



Effect of Different Soil Amendments on Growth Performance and Levels of Copper and Zinc in *Lycopersicon esculentum*

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ABSTRACT

Organic and inorganic amendments are added to soil with the aim of improving the crop yield. The present study investigated the effect of three types of soil amendments; organic (cow dung, chicken droppings) and inorganic (NPK-fertilizer) on trace metals (Cu and Zn) uptake and growth rate performance of greenhouse cultivated *Lycopersicon esculentum*. Thirty-two pot-plants were used for the study, each containing mixture of soil and weighed different amendments for the cultivation of *L. esculentum*. The fruits were harvested at maturity and later analysed for trace metal concentrations using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The study revealed significant differences in heights, stem girths and canopy sizes, as well as the concentrations of Zn. The growth performance and yield results were in the order of NPK fertilizer > chicken droppings manure > cow dung manure > control. The mean concentrations of trace metals indicated that the highest concentrations for trace elements were recorded for both Cu and Zn in *Lycopersicon esculentum* cultivated in cow dung amended soil. There was no significant difference in the concentrations of Cu in tomato fruits across the amendments. However, there was a significant difference in the concentrations of Zn recorded for the *L. esculentum* across the group ($p < 0.05$). The transfer factor of metals for this study showed that tomato fruits did not bioaccumulate the trace metals, and therefore the study revealed that these types of manures and fertilizer are safe to be used as soil amendments without increasing the trace metals elevated levels of phytotoxicity.

INTRODUCTION

Lycopersicon esculentum (tomato) is an important fruit popularly cultivated for its numerous nutritional and health benefits. It has been reported to be cultivated on every continent except Antarctica (Maboko et al. 2015). Tomato is rich in vitamins, organic acids and a wide variety of nutrients that are fundamental to human healthy living and is consequently incorporated into the human daily dietary formulation as fruits and tomato-based products. Therefore, increase in demands for tomatoes among other food crops for consumption due to more informed health awareness has resulted into massive production using diverse types of farming techniques (Dumas et al. 2003). Also, increasing awareness of nutritional and medicinal benefits of antioxidants properties of tomatoes such as vitamin C, lycopene, flavonoids, phenolic acid has shot up the demand for this vegetable crop.

In the effort to increase the availability of this fruit, farmers have employed various farming techniques including the use of organic and inorganic fertilizers (Walker & Bernal 2008). These farming techniques, which include soil amend-

ments, have become a necessity due to the loss of essential nutrients required for good growth and quality yield by food crops in the soil. Soil amendment is one of the many farm techniques explored by farmers in providing solutions to the problem of loss in soil fertility resulting from over-cultivation, industrialization and urbanization.

Although application of fertilizers or manures which is also known as soil amendments is used for the improvement of soil physical, chemical and biological conditions, although it is not without its drawbacks. It has been reported that animal manure contains high metal (Cu, Zn, As, Cd) concentration which causes metal pollution (Sager et al. 2007, Toor et al. 2006, Moral et al. 2008). Also, the discovery that some phosphorus and trace element fertilizers contained elevated quantities of metal like cadmium, arsenic and other metals of environmental concern has instigated research into fertilizer usage of soil amendment (Ajayi et al. 2012).

Therefore, the present study investigated the effect of different soil amendments on the growth rate and fruit yield of *Lycopersicon esculentum*. The study also determined the concentrations of Cu and Zn from the harvested tomato fruits.

MATERIALS AND METHODS

A pot plants experiment was set up in a greenhouse at the production unit of the Department of Biology, Sefako Makgatho Health Sciences University located in the northern part of Pretoria (Tshwane Metropolitan) on a coordinate (25°37'8" S and 28°1'22" E).

Soil and amendments: The experimental design consisted of organic manures such as cow dung, chicken droppings and conventional or inorganic (NPK) fertilizer which were used in the treatment of the soil. Eight pots labelled A1-A8 for cow dung, B1-B8 for chicken droppings, C1-C8 for NPK, and D1-D8 for control, were allocated to each amendment. Each pot of 48cm × 24cm in size was filled with a mixture of soil and 150g of soil manures. The 150g of amendment was chosen according to the experimental design of Yang et al. (2012). The amended soils were watered and turned over at two weeks interval for aeration and proper mineralization of the amendments before transplanting (Ayeni, 2014). Seedlings of *Lycopersicon esculentum* were purchased at a registered nursery (Plantland, Pretoria North, South Africa) and transplanted into the amended and control soils. The plants were watered daily in the afternoon during the planting period.

Data collection: Parameters such as plant growth rate were measured from the first week of seedling transplant for plant height, canopy size and stem girth using meter rule and vernier callipers respectively. Fruit yields data such as total number of fruits per plant and total weight of fruits per plant using a digital electronic sensitive weighing scale were recorded after all the fruits had been harvested at maturity.

