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Response of lettuce to Cd-enriched water and irrigation frequencies

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This pot experiment was an attempt to investigate a broad response of lettuce to different cadmium (Cd) levels of irrigation water (0, 5, 10 and 20 mg l^{-1}) under different irrigation intervals (1, 2 and 4 days). The results showed that increased level of soil Cd through irrigation eventually decreased the yield of lettuce in all cases; however, in some cases yield was increased with lower doses of Cd application. No injury symptoms were observed other than plant height and yield reduction. Shoot dry weight proved to be the most sensitive parameters to the cadmium, especially under water stress conditions. The results also showed that the concentrations of nutrient elements in lettuce shoot were suppressed by water stress. The presence of cadmium in irrigation water did not significantly affect the absorption of nutrient elements by plants except for Fe. Shoot Cd concentration and its uptake decreased with increasing irrigation frequencies and the reverse trend occurred with increasing Cd levels of irrigation water. However, the values were higher than recommended guideline in all conditions. Also, shoot Cd content showed a significant positive correlation with the final accumulated Cd concentration of soil and was expressed by a plateau model under the dry irrigation regime and linear models at other irrigation intervals. Overall, shoot Cd concentration was predicted by using a simple linear regression model regardless of evapotranspiration and transpiration rate of plant.

Key words: Cadmium toxicity; chemical composition; irrigation frequency; lettuce.

INTRODUCTION

With rapid population growth and consequently more food demand, water scarcity is becoming a prime issue for many countries. Among the users of water, agriculture is one of the highest consumers. Treated wastewater could partially overcome the natural shortage of fresh water for agricultural, especially in arid and semi-arid regions. However, such water must be used under strict criteria to minimize human health risk due to pathogenic and toxic pollution of agricultural products (Toze, 2006). Although primary and secondary wastewater treatment processes remove substantial amounts of total solids, the effluent may still contain significant levels of heavy metals, particularly, if it is from industrial sources (Grifferty and Barrington, 2000; Toze, 2006). When this effluent is used for crop irrigation, the heavy metals may be adsorbed by soil particles, taken up by plants through transpiration and/or leached out from the soil profile. Unplanned disposal of industrial effluent has also increased the threat of soil and water resources pollution by heavy metals in developing countries (Khan, 2001; Shayegan and Afshari, 2004).

Cadmium (Cd) has been considered as a major concern of environment, due to its high toxicity to humans as well as plants and its high mobility in soil-water systems (Moral et al., 2002). Toxic effects of Cd on plants include chlorosis, growth inhibition, damage to root tips, reduction in water and nutrient uptake and crop protein synthesis (Dass et al., 1997). It can enrich the food chain, because of its relative high mobility in soil-plant system (Jarvis et al., 1976). However, it can also cause damage to the skeletal system and kidneys and induce cancer in humans (He et al., 2005). It seems that low concentration of Cd has a stimulatory effect on the growth of some

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Table 1. Physico-chemical characteristics of soil used in this study.

Soil properties	Amount/type
Sand (%)	14.1±0.50[a]
Clay (%)	40.4±0.21
Texture	Silty clay
Field capacity (dry weight basis, %)	21.64±0.55
Bulk density (mg m^{-3})	1.42±0.11
OM (%)	1.1±0.05
Soil pH (paste)	7.84±0.21
CEC (cmole kg^{-1})	14.1±0.50
EC_e (dS m^{-1})	0.50±0.11
Background Cd (mg kg^{-1})[b]	0.31±0.09

[a]Mean±S.D. (n=3); [b] DTPA-extractable

plant species (Mahler et al., 1978; Khan and Khan, 1983). Cadmium is easily absorbed and translocated to shoots of many food crops (Page et al., 1972; Turner, 1973; Jarvis et al., 1976; Mumba et al., 2008) and thus may lead to chronic Cd toxicity in humans. Uptake of Cd by plants such as vegetables, included in the daily diet, should be taken into consideration. To minimize the amount of Cd in food crops, information on the origin and environmental behavior of the metal as well as the conditions encouraging its uptake by plants is crucial. Although the ability of lettuce (*Lactuca stiva* L.) to absorb Cd has been demonstrated by some investigations (He et al., 2005; Logan et al., 1997; Mahler et al., 1978; Haghiri, 1973), there is little published information regarding the effect of Cd-enriched irrigation water and irrigation regimes on the growth and chemical composition of this crop. Therefore, the present study was initiated to evaluate the influence of Cd levels in irrigation water and irrigation frequencies on the behavior of lettuce. According to Burken et al. (1996), irrigation intervals and consequently soil moisture and transpiration regimes of the plant affect Cd absorption by plants. The chemical composition may provide information on how nutritional imbalance affects the plants growth and could help describe Cd toxicity details.

