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Research Article

Levels of Trace Metals in Washed and Unwashed leaves of Roadsides *Vernonia amygdalina* obtained in Abak, AkwaIbom State, Nigeria.

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ABSTRACT

This study was carried out to assess the levels of five trace metals (Cd, Cu, Mn, Pb, Zn) in the washed and unwashed leaves of roadsides *Vernonia amygdalina* (bitter leaf), a common edible perennial vegetable grown alongroadsides of three major roads (Abak/IkotEkpene, Abak/IkotAbasi and Abak/Uyo) in Abak and exposed to atmospheric deposits from anthropogenic activities in the area. Samples were collected and analysed using standard procedures. The results revealed that levels of all the trace metals in the unwashed leaves were high compared to the washed leaves which suggest anthropogenic sources contamination. The trace metal levels in the roadsides leaves were in the order: Cu > Zn >Pb>Mn> Cd for each of the three roads. The average levels of the metals in the roadsides plant samples for the three roads were: 133.11± 12.63 and 106.71± 8.73 mg/kg Cu; 67.39± 10.78 and 45.2514.99 mg/kg Zn; 2.33 ± 1.61 and 0.54 ± 0.02 mg/kg Pb; 0.72 ± 0.16 and 0.47± 0.06 mg/kg Mn and 0.54± 0.34 and 0.25 ± 0.14 mg/kg Cd in the unwashed and washed roadsides leaves, respectively. The results were high when compared with those of the control samples obtained in IkotEkang village about 10 km away from the study area. Some of these results were equally high compared with the WHO permissible limits for such trace metals in plants. Hence, anthropogenic activities along the roads in Abak significantly contributed to the elevated levels of trace metal in atmospheric deposits and consequently in the leaves of roadsides *Vernonia amygdalina*. These could inevitably result to health consequences on their consumers if consumed continuously. Such intended hazards on the consumers could be prevented by proper washing of the vegetable leaves before processing and consumption.

Keywords: Trace metals, washed, unwashed,leaves, roadsides, *Vernonia amygdalina*, atmospheric depositions, anthropogenic activities, consumption

INTRODUCTION

Vegetables are part of daily diets in many households, forming an important source of vitamins and minerals required for human health[1]. Vegetables are made up of chiefly cellulose, hemi-cellulose and pectin substances that give them their texture and firmness[2]. Large quantities of pollutants have continuously been introduced into the ecosystems as a result of urbanization and industrial processes. Trace metals are persistent pollutants that can be biomagnified in the food chains, becoming increasingly dangerous to human and wildlife[1].

One of the factors that influence the uptake and bioaccumulation of trace metals in vegetables is atmospheric deposition. Trace metals determination in food stuffs is of vital interest because of their essential or toxic nature. Trace metals analyses are important part of environmental pollution studies[3 - 4]. Some trace metals are essential in plant nutrition, but plants growing in a polluted environment can accumulate trace elements at high concentrations, causing a serious risk to human health[5]. Trace metals emitted from the industries and vehicles may be deposited on the vegetable surfaces during their production, transport and marketing[6]. The contamination of vegetables with trace metals due to atmospheric depositions poses a threat to its quality and safety. Vegetable plants that are exposed to atmospheric deposits from anthropogenic activities like vehicular emission, industrial emission and burning of all forms of wastes dumped along roadsides may be contaminated with trace metals to such a level that could be detrimental to the consumers of such vegetables.

Food safety is a major concern at present. The increasing demand of food safety has accelerated research regarding the risk associated with consumption of food contaminated with trace metals [7]. Trace metals such as Cd and Pb are among the most abundant trace metals and are particularly toxic. The excessive amount of these metals in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases[8 - 9]. Lead (Pb) is well known for its toxicity and adverse effects on human health. Absorption of ingested lead may constitute a serious risk to public health. Some chronic effects of lead poisoning are colic, constipation and anaemia[10]. Trace metal contaminated food can seriously deplete some essential nutrients in the body and further responsible for decreasing immunological defenses, intrauterine growth retardation, impaired psychosocial faculties, disabilities associated with malnutrition and related to high prevalence of upper gastro-intestinal cancer[11 - 12].Serious systemic health hazard can develop as a result of extreme dietary accumulation of trace metals such as Cr, Cd, Ni and Pb[13].

