Changes of photochemical efficiency and epidermal polyphenols content of *Prosopis glandulosa* and *Prosopis juliflora* leaves exposed to cadmium and copper

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**Abstract:** The effect of metals on the photosynthetic activities and epidermal polyphenol content of *Prosopis glandulosa* and *Prosopis juliflora* leaves was investigated by the tissue tolerance test. Foliar tissues of *Prosopis glandulosa* and *Prosopis juliflora* were incubated with Cd<sup>2+</sup> (0.001 M) or Cu<sup>2+</sup> (0.52 M) concentrations for 96 h. The results showed that significant reductions (p < 0.05) of photochemical efficiency in *P. juliflora* leaves were found after 96 h of exposure to 0.52 M Cu<sup>2+</sup> compared with Cd-treatments and controls. In contrast, *P. glandulosa* leaves showed a progressive increase on photochemical efficiency at 72 h after Cu-treatment. The results also showed a significant decrease (p < 0.05) of epidermal polyphenols in *P. juliflora* leaves after 24 h of exposure to 0.52 M Cu<sup>2+</sup> compared with Cd-treatments and control leaves. On the other hand, the values of leaf epidermal polyphenols observed in *P. glandulosa* exposed to copper and cadmium did not show any difference with respect to control. These findings are very important and suggest that these compounds could be considered as a protection mechanism of *P. glandulosa* when is treated with these heavy metals. Finally, the results of bioaccumulation showed that the copper concentration in *P. glandulosa* was higher than the values detected in *P. juliflora* Nevertheless, the cadmium concentration in foliar tissues of *P. juliflora* was significantly higher than *P. glandulosa* after 96 h of exposure to Cu<sup>2+</sup> or Cd<sup>2+</sup>. Therefore, future studies are necessary to elucidate the effects of heavy metals on the biosynthesis of flavonoids and participation of these compounds in the reduction of metal toxicity in *Prosopis* species.

**Keywords:** heavy metals, *Prosopis* species, flavonoids, chlorophyll a fluorescence, bioremediation

**1 Introduction**

As result of a rapid industrialization and urbanization processes in the northwest of Mexico, the release of several contaminants like cadmium and copper in the ecosystem has increased and become a serious problem in this ecoregion of Mexico [1, 2]. These heavy metals can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences in all life forms. In the plants, these elements may induce toxic effects at the cellular level due to alteration of membrane permeability, enzyme inhibition, and induction of antioxidative processes in the plants [3]. Recent studies show the existence of numerous remediation techniques based on chemical or physical principles to clean up the soils from different ecosystems [4]. However, these techniques are characterized by high costs of operation and high levels of energy consumption. Therefore, the use of these technologies is inaccessible in many developing countries. For this reason, the phytoremediation is proposed as a cost effective alternative for the treatment of contaminated soils in different ecosystems [5].
Species of mesquite trees of the genus Prosopis, such as P. glandulosa var. torreyana., and P. juliflora (Sw.) DC., are found in the northern of the Mexico. These species have formed forest extensions known as mezquitales, as one part of desert ecosystems [6]. In recent years, reports have shown that the genus Prosopis, could be considered especially resistant to heavy metals [7,8]. In addition, the translocation mechanisms of heavy metals from the roots to the aerial parts of Prosopis species have been evaluated in order to use these plants in the phytoremediation of contaminated soils [9,10].

In this sense, recent studies suggested that Prosopis species (eg., P. glandulosa and P. juliflora) have developed an exclusion strategy favoring the retention of metals in roots and suggesting the existence of a mechanism of efficient metal uptake and accumulation in the roots [7,11]. Notably, the effect of heavy metals on the physiological status of P. juliflora has been widely investigated but studies of P. glandulosa are scarce [7,12]. Therefore, the overall goal of this work is to evaluate the changes on photochemical efficiency and the total phenolic content of P. glandulosa and P. juliflora leaves exposed to Cd^{2+} and Cu^{2+}; revealing the potential of these species for phytoremediation purposes.

2 Methods

2.1 Seed Collection and Germination

Seeds of Prosopis glandulosa and Prosopis juliflora were donated by the National Forestry Commission of Mexico from a native population in the Mexicali valley, Baja California, Mexico (32°24’6.8394” N, 115°11’51” W). One hundred seeds of each Prosopis species were disinfected with 1% NaOCl (Clorox) for 5 min, and then washed with deionized sterile water. Later, seeds were germinated in sterilized sand (121°C for 2 h during two consecutive days). Seedlings were cultivated with 12 h light:dark photoperiods (>350 μmol m^{-2} s^{-1} photon flux density) in a greenhouse, 60% of relative air humidity temperature and day/night temperatures of 30/32 °C were used. Seedlings were irrigated daily with water and every other week, fertilized with Hoagland solution according to [13].