MATERIALS AND METHODS

Soil source, characterization and preparation

A bulk sample of the top layer (0-20 cm) of a calcareous silty clay soil (fine, mixed, mesic, Typic calcixerepts) was collected from an uncultivated field at Badjgah agricultural experiment station, 16 km north of Shiraz, I. R. of Iran. The soil was then air-dried and passed through a 2 mm sieve and some of its physico-chemical properties were determined (Table 1). The soil was mixed uniformly with 150 mg kg^{-1} N as urea, 50 mg kg^{-1}P as KH_2PO_4, 5 mg kg^{-1} Fe as Fe-EDDHA and 5, 10 and 5 mg of Zn, Mn and Cu kg^{-1}, respectively, as their sulfate salt. The pots were filled with 2 kg of treated soil. The Van Genuchten model (Van Genuchten, 1980) for soil moisture characteristics curve was determined using hanging water column and pressure plates apparatus in order to convert soil moisture to the corresponding matric potential.

Treatments, plant cultivar and cultivation

The seeds of lettuce (*cv* Zarghan, a local cultivar) were planted in prepared pots at a glasshouse under natural light with average day and night temperatures of 37 and 13°C, respectively. Each pot was irrigated with distilled water to near field capacity by weight 21 days after planting and seedlings were then thinned to 3 per pot. Then, irrigation and Cd treatments were initiated. Treatments consisted of 4 Cd levels in irrigation water (0, 5, 10 and 20 mg L^{-1} as $CdCl_2.H_2O$) and 3 irrigation intervals (1, 3 and 4 day) and were arranged in factorial manner in a completely randomized design with three replicates. The amounts of water applied were considered as representative of evapotranspiration, since no water was lost by drainage. In order to measure transpiration, pots without plants were also used in each treatment. The total transpiration was calculated by difference of cumulative applied waters and cumulative evaporation. Irrigation and Cd treatments were continued for 75 days after planting.

Plant harvest and analysis

At harvest, the shoots were cut at the soil surface. The number of leaves, leaf area per pot and average plant height (up to the tip of last emerging leaf) were determined. Leaf area was measured with a leaf area meter (model Windias3, delta-t device). The plant materials were washed with tap and then distilled water, dried to a constant weight at 65°C, weighed and ground with an electric mill to pass a 40-mesh screen. Representative samples were dry-ashed at 550°C for 4 h, extracted with 2 M HCl, filtered through Whatman No. 42 filter paper and analyzed for Cd, Fe, Cu, and Zn by atomic absorption spectrophotometer, K by flame photometer and P by spectrophotometer. The roots were not analyzed, because humans consume only the shoot of this plant.

Data analysis

The collected data were subjected to analysis of variance (ANOVA) for the main and combined effects of irrigation intervals (I) and Cd levels of irrigation water (Cd). Duncan's Multiple Range Test was used to compare the means. The major and significantly affected parameters are presented and discussed. Although, the effect of irrigation treatment was significant on the most parameters, but our focus was on the Cd treatment. So we did not make a comprehensive discussion about the irrigation effect on the measured parameters. Hence, in some cases the data was not showed. Pearson's correlation coefficient (r) between all pairs of data was also calculated. Shoot tissue Cd content as a function of final soil Cd concentrations was showed by using a plateau-type model. linear regression model was also used to predict shoot Cd concentration.

RESULTS AND DISCUSSION

Growth

The results of the ANOVA for the main and combined effects of I and Cd on growth are shown in Table 2. The irrigation treatment had significant effect on all measured growth responses and was the over-ridding contributor to their variations. Moreover, Cd treatment significantly

Table 2. F values (mean squares over error mean squares) and significant levels (P) of the ANOVA for various growth responses of lettuce under three irrigation intervals (I), four cadmium levels of irrigation water (Cd) and their interaction (I×Cd).

Sources of variation	Degrees of freedom	Plant height	Leaf number	Leaf area	Shoot dry weight	Evapotranspiration (ET)	Transpiration (T)/ET ratio
I	2	12.70 P<0.01	55.86 P<0.01	326.68 P<0.01	17.48 P<0.01	7734.98 P<0.01	0.040 P<0.01
Cd	3	3.79 P<0.01	0.55 ns[a]	0.94 ns	11.18 P<0.01	1.74 ns	0.002 ns
I×Cd	6	0.77 ns	1.15 P<0.05	1.56 ns	3.27 P<0.05	4.52 P<0.01	0.001 ns
Experimental error	24	1.38[b]	0.47	2151.31	0.15	6412.33	0.001

[a] Not significant; [b] For experimental error mean squares are presented.

affected plant height and shoot dry weight. The interactive effect of I × Cd treatment also significantly affected shoot dry weight, leaf number and ET. Nearly, all growth parameters were significantly decreased with increasing irrigation intervals (data not shown).