For most of the people, the main route of exposure to trace metals is through the diet except occupational exposures at related industries. The uptake and bioaccumulation of trace metals in vegetables are influenced by a number of factors such as climate, atmospheric depositions, the concentrations of trace metals in soil, the nature of soil on which the vegetables are grown and the degree of maturity of the plants at the time of harvest[14, 5]. Cultivation areas near highways are also exposed to atmospheric pollution in the form of metal containing aerosols. These aerosols can be deposited on soil and absorbed by vegetables, or alternatively deposited on the leaves of vegetables and then absorbed. Voutsa *etal.*[5] have reported high accumulation of Pb, Cr and Cd in leafy vegetables due to atmospheric depositions. Field studies have found positive relationships between atmospheric metal deposition and elevated concentrations of trace metals in plants and top soil[15]. Anthropogenic activities in an area might significantly contributed to the elevated trace metal loads in atmospheric deposits and consequently in the leaves of vegetables grown in the area.

Vernonia amygdalina (bitter leaf) is a common edible perennial vegetable highly consumed by the populace. Bitter leaf helps to cleanse such vital organs of the body like the liver, kidney and is used in the treatment of skin infections such as ringworm, rashes and eczema.Bitter leaf and other vegetables contain both essential and toxic metals over a wide range of concentrations[16].

A lot of studies have been carried out on the levels of trace metals in washed and unwashed samples of some common leafy vegetables such as spinach all over the globe. Data on the effect of washing on the levels of trace metals in the leaves of roadsides *Vernonia amygdalina* in AkwaIbom State in particular and Nigeria in general are not really there or are scare, so there is need for this study. This study was therefore conducted to assess the effect of washing on the levels of five trace metals (Cd, Cu, Mn, Pb, Zn) in the washed and unwashed leaves of roadsides *Vernonia amygdalina* grown along roadsides of three major roads (Abak/IkotEkpene, Abak/IkotAbasi and Abak/Uyo) in Abak, in order to establish the amount of the trace metals deposited on the leaves surfaces with a view to assessingthe relationship between atmospheric depositions and the levels of trace metals contamination of the vegetable as a result of anthropogenic activities like vehicular emission, industrial emission and burning of all forms of wastes along the roadsides. The leaves of *Vernoniaamygdalina* are in high demand in local markets and other areas within the locality because they are part of daily staple food.

MATERIALS AND METHODS

Study Area: The study was carried out in Abak the headquarters of Abak Local Government Area of AkwaIbom State. The area lies between latitudes 4^{o}59'N and4^{o}983'Nand longitudes 7^{o}47'E and 7^{o}783'Ewith an area of 190km^2 (70m^2). The major occupation of the people is farming. There are also automobile mechanics, welders as well as petty traders and timber dealers in the area.

Samples collection: Samples of edible portions (leaves) of roadsides *Vernonia amygdalina* grown along three major roads (Abak/IkotEkpene, Abak/IkotAbasi and Abak/Uyo) in Abak were collected. Samples were randomly collected at a height of 10cm above the soil surface with the help of a stainless knife and pooled together to obtain composites samples for each road. Control samples were equally collected in IkotEkang village about 10 kmfrom the study area.The samples were kept in clean polyethylene bags, properly labeled and transported to the laboratory for analyses.

Samples pretreatment and preparation: Samples pretreatment and preparation were done using standard procedures. Each of the four composites samples were divided into two groups. The first groups were properly washed with clean tap water according to the normal household techniques and the water allowed to drain. The samples were then chopped into small pieces and oven dried at 50 – 60^oC. The dried samples were later ground into powder, properly labeled and set aside for ashing. The second groups were left unwashed and given similar treatments of chopping, drying and grinding.
Exact amount of 1.0 g of each of the washed and unwashed samples was taken separately into different properly labeled crucibles and ashed in a SXL muffle furnace at a temperature of 750 – 850^oC for 4 hours. The crucibles were later removed from the furnace and allowed to cool. The ashed samples were then leached with 5cm^3 of 6MHCl and quantitatively transferred into 50cm^3 volumetric flasks and the volumes made up to the 50cm^{3}marks with distilled water. The solution for the blank determination was treated in a similar manner but without the samples[17]. The samples and the blank solutions were stored in properly labeled plastic reagent bottles for trace metal analyses.

