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Full Length Research Paper

Determination of Zn, Mn, Fe and Cu in spinach and lettuce cultivated in Potiskum, Yobe State, Nigeria

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The concentrations of four heavy metals, Zn, Mn, Fe and Cu were determined in the roots and leaves of lettuce (*Lactuca sativa*) and spinach (*Amaranthus caudatus*) cultivated in Potiskum, Yobe State, Nigeria. The heavy metals were also determined in soil where the vegetables were cultivated. The determination processes were done by Atomic Absorption Spectrophotometer (AAS) techniques. The results show that the heavy metals contents were higher in the soil than the vegetables and also higher in the roots than the leaves in the two vegetables. The levels of Fe obtained in the leaves and roots of spinach were 0.11±0.01 and 0.21±0.05, respectively. The Fe content in the soil was 0.28±0.01. It could be concluded that lettuce and spinach cultivated in Potiskum, Yobe state, Nigeria contained Zn, Mn, Fe and Cu in variable concentrations which may be due to different farming practices adopted in the area to boost productivity. However, the results obtained in this study were lower than the published threshold values (10 to 20.00 mg/kg for Fe and 30 to 300 mg/kg for Pb) considered toxic for plant tissues as well as the critical values or values (Fe: >200-500 mg/kg and Pb: >4 to 30 mg/kg) regarded as excessive. The results were equally lower than the WHO maximum limit (ML) (Pb, 0.3 mg/kg; Zn, 100 mg/kg; Cd, 0.1mg/kg; Mn, 500 mg/kg; Fe, 425 mg/kg; Cu, 73mg/kg; Ni, 67mg/kg and Co, 50 mg/kg) in vegetables. Consumption of these vegetables may not pose health hazards.

Key words: AAS, farming practices, heavy metals, productivity, Potiskum, vegetables.

INTRODUCTION

Long ago, environmental pollution was due to naturally occurring phenomenon such as volcanic eruption. Today, due to the increase in technological innovations, a lot of chemicals are emitted into the environment both in urban and rural areas. These chemicals cause hazards to man and his environment. They are often accumulated in significant concentrations in estuaries, seas, soils and foods (Shagal et al., 2012). Contamination of the soils where plants are grown leads to contamination of plants and animals who feed on them and invariably the health of the human person are at risk of being affected (Willis and Saviry, 1995; Tuzen, 2003).

To ensure increase in productivity, different farming practices are usually adopted by farmers. One of such practices is the application of fertilizers and animal dung to the soils. Irrigation of the crops with all sorts of wastewater is another practice. There is the possibility of over application of such fertilizers or animal dung and wastewater. These may result in the accumulation of certain substances in soils and indeed the crops growing in such soils and may indeed affect the quality of the crops adversely (Anjana and Iqbal, 2006). These substances may have direct or indirect phytotoxic impacts on the crops leading to a decline in yields and threatening food supplies. In addition, the plants may act as a vehicle for transferring these substances into the food chain (Radojevic and Bashkin, 1999).

Heavy metals can be defined as metals having specific gravities greater than 5.0g/cm^3. Most heavy metals are non-biodegradable and persist in the ecosystem (Ademoroti, 1996). Living organism requires some amount of trace elements such as Fe, Pb, Co and Cr, but in excess they may be toxic. Heavy metals contamination is a general term use to describe a condition in people

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having abnormally high levels of toxic metals in their body. Common ones are Hg, Pb, Cd and As. Contaminations of plants by heavy metals are common. Some occur as a result of untreated water used for irrigation, unapproved usage of chemicals, and others as a result of combustion and discharging of fossil fuel from industries, vehicles and other machineries to the soils (Ademoroti, 1996). Discharging of sewage sludge and animals wastes are all sources of heavy metals contamination of soils and plants (Ademoroti, 1996). One of the anthropogenic sources of metals in surface water includes domestic waste and garbage. Therefore the metals find their way by leaches into the soil where plants may be contaminated in the food chain (Shagal et al., 2012). The result is that some plants may represent a serious hazard if consumed as food (Miroslay and Vladimir, 1998).

Plants do not readily absorb large amount of heavy metals, and the amount they do absorb depends on the species and variety of plant, the chemical composition of the soil, the amount of heavy metal and the soil temperature. Intake of vegetables is an important path of heavy metal toxicity to human being. Dietary intake of heavy metals through contaminated vegetables may lead to various chronic diseases (Adefemi et al., 2012). Duruibe et al. (2007) suggested that biotoxic effect of heavy metals depend upon the concentration and oxidation states of heavy metals, kind of sources and mode of deposition.

