

Original Research

Assessment of Heavy Metal Amounts of Spinach Plants (*Spinach Oleracea* L.) Grown on Cd and Chicken Manure Applied Soil Conditions

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Abstract

Although some plants accumulate excessive metal and can grow without any toxic symptoms, the consumption of these plants by humans can be extremely inconvenient for their health. Increasing doses of Cd (0, 10, and 20 mg kg⁻¹ Cd) and chicken manure (0, 500, 1000, and 2000 kg da⁻¹) were applied to the soil to evaluate the effects of cadmium and chicken manure on growth of spinach (*Spinach oleracea* L.) leaves and roots and on some heavy metal concentrations. Cadmium decreased the dry weight amounts of spinach both in leaves and in roots, decreased the amounts of Cd and also other heavy metals. The highest cadmium concentrations were determined at the second dose as 75.04 mg kg⁻¹ in leaves, and 162.17 mg kg⁻¹ in roots. Improved dry weight and decreased Cr, Pb, and Fe amounts were determined with chicken manure application. However it was not found proficient to decrease Cd which was found over the limits of WHO. Because of being highly toxic to humans, health-related limits of cadmium in soils, and in plants consumed by humans have to be carefully controlled in such conditions and the threshold limits must be changed.

Keywords: antagonism, dry weight, health, heavy metals, toxicity

Introduction

Heavy metals; expressed as natural elements with a density greater than 5 g cm⁻³ and an atomic number greater than 20 [1, 2]. Heavy metals as the natural components of rocks which comprise various proportions and forms spread to the environment generally from water, air and soil. Some heavy metal trace elements such as copper (Cu), zinc (Zn), iron (Fe),

manganese (Mn), molybdenum (Mo), nickel (Ni), cobalt (Co); are absolutely necessary for the plants to carry out their growth, development and physiological life functions effectively. However, it has been reported that excessive doses of these elements, and trace amounts of many heavy metals other than this group, including arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) may be toxic to plants [3-5].

In parallel with the increase in the human population and the development of today's technology, the natural distribution pattern and concentrations of heavy metals are significantly increased due to the

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industrial activities such as energy and fuel production, radioactive wastes, mining, sludge applications. Nowadays, anthropogenic activities such as excessive usage of fertilizers and pesticides, and irrigation with wastewater were added in to the list [6]. It is reported that the metal concentration in the soil varies between 1 and 100,000 mg kg⁻¹, and high heavy metal levels cause degradation in ecological balance by causing a decrease in soil, product, yield and quality [7-9]. It has been stated that the presence of heavy metals in plants at toxic levels causes deterioration of many physiological events such as transpiration, stoma movements, water intake, photosynthesis, enzyme activity, germination, protein synthesis, membrane stability, hormonal balance [10]. In addition to the deterioration in plants, due to the accumulation of heavy metals in the bodies of humans and other creatures fed with these plants over time; it is reported that it causes serious damage to the human body and can negatively affect their lives [5, 8, 9, 11].

Of the related heavy metals, Cd has the potential to accumulate in soils, especially as a result of intensive and continuous use of phosphorus fertilizers. And has been indicated that the maximum allowable Cd concentration in agricultural soils in various countries were between 1-20 mg kg⁻¹ [7]. Depending on the high concentrations of cadmium in the soil, there may be also accumulation of cadmium in plants and can negatively affect human health through the food chain [11]. Kabata-Pendias [7] stated that the solubility of the heavy metals depends on some soil factors such as CEC, clay minerals, carbonates and hydrous oxide metals and also their pH affected amounts and kind of organic matter. It was also reported that some agricultural practices have also been tried to soil preventing the mobilization of trace metals and the uptake of metals by plants may be depressed with materials having a high capacity to bind metals such as phosphorites, zeolites, montmorillonites, humic organic matter. [7, 12]. In various studies, it has been reported that some organic materials applied to

the soil reduce the mobility and uptake of heavy metals in the soil [13]. Organic amendments such as animal manure, crop residues and biosolids have been used to immobilize the heavy metals [12]. Chicken manure is also used as an organic fertilizer source due to its high nutrient content, increasing the efficiency, aeration and water holding capacity of the soil [3, 14].

Plants may grow in normal appearance in the soils contaminated with Cd, however its element contents may not be safe for human consumption. Spinach has been used as a test plant in our experiment because of its consumption that has become widespread in recent years, as well as being a vegetable that can significantly affect human health and nutrition due to its hyper accumulator properties. This research was carried out to determine the effects of cadmium and chicken manure applied to the soil on growth of spinach plants and contents of some heavy metals and also to state the concentrations in soil and also in plants were harmful or not for human nutrition.

Materials and Methods

The soil sample from 0-20 cm depth was collected from a field located at the Agricultural Research and Application Centre of Bursa Uludag University (39°35', 40°40' and 28°10', 30°00') in Turkey. Some properties of the soil used in the experiment are shown in Table 1. The soil used in the experiment had a clay texture and a pH of 7.91. Additionally, it had low lime content and Electrical Conductivity (EC). The soil also had adequate concentrations of organic matter and has not a problem dealing with the nutrient elements and heavy metals. (Table 1).

