SAFETY OF SELECTED VEGETABLES GROWN IN URBAN AREAS; CASE OF HEAVY METALS IN DAR ES SALAAM CITY

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ABSTRACT

A study was carried out to assess the safety of selected vegetables grown in urban areas, focusing on a case of heavy metals in Dar es salaam, Tanzania. The study investigated the concentration of three heavy metals, namely Lead (Pb), Cadmium (Cd) and Copper (Cu) in the edible portions of selected leafy vegetables, and the effect of blanching on the possible reduction of heavy metals concentration. The study also compared the levels of metal in selected matured leafy vegetables across their sampling sites and their associated uptake rates of the metals from soil. The selected leafy vegetables were Chinese cabbage (*Brassica rapa*), Spinach (*Spinacia oleracea*), Amaranthus (*Amaranthus spp*), and Pumpkin leaves (*Curcubita moschata*). The heavy metals concentration was measured using an Atomic Absorption Spectrophotometer. The results showed that, Pb and Cd in dry weight (mg/kg) ranged from 4.46 to 10.09 and 0.49 to 0.79 respectively. The concentrations were higher than the permissible levels as per WHO, FAO and TBS for green leafy vegetables. Except for Pb, blanching had no significant reduction effect ($p>0.05$) in the heavy metals concentration in the vegetables. The mean concentration of Pb varied across sites and vegetables ($p< 0.05$), the mean concentration of Cd did not vary across sites but varied across vegetables ($p>0.05$), where the difference was observed between Chinese cabbage and Spinach. The mean concentration of Cu varied across sites and vegetables ($p<0.05$) where the difference was observed between Chinese cabbage and Amaranthus, Pumpkin leaves and Spinach. The uptake of Pb, Cd and Cu concentrations among vegetable varieties was not significantly different ($p>0.05$). This showed that all selected vegetable varieties up took the metals at a similar rate.

DECLARATION

I, Angela Patric Buyamba, do declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution.

Angela Patric Buyamba Date
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The above declaration is confirmed by;

Prof. Chove. B. E. Date
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Dr. Kilima, B. M. Date
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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOLS

µg/g	microgram per gram
AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
As	Arsenic
BAFs	Bioaccumulation factors
Cd	Cadmium
cm	centimeter
Cu	Copper
FAO	Food and Agriculture Organization
Fe	Iron
g	gram
HCl	Hydrochloric acid
Hg	Mercury
HNO_3	Nitric acid
mA	milliampere
mg/kg	milligram per kilogram
Mls	milliliters
MLs	Maximum levels
mm	millimeter
Mn	Manganese
Ni	Nickel
nm	nanometer
ºC	Centigrade
Ppm	parts per million

SD	Standard deviation
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
THQ	Target hazard quotient
TZS	Tanzania Standards
WHO	World Health Organization
Zn	Zinc

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Vegetables constitute an important part of human diet since they contain carbohydrates, proteins, vitamins, minerals and fibers required for human health. They also act as neutralizing agents for acidic substances formed during digestion (Shamar *et al.*, 2009). However, they contain both essential and toxic elements over a wide range of concentrations (Sobukola and Dairo, 2007). Among the toxic elements, heavy metals and some trace elements like Cobalt, Flourine, Iodine and Iron pose biological toxicity and can affect and threaten the health of human being owing to their accumulation and persistence in the compartments of the food chain. Heavy metals constitute a very heterogeneous group of elements which are widely varied in their chemical properties and biological functions. The term heavy metal can be defined as metals which have specific weights more than 5 g cm^{-3} (Raikwar *et al.*, 2008; Hezbullah *et al.*, 2016). Heavy metals are hazardous contaminants in food and the environment and they are non-biodegradable having long biological half-lives (Amin *et al.,* 2013). They are ubiquitous in the environment through various pathways, due to natural and anthropogenic activities (Wilson and Pyatt, 2007). The implications associated with metal contamination are of great concern in agricultural production systems (Kachenko and Singh, 2006) due to their increasing trends in human foods and environment. Metals most often found as contaminants in vegetables include Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Copper (Cu) and Lead (Pb). These metals can pose as a significant health risk to humans, particularly in elevated concentrations above the very low body requirements (Ahmed *et al.*, 2012). These metals could reach food chain through various biochemical process and ultimately biomagnified in various trophic levels and eventually threaten the health of human. Crops and vegetables grown in the contaminated soils have a greater chance of accumulation of heavy metals than those grown in uncontaminated soil (Ratul *et al.*, 2018). Anthropogenic activities, such as mining and industrial processing, are the main sources of heavy metal pollution in the environment. Moreover, the use of large quantities of agrochemicals such as metal-based pesticides and fertilizers also plays an important role in contaminating different foodstuff (Loutfy *et al.*, 2012; Pourang and Noori, 2012). Apart from fertilizer and pesticides, excess amounts of heavy metals from anthropogenic sources that enter into the ecosystem may lead to geo-accumulation and bioaccumulation that pollute the environment and also affect the food chain and pose serious human health risks (Weldegebriel *et al.*, 2012; Saha and Zaman 2013). Heavy metal concentrations in different foods depend on soil composition, water, nutrient balance, as well as metal permissibility, selectivity, and absorption ability (Ahmad and Goni, 2010). The ingestion of heavy metals from different sources (Cu, Ni, Cd and Pb) can seriously cause depletion of some essential nutrients in the human body, which in turn causes a decrease in immunological defences, intrauterine growth retardation (caused by Cd, Mn, and Pb), psychosocial dysfunctions, disabilities associated with malnutrition, and upper gastrointestinal cancer (Iyengar and Nair 2000; Proshad *et al.*, 2018). Although Cu are essential elements, their excessive concentration in food and feed plants are of great concern because of their toxicity to humans and animals (Kabata-Pendias and Mukherjee, 2007).

1.2 Problem Statement and Justification

Heavy metals are natural components of the earth's crust and cannot be degraded nor destroyed. Vegetables take up these metals by absorbing them from contaminated soils, water as well as from deposits on different parts of the vegetables exposed to the air from polluted environments (Sobukola *et al.*, 2010). They enter the human body through contaminated food, water and air. Ingestion of contaminated food is one of the main routes through which heavy metals enter the human body (Grasmück and Scholz, 2005).

Different groups of people practice urban agriculture, including those from low and mid income groups. Poverty, hunger, lack of formal employment opportunities, demand for food proximity to markets and availability of cheap resources such as urban organic wastes and wastewater have stimulated the development of diverse agricultural production systems in and around cities (D'Mello, 2003; Leonard *et al.,* 2012).

In Tanzania, Dar es Salaam in particular, it has been a common practice to cultivate vegetables along the banks of the rivers passing through the city. Waters from these rivers are reported to be polluted by heavy metals, which include Pb, Cu, Zn, Fe, Cr, Cd and Hg (Mashauri and Mayo, 1990; Kihampa and Mwegoha, 2010; Chanzi, 2017). The major sources of these heavy metals are industrial effluents, and indiscriminate disposal of domestic or sewage drainage directed to the rivers untreated or partially treated. Msimbazi River, for instance, which flows through the industrial area, by 1988 had an average sewage and industrial effluents rate of 256 m^3/h with peak values of 606 m^3/h, just before it enters the Indian Ocean (Ak'habuhaya and Lodenius, 1989; Kihampa and Mwegoha, 2010; Chanzi, 2017).

In Msimbazi valley, crops are irrigated with polluted water with heavy metals or exposed to polluted air which can eventually bring hazards to consumers' health (Sawio, 1994; Kibassa *et al.,* 2013; Kayombo and Mayo, 2018). Heavy metals might lead to very serious health effects to consumers of vegetables and other foods grown in urban areas if their concentration levels exceed the permissible limits as stipulated by the World Health

Organization (WHO), Food and Agriculture Organisation (FAO) and Tanzania Bureau of Standards (TBS).

Vegetables grown along the Msimbazi river valley feeds most of markets in the City. The study reported by Leonard *et al.* (2012) established the risk value as well as population at risk through oral exposure to vegetables grown in Msimbazi River valley. Previous studies by Leonard *et al.* (2012) also presented results on vegetable consumption which show that 95.6% of adults, 30.7% of children aged 6-12 years and 12.5% of children aged 2-6 years are at risk of getting Lead carcinogenic effects. Additionally, children aged 2-6 years (5%) followed by children aged 6-12 years (2.8%) and adults (1.2%) are at risk from carcinogenic Cadmium effects. For non-carcinogenic effects, 0.7% adults, 1.18% children aged 6-12 years, and 0.122% children aged 2-6 years are dermal exposed to Copper in vegetables.

