



## Effects of Copper, Zinc, Lead and Cadmium Applied with Irrigation Water on Some Eggplant Plant Growth Parameters and Soil Properties

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### Abstract

Sustainability of agriculture is only ensured by proper soil and water preservation practices. The idea of re-use of wastewater in agriculture is the critical issue regarding with the use and preservation of water resources in today's water scarce conditions. However, heavy metals and trace elements in waste water are important contaminants for plants and soils. Heavy metals create a significant threat on health of environment, plants and eventually on the health of humans and animals. In this study, eggplant seedlings were irrigated with increasing doses of Copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd)-containing irrigation waters to investigate the effects of heavy metal concentrations on plant morphological characteristics (stem length, root length, leaf fresh and dry weight, root fresh and dry weight, leaf area) and soil properties (pH, electrical conductivity, cation exchange capacity, organic matter). While significant changes were not observed in soil properties, effects of heavy metal treatments on plant morphological characteristics were found to be significant ( $p < 0.05$ ).

**Key words:** Copper, zinc, lead, cadmium, eggplant, stress

## Bakır, Çinko, Kurşun ve Kadmiyum'lu Sulama Suyu Uygulamalarının Bazı Patlıcan Genotiplerinin Büyüme Parametrelerine ve Toprak Özelliklerine Etkisi

### Özet

Tarımın sürdürülebilirliği, ancak uygun toprak ve su koruma uygulamaları ile sağlanmaktadır. Tarımda atıksuyun yeniden kullanım fikri, su kıtlığının yaşandığı bu günlerde su kaynaklarının korunması ve kullanımında kritik öneme haiz bir konudur. Ancak, atıksulardaki ağır metaller ve iz elementler bitki ve topraklar için önemli kirleticilerdendir. Ağır metaller, çevre ve bitki sağlığının yanında insan ve hayvan sağlığı üzerinde önemli bir tehdit unsurudur. Bu çalışmada, artan dozlarda Bakır (Cu), Çinko (Zn), Kurşun (Pb) ve Kadmiyum (Cd) içeren sulama sularıyla sulanan patlıcan fidelerinin, bitki morfolojik özellikleri (bitki gövde uzunluğu, kök uzunluğu, gövde yaş ve kuru ağırlığı, kök yaş ve kuru ağırlığı, yaprak alanı) ve toprak özellikleri (pH, elektriksel iletkenlik, katyon değişim kapasitesi, organik madde) üzerinde ağır metal konsantrasyonlarının etkileri incelenmiştir. Ağır metal uygulamalarının toprak özellikleri üzerindeki değişimleri istatistiksel açıdan önemsiz iken, bitki morfolojik özellikleri üzerinde ( $p < 0.05$ ) anlamlı olarak bulunmuştur.

**Anahtar kelimeler:** Bakır, çinko, kurşun, kadmiyum, patlıcan, stres

### Introduction

Turkey is located in a semi-arid climate zone and is not a water-rich country. It is even placed among

the countries suffering from deficits with water resources below the world averages. Since the country passes through a rapid development phase, water resources are polluted at increasing rates

because of industrial, domestic and agricultural activities. It is highly significant to provide a well balance among use-preservation-quality of water resources for a sustainable development.

The world population is expected to increase to about 7.2 and 8.3 billion by the years 2015 and 2030, respectively. Such a scenario requires significant increases in food production. Food production can be raised by cultivating more land, increasing the yields of each crop, growing two or three crops per year over the same piece of land, or by a combination of all these alternatives. However, each one of them has various advantages and disadvantages. There are also some other alternatives requiring more intensive methods of production. For instance, use of fertilizers and pesticides, irrigation, cultivation of high-yield cultivars and mechanization (Wild, 2003). Irrigation is the most important input in agricultural activities to improve both yield and quality. Considering the limited water resources of the country, reuse of low quality or treated waters is essential in agricultural irrigation. However, contaminants in treated waters like heavy metals and trace elements may impose various problems on plants and soils.