No injury symptoms were observed on lettuce foliage. The effect of Cd levels on plant height did not show a clear trend. However, Cd-treated plants were markedly shorter than the control plants (data not shown). Monteiro et al. (2009) reported a significant reduction in the lettuce length, 14 days after exposure to 100 μM Cd in a nutrient solution.

The mean for shoot dry weight slightly increased by 5 mg Cd l^{-1} and dropped significantly at the highest Cd level as 21.9% as compared to the control (Table 3). A significant decline in shoot dry weight of lettuce at 200 mg Cd/kg soil has been observed by John (1973). Kahn and Khan (1983) observed growth stimulation at lower doses of Cd treatment. They believed that a low dose of Cd might be required for normal plant growth. Mahler et al. (1978) and Turner (1973) reported lettuce growth stimulation by adding small amounts of Cd to the root substrate.

The yield depression due to Cd was reported by Haghiri (1973) for several crops including lettuce. His results showed a 60% reduction in lettuce dry weight by adding 10 mg Cd/kg soil. In the present study, yield reductions with the highest Cd level were 18, 11 and 38% at the 1, 2 and 4-day irrigation intervals, corresponding to the accumulation of 161.2, 112.8 and 78.6 mg Cd/kg soil, respectively. The lower depression in lettuce growth in spite of higher soil Cd as compared to the results reported by Haghiri (1973) could be due to the gradual increase of Cd level in the soil.

Cadmium levels had no clear effect on ET rate and T/ET ratio of lettuce (Tables 2 and 3). The variation of these two factors, particularly ET, at each irrigation interval was to some extent in accordance with the variation in shoot dry weight. Transpiration rates were higher at low Cd levels, but decreased with the highest Cd concentration in irrigation water (data not shown). Grifferty and Barrington (2000) stated that high doses of heavy metals can affect plant physiology and reduce transpiration rate and dry matter. Kirkham (1978) reported that trace quantities of Cd (0.01 μg/ml) in nutrient solutions increased turgor pressure and transpiration rates and decreased stomatal resistances in *chrysanthemums*. However, at higher Cd treatments (0.1 and 1 μg/ml), stomatal resistance increased and turgor pressure and transpiration rates decreased.

Among the growth parameters, dry weight followed by plant height was the most sensitive traits to Cd addition. The variations of shoot dry weight were also better correlated with the variations of leaf area (data not shown) and ET rates rather than to other growth parameters. In this work, both growth stimulation and inhibition of lettuce shoot was exhibited, respectively, at lower and higher Cd application rates.

Chemical composition

The results of the ANOVA for the main effects of I and Cd and their interaction on chemical composition of lettuce shoot are shown in Table 4.

Table 3. Shoot dry weight and Evapotranspiration (ET) of lettuce at various irrigation intervals and Cd levels of irrigation water.

Irrigation interval (d)	Cd level of irrigation water (mg L^{-1})				Mean
	0	5	10	20	
		Dry weight (g pot^{-1})			
1	4.34^{a}	4.31^{a}	4.17^{ab}	3.55^{bc}	4.09^{A}
2	3.74^{abc}	3.74^{abc}	4.15^{ab}	3.33^{cd}	3.74^{B}
4	3.69^{abc}	3.98^{abc}	2.73^{de}	2.29^{e}	3.17^{C}
Mean	3.92^{A}	4.01^{A}	3.68^{A}	3.06^{B}	
		ET rate (cm^3 pot^{-1} day^{-1})			
1	107.0^{a*}	106.8^{a}	107.5^{a}	107.0^{a}	107.1^{A}
2	70.9^{c}	73.6^{b}	74.2^{b}	74.8^{b}	73.4^{B}
4	54.3^{d}	53.6^{de}	53.9^{d}	52.0^{e}	53.4^{C}
Mean	77.3^{B}	78.1^{AB}	78.5^{A}	77.9^{AB}	

*Means within each growth parameter followed by the same letter are not statistically different at $P \leq 0.05$. Capital letters are used for the main effects.

Table 4. F values (mean squares over error mean squares) and significant levels (P) of the ANOVA for various chemical composition of lettuce shoot under three irrigation intervals (I), four cadmium levels of irrigation water (Cd) and their interaction (I×Cd).