Research by Kihampa and Mwegoha (2010) showed that due to contaminated surroundings that vegetables are cultivated including the soil, the water used for irrigation and the atmosphere, the vegetables are likely to absorb and uptake these heavy metals. However, the effect of blanching in reduction of heavy metals in leafy vegetables and uptake of metals from the soil by different varieties of vegetables has limited publications.

Blanching can be explained as the mild heat treatment given to fruits and vegetables before further processes like freezing, canning or drying so as to reduce the bulkiness, to aid easy packaging and inactivate enzymes or just for direct consumption (Bamidele *et al.*, 2017). It helps in modifying texture while maintaining the nutritional value of food (Manpreet *et al.*, 2000). The blanching process also helps in eliminating air that could have been trapped in the cells of fruits and vegetables (Bahceci *et al.,* 2004) preserving the colour and flavour (Elisabeth *et al.,* 2001). Due to the advantages that blanching of vegetables has in retaining the nutritional components in vegetables, it could be considered as a better method in preparation of vegetables rather than cooking.

The rationale of this study is to increase awareness and knowledge on the effect of blanching and provide information on the choice of vegetables to consider when practicing urban farming.

1.3 Objectives

1.3.1 General objective

To assess the safety of selected raw and blanched leafy vegetables grown along the Msimbazi River banks in Dar es Salaam, in relation to selected heavy metals, Lead (Pb), Cadmium (Cd) and Copper (Cu) concentration.

1.3.2 Specific objectives

i. To determine the levels of Lead (Pb), Cadmium (Cd) and Copper (Cu) in raw and blanched selected matured green leafy vegetables.
ii. To determine the effects of blanching in reduction of Pb, Cd and Cu in selected matured green leafy vegetables.
iii. Compare the levels of Pb, Cd and Cu in selected matured leafy vegetables across their sampling sites.
iv. Compare the uptake of Pb, Cd and Cu from soil by different selected matured leafy vegetables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Safe Vegetables in Human Nutrition

Food safety is a major public concern worldwide. This is serious problem in the whole world especially in economically poor countries. The production of safe food is an important aspect of food quality assurance as well as human health. During the last few decades, the increasing awareness of food safety has stimulated to do research on the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals residues and toxins (D'Mello, 2003).

Vegetables are valuable source of minerals, vitamins, fibers and contribute significantly to human health. They also have some functional constituents such as protein, vitamins and minerals which have health promoting role. Consumer's demand for quality and safe vegetables is increasing (Sobukola and Dairo, 2007). Vegetables have beneficial role in growth and development of the body, strengthen the immune system and help in disease prevention. Green leafy vegetables contribute sufficient quantity of vitamins and minerals to human diet. They are a good source of iron, calcium, phosphorus, folic acid, riboflavin, ascorbic acid and carotene. The vegetables contain high water content, sugars, protein, starch, fat and energy value (Nile and Park, 2014).

Results show that more than 71% of the 35 respondents use Msimbazi River as a major source of water for vegetable irrigation (Leonard *et al.,* 2012). One of the associated risks is contamination of crops by heavy metals and other toxic chemicals. The major entry is through roots and leaves absorbing the chemicals from contaminated soil, water and air (Zurera *et al.*, 1989). Excessive accumulation in agricultural soils may result not only in soil contamination, but has also consequences for food quality and safety. So, it is essential to monitor food quality, given that plant uptake is one of the main pathways through which heavy metals enter the food chain (Antonious and Kochhar, 2009). According to CODEX STAN 193-1995, vegetables are considered safe and of good quality when their levels of contaminants are within acceptable levels for human consumption that is they will not pose any effects to human health after consumption.

2.2 Sources of Heavy Metals Contaminants in Vegetables (Soil, Water, Air)

The main sources of heavy metal contamination are waste water used for irrigation, the application of metal-based pesticides and fertilizers in soils, industrial emissions and transportation. Wastewater used for irrigation is the major contributor of heavy metals contents of the soil (Mapanda *et al*., 2005).

2.2.1 Soil

Many heavy metals are considered as serious pollutants because of their toxicity nature, persistent and non-degradable conditions in the environment (Nwachukwu *et al*., 2010). Heavy metals in soils are derived from natural components or geological sources as well as from human activities or anthropogenic sources. The residence time of most heavy metals in soil is very long. Heavy metals are the most dangerous contaminants of soil ecosystem as well as food chain all over the world (Zahir *et al*., 2009). There are many sources of heavy metals in soils including (Reichman, 2002): Natural e.g. soil parent material, volcanic eruptions, marine aerosols, and forest fires; Agricultural e.g. fertilizers, sewage sludge's, pesticides and irrigation water; Energy and fuel production e.g. emissions from power stations; Mining and smelting e.g. tailing, smelting, refining and transportation; Automobiles e.g. combustion of petroleum fuels; Urban/industrial complexes e.g. incineration of wastes and waste disposal; and, Recycling operations e.g.

melting of scrap. Metal contamination issues are becoming increasingly common, the occurrence of heavy metals in soils, both natural and polluted, has been the subject of a number of studies (Muller and Anke, 1994; Dudka and Muller, 1999; Caussy *et al*., 2003; Cui *et al*., 2004). Different agricultural practices such as use of fertilizer, manure, weed killers to enhance the crop yield are also heavy metals contributor in the soil. Vegetable contamination with heavy metals is through absorption (from soil) and surface deposition (from polluted air). Some of the general impacts of higher concentrations of heavy metals in soils include retarded plant growth causing problems in nutrient uptake, physiological and metabolic processes.

2.2.2 Water

Irrespective of the importance of urban agriculture, there are various challenges facing urban farmers in the city. One of these challenges is water availability. Due to poor water supply, urban farmers use water from wells and rivers for irrigation and in some cases rainfall during wet season (Dongus, 2001). High concentrations of heavy metals were reported in vegetables from the untreated wastewater irrigated areas (Sinha and Gupta, 2005; Sharma *et al*., 2006). Use of industrial waste water for raising vegetables is very serious issue in Pakistan because these effluents are heavily loaded with harmful metals and metallic compounds (Singh *et al*., 2004). Using wastewater to irrigate agricultural land is one of the ways to reuse the wastewater from urban and industrial areas (Zegi, 2018). In most cases the safety of water used in urban areas is questionable because the sources are always not reliable. There is a possibility that water from these sources might be contaminated by various pollutants, including heavy metals. Dongus (2001) and Quarshie *et al.* (2011) further revealed that in many cases vegetable farmers in Dar es Salaam have complained about contaminated water used for irrigation. This implies that it is likely that irrigated water in the city is contaminated by pollutants from various sources such as manufacturing industries, domestic and agrarian-based pollutants. The situation may be complicated during floods because large amount of heavy metals from different sources are likely to be washed into valleys including those that are used for crops and vegetable production. This is due to the fact that floods, sewerage systems, river flows, air and water vapour all act as the medium for heavy metals distribution. In Msimbazi valley for example, crops are irrigated by polluted water with heavy metals or exposed to polluted air which can eventually bring hazards to consumers' health (Sawio, 1994). When water contaminated by heavy metal is used for irrigation chances are that the concentration of such metals in the soils might be high and eventually absorbed by plants and passed in to human being. If these metals are consumed by human beings, they might cause serious health effect.

2.2.3 Air

Vegetables are also contaminated with heavy metals during transportation and marketing due to polluted air (Agrawal, 2003). Heavy metal pollution of agricultural land and crops in the surrounding area of mining has been considered as a primary environmental concern (Kalali *et al.*, 2011). Vegetable contamination with heavy metals is through absorption (from soil) and surface deposition (Hezbullah *et al.*, 2016).

2.3 Uptake of Heavy Metals by Vegetables

For most garden soils, crop type has proven to be a stronger determinant of the edible crop metal concentration than soil contamination level (Alexander *et al.*, 2006; Waterlot *et al.*, 2013). Morpho-physiological nature of the vegetables greatly influence the quantity of the metal deposited on the plant surface (Khanna, 2011). The plant species possess different potential to remove and accumulate different metals and results in serious health complications when such food stuff is consumed (Zurera *et al.*, 1989).

