[Research]



Physiological responses of *Celtis caucasica* L. and *Robinia pseudoacacia* L. to the cadmium and lead stresses

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ABSTRACT

Afforestation of contaminated areas is considered as a possible strategy for reduction of contaminations. In the present study, the effects of lead (Pb) and cadmium (Cd) were investigated on chlorophyll fluorescence parameters (F_v/F_m , F_o , and F_m), photosynthetic pigments (chlorophyll *a*, *b*, and total chlorophyll), and proline in one-year-old seedlings of *Celtis caucasica* and *Robinia pseudoacacia*. The seedlings were treated 2 times during 10 days, with different concentrations of Pb and Cd (0, 250, 500, 1000 and 2000 mg L⁻¹). Saline solutions containing Pb and Cd were sprayed on the leaves. Chlorophyll fluorescence was measured every other day. Chlorophyll and proline were also measured at the end of experiment period. The results indicated that chlorophyll fluorescence of *C. caucasica* and *R. pseudoacacia* was affected by Pb on the last days and by Cd on the first days. The chlorophyll *a* content of *C. caucasica* at 250 mg L⁻¹ of Pb and the chlorophyll *a* of *R. pseudoacacia* at 1000 and 2000 mg L⁻¹ of Cd increased. With increasing Cd and Pb concentrations, proline of *C. caucasica* increased significantly while proline of *R. pseudoacacia* was not affected by Cd and Pb. Our results suggested fairly similar photosynthetic responses of *C. caucasica* and *R. pseudoacacia* to Cd and Pb concentrations. We concluded that physiological sensitivity of the both species to Pb and Cd were weak and can be used for afforestation in semi-arid areas contaminated by Pb and Cd.

Keywords: Celtis caucasica, Robinia pseudoacacia, Heavy metals, Chlorophyll, Proline

INTRODUCTION

Photosynthesis is one of the most important physiological parameters and it is measurable on the plant ecological studies. Chlorophyll absorbs light energy and drives photosynthesis process that is the basis of animals and human life (Mallick & Mohn, 2003). Hence, the control of damaging effects of the photosynthesis apparatus should be one of the main goals in environmental sciences (Mallick & Mohn, 2003). Photosynthesis can be affected by contamination of water and soil sources and atmosphere expanding in the world.

Heavy metals diffuse in the atmosphere as aerosols, gases and particulates (Bradl, 2005). Sources of heavy metals emitting in the atmosphere are sea salt particles, mineral dusts, extraterrestrial matter, forest fire, volcanic aerosols and industrial sources such as emissions from transportation, coal combustion, and fugitive particulate emissions (Colbeck, 1995). The amounts of estimated emission of cadmium (Cd) and lead (Pb), as heavy metals, in natural are 0.29×10^{-3} and 4×10^{-3} vear-1, tons respectively, that increase up to 5.5×10^{-3} and 400×10^{-3} tons year⁻¹ by anthropogenic activities (Nriagu, 1979). Rahbar Hashemi et al. (2013)reported the seasonal concentrations of heavy metals in the Anzali International wetland by anthropogenic activities about 0.016 mg ml-1 for cadmium, and 0.02 mg ml⁻¹ for copper (Cu) and lead (Pb). Cadmium (Cd), a non-essential element for most of living beings, can be highly phytotoxic (Ralph & Burchett, 1998). Photosystem II (PSII) and photosystem I (PSI) are light harvesting systems which act in light reaction of photosynthesis. Light harvesting complex II (LHCII) in PSII excite upon encountering with light and lose electron. This electron transfers to Q_A

(primary acceptor electron) and then to Q_B (secondary acceptor electron) and flows in the electron transport chain. Reaction center of PSI excites with receiving electron from PSII. These electrons are used to produce ATP and NADPH. PSII function is severely sensitive to Cd, and affects more than PSI (Chugh & Sawhney, 1999; Mallick & Mohn, 2003).

Lead (Pb), a non-essential element, can be toxic in the low concentrations for sensitive species (Ralph & Burchett, 1998). The effect of Pb, on the phytochemistry of *Tithonia diversifolia* exposed to roadside automotive pollution was investigated by Olivares (2003). The author found higher Pb and Ni contents in the leaves from a polluted site (120.79 and 10.20 mg kg⁻¹, respectively) and these concentrations decreased chlorophyll from 9.32 to 9.11 mg g⁻¹. Gülriz *et al.* (2006) clarified that chlorophyll content is related to tree species, seasonal factors, site conditions, and pollution.

