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Bioaccumulation and effect of cadmium in the photosynthetic apparatus of *Prosopis juliflora*

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ABSTRACT

In the present study *Prosopis juliflora* plants grown in hydroponics solution were exposed to 50, 100 and 1000 μM CdCl_2 . The cadmium uptake, transport and toxicity on the photosynthetic activities in the plants were measured at 48 h after starting cadmium treatments. The results showed that the concentration of Cd^{2+} in *P. juliflora* tended to increase with addition of Cd^{2+} to hydroponics solution. However, the increase of Cd^{2+} in roots and leaves varied largely. In this sense, the accumulation of Cd^{2+} in *P. juliflora* roots increased significantly in proportion with the addition of this metal. In contrast a relatively low level of Cd^{2+} transportation index, and bioaccumulation factor were found in *P. juliflora* at 48 h after of treatments. On the other hand the maximum photochemical efficiency of photosystem II (F_v/F_m) and the activity of photosystem II (F_v/F_o) ratios in *P. juliflora* leaf treated with Cd^{2+} not showed significantly changes during the experiment. These results suggested that the photosynthetic apparatus of *P. juliflora* was not the primary target of the Cd^{2+} action. Further studies will be focused in understanding the participation of the root system in *Prosopis* plants with the rhizosphere activation and root adsorption to soil Cd^{2+} under natural conditions.

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The Mexicali Valley is located in northeastern Baja California, south of the Imperial Valley in California. Approximately 70% of the cultivated land in Mexicali is irrigated by gravity flow with water from the Colorado River.[1] In recent years the Mexicali industrial unit such as piping steel, paint making, agriculture, paper mill, fish cultivation, electroplating industries drain their wastewater into the river.[2] As a result of these emissions and the fact that metals are non-biodegradable, heavy metals accumulate in soils affecting the environment, leading to a potential toxic exposure of the population.[3] In the actuality, a variety of techniques (e.g. chemical precipitation and adsorption by activated carbons) have been employed to clean-up the soils and effluents.[4] However, these methods are expensive, require high energy and are not able to completely remove the heavy metals. In contrast phytoremediation is proposed as a cost effective alternative for the treatment of contaminated soils.[5] In this sense, many plant species are able to grow under heavy metals polluted environments.[6] The genus *Prosopis* is characteristic of arid and semiarid zones, and its widespread distribution includes ecosystems in Asia, Africa, and the Americas.[7] The previous studies of *Prosopis juliflora* have focused mainly on the evaluation of ability of these plants as a green solution

to decontaminate soil contaminated with Cd^{2+} . [8] However, previous studies using *P. juliflora* plants have demonstrated that the uptake of heavy metals in excess by plants can initiate a disorder of numerous physiological functions causing damage at the cellular level.[9] Therefore a better understanding of the strategies that are used by individuals of *P. juliflora* for cadmium tolerance will help researchers to develop biotechnological alternatives as the phytoremediation in northwest of Mexico.

In the present study the main objective was evaluated the physiological responses of *P. juliflora* to cadmium (Cd^{2+}) a not essential element.

This assessment was accompanied by analyzing the influence of different concentrations of cadmium on the photosynthetic status and bioaccumulation in *P. juliflora* plants in a short time. We presume that individuals of *P. juliflora* denote a high accumulation of cadmium in response to stress caused by acute exposition.

Materials and methods

Germination of *P. juliflora*

Seeds were donated for the National Forestry Commission of Mexico, in Mexicali, BC, this were surface-sterilized by

soaking in 1% NaOCl (Clorox) for 5 min and finally rinsed with deionized sterile water four times altogether and dried at room temperature. After disinfection, the seeds were germinated in sterilized sand (121 °C for 2 h on two consecutive days), in a greenhouse under the following conditions: temperature range 30–34 °C during the day, 28–30 °C during the night, 12 h light: dark photoperiods and 60% relative air humidity. Once the seedlings developed a few roots, they were transplanted to single pots (0.5 L) containing a commercial potting soil mix combined with quartz sand and peat moss (50% soil, 20% sand and 30% peat moss) sterilized by autoclaving at 121 °C for 2 h. The plants were irrigated daily with water and every other week, fertilized with Hoagland solution.

