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RESEARCH ARTICLE

The phytoremediation effect of *Medicago scutellata* (L.) Mill. on soils under Cd–water stress: a good choice for contaminated dry lands

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Abstract

Recently, due to increased drought risks, need for crops with higher water stress tolerance has increased strongly. Those crops have a wide range of uses such as supplying food as well as land restoration. *Medicago scutellata* (L.) Mill. is a Fabaceae widely cultivated for its capacity to produce high-quality forage. This study was designed as a factorial experiment based on a completely randomized design with three replications. Different cadmium levels were the first factor and included 0, 5, 25, 50, 100, and 125 mg kg^{-1}. Second factor was the drought stress, which had three levels (100, 75, and 50% feed consumption). According to the results, Cd uptake in different organs increased with increasing Cd levels up to 100 mg kg^{-1} while the water stress had a negative effect on Cd uptake by *M. scutellata*. Average concentration of Cd in the leaves, stems, and roots were 63.16, 30.12, and 20.45 mg kg^{-1}, respectively. The high value of translocation factor (TF) confirms the high ability of *M. scutellata* in translocation Cd from root to shoot. Fe, Zn, and K concentration of different organs significantly decreased with increasing Cd level. Fe and Zn concentration increased by increasing water stress levels in all organs and K concentration of roots decreased while in leaves and shoots increased by increasing water stress level. These results indicate that *M. scutellata* has a good ability for eliminating Cd from contaminated soil attribute to its powerful absorption and accumulation for Cd. It also showed a good performance under the co exposure of water stress and Cd indicated by accumulating proline and K in leaves.

Keywords Phytoremediation · Water stress · Animal consumption · Translocation factor

Introduction

From a global view after climate, soil crust is considered as one of the important parts of the human environment. Soil is not only considered as the living environment for terrestrial organism, especially human, but also a unique environment for the other living organisms such as plants. Soil contamination by inorganic pollutants such as heavy metals is one of environmental hazards that threaten human life today. Soil pollution causes loss of vegetation, reducing the plants growth and development, rangeland degradation, soil erosion, and finally desertification. Excessive accumulation of heavy metals in soil reduces biological activity and fertility resulting in reduced yield and quality loss of products that can be dangerous to human health or animal consumption (Stingu et al. 2012).

Cadmium (Cd) is the most prevalent pollution heavy metal in soil which is toxic to human and animal. Cadmium is used in some kinds of batteries, plastic byproducts, fertilizers, and pesticides (Amini et al. 2005). The total amount of Cd in agricultural soils should not exceed 0.4–0.5 mg kg^{-1} and higher values reflect the excessive use of phosphatic fertilizers as well as sewage sludge (Bjelkova et al. 2011; Stingu et al. 2012). During the past 30 to 40 years, using of fertilizers containing phosphoric acid (superphosphate and ammonium phosphate) to agricultural soil in the Iran has increased. Most of agricultural lands in Iran received 100–300 kg superphosphate per hectare annually for more than four decades (Jalali and Khanlari 2008). Amini et al.

Highlights
- Average concentration of Cd in leaves was 63.16 mg kg^{-1}, 6 times more than standard limits.
- Fe, Zn, and K concentration of different organs significantly decreased with increasing Cd level
- It also showed a good performance under the co-exposure of water stress and Cd.
- It cannot be a good choice for phytoremediation due to the further transfer into the food chain

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(2005) assessed 255 top soil samples from central Iran; they reported the total Cd concentration in most of tested samples were more than recommended thresholds of 0.8 mg kg^{-1}. Therefore, ways to remove or degrade this pollutant has been a great concern. Phytoremediation is a green, simple, eco-friendly, sustainable, and cost-effective remediation technology that uses plants to remove and degrade contaminants from the soil. In this regard, *Medicago* species has been widely adopted for the phytoremediation of polluted soils with heavy metals (Lopez et al. 2005; Fan et al. 2008; Wei and Pan 2010). *Medicago sativa* L. has shown the ability to hyperaccumulate heavy metals in its tissues (Angle and Linacre 2005) and to grow well in highly contaminated soils (Amer et al. 2013).