Fluorescence chlorophyll a is used to calculate stress rate at the first days or even at the first hours exposure to stress (Ralph & Burchett, 1998). The first toxic symptom in Halophila ovalis was detected using plasmolysis after 8 days exposure to Cu (10⁻⁴ mol L⁻¹) (Malea *et al.*, 1995a). Whereas chlorophyll fluorescence measurements revealed Cu (5×10⁻³ mol L⁻¹) toxicity within first hours (Ralph & Burchett, 1998). Pulse-Amplitude-Modulation (PAM) fluorometry is carefully connected with the availability of strong, compact light sources for rapid pulse-modulated excitation with sensitive detector systems (Schreiber et al., 1993). It indicates the effects of stress on plant and algae by F_v/F_m (The photosystem II photochemical efficiency) parameter (Jones et al., 1999; Frankart et al., 2003; Lewis et al., 2001; Nielson *et al.*, 2003a). F_{α}/F_m value is a measure of light energy transfer in dark adapted samples or the photochemical quantum yield of open PSII centers (DeEll & Toivonen, 2003; Hanelt & Nultsch, 1995).

The other indicators for assessment of stress in plants are the proline and chlorophyll contents. Proline is an amino acid and unique compound in the protein structure. Since it was first noted to accumulate in wilted ryegrass, accumulation of this amino acid has been observed in a large number of plant species grown under stress conditions. Consequently proline has been used as an indicator of response to the plant stress (Aspinall & Paleg, 1981; Heuer, 1994). Also, it accumulates under a wide range of stress conditions such as high salinity, water shortage, extreme temperatures, nutrient deficiencies, high light intensity, low pH of the growth medium, high level of heavy metals, air pollution, and diseases created by pathogens (Aspinall & Paleg, 1981; Delauney & Verna, 1993; Hare & Cress, 1997).

Chlorophyll content including chlorophyll *a*, *b*, and totall chlorophyll is an important index to evaluate the effects of air pollutantion and heavy metals stress on plants (Joshi & Swami, 2009).

Black locust (*Robinia pseudoacacia* L.) and Hackberry (*Celtis caucasica* L.) are currently being used for green-belt and parks in the big cities, exposing to pollutions of Cd and Pb. Therefore, it is necessary to study the physiological sensitivity of *R. pseudoacacia* and *C. caucasica* to heavy metals stress.

The aim of this work was to find out the physiological responses, i.e., photosynthesis, proline, and chlorophyll content alterations, of *R. pseudoacacia* and *C. caucasica* seedlings to high concentrations of Pb and Cd.

MATERIALS AND METHODS Plant material and growing condition

One-year-old seedlings of R. pseudoacacia and C. caucasica were grown in Alborz Research Station in southwest slope of Alborz mountains in Iran (35° 48′ N, 50° 54′ E and 1300 m a.s.l) with a semi-arid climate. Means annual temperature and rainfall are 13.7 °C and 230 mm, respectively. The seeds were planted in pots (15 cm × 40 cm, diameter and depth) filled with clay, sand and farmyard manure in 2:1:1 proportion, under the same condition in the field, and irrigated daily. The pots were kept under natural photoradiation. The seedlings were successively transferred to the site of experiment after rhizogenesis. After 15 days of acclimation to the new condition, 90 homogeneous plants each species were selected and randomly assigned to five groups. The seedlings were treated with saline solutions containing lead and cadmium. The solution were lead nitrate $(Pb(NO_3)_2)$ and cadmium chloride $(CdCl_2.H_2O)$ in concentrations of 0 (control), 250, 500, 1000, and 2000 mg L⁻¹. Saline solutions containing Pb and Cd were sprayed on the leaves.

Heavy metal experiments

Heavy metal solutions with 250, 500, 1000 and 2000 mg L⁻¹ concentrations were produced by dissolving CdCl₂.H₂O and Pb(NO₃)₂ salts. 10 ml of solution were sprayed to each seedling. The seedlings were treated with different Cd and Pb concentrations during 10 days. There were nine replicates in each treatment.

