

Acta Sci. Pol. Hortorum Cultus, 18(2) 2019, 87–96

a.media.pl ISSN 1644-0692

e-ISSN 2545-1405

DOI: 10.24326/asphc.2019.2.8

ORIGINAL PAPER

Accepted: 21.08.2018

# GROWTH CHARACTERISTICS OF ORNAMENTAL JUDAS TREE (*Cercis siliquastrum* L.) SEEDLINGS UNDER DIFFERENT CONCENTRATIONS OF LEAD AND CADMIUM IN IRRIGATION WATER

Mansoure Hatamian<sup>1</sup>, Abdolhossein Rezaei Nejad<sup>1⊠</sup>, Mohsen Kafi<sup>2</sup>, Mohammad Kazem Souri<sup>3</sup>, Karim Shahbazi<sup>4</sup>

<sup>1</sup> Department of Horticulture, Faculty of Agriculture, Lorestan University, Khorramabad, Iran

<sup>2</sup> Department of Horticultural Sciences, Faculty of Agriculture, University of Tehran, Karaj, Iran

<sup>3</sup> Department of Horticultural Sciences, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

<sup>4</sup> Soil and Water Research Institute, Karaj, Iran

## ABSTRACT

Landscape plantations are significantly water demanding in many parts of the world, particularly in dry regions. Adequate water supply is the main limiting factor behind landscape development, maintenance and beauty in such environments. On the other hand, monitoring the growth response of ornamental and landscape plants to irrigation water containing heavy metals can be useful in management and application of municipal wastewaters to these plantations instead of application in edible vegetable crops production, which is common in many urban areas. In the present study, one year old seedlings of Judas tree (Cercis siliquastrum) were irrigated for two years with water containing 0, 15 or 30 mg  $L^{-1}$  of lead (Pb) in absence or presence of cadmium (0 or 5 mg  $L^{-1}$ ) in a factorial design, and under greenhouse conditions. Heavy metal treatments had no significant effect on leaf SPAD value; however, plants treated with Pb15Cd0 combination had significantly higher SPAD value than Pb30Cd5 combination that showed the lowest leaf SPAD value. Increasing the lead and cadmium levels of irrigation water increased the number of chlorotic and necrotic leaves of plant than control, whereas it reduced the average leaf area and new shoot growth. The control plants or plants treated with lower level of heavy metals showed the highest leaf area and new shoot growth. Leaf photosynthesis rate was significantly reduced in all heavy metal treated plants than control plants, as the lowest amount was in Pb30Cd5 combination treatment. All heavy metal treatments showed higher leaf soluble carbohydrates and proline content than in control plants. Leaf soluble carbohydrates were highest in Pb30Cd0 and Pb30Cd5 treatments, and leaf proline was highest in Pb30Cd5 and Pb15Cd5 treatments. The results indicate that Judas tree is a relatively tolerant species to high concentrations of Pb and Cd in root medium added through the irrigation water, and long term dual application of these heavy metals can have additive harmful effects on plant growth.

Key words: heavy metal toxicity, landscape, proline, soluble carbohydrates, water quality, wastewater irrigation

## INTRODUCTION

Landscape is an important component of the environment in modern human life. In many arid regions, there is no adequate water supply for agriculture production or maintenance of landscape plants. In many parts of Iran, the quantity and quality of water for plant irrigation are becoming an increasingly limiting factor



<sup>&</sup>lt;sup>IM</sup> rezaeinejad.h@lu.ac.ir

[Souri et al. 2018]. On the other hand, in such regions and around metropolitan cities, wastewater represents a significant irrigation source for plantations; however, it has low quality with relatively high levels of heavy metals [Rattan et al. 2005]. Wastewaters may contain different levels of heavy metals; however, they are generally high in lead, cadmium and some other metals such as As, Zn and Cu [Rattan et al. 2005, Singh et al. 2010, Souri et al. 2018]. Plants generally have different responses to heavy metals particularly under high application rates [Ewais 1997, Seregin and Ivanov 2001, Bosiacki and Zieleziński 2011]. Lead and cadmium are the major toxic heavy metals in the environment with a long history of toxicity. Among non-nutrient heavy metals, Cd and Pb pollutions are the most widespread, and most of Pb and Cd contaminations are resulted from human activities [Seregin and Ivanov 2001, Bosiacki 2008].

