

SOIL SALINITY INCREASE Cd UPTAKE OF LETTUCE (*LATTUCA SATIVA* L.)

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Abstract: Soil salinity has been reported to stimulate to Cd uptake of plants, particularly when Cd bioavailability is poor. The present study assessed the effects of two salinity levels of irrigation water (0, 50 mM NaCl) on Cd availability and uptake of different lettuce varieties under growth three levels of Cd (0, 1.5 and 3.0 mg Cd kg⁻¹) pollution level at greenhouse condition. Sixty days after planting, the shoots were harvested, and Cd, Cl⁻ and mineral concentrations of plant were determined. Cadmium application without NaCl treatment positively affected plant dry matter, and the highest yield increase ratio was obtained from Chinese cabbage at 3 mg Cd kg⁻¹ pollution without NaCl salinity but salinity treatment without Cd pollution negatively affected growth and mineral content of lettuce. Increasing levels of NaCl caused increases in the phyto-availability of Cd, as indicated by a significant increase in the leaf concentration of Cd. Plant leaf Cd concentrations were significantly correlated with soil and leaf Cl⁻ concentration. Increasing levels of NaCl under Cd pollution caused increases in the phyto-availability of Cd, Ca, Na, Mn, and Zn mineral content in leaves of lettuce plants except for K, Fe and Cu content. It can be concluded from the study that salinity treatment can increase Cd in the phyto-availability of Cd, so grower must take into consideration Cd level in the soil under salinity stress condition.

Key words: Cadmium, lettuce, mineral content, phyto-availability, soil salinity,

1. INTRODUCTION

Cadmium (Cd) is a non-essential nutrient for plants and is present naturally at various levels in the Earth's crust. Cd tend to accumulate in soils due to industry, agriculture, extensive mining, and military operations and these sources have led to the accelerated release of metals into the ecosystem, causing serious environmental problems and posing health risks to plants and animals, including humans. The mobility and bioavailability, and hence, the potential toxicity of a heavy metal depends upon variety of factors, such as soil pH, type and amount of clay, organic matter and calcium carbonate soil content, and total soil metal content (Hinesly et al., 1982; McLean & Bledsoe 1992; Rieuwerts et al., 1998; Andersen et al., 2002; Adams et al., 2004).

The mechanism of the Cl effect on Cd uptake and accumulation in plants is not well understood. Previously, it has been shown that Cd adsorption to soil constituents is reduced by application of Cl, and this

effect was ascribed to the formation of soluble Cd-Cl complexes (Bingham et al., 1984). Generally, the presence of complexing ligands in soil solution may increase metal retention or greatly increase metal mobility (McLean & Bledsoe, 1992). The common inorganic ligands, complexed with heavy metals, are SO₄⁻², Cl⁻, PO₄⁻³, NO₃⁻, and CO₃⁻² (Scheffer & Schachtschabel, 2002). Among these ligands, chlorides are highly mobile and therefore, under certain conditions, they could be an important factor in the distribution of heavy metals among binding fractions. Therefore, in many arid regions, saline irrigation water containing high level of Cl⁻ might aggravate heavy metal pollution problems, as a result of heavy metals mobilization due to the formation of heavy metals-chloride complexes. Among all heavy metals, Cd can readily form stable complexes with chloride ligands that will affect its adsorption reactions with the soil surface, thereby its mobility in soils and availability to plants (Doner, 1978; McLean & Bledsoe 1992; Norvell et al., 2000).

Table 1. The soil physical and chemical properties used in the experiment.

Soil Properties	Sand	Silt	Clay	N	O.M	CaCO ₃	pH	EC	C
	(%)						(1:2.5 W/S)	(dS m ⁻¹)	g 100g ⁻¹
Mean	20.70	35.90	43.40	0.16	2.25	0.94	6.29	1.79	1.33
Soil Properties	Ca	Mg	K	Na	P	Fe	Zn	Cu	Mn
	(cmol kg ⁻¹)				(kg P ₂ O ₅ ha ⁻¹)	(mg kg ⁻¹)			
Mean	24.90	2.30	0.54	0.12	24.2	1.0	1.8	0.2	3.0

In a study, Smolders & McLaughlin (1996) showed that strengthening the argument that CdCl²⁻ⁿ species play some role in Cd uptake by plants. It is widely believed that chlorocomplexation of Cd increases solubility of Cd in soil with concomitant increases in Cd uptake by roots either as a result of improved Cd transport to roots, improved Cd transport through the root apoplast to sites of Cd uptake, or direct uptake of Cd in form of CdCl²⁻ⁿ species. Several authors have also found that an increase in Cl⁻ concentration in the soil or soil solution resulted in increased Cd availability to plants (Bingham et al., 1983; McLaughlin et al., 1994, 1997; Smolders et al., 1998; Weggler-Beaton et al., 2000; Weggler et al., 2004). The objective of this work, therefore, was to examine the effect of NaCl salinity on the availability and uptake of Cd for different lettuce varieties.

2. MATERIALS AND METHODS

2.1. Plant materials and growing conditions

The study was conducted under greenhouse conditions at The Ordu University, in Turkey, during 2010. Six lettuce varieties (*Lactuca sativa* L. cv Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsaşı) plants were maintained under natural light conditions, a day/night temperature of approximate 27/16°C and 70% relative humidity during the span of the experiment.

2.2. Plant growth

Lettuce seeds were sown into free draining pots filled with 2.0 kg soil (Table 2). Nutrient solution containing N, P, K (20:20:20) was applied into the pots at 15 days intervals. Three seeds were sown initially and after emergence one was left for each pot. Relative humidity and temperature of the greenhouse was recorded using Testo 175-H2 (Testo Electronic, Turkey) data logger. Volumetric water content of the soil was determined daily using a TDR ($r^2 = 0.899$) (Fieldsout TDR-300, Spectrum Technologies Inc., Plainfield, IL). Irrigation water was applied according

to the values gained from TDR readings and available water rates. The EC (electrical conductivity) values (dS/m) of soil samples from the lettuce growing medium at 15 days intervals was examined to determine the effect of the salinity (Fig. 1). All pots were randomized on the benches in the greenhouse. There were three replicates per treatment, 54 pots in total.

2.3. Cadmium and NaCl treatments

Each soil was treated with 0, 1.5 and 3.0mg kg⁻¹ (CdSO₄)₃ 8H₂O. Salinity treatments were established by adding 0, and 50 mM of NaCl to the tap water. The electrical conductivities of these solutions after adding 0, and 50 mM of NaCl were determined with a conductivity meter, Model 470 (Jenway Limited). Salinity treatments were applied 10 days after emergence.

2.4. Growth parameters

Sixty days after sowing, data on plant growth variables, such as head height and head weight were collected.

2.5. Soil and plant analysis

Soil samples were air-dried, crushed, and passed through a 2-mm sieve prior to chemical analysis. Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0) according to Sumner & Miller (1996). The Kjeldahl method (Bremner, 1996) was used to determine total N while plant-available P was determined by using the sodium bicarbonate method of Olsen & Sommers (1982). Electrical conductivity (EC) was measured in saturation extracts according to Rhoades (1996). Soil pH was determined in 1:2 extracts, and calcium carbonate concentrations were determined according to Mclean (1982) and Nelson (1982). Soil organic matter was determined using the Smith-Weldon method according to Nelson & Sommers (1982).