Instrumental analysis: Trace metals (Cd, Cu, Mn, Pb, and Zn) levels in the plant samples solutions were determined as described by AOAC[18] using Atomic Absorption Spectrophotometer (AAS) (UNICAM 939/959 model). The samples solutions were aspirated into the instrument and the absorbance obtained was used to determine the concentrations of the metals in the different samples from calibration curves.

Statistical analysis: Descriptive statistics were performed using Excel 2007 spreadsheet while Pearson correlation were tested using the SPSS statistical software package version 16.0.

RESULTS AND DISCUSSION
The levels of the analysed trace metals in the unwashed and washed plant samples are presented in Table 1. The levels of all the metals in the unwashed roadsides *Vernonia amygdalina* leaves were high compared to the washed leaves which suggest anthropogenic sources contamination. These results were in strong agreement with those reported by[1]in their study "Assessment of Heavy Metals Accumulation in Washed and Unwashed Leafy Vegetables Sector-26 Vashi, Navi Mumbai, Maharashtra" and those reported by [19] in their study "Traffic-Based Heavy Metal Accumulation in *Prunus persica*(L.) Batsch Leaves: Is there any difference between washed and unwashed leaves caused by washingprocedure?" The results were also in agreement with the results of [20]. The metal levels in the roadsides *Vernonia amygdalina* leaves samples were in the order: Cu > Zn >Pb>Mn> Cd for each of the three roads. Accordingly, the average levels of the trace metals in the roadsides *Vernonia amygdalina* leaves samples for the three roads were: 133.11± 12.63 and 106.71± 8.73 mg/kg Cu; 67.39± 10.78 and 45.2514.99 mg/kg Zn; 2.33 ± 1.61 and 0.54 ± 0.02 mg/kg Pb; 0.72 ± 0.16 and 0.47± 0.06 mg/kg Mn and 0.54± 0.34 and 0.25 ± 0.14 mg/kg Cd in the unwashed and washed leaves, respectively. These results were higher than those reported inthe control samples obtained in IkotEkang village about 10 km away from the study area.The results were equally higher than the WHO[21] permissible limits of 73.30 mg/kg Cu, 0.20 mg/kg Cd and 0.30 mg/kg Pb in plants. The levels of Zn and Mn in the vegetable leaves reported in this study were however lower than the WHO[21] permissible limits of 99.4 mg/kg Zn and 500mg/kg Mn, respectively in plants.
Cu with the highest mean level in the *Vernonia amygdalina* leaves samples obtained from the three roadsides in this work, could be attributed to different forms of anthropogenic activities such as the discharge of domestic waste and forest fire as well as wind-blown dust and decaying vegetation [22]. The fact that Cu is used as automobile brake pads makes it possible to link its level in the plant leaves samples to the fumes emitted by the automobile plying the roads. This is in conformity with what was reported by [23]. High level of Cu in biota if not monitored could pose serious health threat such as gastrointestinal bleeding, acute renal failure[21], liver and kidney failure and even death in humans [22,24]. The levels of Cu in the unwashed and washed *Vernonia amygdalina* leaves (for both the study and control samples) obtained in this study were higher than the WHO[21] maximum permissible limit of 73.3 mg/kg Cu in plants.
The level of Zn is the second highest in the *Vernonia amygdalina* leaves samples analysed in this study. Zn is an essential element needed by humans in trace amount. It is also a neurotoxin which has the ability to chelate and deplete the neuronal concentration of glutathione (GHS) when taken in large amount. Zn could cause neuronal cell death in a dose dependent manner[25, 22]. Hooper *et al*[26] reported that Zn also reduces the immune system functions in large amount if it levels are not monitored in the environments.

The levels of Zn in the *Vernonia amygdalina* leaves reported in this study for the unwashed and washed samples were lower than those reported by [27] (105.20 mg/kg in *Nasturtium officinales* obtained from an urban/industrial area) and [28] (194.0 mg/kg in *Petroselinum crispum* obtainedfrom an agricultural area). The levels of Zn reported in this study were generally higher than the maximum value of 4.21 mg/kg reported by [29] in *Vernonia amygdalina* leaves obtained from Challlawa-Yandanko irrigation site. The levels of Zn reported by[30] in unwashed and washed samples of *B. vulgaris*obtained from an urban area in India were 75.23 and 47.70 mg/kg, respectively. The value for the unwashed samples was higher than the result obtained in this study for the unwashed leaves samples of *Vernonia amygdalina* while the value for the washedsamples was within the range of the results obtained for the washed leaves in this study.