Sources of variation	Degrees of freedom	K	P	Fe	Cu	Mn	Zn	Cd	Cd Uptake
I	2	1.71 ns[a]	17.66 P<0.01	3.46 P<0.05	10.29 P<0.01	6.35 P<0.01	4.16 P<0.05	15.91 P<0.01	4.14 P<0.05
Cd	3	0.35 ns	0.25 ns	3.22 P<0.05	2.66 ns	0.4 ns	0.7 ns	20.13 P<0.01	3.77 P<0.05
I×Cd	6	1.07 ns	4.08 P<0.01	2.49 ns	2.82 P<0.05	1.79 ns	2.47 ns	4.34 P<0.01	1.89 ns
Experimental error	24	0.26[b]	0.003	5405.57	0.22	9.78	7.92	16.67	0.001

[a] Not significant; [b] For experimental error mean squares are presented.

Neither I nor Cd and I × Cd treatments influenced potassium content of lettuce. However, variation in other elements shown in Table 4, was mainly and significantly attributed to I treatment, except for the Cd content of lettuce shoot that was mainly affected by Cd treatment. Cd levels also influenced shoot Fe and the uptake of Cd (P<0.05). Interaction of I×Cd affected P, Cu and Cd contents of lettuce shoot significantly (Table 5).

The results indicate that the elemental concentrations in lettuce shoot declined as irrigation interval increased (data not shown). However, such trends were not significant for K. Indeed, the greater nutrient accumulation in the plants was observed under a wet rather than a dry irrigation regime and might be due to higher water supply and consequently, higher T rates.

The phosphorus concentration was significantly lower in Cd-treated plants at the 1-day irrigation interval and did not follow a clear pattern at higher irrigation frequencies (data not shown). Monteiro et al. (2009) reported a significant decrease in P of hydroponically grown lettuce leaves under Cd stress.

According to Krupta et al. (Cited in Monteiro et al., 2009) iron deficiency is a recognized consequence of exposure to other metals, and has implications for various biological processes. In this study, Fe concentration in lettuce was markedly less than that of the control at the lowest level of Cd. However, an insignificant reduction in Fe concentration occurred at higher Cd levels (data not shown). These results were in agreement with those of Wallace et al. (1977) for bush bean. Other researchers reported a decline in the Fe absorption in lettuce leaves (Monteiro et al., 2009); corn shoots (Iwai et al., 1975) and radish (Khan and Frankland, 1983) with increasing levels of Cd. Khan and Khan (1983) also reported that soil Cd application caused an increase in Fe concentration in tomato and a decrease in egg-plant shoots.

The experiment seemed to prove that the impairment of metabolic processes relating to the deficiency of Cu and Mn did not exist, since these elements in lettuce shoot were insignificantly less or equal at 5 mg l^{-1} Cd as compared to the control and were greater at the higher Cd levels (data not shown). Our results were consistent with reports of Ramos et al. (2002) who found an

Table 5. Cadmium concentration and its uptake by lettuce shoots as affected by irrigation and Cd treatments.

Irrigation interval (d)	Cd level of irrigation water (mg L^{-1}) 0	5	10	20	Mean
	Concentration (mg kg^{-1})				
1	6.90^{cd}*	7.37^{cd}	15.17^{b}	31.10^{a}	15.13^{A}
2	1.87^{d}	6.37^{cd}	6.10^{cd}	13.80^{bc}	7.03^{B}
4	3.17^{d}	7.89^{bcd}	6.93^{cd}	9.80^{bcd}	6.95^{B}
Mean	3.98^{C}	7.21^{BC}	9.40^{B}	18.23^{A}	
	Uptake (µg pot^{-1})				
1	30.00^{bc}	32.00^{bc}	62.33^{abc}	110.00^{a}	58.58^{A}
2	8.00^{c}	24.33^{c}	85.67^{ab}	46.33^{bc}	41.08^{AB}
4	11.33^{c}	31.33^{bc}	18.67^{c}	22.33^{c}	20.92^{B}
Mean	16.44^{B}	29.22^{AB}	55.56^{A}	59.56^{A}	

*Means within each plant parameter followed by the same letter are not statistically different at $P \leq 0.05$. Capital letters are used for the main effects.

increase in the Mn content of Cd treated lettuce plants. In contrast, Monteiro et al. (2009) found a significant decrease in Mn content of lettuce leaves treated with Cd under hydroponic conditions.