Table 2 Macro nutrient content of lettuce plants in response to different PGPR treatments under salt stress. Vertical bar indicate the mean \pm SE.

NaCl doses	0 mg kg ⁻¹			3000 mg kg ⁻¹		
Cd doses	0 mg kg ⁻¹	1.5 mg kg ⁻¹	3.0 mg kg ⁻¹	0 mg kg ⁻¹	1.5 mg kg ⁻¹	3.0 mg kg ⁻¹
K %						
Robinson	5.92 \pm 0.62b	6.33 \pm 0.90a	6.31 \pm 0.53a	5.63 \pm 0.42a	4.94 \pm 0.44b	5.35 \pm 0.80a
Funly	6.65 \pm 0.38a	5.36 \pm 0.85b	5.87 \pm 0.10b	4.99 \pm 0.72b	6.18 \pm 0.90a	5.97 \pm 0.37a
Çin marulu	2.98 \pm 0.20c	3.31 \pm 0.16c	3.05 \pm 0.03c	3.51 \pm 0.28c	3.70 \pm 0.45c	3.49 \pm 0.48b
Kasam	6.35 \pm 0.50a	6.19 \pm 0.51a	6.32 \pm 0.54a	4.77 \pm 1.44b	5.11 \pm 0.32ab	5.62 \pm 0.22a
Mangano	6.06 \pm 1.10ab	5.85 \pm 0.26b	6.00 \pm 0.39ab	4.60 \pm 0.66b	3.72 \pm 0.44c	4.10 \pm 0.46b
Arapsacı	6.35 \pm 0.16a	6.38 \pm 0.53a	6.68 \pm 0.16a	5.56 \pm 1.24a	5.08 \pm 0.19ab	4.88 \pm 0.70b
Ca %						
Robinson	1.64 \pm 0.28a	1.04 \pm 0.15c	1.62 \pm 0.30b	2.09 \pm 0.25c	2.14 \pm 0.15c	2.22 \pm 0.14b
Funly	1.76 \pm 0.27a	1.98 \pm 0.31a	1.96 \pm 0.23a	2.93 \pm 0.12a	2.99 \pm 0.58a	2.95 \pm 0.50a
Çin marulu	1.77 \pm 0.58a	1.97 \pm 0.41a	1.61 \pm 0.32b	2.12 \pm 0.12b	1.97 \pm 0.44d	1.77 \pm 0.30c
Kasam	1.17 \pm 0.05b	1.38 \pm 0.05b	1.35 \pm 0.01c	2.56 \pm 0.04ab	2.56 \pm 0.18b	2.17 \pm 0.37b
Mangano	1.07 \pm 0.16b	1.23 \pm 0.15b	1.28 \pm 0.18c	2.83 \pm 0.43a	2.34 \pm 0.13b	2.89 \pm 0.41a
Arapsacı	1.25 \pm 0.10b	1.17 \pm 0.15c	1.27 \pm 0.14c	2.73 \pm 0.55a	2.39 \pm 0.33b	2.47 \pm 0.16a
Na %						
Robinson	0.27 \pm 0.03a	0.16 \pm 0.02b	0.20 \pm 0.01a	0.82 \pm 0.03a	0.84 \pm 0.02ab	0.84 \pm 0.03b
Funly	0.33 \pm 0.02a	0.20 \pm 0.01a	0.20 \pm 0.02a	0.86 \pm 0.04a	0.96 \pm 0.03a	0.96 \pm 0.02a
Çin marulu	0.07 \pm 0.01c	0.11 \pm 0.02c	0.08 \pm 0.02c	0.61 \pm 0.02b	0.62 \pm 0.03c	0.55 \pm 0.03c
Kasam	0.24 \pm 0.01b	0.17 \pm 0.03b	0.22 \pm 0.02a	0.86 \pm 0.04a	0.87 \pm 0.02ab	0.88 \pm 0.02b
Mangano	0.16 \pm 0.02b	0.20 \pm 0.03a	0.20 \pm 0.01a	0.85 \pm 0.03a	0.83 \pm 0.02ab	0.89 \pm 0.03ab
Arapsacı	0.18 \pm 0.02b	0.13 \pm 0.02c	0.17 \pm 0.02b	0.83 \pm 0.03a	0.79 \pm 0.04b	0.87 \pm 0.02b
Zn mg kg ⁻¹						
Robinson	45.7 \pm 7.7b	62.2 \pm 4.1a	55.3 \pm 1.9c	69.5 \pm 4.1b	59.3 \pm 4.3c	66.9 \pm 4.2d
Funly	61.4 \pm 4.8a	52.5 \pm 3.9b	62.8 \pm 1.8b	58.1 \pm 4.2c	85.0 \pm 5.4a	91.0 \pm 5.1a
Çin marulu	19.7 \pm 4.3c	23.0 \pm 3.8c	20.4 \pm 3.1d	22.8 \pm 2.4d	27.5 \pm 3.1d	23.8 \pm 5.3e
Kasam	61.9 \pm 5.3a	68.6 \pm 3.2a	66.7 \pm 3.2b	54.3 \pm 6.0c	73.9 \pm 6.2b	75.5 \pm 6.1c
Mangano	50.3 \pm 5.5b	44.9 \pm 5.2b	50.1 \pm 3.6c	63.4 \pm 4.6b	50.4 \pm 4.8c	56.9 \pm 4.9d
Arapsacı	53.8 \pm 7.0b	61.6 \pm 5.5a	74.3 \pm 1.7a	89.6 \pm 6.5a	86.1 \pm 2.7a	80.4 \pm 5.1b
Mn mg kg ⁻¹						
Robinson	68.0 \pm 3.3a	57.9 \pm 3.7a	59.6 \pm 4.2a	160.3 \pm 7.2b	122.9 \pm 6.0b	131.6 \pm 6.3b
Funly	49.5 \pm 3.4b	57.2 \pm 2.8a	55.2 \pm 3.4ab	133.8 \pm 6.8c	129.3 \pm 6.6b	131.5 \pm 6.7b
Çin marulu	18.3 \pm 2.9c	17.5 \pm 2.9b	15.5 \pm 3.1c	35.5 \pm 6.5d	28.6 \pm 7.1d	27.3 \pm 5.8d
Kasam	62.9 \pm 2.6a	53.5 \pm 3.1a	60.2 \pm 3.2a	123.0 \pm 6.3c	128.5 \pm 6.3b	97.4 \pm 5.7c
Mangano	55.4 \pm 3.1b	50.2 \pm 3.0a	50.5 \pm 3.3b	135.5 \pm 5.0c	96.5 \pm 6.0c	123.2 \pm 6.7b
Arapsacı	61.2 \pm 3.2a	59.2 \pm 2.8a	54.5 \pm 3.6ab	312.2 \pm 5.5a	149.4 \pm 5.9a	174.9 \pm 6.5a
Fe mg kg ⁻¹						
Robinson	288.6 \pm 8.3a	204.3 \pm 6.9ab	244.6 \pm 7.9	248.7 \pm 8.1b	186.4 \pm 5.5b	232.6 \pm 6.0a
Funly	273.7 \pm 6.8a	249.7 \pm 6.0a	260.6 \pm 10.1a	185.4 \pm 7.5c	187.0 \pm 6.0b	201.1 \pm 5.9b
Çin marulu	148.3 \pm 5.9b	210.9 \pm 7.4a	169.5 \pm 12.4c	189.8 \pm 7.8c	160.2 \pm 6.2c	153.3 \pm 5.8c
Kasam	263.0 \pm 7.9a	222.5 \pm 4.7a	219.1 \pm 13.6b	185.1 \pm 7.2c	205.9 \pm 5.9a	214.6 \pm 5.9b
Mangano	187.4 \pm 8.0b	189.3 \pm 5.9b	208.6 \pm 15.3b	170.8 \pm 9.0c	177.0 \pm 6.4b	152.7 \pm 6.0c
Arapsacı	306.7 \pm 10.2a	182.3 \pm 7.2b	227.2 \pm 12.7b	439.9 \pm 8.3a	197.7 \pm 6.1b	216.2 \pm 6.4ab
Cu mg kg ⁻¹						
Robinson	9.9 \pm 0.6b	9.4 \pm 0.8b	10.3 \pm 0.8a	12.9 \pm 1.0a	8.0 \pm 0.3b	9.2 \pm 1.0
Funly	12.1 \pm 0.3a	12.5 \pm 0.7a	13.6 \pm 0.5a	12.5 \pm 1.8a	14.0 \pm 0.5a	12.4 \pm 1.1a
Çin marulu	3.7 \pm 0.6d	4.7 \pm 0.4d	3.3 \pm 0.7d	3.4 \pm 1.3c	3.9 \pm 0.6c	3.0 \pm 0.9d
Kasam	9.3 \pm 0.4b	11.1 \pm 0.9a	8.8 \pm 0.4b	8.6 \pm 1.0b	13.0 \pm 0.7a	9.8 \pm 0.8b
Mangano	7.5 \pm 0.7c	7.7 \pm 0.7c	7.9 \pm 0.8c	8.4 \pm 1.4b	5.7 \pm 0.5c	6.2 \pm 1.1c
Arapsacı	11.9 \pm 0.9a	10.3 \pm 0.7a	11.5 \pm 0.9a	14.1 \pm 0.9a	12.3 \pm 0.4a	9.9 \pm 0.8b