Trace metal analysis of tomato fruit and soil samples: Plant samples harvested at maturity were dried in a hot-air oven for 48 hours at a temperature of 100°C and subsequently ground to fine particles using a homogenizer (blender) for digestion process. Soil samples were also collected and spread in a ventilated room to dry and ground to fine particles for digestion process.

Digestion method and ICP-MS analysis: Open digestion method was used in the digestion process of the samples for trace metals analysis. About 0.5 g of ground plant and soil samples were accurately weighed into a 50 mL conical flask, using a digital electronic sensitive weighing scale. About 3 mL of nitric acid 70% vol, 5 mL of perchloric acid 65% vol and 4 mL of hydrogen peroxide were added to the samples and heated for over 20 minutes until a very clear solution was observed. Deionized water was then added to the solution to the 50 mL mark of the conical flask. The digested solution was further filtered with a Whatman No. 42 filter paper and the samples were then analysed for trace metals content using the ICP-MS.

Transfer factor (TF): The index of soil-plant transfer was calculated using the relationship of the ratio of trace metals in tomato fruit to the concentrations of the trace metals present in the soil ($TF = C_T / C_{soil}$) as described by Olowoyo et al. (2010). The possible human health risk for consuming these tomatoes was calculated by Hazard Quotient (HQM), using the equation $HQM = ADDM / RFDM$ (Nabulo et al. 2010).

TF represents the transfer factor of tomato fruits; C_T is trace metal concentrations in tomato fruits; C_{soil} is trace metal concentrations in the soil.

$$HQM = ADDM / RFDM$$

Where, $ADDM = DI \times MF_T / WB$; $ADDM$ represents the average daily dose ($\mu\text{g/g}$) of the metal; $RFDM$ is the reference dose ($\mu\text{g/g}$) defined as the maximum tolerable daily intake of metal with no adverse effect; DI is the daily intake of plant (0.182 kg/d for adults and 0.118 kg for children) (Olowoyo & Lion 2013); WB is the body weight of examined individuals (55.7 kg for adults and 14.2 kg for children) (Olowoyo et al. (2013).

Statistical analysis: Analysis of variance was performed using the tukeyposthoc test on IBM SPSS 24.0 to evaluate the significant differences in the growth performance as well as the metal concentrations in the soil and in the plant samples.

RESULTS AND DISCUSSION

Growth performance: The results for growth parameters such as plant height mean, canopy size mean and stem girth mean are presented in Table 1. The results obtained from the study ($p < 0.05$) indicated that there were significant differences in the mean plant height and the mean canopy size and stem girth of *L. esculentum* cultivated in the soil treated with different amendments. This observation supports previous studies conducted by Zhang et al. (2010) and Olaniyi & Ojetayo (2012), who reported that nitrogen, phosphorus and potassium stimulate vegetative growth, increase cell size and cellular number resulting from cell division and expansion leading to increase in plant stem girth. The minimum and maximum mean values for plant height (9.5±2.66 cm and 86.38±2.88 cm) were recorded from *L. esculentum* grown in soil amended with chicken droppings and inorganic fertilizer respectively. The minimum and maximum mean values for canopy size (11.6±1.91 cm and 91.88±4.61 cm) were also recorded from *L. esculentum* grown in soil amended with chicken droppings and inorganic fertilizers respectively. Meanwhile, the minimum and maximum mean values for stem girth (3.3±0.2 mm and 18.2±0.45 mm) were both recorded from *L. esculentum* grown in a soil with the same amendment of inorganic NPK fertilizer. It must be noted that the stem girth was not determined at the first day of

Table 1: Mean growth performance of *L. esculentum* grown in different soil amendments.

