Effects of Cadmium, Copper and Zinc Interactions in Soil on their Phytoavailability in Pumpkin (*Cucurbita moschata*)

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Abstract
Experiments were conducted on pumpkin planted and grown in pots under glasshouse conditions to study the interactions between cadmium, copper, and zinc in soil and the effects on their uptake by pumpkin. Known amounts of Cd, Cu and Zn were added to the soil both in single and combined forms before planting, and the possible synergistic or antagonistic interactive effects viz-a-viz their uptake by pumpkin evaluated. The concentrations of the heavy metals both in soil and pumpkins leaves were determined using atomic absorption spectrophotometer (AAS). The results showed various types of interactions among the metals. Generally, it was observed that the addition of Cd to the soil lowers the concentrations of Cu and Zn, while the application of Cu to the soil raises the concentration of Cd and lowers the concentration of Zn. On the other hand, the application of Zn to the soil led to an increase in the concentration of Cd and a decrease in the concentration of Cu. Cu suffers more antagonistic effects in the presence of Cd and Zn. The Transfer Factors for the treated soils were generally lower than that of the untreated soils, this implies that high concentrations of one metal could lower the uptake of another. The generally high Transfer Factor revealed that pumpkin leaves have the ability to accumulate the metals. This calls for concern especially in the case of Cd, which is highly toxic and of no known biochemical importance.

***Keywords*:** cadmium, copper, zinc, interactions, uptake, pumpkin

1.0 Introduction

Heavy metals toxicity is of global concern, however, some heavy metals play important roles in the metabolic regulations of human body [1]. They are non-biodegradable and persistent environmental contaminants which may be deposited on the surfaces and then absorbed into the tissues of vegetables. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils [2].

Cadmium is usually found in soil in the form of Cd^{2+}. This chemical form is the most common form of cadmium, when in excess, can cause brown margins on leaves, curled leaves and brown stunted shoots. Cadmium exposures are also associated with kidney and bone damage in humans. It has also been identified as a potential human carcinogen, causing lung cancer, gastro intestinal cancer, cancer of the pancreas, urinary bladder or prostate [3]. The maximum permissible levels for cadmium in soil and vegetable have been set at 1.00 mg/kg and 0.20 mg/kg respectively [4].

Copper is widely distributed in the body and is essential in all plants and animals. It occurs in the liver, muscle and bone and is transported in the blood stream on a plasma protein called ceruloplasmin [5]. The World Health Organization (WHO) permissible levels of copper in soil and vegetable are 100.00 mg/kg and 73.30 mg/kg respectively [4]. Copper is necessary for normal biological activities of tyrosinase enzyme [6]. It is required as co-factor in different oxidative and reductive enzymes [7]. A low uptake of copper in human consumption can cause a number of symptoms such as growth retardation, skin ailments and gastro intestinal disorders. Copper deprivation in animals contributes to instability of heart rhythm, hyperlipidemia, and increased thrombosis, breakdown of vascular tissue, cardiac hypertrophy and altered corterial function [8]. Copper deficiency is associated with increased oxidative stress, which in turn, leads to low density lipoproteins susceptibility to oxidation [9]. Copper toxicity which is linked to genetic disorders can also be a serious health concern [8].

Zinc is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism [10, 11]. For instance, zinc is required for the proper function of 1,5-deiodinase, the enzyme required for the conversion of thyroxine to the more active form, triiodothyroxine [12]. It is known to be essential for somatic growth of children. Zinc deficiency is a major overlapping public health concern in developing countries. It causes not only growth retardation, but also delays in sexual maturation, hypogonadism and thyroid dysfunction [13].

Vegetables contain some concentrations of elements such as Cd, Cu and Zn that they acquire from cultivated soils in various proportions. The maximum permissible levels for zinc in soil and in vegetables however have been set as 200.00 mg/kg and 99.40 mg/kg respectively [4].