It has been estimated that food of plant origin contributes about half of the mean ingestion of lead, cadmium and mercury (Itanna, 2002). There are number of factors that affect the metals uptake by the plant from the soil such as heavy metal concentration, type of the fertilizer applied, plant growth stage and species (Sharma *et al.*, 2006; Ismail *et al*., 2011).

2.4 Washing and Blanching of Vegetables

Vegetables grow in outside surroundings therefore they are highly subjected to various substances from the environment. Washing of vegetables is the first advised way to treat vegetables before consuming them as just by washing one can reduce bacteria such as *E. coli*, pesticides and even some traces of heavy metals. Studies by Sattar *et al.* (2015) showed various biological washing methods of reducing the concentrations of heavy metals in vegetables, some of the methods employed included tap water wash, various concentrations of radish and ginger solutions where they found out that a significant reduction in heavy metal contents was observed in washed vegetables compared to the unwashed vegetable but washing of vegetables with 8% ginger solution was found to be the most effective.

Traditional practices of vegetables washing to remove dirt and debris before consumption has been assumed to reduce the level of heavy metals and pesticide residues two to three times washing of vegetables with clean water significantly reduces the heavy metals contents (Abel-Rahman *et al.,* 2018). With washing, greater reduction was for lead and cadmium i.e. 75–100% than those for copper and zinc i.e. 27– 55% (Sing *et al.,* 2004).

Blanching is a technique used to partially treat vegetables while retaining their available nutrients. There are various methods used to blanch vegetables, hot water blanching and steam blanching being the most common methods. Studies reported by Bakare *et al.*

(2004) suggested that sometimes when vegetables are blanched the amount of metals in them increase this could be due to the fact that blanching activates certain reactions within the vegetables. Blanching effect is not only limited to elimination of heavy metals other studies by Bonneche` re *et al*. (2012) assessed the effects of washing, hot water blanching, microwave blanching and in-pack sterilization processing on the removal of five pesticide residues (deuteratedethylenethiourea, ethylenethiourea, deltamethrin, 3,5- dichloroaniline, boscalid) in spinach. Results showed that, among various processing, hot water blanching was the most effective way to remove the five pesticide residues by 10–70%, while microwave blanching without water reduced pesticide residues by a maximum of 39%, washing with tap water reduced residues by 10–50%.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

Msimbazi River is located in Dar es Salaam City, Tanzania. It originates from the highlands of Kisarawe in Coast Region. It flows toward the Northeast and enters the Indian Ocean on the Northern part of the City. It is joined by other tributaries and open channels including Sinza, Mambizi and Luhanga streams (Kironde, 2016). The Luhanga stream is fed by Ubungo, Kinyelele, Zimbile, and Kwangula tributaries (Chanzi, 2017).

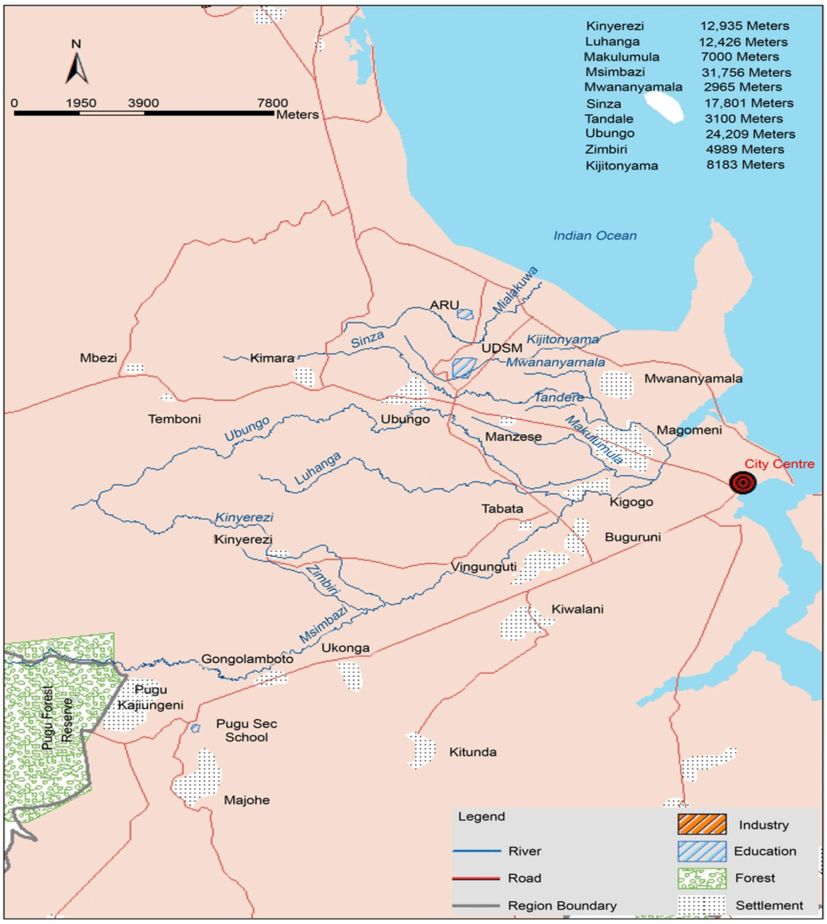

Figure 1: A map showing the Msimbazi River and its main tributaries

Source: Kironde (2016).

This study was carried out from 2 study sites which were chosen based on the type of their surrounding activities along the river and availability of the same varieties of vegetables from both sites at the moment of sample collection. Study site 1 was at Kigogo Sukita where the surrounding areas were different processing industries like the steel industries, food processing industries, paints, dyes and garages. Study site 2 was at Gongo la mboto-Ulongoni where the site was surrounded by mostly residential settlements.

3.2 Data Collection

3.2.1 Sample collection

(a) Vegetable sample collection

The selected leafy vegetables for this study were; Amaranthus (*Amaranthus hybridus*), Pumpkin leaves (*Cucurbita moschata*), Spinach (*Spinacia oleracea*) and Chinese cabbage (*Brassica rapa*). The reason for the selection of these vegetables was the differences in their maturity time as samples were collected at optimum maturity. The edible parts of the samples were collected from two study sites during the dry season. Each samples of vegetable were obtained from three randomly selected plots in triplicates making a total of 24 vegetable samples. The samples were then put in individual polythene bags and transported in a cool box to the SUA laboratory for analysis.

(b) Soil sample collection

Each soil sample was collected from the location where the respective vegetable sample was picked at 0-15cm depth (Kihampa and Mwegoha, 2010), carefully using hand trowel to dig the soil beneath the plant. The 24 soil samples collected were then, put in a polythene bag and transported to the SUA laboratory for analysis.

3.2.2 Sample preparation

(a) Vegetable sample preparation

The stalks were removed from the leafy green vegetable portions. All samples were air dried to reduce moisture content for 72 hours, then transferred in an oven at 70˚C until constant weight. Then, the dried samples were sliced into small pieces and grounded by the use of a laboratory milling machine. The blanched samples were blanched before being dried (TZS, 2003).

(b) Blanching process

The vegetables were washed to remove the dust particles from the field and thereafter wrapped in a muslin cloth and tied, the muslin cloth was dipped in boiling water (95˚C) for 4 minutes to avoid leaching of the metals, then the cloth was removed and the vegetables were put on steel trays and left to drip water for 30 minutes and there after they were oven dried at 65˚C for 24 hours (Hong *et al.*, 2017).

(c) Soil preparation

The soil samples were air dried in a glass house for 5 days, thereafter the samples were grounded by the use of a glass bottle, then they were mixed and sub sampled by the quartering method then, sieved to pass through a 2mm mesh size and subjected for analysis (TZS, 2003).

3.3 Laboratory Analysis

The laboratory analysis was done at the SUA laboratories in the Soil and Geological Sciences Department in Morogoro by the use of Atomic Absorption Spectrophotometer (UNICAM 919 AAS). The procedure for metal analysis of vegetable samples were extracted by employing the ashing method.

(i) Extraction of vegetable samples

The ground sample was accurately measured 3.000 g in a clean, dry and empty crucible. The crucible with its contents was placed in a muffle furnace and ashed at 600 °C for 4 hours. Then, 10 mls of 6N HCl solution was added into the ashed crucible with sample including blank. Then filtered into a 100 mls clean and dried beaker using a Whatman1 (125 mm) filter paper and the filtrate was transferred into 25 mls volumetric flask and topped up to the mark with distilled water then it was subjected for analysis (TZS, 2003).