Reduction in plant growth due to lead or cadmium treatment has been reported in different studies [Ewais 1997, An et al. 2004, John et al. 2008, Małecka et al. 2008, Shafiq et al. 2008]. Reduction in biomass production and its components including leaf area, plant height, photosynthesis and flowering pattern due to lead and cadmium may significantly reduce the nutritional and economical value of edible plants [Rattan et al. 2005, Souri et al. 2018]; however, such effects may be considerably less important in ornamental landscape plants. In addition, cultivation of deep rooted perennial plants can be a new approach for correction of heavy metals or chemicals contaminated soils [Vandecasteele et al. 2005, Bosiacki 2008]. This could be a well adopted approach in sustainable management of environmental pollutions. Nevertheless, in dry regions, adverse climatic conditions including salinity and drought stresses can limit full advantages of such strategies [Frick et al. 1999]. Some plant species, using different mechanisms of precipitation, infiltration, microbial or plant uptake, can significantly improve the soil or water quality, when they are polluted with waste and contaminant materials [Raskin et al. 1997, Krämer 2005].

In many cases, the wastewaters including those produced by industrial activities, are used for fresh vegetable production that is a real threat for human health [Singh et al. 2010]. Application of wastewater to ornamentals and landscape vegetations represents a useful approach for application and management of refined wastewater. However, the heavy metal load of the refined wastewater is also quite important regarding the long term effects on soil quality and plant growth.

Judas tree is a famous ornamental plant in arid and semi-arid regions with good tolerance to low soil water conditions. It has a very deep rooting pattern that represents it as a suitable shrub or tree for arid and semi-arid regions. This plant is a good candidate for strategies regarding the soil correction in dry climates as Xeriscape. Our hypothesis is that besides drought tolerance, the Judas tree is probably tolerant to heavy metal toxicity. However, little information is available on the effects of a long term application of heavy metals *via* irrigation water on growth characteristics of this plant species. In the present study, the growth response of ornamental Judas tree (*Cercis siliquastrum* L.) to different concentrations of lead and cadmium in irrigation water was evaluated.

# MATERIAL AND METHODS

The study was conducted during 2016 and 2017 and under protected greenhouse conditions. A soil mixture of one third leaf litter compost, one third field soil and one third fine sands was prepared. The soil was precisely mixed and passed through 2 mm sieve. The physicochemical characteristics of the soil used in the experiment have been presented in Table 1. Black plastic pots of 30 cm height and 20 cm diameter (about 5 liter volume) were filled with the soil mixture. One year old seedlings of Judas tree (Cercis siliquastrum) were then transferred to the pots on 15 March 2016. For the first three weeks after transplanting, plants were irrigated with general tap water without Pb or Cd treatment. Thereafter, different lead and cadmium combination treatments via irrigation water were applied to plants. Treatments were 0, 15 and 30 mg  $L^{-1}$  lead as lead chloride (PbCl<sub>2</sub>) source and cadmium in 0 and 5 mg  $L^{-1}$  as cadmium chloride (CdCl<sub>2</sub>) source that were arranged in factorial with completely randomized design with four replications. Each replicate was a 5-6 liter volume pot containing one seedling. The treatments were prepared using stock solution of lead (3000 mg  $L^{-1}$ )

Soil texture	Sand (%)	Silt (%)	Clay (%)	EC (dS m <sup>-1</sup> )	pН	Organic carbon (%)	$N \pmod{(\text{mg kg}^{-1})}$	$\frac{P}{(mg kg^{-1})}$	$\frac{K}{(mg kg^{-1})}$
Silty-loam	31	41	28	0.415	7.17	2.4	78.1	15.2	256

Table 1. Physicochemical characteristics of soil used in experiment

and cadmium (1000 mg  $L^{-1}$ ). Plants were irrigated with different volume of water (containing Pb-Cd combination treatments) during their growth period. Generally in April, May and October, plants received two times irrigation per week, and during June, July, August and September, plants received three times irrigation per week. Plants were irrigated on basis of 80% of soil field capacity (FC), and in every threefour months (two times per year), the pots were irrigated with additional well supplied tap water for preventing the salt accumulation in the soil.