Exposure to cadmium

Twenty-two-months old plants showing similar leaf number and size were randomly allocated ($n = 4$) and transferred to individual plastic containers with the following conditions: 10/14 h dark/light period, 28/30 °C day/night temperatures, 60/80% day/night relative humidity and 16 h of light with $>350 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density. Each container was filled up with 500 mL modified quarter strength Hoagland solution with pH 5.5 and continuously aerated. Cadmium was added as CdCl_2 in 0, 50, 100 and 1000 μM concentration to the nutrient solution, separately.

Control plants were transferred to plastic containers with 500 mL of Hoagland solution without CdCl_2 . These exposures were performed in quadruplicates. After 48 h of exposure the plants were uprooted from the plastic containers and leaves, stems and roots collected for further analysis.

Cadmium determination

At the end of each period of exposition (8, 12, 24 and 48 h), the roots were washed with 20 mM EDTA- Na_2 solution to remove root-adsorbed Cd^{2+} for 5 min and then rinsed with distilled water. Five grams of biomass of the roots and aerial part (leaves and stems) were measured with an electronic balance (VELAB VE-204) with an accuracy of 0.01 g. Then the biomass of roots and aerial parts were dried in a forced-air oven for 72 h at 60 °C, followed by 1 day at 70 °C. Subsequently dried roots and aerial parts of each treatment (500 mg) were digested with 10 mL of nitric acid (85% v/v) overnight according to Gonzalez-Mendoza et al. [10] and Estrella-Gómez et al. [11]. Resulting digests were diluted up to 10 mL with deionized water and then Cd^{2+} concentration was determined for each sample by an inductively coupled plasma optical emission spectrophotometer (ICP-OES 400 Perkin-Elmer USA). The detection limit of Cd^{2+} in ICP-OES was of 0.22 mg L^{-1} . All samples were analyzed at $\lambda = 228.8 \text{ nm}$. For quality control and assurance reagent blanks and a Community Bureau of Reference certified

reference material (Sea Lettuce, *Ulva lactuca*) were run in parallel with each batch of samples. Blanks were always below detection levels, and Cd^{2+} concentrations of the certified reference materials were within 91–95% of the certified value. Each sample was run in triplicate to guarantee that the measured absorbencies were constant. Metal concentrations calculated from each replicate absorbance value, was then used to calculate an average metal sample concentration. The concentration of Cd^{2+} in plant tissues is expressed in $\mu\text{g g}^{-1}$ on a dry weight (dw) basis.

Bioaccumulation factor and translocation index

Bioaccumulation factor (BAF) is an index of the ability of the plant to accumulate Cd^{2+} with respect to its concentration in the medium, bioaccumulation factor, was calculated according to Ghosh and Singh [12]. The relative translocation of Cd^{2+} from roots to other parts of the plants, the translocation index (Ti), was calculated according to Singh et al. [13].

Chlorophyll fluorescence measurement

The chlorophyll fluorescence was measured using a Plant Efficiency Analyzer (Hansatech Instruments Ltd, King's Lynn Norfolk PE32 1JL, UK) on completely expanded leaves. The leaves were subjected to a 5 min period of adaptation to darkness under to induce the complete oxidation of the reaction centers using light exclusion clips.[14] The maximum photochemical efficiency of photosystem II (F_v/F_m), and number of active reaction center (F_v/F_o) were determined at 8, 12, 24 and 48 h after exposure to Cd^{2+} . The plants exposed to the cadmium were arranged in a complete randomized design, with four replications and five plants per plot.

Statistical analysis

Data were analyzed with analyses of variance, and mean were comparison test (Tukey's $\alpha = 0.05$) was performed (Statistical Package version 5.5, Statsoft, USA). Significant differences were accepted if $p \leq 0.05$ and data was expressed as mean \pm standard error.