The levels of Pb in the unwashed and washed *Vernonia amygdalina* leaves reported in this study were below the value of 14.37 mg/kg reported by [27]. Other results for Pb, 2.0 mg/kg reported by [28], 1.76 mg/kg reported by [31],0.34 – 0.71 mg/kg reported by [32]and 0.65 mg/kg reported by [29] were in agreement with the results obtained in this study. Pb exists in many forms in the natural sources throughout the world and is one of the most widely and evenly distributed trace metals. Soil and plants can be contaminated by Pb from car exhaust, dust, and gases from various industrial sources[1].Pb^{2+}has been found to be acute toxic to human beings when present in high amount.Soil usually remains a long-term source of Pb^{2+} exposureonce it is contaminated with Pb^{2+}since Pb^{2+} is not biodegradable[1]. Pb in general is known to be toxic to plants, animals, and microorganisms. It is the most common industrial metal that has become widely spread in air, water, soil and food. Indeed, Pb is a non-essential element in plants, animals and microorganisms. Environmental Pb exposure may cause slight deficits in the cognitive development of children[33]. The toxic effect of Pb is nervous system dysfunction of the foetus and infants. In adults, it causes adverse blood effects, reproductive dysfunction, damages to the gastro intestinal track, nephropathies and central as well as peripheral nervous systems[34]. It also causes brain damage, convulsions and behavioural disorders[22].

The levels of Cd reported in the unwashed and washed roadsides *Vernonia amygdalina* leaves samples in this study were higher than the values of 0.03 – 0.05 mg/kg reported in dumpsite plants by [32]. However, the results were in agreement with the values of 0.60 mg/kg reported by [25] and 0.37 mg/kg report by [29]. The Cd level reported in this study was higher than the permissible limit of 0.2 mg/kg stipulated by [21] in the unwashed leaves samples but was within the limit in the washed samples. Cd is a highly toxic, non-essential metal and it does not have a role in biological process in living organisms[35]. Therefore even in low level, Cd could be harmful to living organisms[36]. High levels of Cd in vegetables are carcinogenic and prolong accumulation in foodstuffs leads to chronic effect in kidney, placenta, liver, heart, nervous system, anaemia, renal damage, bone disorder and cancer of the lungs in humans [37, 22].

The levels of Mn reported in the unwashed and washed roadsides *Vernonia amygdalina* leaves samples in this study were below the WHO permissible limit of 500 mg/kg in plants. Mn is an essential element for various biochemical processes. It is essential for the normal bone structure, reproduction and normal functioning of the central nervous system. Its deficiency causes reproductive failure in both male and female[38]. The kidney and the liver are the main storage organs for Mn in the human body.

Effects of Washing on Trace Metal Levels in the *Vernonia amygdalina* Leaves: The levels of trace metals in the washed leaves samples were lower than those in the unwashed samples in this study. Washing the vegetable leaves resulted in lowering the metal contamination in the samples, which indicated that the source of contamination are the air-born particles[1]. This means that a considerable amount of the analysed trace metals (Cd, Cu, Mn, Pb, Zn) might have been originated from atmospheric depositions and could be removed through washing as reported by [39]. As shown in Figure 1, the percentage reduction of the metal levels by washing of the roadsides *Vernonia amygdalina* leaves samples were 77, 54, 35, 31 and 20% for Pb, Cd, Mn, Zn and Cu, respectively. This indicated that the concentration of the contaminants contributed by atmospheric depositions on the plant samples was more in respect of Pb and Cd. This further showed that a considerable amount of Cd and Pb originated to a large extent from atmospheric depositions. Cu and Zn were least contributed by atmospheric depositions and their larger portions might have been transferred to the plants from contaminated soil.

