

Effect of Citric Acid on Cadmium Ion Uptake and Stress Response of Hydroponically Grown Jute Mallow (*Corchorus olitorius*)

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Abstract

This research work investigates the possibility of improving the phytoextraction of Cd by the application of citric acid. For this purpose, plants were grown in hydroponics under controlled conditions. Addition of 1, 5, 10 and 20 mg/L Cd²⁺ significantly decreased the plant growth and biomass, and increased proline contents. The effects were dose dependent with obvious effects at higher Cd²⁺ concentration of 20 mg/L. Application of 5mM citric acid (CA) significantly depressed Cd²⁺ uptake and its accumulation in plant roots and shoots. CA alleviated Cd²⁺ toxicity by reducing oxidative stress. Application of CA also enhanced the antioxidant enzymes activity alone and under Cd stress. The results showed that Cd²⁺ accumulated more in roots than the shoots and application of citric acid depressed Cd²⁺ uptake at all concentrations. Jute mallow (*Corchorus olitorius*) proved to be an effective accumulator for cadmium, however, neither concentration of citric acid showed advantages for phytoextraction of cadmium. The results showed that jute mallow is a potential plant for phytoextraction of Cd without the use of CA as enhancer.

Keywords: Cadmium; Citric acid; Proline; Phytoextraction; Biomass; Jute mallow (*Corchorus olitorius*)

Introduction

Among the heavy metals, cadmium (Cd) is a highly toxic, non-essential and carcinogenic element [1]. In plants, Cd²⁺ enters mainly through root uptake and is transported to above ground parts [2,3]. Higher Cd²⁺ concentration in plants caused several physiological and biochemical disorders including reduced growth and yield, nutrient uptake, changes in chloroplast ultrastructure and initiation of oxidative stress [4,5]. Food chain Cd contamination is the main source of Cd entry to human especially non-smoking general population and is the main constraint for food safety and agricultural land quality [6]. Therefore, different actions can be under taken to remediate Cd contaminated soils including in situ remediation techniques. Plant based remediation techniques are becoming more wide spread as these are environmental friendly and cost effective. Phytoremediation is a biological technique, considered for cleaned up of polluted sites because of its economical, visual advantages and extensive applicability [7,8]. Phytoextraction is a technique which uses plants to hyperaccumulate metals in to harvestable plants. The degree of metal, translocation from root to aerial plant parts depends upon plant species, metal and environmental conditions.

Jute mallow is tolerant to metal stress, has high biomass, the second most important source of fiber after cotton and the ability of jute mallow to give some financial benefit after harvesting makes it a potential plant for phytoextraction [9].

The objectives of the research are; to study the bioaccumulation capability of jute mallow, the stress response of jute mallow to cadmium uptake, to use jute mallow as hyperaccumulator and the possibility of using jute mallow for phytoremediation.

Materials and Methods

Growth conditions and treatments

Eight week old seedlings of Jute mallow (*Corchorus olitorius*) were collected from the Department of Agronomy farm, Bayero University, Kano on Thursday 20th November, 2014 by 9.00 am. After washing with tap water to remove the soil, they were rinsed with deionise water and

replanted in hydroponic solution. They were carefully monitored in a greenhouse for ten days under the conditions: 65% relative humidity, 14 hrs per day, 10 hrs per night under a light intensity of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and average temperatures 39/24°C [10]. Hoagland nutrient solution was used in the experiment which was made of 5 mM KNO₃; 5 mM Ca(NO₃)₂; 1 mM KH₂PO₄; 2 mM MgSO₄; 46.3 μM H₃BO₃; 11.8 μM MnCl₂·4H₂O; 0.7 μM ZnSO₄·7H₂O; 0.32 μM CuSO₄·5H₂O; 0.16 μM H₂MoO₄·H₂O, and 12.5 μM Fe-EDTA. The treatments consisted of five Cd dosages (0, 1, 5, 10, and 20 mg/L) supplied as Cd(NO₃)₂·4H₂O. In the second phase, 5mM of CA were added while varying the concentrations of Cd²⁺ from (0, 1, 5, 10, and 20 mg/L). Each treatment was triplicated and allowed to stand for ten days. The plants were harvested from the hydroponic solution and washed with tap water, rinsed with 1% HNO₃ followed by deionised water and then wiped with tissue paper. They were then dried in an oven at 65°C. The weight of each plant harvested was recorded. The plants were then sorted into roots and shoots.

