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Determination of copper, cadmium and zinc in *abelmoschus esculentus l. moench* using flame atomic absorption spectrophotometry

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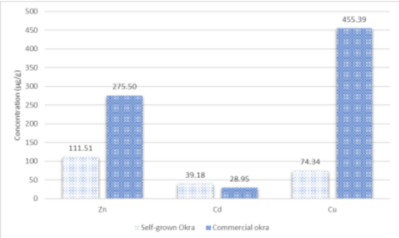
GRAPHICAL ABSTRACT



Comparison Heavy Metals between Self-grown okra and Commercial okra

ABSTRACT

Heavy metals such as cadmium, copper and zinc are important environmental pollutants, particularly in areas where vegetables are irrigated with wastewater. The uptake of these heavy metals especially into the human food chain was done through these leafy vegetables and they may cause harmful effects on health. This study investigates the concentration of heavy metals in Okra (*Abelmoschus esculentus L. Moench*) samples that were planted and compared with those bought from supermarket. The samples were tested for Zn, Cu and Cd determination using Flame Atomic Absorption Spectrometry (F-AAS). The okra samples were digested with 65% nitric acid and diluted to the mark with distilled water. The results obtained show that the mean values of heavy metals studied for commercial okra obtained from the supermarket are generally higher than the okra's that are self-planted. The concentration of Cu, Zn and Cd metals detected in our own planted okra are 74.34 µg/g, 111.51 µg/g and 39.18 µg/g respectively, as compared to the okra obtained from the supermarket with metals concentration of Cu at 455.39 µg/g, Zn 275.50 µg/g and Cd 28.95 µg/g respectively. The results indicated that the concentration obtained from both samples are still above the permissible limit as outlined in Food and Agriculture Organization of United Nations (FOA) and World Health of Organization (WHO) except for concentration of Cd in commercial, and both okra samples are not safe for human consumption.

Keywords: Abelmoschus esculentus L. Moench, *heavy metals, Flame Atomic Absorption Spectrometry, concentration.*

1. INTRODUCTION

Heavy metals and ions are potential environmental contaminants with the potentiality of causing human health problems when consumed overly through food or drink. They are given special attention throughout the world due to their toxic effects even at very low concentrations [1]. Heavy metals such as cadmium, lead and zinc are important environmental pollutant, particularly in areas where vegetables are irrigated with waste water. As such consumption of vegetables by humans and animals pose serious health hazards, although some heavy metals as lead, cadmium and magnesium are important in plant nutrition, many of them do not play any significant role in the plant's physiology. The uptake of these heavy metals especially into the human food chain is done on these leafy vegetables and they have harmful effects on health [2]. Vegetables are considered as neutralizing agents for acidic substances formed during digestion, as human activities increase especially with the application of modern technology, pollution and contamination of human food chain cannot be avoided. Heavy metals in vegetables cannot be underestimated as these food stuffs are related components of human diet. They are literally rich and simply cheaper sources of vitamins. Consumption of these items provides taste and palatability increases appetite, besides providing fiber for digestion and prevent constipation [3]. Heavy metal contamination in food item is one of the most important aspect of food quality assurance; international and national regulation on food qualities have lowered the maximum permissible levels of toxic metals in food item due to an increased awareness of the risk these metals pose to food chain contamination.

Abelmoschus esculentus L. Moench or well known as okra or lady's finger is herbaceous plant that originates from Africa but now widely known and popular at the trophic and warmer part of the temperate zone [6]. In every 100 g consumption of edible portion of okra, it will give 1.9 g of protein, 0.2 g fat, 6.4 g carbohydrate, 0.7 g minerals and 1.2 g fibre to our body [7]. Okra become a favorite vegetable for Malaysian with the total production of 22,295 tonnes in 2005 to 20,400 tonnes as reported by Ministry of Agriculture Malaysia in 2010. Hard seediness of the seed give negative impact especially in agricultural field because this crop will take longer period of time to germinate, hence affect the production of the vegetable. Therefore, to overcome this problem, ways to enhance germination of the seeds including using nitrate, salt and plant hormone (gibberellic acid) are carried out to increase the germination ability of okra [9].