Interactions of heavy metals with one another in soil could have synergistic or antagonistic effects on their mobility and bioavailability, and consequently their uptake by plants. Zhou *et al* [14], studied the joint effects of Cd and other heavy metals (Pb, Cu, Zn and As) on the growth and development of rice plants and the uptake of these heavy metals by rice. The results showed that the growth and development of rice plants were strongly influenced by the double-element combined contamination. Tkalec *et al* [15], investigated the effects of cadmium-zinc interactions on their uptake, oxidative damage of cell macromolecules (lipids, proteins, DNA) and activities of antioxidative enzymes in tobacco seedlings as well as roots and leaves of adult plants. Seedlings and plants were exposed to Cd (10 mM and 15 mM) and Zn (25 mM and 50 mM) as well as their combinations (10 mM or 15 mM Cd with either 25 mM or 50 mM Zn). Measurement of metal accumulation exhibited that Zn had mostly synergistic effect on Cd uptake in roots and seedlings, while Cd had antagonistic effect on Zn uptake in leaves and roots of pumpkin.

This study investigated the effects of cadmium, copper and zinc interactions in soil on their bioavailability and uptake by pumpkin (*cucurbita moschata*).

2.0 Materials and Methods

2.1 Study Site

The soil was collected from the Research Farm of the Federal University of Technology, Minna in the Southern Guinea Savanna ecology. Rainfall pattern is mononodal with the rainy season in Minna, starting in April and ending in October. The soil of the site is classified as Typic Plinthustalf with loamy sand surface soil texture, slightly acidic, low organic carbon, nitrogen and medium phosphorus [16].

2.2 Soil Sampling and Preparation

Surface soil (0-15 cm) samples were collected with an auger, along four diagonal transects, from ten points each, thoroughly mixed and bulked to give four composite samples. Samples were also collected from the four composite samples mixed thoroughly and bulked to give one composite sample for the planting of pumpkin. Sub-samples were also taken for the analysis of the physico-chemical properties and the background concentration of the metals. The sub-sample were air-dried, crushed gently and passed through a 2 mm sieve.

2.3 Analysis of Physico-chemical Properties of Soil

The physico-chemical properties of soil samples which include pH, organic carbon, electrical conductivity and cation exchange capacity were determined according to the method reported by Adeboye *et al*. [17].

2.4 Heavy Metal Application to Soil

To three plastic pots, containing 20 kg of soil each, were added 0.1 mg/kg of Cd, Cu and Zn separately. Similarly, four combined treatments, namely, Cd/Cu, Cd/Zn, Cu/Zn, Cd/Cu/Zn were also carried out triplicates to give a total of 12 pots.

2.5 Planting and Harvesting of Pumpkin
Pumpkin seeds were sown at 2 to 4 seeds per pot and thinned down to 2 plant stands per pot after two weeks. Matured plants were harvested after 30 days of planting. Pumpkin seeds were also sown in three untreated pots as control.

2.5 Determination of Heavy Metals Concentrations in Soil
The background concentrations of Cd, Cu and Zn in the soil as well as concentrations after treatment with single and combined metals were determined using the USEPA Method 3050 B [18]. The determination was carried out using Atomic Absorption Spectrophotometer (Perkin-Elmer, Analyst 200).

2.5 Determination of Heavy Metals Concentrations in Pumpkin Leaves
Samples of pumpkin leaves (2.0g) were weighed into a digestion flask and treated with 9 cm^3 of an acid mixture made up of 1:1:1 mixture of concentrated HNO_3, HCl and H_2SO_4. A blank sample was prepared by applying 9 cm^3 of the acid mixture into an empty digestion flask. The samples were mixed and heated for 30 minutes on an electric hot plate at 80-90 ^oC at which they were brought to boil and a clean solution was obtained. After cooling, the solution was filtered with whatman No. 4 filter paper and transferred quantitatively to a 100 mL volumetric flask and made up to mark with de-ionized water. The solution was then preserved in a universal bottle for further analysis [19]. The determination was carried out using Atomic Absorption Spectrophotometer (Perkin-Elmer, Analyst 200).