Plant height (cm)	CD	11.25±1.41 ^{ab}	15.25±2.48 ^{ab}	20.20±2.12 ^{bc}	24.44±2.68 ^c	31.94±3.44 ^c	47.75±4.52 ^c	50.56±5.13 ^c	52.06±5.35 ^c
	CKD	9.50±2.66 ^b	13.50±2.70 ^b	21.06±2.86 ^b	28.25±2.80 ^b	36.56±1.86 ^b	53.38±3.38 ^b	55.70±3.52 ^b	57.70±3.60 ^b
	NPK	10.63±2.28 ^{ab}	12.94±2.70 ^b	17.44±2.73 ^c	34.20±2.72 ^a	55.63±3.20 ^a	64.50±2.78 ^a	76.00±2.20 ^a	86.38±2.88 ^a
	WA	11.75±1.46 ^a	17.50±2.60 ^a	25.00±3.48 ^a	30.13±40.00 ^b	39.00±6.17 ^b	52.31±5.27 ^b	55.00±5.04 ^b	55.44±4.72 ^{bc}
Canopy size (cm)	CD	13.81±1.22 ^b	23.50±3.02 ^b	25.70±4.00 ^b	28.00±3.27 ^c	33.91±3.32 ^c	39.34±2.62 ^c	42.00±2.83 ^c	50.88±4.45 ^d
	CKD	11.60±1.91 ^b	21.60±2.55 ^b	35.03±4.40 ^a	41.25±6.27 ^b	49.25±7.07 ^b	53.56±7.24 ^b	55.88±7.46 ^b	58.38±7.61 ^b
	NPK	12.03±1.87 ^b	16.53±2.30 ^c	24.03±2.53 ^b	46.56±1.30 ^a	58.25±2.12 ^a	69.81±1.73 ^a	77.38±3.78 ^a	91.88±4.61 ^a
	WA	18.44±6.55 ^a	28.63±3.62 ^a	33.88±4.23 ^a	31.78±5.53 ^c	37.50±3.35 ^c	41.03±3.67 ^c	44.00±3.32 ^c	44.75±3.18 ^c
Stem girth (mm)	CD	ND	4.17±0.43 ^b	5.23±0.70 ^b	5.65±0.78 ^d	6.18±0.54 ^d	6.93±0.65 ^d	7.08±0.56 ^d	7.33±0.60 ^d
	CKD	ND	4.20±0.74 ^b	6.75±0.88 ^a	8.20±1.20 ^b	9.04±1.42 ^b	10.03±1.55 ^b	10.37±1.52 ^b	10.6±1.50 ^b
	NPK	ND	3.30±0.20 ^c	5.11±0.32 ^b	9.56±0.38 ^a	11.72±0.42 ^a	14.03±0.60 ^a	15.96±0.33 ^a	18.2±0.45 ^a
	WA	ND	5.31±0.72 ^a	6.62±0.61 ^a	6.93±0.75 ^c	7.51±0.67 ^c	8.14±0.84 ^c	8.50±0.85 ^c	8.50±0.85 ^c

ND: not determined. Values with same letter(s) within the same row indicate no significant difference ($p>0.05$).

Table 2: The yield performance of *Lycopersicon esculentum* grown in different soil amendments.

Treatment	Total number of fruits	Total fruit weight (kg)
Cow dung	37	6.44
Chicken droppings	56	9.89
NPK	97	17.82
Control	30	5.4

transplanting because of the fragile nature of the seedling stems and susceptibility to damages from any external measuring tools.

The study showed that *L. esculentum* planted in control soil recorded the highest plant height at the first to third week after transplanting. This agreed with the study done by Oyediji et al. (2014) who reported that the initial slow growth rate observed from *A. hybridus* after transplanting could be due to physiological changes by plants in the amended soil. However, from fourth to eight weeks after transplanting, the plant heights for plants grown in soil amended with NPK overtook the ones planted in the soil amended with chicken droppings, control soil and the soil amended with cow dung. Similarly, the canopy size and stem girth recorded for plants grown in soil without amendment were bigger than the ones in the soil amended with cow dung, chicken droppings and NPK during the first to second week after transplanting. The slower growth rate from plants cultivated in pots amended with NPK, chicken droppings and cow dung during early weeks after transplanting agreed with Agbaje et al. (2002); and Ayeni et al. (2012) reports that this could be due to initial physiological changes after transplanting.

The yield performance of the *Lycopersicon esculentum*, which was recorded by the total number and total weight of fruits per treatment, are presented in Table 2. The highest mean yield was recorded for fruits harvested in the soil treated with NPK and the lowest yield was recorded for fruits harvested in soil without amendment. The current study showed

that NPK amended soil produced the highest number of fruits and the highest total fruit weights, followed by chicken droppings amended soil and cow dung amended soil, with the lowest yield recorded for soil without amendment. These observations agreed with previous work done by Mohamed et al. (2016), where the importance of physiological roles of nitrogen, phosphorus and potassium in plant growth and development were noted, and that addition of such micronutrients to plants through soil amendments ensures high productivity.

Trace metals concentration in tomato: The mean concentrations of Cu and Zn in tomato fruits harvested from soil treated with different amendments are presented in Table 3. The highest concentration was recorded for Cu in tomato fruits harvested from cow dung amended soil. The concentrations ranged from $0.13\pm 0.02\mu\text{g/g}$ – $0.30\pm 0.01\mu\text{g/g}$ and there was no significant difference in the concentrations of Cu ($p>0.05$). The highest mean concentration of Cu recorded for *L. esculentum* in this study was well below the recommended safe limits of $40\mu\text{g/g}$ in food by World Health Organization (WHO 2012). Copper serves as a cofactor in many reactions involving iron. It suppresses free radicals and it is involved in collagen synthesis (Demirevska-Kepova et al. 2004). Copper phytotoxicity which is resulted from elevated levels or copper poisoning may cause cirrhosis of liver and in extreme cases lead to death (Kibira et al. 2010).

