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Full Length Research Paper

Assessment of phyto-toxicity potential of lead on tomato (*Lycopersicon esculentum L*) planted on contaminated soils

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Phytotoxic effects of Pb as $Pb(NO_3)_2$ on tomato (*Lycopersicon esculentum*) planted on contaminated soil was assessed in terms of growth, yield and Vitamin C content at various concentrations (300, 600 and 1800 ppm). The residual Pb was also determined in the soil used for plant cultivation and in the experimental plant tissues. Results showed that plant performance significantly reduced with increasing concentrations of Pb contamination. Residual Pb was detected in the tomato roots and shoots, but non in the fruits. Results also showed that Vitamin C content of the tomato was not affected by various concentrations of the Pb contaminants. Pb contamination has adverse effects on tomato production but not on Vitamin C content.

Key word: Lead pollution, Lycopersicon esculentum, phytotoxicity.

INTRODUCTION

Heavy metals are a group of non biodegradable elements with the tendency of bioaccumulation in living systems. They are both industrially and biologically important and include metals such as aluminium, cadmium, zinc, chromium, copper, manganese, nickel and lead (Phipps, 1981; Horsfall and Spiff, 2004). Heavy metals are commonly encountered in industrial wastes and recently, have posed so much environmental concern that cannot be overlooked (Krishnamurti and Naidu, 2000; Guo et al., 2006). Though they occur naturally in rocks, soils and water, environmental contamination via anthropogenic sources due to increased industrialization has resulted in serious problems in the food chain and consequently, the health of organisms, including man (Khairiah et al., 2002; Antunes et al., 2003; Jamal et al., 2006). Also, exponential growth of the world's population over the past 20 years has resulted in environmental build-up of waste products of which heavy metals are of particular concern (Appel and Ma, 2002; Cossich et al., 2002; Vijayaraghavan et al., 2004). Some heavy metals however, at low doses are essential micronutrients for plants (e.g. Cu, Cr, Ni, Zn) but at high doses, may cause metabolic disorders and growth inhibition for most plant species (Mclaughlin et al., 1999; Peralta et al., 2000; Chojnacki et al., 2005). Plants are important component of the ecosystems as they transfer the metals from abiotic into biotic environments (Chojnacki et al., 2005; Richardson et al., 1993; Krupa, 1993; Maksymiec and Baszynski, 1996; Mocquot et al., 1996).

The metals may enter the food chain either through water supplies and aquatic organisms or through arable produce and grazing animals (Thornton, 1991). Excessive concentrations of Pb exhibit noxious effects to plants. It also results in phytotoxicity of cell membrane (William, 1976). Possible causal mechanisms include changes in permeability of cell membrane, reactions of sulphydryl (-SH) groups with cations, possible affinity for reacting which phosphate groups and active groups of ADP and ATP (William, 1976).

Elsewhere, studies have been carried out on heavy metal concentration of outdoor soil-grown tomatoes (Vagn et al., 2001; Kunsch et al., 1994). With the recent advocacy for increased consumption of tomatoes for its lycopene richness (and anti-oxidant activities); the need

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to investigate its potential use in health and environmental concern can not be over-emphasized. The objective of this work therefore was to assess the influence of Pb contamination on the growth, yield and vitamin C content of tomato (*Lycopersicon esculentum*).

MATERIALS AND METHODS

Preparation and characterisation of soil samples

Soil samples were collected at 0 - 30 cm depth from the Fadama Farm, University of Agriculture, Abeokuta, Nigeria and air dried at room temperature. The physico-chemical properties of the soil were determined after soil had been sieved through a 1 mm mesh and representative samples obtained by coning. Soil samples were mixed with distilled water (in a 1: 2 ratio), stirred on mechanical shaker for 15 min and the pH determined using a pH meter (Jenway 3015, Jenway Ltd., Dunmow Essex, England). The organic carbon content and the particle size distribution of the soil samples were determined using the Walkey-Black method and hydrometer method respectively as described by Juo (1988). While, the cation exchange capacity (CEC) of the soil was determined using the neutral ammonium acetate method (Tu et al., 2002). Lead concentrations in the samples were analyzed using acid digestion method followed by quantification with Atomic Absorption Spectrophotometry (AAS) (Fadina and Opeolu, 2003; Opeolu et al., 2006).