As seen in Figure 2, the percentage reduction of Pb was higher in samples from Abak/Uyo and Abak/IkotAbasi Roads with 85 and 80%, respectively.The percentage reduction of Cd was higher in samples from Abak/IkotEkpene and Abak/IkotAbasi Roads with the concentrations of 68 and 65%, respectively while the other metals levels were low and vary according to the anthropogenic activities of the studied sites. This indicated that the atmospheric pollution loads in the area were highly from combustion of leaded fuel, coal, fossil fuel and wearing ofvehicle tires and brake pads as noted by [22, 39- 40]. It had been noted that washing of leaves samples of the roadsides *Vernonia amygdalina* removed about 77% of the Pb contents of the plant samples. This is in agreement with studies by[41]who noted that washing of *C. bursa-pastoris* leaves from a suburban site removed 35.8% of its Pb contents, while washing of the leaves of *Poa annua* from the same site removed only 13.5% of its Pb contents. However, they noted that the washing of *N. oleander* leaves was found to remove 30% of its Pb contents in samples from urban parks, 56% from urban roadsides, and 10% from rural areas.This indicated that proper washing of foodstuff before processing and consumption reduces the imposed pollution risk.

Correlation of Trace Metal Levels in the Vegetable Leaves: Table 2 shows the correlation matrix between the levels of the trace metals in the roadsides *Vernonia amygdalina* leaves. Significant positive correlation relationship was seen between the levels of Cu and Zn in the plant leaves ($r = 0.669$, $p < 001$). The concentration of Cu was also found to be significantly related to the level of Cd ($r = 0.577$, $p < 001$), Pb ($r = 0.784$, $p < 001$) and Mn ($r = 0.772$, $p < 001$) were also strongly related positively. The concentration of Zn showed significant relationship with Cd ($r = 0.624$, $p<.001$), Pb ($r = 0.440$, $p<.001$) and Mn ($r = 0.676$, $p < 001$). There was also a strong positive correlation between Cd and Pb in the plant ($r = 0.714$, $p < 001$). The positive correlations indicated that the elements came from the same sources or are influenced by the same anthropogenic sources.

CONCLUSIONS

Based on the analyses and results, it could be concluded that anthropogenic activities like vehicular emission, industrial emission and burning of all forms of wastes along the roadsides of the three major roads in Abak could significantly contributed to the elevated trace metal loads in atmospheric deposits and consequently in the leaves of the roadsides *Vernonia amygdalina* grown in the area. The levels of the analysed trace metals were high in the unwashed leaves samples compared to the washed. Accordingly, the levels of the analysed metals in the samples obtained from the study roadsides were high when compared with the results of those of the control samples obtained in IkotEkang village about 10 km away from the study area.The levels of some of the analysed trace metals (Cu, Cd and Pb)were equally high when compared with the WHO[21] permissible limits of the trace metals in plants. However the levels of Zn and Mn in the vegetable leaves reported in this study were low compared with the WHO[21] permissible limits of 99.4 mg/kg Zn and 500 mg/kg Mn, respectively in plants. The elevated levels of Cu, Cd and Pb in the vegetable leaves could inevitably result to health consequences on their consumers if consumed continuously. Such intended risks or hazards on the consumers could be prevented by proper washing of the vegetable leaves before processing and subsequence consumption. This study can be used for environmental monitoring based on the analysed trace metals.

Table 1

Levels (mg/kg) of Trace Metals in the Unwashed and Washed Roadsides *Vernonia amygdalina* Leaves

Sample Sites	Samples	Cd	Cu	Mn	Pb	Zn
Study area	Unwashed	0.54 ± 0.34	133.11±12.63	0.72 ± 0.16	2.33 ± 1.61	67.39 ± 10.78
	Washed	0.25 ± 0.14	106.71 ± 8.73	0.47 ± 0.06	0.54 ± 0.02	46.92 ± 14.99
Control	Unwashed	0.07 ± 0.001	124.55 ± 0.007	0.45 ± 0.002	0.25 ± 0.002	41.85 ± 0.002
	Washed	BDL	89.79 ± 0.004	0.42 ± 0.000	0.11 ± 0.003	15.82 ± 0.002
	FAO/WHO (WHO [21])	0.2	73.3	500	0.3	99.4

Above values are means ± standard deviations of triplicate analyses for the three roads; BDL = Below Detection Limit.