Overall, the behavior of Cd stressed lettuce was different with respect to the accumulation of nutrient elements. Cadmium concentrations of lettuce at 2 and 4-day irrigation intervals were substantially lower than the 1-day irrigation regime with 10 and 20 mg Cd l^{-1} (Table 5). Furthermore, lettuce markedly absorbed more Cd under wet irrigation than other irrigation frequencies. Increasing Cd levels of irrigation water increased Cd concentration of lettuce shoot, the differences being more pronounced at the higher Cd levels (Table 5). Cadmium concentrations were also slightly greater at 5 mg Cd l^{-1} compared to 10 mg Cd l^{-1} at 2 and 4-day irrigation intervals. Lettuce shoot cadmium ranged from 1.87 mg/kg, at the 2-day irrigation interval with no Cd application, to 31.10 mg kg^{-1} at the 1-day irrigation regime with 20 mg Cd l^{-1} in irrigation water. The Cd concentration in lettuce under control conditions of the experiment (1.87 to 6.9 mg kg^{-1}) was similar to those grown in urban garden soils (0.2 to 7.0 mg kg^{-1} dry matter) (Kabata-Pendias and Pendias, 1992) and those reported by Gérard et al. (2000) (0.74 to 4.25 mg kg^{-1}). These values were much higher than the levels (0.2 ppm) recommended by European Union for leafy vegetables (Mumba et al., 2008). The higher concentration of Cd in the plants shoot than the permission levels even under no Cd application, point out that there should be continuous monitoring of this metal in the lettuce grown in urban and agricultural soils before it to be harvested and available on the market in each harvest season.

Uptake of Cd (shoot Cd concentration multiplied by shoot dry weight) was increased with the increasing Cd level in irrigation water and a significant difference was observed at 10 and 20 mg l^{-1} Cd as compared with the control conditions. Uptake of Cd was also much higher under wet irrigation than other irrigation regimes. The results of Cd uptake indicate that lettuce could perform more efficient in soil Cd removal under no water stress and light soil pollution for phytoremediation purposes. However, economical use of plants for phytoremediation of polluted soils should be investigated with more research and field evaluations.

Soil water depletion and yield relating to water, soil and plant Cd

The depletion of soil water contents, soil matric potentials, plant and final soil Cd concentrations (Cd concentration of irrigation water multiplied by volume of applied water) and relative shoot dry weights of lettuce as a function of Cd levels at various irrigation frequency are shown in Table 6. As expected, the maximum depletion of soil water contents and minimum soil matric potentials occurred at the 4-day irrigation interval. Increasing Cd levels resulted in higher soil water depletion and lower soil matric potential at the 1 and 2-day irrigation intervals, while a reverse trend was observed at the 4-day irrigation frequency. These patterns were compatible with the ET rates (Table 3). In general, Cd accumulation at the 4-day irrigation frequency was lower than that at other irrigation regimes, due to the lower amount of applied water.

The means for all relative lettuce yields were 92.2, 100.0 and 81.3 % for 1, 2 and 4-day irrigation regimes, respectively (Table 6). Lines/curves of best fit were plotted from data in Table 6. Then the water and final soil Cd concentrations required to reduce shoot dry weight by 10 and 50% and also shoot tissue Cd concentration associated with 10 and 50% yield suppression were

Table 6. Depletion of soil water holding capacity, soil matric potentials, plant and soil Cd concentrations, relative shoot dry weights of lettuce and Cd accumulation index as affected by Cd-enriched water and irrigation intervals.

Irrigation interval (days)	Cd levels (mg l^{-1})	Depletion of soil water holding capacity (% soil matric potential, bar)	Accumulated soil Cd (mg kg^{-1})	Plant Cd (mg kg^{-1})	Relative shoot dry weight (%)	Cd accumulation index
1	0	41.1 (-0.52)	0.0	6.90	100.0	-
	5	41.1 (-0.41)	20.34	7.37	99.3	0.18
	10	41.3 (-0.52)	40.61	15.17	96.1	0.19
	20	41.2 (-0.52)	80.58	31.10	81.8	0.19
2	0	54.5 (-0.96)	0.0	1.87	100.0	-
	5	56.8 (-1.02)	14.16	6.37	100.0	0.22
	10	57.1 (-1.03)	28.14	6.10	111.0	0.11
	20	57.5 (-1.05)	56.38	13.80	89.0	0.12
4	0	83.5 (-3.33)	0.0	3.17	100.0	-
	5	82.5 (-3.27)	10.36	7.89	107.9	0.38
	10	82.9 (-3.24)	20.51	6.93	74.0	0.17
	20	80.0 (-2.85)	39.29	9.80	62.1	0.12

Table 7. Water, soil and plant tissue Cd concentrations for 10 and 50% growth reduction of lettuce shoot at various irrigation intervals.