The plant growth characteristics was recorded at the end of experiment, after two years application of heavy metal treatments. The seedlings were collectively 12 months treated with different levels of Pb and/or Cd in irrigation water. The chlorophyll index of leaves as the Soil and Plant Analysis Development (or SPAD) value of plant leaves was determined with a portable SPAD-meter (Model SPAD-502 Plus, Illinois, USA), from the average of 30 readings of various leaves generally in middle part of plant shoots. The collective new shoot growth of all plant branches was determined using a ruler and expressed in the results as cm per plant. The average plant leaf area was determined using leaf area-meter (Model CI 202, Germany) by recording the average area of 4-5 randomly detached leaves per plant. The number of chlorotic (discolored) and necrotic leaves were counted and recorded per plant. Leaf photosynthesis rates were determined using a portable photosynthesis-meter of Li Cor (Li 6100, Li Cor Co. USA), at full light conditions and at 11 o'clock in the morning from a well developed leaf in the middle part of a new shoot growth. The leaf soluble carbohydrates and proline were determined using anthrone and ninhydrin methods. Proline and soluble carbohydrates were quantified in 95% ethanol extracts of leaves. A sample of 0.5 g of freshly harvested leaves was crushed in five mL of 95% (v/v) ethanol. The supernatant was separated and the remaining lower phase was washed with 5 mL of 70% ethanol and its upper phase added to the previous one over compartment. The extracted solution then was centrifuged at 4500 g for 15 min. The supernatants were collected and stored at 4°C for further determination of proline and soluble carbohydrates. The leaf soluble carbohydrates were then analyzed by reacting 100 µL of diluted extract with 3 mL freshly prepared anthrone (150 mg anthrone + 100 mL 72% (w/w)  $H_2SO_4$ ) and placed in a boiling water bath for 10 min. After cooling, the absorbance of samples was determined at 625 nm using a spectrophotometer following the modified methods of Paquin and Lechasseur [1979]. A standard glucose curve was also used for calculation of carbohydrate content of leaves. The leaf proline concentration was determined using 2 mL of the alcoholic extract, 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. The absorbance of samples was then measured against different standard proline concentrations of 0, 5, 10, 20 and 40 mg  $L^{-1}$  and using spectrophotometer at 520 nm

Data were analyzed using SPSS Software, and comparison of means was performed using LSD test at 5% level.

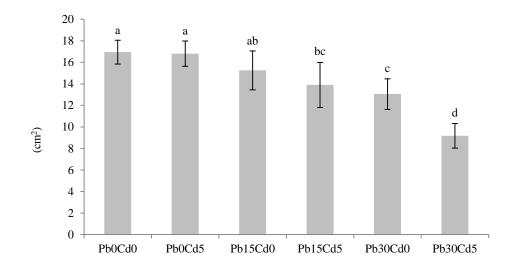
## RESULTS

The results of variance analysis (Tab. 2) showed that the effect of lead levels (factor A) on all plant traits was significant at 1%, whereas the effect of cadmium levels (factor B) for leaf area, leaf carbohydrate and proline concentrations was significant at 1%, and for the new shoot growth, number of chlorotic leaves and net photosynthesis rate, it was significant at 5%, and for leaf SPAD value and number of necrotic leaves, it was not significant. The interaction

effects of Pb and Cd on leaf soluble carbohydrates was significant at 1% and for leaf area, number of necrotic leaves was significant at 5% and for other traits was not significant (Tab. 2).

In the present study, plant growth was restricted by application of lead and cadmium into irrigation water particularly at higher concentrations of dual metals application. The ANOVA of Pb, Cd and their interaction for the new shoot growth was significant at 1%, 5% and not significant, respectively. The mean comparisons showed that the highest length of the new shoot growth was in control plants and in those plants treated with Pb0Cd5 combination that showed no significant difference with combination of Pb15Cd0 or Pb30Cd0 treatment. Significant shorter growth of new shoots was observed in Pb15Cd5, Pb30Cd0, and Pb30Cd5 combination treatments than control plants. The shortest new shoot growth was recorded in plants receiving the highest level of lead and cadmium (Pb30Cd5) treatment (Tab. 2).

