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Physiological Responses of *Azolla caroliniana* Exposure to Cadmium

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Abstract: The objective of this work was to evaluate the effects of cadmium exposure on *Azolla caroliniana* photosynthetic and chlorophyll content. Azolla ferns were exposed to different concentrations of cadmium (0, 0.02, 0.2 and 2 mM) for 72 h. The results showed that treatments with higher cadmium concentration (0.2 and 2 mM) presented clear signs of disturbing photosystem II, since the potential photochemical yields (Fv/Fm) were lower than the control responses. While chlorophyll total contents present a significant decreased with higher cadmium concentration after 24 h of exposure. Finally, our results also demonstrated that chlorophyll fluorescence and chlorophyll total (no destructive methods) analysis can be used as a useful physiological tool to assess early stages of change in photosynthetic performance of *Azolla* in response to cadmium pollution.

Key words: Cadmium · *Azolla* · Photosystem II

INTRODUCTION

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 with water from the Colorado River and the Hardy River also receives brine waste with potentially high concentrations of heavy metals from a geothermal energy plant located at Cerro Prieto in the middle of the agricultural valley, approximately 30 km south of the city of Mexicali. In this sense cadmium is well studied in aquatic environments because it is a particularly toxic heavy metal [1]. Cadmium has no known function in biological systems and even small amounts can be toxic to organisms (5µg/l). Several studies in aquatic systems have show that some plants can accumulate heavy metals and other nutrients from contaminated waters and they may be directed to phytoremediation process [2]. However, selection of plant species for removing metal ions from polluted water will also depend on their growth rate, level of tolerance to heavy metals and concentration of metals in the environment [3]. In this sense, the aquatic fern *Azolla* has been reported to accumulate high concentration of heavy metals and metalloids (3-4 mg L^{-1}) from aqueous media [4-5]. However, even though, it is known that small amounts of metals are accumulated in frond of *Azolla* species, their tolerance and physiological responses to Cd^{+2} have been scarcely studied. The aim of this work was to investigate the changes of photosynthetic activity and chlorophyll content as possible indicators for heavy metal stress in *Azolla* ferns exposed to cadmium.

MATERIALS AND METHODS

Biological Material and Experimental Conditions: One-gram of fresh weight of *Azolla caroliniana*, from the *Azolla* collection Azollatum of Colegio de Posgraduados were placed into plastic containers with 150 mL of Yoshida´s nutrient solution [6]. The pH initial of nutrient solution was 5.5 and electrical conductivity was 876 µS m^{-1} (means four replicates). In addition, a set of

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increased $CdCl_2$ concentrations were applied to the nutrient solution (0.02, 0.2 and 2 mM) and a non-contaminated nutrient solution was used as control. There were four treatments with three replicates from *Azolla caroliniana*. A completely randomized experimental design was set under growth chamber conditions (26°C, 12 h photoperiod, light 280 μmol cm^{-2} s^{-1} and 80% HR; Lab-Line Biotronette®).

Chlorophyll Fluorescence: Chlorophyll fluorescence was measured by a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk PE32 1JL, UK) according to Strasser and Strasser [7]. Reading was collected at 12, 24, 36, 48, 60 and 72 h after exposure to cadmium using three single fronds per treatments. The randomly selected fronds were subjected to a 5 min period of adaptation to darkness under to induce the complete oxidation of the reaction centers. The potential photochemical yield (Fv/Fm) was calculated according to the method of Küpper *et al.* [8].

Chlorophyll Measurement by a Chlorophyll Meter (SPAD-502): For individual fern, three successive readings (depending on the area) in SPAD units were taken by a chlorophyll meter SPAD-502 (Konica Minolta, Osaka Japan) across the whole surface of leaves. The mean of the measurement was calculated using the internal function of the chlorophyll meter.

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

RESULTS AND DISCUSSION

Chlorophyll Fluorescence: Some authors have emphasized on direct effect of Cd on the membrane bound photochemical reactions while others have concentrated on some Calvin's cycle enzymes [9-10]. One of the possible reasons for this disagreement is the fact that results obtained by destructive analyses do not always represent the real situation *in vivo*. In this sense the use of chlorophyll fluorescence represent an alternative method that may be used as an *in vivo* plant stress indicator [11]. The results showed that the *Azolla carolininana* were not affected by concentrations of Cd^{2+} lower than 0.2 mM, however, the treatments with higher doses of cadmium *Azolla* during the first 24 h of exposure shower significant reductions (20-30%) when compared to their respective controls (Fig. 1). This reduction on Fv/Fm may be in part due to the negative effects of Cd on the photochemical reactions (eg., activities of Rubisco and phosphoenolpyruvate carboxylase) and subsequently affected the PSII electron transfer (electron transport is blocked) [12]. Similar results were found by Sanchez-Viveros *et al.* [11]where the suppressed relative yield of potential photochemical yields (Fv/Fm) by heavy metals (*eg.*, Cu) as well as its dynamics in *Azolla* ferns demonstrated a rapid inactivation of PS II induced by the metal.

In summary, the concerted decreases found on chlorophyll fluorescence in plants treated with lower and higher Cd^{+2} concentrations clearly indicate that Cd^{+2} may induce important alterations on the photosynthetic apparatus of *Azolla* ferns.