Measurements of Photosynthetic Parameters

Chlorophyll fluorescence was determined using a PAM_2500 fluorometer (Walz, Germany). Plants were dark-adapted for 30 min to estimate the effect of treatments on photosystem II (PSII) efficiency. The following fluorescence parameters were measured: F_o (the minimum chlorophyll *a* fluorescence after the dark-adaptation), F_m (the maximum chlorophyll *a* fluorescence after the pulse of red light) and F_u/F_m (Kitajima & Butler, 1975; Genty *et al.*, 1989).

Determination of chlorophyll and proline contents

100 mg of fresh plant material was homogenized with 10 ml aceton (80%), then homogenized leaves were centrifuged at 6000 rpm (15 °C) for 10 min. Amounts of supernatants were then supplied to 20 ml. The absorbance at 663 nm and 645 nm determined using extracts was а spectrophotometer (CAIHONG 722 UV/ Spectrophotometer). Chlorophyll *a*, *b*, and total chlorophyll contents were calculated on a fresh weight basis (mg g⁻¹ fw) (Arnon, 1949). Proline content (µg g⁻¹ fw) of the collected determined leaves was spectrophotometrically after Bates et al. (1973).

Statistics

The data was statistically analyzed using an independent T-test and one way variance analysis by SPSS 17.0 to compare paired means between different treatments of Pb and Cd and exposure periods at 5% level of probability.

RESULTS Photosynthesis Lead

 F_v/F_m value of *C. caucasica* reduced significantly on days 7 and 9 in 500 and 1000 mg L⁻¹ treatments by Pb (Fig. 1a). However, F_v/F_m value of R. pseudoacacia reduced significantly in 500 mg L-1 treatment of Pb on day 7 (Fig. 1b). F_o of C. caucasica increased in 500 mg L-1 treatment on days 7 and 9 significantly (Fig. 2a). The results showed a significant increase in 250 mg L⁻¹ treatment of *R. pseudoacacia* on days 7 and 9 (Fig. 2b). F_m was affected by exposure to some concentrations of Pb. There was a significant decrease in F_m of C. caucasica in 1000, and 2000 mg L-1 treatments and a significant increase in 250 mg L⁻¹ treatment on day 7 (Fig. 3a). F_m of R. pseudoacacia was increased significantly in all treatments on the first day. Also, F_m showed a significant increase in 500 and 2000 mg L⁻¹ treatments on day 3, in 250 and 500 mg L⁻¹ treatments on day 5 and 250 mg L⁻¹ treatment of Pb on day 9 (Fig. 3b).

Cadmium

Photosynthesis of C. caucasica was affected by exposure to some Cd concentrations on the first day. F_{v}/F_{m} value in 1000 and 2000 mg L-1 treatments reduced significantly on the first day compared to the control (Fig. 4a). R. pseudoacacia showed a significant decrease in F_v/F_m value in 1000 mg L⁻¹ treatment on the first day and a significant increase in 250 mg L-1 treatment on day 9 (Fig. 4b). Fo of C. caucasica was not affected by exposure to all concentrations of Cd (Fig. 5a). Also, the Cd treatment of 1000 mg L⁻¹ caused a significant increase in F_o of R. pseudoacacia on the first day with respect to other treatments and showed a significant decrease in 500 mg L⁻¹ treatment (Fig. 5b). F_m of C. caucasica showed a significant increase in 250 and 500 mg L-1 treatments of Cd on the first and third days (Fig. 6a). F_m of R. pseudoacacia was affected by 250 mg L-1 treatment of Cd on the first, third and fifth days and showed a significant increase compared to other treatments. Furthermore, F_m decreased significantly on day 7 in 250 mg L⁻¹ treatment compared to the control (Fig. 6b).

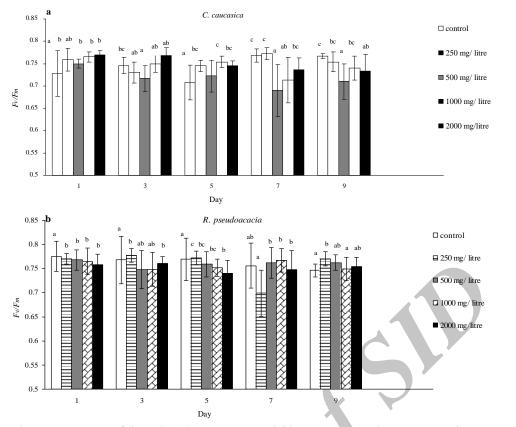


Fig 1. Time course of the F_u/F_m (a) *C. caucasica* and (b) *R. pseudoacacia* leaves up to 9 day spray to different concentrations of Pb (0, 250, 500, 1000 and 2000 mg L⁻¹). Data expressed as mean ± SD; n=9.