The highest mean concentration for Zn was recorded in *L. esculentum* harvested from cow dung amended soil and the minimum mean concentration was recorded in soil with-

Table 3: The mean concentrations of Cu and Zn in the soil and in the *Lycopersicon esculentum* fruit.

Treatments	Cu ($\mu\text{g/g}$)		Zn ($\mu\text{g/g}$)	
	Soil	Plant	Soil	Plant
Cow dung	1.20 \pm 0.10 ^a	0.30 \pm 0.41 ^a	3.00 \pm 1.58 ^a	0.50 \pm 0.24 ^a
Chicken droppings	1.17 \pm 0.11 ^a	0.13 \pm 0.02 ^a	2.41 \pm 1.00 ^a	0.46 \pm 0.14 ^{ab}
NPK	1.30 \pm 0.16 ^a	0.08 \pm 0.03 ^a	3.64 \pm 2.31 ^a	0.38 \pm 0.11 ^{ab}
Control	1.30 \pm 0.16 ^a	0.27 \pm 0.28 ^a	2.36 \pm 2.11 ^a	0.26 \pm 0.11 ^b

Values with different letter(s) in the same row indicate significant difference ($p < 0.05$).

Table 4: The mean values of the soil pH.

Cow dung	Chicken droppings	NPK	Control
7.40 \pm 0.08	7.43 \pm 0.07	7.40 \pm 0.10	7.35 \pm 0.13

Table 5: Transfer factor values of Cu for *Lycopersicon esculentum* harvested from different soil amendments.

Treatment	Cu	Zn
Cow dung	0.003	0.167
Chicken droppings	0.111	0.191
NPK	0.062	0.104
Control	0.208	0.110

out amendments. The concentrations ranged from 0.26 \pm 0.11 $\mu\text{g/g}$ -0.50 \pm 0.24 $\mu\text{g/g}$. There were significant differences in the concentrations Zn in the *L. esculentum* fruits from the different soils. Zinc is reported to be an essential trace element that is involved as a cofactor in several enzymatic reactions and serves as an essential component of nuclear DNA binding protein (Kibria et al. 2012). Studies have established Zn to be required for the normal growth, development of plants and human beings, it is required in minute quantities (Jung 2008, Hashim et al. 2007). The concentration of Zn recorded in this study was below toxic level of 200 $\mu\text{g/g}$ in plant (Alia et al. 2015; Jung 2008).

This study however, observed that highest concentrations the studied trace metals were recorded for the fruits cultivated in the cow dung amended soil. This observation could be due to the area of collection of this cow dung manure. This area which is known for its mining activities has been reported to be polluted by trace metals by Olowoyo et al. (2013).

Soil pH and metals transfer factor: The mean values of the soil pH are presented in table 4. The soil pH values ranged from 7.35 \pm 0.13-7.43 \pm 0.07 and thus showed that the soil used for this study was alkaline. Soil pH has been reported to be a major factor in the availability and uptake of trace metals by plants (Xianjin et al. 2015). It has been reported that metals are mobile in acidic medium than in neutral or alkaline (Wang et al. 2006). This is in accordance with our

study observation which showed that there was a decrease in the level of metal concentration uptake by tomato fruits. This observation is also supported by the recent study conducted by Adamczyk-Szabela et al. (2015), who reported that Zn accumulation was decreased in plants grown under alkaline compared to acidic conditions. The results of transfer factor (TF) for tomato are presented in Table 5. The values of the transfer factor recorded for Cu ranged from 0.003-0.208, and from 0.104-0.191 for Zn. The transfer factor, which is the ratio of the concentration of the metal in the plant to the metal concentration in the soil, is calculated to determine whether a plant bioaccumulates or not (Baylock et al. 2005). The plant with a TF>1 is reported to be enriched with the metal (accumulator), TF that is slightly less than or equal to 1 indicates that the plant is not influenced by the metal, and a TF<1 is an indication of a plant that excludes the uptake of the metal (excluder) (Pilon-Smits 2005). Since, the results of this study indicated TF<1, it showed that *Lycopersicon esculentum* did not bioaccumulate Cu and Zn and hence safe for human consumption, which also agreed with the study of phytoremediation conducted by Sithole et al. (2016).

CONCLUSION

This study concludes that organic manures such as cow dung and chicken droppings can serve as viable alternatives to inorganic or chemical NPK fertilizer in the effort to

balance the production of quality and safe agricultural crops and environmental sustainability without necessarily resulting in elevated levels of trace elements. The present study was conducted in a greenhouse and further in-depth study should be conducted in an open field for extended detailed knowledge on the issue.

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