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Effect of Copper Applications to Soil on Growth and Mineral Contents of Tomato Plants

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Copper-containing fertilizers, fungicides and bactericides are extensively used to control plant diseases in greenhouses in Turkey. Excessive applications of these materials have led to the accumulation of potentially toxic elements and imbalance the mineral nutrition of plants. The objective of this study was to evaluate the effects of Cu applications to soil on growth and mineral contents in different organs of tomato plants grown in greenhouse. For this purpose, tomato seedlings were grown for 10 weeks in a computer-controlled greenhouse and range of Cu was applied to soil as $CuSO_4.5H_2O$ (0, 250, 500, 1000 and 2000 mg kg^{-1}). The results showed that the leaf, stem and root biomass decreased with increasing Cu supply to soil. The N, Mn and Cu contents of plant tissues increased with increasing Cu supply to soil, whereas the P, K, Ca and Fe contents of plant tissues decreased. The Mg content of stem decreased with increasing Cu supply to soil while leaf and root Mg content were not affected by these treatments. The Zn contents of leaf and root decreased with increasing Cu supply to soil but not in the stem. Increasing Cu supply to soil caused a decrease in root growth, reducing uptake nutrient elements such as P, K, Ca, Mg, Fe and Zn. As a result, increasing Cu supply to soil could imbalance the mineral nutrition and inhibit the growth of tomato plants. Therefore, caution should be taken when applying copper in soil.

Key Words: Trace metals, Copper sulphate, Shoot and root biomass, Nutrients, Greenhouse.

INTRODUCTION

Trace metals are considered to be one of the main sources of pollution in the environment, since they have a significant effect on its ecological quality[1]. Lead, copper, manganese, zinc, iron etc. were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution[2]. According to Bowen[3], trace metals such as Cu, Co, Cr (IV), Al, B, As (III), Be, Cd, Mo, Ni, Se (IV) and Ti, even in low concentrations, can be harmful to plants and humans. Metals like iron, copper, zinc and manganese are essential metals since they play an important role in biological system[2]. The decrease in amount of these elements

causes malnutrition in plants. On the other hand, increase in the amount of these elements has hazardous effect[4]. Among the trace metals, copper is present in unpolluted soils in the range of 2 to 40 mg kg^{-1}. Polluted soils, however, contain up to 100 mg kg^{-1} copper[5]. In general, the copper content of the majority of plant species varies between 20 and 30 mg kg^{-1} dry weight[6].

Although copper is an essential element for various metabolic processes, it is only required in trace amounts and becomes toxic at high concentrations[7]. It has been reported that copper interfere with the root uptake of mineral nutrients and with various metabolic functions of essential cations, causing a range of nutritional disorders[8].

An excessive Cu supply usually inhibits root growth before shoot growth[9]. However, this does not mean that roots are more sensitive to high Cu concentrations; rather they are the sites of preferential Cu accumulation when the external Cu supply is large. Zheng *et al.*[10] reported that excessive copper reduced plant root length, root dry weight, total dry weight, root to shoot ratio, leaf area and specific leaf area in three ornamental crops grown in solution culture.

Application of manure obtained from pigs fed with commercial cattle feed increases copper content of soil results in Fe, Zn and Mo deficiencies in plants[11]. Arduini *et al.*[12] reported that the Mn and Zn content of roots decreased in all species (*Pinus pinea* L., *Pinus pinaster* Ait. and *Fraxinus angustifolia* Vahl.) with increasing Cu supply, whereas the Ca and Mg uptake and distribution differed among species.

Lidon and Henriques[13] observed that increasing copper concentrations resulted in an increase in copper level in root and shoots. They also reported that increasing copper in solution from 0.01 to 1.25 mg L^{-1} resulted in 7.4-fold increase in root proton extrusion. It was also observed that Zn uptake decreased with increasing Cu concentrations, while N, P, K, Na, Mg, B, Mo and Al uptake and translocation did not seem to be correlated with Cu treatments.

Copper pollution in soils is caused by not only industrial activities but also agricultural practices. In regions where hop production and vineyards are common, copper accumulation was observed due to applications of chemicals for plant protection purposes. Copper content of these soils may reach 600 mg kg^{-1} [14].

Copper-containing fertilizers, fungicides and bactericides has been used extensively in the greenhouses in the Antalya, Turkey in recent decades. Kaplan[15] found that about 8 % of soils in Antalya, Turkey contained diethylene tetramine pentaacetic acid (DTPA) extractable Cu greater than the critical toxicity level (20 mg kg^{-1}) and the Cu concentration in leaf samples (mean 166.5 mg kg^{-1}) was very high due to the intensive use of copper-containing chemicals.

