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Determination of cu and pb in *solanum melongena l.* by using atomic absorption spectroscopy

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GRAPHICAL ABSTRACT

Home grown *Solanum Melongena L.*

ABSTRACT

The consumption of heavy metals in a large quantity may be harmful to human being. The main ways that these elements can enter human body is by ingestion. These potentially toxic elements can cause several clinical and physiological problems. The aim of this study is to determine the concentration of heavy metals in home grown and commercial Solanum Melongena L.. Home grown Solanum Melongena L. samples were grown and collected at Dusun UTM while commercial Solanum Melongena L. were bought in supermarket in Taman Universiti. Samples were digested using microwave digestion and open reflux acid digestion and lead and copper contents were determined by using Atomic Absorption Spectroscopy. The concentration of lead and copper in home grown and commercial Solanum Melongena L. were compared. All samples of Solanum Melongena L. showed average concentrations of lead and copper and is not within the range (Cu; 40 µg/g, Pb; 5 µg/g) established by International Food Standard, Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). The concentration of lead and copper (µg/g) were determined by AAS for home grown Solanum Melongena L. is in the range between 3.09-3.47 µg/g, 9.72-14.22 µg/g for copper and lead respectively. Meanwhile, the concentration of lead and copper in commercial Solanum Melongena L. are in the range between 4.78-6.10 µg/g for copper and 11.44-15.28 µg/g for lead, respectively. This shows that the concentration of lead in both samples of Solanum Melongena L. are not within the standard.

Keywords: Cu, Pb, heavy metal, eggplant, Solanum Melongena L., atomic absorption spectroscopy

1. INTRODUCTION

The increasing levels of heavy metals contamination in current urban development makes the heavy metals as the main source of pollution in our environment. Heavy metals are kept under environmental pollutant category due to their toxic effects on plants, animals, and human being. According to the U.S. Environmental Protection Agency and the International Agency for Research on Cancer, the high degree of toxicity of As, Cd, Cr, Pb and Hg rank among the priority metals that are of public health significance in which these metallic elements are considered systemic toxicants that are known to induce multiple organ damage even at lower levels of exposure [1]. Studies of heavy metals in ecosystems have indicated that many areas near urban complexes, metalliferous mines or major road systems contain unusual high concentrations of these elements. Specifically, soils in such region have been contaminated from a wide range of sources with Pb, Cd, Hg, As and other heavy metals [2].

Vegetables are essential intake in human nutrition and diet as there are rich in vitamin C, and are important source of carbohydrate, minerals and dietary fiber [3,4]. Heavy metal contamination of soil and human exposure result from anthropogenic activities such as mining, smelting operation, and agriculture that have caused dramatically increased levels of these harmful element. These heavy metals are persistent in nature, therefore, it can get accumulated in soils and plants which will cause absorption of these metal ions by plant roots, and can be accumulated at high levels in the edible part of vegetables or even at low concentration [3, 6]. The amount of heavy metal absorbed by a plant are controlled by four different factors; the concentration and speciation of the metal in soil solution, the movement of metal from the bulk soil to the root surface, the transport of the metal from root surface into the root, and its translocation from the root to the shoot [2]. The total quantity of the ion in the soil will determine the plant uptake of mobile ions present in the soil.

Absorption of metals by plant roots are divided by two processes which are passive and active processes. Passive uptake involves diffusion of ions in the soil solution into the root endodermis while active uptake requires metabolic energy because it occurs against a concentration gradient [7]. These mechanisms can be varies for different metal ions. There is possibility of movement of metal ion throughout the whole plant once the ions have been absorbed through the roots or leaves. The factors that influence the rate and extent of movement within plants include the target metal analyte, plant organ and age of the plant. Mn, Zn, Cd, B, Mo and Se are the list of elements which are readily translocated to the tops of the plant, Ni, Co, and Cu were intermediate and the least extent to be translocated are Cr, Pb and Hg [2]. Vegetables are exposed to heavy metals by various means in many countries and region. So the consumption of these vegetables can cause adverse health effects.

This research will emphasize on the determination of the concentrations of Cu and Pb in home grown and commercial *Solanum Melongena L.* The results were studied and compared. The sources of high concentration level of these elements were studied.