Arid and semi-arid regions comprise more than 60% of Iran (Fakhari and Sadeghi 2016). The average annual rainfall in Iran is about 251 mm and recently, due to increased drought risks, need for crops with higher water stress tolerance has increased strongly (Sadeghi and Robati 2015). *Medicago scutellata* (L.) Mill. is a Fabaceae widely cultivated for it capacity to produce high-quality forage (Fakhari and Sadeghi 2016). Annual *Medicago* is found in almost all regions of Iran. More than 556,000 ha of *Medicago* species are grown as continuous cropping in Iran. This suggests that these plants are appropriate for Iranian pastures. *Medicago* has several annual species, such as *Medicago rigidula* L., *Medicago polymorpha* L., and *Medicago scutellata* L., which can produce high numbers of seeds. It establishes relatively easily but its early growth in autumn is rapid and erect, making it susceptible to overgrazing (Sadeghi and Fakhari 2016). The barrel medic is adapted to different soil texture types, from sandy to clay, and particularly to well-drained neutral-to-alkaline soils with a pH of 6 to 8; it is suited to warm temperate conditions, especially Mediterranean-type climates, has 250–600 mm annual rainfall with dry hot summers and mild moist winters, and is intolerant of winter frosts. It has poor regrowth for a second harvest, but has a good ability to self-regenerate from seeds present in the soil seed bank (Fakhari and Sadeghi 2016). Legumes are a good choice for phytoremediation since they have the capability of N_2 fixation (Amer et al. 2013). A review of current literature indicated that no research, to best of our knowledge, has yet tested the co-exposure of Cd and water stress on the phytoremediation ability of *M. scutellata.* Therefore, the present study was designed with the intention to evaluate the biochemical response of *M. scutellata* to Cd–water stress co-exposures and its utilization potential for phytoremediation.

Materials and methods

Study site and plant material

This research was conducted as a factorial experiment based on completely randomized design in a greenhouse at the College of Agriculture, Shiraz University (29° 43′ N and 52° 35′ W), Shiraz, Iran, during 2014. The temperatures of day and night in the greenhouse were set at 28 and 22 °C. The seeds of *Medicago scutellata* were prepared from Pakan Bazr Co. and stored at 5 °C.

Experimental design

Silty clay loam soil collected from the surface layer (0–30 cm) of soils (fine mixed, mesic Typic Calcixerpets soil) in the College of Agriculture was selected for the pot experiments. Some of soil characteristics are presented in Table 1 and the heavy metals of original soil were as follows: absorbable Cd, 0.2 mg kg^{-1}; absorbable Pb, 0.4 mg kg^{-1}; absorbable Cu, 1.31 mg kg^{-1}; and absorbable Ni, 0.15 mg kg^{-1}.

Silty clay loam soil gathered from the top layer (0–30 cm) of soils in the College of Agriculture was selected for the pot experiments. Some of soil characteristics are presented in Table 1. Soil samples were air dried and grounded to pass 2-mm nylon sieve prior to use. The soils were spiked with sulfuric acid cadmium salt at the rate of 0, 5, 25, 50, 100, and 125 mg kg^{-1} and incubated for 2 weeks. After incubation, each plastic pot was filled with 4 kg of the treated soil. Ten seeds were planted in each pot. After emergence, six plants per pot were kept. Pots were watered at field capacity. Water stress was imposed on the plants 20 days after planting by applying different irrigation regimes (100% as control, 75% and 50% field capacity (FC)) for 3 months. At the end of the trial, different plant organs (roots, shoot, leaves) were separated and sampled for analysis.