Sodium acetate buffered at pH 8.2 (Rhoades 1982), Ammonium acetate buffered at pH 7 (Thomas 1982) was used to determine exchangeable cations. Micro elements in the soils were determined by Diethylene

Triamine Pentaacetic Acid (DTPA) extraction methods (Lindsay & Norvell, 1978). In order to determine the mineral contents of leaves of lettuce, plant samples were collected from fully expanded

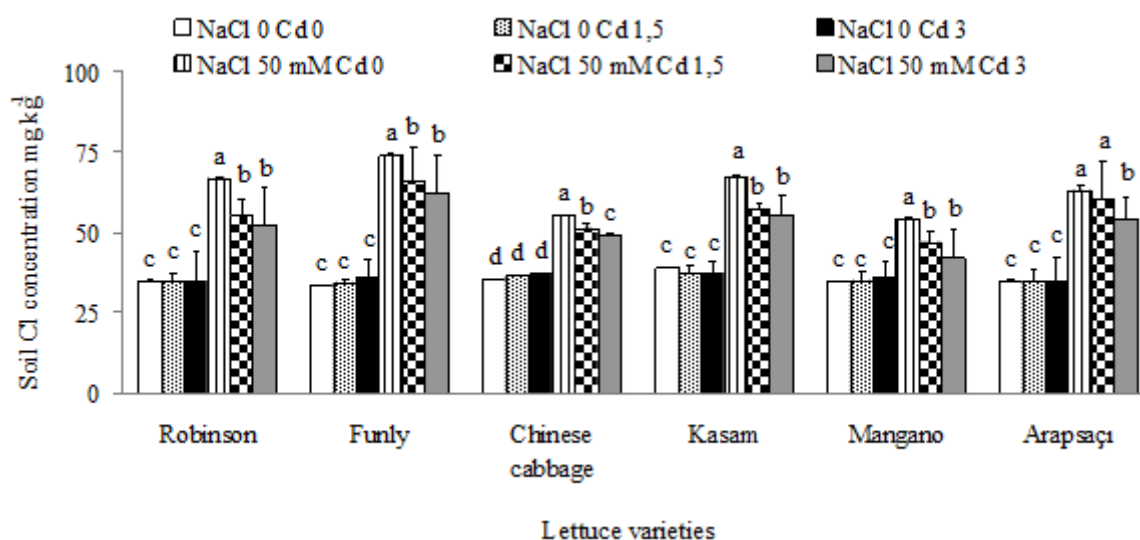


Figure 1. Effects of NaCl (0 and 50 mM) application on soil Cl content Vertical bar indicate the mean \pm SE

leaves at sixty day after sowing, then oven-dried at 68°C for 48 h and ground and passed 1 mm sieve size. K, Ca Mg and Na and micro-elements (Fe, Mn, Zn and Cu) were determined after wet digestion of dried and ground sub-samples using a $\text{HNO}_3\text{-H}_2\text{O}_2$ acid mixture (2:3 v/v) with three step (first step; 145°C, 75%RF, 5 min; second step; 180°C, 90%RF, 10 min and third step; 100°C, 40%RF, 10 min) (Mertens, 2005a). Tissue P, K, S, Ca, Mg, Na, Fe, Mn, Zn, and Cu were determined Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005b). Chlorine (Cl) was determined by titration with silver nitrate (AgNO_3) (Johnson & Ulrich, 1959).

2.6. Statistical Analysis

Each pot was considered as a replicate and all of the treatments were repeated three times. All data were subjected to a two way analysis of variance (ANOVA) and separated by LSD with SPSS 2004. To determine the correlation between leaf Cd concentrations and soil and leaf Cl⁻ concentration, correlation analysis were also done using the SPSS 13.0 software (SPSS Inc., 2004).

3. RESULTS

3.1. Soil salinity and Chlorine (Cl⁻) level

Soil salinity levels were significantly influenced by NaCl application. Soil salinity values of soil increasing with increased NaCl treatment. Electrical conductivities (EC) of the soil solutions were 1.79 dS/cm at the beginning of the study, but these value were 1.82 dS/cm for 0 mM NaCl, and 3.9, 3.3, 3.5, 3.8, 3.4, and 3.7 dS/cm for Robinson,

Funly, Chinese cabbage, Kasam, Mangano, Arapsacı cultivated soil at 50 mM NaCl treatment in the plant harvest period, respectively. Chlorine (Cl⁻) concentration of soil was 36mg kg⁻¹ at the beginning of the study, but these values increased to 55, 66, 57, 47 and 60mg kg⁻¹ for Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı cultivated soil at 50 mM NaCl treatment in the plant harvest period without Cd pollution, respectively (Fig. 1). But these values decreased to 52, 62, 49, 55, 42 and 54mg kg⁻¹ for Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı cultivated soil at 50 mM NaCl with 3mg kg⁻¹ Cd pollution treatment in the plant harvest period, respectively (Fig. 1).

3.2. Effects of NaCl treatments on dry matter of lettuce varieties under growth different Cd pollution level

Increasing levels of NaCl decreased dry matter of lettuce cultivars plant significantly compared to without NaCl treatment, mainly due to osmotic effects on water availability as well as specific toxicity effects of the salt ions. In without NaCl application dry matter of lettuce plants except for Funly and Arapsacı dramatically increased with the Cd pollution, and the highest dry matter yield of was obtained from 3 mg kg⁻¹ Cd pollution levels.