3.0 Results and Discussions
3.1 Physico-chemical Properties of Soil
The soil is classified as loamy Sandy with a pH in water of 6.80. It has an electrical conductivity of 45.39 S/m and an organic carbon of 4.30. The cation exchange capacity was 7.13 Cmol/kg. Low soil pH is a major factor favouring the uptake of heavy metals [20]. The electrical conductivity of soil refers to the ability of soil to conduct electricity. It is an indicator of the total dissolved inorganic substances and other solids. The cation exchange capacity is the ability of the soil to retain cations because of their negatively charged surfaces. It is a surface characteristic that allow heavy metals adsorption on the surface of the soil. A high cation exchange capacity of a soil will translate to high availability of a metal for plant uptake, as it enhances their solubility and mobility [21].

3.2 Heavy Metal Concentrations in Single Metal Treated Soil
The background concentrations of heavy metals in soil is shown in Table 1. The mean concentrations of Cd, Cu and Zn are 11.10 mg/kg, 16.21 mg/kg and 51.45 mg/kg respectively. This shows that the concentration of the metals decreased in the order Zn>Cu>Cd. The mean concentrations of Cu and Zn were below the FAO/WHO maximum permissible level for agricultural soil while that of Cd was above [23].

Heavy metal concentrations in single metal treated soil is shown in Table 1. On application of Cd to the soil sample, the mean concentrations (mg/kg) of both Cu and Zn decreased from 16.21 mg/kg to 13.00 mg/kg for Cu, and from 51.45 mg/kg to 37.11 mg/kg for Zn (antagonistic effect). On the application of Cu, the mean concentration of Cd increased from 11.10 mg/kg to 14.45 mg/kg (synergistic effect) while that of Zn decreased from 51.45 mg/kg to 44.67 mg/kg (antagonistic effect). When Zn was applied to the soil, the concentration of Cd increased from 11.10 mg/kg to 12.12 mg/kg while that of Cu decreased from 16.21 mg/kg to 14.67 mg/kg. Such increase in concentration is due to synergistic interaction while the decrease in concentration is due to antagonistic interaction [21].

Cd, Cu and Zn might be considered as chemically similar metals because they have similar ionic structure and electro negativities. They may therefore influence each other in plant uptake and accumulation. On the other hand they have different ionic radii which may play a role in plant selectivity [22]. The increased uptake of Cd as a result of the addition of Zn in this study might be as a result of competitive transport and absorption interaction between these two ions [22].

Table 1: Heavy Metal Concentrations (mg/kg) in Single Metal Treated Soil

Treatment*	Mean Concentrations of Heavy Metals		
	Cd	Cu	Zn
Cd	12.10 ± 1.21	13.00 ± 2.45	37.11 ± 1.12
Cu	14.45 ± 2.42	25.67 ± 3.12	44.67 ± 4.42
Zn	12.12 ± 2.43	14.67 ± 3.11	52.00 ± 5.21
Background**	11.10 ± 1.11	16.21 ± 2.01	51.45 ± 4.22

* metal added to soil
** concentration in untreated soil

3.3 Heavy Metal Concentrations in Combined Metal Treated Soil

The heavy metal concentrations for combined metal treated soil is shown Table 2. When Cd/Cu combination was applied to the soil, the mean concentrations of Cd (11.10 mg/kg) and Zn (51.45 mg/kg) (background), increased to 13.67 mg/kg and 52.67 mg/kg, respectively (synergistic effect), while that of Cu decreased from 16.21 mg/kg to 15.33 mg/kg (antagonistic effect). On the application of Cd/Zn to the soil, the concentrations of Cd and Zn increased from the background concentrations of 11.10 mg/kg and 51.45 mg/kg to 13.33 mg/kg and 51.67 mg/kg respectively, while that of Cu decreased as compared to the background concentrations, from 16.21 mg/kg to 14.33 mg/kg. When Cu/Zn was applied, the concentrations of Cd increased from 11.10 mg/kg to 11.67 mg/kg and that of Zn from 51.45 mg/kg to 51.67 mg/kg, while that of Cu decreased from 16.21 mg/kg to 13.33 mg/kg. On the application of the three metals, Cd/Cu/Zn, to the soil, the concentrations of Cd increased from 11.10 mg/kg to 14.33 mg/kg and Zn from 51.45 mg/kg to 53.00 mg/kg, while that of Cu decreased from 16.21 mg/kg to 13.33 mg/kg. The interactions of Cu with the other two metals results more in its decrease and possibly reduced mobility and bioavailability.