Determination of Pb Phytotoxicity and Pb concentrations on tomato plants (and soil)

The seeds of *Lycopsersicon esculentum* used for the study were purchased from IAR&T (Moor plantation, Ibadan, Oyo State, Nigeria). Four weeks old seedlings of equal vigour raised in a nursery were selected and transplanted into pots previously filled with ground soil (4.5 kg of the soil per pot sieved with 4 mm pore size sieve). To the transplanted seedlings, 1 g of fertilizer (N: P: K 10: 10: 20) was applied twice per pot, first at transplanting to enhance plant growth and secondly, at flowering to enhance fruit production. The seedlings were irrigated with various concentrations of Pb (300, 600, 1800 ppm) as $Pb(NO_3)_2$ 1 - 2 times per day for 8 weeks. Plant growth was determined by measurement of plant height using a meter rule at transplanting and at 1 week interval after transplant (WAT) for 8 weeks when plants were harvested. The plant branches and number of leaves were counted first at transplanting and at 1 week interval for 8 weeks when plants were harvested, while the number of flowers and fruits were immediately counted at onset of flowering and fruit production. The experiment was carried out in replicates of ten.

At harvest, the best five of each of the ten replicated treatments were selected and their roots, shoots, fruits and soils on which they were grown were analyzed for residual Pb using the Alpha 4 Atomic Absorption Spectrophotometer (AAS) with hollow cathode lamp (Chemtech Analytical, UK).

For determination of residual lead, the plant parts were thoroughly washed with distilled-deionised water and oven-dried at 70℃ for 3 days. Each of the plant part (5 g) were then, weighed, ground and digested using 5 ml conc. HNO_3 in separate 250 ml flasks, properly stirred and 4 ml of 33% H_2O_2 added in a fume cupboard. The mixture was then heated on a hot plate for 8 min, allowed to cool, filtered, washed with 5 ml of HCl: distilled-deionised water (1:1) and made up to 25 ml with distilled-deionised water. The extract was then analyzed using AAS.

After harvest, the soil at 3 cm above the bottom of the pot was collected for Pb analysis. Soluble Pb in the soils from the pots used for cultivation of the selected plants was also detemined. For this purpose, 50 ml of de-ionized water was added to 10 g of the soil sample in 250 ml conical flask and stirred on the mechanical shaker for 15 min. The mixture was filtered and the filterate was analyzed for soluble Pb using AAS.

Analysis of fruits for Vitamin C

Ten milliliters (10 ml) of oxalic acid was added to 10 g of each fruit from the plant samples in a mortar and the mixture crushed into semi liquid slurry using a pestle. The mixture was filtered and the filtrate transferred into 100 ml standard flask and then made up to the mark with oxalic acid and allowed to stand for 2 h to allow for plant vitamin extraction. After 2 h, the clear extract-solution was transferred into a conical flask and covered with aluminium foil to prevent oxidation. 10 ml of the extract was measured into a clean 100 ml standard flask and made to mark with distilled-deionised water. From this extract solution 10 ml was withdrawn and 5 ml 4% potassium iodide solution, 2 ml of 3% v/v acetic acid solution and 10 drops of starch solution added. The vitamin C content of this mixture was then determined titrimetrically using bromosuccinimide. The experiment was carried out in triplicates for each sample (Okiei et al., 2009).

Data analysis

The different data collected were subjected to statistical analyses using SPSS 11.0 version. Tools used include descriptive, analysis of variance (ANOVA) and Duncan multiple range test for statistical significance at 95% confidence level.

RESULTS AND DISCUSSION

Effects of Pb contamination on growth parameters

Results of the effects of Pb contamination on tomato growth and production measured 1 week after transplant for 8 weeks (WAT 1-WAT 8) are shown in Tables 2 - 5. The soil physicochemical characteristics are presented in Table 1. Results showed that all the values for the various parameters determined fall within the ranges reported in literatures indicating that the soil was suitable for cultivation of tomatoes (Huang et al., 2006; Chandra et al., 2008; Al-Lahham et al., 2007). Fertilizer (NPK 10:10:20) was added to the various soils to simulate local practice by farmers, thus providing adequate nutrient to the experimental plants for proper growth and fruit production.