Growth reduction (%)	Irrigation interval (days)		
	1	2	4
	Irrigation water Cd concentration (mg l^{-1})		
10	12.91	19.92	7.18
50	64.57	30.01	23.36
	Soil Cd concentration (mg kg^{-1})		
10	52.36	51.76	4.35
50	261.78	79.16	10.31
	Shoot Cd concentration (mg kg^{-1})		
10	23.59	12.89	6.89
50	50.20	20.25	11.77

calculated (Table 7). It is evident that the 10 and 50% growth suppression of lettuce occurred at lower Cd concentration of water, soil and plant at the 4-day irrigation interval relative to other irrigation frequencies (Table 7). In other words, these values indicate that lettuce is more sensitive to Cd under water stress conditions. The data in Table 7 also shows that lettuce was more tolerant to water Cd levels at the 2-day irrigation regime as compared to the 1-day irrigation regime for the 10% yield decrement. However, on the basis of 50% yield reduction, lettuce was more tolerant

Table 8. Pearson correlation coefficient[a] for parameters[b] measured in this research.

Elements	H	LN	LA	DW	ET	T	K	P	Cd	Zn	Mn	Cu	Fe	Cd_S
H	1													
LN	**0.65**	1												
LA	**0.66**	**0.98**	1											
DW	**0.71**	**0.64**	**0.6**	1										
ET	**0.59**	**0.85**	**0.85**	**0.55**	1									
T	**0.58**	**0.79**	**0.8**	**0.56**	**0.96**	1								
K	0.19	0.36	0.28	0.3	0.33	0.3	1							
P	0.38	**0.7**	**0.64**	**0.51**	**0.65**	**0.59**	0.46	1						
Cd	0.07	0.3	0.28	-0.06	0.45	0.44	0.13	0.2	1					
Zn	0.34	**0.5**	**0.45**	**0.43**	0.26	0.24	0.31	**0.53**	0.03	1				
Mn	0.08	0.31	0.3	0.08	**0.47**	**0.46**	**0.04**	0.37	**0.47**	0.35	1			
Cu	**0.46**	**0.45**	0.39	0.37	**0.53**	**0.58**	**0.26**	**0.44**	**0.52**	**0.47**	**0.52**	1		
Fe	0.31	0.27	0.28	0.05	0.35	0.38	-0.13	0.13	**0.54**	0.4	0.65	**0.73**	1	
Cd_S	-0.1	0.29	0.27	-0.26	0.32	0.33	0.05	0.09	**0.83**	0.03	0.37	**0.46**	**0.47**	1

[a] Bold and underlined figures represent significant values at P≤0.01 and P≤0.05, respectively. [b]Parameters include plant height (H), number of leaves (LN), leaf area (LA), shoot dry weight (DW), evapotranspiration and transpiration rates (ET and T, respectively), shoot elements (K, P, Cd, Zn, Mn, Cu and Fe) and final accumulated Cd of the soil (Cd_s).

to water, soil and plant Cd contents under optimum soil moisture conditions (1-day irrigation interval) as compared to other conditions.

The soil to plant transfer factor is one of the key components of human exposure to metal through the food chain and it is essential to assess this factor in wastewater irrigated crops. This factor is also evaluation in the phytoremediation aspects (Motesharezade et al., 2010). In the present study, cadmium accumulation index (Cd concentration in plant shoot/final accumulated Cd concentration of soil) was calculated (Bjerre and Schierup, 1985; Khan, 2001) at different Cd levels and irrigation intervals (Table 6). This index was greater under lower Cd concentration applied at higher irrigation frequency, which was mainly due to low Cd accumulation in the soil. Such inverse relationship between the transfer factor and total metal concentration in the soil was also reported by Khan *et al.* (2008) and Motesharezade et al. (2010). Gérard et al. (2000) indicated that the Cd concentration factor (Cd in plant/Cd in soil, in mg kg^{-1}) for lettuce was lower in soil containing Cd (8.9 to 25.4 mg Cd kg^{-1} soil) than the control soil (containing 0.6 mg Cd kg^{-1} soil). According to these findings, lettuce grown under water stress conditions and low Cd levels could be more hazardous to the consumers. In viewpoint of phytoremediation, lettuce would perform better under light to moderately Cd-polluted soils.