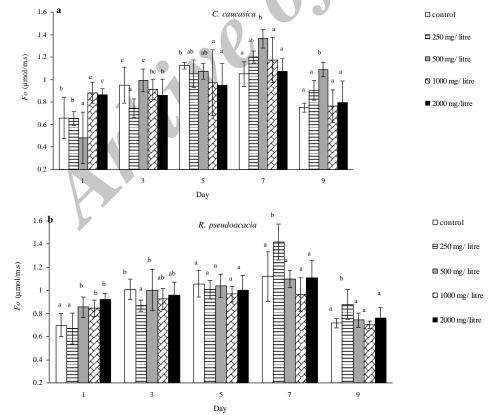


Fig 2. Time course of the *F*_o (a) *C. caucasica* and (b) *R. pseudoacacia* leaves up to 9 day spray to different concentrations of Pb (0, 250, 500, 1000 and 2000 mg L⁻¹). Data expressed as mean ± SD; n=9.

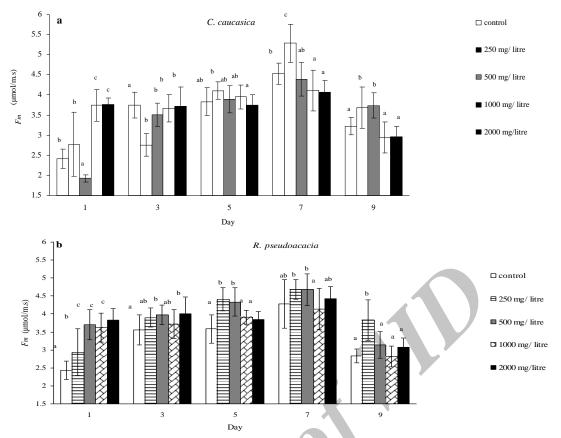


Fig 3. Time course of the F_m (a) *C. caucasica* and (b) *R. pseudoacacia* leaves up to 9 day spray to different concentrations of Pb (0, 250, 500, 1000 and 2000 mg L⁻¹). Data expressed as mean ± SD; n=9.

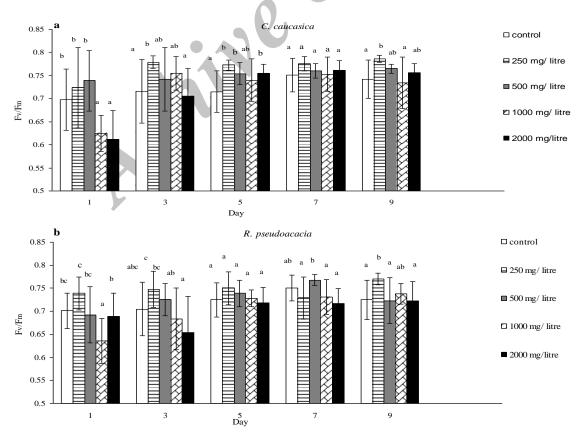


Fig 4. Time course of the F_v/F_m (a) *C. caucasica* and (b) *R. pseudoacacia* leaves up to 9 day spray to different concentrations of Cd (0, 250, 500, 1000 and 2000 mg L⁻¹). Data expressed as mean ± SD; n=9.

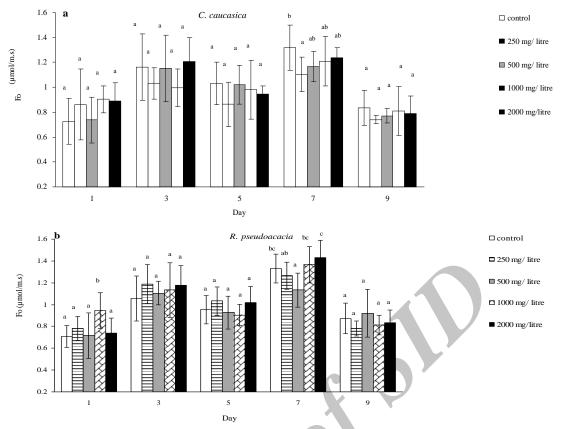


Fig 5. Time course of the *F*_o (a) *C. caucasica* and (b) *R. pseudoacacia* leaves up to 9 day spray to different concentrations of Cd (0, 250, 500, 1000 and 2000 mg L⁻¹). Data expressed as mean ± SD; n=9.