Measurement of heavy metal content and statistical analysis

The roots and shoots of the various plants harvested were ground to fine powder. Based on availability, 0.5 g (root) and 1.0 g (shoot) were used for the analysis. They were weighed into porcelain crucibles and ashed at 450°C in a muffle furnace to constant weight. The ash was dissolved in 0.1 moldm⁻³ nitric acid, filtered and made to mark in a 50 cm³ volumetric flasks. The plants extracts and blank were stored at low temperature before analysis [11].

The concentration of Cd in plant root and shoot were determined by using atomic absorption spectrometer (model PG990). Analysis of

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variance (ANOVA) using the SPSS software was performed to check the accuracy and validity of the results. Data were expressed as mean followed by SD. Statistical significance was assumed at $P < 0.05$.

Determination of proline in plant shoots and statistical analysis

The proline content in the shoots was determined by the method recommended by Bates et al. [12]. The concentration of proline in plant shoots was determined by using UV/Visible spectrophotometer (model T60). Analysis of variance (ANOVA) using the SPSS software was performed to check the accuracy and validity of the results. Data were expressed as mean followed by SD. Statistical significance was assumed at $P < 0.05$.

Results and Discussion

Cadmium concentration and uptake by jute mallow

Cadmium concentrations in roots and shoots (leaf and stem) of Jute mallow plants were significantly increased ($p < 0.05$), when plants were exposed to Cadmium at varied concentrations (1, 5, 10 and 20 mg/L) relative to the control plants (Figure 1). Cadmium concentration in roots and shoots gradually increased with increasing Cd concentration in the Hydroponic treatment.

The concentration was largest in the roots than the shoots correspondingly. Interestingly, application of Citric acid at constant concentration (5mM) significantly decreased ($p < 0.05$), Cd concentrations in roots and shoots as compared to respective only Cd treated plants (Figure 1).

Regardless of the different treatment, the roots parts, were the plant most accumulation areas, while concentrations in other parts were in decrease sequence Root>Shoot.

The changes in translocation factor (TF) and bioaccumulation factor (BCF) against the concentrations of added Cd^{2+} are shown in Figures 2 and 3.

According to Chen et al. [13], translocation factor (TF) is the ratio of the concentration of a metal in the aerial part of a plant divided by the concentration in the root. In this study, the ratio can be used to evaluate the translocation effects of Jute mallow (*Corchorus olitorius*) seedlings replanted in hydroponic solutions. TF varied significantly

with concentration of added Cd^{2+} ($P < 0.05$). Thus, a relatively good fraction of the Cd^{2+} was translocated to the shoots (Figure 2).

The bioconcentration factor (BCF) is the metal concentration in the root divide by the concentration of the solution. The value was used in this research to explain the uptake of Cd^{2+} ion by Jute mallow (*Corchorus olitorius*) from the solution to its root. The BCF showed that the bulk concentrations of Cd^{2+} remained in the roots (Figure 3).

Effects of cadmium and citric acid on proline contents of jute mallow

Proline contents significantly increased ($p < 0.05$), when Plants were exposed to Cadmium at varied concentrations (1, 5, 10 and 20 mg/L) relative to the control plants. Addition of constant concentration of CA (5mM), significantly decreased ($p < 0.05$) the proline content in plants as compared to respective only Cd^{2+} treated plants (Figure 4).