(ii) Analysis of vegetable samples

Working standard solution of metals were prepared from stock standard solutions of Lead, Cadmium and Copper. A standard air-acetylene flame was used in all determinations. The lamp for each metal was inserted into the AAS. The lamp current for Pb, Cd and Cu was 10 mA, 8 mA and 10 mA respectively. The lamp current of each metal was set at 80% and warmed for 30 minutes before analysis started. A wavelength of 217.0 nm for Lead, 228.8 nm for Cadmium and 324.8 nm for copper was set before analysis. Analysis started by reading calibration standards then followed by samples. Blank and reference standards were used to control the quality of the analysis (TZS, 2003).

(iii) Extraction of Soil samples

The method for extraction of metals from soil samples was Aqua regia digestion. The soil was weighed in a pyrex beaker (10 g) followed by addition of 28 mls of Aqua regia solution (1 HCl: 3HNO3) which composed of 7 mls of conc. HCL and 21 mls of conc. HNO3 and then heated on a hot plate until the fumes turned from yellow to whitish, then the sample was left to cool. After cooling the sample was filtered by Whatman 1 (125 mm) into another clean and dry beaker and the filtrate transferred into 25 mls volumetric flask and topped up to the mark with distilled water then it was subjected for analysis (TZS, 2003).

(iv) Analysis of soil samples

Working standard solution of metals were prepared from stock standard solutions of Lead, Cadmium and Copper. A standard air-acetylene flame was used in all determinations. The lamp for each metal was inserted into the AAS. The lamp current for Pb, Cd and Cu was 10 mA, 8mA and 10 mA respectively. The lamp current of each metal was set at 80% and warmed for 30 minutes before analysis started. A wavelength of 217.0 nm for Lead, 228.8 nm for Cadmium and 324.8 nm for Copper was set before analysis. The analysis started by reading calibration standards then followed by samples. Blank and reference standards were used to control the quality of the analysis (TZS, 2003).

3.4 Statistical Analysis

The data were treated statistically using software packages R software and Microsoft Office Excel 2013. Descriptive statistics tool in Microsoft Office Excel 2013 was mainly used to determine the mean concentrations of metals in raw and blanched leafy vegetables. Paired T-test ($p \leq 0.05$) was carried out to examine the blanching effect in reducing heavy metals in selected vegetables. Analysis using two-way analysis of variance (ANOVA) with interaction was carried out to examine the statistical significance of differences in the mean concentration of metals between groups of vegetables in respect to their study sites. One-way ANOVA model was used to compare the uptake of metals in vegetables.

Two- way ANOVA model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

$Y_{ijk} = \text{Re}\,\textit{sponse from } i^{th} \textit{ site, } j^{th} \textit{ vegetable in } k^{th} \textit{ replicate}$

$\mu = \textit{Overall mean}$

$\alpha_i = \textit{Study sites} \qquad i = 1,2$

$\beta_j = \textit{Vegetabletypes} \qquad j = 1,2,3,4$

$(\alpha\beta)_{ij} = \textit{Interraction effect}$

$\varepsilon_{ijk} = \textit{Random error term}$

One-way ANOVA model

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

$Y_{ij} = \textit{response from ith vegetable and jth replicate}$

$\mu = \textit{Overall mean}$

$\tau_i = \textit{Vegetable types} \quad i = 1,2,3,4 \quad j = 1,2,3$

$\varepsilon_{ij} = \textit{error term}$

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Levels of Lead (Pb), Cadmium (Cd) and Copper (Cu) in Raw and Blanched Selected edible parts of Matured Green Leafy Vegetables

4.1.1 Raw vegetable samples

The results of the mean concentrations for Pb, Cd and Cu in leaves of Amaranthus (*Amaranthus spp),* Pumpkin leaves (*Cucurbita moschata*), Spinach (*Spinacia oleracea)* and Chinese (*Brassica rapa*) are presented in Table 1. The values in dry weight (mg/kg) ranged from 4.46 to 10.09, 0.49 to 0.79 and 6.8 to 11.89 for Pb, Cd and Cu, respectively in site 1 and 4.25 to 7.38, 0.61 to 0.92 and 6.6 to 9.45 in site 2. Concentrations of Pb and Cd in vegetables from both study sites were above the permissible levels as per WHO, FAO and TBS (0.3 and 0.2mg/kg respectively). In general, vegetables that were studied from different areas of Europe had a concentration of Pb higher than that of Cd (Nikolic *et al.*, 2014), the same case was shown in the present study and the results aligned with Kihampa and Mwegoha (2010) where their study showed that, with exception to Sweet potato leaves (*Ipomea batata*), other vegetables contained at least two types of heavy metals with high concentrations beyond the permissible values recommended by FAO and WHO for human consumption. However, they reported low levels of Cadmium within permissible limits unlike in the current study. This could be due to the sampling season, cumulative effects as a consequence of increased anthropogenic activities and as a result of population increase accompanied with increased industrial activities such as paint, battery, textile, milling and chemical industries which introduce heavy metals into the river (Kihampa and Mwegoha, 2010).

The current study also shows that there is a cumulative effect of the metals in the vegetables as the mean concentrations of Cu are higher than those previously reported for vegetable in Msimbazi River by Sonawane *et al.* (2013). Additionally, Josephat (2016) reported from a study carried out around Makumbusho that, all vegetables were safe with no risk to human health in amaranthus from consumption. However, a similar study by Kihampa *et al.* (2011) conducted on vegetables grown in the vicinity of the closed dumpsite showed that the levels of Zn, Cr, Pb and Cd were above the permissible levels of heavy metals in food as per FAO/WHO guidelines and Tanzania Bureau of Standards (TBS) permissible levels. The finding showed that vegetables grown along the closed Mtoni dumpsite were not suitable for human consumption. In another study by Manzoor *et al.* (2018) showed that, Pb and Cd in spinach (*Spinacea Oleracea*) and tomato (*Solanumly copersicum*) in India were above the maximum permissible limit according to the international organizations like Food and Agriculture Organization (FAO) and World Health Organization (WHO). Bui *et al*. (2016), studied the contamination of vegetables by heavy metals near mining sites in Northern Vietnam where the soils from the vegetable fields in the mining areas were found to be contaminated with Cadmium (Cd), Lead (Pb), and Arsenic (As), while irrigation water was contaminated with Pb. Average concentrations of Pb and As in fresh vegetable samples collected at the four mining sites exceeded maximum levels (MLs) set by WHO and FAO for Pb (70.6 % of vegetable samples) and As (44.1 % of vegetable samples), while average Cd concentrations in vegetables at all sites were below the MLs of 0.2 mg/kg. Similar findings were reported by Singh *et al*. (2014) for vegetables collected around Dinapur sewage treatment plant in Varanasi, India. The results showed that Pb and Cd concentrations in all vegetables were several folds higher than those reported earlier in India and other countries. Leafy vegetables such as Indian mustard, Amaranthus and Spinach showed higher concentrations of Pb and Cd than roots or tuberous vegetables. This could be due to the fact that leafy vegetables have high translocation, high transpiration and also fast growth rates (Itanna, 2002; Muchuweti *et al.,* 2006). Islam *et al.* (2016) monitored heavy metals like Cr, Ni, Cu, As, Cd, and Pb in potato (*Solanum tuberosum*), red onion (*Allium cepa*), and wild carrot (*Daucus carota*) in Bangladesh where the values of heavy metals reported were higher than the FAO/WHO permissible limits, indicating that the vegetables will pose health risks if consumed.

In the current study, Copper (Cu) concentrations in vegetables from both sites 1 and 2 were within permissible levels as per WHO, FAO and TBS standards (40 mg/kg). Although the concentrations of the Copper established for the vegetables are lower than those permitted by FAO/WHO, what matters in the long run is the quantities consumed and the frequency of intake (Chove *et al.,* 2006). As already noted, there is a cumulative effect on sustained intake of heavy metals, as they are not easily removed from the body.