Heavy metals accumulate in soils and plant roots and then transferred to upper sections of the plants like stem, leaves and fruits. Therefore, a special attention should be paid in selecting the species to be grown over heavy-metal contaminated sites to be irrigated with treated waters (Barman and Bhargava, 1997). On the other hand, plants accumulating high concentrations of heavy metals from contaminated soils can also be used for detoxification/ phytoremediation of metals from soil or growing medium (Sarma, 2011).

Heavy metal contamination caused by natural processes or by human activities is one of the most serious eco-toxicological problems. Long term irrigation can induce changes in the quality of soil as trace element inputs are sustained over long periods. Soil health is endangered when wastewater is used for irrigation of edible plants for prolonged periods (Barman et al., 2000; Singh et al., 2004).

Heavy metal ions like Fe, Cu, Zn, Mn at appropriate concentrations are required for structural and catalytic components of proteins and enzymes as cofactors and they are essential for normal growth and development of plants. However, supra-optimal concentrations of these micronutrients and other heavy metals in plants operate as stress factors (Singh et al., 2002; Singh et al., 2004).

The behavior of some heavy metals in soils does not only depend on the level of contamination as expressed by total concentration, but also on the forms and origin of the metals and the properties of the soils themselves. Soil pH, texture and organic matter contents are mainly important with regard to forms and bioavailability of the heavy metals (Chlopecka et.al., 1996).

In this study, the eggplant seedlings grown under greenhouse conditions were irrigated by increasing concentrations of copper, zinc, lead, and cadmium (the maximum permitted value and twice the limit value) and the effects of heavy metals concentration on morphological characteristics of the plants and soil properties were investigated.

### Materials and Methods

This study was carried out during 2011 by eggplant genotypes of Burdur Central and Burdur Bucak (salt-tolerant) and Kemer and Giresun (not-tolerant) eggplant (*Solanum melongena* L.) genotypes were used as the plant material, Soil, Fertilizer and Water Resources Central Research Institute, Ankara, Turkey. Seeds were sown in viols with 2:1 peat and perlite mixture. After 20 days, seedlings were transplanted into 10 lt pots (25 cm diameter) with a soil mixture (1:1:1 sand, manure, medium textured soil). Then the plants were grown under controlled greenhouse conditions. During the research period, the temperature was 23-25°C and relative humidity was 50-55%. Plants were irrigated with distilled water to bring the deficit moisture to field capacity. Pots were rotated daily for even exposure to solar radiation. There wasn't use any fertilizer during the seedling.

Heavy metal treatments were initiated when the plants had 4-5 actual leaves (about 45 days from sowing). Control plants (A<sub>0</sub>) were not treated with heavy metals and they were only irrigated with distilled water. The other groups were exposed to following heavy metal doses during the experiments:

A<sub>1</sub>= 0.2 mg/l Cu, 0.01 mg/l Cd, 5 mg/l Pb, 2 mg/l Zn (Allowable limit)

A<sub>2</sub>= 0.4 mg/l Cu, 0.02 mg/l Cd, 10 mg/l Pb, 4 mg/l Zn (Twice the allowable limit)

Following 40-days growth under stress conditions, plants were harvested and sampled. Fresh yield per plant (g), dry yield per plant (g), dry matter (%), plant height (cm), root length (cm), root dry weight (g) and leaf area (LA) were determined by using standard methods and techniques to find out the heavy metal damages on plants. Leaf area was measured with a leaf area meter (Licor model LI-3000A).

Following the plant harvest, soil samples were taken from each pot to determine soil pH,

electrical conductivity (EC), lime ( $\text{CaCO}_3$ ), organic matter content, cation exchange capacity (CEC), % calcium (Ca) and magnesium (Mg). EC and pH were determined from saturation paste extract with a pH and EC meter (Richards, 1954);  $\text{CaCO}_3$  was determined with a Scheibler calcimeter (Richards, 1954); organic matter content was determined by

Walkley-Black wet-ashing method (Jackson, 1962), CEC was determined by ammonium acetate method (Richards, 1954).