Results

Cadmium concentration in different tissues of *P. juliflora*

The cadmium concentration in *P. juliflora* increased significantly in roots as Cd^{2+} concentration in the medium increased (Figure 1(b)). In this sense, when plants were exposed to 100 and 1000 μM Cd^{2+} , the concentration of accumulated Cd^{2+} in roots tissues was 0.634 ± 0.004 and $1.43 \pm 0.60 \mu\text{g g}^{-1}$ dry weight, respectively, after 48 h of exposure (Figure 1(b)). In contrast, the Cd^{2+} accumulation

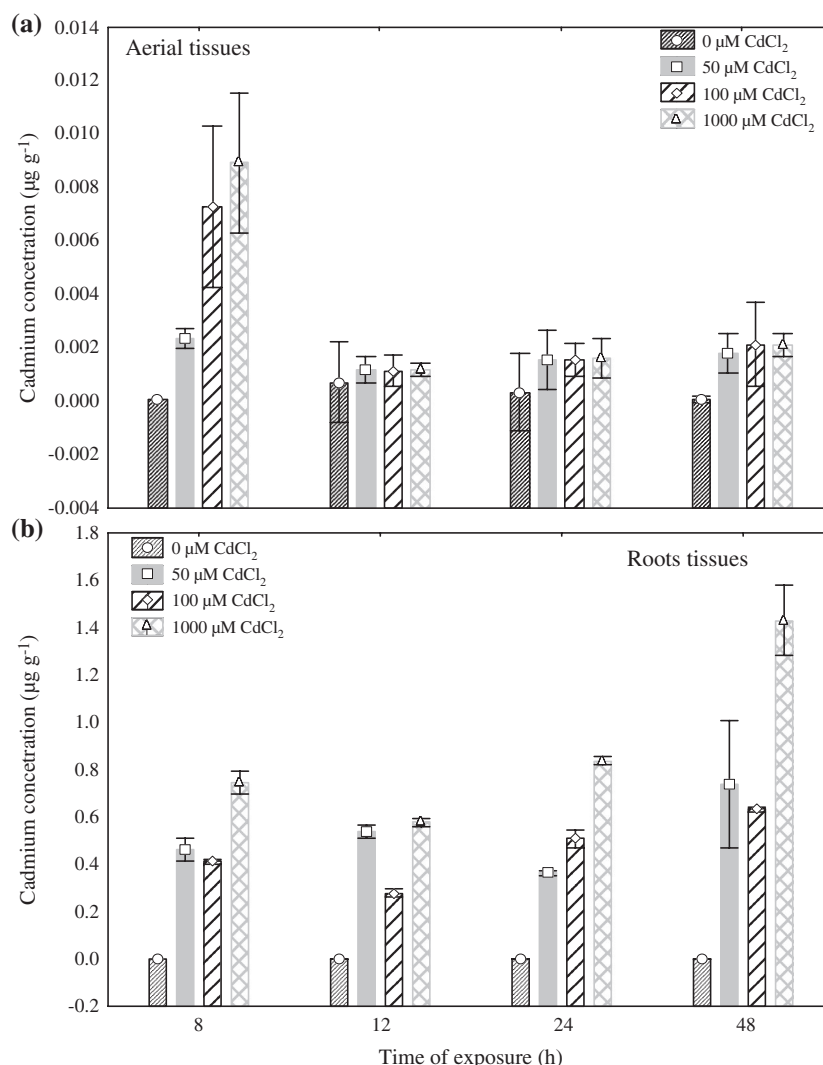


Figure 1. Cadmium concentration ($\mu\text{g g}^{-1}$) in aerial (a) and roots (b) tissues, respectively of *P. juliflora* plants exposed to different concentrations (50, 100 and 1000 μM) under hydroponic conditions during 48 h. Values are mean \pm SD ($n = 4$).

in aerial parts of *P. juliflora* treated with the different doses not showed significantly variation ($p \geq 0.05$) in the accumulation of this metal with respect to low and high doses of Cd^{2+} after 48 h of exposure (Figure 1(a)).

