Research Article

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Interaction of Lead and Nitrate on Growth Characteristics of Ornamental Judas Tree (*Cercis siliquastrum* L.)

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Abstract: Lead is presently a significant heavy metal pollutant that is toxic for all types of life and is of worldwide concern. Various strategies may be applied to increase tolerance of plants to heavy metals, for example nitrogenous compounds have been shown to exert such effects. In the present study combinations of lead levels (0, 15, 30 mgL⁻¹) and nitrate levels (0, 50, 100 mgL⁻¹) applied through irrigation water, were evaluated for interactions affecting growth of ornamental Judas tree seedlings. The treatments were arranged in a randomized factorial design with four replications. The results showed that interactions between lead and nitrate levels on growth traits were not significant; however, the simple effects of lead and nitrate were significant on most morphological traits. The morphological traits of leaf SPAD (Soil and Plant Analysis Development) value, leaf area, new shoots growth and relative water content of leaf were reduced by application of lead (particularly at 30 mgL⁻¹). Nevertheless, application of nitrate particularly at 100 mgL⁻¹ significantly offset the reduced plant growth traits induced by high levels of lead. The results indicate that presence of nitrate can significantly improve plant growth under high lead toxicity.

Keywords: environment, fertilization, heavy metal stress, irrigation, nitrogen, toxicity

1 Introduction

Enhanced heavy metal presence in the environment is a real threat for all types of the life. Heavy metal toxicity is a stress imposed on plants continuously during growth and productive phases. However, some plant species can tolerate high levels of heavy metals and are known as accumulators or excluders (Hall 2002; Pulford and Watson 2002).

Environmental lead pollution arises from various industrial activities that significantly contribute to lead contamination of the soil, water and air (Souri et al. 2018). Plants generally have different responses/tolerances to heavy metals in their root or foliage media (Seregin and Ivanov 2001; Clemens 2006). Nevertheless, a general reduction in plant growth is the commonest response of many plant species to heavy metal toxicity (Aidid and Okamoto 1992; Ewais 1997; Sharma and Agrawal 2005). Heavy metal toxicity also affects morphophysiological traits, as there is generally reduction in biomass production, leaf area and chlorophyll content and plant height. Photosynthesis is also vulnerable to heavy metal toxicity; however, it is often affected after many other basic physiological processes (Marschner 2011; Seregin and Ivanov 2001). Plant species use different physiological mechanisms or adoptive strategies to cope with stressful concentrations of heavy metals such as lead (Clemens 2006). On the other hand, environmental conditions are key factors regarding the toxicity effects of heavy metals. Plant treatment with some biostimulants has been shown to reduce the adverse effects of heavy metals on plant growth. For example, treatment with antioxidants compatible osmolites such as proline; soluble sugars; salicylic acid, and various amino acids including glycine have been shown to reduce lead toxicity effects (Souri, 2016). Various nitrogenous compounds including nitrate can benefit plants under stressful conditions (Marschner, 2011). Nitrate as a nitrogen source or signaling molecule or osmolite can affect many plant physiological processes

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and enable plants to better tolerate stress conditions. Little information is available about the effects of long-term applications and interaction between lead and nitrate on plant growth. The ornamental Judas tree is known for its tolerance to low soil-water status making the species useful to beautify urban landscapes in arid and semi-arid regions. In the present study, the growth of Judas tree seedlings (*Cercis siliquastrum* L.) in response to different combinations of lead and nitrate was evaluated.

2 Material and Methods

This study was conducted during 2016 and 2017 under greenhouse conditions, in which one year old seedlings of Judas tree (*Cercis siliquastrum* L.) were used. The soil was prepared using 1/10 leaf litter, 6/10 field soil and 4/10 fine sands and the components were thoroughly mixed and then passed through 2 mm sieve for better uniformity. The physicochemical characteristics of the soil are presented in Table 1. Plants were transplanted to black plastic pots (one seedling per pot as one replication) with 5-6 liter volume (30 cm height).

For first three weeks after transplanting, plants were irrigated with tap water without lead or nitrate content. Thereafter, different lead and nitrate combination treatments were applied to plants via irrigation water. Lead levels varied from 0, 15 and 30 mg L⁻¹, and nitrate from 0, 50 and 100 mg L⁻¹ were applied to pots through irrigation water. The pots and treatments were arranged in a randomized factorial based design with four replications. Lead was supplied from a lead chloride source (PbCl₂), and nitrate was supplied as calcium nitrate. Plants were irrigated 2-3 times per week (depending on season) from April to end of October and for two consequence years of 2016 and 2017.

