the roadside are contaminated with lead (Aktaruzzaman, et al., 2013; Naser, et al., 2012). Some researchers also identified that the rice and cucumber, which are planted near to the traffic side at Saudi Arabian contained high lead concentration (Ali and Al-Qahtani, 2011). Even though the vegetables might not be treated with chemical pesticide or fertiliser, they are still risky for human consumption due to heavy metal contamination from the polluted air (Ali and Al-Qahtani, 2012).

2.2 Heavy Metal and Consumer Health

Heavy metals can remain in the human body and accumulate into a considerable amount that will bring damage to the body system. Consumption of heavy metal polluted vegetables will disrupt the body chemistry because these metals will not undergo decomposition process within the body. They also have a high affinity towards the body system, which enables them to remain longer in the body. Oral exposure is the most compelling way for those heavy metals to enter the human body and cause disruption (Seidal et al., 1993). The polluted soil and air that transfer the heavy metals into the plants indirectly involve the heavy metals in the food chains that later will harm the consumer (Yang, et al., 2018).

Heavy metals are carcinogenic and able to cause severe disease. The binding of lead to the erythrocyte can remain about 1 month and accumulate in the skeleton for about 20 to 30 years (Järup, 2003). The

introduction of lead into the human body will disrupt haemoglobin and anaemia biosynthesis, an increase of blood pressure, miscarriage, nervous system disruption and brain damage (Rc et al., 2015). Besides, cadmium can cause kidney and skeletal damage. In 1950, the cadmium exposed worker had developed decrease glomerular filtration rate (GFR) (Liang et al., 2012). Furthermore, a case was reported in Japan; people were infected with the *itai-itai* disease caused by the consumption of local rice that uses cadmium-contaminated water for irrigation. Meanwhile, the intake of arsenic can cause an individual to have the gastrointestinal symptom, severe disturbances of the cardiovascular and central nervous system (Järup et al., 2000).

A group researcher's investigates the health of residents at the mine polluted area at Daye, China, based on their consumption of heavy metal contaminated vegetables (Yang et al., 2018). The results show that the total hazard index (HI) and carcinogen risk (CR) for adults and children in the contaminated area is higher than that in the reference area. Besides, the CR of heavy metals for children is significantly higher than that for adults ($p=0.03$). This is because the children are more easily exposed and infected with heavy metals, and they have weaker immunity system than adults. Based on these data, the residents at the mine contaminated area are exposed to a severe health risk with a mean of 77% contribution of arsenic and cadmium.

2.5 Vegetables and Heavy Metal Contamination

The heavy metals that are naturally occurring from plant do not possess many harms as the others that come from chemical or metallic sources (Rc et al., 2015). The presence of heavy metals can be either beneficial to soil or vice versa. They have their role in supporting the growth and development of the plant. For example, copper and zinc are essential for biochemical and physiological functions (Radwan and Salama, 2006). However, these heavy metals are only needed in a certain amount. Otherwise, they will end up act as the killer of the plants. There are also certain types of heavy metals that do not bring any function to plants such as Cd and Pb (Chibuiké and Obiora, 2014). Even though they present in a small amount, their presence will lead to the death of the plant. This scenario is known as heavy metal contamination.

Besides, heavy metals can change the properties of the soil (Chibuiké and Obiora, 2014). Other than providing the nutrient for the plants, the soil can serve as the source of pollutants when it is contaminated (Bradl, 2004). In order to observe either the soil is contaminated or not, it can be identified by measuring the changes of soil microbiological and biochemical properties (Chibuiké and Obiora, 2014). The soil microorganism will be affected from the aspects of its number, diversity and activities (Sobolev and Begonia, 2008). Meanwhile, the degree of toxicity of the heavy metals on the microbes depends on soil temperature, pH, clay minerals, organic matter, inorganic anions and cations and chemical forms of the metal (Chibuiké and Obiora, 2014). When the soil is exposed to long term heavy metal contamination, it affects the microbial activity, including the respiration and enzyme secretion (Sobolev and Begonia, 2008).