Air-dried soil was passed through 4 mm sieve and placed into polyethylene covered plastic pots with an amount of 3.5 kg. The experiment was conducted in randomized plots design with three replicates

Table 1. Some Properties of the soil.

Properties	Amounts	Properties	Amounts
Texture	Clay	Lime, CaCO ₃ , %	4.68
Sand, %	44.76	Organic matter, %	2.21
Silt, %	14.00	Total nitrogen (N), %	0.092
Clay, %	41.24	Available phosphorus (P), mg kg ⁻¹	14.51
pH (1:2.5 soil:water)	7.91	Iron (Fe) mg kg ⁻¹	12.49
EC, µS cm ⁻¹	530.0	Copper (Cu) mg kg ⁻¹	1.56
Sodium (Na) mg kg ⁻¹	175.6	Zinc (Zn) mg kg ⁻¹	0.95
Potassium (K) mg kg ⁻¹	375.5	Manganese (Mn) mg kg ⁻¹	31.64
Calcium (Ca) mg kg ⁻¹	10048	Cadmium (Cd) mg kg ⁻¹	0.02
Magnesium (Mg) mg kg ⁻¹	390.2	Lead (Pb) mg kg ⁻¹	1.82
Boron (B) mg kg ⁻¹	0.65	Chromium (Cr) mg kg ⁻¹	0.02

Table 2. Some properties of the chicken manure* used in the trial.

Properties	Amounts	Properties	Amounts
pH (1:2.5 w/v)	9.82	Iron (Fe) g kg ⁻¹	8.84
EC μ S cm ⁻¹	11.72	Copper (Cu) mg kg ⁻¹	61.41
Organic matter %	42.45	Zinc (Zn) mg kg ⁻¹	376.97
Nitrogen (N) g kg ⁻¹	13.89	Manganese (Mn) mg kg ⁻¹	642.75
Phosphorus (P) g kg ⁻¹	16.13	Boron (B) mg kg ⁻¹	44.48
Sodium (Na) g kg ⁻¹	4.55	Cadmium (Cd) mg kg ⁻¹	0.22
Potassium (K) g kg ⁻¹	23.35	Lead (Pb) mg kg ⁻¹	0.21
Calcium (Ca) g kg ⁻¹	60.73	Chromium (Cr) mg kg ⁻¹	54.00
Magnesium (Mg) g kg ⁻¹	7.49		

*Trade name Batisel which is the commercial product of Yükseller company, Manisa/Turkey.

in greenhouse conditions. Three different rates of cadmium (0, 10 and 20 mg kg⁻¹ Cd) as cadmium sulphate (3CdSO₄.8H₂O) (Extra pure, Merck, Germany) in the form of solution, and increasing doses of chicken manure (0, 500, 1000 and 2000 kg da⁻¹) were applied to the pots in a solid state. The chicken manure used in the experiment is a product of Yükseller company, Manisa/Turkey which is sold as pelleted and under the trade name Batisel. Some properties of the chicken manure were analysed before the experiment and the results were shown on Table 2. Before planting 50 mg kg⁻¹ N, 40 mg kg⁻¹ P, and 50 mg kg⁻¹ K were also added to the pots using ammonium nitrate (NH₄NO₃) (Extra pure, Merck, Germany) and monopotassium phosphate (KH₂PO₄) (Emprove, Merck, Germany) and the pots were incubated for 60 days.

Five spinach (*spinach oleracea* L.) seed cultivars 'Reis F1' were planted in each pot, which were 20 cm in diameter and 18 cm deep, and later on thinning-out was done after germination and three plants were left. The water content of the pots was adjusted to 70% of the field capacity during the experiment. After 45 days, the spinach leaves were cut over the soil level and then the leaves and the spinach roots also removed and were immediately transferred to the laboratory. In order to avoid possible contamination from dust, the samples were washed once in tap water and twice with deionized water. The samples were dried at 70°C for 72 h in a hot air oven (Nuve KD 400, Turkey), weighed and then ground from 0.5 mm sieve using a laboratory mill (Foss CT 193 cyclotec, Denmark).

For the determination of the nutrient concentrations in leaves and roots, the ground samples were digested using a mixture of 3 mL of nitric acid (HNO₃) (65 %, Emplura, Merck) and 3 mL of hydrogen peroxide (H₂O₂) (35 %, Emprove, Merck, Germany) in a microwave oven (Berghof MWS 2, Germany) [15]. Heavy metals (Cd, Pb and Cr), and the nutrients (Fe, Zn, Mn, and Cu) amounts were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES)

(Perkin Elmer Optima 2100DV, USA) [16]. The up-taken amounts were calculated using concentration and dry weight values. Data from all of the experiments were subjected to statistical analysis and the mean values were compared using the least significant difference (LSD) multiple range test with the computer program MINITAB 17.1.0.0 (Minitab Inc., State College, Pennsylvania, USA).