Applications of copper containing fertilizer, pesticides and fungicides to soil have gradually over the years in Mediterranean region and copper accumulation has showed a tendency to increase year by year and yet there has been no study to determine the effects of "copper" on the growth and mineral contents of tomato plants in Mediterranean region. In this study, the author investigated the influence of increasing level of copper applications to soil on the growth and mineral contents in different organs of tomato plants grown in greenhouse.

EXPERIMENTAL

Pot experiments were conducted in a computer-controlled greenhouse located in Antalya, Turkey. The soil used in the experiment was a Xerorthent (Entisol) having the following chemical and physical properties before the application of the treatments; clayey textured (530.4 g kg^{-1} clay, 367.2 g kg^{-1} silt and 102.4 g kg^{-1} sand), pH 6.5 (1:2.5 soil: water ratio) 26.0 g kg^{-1} organic matter, total carbonates equivalent to 44.0 g kg^{-1}, total N 0.18 %, extractable P 110.80 mg kg^{-1}, extractable K 241.8 mg kg^{-1}, extractable Ca 2750 mg kg^{-1}, extractable Mg 541.2 mg kg^{-1}, DTPA-extractable Fe 92.35 mg kg^{-1}, DTPA-extractable Zn 14.80 mg kg^{-1}, DTPA- extractable Mn 295.80 mg kg^{-1} and extractable Cu 15.30 mg kg^{-1}.

Copper was applied to soil as $CuSO_4.5H_2O$ with 24.5% copper. $CuSO_4.5H_2O$ is blue, bright crystal and soluble in water. Tomato (*Lycopersicon esculentum* (L.) Mill. Cv. F144) was selected for this study as a test plant. The seedlings of tomato were obtained from the West Mediterranean Agricultural Research Institute, Antalya, Turkey.

The soil passed through a 4 mm sieve. 4 kg of air-dried soil were mixed with 1 kg of a mixture containing 75 % turf and 25 % perlite to improve the texture of the soil. Only one tomato seedling was planted to each pot. Copper was applied to soil in five different levels (0, 250, 500, 1000 and 2000 mg kg^{-1}). In this study, treatments were selected based on the results of Kaplan's study[15] conducted that in Mediterranean region where greenhouse tomato growing is intensive. The pots, which were sited in a greenhouse, were arranged in a randomized plot design with four replicates.

Processes during and at the end of the experiment period

Pots were incubated for 2 weeks after the addition of $CuSO_4.5H_2O$ and before planting. The seedlings were allowed to grow for a period of 10 weeks. All pots were fertilized once in a week with mono ammonium phosphate, potassium nitrate, ammonium nitrate and magnesium sulphate. Total amounts of nutrients provided to each pot were: 144 kg Nha^{-1}, 108 kg P_2O_5 ha^{-1}, 143 kg K_2O ha^{-1} and 17 kg MgO ha^{-1}. Pots also received 2.16 kg Fe ha^{-1}, 2.16 kg Mn ha^{-1}, 0.81 kg Zn ha^{-1}, 0.27 kg B ha^{-1} and 0.05 kg Mo ha^{-1}. Fertilizers were applied based on the local recommendation for tomato fertilization.

From the 4th week after planting, plant height was measured weekly. Plants were harvested 10 weeks after the start of the treatments. At harvest, plants were washed by distilled water and separated into leaf, stem and root and dried in a forced air oven at 65ºC to a constant weight. After drying; leaf, stem and root dry weights were recorded. The leaf, stem and root samples were ground separately in a stainless mill to pass through a 20 mesh screen and kept in clean polyethylene bags for analysis.

Chemical and statistical analysis

The soil used in the experiment was chemically analyzed after they had been air-dried and passed through a 2 mm sieve. Total carbonates were determined according to the calcimeter method of Nelson[16]. Soil texture was determined by hydrometer method[17] and organic matter by the Walkley-Black[18]. Extractable P content was extracted by $NaHCO_3$[16] and determined by a molybdate colorimetric method[19], extractable K, Ca and Mg were extracted with ammonium acetate and determined by atomic absorption spectrophotometry[20]. Soil Fe, Mn, Zn and Cu were extracted with diethylene tetramine pentaacetic acid (DTPA)[21] and then determined in the obtained extract by atomic absorption spectrophotometry.