Correlation between measured parameters

The results of the linear correlation (Pearson correlation coefficient, r) between all growth parameters, elements detected in soil and plants are presented in Table 8. According to Table 8, there was no significant negative correlation between the measured parameters. Final accumulated soil Cd concentration (Cd_s) showed a strong correlation (*P≤0.01*) with the shoot Cd (the highest correlation, r=0.83), Cu and Mn concentration in plant shoot. In addition, the T rate of lettuce plants had a correlation with Cd_s (*P≤0.05*). Plant height had a significant correlation with P and Zn contents of shoot tissues at *P≤0.05* level of probability, but at *P≤0.01* with Cu concentration of shoots. Shoot dry weight of lettuce showed slightly stronger correlation with T rather than ET rates (*P≤0.01*) and was also correlated with P, Zn (*P≤0.01*) and Cu (*P≤0.05*) contents of plants shoot. The ET and T rates of plants presented significant correlation with K (only for ET), P, Mn, Cu, Fe and even Cd contents of plants shoot. The amount of Cu in shoot tissue was found to have a significant correlation with the amounts of all measured parameters. There was a significant correlation between Cd in plants and Cu, Mn, and Fe levels of lettuce shoot (*P≤0.01*). John et al. (1972) also found that concentrations of Cd in plants grown on Cd-treated soils had a significant positive correlation with Fe, Zn and Cu levels in the same plant part.

Plant tissue Cd concentration model

Plant uptake of heavy metals is a function of plant species, individual trace elements and soil characteristics. It may follow many different rate response functions including linear, asymptotic, no response, or even negative (Page et al., 1987). A hypothesis has been