Analysis and measurement

The harvested plant parts were washed completely with distilled water, dried at 70 °C, and weighed. For the measurement of mineral elements, plant samples were dried at 550 °C for 4 h to obtain ashes, then extracted with 2 M HCl, filtered through Whatman No. 42 filter paper, and analyzed for Cd, Fe, and Zn by an atomic absorption spectrophotometer (model Perkin Elmer 4110) (Azizian et al. 2011). Potassium concentrations were determined by flame photometry (INESA, FP6410, Shanghai, China). Available metals were determined using diethylene-triamine-pentaacetic acid (DTPA) buffered at pH 7.3. Cadmium was determined by atomic absorption spectrophotometry (AAS).

For the proline measurements, plant's part samples were grounded in liquid nitrogen. The concentration of proline was estimated based on the acid-ninhydrin method (Bates et al.

Table 1 Chemical and physical properties of the pot soil

Soil texture	Sand (%)	Silt (%)	Clay (%)	EC (dS m^{-1})	pH
Silt clay loam	16	48	36	0.9	7.1

Table 2 Analysis variance of the effect of cadmium [Cd] and water stress on measured factors of *M. scutellata*

Source of variance	Degree of freedom	Mean squares							
		Root [Cd]	Stem [Cd]	Leaves [Cd]	Soil [Cd]	TF	Root [Fe]	Stem [Fe]	Leaves [Fe]
Cd	5	2674.38**	4204.12**	24905.90**	1050.85**	23.13**	1076071.1**	294258.6**	47704.8**
Water stress	2	780.22**	531.57**	7035.04**	6.97**	0.49 ^{ns}	174645.08**	41365.02**	62278.5**
Cd × water stress	10	133.77**	78.70**	931.92**	2.30**	3.10**	5231.34**	5667.20**	1472.6**
Error	36	1.16	0.52	13.67	0.046	2.08	138.88	67.48	36.78
Coefficient variance		5.27	2.395	5.85	2.41	29.26	1.65	1.74	1.07
Source of variance	Degree of freedom	Mean squares							
		Root [Zn]	Stem [Zn]	Leaves [Zn]	Root [K]	Stem [K]	Leaves [K]	Proline	Crude protein
Cd	5	1854.09**	1367.68**	1586.45**	3860296.3**	6408185.19**	1798611.11**	2.30**	289.8**
Water stress	2	1334.8**	973.45**	351.33**	7744629.63**	2667407.41**	10371666.6**	162**	66.06**
Cd × water stress	10	80.06**	48.45**	12.38**	56407.41^{ns}	12074.07**	55444.44**	1.93^{ns}	0.07
Error	36	1.35	1.03	0.67	12592.59	8703.7	11666.7	1.64	0.17
Coefficient variance		2.64	2.45	2.23	2.81	2.91	1.98	11.69	2.59

TF translocation factor, *ns* non-significant

* Significant at ($p \leq 0.05$)

** Significant at ($p \leq 0.01$)

1973) by spectrophotometer with minor modifications (Fakhari and Sadeghi 2016).

Crude protein was estimated from nitrogen by multiplying total N by 6.25, according to the Kjeldahl method described in

Table 3 Cadmium concentration (mg kg^{-1}) concentration in different organs (root, shoot, leaves) of *M. scutellata* at various water stress and cadmium levels