Dried plant samples (leaf, stem and root) of 0.5 g each were digested with 10 mL $HNO_3/HClO_4$ (4:1) acid mixture on a hot plate. The samples were then heated until a clear solution was obtained. The same procedure was repeated several times. The samples were filtered and diluted to 100 mL using distilled water. Concentrations of K, Ca, Mg, Fe, Zn, Mn and Cu in the digestates were determined by using atomic absorption spectrophotometry[22]. Phosphorus was measured by spectrophotometry[23] and N was determined by a modified Kjeldahl procedure[22].

Statistical analysis was carried out using the MSTAT-C software. Means were compared by analysis of variance (ANOVA) and the LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

The leaf, stem and root dry weights and plant height of tomato plants were significantly lower than of control plants at concentrations higher than 500 mg kg^{-1} copper (Fig. 1). The copper concentrations used in this experiment reduced the dry weights of leaf, stem and root and plant height. In addition, it seems that the root dry weight was much lower than in the stem and leaf dry weights. For example, the root dry weight when grown in 500 mg kg^{-1} copper treatment was ≈ 5 times lower than that in the stem tissue and ≈ 10 times lower than that in the leaf tissue. The decreased root dry weight indicates that plant roots are more sensitive to copper toxicity than leaf and stem tissue in tomato plants. This is agree with other reports on excessive copper depressing root[12,10], leaf growth[24] and shoot growth[25] in other species.

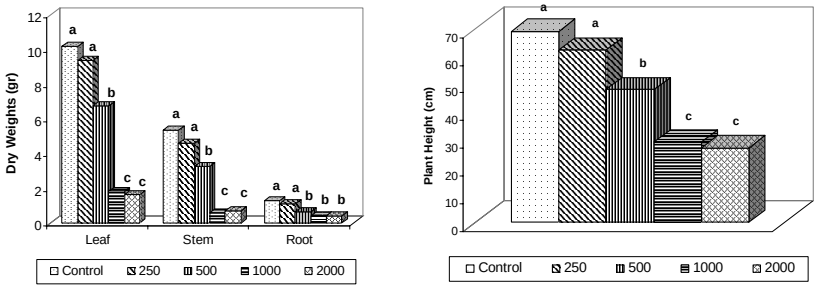

Fig. 1. Effects of increasing Cu applications to soil on the leaf, stem and root dry weights (g) and plant height (cm) of tomato plants. Means with a common letter are not different ($p \leq 0.05$) by LSD-test

Nitrogen contents of leaf, stem and roots increased at all copper treatments. The highest N contents of plant tissues was obtained at 2000 mg kg^{-1} copper (Table-1). The increasing copper treatment to soil influenced the uptake of N whose content markedly increased in the plant tissue of tomato plants. It is known that copper has a strong affinity for the N atom of amino acids[26]. Therefore, positively significant correlation between copper and N was observed. In the present study, it was found that copper was strongly correlated with N in leaf, stem and root ($r = 0.804$, 0.811 and 0.878 at $p < 0.001$, respectively). A similar response was reported by Rautio *et al.*[27], Parat *et al.*[28] and Wang *et al.*[29].

Increasing level of copper treatment to soil tended to decrease the phosphorus contents of leaf and stem but not in the phosphorus content of roots. The phosphorus contents of stem and leaf were significantly lower than that of control plants at concentrations higher than 250 mg kg^{-1} copper (Table-1). Leaf and stem dry mass decreased due to the fact that root dry weight decreased with increasing copper treatments to soil. As a result, an decrease in phosphorus contents of leaf and stem by increasing copper treatments seem to be due to decrease of the phosphorus uptake in roots. These results are supported by other authors[10,30].

Potassium contents of leaf, stem and roots decreased with increasing level of copper treatment to soil. Leaf and stem potassium contents were significantly lower than that of control plants at concentrations higher than 1000 mg kg^{-1} copper while root potassium content was significantly decreased at concentrations higher than 500 mg kg^{-1} copper (Table-1). The increasing copper treatment to soil influenced the uptake of potassium whose content markedly decreased in the plant tissues of tomato plants. A reduction in potassium contents of plant tissues seems to be due to inhibition of potassium uptake caused by high level of copper treatment to soil. A similar response was observed in cereal, citrus seedlings, phragmites australis, zea mays and cowpea treated with copper[30-33].