The effects of Pb toxicity on plant performance are presented in Tables 2 - 3. Results showed that the plant performance generally depreciated with increased concentration of Pb. Results also showed that the number of leaves in each plant decreased with increase in concentration of Pb contamination (Table 2). For instance, at 600 ppm the number of leaves of the plant was 148.6 ± 29.03 and at 1800 ppm, the leaves decreased to 113.2 ± 10.95. It has been reported that synergistic effect is exerted with more nitrate supply from $Pb(NO_3)_2$ used to irrigate experimental plants, thus explaining the lowered effect of the Pb on the experimental plants in this study at 600 ppm. (Fargasova, 2001;

Table 1. Physicochemical properties of experimental soil

Parameter	Value
pH	6.56
Organic carbon	2.5%
Zn^{2+}	3.9mg/kg
Pb^{2+}	35.87mg/kg
Sand	81.8%
Clay	10.4%
Silt	7.8%
Ca^{2+}	1.36Cmol/kg
Mg^{+}	1.25Cmol/kg
K^{+}	0.77Cmol/kg
Na^{+}	0.59Cmol/kg
H^{+}	0.04Cmol/kg
Al^{3+}	0.1Cmol/kg
Textural class	sandy loam

Table 2. Effect of lead on the number of leaves.

TRT	WAT 1	WAT 2	WAT 3	WAT 4	WAT5	WAT 6	WAT 7	WAT 8
300	23.40 ± 6.73b	34.60 ± 3.03a	61.60 ± 7.90b	98.80 ± 16.86c	143 ± 30.56d	162.40 ± 28.63d	162.40±28.63d	148.60±29.23d
600	20.00 ± 1.26a	38.20 ± 8.90b	55.60 ±13.98a	77.80 ± 16.3b	98.6 ± 12.41a	122.20 ± 12.58a	122.20±12.58a	113.20±10.95a
1800	18.80 ± 2.85a	37.80 ± 4.63b	53.40 ± 6.88a	72.40 ± 14.5a	114 ± 39.32b	130.60 ± 36.83b	133.40±35.93b	119.80±33.59b
CTL	28.20 ± 2.42c	52.60 ± 6.53c	73.20 ± 8.88c	103.60 ± 8.62d	139.8 ± 7.65c	154.20 ± 9.54c	154.20±9.54c	141.20±10.02c

Means followed by the same letter within a column do not differ significantly according to DMRT at p = 0.05; TRT= Treatment; WAT= Weeks after transplanting; CTL= Control.

Table 3. Effect lead of on the number of branches.

TRT	WAT 1	WAT 2	WAT 3	WAT 4	WAT5	WAT 6	WAT 7	WAT 8
300	5.60 ± 1.40a	8.00 ± 0.84a	10.40 ± 0.93a	14.00 ± 1.92b	22.60 ± 4.13c	26.6 ± 3.16c	26.6± 3.16c	26.6 ± 3.16c
600	5.40 ± 0.40a	8.00 ± 1.70a	10.60 ± 2.20a	13.20 ± 1.93b	19.00 ± 3.29b	23.2 ± 2.87b	23.2 ± 2.87b	23.2 ± 2.87b
1800	4.40 ± 0.24a	7.00 ± 0.32a	9.00 ± 0.84a	10.00 ± 0.84a	17.00± 6.52a	20.0 ± 6.33a	20.40 ± 6.25a	20.40 ± 6.25a
CTL	7.00 ± 1.05b	11.00± 1.18b	13.00 ± 1.22b	14.00 ± 1.14b	19.40 ± 4.28b	25.60 ± 3.26c	25.60 ± 3.26c	25.60 ± 3.26c

Means followed by the same letter within a column do not differ significantly according to DMRT at p =0.05; TRT= Treatment; WAT= Weeks after transplanting; CTL= Control.

Table 4. Effect of lead on stem height.

TRT	WAT 1	WAT 2	WAT 3	WAT 4	WAT5	WAT 6	WAT 7	WAT 8
300	6.98 ± 1.42a	13.60 ± 1.42a	17.62 ± 1.60a	25.24 ± 3.13c	32.86 ± 3.91d	38.00 ± 4.25b	38.04 ± 4.26c	38.04 ± 4.26c
600	6.50 ± 0.73a	14.12 ± 3.63a	17.82 ± 4.37a	20.40 ± 5.32a	26.68 ± 5.08a	32.02 ± 5.49a	32.02 ± 5.49b	32.02 ± 5.49b
1800	7.20 ± 0.95a	15.16 ± 3.02b	18.46 ± 3.15a	23.18 ± 2.85b	28.90 ± 3.89b	31.92 ± 3.73a	33.98 ± 3.32b	33.98 ± 3.32b
CTL	10.98 ± 1.35b	17.08 ± 2.97c	20.16 ± 2.03b	24.10 ± 1.56c	29.10 ± 1.71c	31.78 ± 1.89a	31.58 ± 1.78a	31.78 ± 1.89a

Means followed by the same letter within a column do not differ significantly according to DMRT at p = 0.05; TRT= Treatment; WAT= Weeks after transplanting; CTL= Control.