Heavy metals like Cu, Co, Zn, Fe, and Mn at low concentrations are essential metals for enzymatic activities and many biological processes. Other metals such as Cd and Hg have no known essential role in the body of living organisms and are toxic even at low concentrations. The essential metals also become toxic at high concentration (Jonathan and Maina, 2009). Food chain contamination by heavy metals has become a burning issue in recent years because of their potential accumulation in biosystems through contaminated water, soil and air (Uwah et al., 2009). Continue eating of crops that contain high levels of heavy metals may cause serious health problems. For example, cadmium is readily accumulated by plants and may get to levels which are poisonous to the plants and consequently posing a threat to animals and humans that consume the plants (Radojevic and Bashkin, 1999). Severe contamination of Cd may result in pulmonary effects such as *emphysema*, *bronchiolitis* and *alveolitis.* Renal effect may also result due to subchronic inhalation of Cd (European Union, 2002). Lead toxicity causes reduction in haemoglobin synthesis, disturbance in functioning of kidney, joints, reproductive and cardiovascular systems and chronic damage to the central and peripheral nervous systems (Ogwuegbu and Muhanga, 2005).

Uwah et al. (2011) studied the heavy metal levels in spinach (*A. caudatus*) and lettuce (*L. sativa*) grown in Maiduguri, Nigeria. They noted that agronomic practices such as application of fertilizers and use of waste water can affect bioavailability and crop accumulations of heavy metals and that the vegetable and soil samples studied contained variable levels of heavy metals (As, Cd, Cu, Fe, Mn, Pb and Zn). In a related study, Uwah et al. (2012) investigated the heavy metal levels in roots and leaves of some vegetables grown in Gwoza, Borno state, Nigeria. They noted the vegetables (spinach, groundnut and bean) obtained in Gwoza contained some heavy metals (Co, Cr, Pb, and Fe) in variable concentrations due to intensive and different farming activities such as application of fertilizers and irrigation with wastewater in the area.

In another study, Adefemi et al. (2012) studied the level of some heavy metals (Cd, Co, Cr, Ni, Cu, Fe, Mn, Pb and Zn) in some edible plants collected from selected dumpsites in Ekiti State, Nigeria. They pointed out that in the plants (*A. cruentus* and *Talinum fruticosum*) studied; the results showed variations in the metals accumulation and that the results showed that the plants were highly contaminated with heavy metals. They noted that in Atikankan and Aba – Egbira, Co was actively transported to the leaves of *A. cruentus* where it was accumulated. They also noted from the results that *A. cruentus* and *T. fructicosum* have an unusual ability not only to accumulate heavy metals in their roots but also translocate them to the edible parts. They concluded that consumption of these vegetables with elevated levels of heavy metals may cause related health disorders. In a similar study, Bioaccumulation of trace metals concentration in some vegetables grown near refuse and effluent dumpsites along Rumude-Doubeli bye-pass in Yola North, Adamawa State, Shagal et al. (2012) noted that the three vegetable samples (*Amaranthus* spp, *Hibiscus sabdariffa* and *Lactuca sativa*) all contain the investigated trace metals (Cd, Cu, Pb,Mn and Zn) in variable concentrations. They pointed out that Pb which is toxic and hazardous to man was found to be within the range of 5.02 to 8.80 mg/kg in the samples and that Cd had its lowest concentration of 10.00 mg/kg in *L. sativa* and highest concentration of 16.67 mg/kg in *H. sabdariffa*. They concluded that these values were higher than the maximum permitted level proposed by FAO/ WHO guidelines and that all the vegetables studied were contaminated by Cd and Pb thus rendering the plants toxic to consumers.

Potiskum, Yobe state, north eastern region of Nigeria lies on the geographical coordinates of 11° 42' 42" N, 11° 4' 10" E is known for its light annual rain falls. In this area, the use of polluted water for growing of vegetables is a common practice as done in the immediate surroundings of big cities in Nigeria (Uwah, 2009; Uwah et al., 2011). Although this water is considered to be a rich source of organic matter and plant nutrients, it also contains sufficient amounts of soluble salts and heavy metals like Fe, Mn, Cu, Zn, Pb, Ni, Sn, Hg, Cr, As, Al (Kirkhan, 1983; Uwah, 2009; Uwah et al., 2011). When such water is

Table 1. Heavy metal levels (mg/kg) in soil as well as in the roots and leaves of spinach obtained in Anguwan Jaji, Potiskum, Yobe State.

Spinach /Soil	Heavy metals (mg/kg)			
	Zn	Mn	Fe	Cu
Leaves	$0.08^{a}\pm0.01$	$0.02^{a}\pm0.006$	$0.11^{a}\pm0.01$	$0.03^{a}\pm0.01$
Roots	$0.21^{a}\pm0.01$	$0.13^{b}\pm0.007$	$0.21^{a}\pm0.05$	$0.14^{b}\pm0.01$
Soil	$0.25^{a}\pm0.01$	$0.18^{c}\pm0.01$	$0.28^{a}\pm0.01$	$0.18^{c}\pm0.02$

The above values are means of replicate values (n = 3). Within column, means with different alphabets are statistically different (p<0.05).

used for cultivation of crops for a long period, these heavy metals may accumulate in soil and may be toxic to the plants and also cause deterioration of soil (Kirkhan, 1983).