Statistical analysis was carried out according to complete randomized block design and means were compared by LSD test at  $p < 0.05$  level.

**Table 1.** Soil properties

Genotype	Treatment	pH	EC dS/m	CEC meq/100g	ESP	Ca (%)	Mg (%)	$\text{CaCO}_3$ (%)	OM (%)
Burdur Central	A <sub>0</sub>	7.6	4.8	33.4	3.3	74.7	15.7	19.5	2.9
	A <sub>1</sub>	7.8	4.2	33.6	4.1	77.9	15.6	20.3	2.6
	A <sub>2</sub>	8.0	4.3	33.5	3.7	71.4	17.4	19.9	2.8
Burdur Bucak	A <sub>0</sub>	7.9	4.2	33.3	3.8	72.2	17.6	20.4	2.5
	A <sub>1</sub>	7.7	3.6	33.6	4.3	75.6	16.4	19.4	2.9
	A <sub>2</sub>	7.9	3.8	33.5	3.6	72.7	14.4	19.7	2.6
Kemer	A <sub>0</sub>	7.4	4.7	33.6	3.5	76.6	15.3	19.9	2.8
	A <sub>1</sub>	7.7	4.8	33.8	3.7	74.4	15.3	20.8	2.7
	A <sub>2</sub>	7.9	5.0	33.5	4.2	72.4	15.1	20.1	2.4
Giresun	A <sub>0</sub>	7.9	3.9	30.6	4.4	77.8	15.6	19.9	2.5
	A <sub>1</sub>	7.8	4.6	33.5	3.9	73.3	16.1	20.4	2.7
	A <sub>2</sub>	7.8	4.8	33.4	3.8	72.2	17.3	20.6	2.7

## Results and Discussion

### Soil Properties

Soil pH is a significant parameter for bio-availability of heavy metal as well as plant nutrient uptake from the soil. Table 1 presents pH values of soil samples. Soil pH values varied between 7.4 and 8.0 and there was slight increase in pH values with the treatments. The greatest increase was observed in tolerant-genotype A<sub>2</sub> of Burdur Bucak with 5.9%. However, the increase was not statistically significant. Soil pH value may have direct impacts on heavy-metal induced effects in soils. Particularly in increased acidity or solubility conditions, toxic effects of heavy metal treatments may also increase. In general, heavy metal cations are highly mobile under acidic conditions and increasing pH values through lime treatments usually reduces their bio-availability. However, molybdate anions become more available with increasing pH values (Alloway, 1995).

While electrical conductivity (EC) values of soils decreased in salt-tolerant genotypes, they tended to increase in sensitive genotypes. EC values varied between 3.6 - 5 dS/m. A<sub>0</sub> treatment of the genotype Burdur Bucak had the lowest value with 3.6 dS/m and A<sub>2</sub> treatment of Kemer had the highest value with 5 dS/m.

It was observed that the resistant-genotypes were able to uptake plant nutrients until the threshold heavy metal concentrations. In other words, A<sub>1</sub> treatment was less effective than A<sub>2</sub> treatment. Variations in soil EC values also

prove this case. The reason for high EC values was chemical fertilizer applications to pot upper soil layer and manure. As we all know, fertilizers are also a sort of salt. High nutrient levels in soils increase the EC values. Cation exchange capacity (CEC) values of experimental soils varied between 33.3 - 33.6 meq/100 g and heavy metal treatments did not result in any variations in those parameters. ESP is also another significant parameter in plant production and the values of the present study varied between 3.3 and 4.4. Significant differences were not observed in ESP values of the treatments. Rakesh Sharma and Raju (2013) reported similar soil characteristics and insignificant correlations between soil characteristics and heavy metal concentrations.