Dry matter yield of Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı were 3.1, 3.1, 3.7, 3.3, 4.5, and 4.9 g plant⁻¹, respectively. This increased to 4.85, 6.93, 4.92 and 7.02 g plant⁻¹ for Robinson, Chinese cabbage, Kasam, and Mangano but decreased to 2.9 and 3.3 g plant⁻¹ for Funly and Arapsacı at 3 mg kg⁻¹ Cd without NaCl, respectively (Fig. 2).

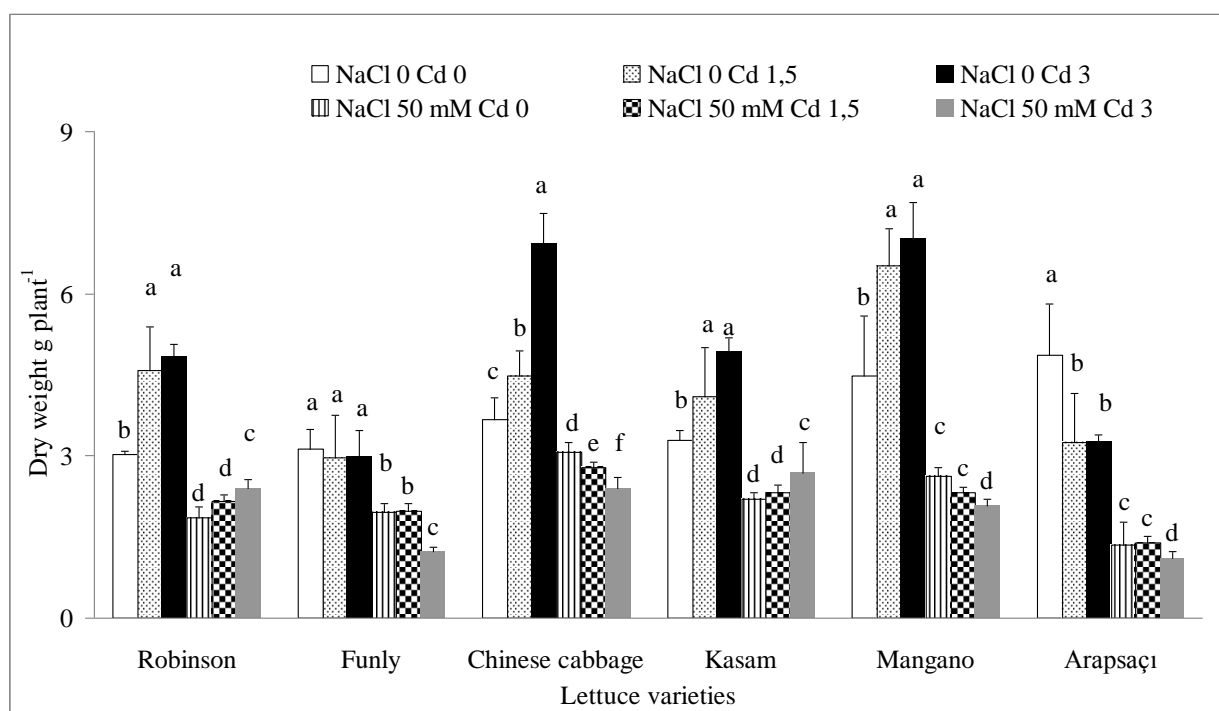


Figure 2. Dry matter yield of lettuce varieties in response to different Cd and NaCl treatments. Vertical bars indicate the mean \pm SE.

The highest yield increasing were obtained from Chinese cabbage (89%), and followed by Robinson (61%) and Mangano (57%) at 3 mg kg⁻¹ Cd pollution, compared to without NaCl treatments and non Cd pollution condition. On the other hand, dry matter yield of lettuce except for Robinson and Kasam was negatively affected with increasing Cd pollution level with 50 mM NaCl treatment application.

At 50mM NaCl without Cd pollution, dry matter yield of Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı were 1.9, 2.0, 6.1, 2.2, 2.6 and 1.3 g plant⁻¹, respectively. This decreased to 1.2, 2.4, 2.0 and 1.1 g plant⁻¹ for Funly, Chinese cabbage, Mangano and Arapsacı at 3mg kg⁻¹ Cd pollution with 50 mM NaCl, but increased to 2.4 and 2.7 g plant⁻¹ for Robinson, and Kasam, respectively (Fig. 2). The highest decrease ratio of dry matter yield were obtained from Funly (37%), and followed by Chinese cabbage (28%), Mangano (20%) and Arapsacı (18%) at 3mg kg⁻¹ Cd pollution and 50 mM NaCl, compared to without Cd pollution.

3.3. Effects of NaCl treatments on Cd and up Cl⁻ take of lettuce under growth different Cd pollution condition

The changes in soil solution Cd level with NaCl treatment applications, and it was reflected in the phyto-availability of Cd. Results showed that

increasing levels of NaCl caused increases in the phyto-availability of Cd, as indicated by a significant increase in the leaf concentration of Cd (Fig. 3A). The highest increase in the availability of Cd to plants was pronounced when the soils treated with the highest Cd pollution level (3mg kg⁻¹) were irrigated with the highest NaCl level (50 mM). Without NaCl application, Cd contents of Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı were 1.2, 1.1, 0.13, 1.1, 0.8 and 1.2mg kg⁻¹, respectively, but increased to 77, 88, 9, 84, 37 and 70mg kg⁻¹ at 3mg kg⁻¹ Cd pollution with 50 mM NaCl treatment, respectively (Fig. 3A).

Chlorine concentrations of lettuce varieties were also increased with increase NaCl application. In without NaCl and non Cd pollution treatment, Cl⁻ contents of Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı were 13.3, 12.9, 13.7, 12.7, 13.0, and 11.0mg kg⁻¹, respectively. These figures increased to 23.9, 22.9, 22.7, 22.0, 21.0, and 22.0mg kg⁻¹ for Robinson, Funly, Chinese cabbage, Kasam, Mangano, Arapsacı at 3mg kg⁻¹ Cd pollution with 50 mM NaCl, respectively (Fig. 3B). Plant leaf Cd concentrations were significantly correlated with soil and leaf Cl⁻ concentration (Figs. 4 and 5). Increasing levels of NaCl caused increases in the phyto-availability of Cd, as indicated by a significant increase in the leaf Cd concentration.