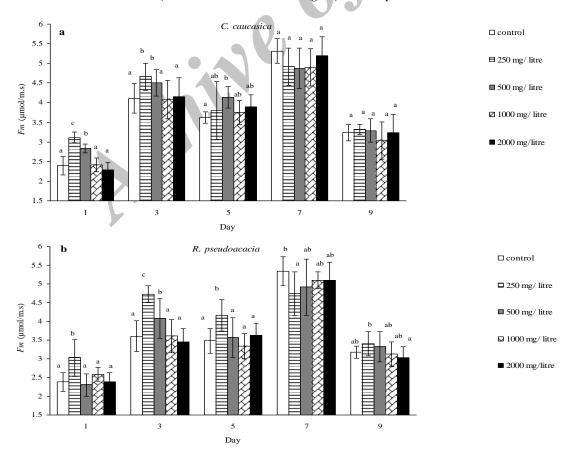


Fig 6. Time course of the *F*_m (a) *C. caucasica* and (b) *R. pseudoacacia* leaves up to 9 day spray to different concentrations of Cd (0, 250, 500, 1000 and 2000 mg L⁻¹). Data expressed as mean ± SD; n=9.

Chlorophyll content Lead

C. caucasica showed a significant increase in chlorophyll *a* and total chlorophyll contents in 250 mg L⁻¹ treatment of Pb compared to the control and other treatments. While, chlorophyll *a*, *b*, and total chlorophyll contents of *R. pseudoacacia* were not affected by exposing to all concentrations of Pb (Fig. 7).

Cadmium

C. caucasica showed no significant difference in chlorophyll contents in responses to Cd treatments. However, chlorophyll *a* and total chlorophyll in *R. pseudoacacia* increased significantly in 1000 and 2000 mg L⁻¹ treatments of Cd compared to the control (Fig. 8).

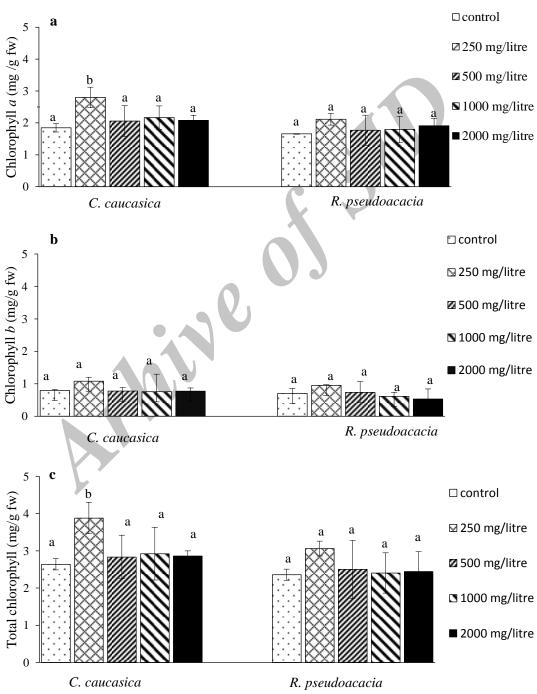


Fig 7. Effects of different concentrations of Pb on (a) chlorophyll *a*, (b) chlorophyll *b*, and (c) total chlorophyll of *C. caucasica* and *R. pseudoacacia* leaves. Values are mean ± SD and bars indicate standard errors.