TABLE-1
EFFECTS OF INCREASING COPPER APPLICATIONS TO SOIL ON MACRONUTRIENTS CONTENTS OF LEAF, STEM AND ROOT SAMPLES[1]

Cu Treatments	N (%)			P (%)			K (%)			Ca (%)			Mg (%)		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control	4.57c	4.36c	4.29d	0.61a	0.41a	0.24	3.76a	5.06a	2.06a	2.25a	1.57a	1.51	0.55	0.89a	2.81
250 mg kg^{-1}	4.66b	4.40bc	4.42c	0.49b	0.33b	0.24	3.95a	5.57a	2.05a	2.16a	1.65a	1.58	0.55	0.56b	1.96
500 mg kg^{-1}	4.66b	4.41bc	4.38c	0.50b	0.31b	0.21	3.46a	5.17a	1.77ab	2.06a	1.49ab	1.46	0.57	0.62b	1.95
1000 mg kg^{-1}	4.72ab	4.48b	4.53b	0.27c	0.10c	0.17	2.17b	2.08b	1.29bc	1.74ab	1.27b	1.73	0.59	0.57b	1.88
2000 mg kg^{-1}	4.76a	4.66a	4.79a	0.24c	0.12c	0.20	1.84b	0.84c	1.16c	1.22b	0.77c	2.02	0.75	0.45b	1.76
ANOVA[2]	**	**	**	**	**	ns	**	**	**	*	**	ns	ns	**	ns

[1]Data are means of 4 measurements. Treatments wIth no common letters in a column are significantly different ($p < 0.05$, LSD test).
[2]Significance levels: $**p < 0.01$, $*p < 0.05$, ns: not significant.

TABLE-2
EFFECTS OF INCREASING COPPER APPLICATIONS TO SOIL ON MICRONUTRIENTS CONTENTS OF LEAF, STEM AND ROOT SAMPLES[1]

Cu Treatments	Fe (mg kg^{-1})			Mn (mg kg^{-1})			Zn (mg kg^{-1})			Cu (mg kg^{-1})		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control	143.5a	56.5a	6617.5a	176.0b	53.8c	181.3c	38.7c	39.1	54.9b	12.0b	8.0c	25.0d
250 mg kg^{-1}	125.5b	46.5b	4662.0b	190.3b	65.2c	273.9bc	77.15b	47.0	147.1a	31.0a	16.8b	262.5c
500 mg kg^{-1}	105.3c	42.5b	3707.5c	260.3b	78.9c	308.9bc	127.1a	48.2	68.0b	32.5a	19.0b	304.5c
1000 mg kg^{-1}	91.5d	33.5c	3354.0d	548.8a	193.0b	352.6b	33.2c	52.2	43.9b	31.5a	17.0b	544.0b
2000 mg kg^{-1}	79.8e	21.0d	3343.5d	503.2a	284.8a	524.2a	29.1c	64.5	44.9b	33.0a	27.5a	855.0a
ANOVA[2]	**	**	**	**	**	**	**	ns	**	**	**	**

[1]Data are means of 4 measurements. Treatments with no common letters in a column are significantly different ($p < 0.05$, LSD test),
[2]Significance levels: $**p < 0.01$, ns: not significant.

Increasing level of copper treatment to soil had no significant influence in the calcium content of roots. Leaf and stem calcium contents decreased with copper treatment to soil at concentrations higher than 1000 mg kg^{-1} and 500 mg kg^{-1} copper, respectively (Table-1). The increasing copper treatment to soil did not seem to influence the calcium uptake in roots, probably because other divalent cations did not compete for spesific Ca^{2+} channels[8]. However, the calcium content of leaf and stem decreased with increasing level of copper treatment to soil. Under the normal conditions; the plant calcium requirement increases with increasing external contents of heavy metals. In order to protect roots against the adverse effects of high content of various other cations (Cu, Al, Mn etc.) in the soil solutions, the Ca^{2+} concentrations required for optimal growth has to be much higher in soil solutions than in balanced flowing nutrient solutions and an increase in the concentration of Ca^{2+} in the external solution leads to an increase in the calcium content in the leaves[34,35], but any factor which prevents the growth of new roots may be expected to prevent calcium uptake[26]. In the present study, the calcium contents of leaf and stem decreased due to the fact that root growth decreased with increasing level of copper treatment to soil. A reduction in the leaf and stem calcium contents was also observed in ornamental crops, *F. angustifolia* seedlings, citrus seedlings and cow pea[12,30,10,33].

Copper treatments to soil differently influenced the magnesium contents of plant tissues. The magnesium contents of leaf and roots were not affected by copper treatment to soil. The stem magnesium content was significantly lower than that of control plants at concentrations higher than 250 mg kg^{-1} copper (Table-1). Magnesium is one of the essential elements for plant growth, but its rate of uptake can be strongly depressed by other cations and it was often reported that heavy metals reduce the root, stem and leaf content of magnesium in the seedlings, citrus seedlings[12,30,36]. However, the magnesium contents of leaf and root were unaffected in tomato plants treated with copper. In contrast, the stem magnesium content decreased with increasing level of copper treatment to soil. A similar response was reported in miniature rose plant and cow pea by Zheng *et al*.[10] and Kopittke and Menzies[33].