The ANOVA of Pb, Cd and their interaction effect for leaf SPAD value was not significant (Tab. 2). The result of mean comparison (Tab. 3) showed that Pb, Cd or their dual combinations had no significant effect on leaf SPAD value than control plants; however, plants treated with Pb15Cd0 combination had significantly higher SPAD value than Pb30Cd5 combination that showed the lowest leaf SPAD value. The ANOVA of Pb, Cd and their interaction for number of chlorotic leaves per plant was significant at 1%, 5% and not significant, respectively (Tab. 2). Comparison of means showed that the number of chlorotic leaves per plant was increased with increasing lead and cadmium levels of irrigation water, as the highest number of chlorotic leaves was in those plants treated with combination treatment of highest level of lead and cadmium (Pb30Cd5) that showed no significant difference with Pb30Cd0 treatment. The lowest number of chlorotic leaves per plant was observed in those plants of control without heavy metal application (Tab. 3). The ANOVA of Pb, Cd and their interaction for the number of necrotic leaves per plant was significant at 1%, not significant and at 5%, respectively (Tab. 2). Comparison of means showed that application of heavy metals significantly increased leaf necrotic symptoms than control plants (Tab. 3). The number of necrotic leaves per plant was highest in those plants treated with Pb30Cd0 treatment that showed no significant difference to plants treated with Pb30Cd5 or Pb15Cd5 combination.



**Fig. 1.** Average leaf area of plants treated with Pb and Cd in irrigation water. Data are average of four replications  $\pm$ SD. The columns with at least one common letter are not significantly different at 5% level of LSD test

Source of variation	df	Leaf SPAD value	New shoot growth	Leaf area	No of chlorotic leaves	No of necrotic leaves	Leaf soluble sugars	Leaf proline	Net photo- synthesis
Factor A (Pb)	2	217.6 <sup>ns</sup>	354.5**	67.31**	6.29**	5.54**	437309.17**	8.94**	0.84**
Factor B (Cd)	1	60.48 <sup>ns</sup>	$204.16^{*}$	19.26**	$1.50^{*}$	0.37 <sup>ns</sup>	125744.32**	15.03**	$0.48^{*}$
$\mathbf{A} \times \mathbf{B}$	2	42.50 <sup>ns</sup>	68.166 <sup>ns</sup>	$7.23^{*}$	0.12 <sup>ns</sup>	$0.87^{*}$	50520.97**	0.24 <sup>ns</sup>	$0.06^{ns}$
Error	15	86.47	34.10	1.71	0.33	0.21	7996.91	0.84	0.05
CV		14.43	16.10	9.24	26.64	36.26	10.82	18.41	18.86

**Table 2.** Analysis of variance of plant growth traits

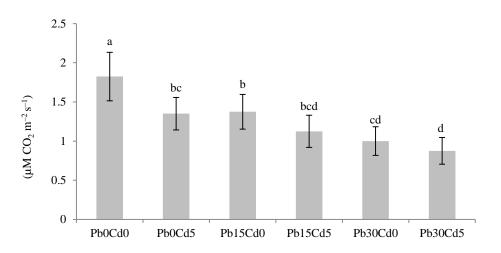
\*\*,\* and ns mean significant effect at 1%, 5% and not significant effect, respectively

**Table 3.** Leaf SPAD value, the new shoot growth and the number of chlorotic or necrotic leaves per plant under cadmium, lead or their dual combinations

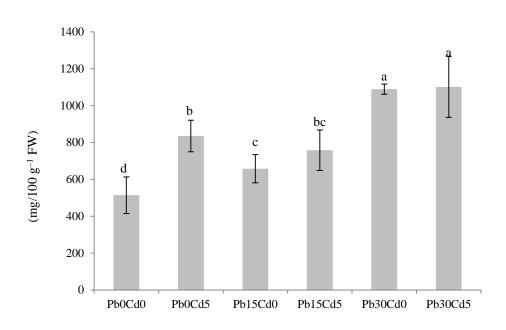
	Leaf SPAD value	New shoot growth $(\text{cm pot}^{-1})$	Number of chlorotic leaves	Number of necrotic leaves
Pb0Cd0	66.5 ±8ab	43 ±7a	1.3 ±0.5d	0 ±0d
Pb0Cd5	67.8 ±8ab	43.5 ±3a	1.5 ±0.6cd	0.8 ±0.5c
Pb15Cd0	71.6 ±11a	39 ±7ab	1.8 ±0.4cd	1.3 ±0.5bc
Pb15Cd5	63.7 ±5ab	32 ±5bc	2.3 ±0.5bc	1.8 ±0.5ab
Pb30Cd0	59.8 ±9ab	35.5 ±6ab	2.8 ±1.0ab	2.3 ±0.5a
Pb30Cd5	57 ±7b	24.5 ±4c	3.5 ±0.6a	1.8 ±0.5ab