Plants were irrigated based on a water content of 80% of soil field capacity (FC). Two times per year pots were irrigated with additional Well-water, to prevent salt accumulation in the soil.

Some growth characteristics were recorded after the second year of treatments application. The leaf SPAD value (The Soil and Plant Analysis Development) was determined using SPAD meter (Model SPAD-502 Plus, Illinois, USA), and the average of 30 readings/pot of various

leaves in the middle part of plant shoots was recorded. The sum of new plant-shoot growth was determined using a ruler and expressed in the results as cm per plant. The average plant leaf area was determined using a leaf area meter (Model CI 202, Germany), by recording the area of 4-5 randomly detached leaves from the middle of new shoots. This value is presented in the results as a single leaf area. The number of necrotic points on leaves was counted for all plant leaves and the average per single leaf is presented in the results. The leaf water content was determined with leaf blade discs. The samples were first weighed to determine their fresh weight (FW) and then they were hydrated to full turgidity for 24 h on distilled water, in a closed Petridish. The samples were then well dried off with tissue paper and immediately weighed to obtain their fully turgid weight (TW). The samples were oven-dried at 70 °C and weighed to determine their dry weight (DW). The leaf water content (%) was estimated from the following equation:

$$\text{Leaf water content} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}).$$

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

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

The results of analysis of variance (Table 2) show that the interaction effects of lead levels and nitrate levels on growth traits were not statistically significant. However, the simple effect of factor A (lead levels) on leaf SPAD value, plant leaf area, necrotic points on leaves, sum of new shoots growth and leaf water content was significant at 1%. The simple effect of factor B (nitrate levels) was not significant on leaf SPAD value, but it was significant for all remaining traits.

Comparison of means for leaf SPAD value (Figure 1) showed that increasing the lead levels resulted in significantly lower leaf SPAD values; whereas there was no significant difference among nitrate levels regarding this trait. The lead and nitrate interactions showed that the highest leaf SPAD value occurred in plants treated with

Table 1: Some physicochemical characteristics of the soil used in the experiment

Soil texture	Sand (%)	Silt (%)	Clay (%)	EC (dS m ⁻¹)	pH	Organic carbon (%)	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)
Silty-loam	31	45	24	0.415	7.17	2.45	78.1	15.2	256

Table 2: Analysis of variances of some growth factors of Judas tree plants

Source of Variation	df	Leaf SPAD value	Leaf area	Necrotic points of leaf	New shoot growth	Leaf water content
Factor A(Pb)	2	898.3**	38.9**	25.0**	952.6**	661.8**
Factor B (NO ₃)	2	41.5 ^{ns}	73.7**	15.9**	448.2*	435.5**
A*B	4	26.2 ^{ns}	2.8 ^{ns}	0.597 ^{ns}	6.7 ^{ns}	16.5 ^{ns}
Error	24	56.9	2.18	0.56	60.0	37.6
CV		10.7	13.3	36.3	15.2	12.1

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

highest nitrate levels without lead (A1B3). Increased lead levels significantly reduced leaf SPAD value compared to control plants. Nevertheless, the lowest leaf SPAD value was in those plants treated with highest lead level and concurrently 50 mgL⁻¹ nitrate (Figure 1). Adverse effects of heavy metal toxicity on leaf chlorophyll content have been also reported in other studies (Aidid and Okamoto 1992; Vandecasteele *et al.* 2005). Leaf chlorophyll content

is a rather sensitive leaf-trait that is vulnerable to many environmental stimuli including, salinity; drought; cold; heat; nutrient deficiencies and heavy metal toxicities (Marschner 2011; Souri 2016). Leaf greenness is a function of chlorophyll biosynthesis and chlorophyll degradation. In the present study, high lead level in the root medium that was applied during two years via irrigation water probably resulted in lead toxicity and induction of higher ethylene

Figure 1: Leaf SPAD value of plants under lead and nitrate interaction treatments. Treatments indicate lead levels (A: 0, 15, 30 mgL⁻¹) and nitrate levels (B: 0, 50, 100 mgL⁻¹). Data are average of four replications $\pm$ SD. Comparison of means was done at 5% level of LSD test

production/accumulation that resulted in chlorophyll degradation (Marschner 2011). Lead can disturb many leaf physiological functions including protein and chlorophyll biosynthesis. It can also perturb many physiological functions of nutrient elements involved in biochemical reactions such as photosynthesis (Seregin and Ivanov 2001; Clemens 2006).