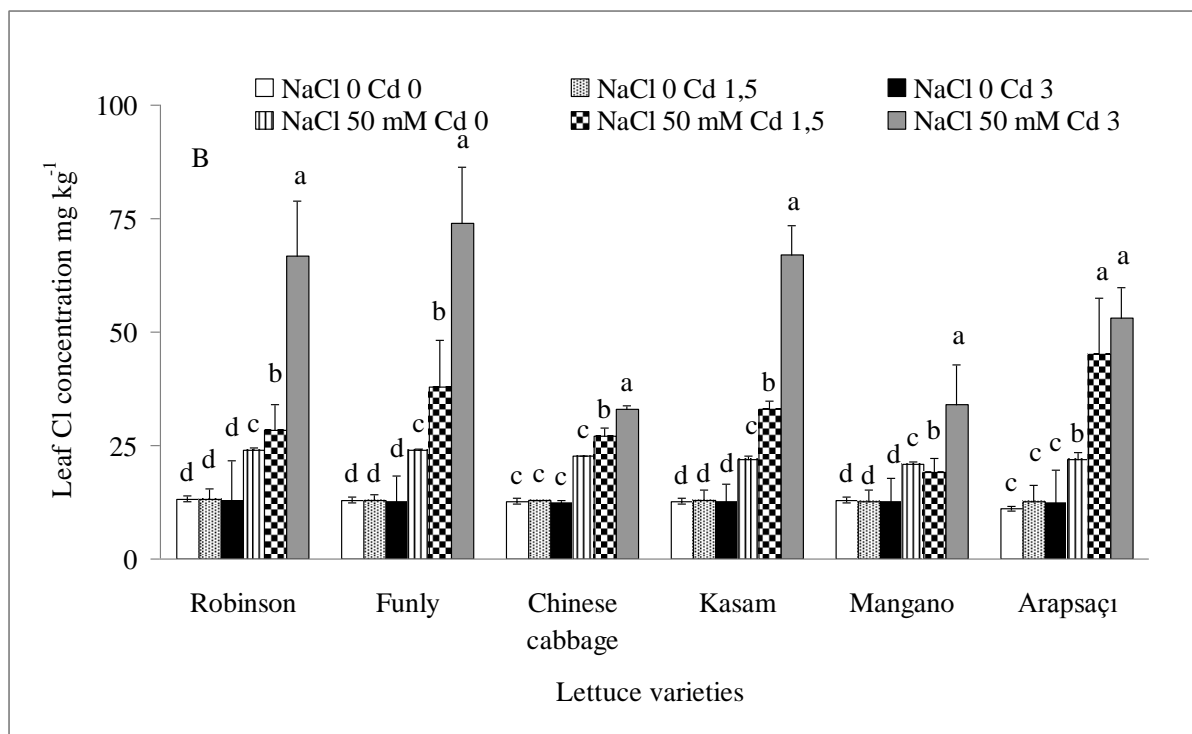
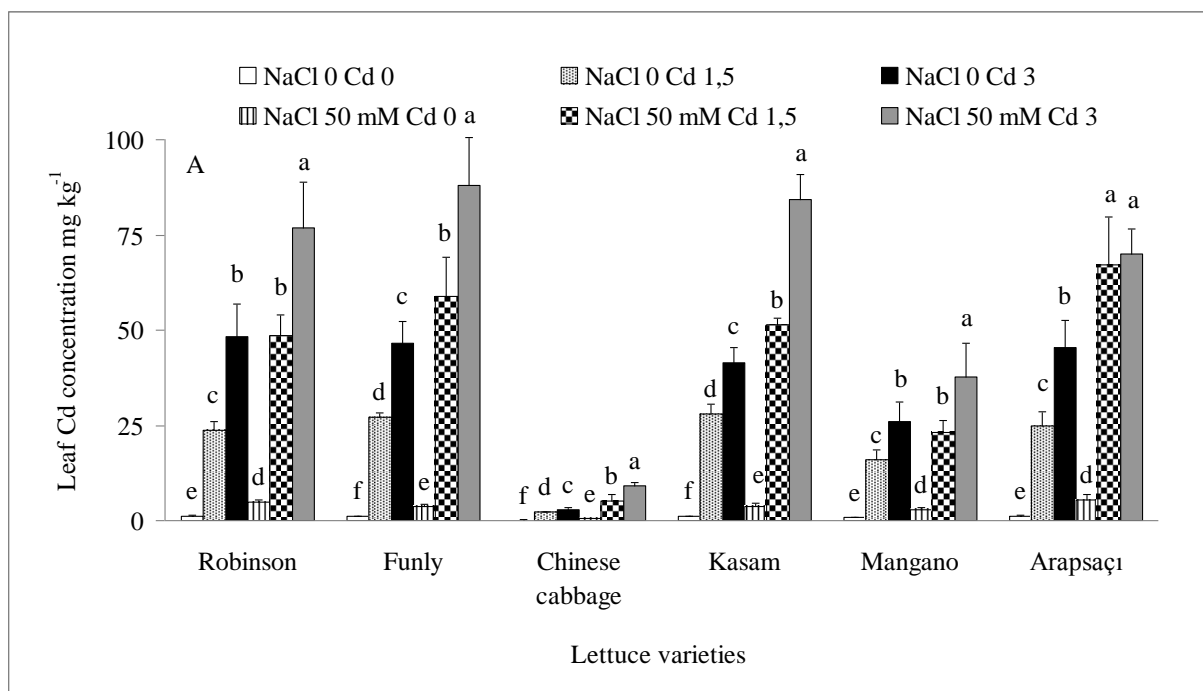


Figure 3. Effects of different salinity levels on Cd (Fig.A) and Cl (Fig.B) concentration of lettuce varieties

3.4. Effects of NaCl treatments on nutrient element content of lettuce under growth different Cd pollution condition

The concentrations of K, Ca, Na, Fe, Mn, Zn, Cu and Cd and Cl content in lettuce in response to NaCl treatments under different Cd pollution levels are shown in table 2 and figures 2 and 3. The results showed that salinity and Cd pollution significantly

affected ionic compositions of leaves (Table 2).

In this study, external NaCl salinity application to 50 mM under 3mg kg⁻¹ Cd pollution condition increased the Ca, Na, Mn, and Zn mineral content in leaves of lettuce plants except K, Fe and Cu content. Salt stress resulted in an increase of Ca, Na, Mn, and Zn in leaves of lettuce. Plants treated with Cd generally gave greater mineral content than the non-treated plants under both NaCl treatment

and absence of NaCl treatments. Cd pollution significantly decreased the K, Fe and Cu content in lettuce. The maximum values for Ca, Na, Mn, and Zn mineral contents were obtained with 50 mM NaCl and 1.5 mg kg⁻¹ Cd pollution level from Funly varieties and followed by Robinson (Table 2).

4. DISCUSSION

Soil EC and Cl content were significantly increased by increasing NaCl levels. As expected, Na⁺ and Cl⁻ concentrations increased proportionally

with increased NaCl levels. Moreover, the irrigation with saline water caused a significant increase of solubility soil Cd and plant uptake. The significant increases in the plant Cd concentration could be related to the displacement of Cd from soil exchange sites by Na of the saline irrigation water, and partly to the salt-induced pH drop as well as to the formation of soluble ligand complexes of these elements (Khattak et al., 1989; Krishnamurti & Huang, 1992; Ghallab et al., 2007).