2. EXPERIMENTAL

2.1 Preparation of compost

Compost pile was started on bare earth to allow worms and other beneficial organism to aerate the compost. Twigs or straw were laid first in a few inches deep to help in drainage and aerate the pile. Then, compost materials were added in layers with alternating moist and dry material before adding horse manure into the pile. The compost pile was watered to retain the moisture of the compost. Next, compost pile was covered with plastic sheeting to retain the moisture and heat. The compost pile was turned every five to seven days with a shovel. Water was added when needed. The temperature of the compost in the first few weeks is about 140°F. After about four weeks, less heat will be produced and compost will maintain lower temperature. For the next four more weeks, the pile will no longer produce heat after turning and the volume will be about one third of original. The pile was allowed to cure for four more weeks before using.

2.2 Planting *Solanum Melongena L.*

The seeds were sowed about 1/4 inch deep with compost in cell containers. The seeds were watered every day. The seeds started to germinate after two weeks. Once the seeds had grown the third leaves, the seedlings were transplanted into a polybag which are placed in a full sun area. The fruits were harvested after 50 days from the transplant date.

2.3 Chemicals and reagents

Concentrated nitric acid (65% HNO3), hydrochloric acid (HCl), hydrogen peroxide (H2O2) were purchased from QreC (Asia) Sdn. Bhd. (Selangor, Malaysia) Stock standard solutions containing 1000 mgL-1 of Pb was purchased from Scharlau (Sentmenat, Spain), Cu was purchased from Merck (Darmstadt, Germany) were used for preparing working standards of 0.3, 0.6, 0.9, 1.2, 1.5 mgL-1 and 1.0, 2.0, 3.0, 4.0, 5.0 mgL-1 for Cu, and Pb. Working solutions were prepared using deionized water from Sartorious (Goettingen, Germany).

2.4 Collection, preparation and preservation of samples

Home grown samples of Solanum Melongena L. and soil sample were collected at Dusun UTM, Skudai, Johor, while commercial Solanum Melongena L. samples were bought in local market in Taman Universiti, Skudai, Johor, prepared and preserved in the laboratory until further analysis.

In microwave acid digestion, triplicates of 0.5 g of sample material was weighed into weighing cup and placed into the digestion vessel. To each vessels, a 7 mL concentrated nitric acid, HNO3 and 2 ml of hydrogen peroxide, H2O2 were added (conducted in the fume hood). Then, the sealing disc was placed onto the locking lid before closing the vessel. The digestion vessels with less tighten lid were placed in the fume hood for 30 minutes. The microwave was set up with condition as shown in Table 3.2. The vessels were placed according to the instrument's instruction. Then the caps were tightened and locked before the digestion process starts. After digestion process completed, the vessels were cooled down for 10 minutes in the fume hood to release gas. The samples were filtered by using HmbG syringe filter 25 mm 0.45 µM with disposable 20 mL/CC syringe. Ultrapure water were added to a final volume of 25 mL. The samples were stored at 4°C until analysis.

Meanwhile, in open reflux acid digestion, a triplicates of 0.50 g chopped Solanum Melongena L. sample was weighed and transferred into conical flasks. To each replicate, a 5 mL of 65% HNO3 was added, and once filter funnel was put in place, the content was refluxed on a hot plate for 2 hours conducted in the fume hood. The digested mixture was then evaporated 5 to 10 minutes and cooled for another 5 minutes. Then another 2 mL of a 65% HNO3 was added. The content was refluxed for a further 2 hours in the fume hood. The cooled digest was filtered by using a Smith 125 mm filter paper into a 50 mL vial and diluted to the mark of 25 mL with ultrapure water.

A triplicates of 2.0 g portion of soil sample was weighed into 50 mL conical flask. To each replicates, 10 mL of 1:1 HNO3 was added into it. The flask was placed on a hot plate, fitted with filter funnel and refluxed at 95°C for 15 minutes in the fume hood. The digestate was cooled and 5.0 mL of concentrated HNO3 was added into it. The solution was refluxed for further 30 minutes at 95°C in the fume hood. The last step was repeated until the solution was reduced to about 5 mL without boiling (by only partially covering the beaker). The sample was cooled again and 2 mL of ultrapure water and 3 mL of H2O2 were added to it. The sample was then refluxed gently in the fume hood to start the peroxide action with the flask covered. Then, 5 mL of concentrated HCl and 10 mL of ultrapure water were added and the sample was refluxed in the fume hood for an additional 15 minutes without boiling. The sample was cooled and filtered through a Smith 125 mm filter paper into a 50 mL vial and diluted to the mark of 25 mL with ultrapure water. The sample was kept in the refrigerator until analysis.