4.1.2 Blanched vegetables sample

The results of the mean concentrations for Pb, Cd and Cu in blanched leaves of Amaranthus (*Amaranthus spp),* Pumpkin leaves (*Cucurbita moschata*), Spinach (*Spinacia oleracea)* and Chinese (*Brassica rapa*) are presented in Table 1. The values in dry weight (mg/kg) ranged from 3.21 to 8.42, 0.54 to 0.74 to and 6.4 to 11.28 to Pb, Cd and Cu, respectively in site 1 and 3.63 to 5.71, 0.43 to 0.88 and 6 to 10.47 in site 2 compared to the values of raw vegetables in dry weight (mg/kg) that ranged from 4.46 to 10.09, 0.49 to 0.79 and 6.8 to 11.89 for Pb, Cd and Cu, respectively in site 1 and 4.25 to 7.38, 0.61 to 0.92 and 6.6 to 9.45 in site 2. This shows that blanching slightly reduced the amount of metals in vegetables. However, it did not have so much effect in reduction of the heavy metals in vegetables and this could be due to the fact that blanching is mainly done to an extent where nutrients are still reserved in vegetables, unfortunately as nutrients are being reserved so are the harmful metals. According to Medine *et al*. (2007) the mineral and dietary fiber content of vegetables tend to be more resilient against loss from processing than vitamins, the report further stated that approximately 78 to 91 percent of minerals are retained after blanching. They further explain that when blanching in hard water, the uptake of calcium, potassium and sodium from the water far exceeds the potential mineral loss from the processing.

Table 1: Mean concentrations and Standard deviation (SD) of heavy metals in raw and blanched vegetables from the two study sites

	Raw vegetables (Mg/Kg)						**Blanched vegetables (Mg/Kg)**					
	Site 1 Mean ± SD			**Site 2 Mean ± SD**			**Site 1 Mean ± SD**			**Site 2 Mean ± SD**		
Vegetable types	**Pb**	**Cd**	**Cu**	**Pb**	**Cd**	**Cu**	**Pb**	**Cd**	**Cu**	**Pb**	**Cd**	**Cu**
Amaranthus Spp	10.09 ± 0.36	0.72 ± 0.22	11.89 ± 0.61	4.25 ± 1.09	0.79 ± 0.04	8.43 ± 1.27	8.42 ± 1.3	0.67 ± 0.17	11.28 ± 0.61	3.63 ± 1.88	0.72 ± 0.14	8.34 ± 1.27
Pumpkin Leaves	6.96 ± 4.78	0.49 ± 0.14	11.69 ± 0.7	4.46 ± 0.78	0.67 ± 0.24	9.45 ± 0.61	6.34 ± 3.55	0.38 ± 0.17	11.08 ± 1.27	4 ± 1.3	0.65 ± 0.4	9.43 ± 0.93
Spinach	5.5 ± 2.87	0.79 ± 0.2	11.28 ± 1.06	5.92 ± 2.01	0.92 ± 0.03	8.03 ± 1.96	5.38 ± 2.25	0.72 ± 0.03	11.18 ± 0.7	5.71 ± 2.52	0.88 ± 0.18	7.01 ± 1.61
Chinese	4.46 ± 0.95	0.47 ± 0.14	6.8 ± 1.27	7.38 ± 2.72	0.61 ± 0.31	6.6 ± 1.27	3.21 ± 0.95	0.45 ± 0.07	6.4 ± 1.61	4.5 ± 0.4	0.57 ± 0.46	6.5 ± 1.8

Allowed permissible levels as per WHO, FAO, TBS Standards Lead (Pb) = 0.3mg/kg, Cadmium (Cd) = 0.2 mg/kg, Copper (Cu) = 40mg/kg

4.2 Effects of Blanching in Reduction of Lead (Pb), Cadmium (Cd) and Copper (Cu) in Selected Matured Green Leafy Vegetables

From Figure 2 the average concentration of Pb in raw vegetables is slightly higher than that in blanched vegetables. The average concentrations of Cd for both raw and blanched vegetables are almost the same. The average concentration of Cu in blanched vegetables is slightly higher than that in raw vegetables.

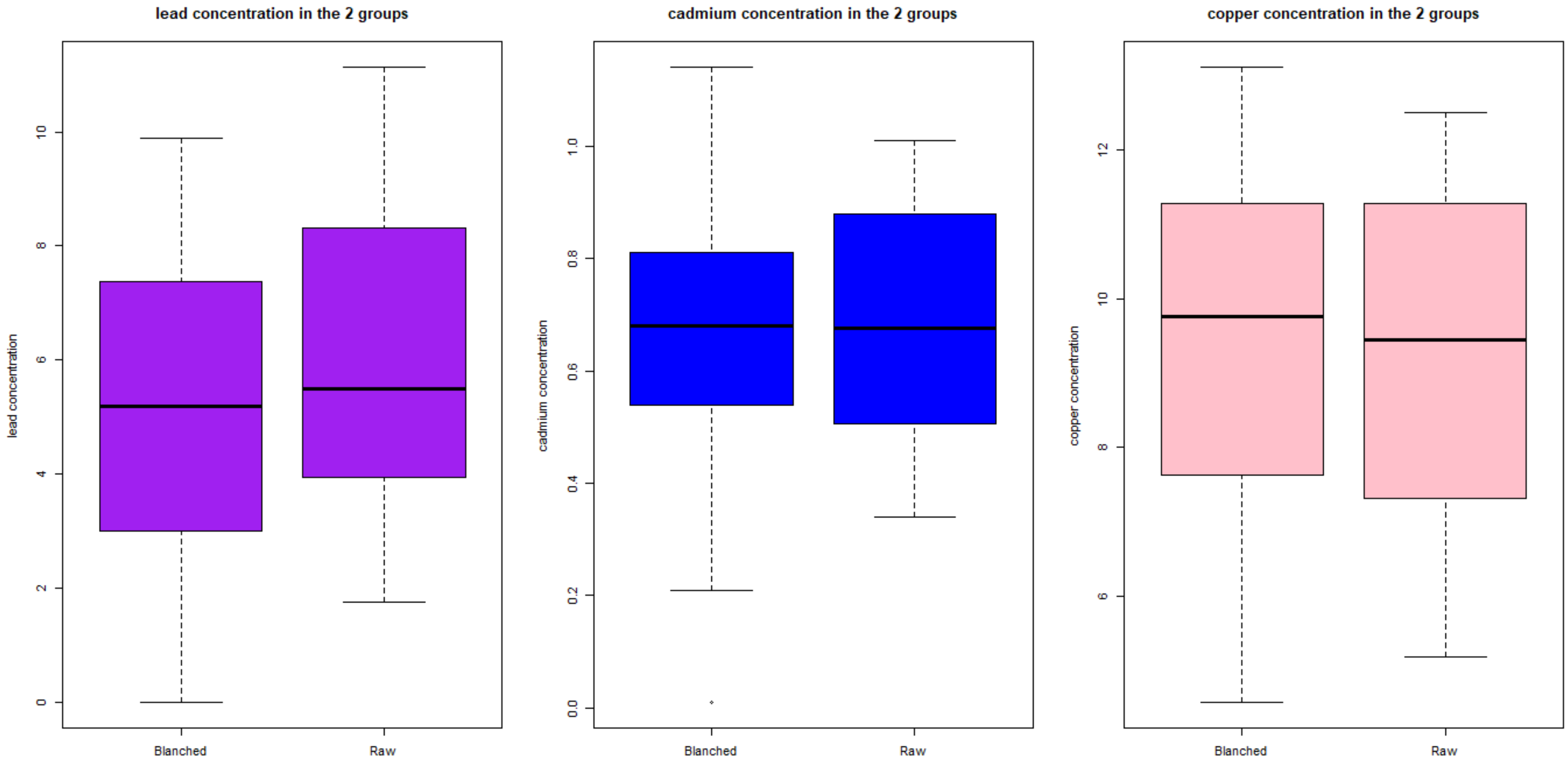

Figure 2: Box plots showing the average of the metal concentrations between the blanched and raw vegetables

The values from Table 2 indicate that the mean concentration of Pb in raw and blanched vegetables are significant different ($p<0.05$), this shows that there is a significant reduction of Pb through blanching of the vegetables. The mean concentration of Cd and Cu in raw and blanched vegetables are not significant different ($p>0.05$), this shows that there is no significant reduction of the two metals through blanching the vegetables. Blanching has been reported not only in reduction of heavy metals but also pesticides and other toxic elements from fruits and vegetables. This reduction could be due to degradation of the toxic substance or washing and leaching of the toxins into the blanching water (Bamidele *et al.,* 2017).