Table 5. Number of flowers (Mean±SE)

Treatment	WAT 5	WAT 6	WAT 7	WAT 8
300	8.80±2.22b	10.08±2.58c	16.20±4.02d	16.80±4.53c
600	4.80±3.43a	5.20±3.25a	10.20±1.43a	10.60±5.496a
1800	5.00±2.76a	8.20±3.51b	14.00±7.07b	14.20±3.320b
CTL	8.20±2.50b	10.20±2.86c	15.70±5.13c	16.50±4.32c

Means followed by the same letter within a column do not differ significantly according to DMRT at p = 0.05; WAT:=Weeks after transplanting CTL= Control

Stevens et al., 2003). Table 3 shows the effect of Pb contamination on the number of branches of the experimental plants. Results showed that the number of branches of each plant reduced with increase in contamination from 300 - 1800 ppm. Though the number of branches varies between each treatment, at harvest, there was no statistical difference in the numbers between control and 300 ppm treatment and those of 600 and 1800 ppm treatments. The observations are consistent with the findings of Fargasova (2001) who reported that Pb significantly inhibited growth of *Sinapis alba* L. The effect of Pb contamination on stem height (plant growth) of experimental plants is presented in Table 4. Results showed that there was no significant difference in stem height in all the different concentrations of Pb contaminations studied. However, it was observed that the plant heights of the control plants were significantly higher than all the test plants. Other workers have suspected the influence of anionic radicals in metallic salts to reduce metal toxicity on plants (Stevens et al., 2003). Thus, the higher levels of Pb in the irrigation water might have antagonistic influence on nitrate availability resulting in poorer growth Influence of Pb toxicity on yield and vitamin C content.

Pb toxicity effect was pronounced on flower production of experimental plants. The numbers of flower produced were lower at the 600 and 1800 ppm contamination levels. There were significant differences in number of flower amongst the treatments with the control plants having the highest number (Figure 1). The differences were not statistically significant at $p \leq 0.05$. Results also showed that Vitamin C content of fruits was not significantly different for all treatments (figure 5) and Pb was not detected in all the fruit samples investigated.

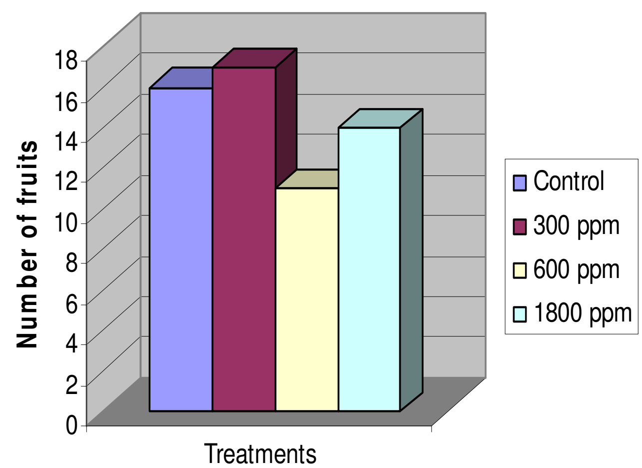

Figure 1. Number of Fruits on Plants

This is an indication that the Pb contamination had little or no influence on the vitamin C and fruit production in tomato.