W.S.L (FC%)	Cadmium level (mg kg^{-1})						
	0	5	25	50	100	125	Mean
Cd in roots							
50%	0.400k	5.466j	10.366i	14.866g	27.566e	27.133e	14.295C
75%	0.866k	9.366i	12.533h	15.166g	41.333c	38.766d	19.666B
100%	1.083k	10.566i	15.330g	23.266f	59.200a	54.966b	27.394A
Mean	0.783F	8.466E	12.733D	17.776C	42.7A	40.288B	
Cd in shoot							
50%	0.466l	10.133j	13.566i	32.400f	41.220d	55.533b	25.552C
75%	1.206kl	13.490i	19.233g	37.133e	45.666c	55.433b	28.695B
100%	1.80k	15.366h	33.133f	45.920c	64.466a	56.110b	36.134A
Mean	1.157F	12.998E	21.978D	38.487C	50.451B	55.693A	
Cd in leaves							
50%	0.533k	14.333j	18.770j	78.366e	115.510d	56.633g	47.359C
75%	2.190k	17.750j	49.433h	81.750e	127.910c	61.766fg	56.803B
100%	3.110k	28.990i	66.280f	156.530b	180.780a	76.280e	85.332A
Mean	1.948F	20.362E	44.830D	105.551B	141.403A	64.896C	
Cd in soil							
50%	0.240l	2.333j	5.300g	8.300e	8.700d	31.933a	9.468A
75%	0.173l	2.100jk	4.833h	7.760f	8.330e	30.616b	8.970B
100%	0.107l	1.7830k	4.366i	7.600f	8.217e	27.310c	8.230C
Mean	0.173F	2.072E	4.833D	7.889C	8.417B	29.953A	

W.S.L water stress level

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

Table 4 The effect of cadmium [Cd] and water stress on Translocation factor (TF) of *M. scutellata*

W.S.L (FC%)	Cadmium level (mg kg^{-1})						
	0	5	25	50	100	125	Mean
50%	1.66bcdef	1.92abcde	1.29fgh	2.18ab	1.49defgh	2.02abc	1.76A
75%	1.61cdefg	1.45efgh	1.52cdefgh	2.37a	1.11gh	1.43efgh	1.58A
100%	1.76bcdef	1.46defgh	2.17ab	1.97abcd	1.09gh	1.01h	1.58A
Mean	1.680B	1.61B	1.66B	2.17A	1.23C	1.49CB	

W.S.L water stress level

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

the Association of Official Analytical Chemists (2002). The translocation factor (TF) from root to shoot was calculated as follows (Su et al. 2014):

$$\mathrm{TF} = \frac{[\mathrm{Cd}]\mathrm{shoot}}{[\mathrm{Cd}]\mathrm{root}}$$

Statistical analysis

This experiment was conducted as a factorial experiment based on a completely randomized design with three replications. Analysis was performed with SAS Program Version 9.1.3 (2004). Different cadmium levels were the first factor and included 0, 5, 25, 50, 100, and 125 mg kg^{-1}. Second factor was the water stress, which had three levels (100, 75, and 50% FC).

Results

Some physiochemical properties of the selected soil for the pot experiment are presented in Table 1. The tested soil has a medium texture (silty clay loam) with no acidity and salinity problem and its cadmium (Cd) level was very low.

ANOVA (Table 2) showed that the interaction effect of the cadmium application and water stress ([CD] × water stress) was significant on all the measured factors of *Medicago scutellata*, with the exception of potassium concentration in root and proline content, at 1% probability level. However, the main effect of cadmium application and water stress was significant at 1% probability level about potassium concentration in root and proline content (Table 2).

According to Table 3, with increasing the level of cadmium of up to 100 mg kg^{-1}, Cd concentration in roots, stems, and

Table 5 Iron [Fe] concentration in different organs (root, shoot, leaves) of *M. scutellata* at various water stress and cadmium levels

W.S.L (FC%)	Cadmium level (mg kg^{-1})						
	0	5	25	50	100	125	Mean
Fe in root							
50%	1228.36a	1052.66c	1001.33d	645.86i	495.06jk	499.00j	820.38A
75%	1226.66a	947.86e	836.00g	476.33kl	284.33m	303.30m	679.08B
100%	1074.00b	911.43f	783.96h	475.36l	256.00n	284.33m	630.85C
Mean	1176.34A	970.65B	837.76C	532.51D	345.13F	362.22E	
Fe in shoot							
50%	887.133a	682.433c	512.066g	355.433j	338.333j	351.00ij	521.067A
75%	720.366b	616.666e	501.966g	341.066j	321.666k	311.333kl	468.844B
100%	637.33d	578.633f	414.266h	311.366kl	304.133l	306.21l	425.322C
Mean	748.278A	625.911B	476.1C	335.956D	321.378E	322.844E	
Fe in leaves							
50%	710.966a	692.4b	662.4c	618.533e	530.733j	534.00ij	624.839A
75%	659.566c	633.4d	541.933hi	509.400k	495.066l	500.033kl	556.567B
100%	586.433f	570.666f	550.533h	501.533kl	414.233m	423.00m	507.733C
Mean	652.322A	632.156B	584.956C	543.156D	480.011E	458.678E	