In this study, the roots and leaves of lettuce (*L. sativa*) and spinach (*A. caudatus*), largely cultivated in Potiskum, Yobe State, Nigeria, were investigated to determine the levels of some heavy metals (Zn, Mn, Fe and Cu) in the vegetables. This became necessary considering the importance of these and other vegetables to health and the fact that many people have now resulted to eat vegetables for their healthy living. Indeed, vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, as well as vitamins, minerals and trace element (Abdola and Chmtelnicka, 1990). The levels of the heavy metals were also determined in soil where the vegetables are cultivated.

MATERIALS AND METHODS

Samples and sampling

Vegetable samples (roots and leaves of spinach and lettuce) and soil within the area were collected in October, 2011 from vegetable farms in Anguwan Jaji, Potiskum, Yobe state, where the vegetables are cultivated with the use of fertilizers, animal dung and all sort of available wastewater. The samples were collected using the techniques described by Radojevic and Bashkin (1999). The samples were homogenised to obtain representative samples and were properly labelled and transported to the laboratory in clean polythene bags for analyses.

Digestion and analysis of samples

Soil and sliced vegetable samples were dried in an oven at 105°C for 24 h until they were brittle and crisp (USEPA, 1996). A portion (2 g) of dried, disaggregated and sieved vegetable and soil samples were placed separately in 100 cm^3 Teflon beakers and then digested with 15 cm^3 concentrated nitric acid at 550°C for about 3 h, the digests were then filtered into a 100 cm^3 volumetric flask (USEPA, 1996; Ademoroti, 1996). Levels of Fe, Co, Pb and Cr in the vegetable and soil samples digest were analysed using an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer (AAS) equipped with an air - acetylene burner. The heavy metals concentrations in the vegetable and soil samples were calculated using:

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L)} \times V}{M} \quad (1)$$

Where:
V = Final volume (100 cm^3) of solution after digestion
M = Initial weight (2 g) of sample measured.

Transfer Factors (TF) for Heavy Metals from Soil to Vegetables

Transfer factor is the ratio of the concentration of heavy metals in a plant to the concentration of heavy metals in soil. The transfer factors (TF) for each heavy metal were computed based on the method described by Harrison and Chirgawi (1989) according to the following formula:

$$TF = Ps \text{ (mg/kg dry wt)}/St \text{ (mg/kg dry wt)} \quad (2)$$

Where:
Ps is the plant- metal content originating from the soil
St is the total heavy metal contents in the soil.

Data analyses

The data collected were in replicates of three. Data collected were subjected to statistical tests of significance using analysis of variance (ANOVA) and the Student t-test at p<0.05 to assess pairs of results in the vegetables and soils. That was, to assess significant variation in the levels of the heavy metals in the vegetables as well as in soils. Probabilities less than 0.05 ($p < 0.05$) were considered statistically significant. All statistical analyses were done by SPSS software for windows.

RESULTS AND DISCUSSION

Levels of the heavy metals obtained in the vegetable and soil samples are shown in Tables 1 and 2. From the

Table 2. Heavy metal levels (mg/kg) in soil as well as in the roots and leaves of lettuce obtained in Anguwan Jaji, Potiskum, Yobe State.

Lettuce /Soil	Heavy metals (mg/kg)			
	Zn	Mn	Fe	Cu
Leaves	0.06^{a}±0.004	0.06^{a}±0.005	0.07^{a}±0.005	0.02^{a}±0.004
Roots	0.19^{b}±0.007	0.16^{b}±0.01	0.08^{a}±0.1	0.15^{b}±0.002
Soil	0.25^{c}±0.01	0.18^{c}±0.01	0.28^{b}±0.01	0.18^{c}±0.02

The above values are means of replicate values (n = 3). Within column, means with different alphabets are statistically different (p<0.05).