2. EXPERIMENTAL

The compost pile was layered on ground to allow worms and other beneficial organism to aerate the compost. Organic waste such as dried leaves, grass and plant trimmings was put down in layer a few inches deep. Twigs, stalks and chipped were placed at bottom. Then, compost materials were added in layers with alternating moist and dry material before adding horse manure into the pile. The compost pile was sprinkled water over it to retain the moisture of the compost. Next, compost pile was covered with plastic sheeting to retain the moisture and heat. After a week, the pile was turned to provide oxygen with a garden folk. The compost pile was turned every seven days. 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. When the compost no longer gives off heat and becomes dry, brown, and crumbly, it was ready to feed to the plantation.

Compost used in planting *Abelmoschus esculentus L. Moench*

2.2 Planting *Abelmoschus esculentus L. Moench*.

Okra seeds was purchased from supermarket in Taman Universiti. The seeds were grown with compost in polybag. Okra seeds were planted about 1-2 cm deep in a single polybag and placed in full sun area. The okra was seeds watered every morning to keep it growing. The plantation was planted with compost, and boosted at low concentration of hormone. The rate of compound fertilizer NPK (15:15 :15) 100 kg N ha−1, 40 kg P ha−1, and 60 kg K ha−1; for was used. The fertilizer was applied twice to each polybag at 3 and 6 weeks after planting. The fruits were harvested after 60 days from planted date.

Figure 3.2: Okra after a week

2.3 Chemicals and reagents

Concentrated nitric acid (65% HNO_3), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) were purchased from QreC (Asia) Sdn. Bhd. (Selangor, Malaysia) Stock standard solutions containing 1000 mgL^{-1} of Zn was purchased from Scharlau (Sentmenat, Spain), Cu was purchased from Merck (Darmstadt, Germany) and Cd was purchased from from Sigma-Aldrich (St. Louis, Missouri, United States). The metals were used for preparing working standards of 0.3, 0.6, 0.9, 1.2, 1.5 mg/L, 0.2, 0.4, 0.6, 0.8 mg/L, 1.0 and 0.2, 0.4, 0.6, 0.8, 1.0 mg/L for Cu, Zn and Cd respectively. Working solutions were prepared using deionized water from Sartorious (Goettingen, Germany).

2.4 Collection, preparation and preservation of samples

The okra was collected from the farm in Dusun UTM, Skudai, Johor and also bought from the local supermarket in Taman Pulai, Johor. The okra was plucked from the stem, brought to the laboratory and washed with de-ionized water; the okra then dried at room temperature until it was ready for grinding.

2.3 Digestion procedure

2.3.1 Open reflux acid digestion of Okra samples

A tripilicates of okra sample was weighed 0.5 g into three separate conical flask. Then 5 mL of 65% HNO_3 was added and filter funnel fitted on the conical flask. The content was refluxed on hot plate for 2 hours. The digested mixture was then evaporated and cooled. Then another 2 mL of 65% HNO_3 was added. The content was refluxed. The cooled digest was filtered by using a Smith 125 mm filter paper into a 50 mL vial and diluted to the mark with 50 mL of deionized water.

2.4 Instrumental analysis

The concentration of Zn, Cd and Cu was determined by a Perkin Elmer PinAAcle 900T Flame Atomic Absorption Spectrophotometer (Waltham, Massachusetts, United States). FAAS provided with background correction of deuterium and hollow cathode lamp (HCL) for the measurement of absorbance at wavelength and conditions as tabulated in Table 2.1. It was conducted using air/acetylene flame. F-AAS provides rapid metal analysis with great selectivity and allows the analysis of elements at the ppm level.

Table 2.1: FAAS instrument's conditions (Perkin Elmer, 1996)

Elements	Wavelength (nm)	Slit (nm)	Char. Conc. (mg/L)	Sensitivity Check (mg/L)	Linear to (mg/L)
Cu	324.75	2.7/0.8	0.025	1.3	1.6
Pb	283.31	2.7/1.05	0.18	8	10

2.4.1 Preparation of Standard Solution for F-AAS Analysis

For F-AAS analysis, 5 calibration solutions were prepared with various concentration range based on linear range as suggested for respectively metals. The series of standard solution were prepared for each metal. 0.3 ppm to 1.5 ppm for Cu, 0.2 ppm to 1.0 ppm for Zn, and 0.2 ppm to 1.0 ppm for Cd. All the standard solutions were prepared in 25 mL of Volumetric flask from 1000 ppm of standard for each Cu, Zn, and Cd. Appropriate working standards were prepared for each of the metal solution by serial dilution of the intermediate solutions using deionized water. Then, for determination of each metals through F-AAS analysis, a calibration curve was constructed by using the metal's standard solution. By interpolation, the concentrations of the metals in sample digests were determined.