Iron contents of plant tissues decreased with increasing level of copper treatment to soil. The iron contents of plant tissues were significantly lower than those of control plants at all copper treatments (Table-2). The presence of copper in the soil strongly influenced the uptake and internal transport of iron, whose content markedly decreased in the plant tissues (leaf, stem and root). Heavy metals, in particular copper, are known to be displace iron from chelate complexes forming corresponding heavy metal chelates. This may be important in limiting iron uptake and utilization,

either by reducing iron chelate translocation to roots or within the plant itself by the effect of the heavy metal on centres of physiological activity for iron[26]. Lidon and Henriques[37] reported that shoot and root iron contents decreased with increasing rate of copper. Shoot iron content decreased at > 0.05 mg copper L^{-1}, while in the root iron content decreased above this level of copper. Similarly, Alva and Chen[7] found that increase in concentrations of external copper decreased the uptake iron and zinc by the citrus seedlings.

Increasing level of copper treatment to soil tended to increase manganese contents of plant tissues. The manganese contents of plant tissues were significantly higher than that of control plants at concentrations higher than 1000 mg kg-1 copper in leaf and stem and at 250 mg kg^{-1} in root (Table-2). The scientific reports about manganese contents of plant tissues with increasing level of copper treatment differ from each other. Some authors reported that Mn contents of root, stem and leaf decreased with increasing copper treatments[7,10,12,27] while other authors found that high copper supply increased the content of manganese in all of the plant organs[38]. Data obtained in the present study support finding reported by Pinc and Scholz[38].

Increasing level of copper treatment to soil had no significant influence in the zinc content of stem. Leaf and root zinc contents initially increased and then decreased with copper treatments to soil (Table-2). The increasing copper treatment to soil influenced the uptake of zinc whose content markedly increased and then decreased in leaf and root, but not seem to influence the zinc uptake in stem. This result may be explained with the effect of SO_4^{2-} in the structure of $CuSO_4.5H_2O$ applied as copper supply. Due to the fact that SO_4^{2-} released from the decomposition of $CuSO_4.5H_2O$ lead to a decrease soil pH, extractable zinc increased in the soil[39]. In the present study; soil pH decreased from 6.50 to 5.43, extractable zinc increased from 13.0 to 19.15 mg kg^{-1}. Our results are similar to Zabunoglu and Brohi[39]. However, because of antagonism effect between zinc and copper[26,35], zinc contents of plant tissues decreased with increasing level of copper treatment. Morishita *et al*.[40] determined that the level of copper, manganese and zinc in the roots of rice and tomato increased with increasing rates of copper, manganese and zinc, respectively, in the culture solution but in tomato they reached a maximum and then fell. The same researchers informed that the sulphur content of the roots played the important role in the transport of the heavy metals. Lidon and Henriques[13] reported that zinc uptake decreased with increasing copper concentrations.

Copper contents of plant tissues increased with increasing level of copper treatment to soil. The copper contents of plant tissues were significantly higher than those of control plants at all copper treatments. Copper

content in the root was much higher than in the stem and leaf tissues. For example, root tissue copper content when grown in 250 mg kg^{-1} copper treatment was > 8 times higher than that in the leaf tissue and > 15 times higher than that in the stem tissue (Table-2). Rhoads *et al.*[41] pointed out that tissue copper content increased (particularly roots) with increasing copper rate. Quarilli *et al.*[42] reported that copper accumulation increased with increasing copper concentration in 17 d old tomato seedlings grown in nutrient solutions containing copper at 0.5 or 50 μM. In the present study, copper content in the root system was much higher than in the stem and leaf tissues. Much higher copper retention in roots than in leaves and stems was reported in other studies as well[10, 24,30].

In conclusion, soil copper treatment, especially aiming to control plant diseases, showed different effects on mineral contents and negatively affected on growth of tomato plants. It is known that plants should have well developed root system in order to uptake nutrient elements[35]. However, increasing copper treatments to soil caused a decrease in root growth, reducing uptake of nutrient elements such as phosphate, potassium, calcium, manganese, iron and zinc. Above results show that nutrient uptake and transport are very sensitive to copper and increasing rates of copper to soil could imbalance the mineral nutrition and inhibite the growth of tomato plants by decreasing root growth. Therefore, caution is advisable to avoid over application of copper in the soil.

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