Table 2: Mean Concentrations of Heavy Metals (mg/kg) in Combined Metal Treated Soil

Treatment*	Mean Concentrations of Heavy Metals		
	Cd	Cu	Zn
Cd/Cu	13.67 ± 2.14	15.33 ± 2.12	52.67 ± 1.01
Cd/Zn	13.33 ± 1.12	14.33 ± 3.45	51.67 ± 2.23
Cu/Zn	11.67 ± 2.55	13.33 ± 4.11	51.67 ± 4. 12
Cd/Cu/Zn	14.33 ± 2.44	13.33 ± 1.12	53.00 ± 1.12
Background**	11.10 ± 1.11	16.21 ± 2.01	51.45 ± 4.22

* metal added to soil
** concentration in untreated soil

3.4 Heavy Metal Concentrations in Pumpkin from Single Metal Treated Soil

The mean concentrations of heavy metals in pumpkin leaves from single metal treated soil is shown in Table 3. The uptake of the heavy metals by pumpkin leaves was in agreement with the results of their interactions in soil viz-a-viz their possible mobility and bioavailability. For instance, when Cd was applied to the soil, the concentrations of Cu and Zn in pumpkin leaves decreased; from 22.00 mg/kg to 13.67 mg/kg and from 51.67 mg/kg to 43.33 mg/kg, respectively. This is in agreement with the result obtained by Moustakas *et al.* [24], in which they reported a significant inhibitory effects of Cd on Zn concentrations in the leaves of pot marigold.

Similarly, when Cu was applied to the soil, the concentrations of Cd in pumpkin leaves decreased from 15.67 mg/kg to 14.00 mg/kg while that of Zn decreased from 51.67 mg/kg to 44.67 mg/kg. When Zn was applied to the soil, the concentrations of Cu decreased from 22.00 mg/kg to 20.33 mg/kg. However, that of Cd in pumpkin leaves increased from 15.67 mg/kg to 16.67 mg/kg. This study revealed a clear inhibitory or antagonistic effect between Cu and Cd and between Zn and Cu, on their uptake by pumpkin. The effect of Zn on Cd is synergistic, meaning that elevated level of Zn in soil will enhance the uptake of Cd by pumpkin.

3.5 Heavy Metal Concentrations in Pumpkin from Combined Metal Treated Soil

Heavy metal concentrations in pumpkin from combined metal treated soil is shown in Table 4.

Table 3: Mean Concentrations of Heavy Metals (mg/kg) in Pumpkin from Single Metal Treated Soil

Treatment*	Mean Concentrations of Heavy Metals		
	Cd	Cu	Zn
Cd	11.00 ± 2.33	13.67 ± 2.11	43.33 ± 2.30
Cu	14.00 ± 3.11	17.00 ± 1.45	44.67 ± 4.46
Zn	16.67 ± 4.21	20.33 ± 1.22	52.00 ± 5.11
Control**	15.67 ± 2.43	22.00 ± 3.14	51.67 ± 4.47

* metal added to soil
** mean concentrations of heavy metals in pumpkin from untreated pots

Table 4: Mean Concentrations of Heavy Metals (mg/kg) in Pumpkin from Combined Metal Treated Soil

Treatment*	Mean Concentrations of Metals (mg/kg)		
	Cd	Cu	Zn
Cd/Cu	13.33 ±4.77	19.00 ±2.34	45.00 ±4.43
Cd/Zn	12.67 ±2.37	15.67 ±3.86	53.67 ±3.36
Cu/Zn	12.67 ±1.12	12.33 ±2.55	46.33 ±3.11
Cd/Cu/Zn	12.67 ±1.32	16.00 ±1.35	36.67 ±3.27
Control**	15.67 ±2.43	22.00 ±3.14	51.67 ±4.47