Treatments did not yield any significant variations in soil Ca and Mg contents. What is significant for soil is the ratio of Ca and Mg to each other. If the Ca/Mg ratio is near or less than 1, the uptake and translocation of Ca from soil-water to the above-ground parts of the growing crop is diminished due to antagonistic effects of high magnesium or competition for absorption sites to such an extent that less calcium is absorbed. A calcium deficiency may then be experienced at a higher calcium concentration in the applied water or in soil-water than would occur if the Ca/Mg ratio were higher. Although not definitely confirmed, it can be anticipated that irrigation water with a similar ratio ( $\text{Ca/Mg} < 1$ ) will produce a similar effect if a readily available source of calcium is not present in the soil (Ayers and Westcot, 1994).

Experimental treatments did not also resulted in significant variations in soil organic matter contents. However, metal association with organic materials can affect their chemical state and availability in natural waters. Among other characteristics, organic materials mask metal toxicity to phytoplankton, increase metal solubility and act as metal buffers via their so-called complexation capacity (Shuman and Cromer, 1979). The concentration of Pb, Fe and Mn appeared to be negatively correlated with organic matter and conductivity of the soil. Statistical analysis showed the nonlinear relationship of all soil properties to the heavy metals in soil (Deka and Sarma, 2012).

### Plant Characteristics

A healthy life cycle of a plant basically depends on balanced uptake of essential nutrients and uptake of toxic elements up to non-toxic levels. If above mentioned balance is destroyed unilateral, developmental disorders may exist and the plant may not complete its life cycle sometimes. In other words, if plant is exposed to high concentrations of any nutrient, the anion-cation balance may be destroyed and some metabolic processes may be negatively affected.

**Table 2.** Effects of heavy metal treatments on plant morphological characteristics

Genotype	Treatment	Fresh Weight of Plant (g/plant)	Dry Weight of Plant (g/plant)	Fresh Weight of Root (g/plant)	Dry Weight of Root (g/plant)	Plant height (cm)	Root Length (cm)	LA (cm <sup>2</sup> )
Burdur Central	A <sub>0</sub>	217 efg	37 a	36.7 cd	5.0 b	100.0 a	20.00 b	306.33 c
	A <sub>1</sub>	213 fg	27 bcd	33.3 cd	4.7 bc	95.3 b	19.33 bc	303.67 c
	A <sub>2</sub>	203 gh	23 cd	30.0 d	4.3 bc	90.0 de	18.67 bc	256.47 e
Burdur Bucak	A <sub>0</sub>	277 a	37 a	40.0 c	4.7 bc	93.0 c	21.67 a	341.37 b
	A <sub>1</sub>	273 a	33 ab	36.7 cd	4.3 bc	89.3 e	20.00 b	275.37 d
	A <sub>2</sub>	263 ab	23 cd	33.3 cd	4.0 cd	84.0 f	19.33 bc	250.00 ef
Kemer	A <sub>0</sub>	250 bc	37 a	40.0 c	6.0 a	98.0 a	18.00 c	307.27 c
	A <sub>1</sub>	230 de	30 abc	30.0 d	5.0 b	90.0 de	15.50 de	238.97 fg
	A <sub>2</sub>	220 ef	23 cd	20.0 e	4.7 bc	84.0 f	14.00 de	212.33 h
Giresun	A <sub>0</sub>	240 cd	30 abc	73.3 a	4.3 bc	92.0 cd	18.33 c	405.00 a
	A <sub>1</sub>	197 h	23 cd	66.7 a	3.3 d	85.7 f	15.67 d	230.63 g
	A <sub>2</sub>	213 fg	20 d	50.0 b	2.0 e	75.3 g	14.67 e	200.00 h
LSD		14.00	8.80	9.80	0.86	2.08	1.54	17.17

LS: Level of Significance; \*\* P<0.05; Means with the same letter in a column are not significantly different from each other according to the LSD test at P<0.05