Results and Discussion

Effects of Cadmium on Dry Weight Amounts

Increasing doses of cadmium applied to the soil significantly affected the leaf and root dry weight yield of spinach plants ($p < 0.01$). With increasing cadmium doses, the leaf and root dry weight yield of the plant was negatively affected, and the lowest leaf (2.77 g pot⁻¹) and root (0.52 g pot⁻¹) dry weight yield was obtained from the highest dose of cadmium (Cd2) (Table 3). Significant decreases on the growth of spinach plants due to high concentrations of cadmium in our research findings have been supported with the findings of the previous studies done with various leafy vegetables. Chetan and Ami [17] reported decreases on growth parameters of spinach plant grown under two doses of Cd as compared to control. Canal and Bozkurt [18] reported decreases on plant height, plant weight, and number of leaves of lettuce grown under toxic Cd conditions. Monteiro et al. [19] also reported a reduction in growth and biomass production in lettuce. Anwar et al. [20] reported reduced biomass at leafy vegetables such as mint, coriander and fenugreek with the application of sewage water containing high amounts of Cd. Decreased dry matter yield as mentioned in the previous trial results, in our experiment is thought to be caused by the deterioration in the uptake of plant nutrients in the presence of Cd and their function within the plant [21].

Table 3. Effects of increasing doses of cadmium and chicken manure on dry weight of leaf and root of spinach.

	Plant Parts	Cadmium Doses (mg kg ⁻¹)	Chicken Manure (kg da ⁻¹)													
			CM0			CM1			CM2			CM3			Mean	
Dry weight (g pot ⁻¹)	Leaves	Cd0	7.12	a	B	7.44	a	B	11.38	a	A	10.57	a	A	9.13	a
		Cd1	4.55	b	AB	3.64	b	B	4.45	b	B	6.68	b	A	4.83	b
		Cd2	2.02	c	A	2.46	b	A	3.33	b	A	3.28	c	A	2.77	c
		Mean	4.57	B		4.51	B		6.39	A		6.84	A			
	A _{LSD<0.01}		1.274	B _{LSD<0.01}			1.104			AxB _{LSD<0.01}			2.207			
	Roots	Cd0	2.46			4.09			2.98			3.79			3.33	a
		Cd1	0.63			0.58			0.63			1.41			0.81	b
		Cd2	0.34			0.40			0.65			0.70			0.52	b
	Mean		1.14			1.69			1.42			1.97				
	A _{LSD}		ns	B _{LSD<0.01}			1.231			AxB _{LSD}			ns			

The differences between values by different letters are significant. Capital letters for each row and small letters for each column. ns: not significant

Effects of Cadmium on Cadmium Amounts

With the applied cadmium doses statistically significant increases ($p < 0.01$) were found on the cadmium concentrations and on the up-taken Cd amounts in the leaves and in the roots of the spinach plant. The average Cd concentration of the plants in the applications without cadmium (Cd0) was determined as 0.58 mg kg⁻¹ in leaves and 1.23 mg kg⁻¹ in roots (Fig. 1). These values increased with cadmium applications and were found as 65.71 mg kg⁻¹ and 75.04 mg kg⁻¹ in leaves, and values in the roots increased more than in the leaves and were found as 108.10 mg kg⁻¹ and 162.17 mg kg⁻¹ respectively. It was observed that cadmium accumulated in the roots, and cadmium concentration was found higher in the roots than leaves (Fig. 1). The results showed the ability of spinach to accumulate the cadmium element in high amounts. Elevated plant Cd concentrations parallel with increased Cd doses were also reported in some studies [22, 23]. Various researches indicated the sufficiency level of Cd in mature leaf tissues as 0.05-0.2 mg kg⁻¹, 0.013-0.22 mg kg⁻¹ for cereal grains, 0.07-0.28 mg kg⁻¹ for grasses and legumes and excessive or toxic range as 5-30 mg kg⁻¹ and tolerable limit for agronomic crops as 0.05-0.5 mg kg⁻¹ [7, 10]. The permissible Cd level in plants was limited as 0.02 mg kg⁻¹ by World Health Organization (WHO) [12]. It was also pointed out that the daily intake of Cd was limited as 1 µg Cd per kg⁻¹ of the body weight and equivalent to 70 µg for a body weight of 70 kg⁻¹ according to WHO reports [24]. The toxic effects of Cd can be observed in people who regularly consume plants which contain Cd more than 3 mg kg⁻¹ in their tissues [25]. In our research, while the spinach plants which were grown under normal soil conditions had suitable Cd concentrations in their

leaves, it was determined that the leaves grown under Cd applied soils had the Cd concentrations over this dedicated limits. Once Cd is absorbed by this edible plant parts it causes disorders in kidneys and bones, damages in reproductive, nervous and cardiovascular systems and also leads to cancer [26- 28]. So regularly consumption of these plants might be dangerous for human health.

Since the accumulation of cadmium in the roots adversely affects root development, and the up-taken amount of cadmium was detected more in the leaves rather than in the roots. The lowest up-taken amount of cadmium in leaves (5.24 µg pot⁻¹) and in roots (3.62 µg pot⁻¹), were obtained from the control dose (Cd0) where cadmium was not applied (Table 4). The highest up-taken cadmium amount was obtained from the first dose of cadmium (Cd1), in being leaves (314.42 µg pot⁻¹) and in the roots (89.36 µg pot⁻¹). The amount decreased at the highest dose of Cd in relation to the decrease in dry weight and found as 209.92 µg pot⁻¹ in leaves and 84.74 µg pot⁻¹ in the roots. These findings were in correspondence with the findings of Dotaniya et al. [29] which reported the Cd uptake of spinach in leaves as 180 and 200 µg pot⁻¹ in 1 and 2 mg kg⁻¹ Cd doses. However the up-taken amounts may show differences due to the soil factors such as Cd concentration in soil, pH, level of organic matter and also plant species and varieties [10, 11].