The results also showed that plant leaf area (Figure 2) was reduced by increasing lead levels in irrigation water, whereas it was increased by increasing nitrate levels of irrigation water. Interaction of lead and nitrate levels showed that the highest leaf area was in those plants treated with highest nitrate level without lead application (A1B3), while the lowest leaf area was in those plants treated with highest lead levels and lowest levels of nitrate (A3B1). Application of nitrate allowed recovery of the leaf area reduced by high lead concentrations in irrigation water, as the leaf area of those plants with

highest levels of lead and nitrate application (A3B3) was similar to those plants that received no lead and nitrate (A1B1) (Figure 2). Reduction in leaf area and plant growth has been also reported in other studies (Ewais 1997; Li et al. 2009; Nagajyoti et al. 2010). Leaf area expansion is a function of cell division and cell enlargement. These two phenomena are influenced by different internal and external factors including phytohormones and osmolite levels of leaf meristematic cells (Marschner 2011; Souri 2016). Heavy metal ions exert toxic effects on cell division; however, it has been shown that cell enlargement is even more vulnerable to heavy metal levels (Sharma and Agrawal 2005; Clemens 2006). Reduction in cation nutrient elements such as K, Ca, Zn and Mn due to lead toxicity could also play a role in reduced leaf area induced by application of high lead levels (Hall 2002; Sharma and Agrawal 2005; Marschner 2011). On the other hand, increasing leaf area associated with higher nitrate level in

Figure 2: The leaf area of Judas tree plants under lead and nitrate interaction treatments. Treatments indicate lead levels (A: 0, 15, 30 mgL⁻¹) and nitrate levels (B: 0, 50, 100 mgL⁻¹). Data are average of four replications $\pm$ SD. Comparison of means was done at 5% level of LSD test

irrigation water could be due to different roles of nitrate in plant cell metabolism. Nitrate is a very suitable nitrogen source for biosynthesis of many primary and secondary metabolites including amino acids and proteins. Nitrate can also act as a semi-hormonal compound or signaling molecule in rather low concentrations. In addition, nitrate is an important osmolyte in plants with significant influence on cell turgidity and enlargement (Marschner 2011). It has been shown that higher nitrate or nitrogenous compounds can reduce heavy metal toxicity in plants (Marschner 2011; Sourì 2016).

Determination of necrotic points on plant leaves (Figure 3) showed that the highest frequency of necrotic points occurred on leaves of those plants treated with highest lead and lowest nitrate levels (A3B1), whereas the lowest frequency of necrotic points were seen on leaves of those plants without lead treatment but with highest levels of nitrate (A1B3). Increase in the lead levels of irrigation water significantly increased necrotic points of leaves and application of either 50 or 100 mg L⁻¹ nitrate significantly reduced the necrotic points on plant leaves. Leaf chlorosis and necrosis have been also reported in plants under lead or other heavy metals toxicity (Eweis 1997; Seregin and Ivanov 2001; Li et al. 2009). Highly visible leaf necrosis could exclude Judas trees from being planted under high lead conditions. Oxidative stress has frequently been reported under heavy metals toxicity, particularly lead, finally resulting in leaf necrotic points (Aidid and Okamoto 1992; Vandecasteele et al. 2005; Kachout et al. 2009).

Increasing the lead levels of irrigation water significantly reduced the new shoots growth of plants

(Figure 4). On the other hand, application of nitrate in irrigation water significantly increased the growth of new shoots; however, the slope of shoot growth by nitrate application was sharper than the slope of reduction of shoot growth by lead application (Figure 4). The lead and nitrate interaction effects showed that the highest shoot growth rate was in those plants treated with highest nitrate level without lead application (A1B3). Application of nitrate particularly at 100 mg L⁻¹ significantly repaired reduced shoot growth induced by high lead levels (Figure 4). Reduction in plant growth and plant height has been also reported under lead or other heavy metals (Kachout et al. 2009; Li et al. 2009). Lead can significantly disturb the functions of meristem cells leading to restricted growth characteristics (Aidid and Okamoto 1992; Seregin and Ivanov 2001). The growth of new shoots is an indicator of plant biomass production, and it has been shown that lead toxicity can significantly reduce plant biomass production (Krämer 2009; Nagajyoti et al. 2010). Reduced rate of photosynthesis may have contributed to reduced growth of new shoots in the second year of lead and/or nitrate application. High levels of lead have been shown to reduce water and nutrient uptake rates of plants. There might be also changes in plant phytohormones levels. As a result of lead stress ethylene accumulates and cytokinins- and gibberline-like substances are generally reduced (Hall 2002; Poschenrieder and Barceló 2004; Marschner 2009).