W.S.L water stress level

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

Table 6 Zinc [Zn] concentration in different organs (root, shoot, leaves) of *M. scutellata* at various water stress and cadmium levels

W.S.L (FC%)	Cadmium level (mg kg^{-1})						
	0	5	25	50	100	125	Mean
Zn in root							
50%	81.366a	56.633c	51.433d	39.666f	28.033i	57.500c	52.439A
75%	59.610b	56.966c	47.133e	37.533g	26.366ij	39.4fg	44.503B
100%	56.500c	45.733e	32.600h	25.133j	19.780k	31.65h	35.233C
Mean	65.828A	53.111B	43.722C	34.111D	24.728E	42.85C	
Zn in shoot							
50%	72.233a	54.8cd	48.566f	38.200h	26.33k	53.233de	48.889A
75%	57.400b	53.100e	43.466g	36.033i	22.5l0f	35.330i	41.306B
100%	55.133c	44.833g	30.566j	25.900k	18.43m	35.233j	34.183C
Mean	61.589A	50.911B	40.867C	33.378E	22.411F	39.6D	
Zn in leaves							
50%	64.833a	50.533d	42.800e	33.266g	22.466k	34.866f	41.461A
75%	56.066b	42.666e	32.566gh	31.533h	19.533l	32.133gh	35.750B
100%	53.100c	41.633e	29.466i	27.666j	16.330m	28.500ij	32.767C
Mean	58.00A	44.944B	34.944C	30.80E	19.433F	31.833D	

W.S.L water stress level

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

leaves increased but then decreased at the level of 125 mg kg^{-1} and the Cd concentration of different organs (roots, stems, and leaves) reduced with increasing water stress level from 100 to 50% FC (Table 3). The highest Cd average concentration of root was observed at 100 mg kg^{-1} and 100% FC which increased 54-fold compared with the control (0 mg kg^{-1} cadmium and 100% FC). Cadmium concentration of roots from 100 to 125 mg kg^{-1} decreased 7% (Table 3). The highest Cd average of shoot and leaves belonged to 100 mg kg^{-1} and 100% FC which increased 35-fold and 58-fold compared with the control, respectively (Table 3). The average concentrations of Cd in leaves, stems, and roots were 63.16, 30.12, and

Table 7 Potassium [K] concentration in different organs (root, shoot, leaves) of *M. scutellata* at various water stress and cadmium levels

W.S.L (FC%)	Cadmium level (mg kg^{-1})						
	0	5	25	50	100	125	Mean
K in root							
50%	3800c	3733.33c	3033.33f	2400.00h	153.33j	2533.33h	2838.89C
75%	4000b	4033.33b	3466.66d	2833.33g	1833.33i	2800.00g	3161.11B
100%	4466.66a	4533.33a	3800.00c	3133.33f	2400.00h	3300.00e	3605.56A
Mean	4088.89A	4100A	3433.33B	2788.89C	1922.22D	2877.78C	
K in shoot							
50%	5400.00a	5433.33a	4600.00c	4166.66e	3766.66f	4400.00d	4627.78A
75%	4833.33b	4766.66bc	3766.66f	3600.00f	3000.00gh	4100.00e	4011.11B
100%	4100.00e	4066.66e	3000.00gh	2900.00h	2700.00i	3133.33g	3316.67C
Mean	4777.78A	4755.56A	3788.89B	3555.56C	3155.56D	3877.78B	
K in leaves							
50%	7566.66a	7600.00a	7033.00b	5400.00e	3800.00j	5900.00d	6216.67A
75%	6800.00c	6766.66c	6000.00d	4800.00g	3033.33k	5000.00f	5400.00B
100%	6033.00d	6000.00d	5066.66f	4100.00i	2500.00l	4500.00h	4700.00C
Mean	6800.00A	6788.89A	6033.33B	4766.67D	3111.11E	5133.33C	