Table 2: The effect of blanching on Lead (Pb), Cadmium (Cd) and Copper (Cu) in vegetables

	Pb			Cd			Cu		
Vegetable group	**Mean**	**Mean difference**	**t-value (p-value)**	**Mean**	**Mean difference**	**t-value (p-value)**	**Mean**	**Mean difference**	**t-value (p-value)**
Raw samples	6.13			0.68			9.27		
Blanched samples	5.25	0.88	2.85 (0.01*)	0.67	0.02	0.39 (0.7)	9.25	0.03	0.11 (0.91)

*Significant levels: p< 0.05 0 '***' 0.001 '**' 0.01 '*' 0.05*

Further studies by Bakare *et al*. (2004) Blanching had an effect on the availability of Cd and Pb but not Hg in Amaranthus, irrespective of the site location. Cd increased more than three-fold in Amaranthus after boiling than when it was fresh, whereas there was a slight decrease in the amount of Pb.

4.3 Levels of Lead (Pb), Cadmium (Cd) and Copper (Cu) in Selected Leafy Vegetables between their Sampling Sites

From Figure 3 the average concentration of Lead and Copper in site 1 is higher than that in site 2 whereas the average concentration of Cadmium is higher in site 2 compared to that of site 1.

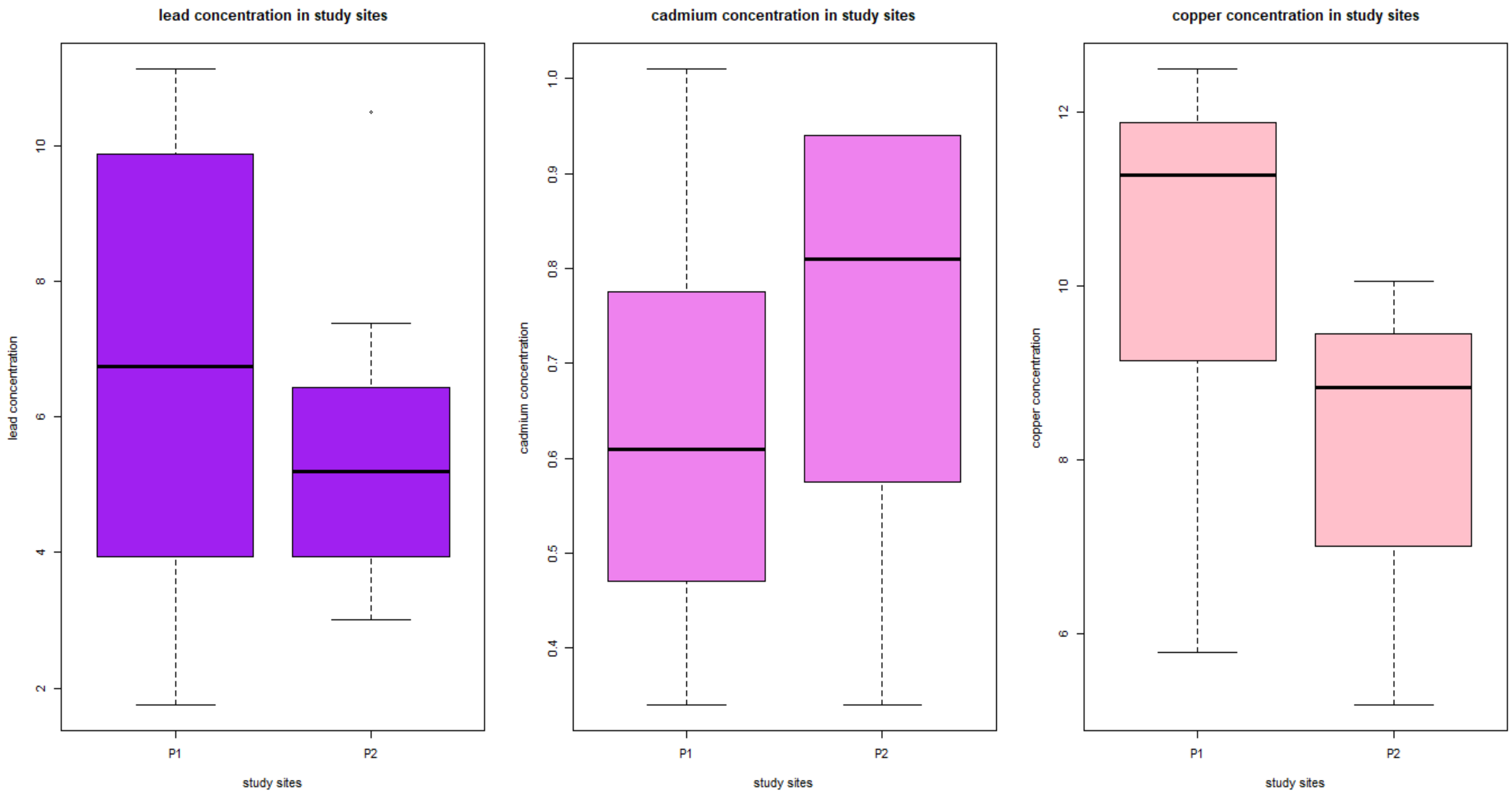

Figure 3: Box plots showing the average concentration of metals between study sites

From Figure 4 the average concentration of Lead in vegetables is in the order that *C.moschata* contains the lowest concentration of Pb and *Amaranthus spp* has the highest concentration of Pb compared to the rest of the vegetables and the average concentration of Cadmium in vegetables is in the order *B.rapa* has the lowest concentration of Cd compared to the rest of the vegetables and *S.oleracea* contains the highest concentration of Cd, these results aligned with the results found in Bangladesh where Cd, Ni, and Cr was reported to be the highest in spinach while amaranth showed highest concentration in Pb and Co (Naser *et al.,* 2011). The difference in level of heavy metal contamination between spinach and amaranth species was due to their morpho-physiological differences in terms of heavy metal content, exclusion, accumulation, foliage deposition and retention efficiency (Carlton-Smith and Davis, 1983; Naser *et al.*, 2011). The average concentration of Copper in vegetables were in the order that *B. rapa* has the lowest concentration of Cu compared to the rest of the vegetables and *C.moschata* contains the highest concentration of Cu.