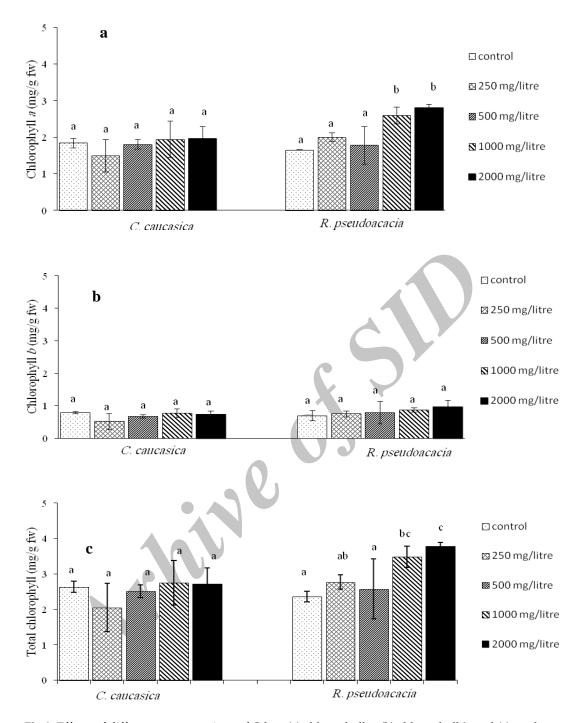


Fig 8. Effects of different concentrations of Cd on (a) chlorophyll *a*, (b) chlorophyll *b*, and (c) total chlorophyll of *C. caucasica* and *R. pseudoacacia* leaves. Values are mean ± SD and bars indicate standard errors.

Proline content

Lead

Proline content of *C. caucasica* increased with increasing Pb concentrations and showed significant differences in 1000 and 2000 mg L⁻¹ treatments compared to the control. Proline content of *R. pseudoacacia* showed no significant difference in Pb concentrations of (Fig. 9a).

Cadmium

C. caucasica was affected by exposure of Cd concentrations . Proline content increased significantly in all of the treatment compared to the control. Also, *R. pseudoacacia* showed a significant decrease in 2000 mg L^{-1} treatment (Fig. 9b).

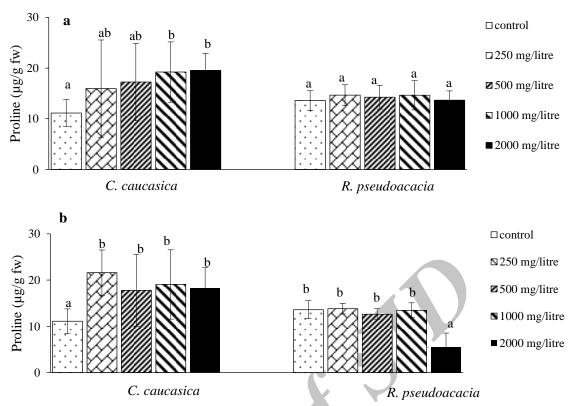


Fig 9. Effects of different concentrations of (a) Pb, and (b) Cd on proline of *C. caucasica* and *R. pseudoacacia* leaves. Value is mean ± SD and bars indicate standard errors.

DISCUSSION

The F_v/F_m value is an indicator of the photosynthetic efficiency of plants. This variable can be a good indicator of the photosynthetic performance of the apparatus and shows the ability of the plants to tolerate environmental stresses (Maxwell & Johnson, 2000). In our study, F_v/F_m value for the control treatment and for 250, 500, 1000 and 2000 mg L-1 treatments of Cd and Pb for both C. caucasica and R. pseudoacacia was slightly lower than the optimal value, i.e., F_v/F_m =0.83 (Bjorkman & Demmig, 1987; Johnson et al., 1999) (Figs. 1a, b and 4a, b). Both Pb and Cd revealed degrees of toxicity, as indicated by the decline in F_v/F_m value of C. caucasica and R. pseudoacacia. The higher concentrations of Pb and Cd (1000 and 2000 mg L⁻¹) had greater apparent stress response in C. caucasica in terms of F_v/F_m value (Figs. 1a and 4a). Chlorophyll fluorescence parameters were affected by Pb on last days (7 and 9) and by Cd on first days (1 and 3). Previous showed that chlorophyll studies fluorescence was reduced by exposure of Cd. For example chlorophyll fluorescence

was reduced significantly at 10 μ mol ml⁻¹ in *Focus vesiculosu*, *Cladophora rupestris*, *Palmaria palmate* and *Polysiphonia lanosa* (Baumann *et al.*, 2009) and F_v/F_m value was reduced at 93 μ M CdCl₂ in *Scenedesmus armatus* (Tukaj *et al.*, 2003). Domongues *et al.* (2008) reported that F_v/F_m value decreased in 200 mg L⁻¹ treatment of Cd in Holm oak (*Quercus ilex*) seedlings.