2.2 Preparation of Leaf Cultures and Heavy Metal exposure

Leaves of P. glandulosa and P. juliflora from seedlings were collected. Groups of five leaves were randomly allocated and transferred to Petri dishes (n = 4) supplemented with 10 ml of a solution prepared with 0.001 M of cadmium chloride (CdCl_{2}) and 0.52 M of copper sulfate (CuSO_{4} \cdot 5H_{2}O), according to a previous study [14]. Control leaves of plants were transferred to plastic Petri dishes containing 10 ml of bidistilled water. The Petri dishes were incubated for 96 h as earlier described [13].

2.3 Determination of Physiological Parameters

Determinations of leaves chlorophyll (Chl) and polyphenol contents (Phen) were done using the Dualex sensor (FORCE-A, Orsay, France) according to [15]. In the case of pigment analysis, all measurements were carefully conducted with the leaves adaxial side facing the light source. The content of polyphenols and leaf chlorophyll were expressed as Dualex units. Readings were taken at 0, 24, 48, 72 and 96 h after exposure to copper and cadmium using four leaves per treatment. In addition, chlorophyll fluorescence was measured by a Chlorophyll Fluorometer (OS-30p, OPTI-SCIENCE, USA) according to [16]. Readings 0, 24, 48, 72 and 96 h after exposure to copper and cadmium using four leaves per treatment using light exclusion clips. The potential photochemical yield (Fv/Fm) was calculated according to the method of [17].

2.4 Heavy Metals Determination

At the end of bioassays, the leaves were washed with a 15 mm EDTA-Na_{2} solution for 3 min to remove the cadmium and copper, and then rinsed with distilled water. Five grams of leaf samples (Cu^{2+} and Cd^{2+}) were dried in a forced-air oven for 72 h at 65 °C. After drying, the samples of each treatment were homogenized and milled. Leaf material (500 mg) was digested with 10 mL of nitric acid (85 % v/v) overnight according to [14]. Digested samples were diluted up to 10 mL with deionized water, then the cadmium and copper concentrations were determined for each sample using an inductively coupled plasma optical emission spectrophotometer (ICP-OES 400 Perkin-Elmer USA) at λ= 324.8 (Cu^{2+}) and 228.8 nm (Cd^{2+}), respectively. Each sample was run in triplicate to ensure accuracy. Metal concentrations calculated from each replicate absorbance value, was then used to calculate an average metal sample concentration. The concentration of both metals in leaves sample was expressed in ppm.
2.5 Statistical Analysis

Data were evaluated by One-way analyses of variance (ANOVA) and the differences between means were determined by Tukey-Kramer Test ($P < 0.05$) using the statistical package SAS (Version 9.0, SAS Institute, 2002).

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

3 Results

3.1 Leaf Chlorophyll (Chl) Content

In the present study, the exposure of *P. glandulosa* leaves to Cu$^{2+}$ and Cd$^{2+}$ (0.52 M Cu$^{2+}$ and 100 µM Cd$^{2+}$, respectively), did not show significant changes in the Chl values during all experiment with respect to control (Fig. 1). On the other hand, the effect of cadmium on *P. juliflora* leaves showed a significant reduction of Chl after 96 h of exposure.

(Fig. 1). In contrast, the chlorophyll in the *P. juliflora* leaves immersed in a copper solution did not exhibited significant changes compared with the leaves immersed in water (Fig. 1).

3.2 Epidermal Polyphenols (EPhen) and Chlorophyll a Fluorescence

Content of EPhen in the *P. glandulosa* leaves of control treatments did not show any changes during the experiment (Fig. 2). However, when this species was exposed to a cadmium solution, it showed an increase after 48 h of exposure to metal. On the other hand, the measurements of EPhen in the *P. glandulosa* leaves treated with copper did not show significant changes during the experiment (Fig. 2). In contrast, our results showed changes in the physiological responses of *P. juliflora* exposed to copper suggesting that the time after stress application is a key factor (Fig. 2). In this sense, the EPhen content decreased in two periods of time: 24 to 48 h...
In the present study, the measurement of Fv/Fm in P. glandulosa leaves treated with copper showed a decrease (p<0.05) from 24 to 48 h followed by a progressive rise from 72 to 96 h (Fig. 2). Finally, the Fv/Fm values observed in the P. glandulosa leaves treated with cadmium (0.001 M) did not show significant changes compared with the control (Fig. 3).