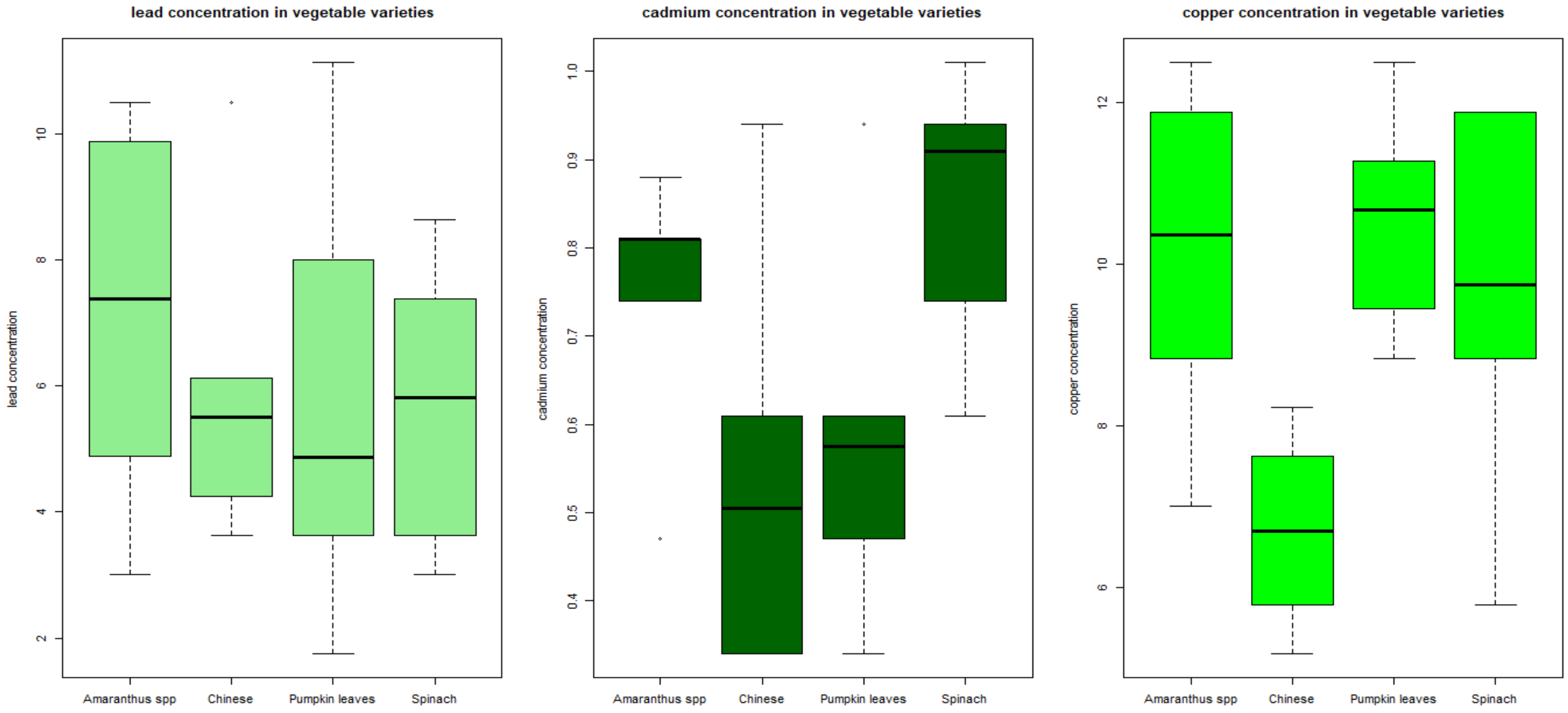

Figure 4: Box plots showing the average concentration of metals in vegetable types

The comparison in metal concentration between study sites and vegetable types is indicated in Table 3, where the results for the three heavy metals are presented. The study sites are not significantly different ($p<0.05$) this shows that the mean concentration of Pb does not vary across sites. Results show that the vegetable types are not significantly different ($p>0.05$) this indicates that the mean concentration of Pb does not vary across the vegetable types. But there is a significant interaction effect between the vegetable types and the study sites ($p<0.05$) this shows that the mean concentration of Pb vary across vegetables and sites as shown in Figure.5.

Table 3: **Comparison in metal concentration between study sites and vegetable types**

	Metals					
	Pb		Cd		Cu	
Parameters	**mean ± SD**	**F-value (P-value)**	**mean ± SD**	**F-value (P-value)**	**mean ± SD**	**F-value (P-value)**
Study sites	6.128 ± 2.66	1.645 (0.22)	0.683 ± 0.209	2.767 (0.12)	9.272 ± 3.118	22.753 (0.000209 ***)
Vegetable types	6.13 ± 2.824	0.519 (0.68)	0.91 ± 0.180	3.632 (0.0359 *)	9.272 ± 1.716	13.345 (0.000127 ***)
Study sites* vegetable types	6.12 ± 1.965	3.747 (0.0326 *)	0.683 ± 0.166	0.088 (0.9657)	9.2 ± 1.094	2.408 (0.105)

*Significant levels: p< 0.05 0 '***' 0.001 '**' 0.01 '*' 0.05*

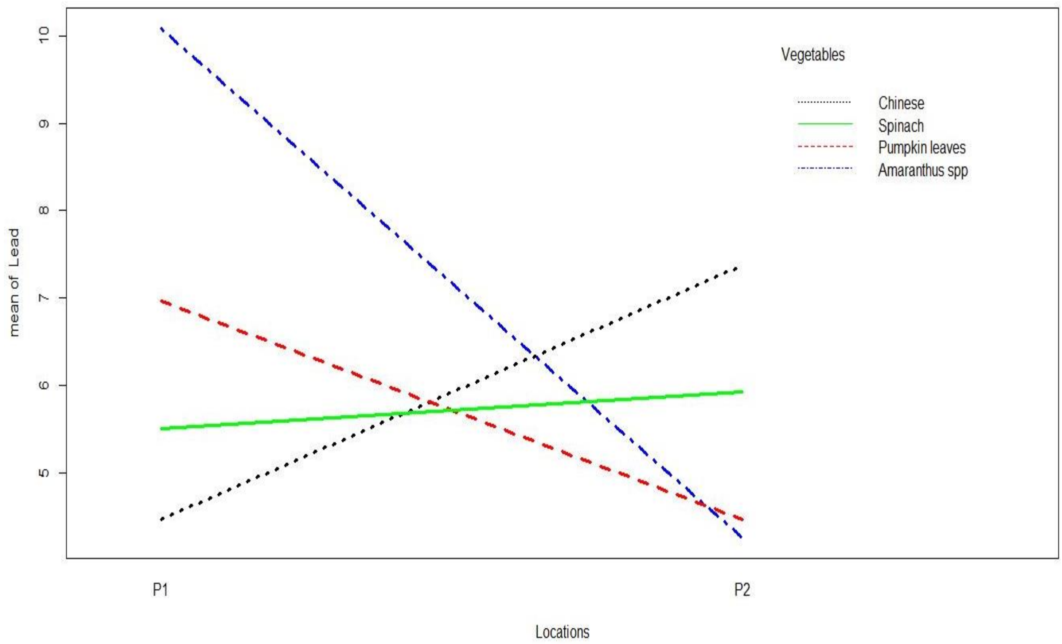

Figure 5: **Plot showing interaction effect between vegetable types and study sites**

The levels of Cd concentration in the two study sites is not significantly different ($p>0.05$) this shows that the mean concentration of Cd does not vary across sites. Vegetable types are significantly different ($p<0.05$) where the difference is seen between Chinese and spinach vegetables as shown in Table 4. There is no significant interaction effect between the vegetable types and the study sites ($p>0.05$).

The level of Cu concentration in the two study sites is significantly different. The level of Cu concentration among vegetable varieties is significantly different where the difference is seen between Chinese and Amaranthus, Pumpkin leaves and spinach as shown in Table 4. There is no significant interaction effect between the vegetable types and the study sites ($p>0.05$).

Table 4: Mean and Standard deviation (SD) of vegetables in their study sites

		Cd	Cu
Variable	**Vegetable type**	**Mean ± SD**	**Mean ± SD**
Vegetables	Amaranthus	0.75±0.15[abc]	10.16±2.09[bcd]
	Chinese	0.54±0.22[ad]	6.71±1.14[a]
	Pumpkin leaves	0.59±0.2[bd]	10.57±1.36[c]
	Spinach	0.85±0.15[cb]	9.65±2.27[dc]
Sites	Site 1		10.41±2.33[a]
	Site 2	N/A	8.13±1.58[b]

The mean ± SD along the same column bearing similar letter(s) are not significantly different at 5% level of probability
N/A- not applicable

4.4 Comparison of the Uptake of Pb, Cd and Cu from soil by different Selected Matured Leafy Vegetables

Figure 6 presents the uptake levels by the vegetables from the soil for the three metals; On average uptake concentration of lead in vegetables is in the order that *B.rapa* uptakes Pb more than the other vegetables and *S.oleracea* uptakes it less compared to the other vegetables. On average uptake concentration of cadmium in vegetables is in the that *S.oleracea uptakes Cd more* than the other vegetables and *C. moschata* uptakes it less compared to the other vegetables. On average uptake concentration of copper in vegetables were in the order that *B.rapa* uptakes Cu more than the other vegetables and *Amaranthus spp* uptakes it less compared to the other vegetables.