Effects of Cadmium on Some Other Heavy Metal Amounts

The effect of increasing cadmium doses applied to the soil, on the concentrations and the up-taken amounts of Cr with leaves and roots of the spinach plant was found statistically significant ($p < 0.01$).

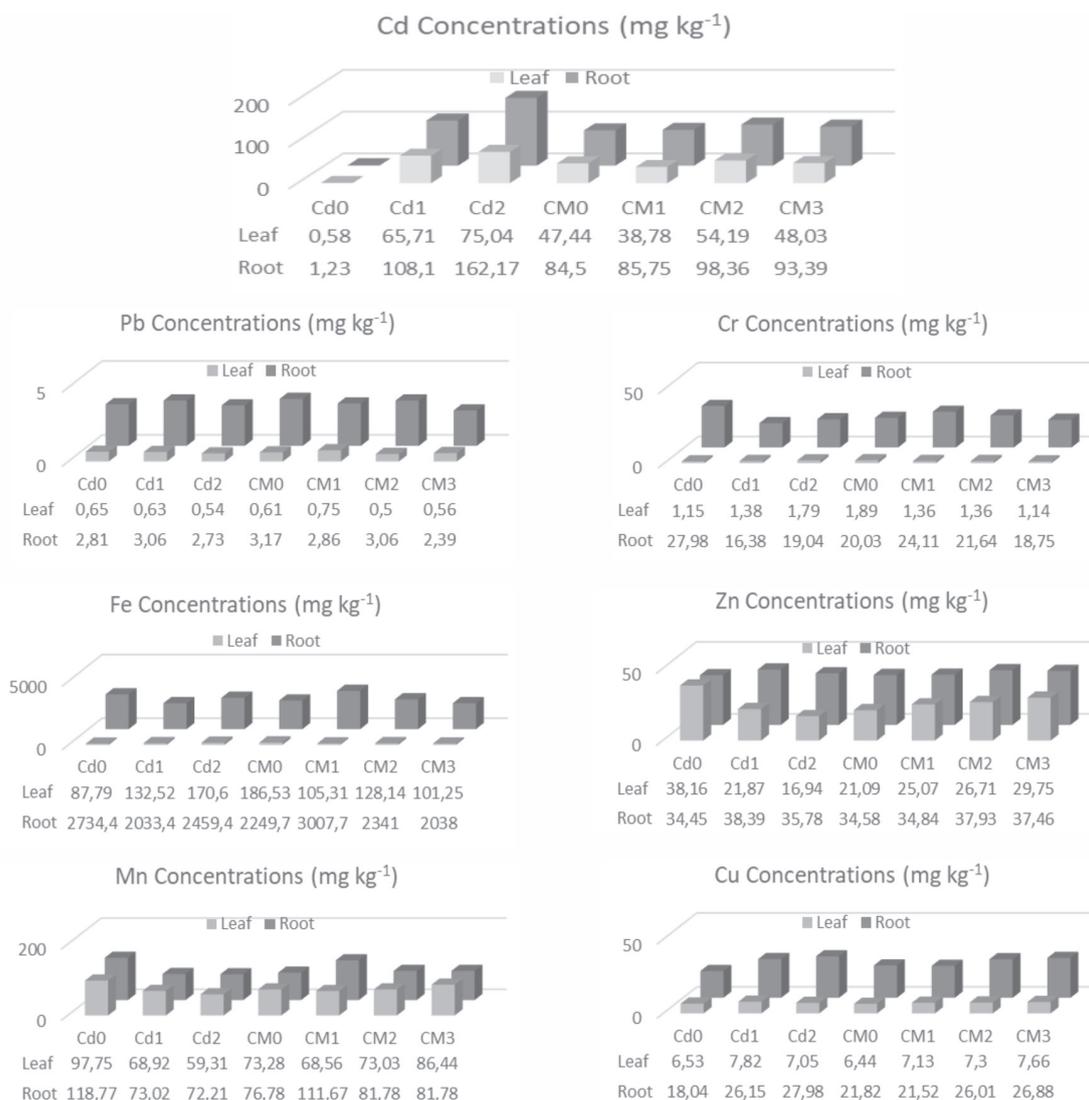


Fig 1. Effects of increasing doses of cadmium and chicken manure on some heavy metal contents of leaf and root of spinach.