For example, disruption of the denitrification cycle can occur due to heavy metal exposure as the cycle involves the action of soil microorganisms (Chibuiké and Obiora, 2014). Briefly, denitrification is a natural microbial process that converts nitrate into dinitrogen during anaerobic respiration. The metal inhibits the action of nitrous oxide reductase resulting emission of nitrous oxide that can damage the ozone layer. Suppression of denitrification in the soil will cause the nitrogen cannot be converted and flush into the environment, causing nutrient pollution. This nutrient pollution will then lead to eutrophication and algal blooms, which will give negative impacts on agricultural needs (Chibuiké and Obiora, 2014). Therefore, the development of healthy plants can be affected due to loss of their needed compound when heavy metal contaminates the soil.

The availability of heavy metals in plants is related to soil contamination because plants can take up the element in the soil (Nergiz and Ergonul, 2014). Plants usually absorb several compounds from the soil that are beneficial for their growth and survival. However, if those heavy metals

contaminate the soil, the plants are not able to efficiently separate the needed compounds from the toxic elements; thus, they take up both beneficial and harmful compounds into their tissues. Plants usually only take up one or two types of heavy metals from the soil solution. However, it depends on several factors such as metal forms, plant species and parts and soil properties.

The pH of the soil will determine the solubility of the heavy metal to be taken up by the plant, and it varies according to the types of metal (Tangahu et al., 2011). The higher the concentration of the heavy metal in the soil, the higher the rate of uptake by the plants. However, when the uptake is excessive, it will cause the roots of the plants to be injured and lowered the absorbing ability. Therefore, in order to determine the heavy metal contamination, the measurement of heavy metal content solely in the seeds are not accurate. This is because the root acts as the barrier of the heavy metal's translocation (Rc et al., 2015).

A study shows that the leafy vegetables such as spinach, amaranth and potato leaf are profoundly affected by heavy metals contamination compared to other types of vegetables such as cowpeas and pepper (Yang et al., 2018). The heavy metals do not only come from the soil but also released by smelting waste gases. Thus, the roots and fruits type vegetable has a lower level of contamination than leafy vegetable because leafy vegetables are contaminated through two ways, soil and waste gases. Besides, the edible part of the leafy vegetables shows the highest level of heavy metals. Thus, they concluded that heavy metal contamination also depends on the difference in the growth stage (Yang et al., 2018).

Moreover, a study reported that Jews mallow and arugula have a higher level of manganese than kidney beans, haricot and peas (Ali and Al-Qahtani, 2012). They also reported that the Cu also presented the highest in Jews mallow, spinach and arugula followed by stem and roots vegetables. The lowest amount of Cu was observed in cereals and fruit type vegetable. Based on the result, they concluded that leafy vegetable has a higher potential for toxic metal accumulation compared to the other parts of the plants. The cadmium from the fertiliser has a higher capacity to be absorbed by spinach, cabbage and broad-leaved mustard (Kayastha, 2007). Another study that supports this result is the study when they figured out that the level of Cu is higher in leafy vegetables than non-leafy vegetable species collected in Turkey (Aksoy and Demi, 2005).

2.6 Heavy Metal Contamination Incidents In Vegetable

In 1985, the tailing dam in Chenzhou had collapsed causing 400 metres crops cultivated farmland covered with mining waste spills. The researchers investigated the quality of several agricultural products, including cereal, pulses and vegetables at the affected area seventeen years later. The results showed that the soils collected from several affected regions were still heavily polluted with arsenic, cadmium, zinc, lead and copper. Besides, the edible leaves and stems of crops contained the highest concentration of heavy metal rather than seeds or fruit. Ipomoea is the most contaminated vegetables among all the samples. The concentration of cadmium and lead in ipomoea exceeded the maximum permissible levels by 6.6 and 8.5 times, respectively. Meanwhile, taro recorded the highest concentration of zinc and cadmium. Therefore, the crops grown in Chenzhou mine waste affected area were still not safe for consumption (Liu et al., 2005).