BAF and translocation index of Cd^{2+}

In the present study, the results showed that BAF in root was significantly ($p < 0.05$) higher than aerial parts (stem and leaves) after 48 of exposure to cadmium (Table 1). The BAF in roots showed a significantly increased at 1000 μM (70.85 ± 2.40) at the end of the experiment with respect to 100 (31.7 ± 1.02) and 50 μM of Cd^{2+} (35.93 ± 5.15), respectively (Table 1). In contrast, BAF values for plants exposed to 50, 100 and 1000 μM Cd^{2+} did not show any significant ($p \geq 0.05$) changes at end of experiment (Table 1). On the other hand, the translocation index (Ti) found for *P. juliflora* were considerably low and the lowest index (0.0%) was found with 50 μM , followed by 0.13 and 0.63% for the doses 1000 and 100 μM Cd^{2+} respectively (Table 1).

Chlorophyll fluorescence measurement

The size and number of active reaction center (F_v/F_o) and the maximum photochemical efficiency of photosystem II (F_v/F_m) of *P. juliflora* not showed significantly changes after exposure to 50–1000 μM Cd^{2+} during all period of exposition to metal (Figure 2(a) and (b)).

Discussion

In order to maintain the absorption of essential metals within physiological limits and minimize their negative effects, many mesquite species have evolved a complex network of homeostatic mechanisms in roots that serve to control the uptake, accumulation, trafficking and detoxification of metals.[15,16] In this sense, our result revealed that the amount of cadmium accumulated in the roots of *P. juliflora* indicated that this species have the ability to take up cadmium from the solution and to accumulate this metal in their roots principally. This result might be related to the presence of exclusion and sequestering processes such as production of

Table 1. BAF and translocation index (Ti) of cadmium in *P. juliflora* during 48 h exposure.

Doses (μM)	BAF		Ti
	Roots	Aerial part	Aerial part/roots
50	35.93 ± 5.15^a	0.00 ± 0.00^a	0.0002 ± 0.0003^a
100	$31.70 \pm 1.02^{a,b}$	0.22 ± 0.03^b	0.0063 ± 0.001^b
1000	70.85 ± 2.40^c	0.13 ± 0.02^b	0.0013 ± 0.0002^a
LSD	124.55	74.10	44.41

Note: Data are means \pm SD ($n = 4$). Those with different superscript letter (a, b and c) in the same column are significantly different (LSD, $p < 0.05$).