Chromium concentrations and the up-taken amounts in the roots were found higher than leaves. Along with the increased cadmium doses, the up-taken amount of Cr by the leaves and roots of the plant was decreased. While the highest chromium uptake in the leaf (10.23 $\mu\text{g pot}^{-1}$) and in the root (106.43 $\mu\text{g pot}^{-1}$), was obtained from the control dose (Cd0) where cadmium was not applied, the lowest Cr amount in the leaves (4.67 $\mu\text{g pot}^{-1}$) and in the roots (9.52 $\mu\text{g pot}^{-1}$) was obtained from the highest dose of cadmium (Cd2). While the highest Cr concentration in the roots (27.98 mg kg^{-1}) was found at the control dose (Cd0) and decreased with increasing cadmium doses, owing to the competition of Cr with Cd (Fig. 1). The findings of Dotaniya et al. [29] also confirm the competition between Cd and Cr. In their research results decreased Cr concentrations and uptake were reported in both roots and in the leaves of spinach and they concluded as a higher Cd amount had an antagonistic effect over Cr.

The effect of increasing cadmium doses applied to the soil, on the up-taken amounts of Pb of leaves

and roots of the spinach plant was found statistically significant ($p < 0.01$). The highest up-taken Pb was obtained in the leaves (5.37 $\mu\text{g pot}^{-1}$) and in the roots (9.28 $\mu\text{g pot}^{-1}$), from the control dose (Cd0) where cadmium was not applied (Table 4). Along with the increased cadmium doses, the up-taken amount of Pb by the plant's leaves and roots decreased, and the lowest up-taken Pb amount, in leaves (1.42 $\mu\text{g pot}^{-1}$) and in roots (1.30 $\mu\text{g pot}^{-1}$) was obtained from the highest cadmium dose (Cd2). Lead also accumulated in the roots. Even though there was inhibited root development due, the excessive concentration in the roots, the up-taken amount of Pb at the Cd applied pots, was found more in the leaves rather than in the roots. Similar to the negative effect of Cd on Pb uptake in our study, its competition with elements such as K, Ca, Mg, Fe, Mn, Cu, Zn and Ni was also reported [30].

The effect of increasing cadmium doses applied to the soil on the Fe concentrations of leaves and roots of the spinach plant and their up-taken amounts was found statistically significant ($p < 0.01$). Concentrations of Fe

Table 4. Effects of increasing doses of cadmium and chicken manure on Cd, Cr, and Pb uptake of leaf and root of spinach

	Plant Parts	Cadmium Doses (mg kg ⁻¹)	Chicken Manure (kg da ⁻¹)									
			CM0		CM1		CM2		CM3		Mean	
Cd (µg pot ⁻¹)	Leaves	Cd0	3.56		5.58		5.69		6.12		5.24	c
		Cd1	285.95		205.09		355.68		410.96		314.42	a
		Cd2	168.38		146.39		253.30		271.59		209.92	b
		Ort	152.63	BC	119.02	C	204.89	AB	229.55	A		
	A _{LSD<0.05}		76.436		B _{LSD<0.01}		89.649		AxB _{LSD}		ns	
	Roots	Cd0	3.11		3.09		3.75		4.53		3.62	b
		Cd1	60.90		64.67		76.84		155.02		89.36	a
		Cd2	50.86		55.50		115.01		117.58		84.74	a
		Ort	38.29	B	41.09	B	65.20	AB	92.38	A		
	A _{LSD<0.05}		37.105		B _{LSD<0.01}		43.519		AxB _{LSD}			
Cr (µg pot ⁻¹)	Leaves	Cd0	8.78		9.32		12.24		10.57		10.23	a
		Cd1	7.99		4.65		6.38		7.67		6.67	b
		Cd2	5.37		3.89		5.33		4.10		4.67	b
		Ort	7.38		5.95		7.98		7.45			
	A _{LSD}		ns		B _{LSD<0.01}		2.291		AxB _{LSD}		ns	
	Roots	Cd0	54.39		164.73		98.98		107.61		106.43	a
		Cd1	8.56		9.39		13.71		24.10		13.94	b
		Cd2	7.09		6.55		12.66		11.77		9.52	b
		Ort	23.35		60.22		41.78		47.83			
	A _{LSD}		ns		B _{LSD<0.01}		54.957		AxB _{LSD}		ns	
Pb (µg pot ⁻¹)	Leaves	Cd0	3.79		6.67		6.57		4.45		5.37	a
		Cd1	2.28		2.36		2.51		5.18		3.08	b
		Cd2	1.28		1.64		1.11		1.64		1.42	b
		Ort	2.45		3.56		3.40		3.76			
	A _{LSD}		ns		B _{LSD<0.01}		0.003		AxB _{LSD}		ns	
	Roots	Cd0	7.79		14.02		6.76		8.53		9.28	a
		Cd1	1.63		1.21		1.94		3.75		2.13	b
		Cd2	0.85		1.07		1.76		1.52		1.30	b
		Ort	3.43		5.43		3.49		4.60			
	A _{LSD}		ns		B _{LSD<0.01}		3.409		AxB _{LSD}		ns	

The differences between values by different letters are significant. Capital letters for each row and small letters for each column. ns: not significant

in the leaves increased with the Cd doses and the highest Fe (170.6 mg kg⁻¹) was found in Cd2. However, in the roots (2734.44 mg kg⁻¹) the highest Fe was observed in Cd0, because of the competition with Cd, the amounts of Fe decreased with Cd doses (Fig. 1). Low concentrations of iron in Cd0 in the leaves, were dedicated to the result of dilution effect. The antagonistic relationships between Cd and Fe uptake as well as the effects of