Also, on March 11, 2011, the Tohoku region in Japan was struck with a tsunami due to the 9.0 magnitude of the earthquake which causes the spread of radioactive material from Fukushima Daiichi Nuclear Power Station. The impact of the nuclear accident affected the agricultural industry at Fukushima badly. The radioactive waste potentially spread the heavy metals to the affected areas as occurred in the Savannah River Site (Carl, 2001). In the year of the earthquake, the vegetables, for examples, spinach, grown at Fukushima exceeded the reference value for radioactive mineral. They were considered safe only three years after the disaster. The spinaches were no longer exceed the reference value for radioactive material and safe for human consumption.

3. HEAVY METAL DETECTION

3.1 Atomic Absorption Analysis (AAS)

AAS is an effective method to determine a very low concentration of metals. It is fast and straightforward to determine the presence of metals in the samples. It has two different techniques which are flame AAS (FAAS) and electrothermal AAS (ETAAS). FAAS will continuously send the absorption signal while for ETAAS, the signals will be in discontinuous trend, which needs two to four minutes per sample. The sample must be diluted usually with dilute acid or xylene solution. The sample preparation takes more extended period because AAS cannot directly analyse the solid sample. Instead, the samples must be in liquid form. This sample will be introduced to heat for vaporisation of atoms, leading to changes in sample state from liquid to gas.

Conventional AAS (FAAS) requires high temperature (23000c) to vaporise the liquid sample. Then, the concentration of elements will be measured by the absorption of electromagnetic radiation by the atoms at a specific wavelength. AAS offers limited interferences and high sensitivity in its detection. However, the exposure of the sample to heat might cause chemical interference due to the formation of oxides in the flame. This chemical interference can be corrected by modifying the sample dilution. Hence, AAS is not recommended to analyse samples containing a large number of elements as it only can analyse one element at one time. Thus, the analysis will be longer. It is also not used to detect light elements such as H, C, N, O, P and S, halogens and inert gases.

3.2 Neutron Activation Analysis (NAA)

Neutron Activation Analysis (NAA) is an instrument that use irradiation system to detect trace elements. The sample is introduced to neutrons in a nuclear sample. The nucleus in the sample absorbed the neutrons and converted into a radioactive nucleus. These radioactive nuclei will emit specific gamma rays which are used as an indication of elements that presents in the sample. The samples are transparent to the probe (neutron) and analytical signal (gamma-ray). Thus, it eliminates matrix interference. However, other interferences might arise. The different component of the sample can deliver a similar gamma-ray signal that will cause inaccurate detection. NAA is a highly sensitive method, but the problem occurs when some elements are unable to absorb the neutron. Consequently, the undetected component will be considered as absent in the sample. Besides, NAA does not require any sample pre-treatment that possess contamination risk to the sample. It is also capable of reading the multi-element analysis in one run (Parry, 2005).

3.3 X-ray Fluorescent Spectrometer (XRF)

The heavy metal elements can also be detected by using X-ray Fluorescent Spectrometer (XRF). The sample will be introduced to X-ray beams irradiation to excite the atoms. When these atoms are excited, they will become unstable and emit energy called ad fluorescent radiation. The fluorescent radiation reflects the characteristics of a transition between specific electron orbitals in an element. Thus, the identity of that element can be identified (Wirth, K. & Barth, A., n.d.). There are many types of XRF, including direct *in-situ* portable X-ray fluorescence (PXRF), energy dispersive X-ray fluorescence (EDXRF) and total reflection X-ray fluorescence (TXRF).

The combination of different types of XRF allows a comprehensive analysis of bulk and particulates samples. XRF is a fast, non-destructive and multi-element analysis method (Bamford et al., 2004). It can analyse large numbers of both major and trace elements. However, it is not suitable to analyse the elements with atomic number less than 11. Also, XRF sometimes requires other instruments to further analyse the same elements but with different characteristics. For examples, the presence of isotopes cannot be differentiated by XRF. Instead, other techniques are needed like TIMS or SIMS to characterise the isotopes. Besides, XRF cannot identify the same elements with different valence state of ions, but this can be done via wet chemical analysis or Mossbauer spectroscopy (Wirth, K. & Barth, A., n.d.).