Leaf water content was not affected by application of 15 mg L⁻¹Pb in irrigation water during two years; however, application of 30 mg L⁻¹Pb significantly reduced leaf water content (Figure 5). On the other hand, increase in the application rate of nitrate in irrigation water significantly

Figure 3: Number of necrotic points on leaves of Judas tree plants under lead and nitrate interaction treatments. Treatments indicate lead levels (A: 0, 15, 30 mg L⁻¹) and nitrate levels (B: 0, 50, 100 mg L⁻¹). Data are average of four replications $\pm$ SD. Comparison of means was done at 5% level of LSD test

Figure 4: Sum of new shoots growth of Judas tree plants under interaction of lead and nitrate applications. Treatments indicate lead levels (A: 0, 15, 30 mg L⁻¹) and nitrate levels (B: 0, 50, 100 mg L⁻¹). Data are average of four replications $\pm$ SD. Comparison of means was done at 5% level of LSD test

increased leaf water content. The interaction effects of lead and nitrate in irrigation water showed that the highest leaf water content was in those plants treated with no lead but receiving the highest nitrate level (A1B3). The lowest leaf water content was in those plants treated with the highest lead level and lowest nitrate level (A3B1). The results showed that only under application of 15 mg L⁻¹Pb in irrigation water was nitrate at 100 mg L⁻¹ able to increase leaf water content significantly (Figure 5). Leaf water content is a very important factor in leaf biochemical and metabolic efficiency, as low level of leaf water content generally restricts leaf photosynthesis and its biochemical reactions (Poschenrieder and Barceló 2004; Sourì 2016). One of the toxicity effects of heavy metals including lead on plant physiology is exerted on water uptake kinetics by roots and their water conductivity (Seregin and Ivanov 2001). On the other hand, nitrate can increase the water content of leaf tissue mainly due to its osmotic effect

(Marschner 2011). Nitrate is well known for inducing fleshy and watery tissues. Such effects, but with limited dimension, can also be proposed on Judas tree seedlings under high lead stress in the present study.

Soil fertility levels can influence heavy metal toxicity (Seregin and Ivanov 2001; Vandecasteele et al. 2005). It has been shown that high levels of some nutrients such as K, Ca and Zn can significantly reduce the toxicity and damages induced by heavy metals (Aidid and Okamoto 1992; Seregin and Ivanov 2001). However, in this study the results of soil analysis show that the soil contained a moderate level of fertility, with acceptable amounts of available major nutrients: nitrogen, phosphorus and potassium.

Heavy metals can significantly change leaf characteristics including water conductance, photosynthesis and transpiration rates. These leaf traits are among the most important metabolic processes

Figure 5: Leaf water content of Judas tree plants under long term (two years) lead and nitrate interaction treatments. Treatments indicate lead levels (A: 0, 15, 30 mg L^{-1}) and nitrate levels (B: 0, 50, 100 mg L^{-1}). Data are average of four replications $\pm$ SD. Comparison of means was done at 5% level of LSD test

(Ewais 1997; Seregin and Ivanov 2001). Application of heavy metals has been shown to reduce many plant growth parameters including leaf morphophysiological characteristics (Ewais 1997; Krämer 2009; Van der Ent et al. 2013). In *Jatropha* trees, cadmium was more toxic than lead on plant dry matter production; however, no additive effects were found on plant dry matter in the presence of both pollutants. Heavy metals may have some indirect toxicity effects such as metal-induced water stress, Mg, Mn and Fe deficiency, and the stomatal closure that restricts the amount of available CO_2 and photo-assimilation. Nevertheless, the inhibition of key enzymes, particularly in nitrogen metabolism and restricted nutrient uptake, and membrane disturbance are among direct effects of heavy metal in plant metabolism (Seregin and Ivanov 2001).

4 Conclusion

In the present study, high amounts of lead were applied to plants through irrigation water. Presence of lead at either 15 or 30 mg L^{-1} , significantly reduced plant morphological traits and leaf water content compared to control plants. However, application of nitrate at 50 mg L^{-1} and particularly 100 mg L^{-1} significantly offset growth retardation induced by high levels of lead. The results confirm the value of the ornamental Judas tree as a relatively lead-tolerant species.

Conflict of interest: Authors declare no conflict of interest.

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