Fe on the uptake of Cd by the plant and transport from the roots to the shoots are also important. Several studies on cadmium stress showed impaired Fe translocation from roots to shoots and also decreased concentrations in the plant with Cd application [21, 31, 32]. Along with the increased cadmium doses, the up-taken amount of iron by the leaves and roots of the plant decreased. While the highest iron uptake in the leaf

(770.90 $\mu\text{g pot}^{-1}$) and in the root (10323.0 $\mu\text{g pot}^{-1}$), was obtained from the control dose (Cd0) where cadmium was not applied, the lowest iron up take in the leaves (424.53 $\mu\text{g pot}^{-1}$) and in the roots (1288.7 $\mu\text{g pot}^{-1}$) was obtained from the highest dose of cadmium (Cd2) (Table 5). Although the accumulation of cadmium in the roots affected the root development more than leaves, and over accumulation of iron in the roots caused the up-taken amount of iron to be found higher in the roots compared to the leaves.

The effect of increasing cadmium doses applied to the soil, on the up-taken amounts of Zn of leaves and roots of the spinach plant was found statistically significant ($p < 0.01$), however the effect, was not significant on the concentrations of the roots. Along with the increased cadmium doses the up-taken amount of Zn by the plant's leaves and roots decreased suggesting the Cd/Zn antagonism [10, 21, 33]. The highest up-taken Zn was obtained in the leaves (327.35 $\mu\text{g pot}^{-1}$) and in the roots (112.12 $\mu\text{g pot}^{-1}$), from the

Table 5. Effects of increasing doses of cadmium and chicken manure on Fe, Zn, Mn, and Cu uptake of leaf and root of spinach

	Plant Parts	Cadmium Doses (mg kg ⁻¹)	Chicken Manure (kg da ⁻¹)								Mean	
			CM0		CM1		CM2		CM3			
Fe ($\mu\text{g pot}^{-1}$)	Leaves	Cd0	945.28		622.07		809.56		706.70		770.90	a
		Cd1	712.01		402.80		748.16		745.38		652.09	ab
		Cd2	513.01		314.71		462.75		407.65		424.53	b
		Ort	723.43		446.53		673.49		619.91			
	A _{LSD}		ns		B _{LSD<0.01}		238.644		AxB _{LSD}		ns	
	Roots	Cd0	5845.8		21363.4		6818.8		7264.1		10323.0	a
		Cd1	1120.5		1158.7		1651.2		2982.1		1728.1	b
		Cd2	827.56		924.74		1776.8		1625.8		1288.7	b
		Ort	2598.0		7815.6		3415.6		3957.3			
	A _{LSD}		ns		B _{LSD<0.01}		5962.952		AxB _{LSD}		ns	
Zn ($\mu\text{g pot}^{-1}$)	Leaves	Cd0	236.72		296.06		300.86		475.75		327.35	a
		Cd1	59.36		72.11		87.74		165.06		96.07	b
		Cd2	24.53		32.55		63.47		64.47		46.26	b
		Ort	106.87	B	133.57	B	150.69	AB	235.09	A		
	A _{LSD<0.01}		96.604		B _{LSD<0.01}		83.662		AxB _{LSD}		ns	
	Roots	Cd0	97.24		131.96		96.67		122.59		112.12	a
		Cd1	21.74		21.91		26.79		57.72		32.04	b
		Cd2	10.26		14.03		24.36		27.97		19.16	b
		Ort	43.08		55.97		49.28		69.43			
	A _{LSD}		ns		B _{LSD<0.01}		42.523		AxB _{LSD}		ns	
Mn ($\mu\text{g pot}^{-1}$)	Leaves	Cd0	668.30		725.13		837.98		1173.67		851.27	a
		Cd1	208.61		228.12		225.02		562.73		306.12	b
		Cd2	107.95		109.05		206.86		212.52		159.09	b
		Ort	328.29	B	354.10	B	423.29	B	649.64	A		
	A _{LSD<0.05}		208.180		B _{LSD<0.01}		244.165		AxB _{LSD}		ns	
	Roots	Cd0	241.14		859.92		302.37		324.84		432.07	a
		Cd1	41.01		41.82		48.16		118.94		62.48	b
		Cd2	21.17		27.18		53.68		54.09		39.03	b
		Ort	101.11		309.64		134.74		165.96			
	A _{LSD}		ns		B _{LSD<0.01}		217.854		AxB _{LSD<0.05}			

Table 5. Continued.

Cu ($\mu\text{g pot}^{-1}$)	Leaves	Cd0	42.62		49.41		51.96		75.18		54.79	a
		Cd1	25.83		28.60		29.44		53.22		34.27	b
		Cd2	10.14		14.74		26.32		25.90		19.27	b
		Ort	26.20	B	30.92	B	35.91	AB	51.43	A		
	A _{LSD<0.01}		19.419		B _{LSD<0.01}		16.818		AxB _{LSD}		ns	
	Roots	Cd0	49.60		64.37		52.33		68.48		58.70	a
		Cd1	14.60		12.61		20.93		41.05		22.29	b
		Cd2	7.94		10.89		19.81		21.88		15.13	b
		Ort	24.04		29.29		31.02		43.80			
	A _{LSD}		ns		B _{LSD<0.01}		22.598		AxB _{LSD}		ns	

The differences between values by different letters are significant. Capital letters for each row and small letters for each column. ns: not significant

control dose (Cd0) where cadmium was not applied, and the lowest up-taken Zn amount, in leaves ($46.26 \mu\text{g pot}^{-1}$) and roots ($19.16 \mu\text{g pot}^{-1}$) was obtained from the highest cadmium dose (Cd2). Although Zn concentrations were found high in the roots than in leaves, the up-taken Zn amounts were found high in the leaves because of the inhibited root dry weight and being not too high concentrations of Zn in the roots as compared with Fe (Table 5).