3.4 Inductively Coupled Plasma Mass Spectrometry (ICPMS)

Inductively Coupled Plasma Mass Spectrometry (ICPMS) is a combination of ICP and MS that is used to detect the low concentration of an analyte in the sample. ICPMS is equipped with a concentric nebuliser, a quartz torch with quartz injector tube and cyclonic spray chamber. Food samples typically need to be digested from solid into the liquid sample (Bressy et al., 2013). The liquid samples are introduced into the ICP through its nebuliser. Then, the liquid sample is nebulised to produce the aerosol that is transported by the high temperature of Argon gas into the plasma torch (Bazilio and Weinrich, 2012). Ions will be produced along the process and will be detected by mass spectrometry (MS) based on mass to charge ratio (Ilego, 2012). ICPMS is a rapid and sensitive technology which can detect the presence of an analyte in the samples as low as 1 part per trillion (Ilego, 2012). The inductive argon coupled plasma ICP serves as the high-temperature ion source. The argon ICP can ionise most metals at 80% to more than 90% efficiency (Elliot et al., 2007).

ICPMS is widely used in heavy metal detection as it is a rapid method compared to the other techniques. The multi-element of ICPMS allows the determination of many elements in a shorter time. For examples, 70 elements can be analysed in less than 2 minutes by using less than 2 ml of solution at an uptake rate of 1 ml min⁻¹ (Beauchemin, 1999). However, even though ICPMS can ionise the sample, there is a possibility that an element will be only 50% ionised causing the detection limit to reduce by a factor of 2 compared to totally ionised element. Similarly, the detection limit will decrease when only one isotope of a multi-isotope element is detected. Besides, if the sample is tested with a conventional ICPMS that consists of only nebuliser and spray chamber, only 2% of the sample can reach the detector. Mass discrimination can occur when a quadrupole-based instrument separates the ions. This is because the transmission of ions is not uniform over the mass range.

Moreover, the plasma sampling position might also affect the detection capability of ICPMS. The chemical form of element in a solution can influence the sample when it undergoes the various process in the plasma. Thus, the maximum of the ion density will be affected (Beauchemin, 1999). Besides, ICPMS is preferred due to its sensitivity. The detection limits are 10-100 times superior to ICPAES. It can detect and analyse both metal and non-metals element including sulphur, phosphorus, silicon and all types of halogen except fluorine (Elliot et al., 2007). ICP is an efficient elemental ion source for MS since most of the elements in the periodic table are easily ionised (Beauchemin, 1999). However, ICPMS is not able to directly analyse 5 elements which are hydrogen, helium, fluorine, neon and argon. Hydrogen, helium, fluorine and neon cannot be detected because of their ionisation potentials that are higher than argon that is responsible for the conversion of liquid particles into ions. Meanwhile, argon itself cannot be measured in an argon plasma (Elliot et al., 2007).

The liquid form of the sample that is introduced to the nebuliser presents a reasonable control over homogeneity and ease of calibration. The sample is then delivered to the nebuliser by using a peristaltic pump which can reduce the physical interference such as the changes in the sample's viscosity. However, the solution of the sample might also cause inaccurate detection due to dissolve solid in that solution. In the plasma, the sample will travel through a component called orifice, which is small and can be easily clogged due to the dissolve solid. ICPMS can tolerate 0.2% concentration of dissolved solids, but the presence of dissolve solid can cause concomitant element which the analyte signal is suppressed or enhanced by the presence of other elements (Beauchemin, 1999).

4. CONCLUSION

The marketed vegetables are not surely confirmed that they are free from heavy metals. Several studies have shown that leafy vegetables are the heaviest metal contaminated plant. In order to ensure the quality of the vegetables, various techniques can be used to identify the presence of toxic elements in the samples. Based on the comparisons between those detection instruments, ICPMS is the best option among the others as it is highly sensitive, able to detect a wide range of elements simultaneously and low rate of interference.

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