3. RESULTS AND DISCUSSION

3.1. Comparison of microwave acid digestion with open reflux acid digestion

The time taken for the decomposition process in microwave acid digestion is shorter than open reflux acid digestion where it only takes around one hour to complete the digestion process. Meanwhile, in open reflux acid digestion, it took around three to four hours for the sample to digest. Even with that time, the sample may not be digested completely, and more acids are needed to break down the organic material in the sample. However, in microwave acid digestion, there is no need for the further addition of the acid during the digestion process. If we were to compare the efficiency of the digestion process and time consumed, microwave acid digestion is a better method to digest the sample.

3.2. Concentrations of heavy metals in *Solanum Melongena L.*

The analysis has been done by using FAAS in the determination of Cu and Pb in home grown and commercial *Solanum Melongena L.* The concentrations of Cu and Pb in home grown and commercial samples were compared. Then, the analysis was extended to the soil sample to investigate the sources of heavy metals presence in home grown *Solanum Melongena L.* samples. The mean concentrations are in µg/g since the samples studied are solid samples. The samples were analysed with FAAS and the results were calculated and shown in Table 3.2.2 and 3.2.3. The LOD and LOQ were shown in Table 3.2.1. The concentration of Pb and Cu in home grown *Solanum Melongena L.* are in the range between 3.09-3.47 µg/g for Cu, 9.72-14.22 µg/g for Pb. Meanwhile the concentration of Pb and Cu in commercial *Solanum Melongena L.* are in the range between 4.78-6.10 µg/g and 11.44-15.28 µg/g for Cu and Pb respectively.

Table 3.2.1: Equation, correlation values of the calibration curves, LOD, LOQ of heavy elements studied

Elements	Equation	Correlation	LOD (ppm)	LOQ (ppm)
Cu	y = 0.1123x	0.9977	0.015	0.047
Pb	y = 0.0196x	0.9999	0.021	0.065

Table 3.2.2: Concentration of heavy metals in home grown *Solanum Melongena L.*

Samples	Cu (µg/g)	Pb (µg/g)
Replicate 1	3.09	14.22
Replicate 2	3.24	11.11
Replicate 3	3.47	9.72
Mean±standard deviation	3.27±0.16	11.68±1.88
%RSD	4.89	16.10

Table 3.2.3: Concentration of heavy metals in commercial *Solanum Melongena L.*

Samples	**Cu (µg/g)**	**Pb (µg/g)**
Replicate 1	4.78	12.86
Replicate 2	6.10	11.44
Replicate 3	5.56	15.28
Mean±standard deviation	5.48±0.54	13.19±1.59
%RSD	9.85	12.05

The mean values of Cu and Pb contents in *Solanum Melongena L.*studied are as shown in Table 3.2.2 and 3.2.3. It was found out that the concentration of both heavy metals, which are Cu and Pb are higher in the commercial samples compared to in home grown samples. This may be probably due to the usage of chemical fertiliser and pesticide on production of crops. In fact, Sinclair and Pressinger [9] argued that approximately 75% of grocery store farming product had been tested positive to have pesticide residue. This means that the farmers had been using excessive pesticides to get rid of insects. The presence of heavy metals in pesticides in the treatment of vegetables has caused heavy metals pollution in the vegetables [10]. This can be proved by the work of Chiroma *et al.* (2007), where the study was to investigate the heavy metals content in spinach treated and untreated with pesticide. It was found out that the concentrations of Cd, Pb and Cu in stem, leaves, and roots of the spinach treated with pesticides are higher compared to the untreated samples. Copper sulphate and iron sulphate have the most significant concentration of Pb. This shows that the application of pesticide on spinach increases the Cd, Pb and Cu concentrations. Thus, the concentration of heavy metals in commercial sample is higher than in home grown sample may be due to the usage of pesticide used on plants. The common pesticides found in *Solanum Melongena L.* are endosulfan sulfate and endosulfan II.

It was established that the concentrations of the heavy metals determined in the tested *Solanum Melongena L.*in both home grown and commercial samples were higher in Pb but lower in Cu than the admissible concentration regulated by the International Food Standard and Malaysian Food Regulation 1985. This may be due to the heavy metals deposition from the area surrounding the plants such as soil and water. Thus, further analysis of concentrations of heavy metals in soil has been done to investigate the probable sources of heavy metals presence in *Solanum melongena L.* samples.