Data are average of four replications  $\pm DS$ 

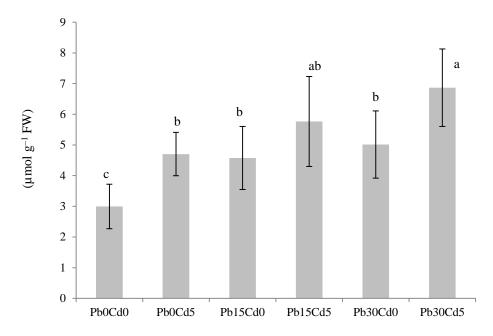
In each column, means containing similar letters are not significantly different at 5% level of LSD test



**Fig. 2.** Leaf photosynthesis rate in plants under treatment with Pb and Cd in irrigation water. Data are average of four replications  $\pm$ SD. The columns with at least one common letter are not significantly different at 5% level of LSD test



**Fig. 3.** Leaf soluble sugars in plants treated with Pb and Cd in irrigation water. Data are average of four replications  $\pm$ SD. The columns with at least one common letter are not significantly different at 5% level of LSD test



**Fig. 4.** Leaf proline concentration in plants treated with Pb and Cd in irrigation water. Data are average of four replications  $\pm$ SD. The columns with at least one common letter are not significantly different at 5% level of LSD test

The ANOVA of Pb, Cd and their interaction for the average leaf area was significant at 1%, 1% and 5%, respectively (Tab. 2). Comparison of means showed that the average plant leaf area was generally reduced by increasing the application levels of heavy metal (Fig. 1). The Pb15Cd5, Pb30Cd0, and Pb30Cd5 treatments showed significant lower leaf area than control plants. The highest leaf area was in control plants and those plants treated with Pb0Cd5 treatment, which showed no significant difference with Pb15Cd0 treatment. The significant lowest leaf area was in plants treated with the highest lead and cadmium levels (Pb30Cd5).

The ANOVA of Pb, Cd and their interaction was significant at 1%, 5% and not significant, respectively for leaf photosynthesis rate (Tab. 2). Comparison of means showed that leaf photosynthesis rate was reduced in all heavy metal treated plants than control plants that showed the highest photosynthesis rate (Fig. 2). Leaf area and leaf photosynthesis in Pb0Cd5 and Pb15Cd0 treatments were also significantly higher than those photosynthesis measured in Pb30Cd5 treatment that showed the lowest photosynthesis rates.

Determination of leaf soluble carbohydrates (Fig. 3) and proline (Fig. 4) concentrations showed that there was a general increase in these two traits with higher levels of lead and cadmium in irrigation water. All heavy metal treatments showed higher leaf soluble carbohydrates and proline content than control plants (without heavy metal application). The highest leaf soluble carbohydrates were in Pb30Cd0 and Pb30Cd5 treatments that showed significant difference with other treatments, whereas the highest leaf proline was in plants treated with Pb30Cd5 combination that showed no significant difference with combination treatment of Pb15Cd5. The significant lowest amount of leaf soluble carbohydrates and proline was in control plants without heavy metal application.

# DISCUSSION

In the present study, the new shoot growth and leaf area of plants were adversely affected particularly by higher concentration of heavy metals. Restriction in plant growth characteristics due to high rate of heavy metal application has been also reported in other studies [Ewais 1997, John et al. 2008, Shafiq et al. 2008, Bosiacki and Zieleziński 2011]. Lead and cadmium are the two major and environmentally abundant heavy metals that have distinct toxicity effects on many biological systems. In the present study; however, lead was used at considerably higher concentrations than cadmium, and the reduction in growth traits seems to be due to the adverse additive effects of Pb and Cd. Such adverse additive effects of dual application of cadmium and lead were also reported on plant growth [An et al. 2004]. Lead and cadmium are non essential metals that particularly at high concentrations can severely inhibit the cell metabolic functions [Seregin and Ivanov 2001, Benavides et al. 2005, Lux et al. 2010]. The reduced chlorophyll content and photosynthesis besides other toxic effects of high levels of Cd and Pb on cell division and cell elongation can result in restricted shoot growth. In addition, changes in physiological processes due to deleterious effects of lead and cadmium on membrane functions, enzyme activity, and increased level of oxidative stress due to hydroxyl radicals, superoxide anions and hydrogen peroxide [Shafiq et al. 2008, Yan et al. 2010, Yusuf et al. 2010], can justify the reduced growth due to high levels of Pb and Cd application via irrigation water in the present study.