2.5 LOD and LOQ

LOD is the lowest concentration of analyte that can be detected approximately by calculation and needless to be quantified as a true value. On the other hand, LOQ is the lowest concentration of analyte that can be quantitatively determined. The formula of both LOD and LOQ are shown below.

$$LOD = \frac{(3\ x\ SD)}{slope\ of\ graph} \qquad \text{Equation 3.1}$$

$$LOQ = \frac{(10\ x\ SD)}{slope\ of\ graph}$$ Equation 3.2

2.6 Precision

Precision represents the repeatability and reproducibility of analytical measurement. This can be obtained by calculating the relative standard deviation (RSD) as shown in the formula below.

$$RSD = \frac{SD\ of\ sample}{mean\ of\ sample} x100\%$$ Equation 3.4

3 RESULTS AND DISCUSSION

3.1 Analysis of Commercial okra and Self-plant okra for Metal Level

This study showed that self-plant okra and commercial okra samples contained of Cd, Zn and Cu. Table 3.1 shows the mean concentrations of Cd, Zn and Cu in both samples. The samples were analyzed using flame atomic absorption spectrometry for the determination of Cd, Zn and Cu at their corresponding wavelength of 228.8, 213.9 and 324.8 nm, respectively. It was observed that Zn was highest in self-plant okra as compared to the other metals. Level of metals in self-plant okra were following order: Zn>Cu>Cd. The results also indicated that sample of commercial okra contained high levels of Cu followed by Zn and Cd. Level metals in commercial okra were following order of: Cu>Zn>Cd. The comparison heavy metals in both samples are summarized in Figure 31.

Table 3.1: Metal Concentrations (µg/g) of Heavy Metals in Self-plant okra and Commercial okra

Samples	Cd	Zn	Cu
Self-grown okra	39.18	111.51	74.34
Commercial okra	28.95	275.50	455.39

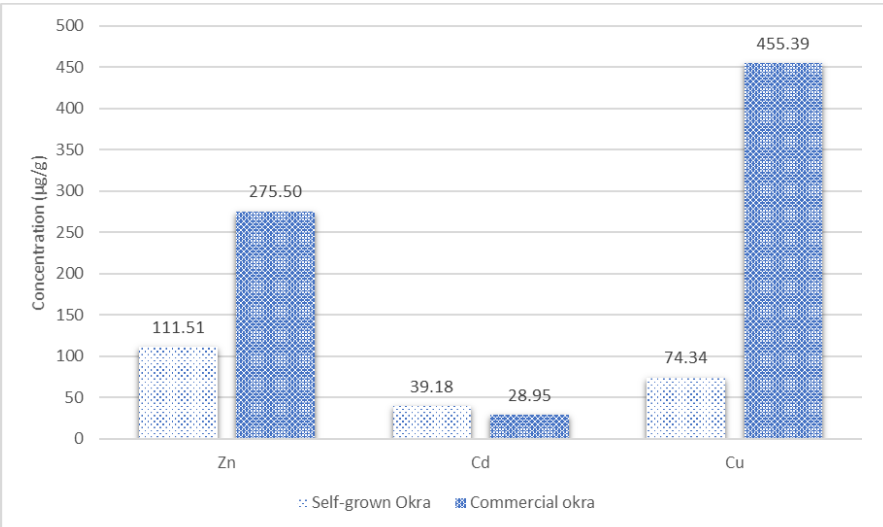


Figure 3.1: Comparison Heavy Metals between Self-grown okra and Commercial okra

3.2 Instrumental Calibrations

The data qualities obtained from FAAS for metal analysis are highly affected by the calibration and standard solutions preparation procedures. The instrument was calibrated using a series of standard working solutions of each of the metals prepared freshly by appropriate dilution of the intermediate standard solutions.

The intermediate standard solutions were prepared from stock solution of each metal. The concentrations of the intermediate standards, working standard solutions and values of correlation coefficients of the calibration graphs for the three metals of interest is presented in Table 4.1 and their calibration graphs in Figure 3.2,3.3 and 3.4.