Residual Pb in soil and plant tissues

Results of the study on residual Pb in the soil samples and plant tissues investigated showed that residual Pb was detected in the roots and shoots of the experimental plants; concentrations were lowest for control and highest for 1800 ppm contaminated soils. The same trend was observed in the experimental soils after harvest (Figures 2, 3 and 4). This implies that Pb uptake by plants was

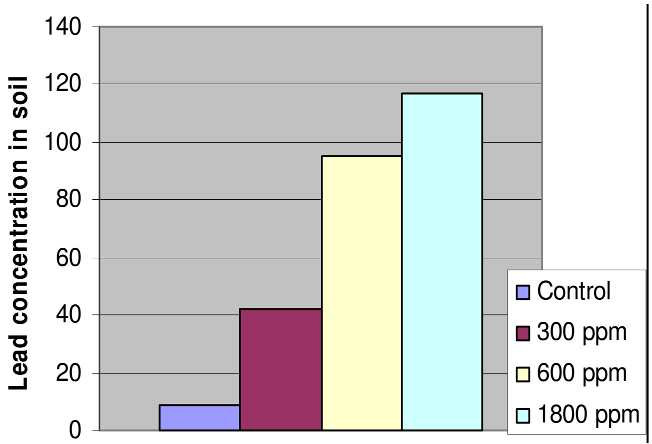

Figure 2. Residual concentration of lead in soil after harvest.

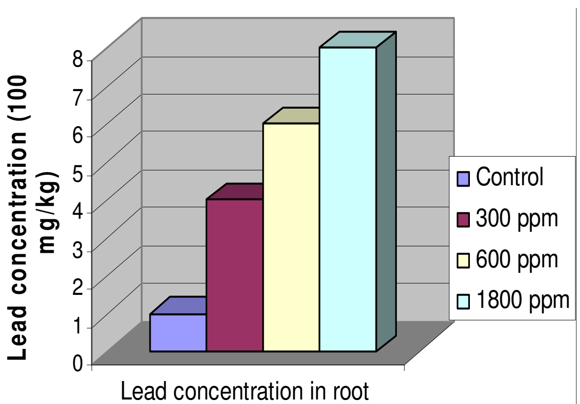

Figure 3. Residual concentration of lead in root after harvest.

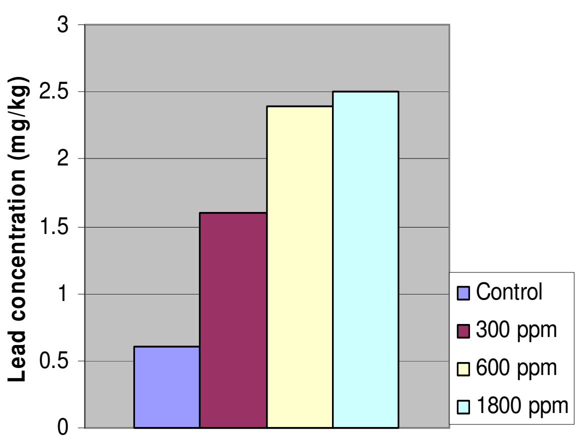

Figure 4. Residual concentration of lead in shoots

dependent on the amount available in the soil. Element removal from soil has been reported to be dependent on content in soil (Vyslouzilova et al., 2003; Grytsyuk et al.,

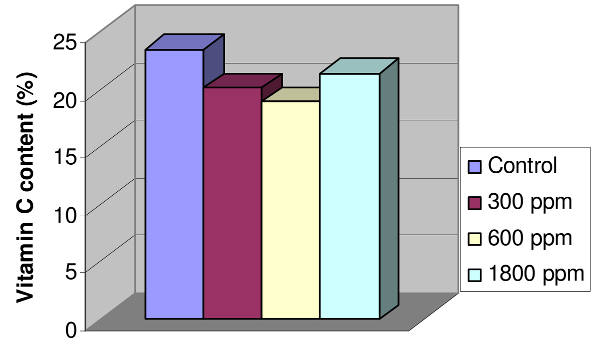

Figure 5. Vitamin C content of harvested fruits

2006; Soyingbe et al., 2007). The higher levels of Pb in the plant roots of this study are consistent with the findings of Fargasova (2001) who had similar results. High levels of Cd, another divalent heavy metal have also been reported to be present in roots of tomato grown in a controlled environment in hydroponics than in shoots (Lopez-Milan et al., 2009).

Conclusion

Pb contamination was found to have adverse effects on growth parameters of tomato. The effects were pronounced on number of leaves, branching and plant height. Yield and nutrient quality factors (numbers of flowers, fruit and vitamin C content) were not affected significantly. Pb contamination has adverse effects on tomato production but not on Vitamin C content. Similar field experiments and a wider survey of impact of Pb contamination on tomato and other vegetable crops needs to be carried out.

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