Expanded leaf area with good greenness can guarantee adequate photosynthesis activity. Plant leaf area is influenced by many factors, and it is a function of cell division and cell enlargement [Marschner 2011]. In the present study, high application rates of Pb and Cd could restrict the leaf area expansion by different mechanisms. The inhibition or any damage to leaf photosynthesis, enzyme activity, meristem cell division, water absorption, nutrient uptake and cell enlargement are among those that may collectively result in restricted leaf area under heavy metal treatments [Seregin and Ivanov 2001, Shafiq et al. 2008, Lux et al. 2010]. Changes in phytohormones activities such as cytokinins and gibberellins due to high application rate of Pb and Cd could also play a role in reduced leaf area.

Leaf SPAD value as greenness index of plant leaves is one of the best indicators of general plant health conditions, that is influenced by many environmental and stress factors. However, in the present study, there was slight effect of heavy metals on leaf SPAD value, indicating relative tolerance of seedlings to heavy metal applications. The reduction in leaf greenness seemed to be more in form of pointed chlorosis that probably changed to necrotic points particularly the outer edge of leaves. Reduction in leaf SPAD value or chlorophyll content has been also reported in other studies [Ewais 1997, Seregin and Ivanov 2001]. Leaf chlorophyll is the context in which metabolic reactions are taking place, and any decrease or damage to it can significantly impair plant photo assimilation production. Leaf chlorosis and necrosis points have been also reported in other plant species under heavy metal treatments [Benavides et al. 2005, Mangkoedihardjo and Surahmaida 2008]. On the other hand, increase in the necrosis trend of leaves with increasing the Pb and Cd levels could be a mechanism of heavy metal tolerance (exclusion) in this plant species.

Our results showed that leaf photosynthesis rate was reduced under higher heavy metal application. The reduction in photosynthesis process and plant biomass production has been widely reported under heavy metal treatment [Ewais 1997, Islam et al. 2008, John et al. 2008, Bosiacki and Zieleziński 2011]. Leaf is the center of plant metabolic reactions, the main to be photosynthesis and many related biochemical reactions. Leaf photosynthesis is a multi-reaction process that is occurring in chloroplasts, and is completely dependent on a normal functioning of other cell organelles. Heavy metals may interrupt the process at various levels or stages. Many enzymes involved in photosynthesis process, are those sensitive to the toxic presence of heavy metals in the cell medium [Seregin and Ivanov 2001, Benavides et al. 2005, Clemens 2006]. Photosynthesis is the generating force for many metabolic reactions taking place in plant tissues. Any damage to leaves and phytoassimilation process can adversely affect plant growth and tolerance. Heavy metals generally manifest high affinity for sulfur-containing ligands and strong bind formation [Seregin and Ivanov 2001].

When heavy metals enter the cell, they interact with SH-groups, inactivate many enzymes and disturb many metabolic processes [Seregin and Ivanov 2001].

Our results showed that all heavy metal treatments significantly increased the leaf soluble carbohydrates and proline concentration than control plants. The soluble sugars and proline amino acid are the two major metabolites involve in higher plant tolerance to environmental stresses. There is a general increase in these two cell components under adverse climatic conditions and heavy metal toxicity [Marschner 2011]. The increase in these two metabolites under application of heavy metals has been reported in other studies [Seregin and Ivanov 2001, John et al. 2008]. Heavy metal toxicity also affects the water relationship of plant tissues and inhibits enzymes activities [Seregin and Ivanov 2001]. It can also induce oxidative stress through increased levels of hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide [Yan et al. 2010, Yusuf et al. 2010]. Elevated levels of leaf soluble carbohydrates and proline are probably involved in inactivation and complex formation of Pb and Cd in various plant tissues particularly in leaves [Brown and Brinkmann 1992]. In duckweed plants (Lemna polyrrhiza L.), low concentration  $(10 \text{ mg L}^{-1})$  of Pb and Cd caused an increase in leaf proline, protein and sugars, but at higher concentrations (above 30 mg  $L^{-1}$ ) decrease in these traits was observed [John et al. 2008].