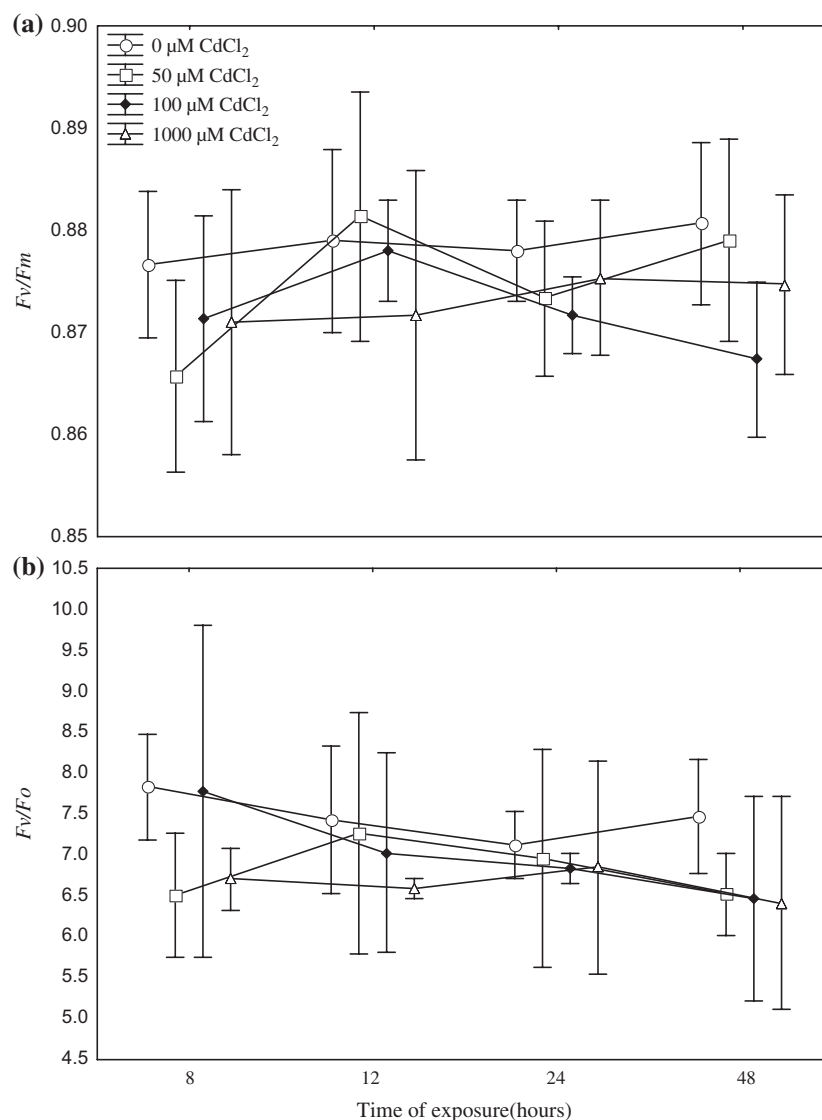


Figure 2. Fluctuations of various fluorescent parameters for *P. juliflora* after 48 h exposure to 50, 100 and 1000 μM Cd²⁺: (a) The maximum photochemical efficiency of photosystem II (F_v/F_m), and (b) number of active reaction center (F_v/F_o). Values are mean \pm SD ($n = 4$).

organic acids, antioxidative replay, cell-wall lignification or suberization that moderate the uptake Cd²⁺ by roots and induce their accumulation in tissues.[10] Some of these processed have been observed in root system of *Avicennia germinans* and *Salix jiangsuensis* J172 exposed to Cd²⁺, where the metal was found mainly on the outer surface of the rhizodermis and in the cell walls of the rhizodermis and cortical cells.[10,17] The higher values of Cd²⁺ found in roots and the low value for aerial parts/root translocation index found in *P. juliflora* exposure during

48 h, suggest a metal exclusion mechanism might be operating, as described by Baker [18] and Boularbah et al. [19]. Similar results were observed by Senthilkumar et al. [20] who reported that *P. juliflora* plants accumulated metals (e.g. Cu and Cd) from contaminated soils, thus reaching higher metal concentrations in roots rather than stems. Finally, considering the accumulation efficiency and tolerance of *P. juliflora* to Cd²⁺, this plant can be explored further for the phytostabilization of the heavy metals (e.g. cadmium) in arid and semi-arid areas.

In other hand, the chlorophyll *a* fluorescence represents an excellent screening tool for evaluation of heavy metal stress in aerial parts of plants.[21,22]

However, in the present study the changes observed in *Fv/Fm* and *Fv/Fo* as result of exposition to Cd^{2+} demonstrated that under our conditions experimental these parameters not showed a rapid inactivation of photosystem II in *P. juliflora* leaves. The presence stable values of the *Fv/Fo* and *Fo/Fv* chlorophyll fluorescence parameter in *P. juliflora* could indicate the stability of the thylakoid structure and efficient electron flow through the photosystems according to Pajević et al. [23]. Similar results were observed by Nikolić et al. [24] who reported that chlorophyll fluorescence parameters not showed considerably changed in three willow species treated with Cd^{2+} under hydroponic culture. In this sense, Vassilev et al. [25] mention that an minimal influence of Cd^{2+} on the chlorophyll fluorescence parameters may occur along with obvious disorders in thylakoid membranes and chloroplasts, implying that others metabolic process, apart from primary photochemical reactions, can also be affected.

Conclusion

The results presented in this research demonstrate that exposure of *P. juliflora* plants to excess Cd^{2+} not causes specific negative effects on the photosystem II. Finally, the present study showed that *P. juliflora* is a promising prospect for heavy metals phytostabilization purposes occurring in arid and semi-arid areas in the northwest Mexico.

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