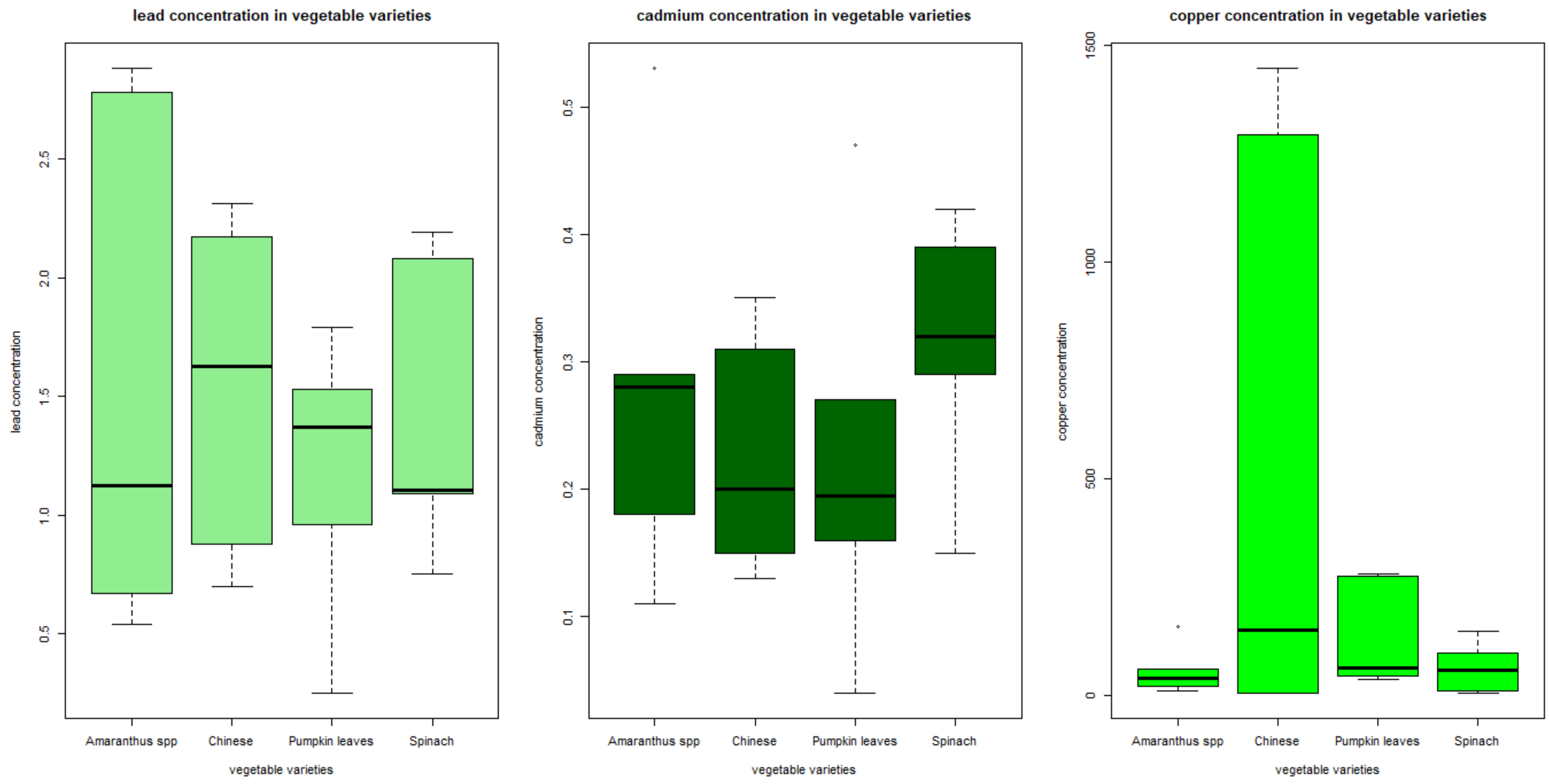

Figure 6: Box plots showing the average concentration of metals uptake in vegetable types

From Table 5 the uptake of Pb, Cd and Cu among vegetable varieties is not significant different ($p>0.05$) this shows that all vegetable varieties uptake the metals at very close rates. Vegetables have a very well-organized mechanism to uptake essential micronutrients from the soil. The root system of the vegetables in association with chelating agents and redox reactions can solubilize and uptake micronutrients at even very low ppm. The efficient mechanisms of vegetables help in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements (Manzoor *et al.,* 2018). Zhou *et al.* (2016) studied 22 vegetables for heavy metals accumulation and assessed the health risk factors of consuming the vegetables by target hazard quotient (THQ) method. Leafy vegetables accumulated more heavy metals as compared to melon vegetables, which is an indication that cultivation of melon vegetables is suitable for soil contaminated with heavy metals (Manzoor *et al.,* 2018). Ning *et al.* (2015) found highest levels of heavy metals in leaf lettuce (*Lactuca sativa*) 125.52µg/g dry weight and bitter lettuce (*Lactuca virosa*) 71.2 µg/g dry weight. Vegetable species differ widely in their ability to take up and accumulate heavy metals, even among cultivars and varieties within the same species (Zhu *et al.,* 2007; Säumel, 2012). Edible amaranth, spinach, and caraway had higher concentrations and bioaccumulation factors (BAFs) of Pb, Cd, Zn, and as and were classed as “high accumulators (Bamidele *et al.*, 2017). Studies of Pb mobility in leafy vegetables have shown the partitioning of Pb greatest in roots, followed by a decreasing concentration gradient in above ground biomass (Rahlenbeck *et al.,* 1999; Finster *et al.,* 2004). Hence, from Boolaroo the elevated concentrations of Pb in vegetables may be a result of foliar uptake of aerial Pb deposits originating primarily from the nearby smelter (Anthony and Balwant, 2005). The variation of mean concentrations of the metals in soils at different growth stages of three vegetables plots was non-significant (Naser *et al.*, 2011). This might be due to higher mobility of Cd with a natural occurrence in soil (Alam *et al.*, 2003).

Table 5: Metal Uptake by different Vegetable varieties from the study sites

Metals	Parameters	Vegetable varieties			
		Amaranthus	**Chinese**	**Pumpkin**	**Spinach**
Lead	mean ± SD	1.52 ± 1.04	1.55 ± 0.66	1.21 ± 0.55	1.39 ± 0.6
	F-value(p-value)	0.263 (0.851)			
Cadmium	mean ± SD	0.28 ± 0.14	0.22 ± 0.09	0.22 ± 0.14	0.32 ± 0.09
	F-value(p-value)	0.85 (0.483)			
Copper	mean ± SD	55.21 ± 53.49	673.25 ± 509.49	127.53 ± 117. 59	63.15 ± 55.98
	F-value(p-value)	2.371 (0.101)			

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

This study evaluated the concentrations of heavy metals in green leafy vegetables from areas used for urban agriculture in Dar es Salaam and assessed their safety based on the permissible limit by WHO, FAO and TBS. Except for Copper other metals (Lead and Cadmium) concentrations in both raw and blanched vegetables were above the permissible level established by all the mentioned standards suggesting that heavy metal pollution in these areas might pose health risks to humans. Blanching has shown a significant reduction of Lead and not for the other two metals (Cadmium and Copper) but still Lead was above the allowable permissible levels.

From the study the levels of Lead did not vary between study sites or among vegetables. The study further showed that Copper concentration was higher in site 1 compared to site 2, Cadmium was higher in site 2 compared to site 1. The study revealed that despite the differences in the surrounding activities from both study sites still all vegetables were highly polluted. The present study revealed that there was high concentration of heavy metal in soils and vegetables therefore the uptake of Lead, Cadmium and Copper concentrations among vegetable varieties showed that all vegetable varieties featured similar metal uptake rates.

The study findings are of significant importance to farmers or individuals who are practicing urban farming; it provides them with information on the choice of vegetables to consider when practicing urban farming. Also, the study helps in creating or increasing awareness and knowledge on the treatment effect (blanching) of vegetables.

5.2 Recommendations

i. Although Copper was within permissible levels the accumulative effect must be taken into consideration. If proper actions are not taken, then with time it might exceed the permissible levels. Therefore, further research should be carried out to monitor the accumulative effect of the heavy metals in vegetables that are grown within contaminated surroundings.

ii. It is recommended that other treatment effects could be applied to reduce metals at a better rate compared to the one used in this study. Singh and Prasad (2011) stated that, a promising, relatively new technology for removal of heavy metal from contaminated sites is phytoremediation. They also suggested that, research related to relatively new technology should be promoted and emphasized and expanded in developing countries where heavy metal pollution has already touched alarming level. Example, the levels we are currently experiencing in Dar es Salaam.

iii. Therefore, in highly contaminated areas like Msimbazi river valley it is recommended that the government should set policies to ensure that only treated or partially treated waste is discharged in the river so as the water used for irrigation is less contaminated

iv. Also, growth of low accumulator vegetables example Melon vegetables, Indian grass or Willows would be more suitable rather than high accumulators like leafy vegetables in such highly polluted areas.

v. Since the study was carried out during the dry season only then it is limited to seasonal variations. Therefore, I would recommend for other researchers to carry a similar study during the wet season or both seasons for comparison.

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