Table 1, the heavy metal levels in the leaves of spinach ranged from 0.02 ± 0.006 mg/kg Mn to 0.11 ± 0.01 mg/kg Fe. In the roots, the Heavy metal levels ranged from 0.13 ± 0.007 mg/kg Mn to 0.21 ± 0.05 mg/kg Fe. In the lettuce (Table 2), the heavy metal levels ranged from 0.02 ± 0.004 mg/kg Cu to 0.07 ± 0.005 mg/kg Fe in the leaves, while in the roots, the heavy metal levels ranged from 0.08 ± 0.1 mg/kg Fe to 0.19 ± 0.007 mg/kg Fe. In Tables 1 and 2, the heavy metal levels in the soil samples ranged from 0.18 ± 0.01 mg/kg Mn to 0.28 ± 0.01 mg/kg Fe. The results revealed that the roots and leaves of spinach and lettuce obtained in Potiskum, Yobe state contained Zn, Mn, Fe and Cu in varied concentrations may be due to the peculiar type of agricultural practices in the area, such as the use of polluted water for growing of vegetables and the application of fertilizers and animal dung. In Table 1, statistical test of significance using ANOVA and t- test revealed no significant differences ($p < 0.05$) between the levels of Zn in the soil as well as in the roots and leaves of spinach. The results also revealed no significant differences ($p < 0.05$) between the levels of Fe in the soil as well as in the roots and leaves of spinach. There were however, significant differences ($p > 0.05$) in the concentrations of Mn as well as Fe in the soil and the leaves and roots of spinach. In Table 2, all the elements analysed showed significant differences ($p > 0.05$) in the soil as well as the leaves and roots of lettuce. The only exception was Fe in the leaves and roots of lettuce which gave no significant difference ($p< 0.05$). The results simply explained that the heavy metals accumulated in the soils are transported to the vegetables through their roots by the process of absorption. In Tables 1 and 2, the order of contamination of the heavy metals in both the soil and vegetable samples were: Fe> Zn > Cu > Mn. The exception was in the leaves of lettuce in which the heavy metal levels were in the order of: Fe > Zn = Mn > Cu. The results were in agreement with previous studies by Uwah *et al.,* 2009; Uwah, 2009 , Uwah *et al.,* 2011; Uwah *et al.,* 2012 as well as Adefemi *et al.,* 2012. The values obtained in this study were lower than the published threshold values considered toxic for plant tissues as well as the critical values or values regarded as excessive. The results were equally lower than the WHO maximum limit (ML) of heavy metals in vegetables. The values considered as toxic for some of the metals are: 10 to 20.00 mg/kg for Fe and 30 to 300 mg/kg for Pb (Kabata-Pendias and Pendias, 1984). The critical values or values regarded as excessive are; Fe: >200 - 500 mg/kg and Pb: >4 - 30 mg/kg (EC-UN/ECE, 1995a). The heavy metal levels were also lower than the WHO maximum limit (ML) of heavy metals in vegetables. The WHO maximum limit (ML) of some metals in vegetables are: Pb, 0.3mg/kg; Zn, 100 mg/kg; Cd, 0.1mg/kg; Mn, 500 mg/kg; Fe, 425 mg/kg; Cu, 73 mg/kg; Ni, 67 mg/kg and Co, 50 mg/kg (FAO/WHO, 2001).

Transfer Factors (TF) of the Heavy Metals from Soil to Vegetables

The transfers of the heavy metals from soil to the vegetables are presented in Tables 3 and 4. Transfer factor is one of the key components of human exposure to heavy metals through the food chain. Transfer factors were computed for the heavy metals to quantify the relative differences in bioavailability of metals to vegetables or to identify the efficiency of a vegetable species to accumulate a given heavy metal. These factors were based on the root uptake of the heavy metals and not the foliar absorption of atmospheric metal deposits (Lokeshwari and Chandrappa, 2006; Awode et al., 2008). In Tables 3 and 4, the Transfer Factors of all the heavy metals in the vegetable parts were less than 1. It is easy for plants species with TF > 1 to translocate metals from roots to shoots than those which restrict metals in their roots (those with TF less than 1). Variations in transfer factors among different vegetables may be attributed to differences in the concentration of metals in soil and differences in element uptake by different vegetables (Cui et al., 2004).

CONCLUSION

Based on the analyses and the results, it could be concluded that, the roots and leaves of spinach and lettuce obtained in Potiskum, Yobe state contained Zn, Mn, Fe and Cu in varied concentrations due to the peculiar type of agricultural practices in the area, such as the use of polluted water for growing of vegetables and the application of fertilizers and animal dung. The values

Table 3. Transfer factors (TF) of the heavy metals from soil to spinach.

Spinach	Heavy metals			
	Zn	Mn	Fe	Cu
Leaves	0.32	0.11	0.39	0.16
Roots	0.84	0.70	0.75	0.77

Table 4. Transfer factors (TF) of the heavy metals from soil to lettuce.

Lettuce	Heavy metals			
	Zn	Mn	Fe	Cu
Leaves	0.24	0.33	0.25	0.11
Roots	0.75	0.88	0.28	0.83

obtained in this study were lower than the published threshold values considered toxic for plant tissues as well as the critical values or values regarded as excessive. The results were equally lower than the WHO maximum limit (ML) of heavy metals in vegetables. Consumption of these vegetables as food may not constitute possible health hazards at the time of the study. These results may serve as a base line data for determination of heavy metals in vegetables in the area.

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