 F_o values increased by increasing Cd concentration in *Zea mays* L. (Yasemin *et al.*, 2008). It agrees with our results that F_o increased in *R. pseudoacacia* by exposure to 1000 mg L⁻¹ Cd (Fig. 5b). In this study, F_m of *C. caucasica* in 250 and 500 mg L⁻¹ of Cd and *R. pseudoacacia* in 250 and 500 mg L⁻¹ of Pb and Cd increased significantly (Figs. 3b and 6a, b). F_m of *Halophila ovalis* was increased slightly after 72 hours by exposure to Pb concentrations (1 to 10 mg L⁻¹) (Ralph & Burchet, 1998). It has been reported also that F_m was decreased by metals (Mallick & Mohn, 2003; Ralph & Burchet, 1998; Tukaj *et al.*, 2003).

 F_o is an emission from the excited chlorophylls in PSII antenna competing to excitation energy transfer to RCII (Photosystem II reaction centers), which takes place before the excitations energy reach the reaction center (Mallick & Mohn, 2003). Also, F_o fluorescence derives from chlorophyll *a* antennae attached with the PSII light-harvesting complex (Karukstis, 1991), therefore it was obvious that the efficiency of energy transfer from this complex to the PSII reaction center was affected by Cd and Pb stress.

 F_m as the maximal fluorescence yield was increased by Cd and Pb toxicity on day 7. F_m emission indicates the state of PSII when all Q_A molecules are in a reduced state in RCII (Schreiber *et al.*, 1993). F_m increased in 250 and 500 mg L⁻¹ of Cd and Pb in *R. pseudoacacia* and in contrary, in 500, 1000 and 2000 mg L⁻¹ of Cd concentrations in *C. caucasica* F_m was decreased. The increase of F_m was higher than F_o .

Under normal conditions, Q_A is kept oxidized by transferring electrons to NADP and finally to CO₂ via Q_B , the plastoquinone pool and PSI, thus F_v/F_m value remains fairly high. If reoxidation of Q_A is restricted by decrease or slight block of electron transport from PSII to PSI by any of the stress factors, F_v/F_m value may decrease (Mallick & Mohn, 2003).

Catronia et al. (2004) has reported that total chlorophyll concentration decreased under Cu and herbicide stresses. In the other studies chlorophyll content were reduced under Cd toxicity in Atriplex halimus (Nedjimi & Daoud, 2006). Cd occupies position of Mg in chlorophylls structure, leading to chlorophyll destruction (Küpper et al., 1998, 2002) against with what we detected. We observed that chlorophyll a and total chlorophyll increased significantly in C. caucasica in 250 mg L⁻¹ of Pb (Figs. 7a and c) and had significant increase in 1000 and 2000 mg L-1 of Cd in R. pseudoacacia (Fig. 8a,c). Our result were in agreement with several research revealed that increase in chlorophyll contents under air pollution. Tripathi and Gautam (2007) reported the increase (12.8%) in chlorophyll content of Mangifera indica leaves subjected to air pollution. Sevyednejad et al. (2009a) have reported increases in chlorophyll a, b, and total chlorophyll Albizia lebbeck in and Callistemon citrinus under air pollution. Cd toxicity causes leaf chlorosis that is one of the most commonly observed

consequences (Skórzyńska & Baszyński, 1997). It has been proved that the first indicator of flour effects on plant is chlorosis (Kendrickk *et al.*, 1956). By increase in the content of chlorophyll, *C. caucasica* and *R. pseudoacacia* exposed to the concentrations of Cd and Pb can tolerate heavy metal stress not suffering from damages of physiological and biochemical changes.