* metal added to soil
** mean concentrations of heavy metals in pumpkin from untreated pots

From Table 4, when Cd/Cu was applied to the soil, the concentrations of Cd, Cu and Zn in pumpkin leaves decreased compared to that of the untreated pots (control). On the application of Cd/Zn to the soil, the concentrations of Cd and Cu in pumpkin leaves decreased while that of Zn increased. When Cu/Zn was applied to the soil, the concentrations of Cd, Cu and Zn in pumpkin leaves decreased. On the application of the three metals, Cd/Cu/Zn, to the soil, the concentrations of Cd, Cu and Zn in pumpkin leaves decreased. Generally there exists a predominant inhibitory effects of the metals on their uptake by pumpkin.

3.6 Soil-Pumpkin Transfer Factors for Single Metal Treated Soil

The TF value of a heavy metal gives an indication of its mobility in the soil. It is calculated as:

$$TF = \frac{Concentration\ of\ metal\ in\ pumkin}{Concentration\ of\ metal\ in\ soil}$$

A high TF value indicates high rate of transfer of the metal into the plant tissue. This may also involve a risk of human food chain accumulation. The result of the soil-pumpkin transfer factors for single metal treated soil is shown in Table 5, while that of combined metal treated soil is shown in Table 6.

The TF results for the single metal treatment (Table 5) show that Cd was the most mobile heavy metal among the group while Cu the least. The order of decreased mobility is Cd>Zn>Cu. For the combined metal treatment Cu was the most mobile heavy metal among the group while Zn was the least.

Table 5: Soil-Pumpkin Transfer Factors for Single Metal Treated Soil

Treatment*	Transfer Factors		
	Cd	Cu	Zn
Cd	0.91	1.05	1.17
Cu	0.97	0.66	1.00
Zn	1.38	1.39	1.00
Mean TF	1.09	1.03	1.06
Control**	1.41	1.36	1.00

* metal added to soil **TF for untreated soil

Table 6: Soil-Pumpkin Transfer Factors for Combined Metal Treated Soil

Treatment*	Transfer Factors		
	Cd	Cu	Zn
Cd/Cu	0.98	1.23	0.85
Cd/Zn	0,95	1.09	1.04
Cu/Zn	1.09	0.92	0.90
Cd/Cu/Zn	0.88	1.20	0.69
Mean TF	0.98	1.11	0.87
Control**	1.41	1.36	1.00

* metal added to soil **TF for untreated soil

The order of decreased mobility is Cu>Cd>Zn. The Transfer Factors were general lower in the treated soil compared to the untreated soil (control). This shows that the high concentrations of one metal is capable of limiting the uptake of another. This is in agreement with the work of Zhou, *et al* [14]. They reported that due to interactions, uptake of Pb, Cu and Zn by rice plant was inhibited by the presence of Cd.

4.0 Conclusion

This study was carried out to examine the various interactions between cadmium, copper and zinc both in soil and in pumpkin leaves. Generally, it was observed that the addition of Cd to the soil led to a decrease in the concentration of Cu and Zn. It was also observed that the application of Cu to the soil, led to an increase in the concentration of Cd and a decreased in the concentration of Zn. However, the application of Zn to the soil led to an increase in the concentration of Cd and a decrease in the concentration of Cu. In the combined form, the application of Cd/Cu to the soil led to an increase in the concentration of Cd and Zn and a decreased in the concentration of Cu. The addition of Cd/Cu/Zn to the soil led to an increase in the concentration of Cd and Zn and a decrease in the concentration of Cu. Generally, the uptake of the metals by pumpkin revealed an inhibitory or antagonistic effects of the metals. Bioavalability of a metal enhances its uptake by plants [21]. The result of the transfer factors shows Cd was the most phytoavailable of the three metals. It also revealed that pumpkin leaves have the ability to accumulate the metals. A proper and continuous monitoring of heavy metals in soils for the cultivation of pumpkin is recommended.

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