Fodor (2002) suggested an interesting stepwise model for the action of heavy metals in plants. Initially, there are interactions with other ionic components present at the locus of entry into the plant rhizosphere that subsequently have consequences for the metabolism. This is followed by an impact on the formation of reactive oxygen species (ROS) in the cell wall and an influence on the plasmalemma membrane system (stage 1). At stage 2, the metal ion reacts with all possible interaction partners within the cytoplasm, including proteins, other macromolecules and metabolites. Stage 3 is mainly related to the factors that influence homeostatic events, including water uptake, transport and transpiration. At this stage, symptoms start to develop, and they become visible at stage 4

according to Fodor's model. As an example, the chlorophyll and, usually to a smaller degree, carotenoid contents decrease, which have obvious consequences for photosynthesis and plant growth (Barcelo and Poschenrieder 2004). The death of the plant cell occurs at stage 5. This model has the advantage that visible effects are linked to metabolic events that are influenced by the metal ion of interest (Appenroth, 2010).

Variations in fresh and dry weights of stems and leaves with heavy metal treatments are provided in Table 2. Investigated plant morphological characteristics, except for root dry weight and root length, were found to be significant at p < 0.05 level. Poschenrieder and Barcelo (2004) revised the old concepts that (i) heavy metals have a direct effect on stomata closure and (ii) that the roots simply act as an

osmometer, producing a hydraulic signal. Instead, roots can influence the water content via chemical signals, especially abscisic acid. Moreover, water transport appears to be modulated by an impairment of aquaporins, which is one of the earliest responses to heavy metals in plants. Many well-described physiological (or toxicological) responses may not have direct effects but consequences of fast responses in the roots (Appenroth 2010).

Parallel to plant growth and development, heavy metal treatments yielded significant differences in plant fresh and dry weights as compared to the control treatment. Such differences were more distinctive. In general, A<sub>1</sub> and A<sub>2</sub> treatments yielded decreasing values in investigated parameters of all genotypes. Statistical analysis revealed significant differences in investigated parameters of the treatments at  $p < 0.05$  level.

Previous studies proved that high heavy metal doses might have significant effects on water uptake and transport in plants. While A<sub>1</sub> treatments resulted in 7.2% decrease in plant fresh weights and 18.1% decrease in plant dry weights, such ratios were respectively reported as 8.52 and 36.36% in A<sub>2</sub> treatments. Among the genotypes, salt-tolerant Burdur Bucak and Burdur Central had lower heavy metal-induced variations the tolerant genotypes. Gratao et al. (2008) pointed out that the Cd accumulation on plant leaves had negative impacts on plant development and reported decreasing fresh and dry plant weights with increasing Cd doses. Dinakar et al. (2008) indicated that Cd stress lead to protein degradation through amino acid metabolism resulting in decreased plant growth. John et al. (2009) reported decreasing plant growth, carotenoid and chlorophyll contents with increasing stress conditions created by different heavy metals (e.g. Cu, Cd, Pb).

Proline accumulates under Cd stress. However, the accumulation does not occur directly as a response to the presence of Cd but because of disturbed water balance by the excess of Cd. One way to investigate the specificity of the stress caused by an excess of a heavy metal ion is to apply the microarray strategy to mRNA-related cDNAs in order to compare the effects of different heavy metals with those of other stress signals, e.g. water deficiency stress (Clemens, 2006; Appenroth, 2010).

Different heavy metal stress levels yielded varying decreases in root fresh and dry weights. While A<sub>1</sub> treatment resulted in 8-25% decreases in root fresh weights, A<sub>2</sub> treatment resulted in 15-50% decreases. With regard to genotypes, the least

variation was observed in A<sub>1</sub> treatment of Burdur Bucak (8.3%) and the largest variation was observed in A<sub>2</sub> treatment of Kemer. Root dry weights generally exhibited parallel trends with root fresh weights. As compared to the control plants, the greatest decrease in root dry weights was observed in A<sub>2</sub> treatments. Root dry weights of sensitive genotypes were more effected and especially the decrease in dry root weight of the genotype Giresun was 39% in A<sub>1</sub> and 62% in A<sub>2</sub> treatment. Kopittke and Menzies (2006) reported significant decreases in stem and root development and weights with Cu<sup>+</sup> toxicity.