W.S.L water stress level

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

Table 8 Proline and crude protein content of *M. scutellata* at various water stress and cadmium levels

W.S.L (FC%)	Cadmium level (mg kg^{-1})						
	0	5	25	50	100	125	Mean
Proline (μM/g dry weight)							
50%	11.37c	12.31c	12.81bc	14.50ab	14.833ab	15.13a	13.495A
75%	7.53de	11.40c	12.11c	12.90bc	13.13abc	13.14abc	11.747B
100%	6.47e	6.86de	7.166de	8.267d	8.300de	8.83d	7.651C
Mean	8.459D	10.19C	10.69BC	11.889AB	12.089A	12.456A	
Crude protein (%)							
50%	19.76c	19.20c	17.50d	13.43f	8.26i	6.46j	14.106C
75%	21.43b	21.60b	19.160c	15.16e	10.33h	7.96i	15.870B
100%	23.30a	23.60a	21.30b	17.10d	12.56g	10.16h	17.933A
Mean	21.50A	21.178A	19.32B	15.23C	10.39D	8.20E	

W.S.L water stress level

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

20.45 mg kg^{-1}, respectively. In other words, the average concentrations of Cd in leaves and shoots were 3.08 and 1.47 times higher than roots, respectively. The highest amount of Cd in soil was observed at 125 mg kg^{-1} Cd under 50% FC which was 298 times higher than control (0 mg kg^{-1} cadmium and 100% FC) (Table 3).

The highest translocation factor (TF) was observed at the concentration of 50 mg kg^{-1} under 75% FC while the lowest one, with 50% reduction, was observed at 125 mg kg^{-1} under 100% FC (Table 4). In all treatments, the TF was more than 1 which reflected the high ability of *M. scutellata* in translocation Cd from root to shoot (Table 4).

According to Table 5, the average of iron (Fe) concentration in different organs (root, shoot, and leaves) decreased with the increasing Cd level from 0 to 125 mg kg^{-1}. Under water stress, the Fe concentration of different organs significantly increased. In all tested organs, the highest Fe concentration belonged to the 0 mg kg^{-1} Cd and 50% FC while the lowest one was observed at 100 mg kg^{-1} and 100% FC (Table 5).

Table 6 showed that Zinc (Zn) concentration in different organs increased significantly with the increasing water stress level from 100 to 50% FC. Similar to Fe concentration, in all organs (roots, shoots, and leaves), the highest Zn concentration belonged to the 0 mg kg^{-1} Cd under 50% FC while the lowest one was observed at 100 mg kg^{-1} and 100% FC. Also, the average of Zn concentration in different organs decreased with increasing Cd level from 0 to 125 mg kg^{-1} (Table 6).

Potassium (K) concentration of different organs significantly decreased with the increasing Cd level up to 100 mg kg^{-1} but increased at 125 mg kg^{-1} compared with 100 (Table 7). K concentration of roots decreased while in leaves and shoots increased by increasing water stress level. The highest amount of K concentration in all organs belonged to the 5 mg kg^{-1} Cd under 50% FC while the lowest one was observed at 100 mg kg^{-1} Cd and 100% FC in the case of shoots and leaves and 100 mg kg^{-1} and 50% FC in the case of roots (Table 7).

According to Table 8, proline content increased significantly with the increasing Cd level and water stress. The highest amount of proline content was observed at 125 mg kg^{-1} Cd under 50% FC while the lowest one belonged to the control treatment. Unlike proline, the percentage of crude protein decreased significantly with the increasing Cd level and water stress. The highest percentage of crude protein was observed in control treatment while the lowest belonged to 125 mg kg^{-1} Cd and 50% FC. By consuming Cd, protein content decreased significantly (Table 8).