Increase of free proline content in response to various environmental stresses in plant has been frequently reported (Levitt, 1972). Environmental stress for example, high and low temperatures, drought, air and soil pollutions causes excess Reactive Oxygen Species (ROS) in plant cell, reacting extremely and produces cytotoxic to all organisms (Pukacka & Pukacki, 2000). It has been reported that proline is one of the most universal polyfunctional substance to protect plants under various stresses (Ashraf & Foolad, 2007). In the present study, proline increased significantly in leaves of C. caucasica in Cd and Pb concentrations compared to the control (Figs. 9a and b). Dinakar et al. (2008) reported that proline levels increased in Arachis hypogaea seedlings tissue (leaves and roots) during 25 days increasing Cd concentrations. with Accumulation of free proline in response to heavy metal stress is an usual reaction among plants (Costa & Morel, 1994). Schat et al. (1997) suggested that proline accumulation in plant tissues under Cd stress is due to decrease of the plant water potential and this accumulation can be related to the water equilibrium. However, the resistance of trees to environmental stress in the field should be considered in afforestation efforts. The weak physiological responses of C. caucasica and R. pseudoacacia to Pb and Cd contamination showed the suitability of both species for afforestation in contaminated areas by Pb and Cd.

CONCLUSION

In conclusion, our study showed physiological responses of *C. caucasica* and *R. pseudoacacia* were weakly affected by Pb and Cd contaminations. Chlorophyll fluorescence responses of *C. caucasica* and *R. pseudoacacia* to Pb and Cd were similar. Both species were affected by 250 and 500

mg L⁻¹ of Pb and 1000 and 2000 mg L⁻¹ of Cd, respectively. The effects of different functions of heavy metals on plant organisms were found to be attributed to the differences among concentrations as well as time of affection by Pb and Cd. Detection of weak physiological responses of *C. caucasica* and *R. pseudoacacia* confirmed the possibility of afforestation programs in polluted areas by using Pb and Cd. The resistance to the other environmental stresses, however, should be considered.

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فعاليت فيزيولوژيک داغداغان (.Celtis caucasica L)

و اقاقیا (Robinia pseudoacacia L.) تحت تاثیر تنش عناصر سرب و کادمیوم

(تاريخ دريافت:5/2/29 - تاريخ پذيرش: 92/7/20)

چکیدہ

امروزه جنگلکاری در مناطق آلوده به فلزات سنگین به منظور کاهش آلودگی مدنظر قرار می گیرد. در این تحقیق اثرات سرب و کادمیوم بر روی پارامترهای فلورسانس کلروفیل ($F_{m} F_{0}.F_{v}/F_{m}$)، رنگدانههای فتوسنتزی (کلروفیل B، d و کلروفیل کل) و پرولین در نهالهای یکساله داغداغان و اقاقیا مطالعه شدند. نهالها در طی ده روز، دو مرتبه با غلظتهای مختلف سرب و کادمیوم (صفر، 250، 500، 1000 و 2000 میلی گرم بر لیتر) تیمار شدند. محلول نمکی حاوی سرب و کادمیوم بر روی برگها اسپری شد. فلورسانس کلروفیل به صورت یک روز در میان و محتوای رنگدانههای کلروفیل و پرولین در آخرین روز تیمار اندازه گیری شدند. نتایج نشان داد که فلورسانس کلروفیل داغداغان واقاقیا در روزهای پایانی تحت تاثیر سرب قرار گرفت و کادمیوم فلورسانس کروفیل هر دو گونه را در روزهای شروع تیمار تحت تاثیر قرار داد. محتوی کلروفیل B داغداغان در تیمار 200 میلی گرم بر لیتر سرب و اقاقیا در تیمار 1000 و 2000 میلی گرم بر لیتر کادمیوم افزایش یافت. با افزایش غلظت سرب و کادمیوم مورم بر لیتر سرب و اقاقیا در تیمار 1000 و 2000 میلی گرم بر لیتر کادمیوم افزایش یافت. با افزایش غلظت سرب و کادمیوم مولی مختلف سرب و کادمیوم را در روزهای شروع تیمار تحت تاثیر قرار داد. محتوی کلروفیل B داغداغان در تیمار در غلظت مولین در دافتاین به طور معنی داری افزایش یافت در حالی که غلظت پرولین در اقاقیا تفاوت معنی داری را در غلظت مولین در دافتایان به طور معنی داری افزایش یافت در حالی که غلظت پرولین در اقاقیا نسبتا مشابه است و مختلف سرب و کادمیوم نشان نداد. نتایج ما نشان داد که پاسخهای فتوسنتزی داغداغان و اقاقیا نسبتا مشابه است و میلیت کولین در داغداغان به سرب و کادمیوم ضعیف است و بنابراین هر دو گونه قابلیت کاشت در مناطق آلوده به سرب و کادمیوم را دارند.

* مولف مسئول