Metal	Concentration of intermediate Standard (mg/L)	Concentration of working standard (mg/L)	Correlation Coefficient of calibration curves
Zn	10	0.2, 0.4, 0.6, 0.8 and 1.0	0.9998
Cd	10	0.2, 0.4, 0.6, 0.8 and 1.0	0.9950
Cu	10	0.3, 0.6, 0.9, 1.2 and 1.5	0.9979

Table 4.1: Concentration Values of Working Intermediate Standard Solutions, Working Standard Solutions and Correlation Coefficient Of Calibration Graphs

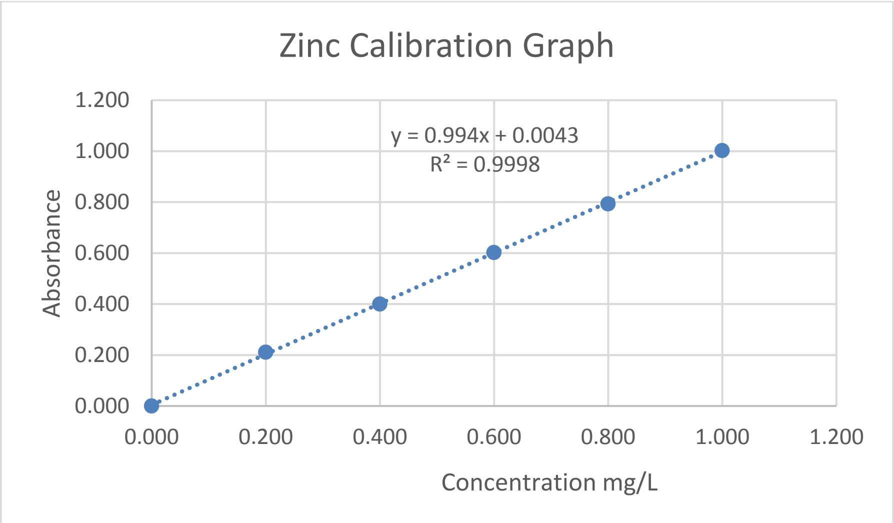


Figure 3.2: Plot of Absorbance versus Concentration for Zinc

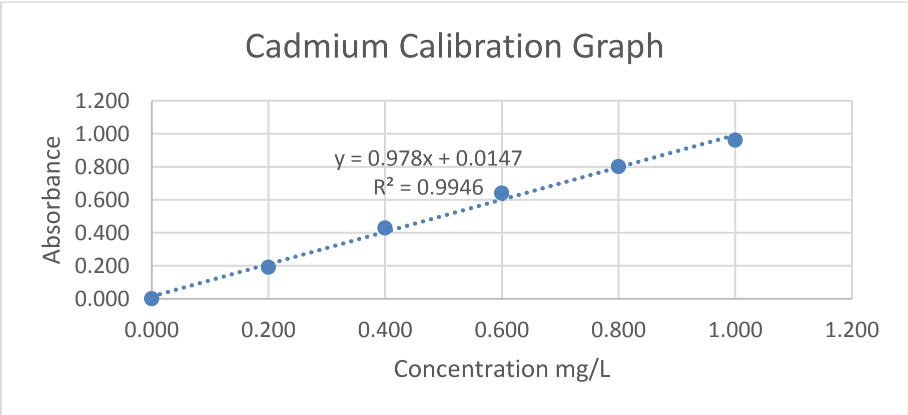


Figure 3.3: Plot of Absorbance versus Concentration for Cadmium

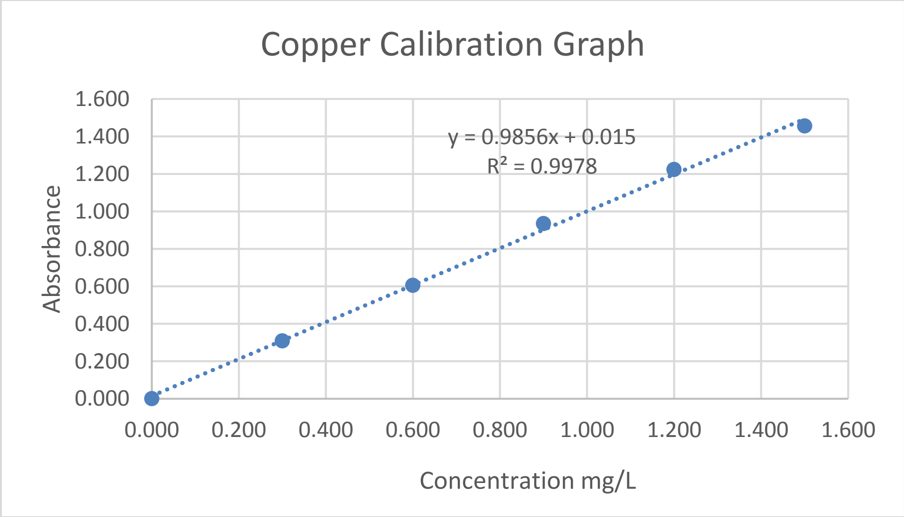


Figure 3.4: Plot of Absorbance versus Concentration for Copper

3. 3. Method of Performance and Method of Validation

3. 3. 1. Precision

The results of percent relative standard deviation for the studied metals were presented in Table 4.3 below.

Table 4.3: Mean±SD, n=3mg/l and %RSD of Zn, Cd and Cu in okra samples

Metal type	**Mean & RSD%**	**Self-plant Okra**	**Commercial Okra**
Zinc	Mean %RSD	111.51±0.012 4.12	275.50±0.007 2.05
Cadmium	Mean %RSD	39.18±0.004 13.32	28.95±0.001 3.12
Copper	Mean %RSD	74.34±0.245 48.36	455.39±0.002 2.23

3.3 LOD and LOQ

Table 3.4 shows the linearity, LOD and LOQ for each metal. The linearity of each metal was good and acceptable.

Table 3.4: Linearity, LOD and LOQ

Metal	**r^2**	**LOD (ppm)**	**LOQ (ppm)**
Zn	0.9940	0.0198	0.0600
Cd	0.9780	0.1016	0.3078
Cu	0.9856	0.097	0.2940

3.3 Food and Metal Contamination

Table 3.5 shows the permissible levels of soil and food concentration limits for Cd, Zn and Cu. It has been found that concentration of heavy metals in both samples analyzed was above the permissible limit of WHO (World Health Organization) standards and Food limit (mg/kg) by Malaysia Food Act 1983 and Food Regulations 1985 except for Cd in commercial okra almost reach the permissible limit.

According to Chuck (2016), presence of high amounts of Zn and Cu in food is enough to pose health problems, as it has been reported that the major source of these metals are available in almost all urban environmental soils [11]. The acute exposure of Zn can cause tachycardia, vascular shock, dyspeptic nausea, pancreatic disorder, diarrhea