Excessive amount of toxic metals including Pb and Cd usually causes reduction in plant growth [Seregin and Ivanov 2001, Shafiq et al. 2008], as was shown in the present study; however, with increasingly large amount of applied Pb and Cd in irrigation water during two growing years, the dimension of growth reduction was minor compared to other studies, in which heavy metals generally have been applied once and in a given concentration. It has been shown that uptake of Pb and Cd heavy metals are concentration and time dependent, as treatment with 1, 10 and 20 mg  $L^{-1}$  of Cd and Pb showed synergistic relationship, while application of 30 and 40 mg  $L^{-1}$ showed antagonistic relationship during the metal uptake [John et al. 2008]. This indicates that Judas tree represents a good candidate to be irrigated with

wastewater containing some levels of heavy metals. In Jatropha plants (Jatropha curcas L.), a significant positive effect on plant dry matter was found at low but not under high concentrations of cadmium and lead [Mangkoedihardjo 2008]. Effects of Cd, Ni and Pb on growth, chlorophyll and protein content of potted Cyperus difformis, Chenopodium ambrosioides and Digitaria sanguinolis showed that the three heavy metals inhibited the shoot growth (less biomass, chlorophyll and protein content), but they were less suppressive to root growth [Ewais 1997]. Nevertheless, in cucumber, binary metal combinations of Cd, Pb and Cu produced all three types of interactions (antagonistic, additive, and synergistic responses), depending on the combinations, and ternary combinations resulted in a decreased toxicity of heavy metals [An et al. 2004].

Various metabolic disorders arise under Pb and Cd treatments, and it is usually difficult to tell which disorders are of primary and which are of secondary origin [Seregin and Ivanov 2001]. Changes in physiological processes due to deleterious effects of lead and cadmium on membrane functions, enzyme activity, and increased level of oxidative stress induced by hydroxyl radicals, superoxide anions and hydrogen peroxide [Yan et al. 2010, Yusuf et al. 2010] can justify the reduced growth under high levels of Pb and Cd application via irrigation water in the present study. Changes in membrane lipids, cell structure, cell organelles such as mitochondria and peroxisomes have been also reported under heavy metal application in various plant species [Islam et al. 2008, Małecka et al. 2008, Yan et al. 2010]. Nevertheless, it has been found that cadmium and lead do not specifically affect the particular processes, but instead they exert non-specific toxic effects on cell metabolism [Seregin and Ivanov 2001]. When bound on the cell surface and within the cells, Cd and Pb ions interact with the functional groups of proteins, nucleic acids, polysaccharides, etc. and substitute for other metal ions already bound to these functional groups [Seregin and Ivanov 2001].

Water is an increasingly limiting factor for plant growth in arid regions. On the other hand, wastewater represents a significant irrigation water source, but it has relatively high levels of heavy metals and other pollutants. Application of wastewater to ornamental and landscape plants, rather than vegetable cultivation, represents a useful approach in application of refined wastewater [Bosiacki 2008]. However, those plants must be tolerant to high levels of heavy metals in irrigation water or in soil. Compared to herbaceous species, fast-growing trees have several advantages (including deeper root system, high productivity and transpiration activity) to correct the heavy metal polluted soils [Pulford and Watson 2002]. Plant species with high uptake rate can be applied for biological remove of contaminants in soils or watersheds [Pulford and Watson 2002]. So, application of wastewater to perennial ornamental plants can prevent the entrance of heavy metals into food chain while increasing the beauty of the landscape and environment. In the present study, promising results of plant growth and tolerance were observed with Judas tree seedlings exposed to irrigation water containing different levels of Pb and Cd during two years growth period.

## CONCLUSIONS

In the present study, the growth characteristics of Judas tree seedlings were reduced by both Cd and Pb or their dual application in irrigation water. The binary combination of Cd and Pb particularly at higher lead levels significantly reduced the plant growth indices of leaf area, the new shoot growth and leaf photosynthesis rate, whereas it increased the leaf soluble carbohydrates and proline concentration. High levels of Cd and particularly Pb were added to soil (root medium) of seedlings during 12-13 months irrigation practice in two consequence years. Compared to other studies with single application of a given heavy metal (Pb and/or Cd), our results indicate that Judas tree is probably a good perennial shrub or tree, suitable for cultivation under wastewater irrigation that generally contains high levels of heavy metals.

#### ACKNOWLEGDEMENTS

This research was conducted as a part of PhD dissertation and financially supported by funds provided by Lorestan University, Khorramabad, Iran.