The result of correlation analysis (Table 9) showed that the highest correlation coefficient was observed between Zn concentrations in roots with Zn concentration in shoot (0.991**). Protein had negative and significant correlation with proline and Cd concentration in different organs; however, it showed a positive and significant correlation with other measured elements (Fe, Zn, and K). K showed a negative and significant correlation with Cd and positive and significant correlation with Zn and Fe concentration in different organs. Fe and Cd showed a negative and significant correlation within different organs (Table 9).

Discussion

In this study, the effect of different Cd levels of soil on the biochemical response of *Medicago scutellata* was investigated under water stress. According to the results, Cd uptake in different organs increased with increasing Cd levels up to 100 mg kg^{-1} while the water stress had a negative effect on Cd uptake by *M. scutellata*. These results are supported by

Table 9 Correlation coefficient between the measured variables

	TF	Protein	Proline	K in leaves	K in shoot	K in root	Zn in leaves	Zn in shoot	Zn in root	Fe in leaves	Fe in shoot	Fe in root	Cd in leaves	Cd in shoot	Cd in root
Cd in root	0.300*	− 0.744**	0.087ns	− 0.812**	− 0.690**	− 0.612**	− 0.798**	− 0.762**	− 0.732**	− 0.864**	− 0.771**	− 0.900**	0.759**	0.906**	
Cd in shoot	− 0.135ns	− 0.825**	0.231ns	− 0.811**	− 0.707**	− 0.698**	− 0.881**	− 0.893**	− 0.705**	− 0.865**	− 0.901**	− 0.965**	0.816**		
[Cd] leaves	− 0.115ns	− 0.559**	0.072ns	− 0.925**	− 0.509**	− 0.640**	− 0.881**	− 0.893**	− 0.705**	− 0.778**	− 0.801**	− 0.833**			
Fe in root	0.178ns	0.810**	− 0.254ns	0.868**	0.751**	0.725**	0.755**	0.843**	0.815**	0.893**	0.931**				
Fe in shoot	0.035ns	0.726**	− 0.252ns	0.821**	0.783**	0.708**	0.928**	0.876**	0.869**	0.836**					
Fe in leaves	0.241ns	0.571**	0.093ns	0.875**	0.877**	0.459**	0.844**	0.828**	0.815**						
Zn in root	0.145ns	0.452**	0.045ns	0.904**	0.890**	0.570**	0.928**	0.991**							
Zn in shoot	0.168ns	0.501**	− 0.001ns	0.926**	0.894**	0.618**	0.946**								
Zn in leaves	0.156ns	0.627**	− 0.190ns	0.894**	0.845**	0.710**									
K in root	0.084ns	0.855**	− 0.647ns	0.657**	0.393**	1									
K ni shoot	0.145ns	0.320*	0.245ns	0.393**											
K in leaves	0.227ns	0.569**	0.017ns												
Proline	0.168ns	− 0.606**													
Protein	0.155ns														

* Correlation is significant at $p \leq 0.05$

** Correlation is significant at $p \leq 0.01$

Shen et al. (2002) who founded that Cd uptake of corn (*Zea mays* L.) increased with the increasing Cd level of soil. Azizian et al. (2011) also found the same results about Cd concentration of corn (*Zea mays* L.) and oat (*Avena sativa* L.) under different irrigation treatments. Gardea-Torresdey et al. (2000) reported that nickel uptake of (*Medicago sativa* L.) increased with increasing soil nickel and shoots showed to have more nickel concentration than the roots. In contrast, Bjelkova et al. (2011) observed that the most Cd was accumulated by roots in linseed (*Linum usitatissimum* L.) cultivars. These phenomena may be due to a difference in chemical functional groups in the plants species roots and shoots.