and damage of hepatic parenchyma [12]. Okra also contaminated with Cd which is the toxicity as the level is greater than the safe limits. The consumers must be prevented from their use. The accumulation of Cd in both samples are the lowest than other metals. Cd has been reported is highly mobile metal zone and it is easily absorbed by the plants [13]. Since the concentration of Cd in self-grown okra was above the limit and can result in health effects such as lung problems which include pneumonitis and pulmonary edema, liver injury, convulsions.

Table 3.5: Permissible levels of Zn, Cu and Cd metals in food standards

Heavy metals	**Food limit (mg/kg) by Malaysia Food Act 1983 and Food Regulations 1985**	**Food limit (mg/kg) by FAO/WHO**
Zn	40	60
Cu	30	40
Cd	30	40

4. CONCLUSION

In this study, the accumulation of Cu, Zn and Cd showed varied between both samples. For samples from self-grown, concentration of Cu, Zn and Cd is 48.36 µg/g, 111.51 µg/g and 39.18 µg/g respectively. For commercial okra, concentration of Cu, Zn and Cd is 455.39 µg/g, 275.50 µg/g and 28.95 µg/g respectively. The calibration graph, linearity showed 0.999777, 0.995029 and 0.997863 for Zn, Cd and Cu respectively. The concentration of commercial okra and self-grown showed it were high and above permissible limits by FOA/WHO as well as Malaysia Food Act 1983 and Food Regulations 1985 except for Cd in commercial okra. Farmers encouraged to use well water for irrigation in their gardens. Farmers should aware of this contamination that could endanger the health of consumers hence the need for relevant authorities to check and regulate practice that lead to okra and other plant contamination. To avoid the entrance of metals into the food chain, municipal or industrial waste should not be drained into rivers and farmlands without prior treatment. In addition to treating the discharge that enters into the farms, it is imperative to utilize alternative measures of cleaning up the already contaminated substrates. The continuous monitoring of the soil, plant and water quality along with preventing metals from entering the vegetables are prerequisites for the prevention of potential health hazards to human beings.

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