3.3 Heavy Metals Concentration in P. Juliflora and P. glandulosa

The bioaccumulation of cadmium and copper of both Prosopis species are shown in Table 1. The results of bioaccumulation showed that the copper concentration of P. glandulosa was higher than P. juliflora (Table 1). Nevertheless, the cadmium concentration in foliar tissues of P. juliflora was significantly higher than P. glandulosa after 96 h of exposure.

4 Discussion

In the present study, our results show that P. glandulosa appear to have a higher metal tolerance (e.g., copper) than P. juliflora. In this sense, the reduction of Fv/Fm values observed in P. juliflora could be explained by negative effects of copper on the structure and composition of reaction center complex (RC) of pigment-depleted Photosystem II reaction centers (PS II-RCs) [17]. In addition, the differences observed in the Fv/Fm values among these Prosopis species treated with copper may be related to capacity of the P. glandulosa leaves for acquisition and transport of metal. For example, no symptom of necrosis or chlorosis were observed in the leaves of P. glandulosa, suggesting that the dose of Cu²⁺ evaluated was not severely phytotoxic compared to P. juliflora. Similar results were observed in willow plants exposed to heavy metals, where the genetic variation was related with the tolerance capacity [18]. On the other hand, several studies show that the plants possess a sophisticated and interrelated
network of biochemical strategies to reduce the negative impact of heavy metals on the physiological processes; such as mechanisms of induction of chelating agents (eg., phytochelatins), the production of specific amino acids (eg., proline) and plant secondary metabolites as flavonoid and phenolic compounds [19,20]. In this sense, recent studies have suggested that the epidermal polyphenols (EPhen) content in plants, mainly flavonoids, may act as chelating agents of metal ions reducing the presence of free radicals, and protect against oxidative stress [21]. Our results showed that the synthesis of EPhen was negatively affected when *P. juliflora* was treated with increasing copper doses. Therefore, the low values of EPhen in *P. juliflora* could be explained by impairment of antioxidative system due to the exposure to copper reducing the synthesis of new polyphenols. Similar results have been reported in *Euglena gracilis*, which is not able to counteract the effects of exposure to cadmium [22]. On the other hand, the values of EPhen observed in *P. glandulosa* exposed to copper and cadmium did not show differences compared with the control. This is very important and suggests that these compounds could be considered as a protection mechanism of *P. glandulosa* when is treated with heavy metals. Notably, studies have suggested that flavonoids may act as antioxidants by donating electrons to guaiacol-type peroxidases (GuPXs) for the detoxification of 

H$_2$O$_2$ produced under conditions of heavy metals stress [23].

Additionally, the EPhen might cooperate with different antioxidant metabolites (ascorbate cycle, proline or glutathione) to scavenge the hydrogen peroxide which leaks out from mesophyll cells where it is produced in response to heavy metals [21]. In our study, both *Prosopis* species accumulated copper in their leaves but *P. glandulosa* was characterized by a high concentration of this metal. These results might be related with the presence of exclusion processes that moderate the metal uptake and induce their accumulation [24]. In contrast, the presence of low values of cadmium found in *P. glandulosa* and high values for *P. juliflora* during 96 h of exposure, suggest that a metal exclusion mechanism might be operating, as previously described [25]. Therefore, our study suggest that differences in the heavy metal accumulation by *Prosopis* species may be result of an integrated network of multiple physiological responses due to genetic factors. Some of these processes have been observed in *Euglena gracilis* exposed to cadmium where the heavy metal induced changes in the production of total phenolic compounds [22].

5 Conclusion

In conclusion, copper produces a variety of toxic effects on photosynthetic activity of *P. juliflora* but not of *P. glandulosa*. This suggests that the tolerance to heavy metals by *P. glandulosa* and *P. juliflora* can depend on multiple factors, such as genotype, growth stage, concentration, and exposure time to heavy metals.

Hence, future studies are necessary to elucidate the effects of heavy metals in the biosynthesis of flavonoids and participation of these compounds in the reduction of metal toxicity in *Prosopis* species. Additionally, we propose the determination of epidermal polyphenols as suitable parameters for plant test systems, which can be utilized for successful screening for higher heavy metal tolerance among *Prosopis* plants.

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