Suitable plants for phytoremediation should be able to prevent the transportation of heavy metals to the aerial parts and keep them at the root level thus preventing them from entering to the food chain (Stingu et al. 2012), or they are grown for industrial, non-food purpose such as using in textile or furniture industry. In our study, the average concentrations of Cd in leaves and shoots were 3.08 and 1.47 times higher than roots, respectively. The high value of translocation factor (TF) confirms the high ability of *M. scutellata* in translocation Cd from root to shoot. Since the main application of *M. scutellata* is for forage production for feeding animals, this factor should be taken into consideration. Studies have shown that cattle which graze on metal contaminated plants will accumulate the toxic metals in their bodies which could then be passed to humans (Chamberlain and Miller 1982). The standard limit of Cd is 0.1 mg kg^{-1} of the weight of the plant for human consumption and 10 mg kg^{-1} for animal consumption, while in our study, it was 63.16 mg kg^{-1} just in leaves.

In our study, Cd concentration of different organs (roots, stems, and leaves) reduced with increasing water stress level; similar findings were reported by Angle et al. (2003) who observed higher metal uptake as well as biomass production at higher soil moisture.

Fe, Zn, and K concentration of different organs significantly decreased with the increasing Cd level. Wong et al. (1986) observed a significant reduction in Zn and Fe by increasing Cd level and stated it can be due to the antagonistic effects of accumulation of Cd in plant tissues on the uptake of these essential metals. Yildiz (2005) reported that Zn concentration in tomato (*Solanum lycopersicum* L.) and corn (*Zea mays* L.) plant decreased by increasing Cd level. Also, similar findings were reported by Veselov et al. (2003) and Sandalio et al. (2001) in wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) plants. Cd toxicity in plants can be due to the interaction with essential nutrients for the plant which cab affect the balance of nutrients and reduced plants fertility. Cadmium and zinc are chemically similar; hence, they are competing with each other for absorbing by plants (Nazar et al. 2012).

Fe and Zn concentration increased by increasing water stress level in all organs and K concentration of roots decreased while in leaves and shoots increased by increasing

water stress level. These results are in line with the findings of Alizadeh (2010) in corn (*Zea mays* L.). Osmotic advantage of K in improving cell water saturation under water stress is well documented which can explain the reason of its translocation from roots to the aerial parts under water stress (Cakmak 2005; Zhao 2000).

According to the result, proline content increased significantly with increasing Cd level and water stress. Proline acts as an osmolyte as well as an osmoprotectant under stressed condition which helps in protecting of enzymes, biological membranes, and photosynthetic apparatus from oxidative damages (Batish et al. 2006). Since proline supplies energy for growth and survival, its accumulation during stress helps the plant to tolerate the stressed condition better and easier (Gill and Toteja 2010). The observations made in this study are parallel with that in earlier studies (Shah et al. 2001; Mehta and Gaur 1999; Parida et al. 2008). According to the results, protein content decreased significantly by increasing Cd level. Cd alters the conformation of proteins such as enzymes and transporter proteins due to its strong affinity as ligands to sulfhydryl and carboxylic group (Nazar et al. 2012). Studies have shown the activity of reactive oxygen species during stress can damage biological molecules such as proteins and lipids (Molassiotis et al. 2006).

Conclusion

The forage crop *M. scutellata* can be considered as a Cd accumulator plant species. It also showed a good performance under the co-exposure of water stress and Cd indicated by accumulating proline and K in leaves. Its translocation factor was more than 1 which reflected the high ability of *M. scutellata* in translocation Cd from root to shoot. The amount of Cd accumulation in leaves is about 63 mg kg^{-1} which is 6 times more than standard limit for animal consumption. Since the main application of *M. scutellata* is for forage production for feeding animals and it does not have any remarkable non-food uses, it cannot be a good choice, due to the further transfer into the food chain. However, the ability enhancement of Cd translocation to *M. scutellata* shoot in higher concentrations of soil Cd indicates a great performance of the plant for Cd phytoextraction and could be introduced as Cd hyperaccumulator plant.

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