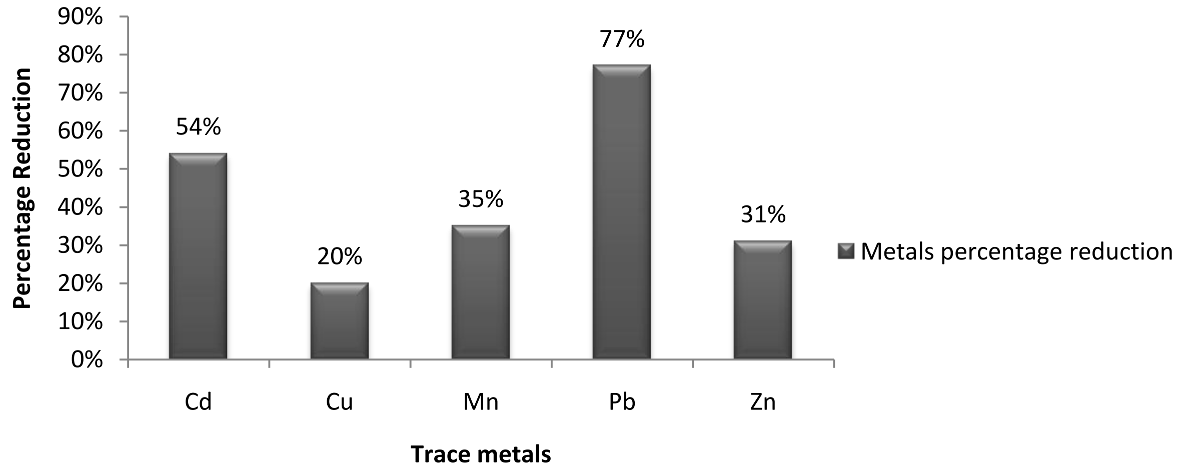

Figure 1
Effects of Washing on Trace Metal Levels in the Roadsides *Vernonia amygdalina* Leaves

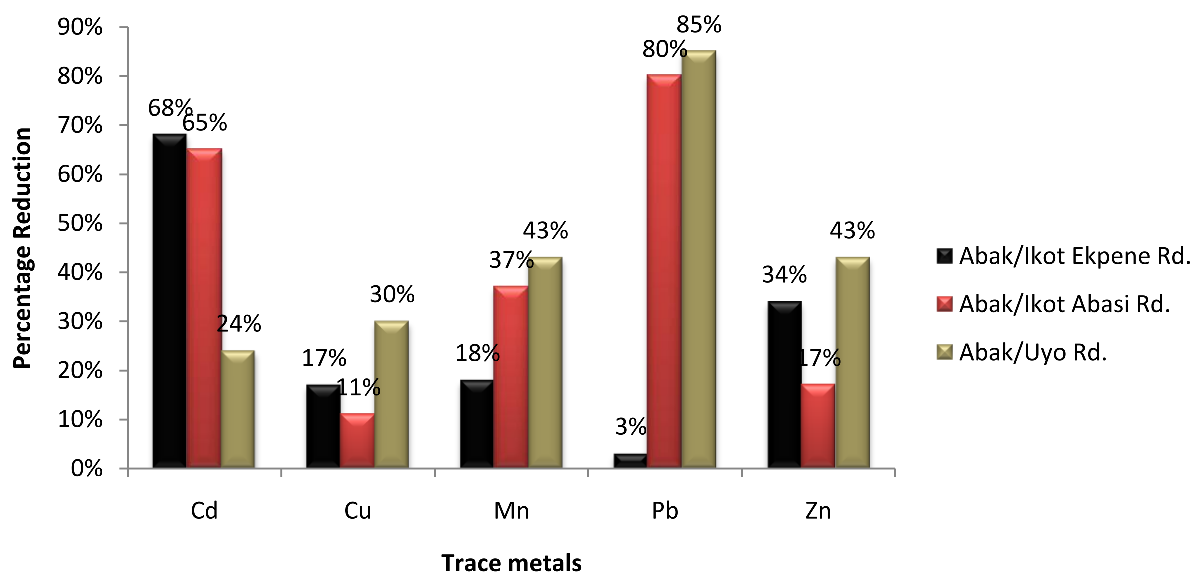

Figure 2
Percentage Reduction of the Trace Metals Levels by Washing of the Vegetable Leaves.

Table 2
Correlation Matrix for the Trace Metal Levels in the Vegetable Leaves

	Cu	Zn	Cd	Pb	Mn
Cu	1				
Zn	0.669**	1			
Cd	0.577**	0.624**	1		
Pb	0.784**	0.440**	0.714**	1	
Mn	0.772**	0.676**	0.892**	0.881**	1

**Correlation is significant at the 0.01 level of significance (2 tailed).

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