The effect of increasing cadmium doses applied to the soil, on the Mn concentrations and on the up-taken amounts of Mn of leaves and roots of the spinach plant was found statistically significant ($p<0.01$). Along with the increased cadmium doses, concentrations and the up-taken amount of Mn by the plant's leaves and roots decreased. The highest up-taken Mn was obtained in the leaves ($851.27 \mu\text{g pot}^{-1}$) and in the roots ($432.07 \mu\text{g pot}^{-1}$), from the control dose (Cd0) where cadmium was not applied, and the lowest up-taken Mn amount, in leaves ($159.09 \mu\text{g pot}^{-1}$) and roots ($39.03 \mu\text{g pot}^{-1}$) was obtained from the highest cadmium dose (Cd2). Manganese concentrations in the roots were also found higher than in leaves. The highest Mn concentration ($118.77 \text{ mg kg}^{-1}$) was found at the control dose (Cd0) and tended to decrease with increasing cadmium doses and least concentration was found at the second dose of Cd (Cd2) (Fig. 1). The results presented show the competition of Mn with cadmium clearly. Nazar et al. [10] and Rahman et al. [34] also reported the antagonism between Cd and Mn, related to the shared transporting sites and confirm the findings of us.

The average Cu concentration of plants in the applications without cadmium (Cd0) was determined as 6.53 mg kg^{-1} in leaves and 18.04 mg kg^{-1} in roots (Fig. 1). These values increased with cadmium applications and were found as 7.82 mg kg^{-1} and 7.05 mg kg^{-1} in leaves, and the values in the roots increased more than in leaves and were found as 26.15 mg kg^{-1} and 27.98 mg kg^{-1} . It was observed that Cu also accumulated in the roots,

and Cu concentration was found higher in the roots than leaves (Fig. 1). Since the accumulation of cadmium in the roots adversely affects root development, the up-taken amount of Cu was detected a little bit more in the leaves rather than in the roots. The highest up-taken amount of Cu in leaf ($54.79 \mu\text{g pot}^{-1}$) and in root ($58.70 \mu\text{g pot}^{-1}$), were obtained from the control dose (Cd0) where cadmium was not applied (Table 5). The lowest up-taken Cu amount was obtained from the second dose of cadmium (Cd2), both in leaves ($19.27 \mu\text{g pot}^{-1}$) and in the roots ($15.13 \mu\text{g pot}^{-1}$). Wu and Zhang [35] reported a reduction on Cu amounts in barley due to the addition of Cd to the growing medium. Competition of cadmium with the elements K, Ca, Mg, Fe, Mn, Cu, Zn, and Ni were reported in researches done with different plants [30]. In addition, high cadmium in the plant, along with other microelements, caused chrome and lead amounts to decrease and antagonistic relationship of Cr and Pb's with Cd has been also revealed in our study. Cd and not only the nutrients but also the other heavy metals compete for the same transporters, therefore under heavy Cd toxic conditions the optimization of nutrient elements could prevent the nutrients deficiencies and also reduce the accumulation of Cd [10].

Effects of Chicken Manure on Dry Weight Amounts

Increasing doses of chicken manure applied to the soil and its interaction with Cd have also statistically significant effect ($p<0.01$) on the dry weight yield of spinach leaves. However, its effect on root dry weights was not found significant. The highest leaf dry weight yield (6.84 g pot^{-1}) was obtained from the highest application dose of chicken manure (CM3) (Table 3). According to the interactions of cadmium and the chicken manure; the highest leaf dry weight yield

(11.38 g pot⁻¹) was obtained from spinach plants in pots (Cd0xCM2), where no cadmium applied and the second dose of chicken manure. In contrast, the lowest leaf dry weight yield (2.02 g pot⁻¹) was obtained from spinach plants in pots (Cd2xCM0), where no chicken manure was applied and the highest level of cadmium. Chicken manure is a good source of nutrient elements and serves as a soil amendment material improving soil physical, chemical and also the biological properties [36]. Humic substances in chicken manure exhibit behaviours similar to growth hormones in plants, increase the intake of plant nutrients, promote plant growth and have a positive effect on increasing the amount of dry matter [3, 12, 14]. Humic compounds may be an important alternative in terms of increasing nutrient availability and regulating the toxicity problems in some areas. It has been stated that humic acids are effective on plant growth and development, and when applied in small quantities, it positively affects the development of plants. However, when applied in large quantities, ineffective or even quite negative effects on development were also reported [37].