## REFERENCES

- An, Y.J., Kim, Y.M., Kwon, T.I., Jeong, S.W. (2004). Combined effect of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. Sci. Total Environ., 326(1–3), 85–93.
- Benavides, M.P., Gallego, S.M., Tomaro, M.L. (2005). Cadmium toxicity in plants. Braz. J. Plant Physiol., 17, 21–34.
- Bosiacki, M. (2008). Accumulation of cadmium in selected species of ornamental plants. Acta Sci. Pol. Hortorum Cultus, 7(2), 21–31.
- Bosiacki, M., Zieleziński, Ł. (2011). Phytoextraction of nickel by selected species of lawn grasses from substrates contaminated with heavy metals. Acta Sci. Pol. Hortorum Cultus, 10(3), 155–173.
- Brown, G., Brinkmann, K. (1992). Heavy metal tolerance in *Festuca ovina* L. from contaminated sites in the Eifel Mountains, Germany. Plant Soil, 143(2), 239–247.
- Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie, 88, 1707–1719.
- Ewais, E.A. (1997). Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. Biol. Plantar., 39(3), 403–410.
- Frick, C.M., Germida, J.J., Farrell, R.E. (1999). Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. In technical seminar on chemical spills. Environment Canada, 105–124.
- Islam, E., Liu, D., Li, T., Yang, X., Jin, X., Mahmood, Q., Tian, S., Li, J. (2008). Effect of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*. J. Hazard. Mater., 172, 479–484.
- John, R., Ahmad, P., Gadgil, K., Sharma, S. (2008). Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ., 54(6), 262.
- Krämer, U. (2005). Phytoremediation: novel approaches to cleaning up polluted soils. Curr. Opin. Biotechnol., 16(2), 133–141.
- Lux A., Martinka M., Vaculík M., White P.J. (2010). Root responses to cadmium in the rhizosphere: A review. J. Exp. Bot., 62, 21–37.
- Małecka, A., Piechalak, A., Morkunas, I., Tomaszewska, B. (2008). Accumulation of lead in root cells of *Pisum sativum*. Acta Physiol. Plant., 30, 629–637.
- Mangkoedihardjo, S. (2008). Jatropha curcas L. for phytoremediation of lead and cadmium polluted soil. World Appl. Sci. J., 4(4), 519–522.

- Marschner H. (2011). Marschner's mineral nutrition of higher plants. Academic Press, London.
- Paquin R., Lechasseur P. (1979). Observations sur une method de dosage de la proline libre dans les extraits de plantes. Can. J. Bot., 57, 1851–1854.
- Pulford, I.D., Watson, C. (2002). Phytoremediation of heavy metal-contaminated land by trees – A review. Environ. Intern., 29, 529–540.
- Raskin, I., Smith, R.D., Salt, D.E. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. Curr. Opin. Biotechnol., 8(2), 221–226.
- Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K., Singh, A.K. (2005). Long-term impact of irrigation with sewage effluents on heavy metals content in soils, crops and groundwater – a case study. Agr. Ecosys. Environ., 109, 310–322.
- Seregin, I.V., Ivanov, V.B. (2001). Physiological aspects of cadmium and lead toxic effects on higher plants. Russian J. Plant Physiol., 48(4), 523–544.
- Shafiq, M., Iqbal, M.Z., Mohammad, A. (2008). Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. J. Applied Sci. Environ. Manag., 12(3).
- Singh, A., Sharma R.K., Agrawal M., Marshall, F.M. (2010). Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. Tropical Ecol., 51, 375–387
- Souri, M.K., Alipanahi, N., Hatamian, M., Ahmadi, M., Tesfamariam, T. (2018). Elemental profile of heavy metals in garden cress, coriander, lettuce and spinach, commonly cltivated in Kahrizak, South of Tehran – Iran. Open Agric., 3(1), 32–37.
- Száková, J., Tlustos, P., Vyslouzilová, M., Pavlíková, D. (2003). Fluctuation of mobile portions of soil As, Cd, Cu, Pb, and Zn during vegetation of willows. Chem. Inż. Ekol., 10, 975–982.
- Vandecasteele, B., Meers, E., Vervaeke, P., De Vos, B., Quataert P., Tack, F.M.G. (2005). Growth and trace metal accumulation of two *Salix* clones on sedimentderived soils with increasing contamination levels. Chemosphere, 58, 995–1002.
- Yan, Z.Z., Ke, L., Tam, N.F.Y. (2010). Lead stress in seedlings of *Avicennia marina*, a common mangrove species in South China, with and without cotyledons. Aquat. Bot., 92, 112–118.
- Yusuf, M., Fariduddin, Q., Hayat, S., Ahmad, A. (2011). Nickel: an overview of uptake, essentiality and toxicity in plants. Bull. Environ. Contam. Toxicol., 86, 1–17.