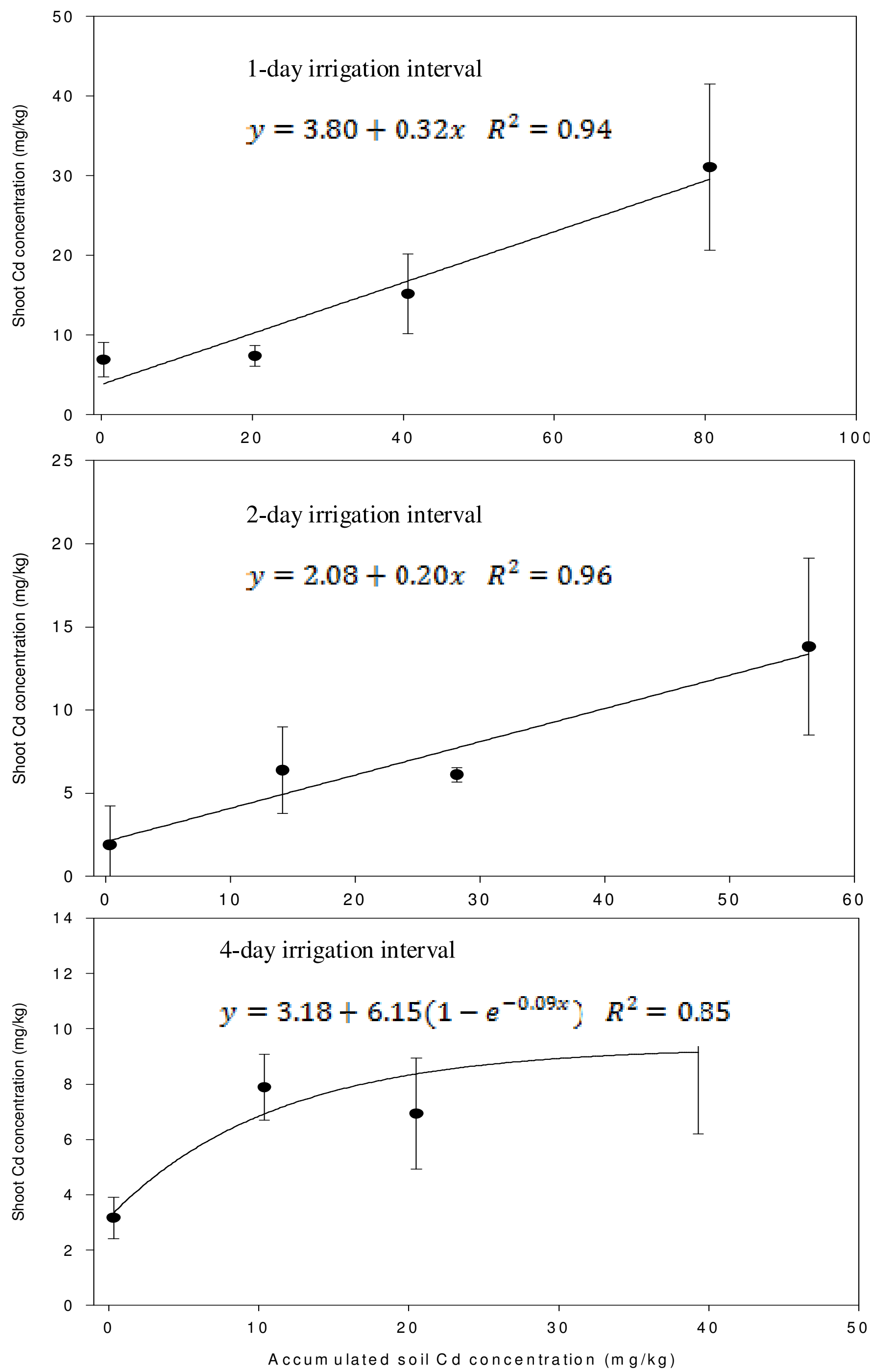

Figure 1. Cadmium concentration in lettuce shoot in relation to accumulated Cd in the soil (Cd concentration in irrigation water multiplied by volume of applied water) at various irrigation intervals (points show mean ± SD).

proposed to explain trace element uptake by various crops in which the uptake does not follow a linear trend with trace element application rate, but, would rather approach a maximum (plateau) as metal loading increased (Corey et al., 1987). We examined the above mentioned models for Cd concentrations in lettuce shoot as a function of final soil Cd concentrations (Cd concentration in irrigation water multiplied by volume of applied water) under different irrigation regimes (Figure 1). Grifferty and Barrington (2000) believed that plant uptake of trace metal could be different under various transpiration regimes. As Figure 1 shows the cadmium concentration in lettuce under a dry irrigation regime exhibited a plateau-type response that could be modeled with the Mitscherlich plateau equation (Logan and Chaney, 1987) as:

$$y = a + b(1 - e^{-cx}) \quad (1)$$

Where *y*=shoot metal concentration (mg kg^{-1}), *x*=soil metal concentration (mg kg^{-1}), *a*= *y* intercept or background plant tissue concentration where x=0 (mg kg^{-1}), *b*= asymptote (plateau) plant shoot concentration (mg kg^{-1}) above background and *c*= slope of the curve in the region between the asymptote and intercept. According to Figure 1 the maximum Cd concentration in lettuce would be 9.32 mg kg^{-1} at the 4-day irrigation interval. Lettuce had a linear Cd concentration response to increasing soil Cd levels by irrigation at 1 and 2-day irrigation frequencies:

$$y = a + b(x) \quad (2)$$

Where *b*=slope of the response line and other parameters as described before. It is evident from Figure 1 that the background Cd concentration of lettuce at the 1-day irrigation interval was higher than at the 2-day irrigation regime (line intercept). Also, Cd accumulation rate in lettuce was greater under the 1-day irrigation frequency than that obtained at the 2-day irrigation regime (slope of the line). Logan et al. (1997) showed that Cd concentration in lettuce linearly increased with sludge application, whereas a plateau-type response was observed for corn.

Simple regression models were tested using suitable correlated parameters (Cd_s (mg l^{-1}), ET and T (l)), according to Table 8, to predict shoot Cd concentration (*Cd*, mg kg^{-1}). Cadmium levels of irrigation water (Cd_w, mg l^{-1}) were also included in the models. The following best model according to the stepwise multiple regression analysis was obtained:

$$Cd = 3.474 + 0.486Cd_s - 0.713Cd_w$$

n=36, R^2=0.752, SE=4.22 (3)

This model explains 75.2% of the variance in the Cd concentration of lettuce. The ET and T terms were insignificant variables in the regression, suggesting that there were no real differences between irrigation treatments and consequent transpiration regimes in Cd uptake by lettuce.

Conclusion

The results of this study indicated that the presence of Cd in irrigation water could reduce lettuce growth. However, such reduction was not prominent due to the gradual increase in soil Cd, especially under optimum irrigation conditions. Moreover, no Cd toxicity symptoms were observed in the plant foliage. Lettuce was more sensitive to the application of Cd under water stress since growth suppression occurred at lower Cd levels in the water, soil and plant Cd concentrations. Shoot dry weight of lettuce was the most sensitive of the traits to Cd application through irrigation. In some cases, the application of cadmium in lower doses had a stimulating effect on lettuce growth. Moreover, Cd concentration of lettuce shoot was predicted by using a simple linear regression model regardless of ET and T regimes. The findings of this study also support the fact that lettuce could absorb considerable amounts of Cd higher than allowed values without any visual injury symptoms that could be a health risks to the consumers. In addition, there is need to extend the study for evaluating Cd uptake in field conditions.

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