Effects of Chicken Manure on Cd Amounts

The effect of increasing doses of chicken manure applied to the soil on the up-taken amount of cadmium of the leaves and roots of the spinach plant was found statistically significant ($p < 0.05$). In parallel with the increasing chicken manure applications, concentrations and the up-taken amount of cadmium by the plant has increased (Fig. 1 and Table 4). The highest cadmium up take was obtained from the highest application dose of chicken manure (CM3) in the leaf (229.55 $\mu\text{g pot}^{-1}$) and in the root (92.38 $\mu\text{g pot}^{-1}$) (Table 4). Stimulating effect of humic substances on plant growth is associated with increasing the intake of macro nutrients. In addition to macro and micro plant nutrients, poultry manure can also contain many heavy metals depending on the production and management process of the poultry and the amount of As, Cd, Cu and Mn of the soils may increase compared to the control with chicken manure applications [38]. It has been reported that some organic materials applied to the soil reduce the mobility and uptake of heavy metals in the soil by increasing the surface charge and supplies the retention of Cd in the soil [12]. The ability of soils to absorb these trace elements depends on their ability to form pH-dependent surface loads of organic matter, cation exchange capacities, clay minerals and hydrated iron and manganese oxides [39, 40, 41]. Organic amendments including humic substances, increase the surface charge and can form complexes with metal cations, and organic bond fractions and therefore increase intake or, conversely compete with roots and reduce the uptake [12, 28].

Effects of Chicken Manure on Some Heavy Metal Amounts

The effect of increasing doses of chicken manure applied to the soil, on the Cr, Pb and Fe uptake of the leaves and the roots of the spinach plant was not statistically significant. In contrast, it was found significant on the concentration of Cr in the leaves (Data not shown). Although the highest Cr concentration was found (1.89 mg kg⁻¹) at control pots (CM0) tended to decrease with the increasing chicken manure and was found least (1.14 mg kg⁻¹) at the highest dose (CM3) (Fig. 1). The effect of increasing doses of chicken manure applied to the soil, on the concentrations of Pb of the leaves and the roots of the spinach plant was not found statistically significant. However, the concentrations of Fe were found significant both in leaves and roots. The highest Fe concentration was found (185.53 mg kg⁻¹) in control pots (CM0) tended to decrease with the increasing chicken manure and found least in leaves (101.25 mg kg⁻¹), and in roots (2038 mg kg⁻¹) at the highest dose (CM3) (Fig. 1).

The effect of increasing doses of chicken manure applied to the soil on the up-taken amount of Zn, Mn, and Cu in the leaves of the spinach plant was found statistically significant ($p < 0.05$) but not significant in the roots. In parallel with the increasing chicken manure applications, the amount of up-taken Zn, Mn, and Cu by the plant has increased. The highest up-taken amounts were obtained from the highest application dose of chicken manure (CM3) in the leaves as (235.09 $\mu\text{g pot}^{-1}$), (649.64 $\mu\text{g pot}^{-1}$), and (51.43 $\mu\text{g pot}^{-1}$) respectively (Table 4). Rhizosphere is an important environment on building the relations between nutrients, heavy metals and roots. Nutrient elements and metals may precipitate, absorb or form complexes with organic compounds depending to the rhizosphere pH, redox potential and chelating capacity. The solubility and availability of the Cd and other heavy metals could be reduced if they bound in to chelate complex [10]. Interaction between metal and solid phase of humic acid may decrease availability of heavy metals and various research results were reported dealing with the decreased heavy metal uptake by plants with the applications of organic amendments [13]. Kamari et al [42] also reported the immobilization of heavy metals in soil with manure applications in some studies and depended on the effectiveness of stabilization to the nature of contaminants, physical and chemical characteristics of the amendment and also the type of soil. On the other hand formation of organa-metal compounds may increase the solubility of heavy metal and availability in plants [37]. Parallel to the findings of us, Arroyo et al. [43] also pointed increased concentrations of Cu in plants grown on the poultry manure amended soils.

Conclusions

Cadmium concentrations and the up-taken amounts increased with the addition of Cd to soil. This situation led degradation on the dry weight of spinach both in leaves and in roots and also decreased the amounts of other heavy metals. Cd and also Pb, Cr, Zn, Fe, Mn and Cu were accumulated in spinach roots rather than leaves. Adding chicken manure to the soil under toxic conditions of Cd improved the dry weight of the plants, and lessens the amounts of Cr, Pb, and Fe but not found proficient to decrease Cd, Zn, Mn, and Cu amounts. High amounts of Cd in the soil was not only accumulated Cd in spinach roots, but also raised the amounts in the leaves. However, no visual chlorotic or necrotic deformation and toxicity symptoms were found because of its tolerance to toxicity. The tolerance and normal appearance of the plants to elevated concentrations of trace metals in soils may result a health risk to humans and also to animals which consumes these plants. Because of the Cd amounts found over the limits of WHO, consumption of these spinach plants by humans were found dangerous for their health. In case of trace elements like Cd that are easily tolerated by plants such as spinach and highly toxic to humans, health-related limits of these elements in soils, and in plants consumed by humans have to be carefully controlled and the threshold limits must be changed.

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Conflict of Interest

The authors declare no conflict of interest.

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