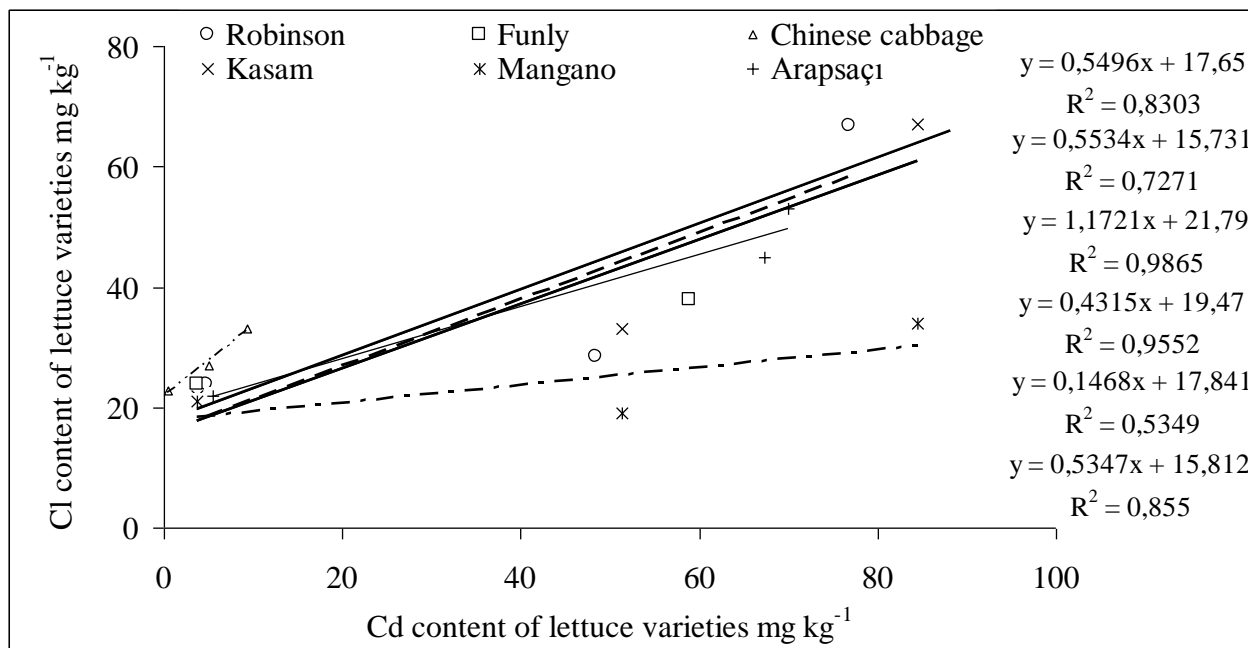


Figure 4. Relationship between leaf Cd and Cl⁻ content of lettuce varieties in response to different Cd and NaCl treatments

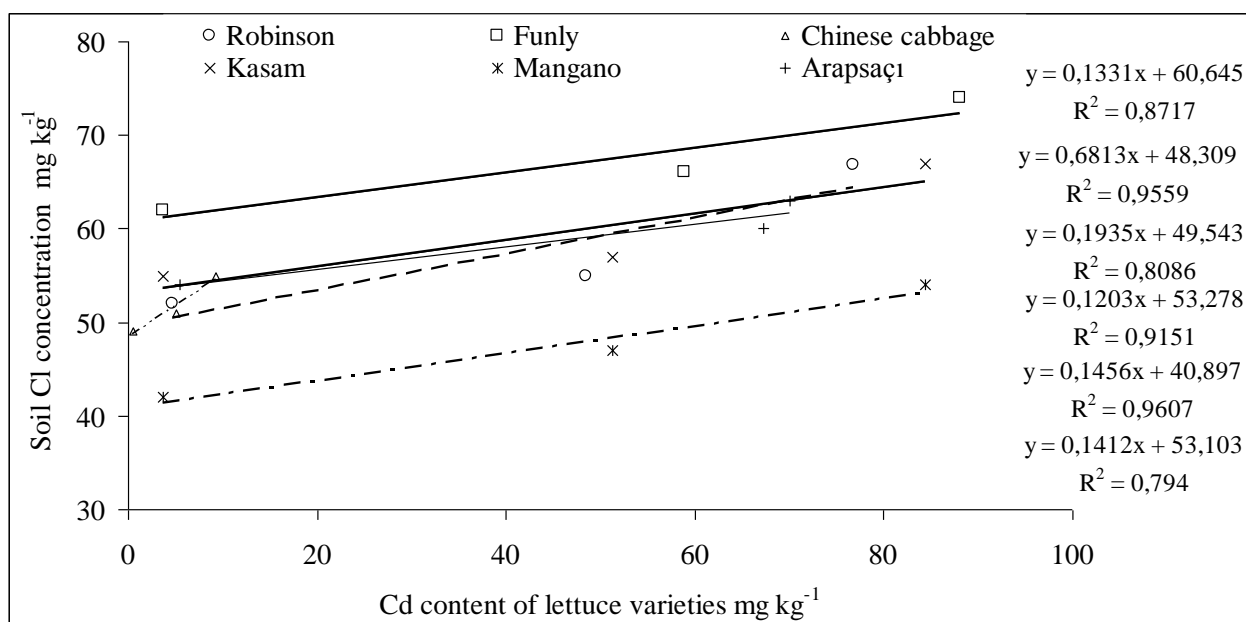


Figure 5. Relationship between leaf Cd content of lettuce varieties and soil Cl⁻ content in response to different Cd and NaCl treatments

High level salinity stress caused to decreasing dry matter of lettuce cultivars plant compared to without NaCl. On the other hand dry matter of lettuce plants dramatically increased with the Cd pollution, and the highest dry matter yield of were obtained from 3 mg kg⁻¹ Cd pollution doses without NaCl application. However, dry matter of lettuce significantly decreased at 50mM NaCl application under 3mg kg⁻¹ Cd pollution level. These results were related to plant Cd and Cl levels, the highest Cd levels of all the lettuce cultivar were obtained from 50mM NaCl treatment under Cd pollution condition. Our findings are concordant with sunflower (*Heliantus annus* L.), kenaf (*Hibiscuscannabinus*), sorghum (*sorgum vulgare*), wheat (*Triticum turgidum* L.), radish (*Raphanus sativus* L.) physalis (*Physalis ixocarpa*), broccoli (*Brassica oleracea* L., var. italica), and strawberry (*Fragaria×ananassa*) plants are relatively sensitive to salt stress (Hattori et al., 2006; Özkutlu et al., 2007; Yıldırım et al., 2008; Estringü et al., 2011, Yıldırım et al., 2011, Karlıdağ 2011a and 2011b) and it has been shown to reduce leaf number, leaf area, shoot dry weight and number of crowns and yield of plants. Reduction in the plant growth has been attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of the ionic imbalance and decrease in many metabolic activities (Kumar et al., 2005).

The present study showed that increasing levels of NaCl caused increases in the phyto-availability of Cd, as indicated by a significant increase in the leaf concentration and the uptake of Cd. The highest increase in the availability of Cd to plants was pronounced when the soils treated with the highest Cd pollution level (3mg kg⁻¹) were irrigated with the highest NaCl level (50mM). In the present study also show that Cd concentrations of lettuce varieties were significantly correlated with soil and leaf Cl⁻ concentration (Figs. 4 and 5). Increasing levels of NaCl caused increases in the phyto-availability of Cd, as indicated by a significant increase in the leaf Cd concentration. Cadmium uptake by plants may be further increased by application of large amount of Cl⁻ to the soil. Our findings are concordant with Bingham et al., (1983) and Norvell et al., (2000) suggest that mobilization of Cd by NaCl salinity can be explained by the formation of soluble Cl⁻ complexes of Cd and formation of complexes with chloride tends to shift Cd from the solid to the solution phase, thereby enhancing solubility and mobility of Cd. Sodium may also be implicated in enhancing Cd solubility due to Na in irrigation water displacing Cd from soil exchange sites into soil solution. Many studies, however, demonstrated that the effect of NaCl on increase of Cd solubility was mainly due to the formation of CdCl_n²⁻ⁿ