Decreases were also observed in stem and root lengths with heavy metal treatments. The decrease in stem length was 5.91% in A<sub>1</sub> treatment and 13% in A<sub>2</sub> treatment. While the decrease ratio varied between 3-4% in A<sub>1</sub> and between 9-10% in A<sub>2</sub> treatments of tolerant-genotypes, the ratio was between 6-8% in A<sub>1</sub> and 14-18% in A<sub>2</sub> treatments of sensitive-genotypes. The variations in root lengths were generally parallel to variations in stem length. The decrease ratio in root lengths was between 3-10% in both treatments of tolerant-genotypes and between 13-22% in sensitive genotypes. In all genotypes, A<sub>2</sub> treatments resulted in higher decreases in root lengths. Tanyolaç et al. (2007) reported significant decreases in stem and root lengths with toxic copper accumulation and indicated higher decrease rates in sensitive genotypes.

As it was in many other stress factors, the first morphological change and decrease was observed in leaf area in heavy metal stress, too. The highest leaf area was observed in A<sub>1</sub> treatment of Kemer and Giresun genotypes (22- 43 %) and the decrease ratio in leaf area of tolerant-genotypes was between 0.87 - 19 %. On the other hand, A<sub>2</sub> treatments increased the wane ratio in leaf area of all genotypes and this ratio has reached up to 30- 50 % in sensitive genotypes. Lagriffoul et al. (1998) indicated that Cd accumulation in plants might negatively affect the respiration, photosynthesis and water uptake and reported Cd-induced decreases in leaf area. Sandalio et al. (2001) reported Cd-induced plant growth inhibitions in peas. Tabaldi et al. (2007) showed that metal treatment might affect cell and/or mitochondrial membrane permeability, lysosome membrane stability, protein unfolding and/or precipitation, enzyme inhibition, irreversible conformational changes and mutations in nucleic acids.

Concentrations of heavy metals are relevant in the sub-millimolar or even sub-micromolar range. Thus, direct osmotic effects can be excluded. Some of the effects mentioned here are

common to many heavy metals, such as an influence on membrane transport and an inhibition of root growth and enzyme activities. Early effects may be weakly or strongly connected with water relations. It is relevant to focus here solely on the role of stomata and possible effects of heavy metals. In contrast to earlier results, it is now assumed that the primary effects of heavy metals in whole plants are not directly connected with the induction of stomata closure, but that early effects in roots (e.g. disturbed nutrient absorbance) may be responsible for changes in transpiration (Poschenrieder and Barcelo, 2004; Appenroth, 2010).

### Conclusions

Heavy metals exert a significant threat on health of environment and plants and consequently on health of humans. Even though plants are selective in ion intake, if the ratio of essential nutrients increases, they may uptake some heavy metals through passive means. In other words, plants may uptake the elements in their surroundings even in small quantities regardless of the essentiality of the element for plant growth. Mostly the accumulated elements are up taken by the plants. If the heavy metals accumulated in soil are in the form of free ions, they can both trans-located into plants and ground waters. Thus, they can easily spoil up the water quality, may destroy microorganisms and eventually get into food chain and create threats on human and animal health. The toxicity levels of heavy metals may vary based on concentration, form of existence and type of species they affect.

Application of copper, zinc, lead and cadmium to soil by irrigation for a short period of time did not influence soil properties. The reason for that is the buffering nature of soil. In other words, applied heavy metals can be tolerated by soil for a short period. The situation in plants is quite different from soil. Exposure of all the genotypes to heavy metals above threshold levels resulted in changes in some morphological characters. The effects were more apparent in plants with low salt tolerance. The genotypes with salt tolerance possess better defense mechanisms against stress and therefore were less affected by the applications with threshold levels. The threshold value for eggplant should be taken into account when irrigation water with copper, zinc, lead and cadmium is used.

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