Chlorophyll Content: In the present investigation, Cd^{+2} toxicity in *Azolla* fronds were assessed by a chlorophyll meter (SPAD-502). The chlorophyll meter or SPAD meter is a simple, portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves. In this sense, our results indicate that the concentration of chlorophyll content (units SPAD) in *Azolla* fronds was decreasing with the increasing concentration of Cd at 36 h after of treatment (Fig. 2). Furthermore, there was a significant difference between the treated groups and the control group ($P < 0.02$) at final of experiment (Fig. 2). This results can be due at that Cd can cause disorganization of chloroplasts leading to a reduction of the photosynthetic pigments by chlorophyll biosynthesis inhibition that leading at chlorophyll contents low [13-14]. On the other hand diverse studies reported that the decline in chlorophyll content might be caused by a reduction in the synthesis of chlorophyll, possibly by increasing chlorophyllase activity, by disorderness of chloroplast membrane and by inactivation of electron transport in photosystem I [15]. Similar results were found by Arunakumara and Zhang [16] where the cells of *Synechosystis* sp. PCC6803 (unicellular cyanobacterium) exposed to different dose of Cd showed a rapid reductions of chlorophyll total.

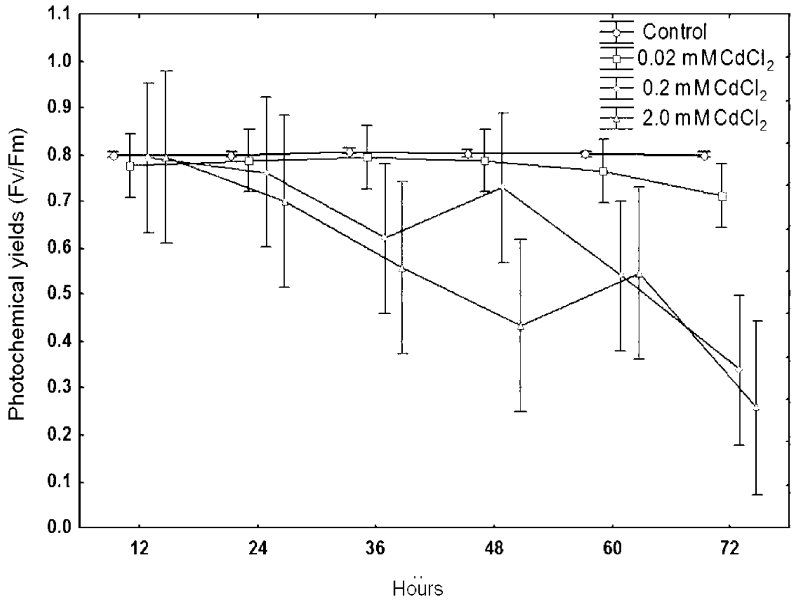

Fig. 1: Time course response of potential photochemical yields of PSII measured in fronds of *Azolla caroliniana* exposed to 0.02, 0.2, 1, 2 mM Cd^{+2} during an exposure period of 72 h. Means ± Standard error. *n*=3.

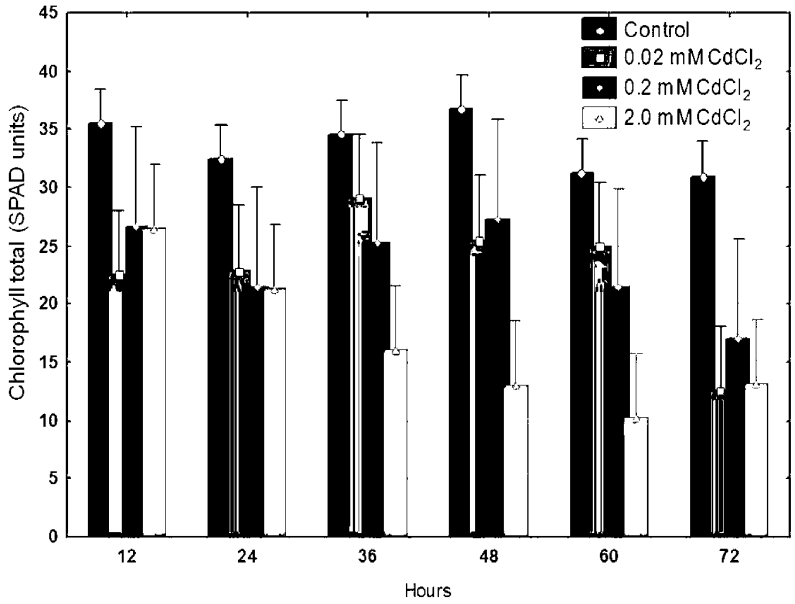

Fig. 2: Variation of Chlorophyll content (SPAD units) in fronds of *Azolla caroliniana* exposed to 0.02, 0.2, 1, 2 mM Cd^{+2} during an exposure period of 72 h. Means ± Standard error. *n*=3.

CONCLUSION

Our results suggest that one of the negative effects of cadmium, bases on chlorophyll fluorescence and Chlorophyll total tests, may be related to a rapid inactivation of PSII. Additionally, the used of chlorophyll fluorescence and chlorophyll total tests may be used as physiological indicators' to understand in part, the main mode of action of cadmium on the photosynthetic apparatus of *Azolla* ferns in the ecosystem.

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