complexes and not to ion exchange of Na for Cd on soil surfaces (Smolders et al., 1998; Khoshgoftar et al., 2004), because monovalent cations such as Na are relatively inactive at displacing cations such as Cd, even at relatively high concentration of Na. The stimulatory effect of on Cl⁻ increasing Cd availability to plants has been found in a number of glasshouse experiments using soil as the growth medium (Smolders et al., 1998; Weggler-Beaton et al., 2000; Weggler et al., 2004; Usman et al., 2005; Khoshgoftar et al., 2004, 2006). The effect of NaCl salinity on increasing the phyto-availability of Cd is due to the enhanced solubility of Cd in soils through the formation of soluble Cl⁻ complexes of Cd. These complexes are less strongly sorbed to the soil than the free Cd²⁺ ion and hence, increasing the mobility of Cd to the soil-root interface (Weggler-Beaton et al., 2000). Other explanations are related to the fact that CdCl₂ complexes in the soil solution are also available for plant uptake as well as osmotic stress effects on root functions (Smolders et al., 1998; Weggler-Beaton et al., 2000; Khoshgoftar et al., 2004). Although uncomplexed free metal ion in soil solution is considered as the most available form taken up by the plant, there is increasing evidence that also some metal complexes, such as chloro complexes, can be absorbed by the plant root, although at a lower efficiency (Smolders & McLaughlin, 1996).

External NaCl application under Cd pollution condition increased the Ca, Na, Mn, and Zn mineral content in leaves of lettuce plants except K, Fe and Cu content regardless of treatments (Table 2). Mineral contents of the leaves of the lettuce plants except Ca, Na, Mn, and Zn drastically decreased under salt stress. Salt stress raised the Ca, Na, Mn, and Zn content regardless of the treatments. NaCl salinity not only reduces K contents of plant leaves, but reduces Fe and Cu transport and mobility to growing regions of the plants (Grattan & Grieve, 1999). It has been shown that high concentrations of NaCl in the soil solution may disorder nutrient-ion activities, causing the plants to be susceptible to osmotic and specific-ion injury as well as to nutritional disorders that result in reduced yield and quality (Grattan & Grieve, 1999; Essa, 2002).

5. CONCLUSION

The results indicated that sodium chloride salinity induced changes in the solubility and speciation of Cd in the soil solution. The irrigation with saline water resulted in an increase in the availability of Cd for lettuce plants as a result of increasing the total concentration of Cd in the soil solution as well as the high affinity of Cd to form Cd-chloro complexes. The

results suggest that concentration Cl^- could be considered as an important factor in determining the phyto-available Cd in the soil. Therefore, a good management of Cl^- should be taken into account if Cl^- levels are elevated in the soil and/or in the irrigation water. Cd concentrations were significantly correlated with soil and leaf Cl^- concentration. Cadmium uptake by plants may be further increased by the application of a larger amount of Cl^- to the soil. It might be possible to increase the amount of Cd absorbed by plants by applying Cl^- and adjusting the soil pH, as well as shortening the time required for plants to clean up Cd contaminated soil.

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REFERENCES

- Adams, M. L., Zhao, F. J., McGrath, S. P., Nicholson, F. A. & Chambers, B. J., 2004. Predicting cadmium concentrations in wheat and barley grain using soil properties. *Journal of Environmental Quality*, 33, 532–541.
- Andersen, M. K., Refsgaard, A., Raulund-Rasmussen, K., Strobel, B. W. & Hansen, H. C. B., 2002. Content, distribution, and solubility of Cd in Arable and forest soils. *Soil Sci. Soc. Am. J.* 66, 1829–1835.
- Bingham, F. T., Strong, J. E. & Sposito, G., 1983. Influence of chloride salinity on cadmium uptake by Swiss chard. *Soil Science*, 135, 160–165.
- Bremner, J.M., 1996. Nitrogen-total. pp1085–1121. *Methods of soil analysis*. Part III. Chemical Methods (Bartels J. M. and Bigham, J. M. eds). 3rd Ed., ASA SSSA Publisher, Agronomy. No: 5 Madison, WI, USA
- Doner, H. E., 1978. Chloride as a factor in motilities of Ni(II), Cu(II), and Cd(II) in soil. *Soil Science Society of American Journal*, 42, 882–885.
- Essa, T.A., 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. *Journal of Agronomy and Crop Science*, 188, 86–93.
- Esringü, A., Kant, C., Yildirim, E., Karlidag, E. & Turan, M., 2011. Ameliorative effect of foliar nutrient supply on growth, inorganic ions, membrane permeability and leaf relative water content of *Physalis* (*Physalis ixocarpa*) plants under salinity stress. *Communications in Soil Science and Plant Analysis*, 42, 408–423.
- Ghallab, A., Rabie, A. & Usman, A., 2007. Effect of Sodium Chloride-induced Salinity on Phyto-availability and Speciation of Cd in Soil Solution. *Water, Air and Soil Pollution*, 185, 43–51.
- Grattan S.R. & Grieve, C.M., 1999. Salinity-mineral nutrient relations in horticultural crops. *Scientia Horticulturae*, 78, 127–157.
- Hinesly, T.D., Redborg, K.E., Ziegler, E.L. & Alexander, J.D., 1982. Effect of soil cation exchange capacity on the uptake of cadmium by corn. *Soil Science Society of America Journal*, 46, 490–497.
- Hattori, H., Kuniyasu, K., Chiba, K. & Chino, M., 2006. Effect of chloride application and low soil pH on cadmium uptake from soil by plants. *Soil Science and Plant Nutrition*, 52, 89–94.
- Johnson, C. M. & Ulrich, A., 1959. Analytical methods for use in plant analysis. In: *California Agricultural Experiment Station Bulletin*, Vol. 766, pp. 26–78. Berkeley, CA: University of California.
- Karlidag, H., Yildirim, E. & Turan, M., 2011a. Role of 24-epibrassinolide in mitigating the adverse effects of salt stress on stomatal conductance, membrane permeability, and leaf water content, ionic composition in salt stressed strawberry (*Fragaria x ananassa*). *Scientia Horticulturae*, 130, 133–140.
- Karlidag, H., Esitken, A., Yildirim, E., Donmez, M.F. & Turan, M., 2011b. Effects Of Plant Growth Promoting Bacteria (PGPB) On Yield, 5 Growth, Leaf Water Content, Membrane Permeability And Ionic 6 Composition Of Strawberry Under Saline Conditions. *Journal of Plant Nutrition*, 34, 34–45.
- Khattak, R. A., Jarrell, W. M. & Page, A. L., 1989. Mechanism of native manganese release in salt-treated soils. *Soil Science Society of American Journal*, 53, 701–705.
- Khoshgoftar, A. H., Shariatmadari, H., Karimian, N., Kalbasi, M., S.E.A.T.M. van der Zee, & Parker, D.R., 2004. Salinity and zinc application effects on phytoavailability of cadmium and zinc. *Soil Science Society of American Journal*, 68, 1885–1889.
- Khoshgoftar, A. H., Shariatmadari, H., Karimian, N., Kalbasi, M. & S.E.A.T.M., van der Zee, 2006. Cadmium and zinc in saline soil solutions and their concentrations in wheat. *Soil Science Society of American Journal*, 70, 582–589.
- Krishnamurti, G. S. R. & Huang, P.M., 1992. Dynamics of potassium chloride induced manganese release in different soil orders. *Soil Science Society of American Journal*, 56, 1115–1123.
- Kumar, R., Goyal, V. & Kuhad, M.S., 2005. Influence of fertility–salinity interactions on growth, water status and yield of Indian mustard (*Brassica juncea*). *Indian Journal of Plant Physiology* 10, 139–144.
- Lindsay, W.L. & Norvell, W.A. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of American Journal*, 42, 421–428.
- McLaughlin, M. J., Palmer, L. T., Tiller, K. G., Beech, T. A. & Smart, M. K., 1994. Increased soil salinity causes elevated cadmium concentrations in field-grown potato tubers. *Journal of Environmental Quality*, 23, 1013–1018.
- McLaughlin, M. J., Tiller, K. G. & Smart, M. K., 1997. Speciation of cadmium in soil solution of saline/sodic soils and relationship with cadmium concentrations