3.3. Concentrations of heavy metals in soil samples

The soil samples were also analysed with FAAS and the result were calculated and tabulated in Table 3.3. The concentration of Pb and Cu in soils are in the range between 10.90-19.49 µg/g and 5.45- 7.38 µg/g for Cu and Pb respectively. The mean values with standard deviations of Cu and Pb are 15.29±3.51 µg/g and 6.44±0.79 µg/g respectively. This shows that Cu has higher concentration in soil compared to Pb.

Table 3.3: Concentration of heavy metals in soil

Samples	**Cu (µg/g)**	**Pb (µg/g)**
Replicate 1	19.49	7.38
Replicate 2	15.47	6.48
Replicate 3	10.90	5.45
Mean	15.29±3.51	6.44±0.79
%RSD	22.95	12.22

The high concentration of Pb in *Solanum Melongena L.* samples may be due to the deposition of the elements from the surrounding into the plants. When lead is released to the air from industrial sources or vehicles, it may travel long distances before settling to the ground, where it usually sticks to soil particles [11].

The accumulation of heavy metals in the soil will influence the rate of diffusion from the soil to the plant [2]. The uptake of heavy metals by plants can be determined with bioaccumulation factor (BAF) [12]. BAF is defined as the ratio of the metal concentrations in vegetables to the metal concentrations in soil. This can be used to estimate the ability of vegetables to accumulate metals in the plant. BAF of Pb is higher than Cu shown in Table 3.5 which implies that the ability of the vegetables to uptake the Pb metals from soil is higher than Cu. Thus, as resulted, the concentration of Pb in *Solanum Melongena L.* is higher than Cu. The consumption of Pb in long term may lead to brain and kidney damage and possibly cause death.

Table 3.3: BAF of Cu and Pb

Elements	**Concentration of home grown sample (µg/g)**	**Concentration of soil sample (µg/g)**	**BAF**
Cu	3.27	15.29	0.2136
Pb	11.68	6.44	1.8145

3.3 Comparison with International Food Standard

In every different type of food, there are certain limit that had been set up by various organization in different country that advise the consumers on the concentration of metal that are safe to be consumed. According to the FAO/WHO Codex alimentarius Commission 1984 and Malaysian Food Regulation 1985, the standard limit of concentrations allowed in solid type of food is as shown in Table 3.6.

Table 3.4: Permissible limits of heavy metals according to International Standard

Elements	**Maximum permissible limit of elements**	
	Malaysian Food Regulation 1985 (µg/g)	FAO/WHO Codex alimentarius Commission 1984 (µg/g)
Cu	30.00	40.00
Pb	2.00	5.00

Based on the results obtained, the concentrations of Pb in *Solanum Melongena L.* samples in both home grown and commercial are higher than limits allowed by International Food Standard and Malaysian Food Regulation while the concentration of Cu is in the permissible limit. The consumption of this vegetables in a long period of time may affects our health as the heavy metals ingested will also accumulates in our body until a certain limit in which will affects our organ, long term of consumption of lead may lead to high lead levels can severely damage the brain and kidneys and possibly cause death, miscarriage in pregnant women, damage organs responsible for sperm production in men. In order to prevent from consuming heavy metals from vegetable, the fruits or vegetables can be wash by wash solution to remove pesticide residue from vegetables.

4. CONCLUSION

The calibration graphs were linear in the range from 0.2-1.0 ppm and 1.0-5.0 ppm with correlation coefficients of 0.9977 and 0.9999 for Cu and Pb respectively. The limit of detection (LOD) is 0.015 for Cu and 0.021 for Pb while limit of quantification (LOQ) is 0.047 and 0.065 for Cu and Pb respectively. The concentrations of heavy metals presence in home grown *Solanum Melongena L.* samples are in the range between 3.09-3.47 µg/g for Cu and 9.72-14.22 µg/g for Pb. Meanwhile the concentration of Pb and Cu in commercial *Solanum Melongena L.* are in the range between 4.78- 6.10 µg/g and 11.44-15.28 µg/g for Cu and Pb respectively. The high concentration of Pb and Cu in commercial samples compared to home grown samples are due to the excessive amount of pesticide used to treat the crops from pest. The study shows that the concentration of Cu and Pb in *Solanum Melongena L.* are not within the permissible limit allowed by Malaysian Food Regulation 1985 and International Food Standard, Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). The study showed that the concentration of Pb in home grown and commercial *Solanum Melongena* are not within the standard.

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