- in potato tubers (Solanum tuberosum L.)*. Australian Journal of Soil Research, 35, 183–198.
- McLean, J. E. & Bledsoe, B. E.**, 1992. *Behaviour of metals in soils* (EPA Ground Water Issue, EPA 540-S-92-018:25 pp). Washington, USA: Environmental Protection Agency.
- McLean, E.O.**, 1982. *Soil pH and lime requirement*, p.199-224. Methods of soil analysis. Part II. Chemical and microbiological properties (Page, A.L., R.H. Miller and D.R. Keeney eds). 2nd Ed., ASA SSSA Publisher, Agronomy. No: 9 Madison, WI, USA.
- Mertens, D.**, 2005a. *AOAC Official Method 922.02*. Plants Preparation of Laboratory Sample. Official Methods of Analysis, 18th edn. Horwitz, W., and G.W. Latimer, (Eds). Chapter 3, pp1-2, AOAC-International Suite 500, 481. North Frederick Avenue, Gaithersburg, Maryland 20877-2417, USA.
- Mertens, D.**, 2005b. *AOAC Official Method 975.03*. Metal in Plants and Pet Foods. Official Methods of Analysis, 18th edn. Horwitz, W., and G.W. Latimer, (Eds). Chapter 3, pp 3-4, AOAC-International Suite 500, 481. North Frederick Avenue, Gaithersburg, Maryland 20877-2417, USA.
- Nelson, R.E.**, 1982. *Carbonate and Gypsum*. Methods of soil analysis. Part II. Chemical and microbiological properties (Page, A.L., R.H. Miller and D.R. Keeney eds). 2nd Ed., ASA SSSA Publisher, Agronomy. No: 9 Madison, WI, USA p.181-196.
- Nelson, D.W. & Sommers, L.E.**, 1982. *Organic Matter*, Methods of soil analysis. Part II. Chemical and microbiological properties (Page, A.L., R.H. Miller and D.R. Keeney eds.). 2nd Ed., ASA SSSA Publisher, Agronomy. No: 9 Madison, WI, USA. p.574-579.
- Norvell, W. A., Wu, J., Hopkins, D. G. & Welch, R. M.**, 2000. *Association of cadmium in durum wheat grain with soil chloride and chelate-extractable soil cadmium*. Soil Science Society of American Journal, 64, 2162–2168.
- Olsen, S.R. & Sommers, L.E.**, 1982. *Phosphorus*, p.403-427. Methods of soil analysis. Part II. Chemical and microbiological properties (Page, A.L., R.H. Miller and D.R. Keeney eds.). 2nd Ed., ASA SSSA Publisher, Agronomy. No: 9 Madison, WI, USA.
- Özkutlu, F., Öztürk, L., Erdem, H., McLaughlin, M. & Çakmak, I.**, 2007. *Leaf-applied sodium chloride promotes cadmium accumulation in durum wheat grain*. Plant and Soil, 290, 323-331.
- Rhoades, J.D.**, 1982. *Cation Exchange Capacity*, p.149-157. Methods of soil analysis. Part II. Chemical and microbiological properties (Page, A.L., R.H. Miller and D.R. Keeney eds.). 2nd Ed., ASA SSSA Publisher, Agronomy. No: 9 Madison, WI, USA.
- Rhoades, J.D.**, 1996. *Salinity*: Electrical conductivity and total dissolved solids, p 417-436. In: Methods of soil analysis. Part III. Chemical Methods (Bartels, J.M., and J.M. Bigham eds.) 2nd Ed. ASA SSSA Publisher Agronomy. No: 5 Madison WI, USA.
- Rieuwerts, J.S., Thornton, I., Farago, M.E. & Ashmore, M.R.**, 1998. *Factors influencing metal bioavailability in soils: Preliminary investigations for the development of a critical loads approach for metals*. Chemical Speciation Bioavailability, 10, 61–75.
- Scheffer, F. & Schachtschabel, P.**, 2002. *Lehrbuch der Bodenkunde*. 15. Auflage. Berlin: Spektrum Akademischer Verlag Heidelberg.
- Smolders, E. & McLaughlin, M. J.**, 1996. *Chloride increases cadmium uptake in Swiss chard in a resin-buffered nutrient solution*. Soil Science Society of American Journal, 60, 1443–1447.
- Smolders, E., Lambergts, R.M., McLaughlin, M.J. & Tiller, K.G.**, 1998. *Effect of soil solution chloride on cadmium availability to Swiss chard*. Journal of Environmental Quality, 27, 426–431.
- SPSS Inc.** 2004. *SPSS Inc. SPSS® 13.0 Base User's Guide*, Prentice Hall.
- Sumner, M.E & Miller, W.P.**, 1996. *Cation Exchange Capacity and Exchange Coefficients*. In: Methods of Soil Analysis. Part III. Chemical Methods. 2nd Edition. Agronomy. No:5 Madison, Wisconsin, USA, pp 1201-1230.
- Thomas, G.W.**, 1982. *Exchangeable Capacity*,. Methods of soil analysis. Part II. Chemical and microbiological properties (Page, A.L., R.H. Miller and D.R. Keeney eds.). 2nd Ed., ASA SSSA Publisher, Agronomy. No: 9 Madison, WI, USA. p.159-164.
- Usman, A.R.A., Kuzyakov, Y. & Stahr, K.**, 2005. *Effect of immobilizing substances and salinity on heavy metals availability to wheat grown on sewage sludge-contaminated soil*. Soil and Sediment Contamination, 14, 329–344.
- Yildirim, E., Donmez, M.F. & Turan, M.**, 2008. *Use of bioinoculants in ameliorative effects on radish (Raphanus sativus L.) plants under salinity stress*. Journal of Plant Nutrition, 31, 2059 – 2074.
- Yildirim, E, Karlidag, H., Turan, M., Dursun, A. & Goktepe, F.**, 2011. *Growth, nutrient uptake and yield promotion of broccoli by Plant Growth Promoting Rhizobacteria with manure*. HortScience, 46, 932-936.
- Wegglar, K., McLaughlin, M. J. & Graham, R.D.**, 2004. *Effect of chloride in soil solution on the plant availability of biosolid-borne cadmium*. Journal of Environmental Quality, 33, 496–504.
- Wegglar-Beaton, K., McLaughlin, M. J. & Graham, R.D.**, 2000. *Salinity increases cadmium uptake by wheat and Swiss chard from soil amended with biosolids*. Australian Journal of Soil Research, 38, 37–45.

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