Discussion

In the present study, Cd^{2+} induced oxidative stress as shown by increase in proline production (Figure 4). Larger production of MDA under cd stress has also been reported in *B. napus L.* seedlings [14] and tomato [15]. The result suggested that reactive oxygen species (ROS) might be produced in Jute mallow plant under Cd^{2+} stress and cause lipid peroxidation, as evidenced by the increased of shoots proline.

Exposure of plants to non-redox reactive metals also resulted in oxidative stress as indicated by lipid peroxidation, (H_2O_2) accumulation, and an oxidative burst. Available data suggest that Cd, when not detoxified rapidly enough, may trigger, via disturbance of the redox control of the cell, a sequence of reactions leading to growth inhibition, stimulation of secondary metabolism, lignification, and finally cell death.

By contrast, CA plus Cd^{2+} addition significantly ($p < 0.05$), prevented proline accumulation, when compared with the Cd^{2+} treatment alone, suggesting a protective role of CA in preventing oxidative stress in Jute mallow. Cd^{2+} cannot directly produce ROS [16]. Plants usually face oxidative damage when in contact with Cd^{2+} and other metals [17].

The Cd^{2+} concentration and uptake by Jute mallow increased in roots and above ground parts with increasing Cd^{2+} levels in the nutrient solution (Figure 1). These results are in line with the previous findings in some plants like *B. napus L.* [18,19].

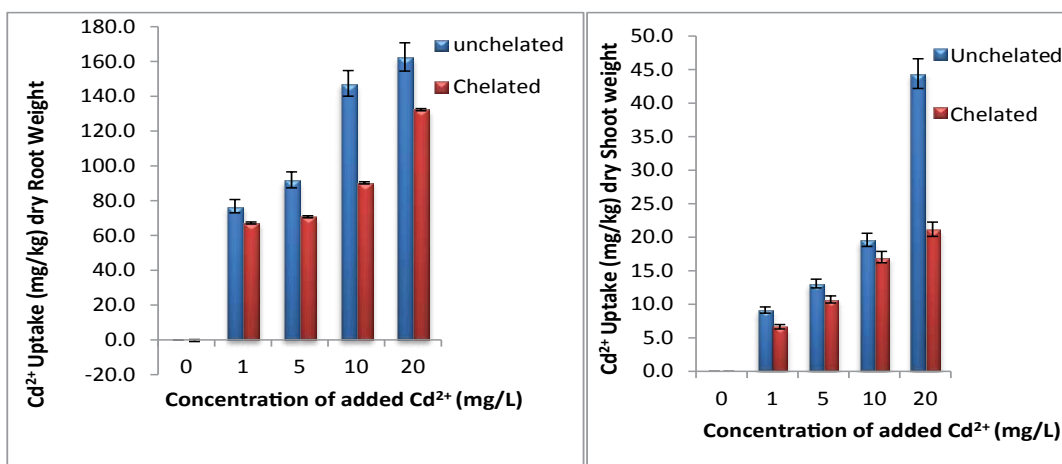


Figure 1: Total Cd^{2+} uptake by Unchelated (treatment without citric acid) and Chelated (treatment with citric acid (5mM)) hydroponically grown Jute mallow (*Corchorus olitorius*).

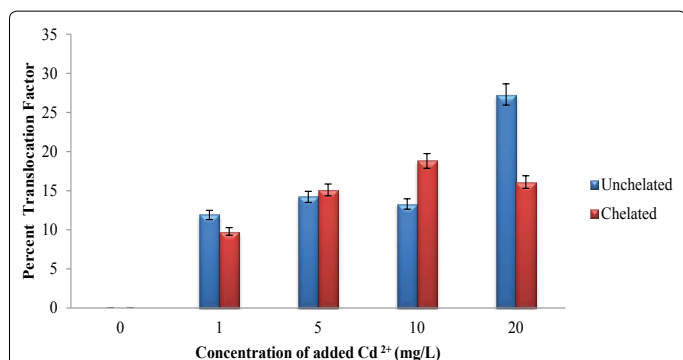


Figure 2: Translocation Factor (T.F) of Cd²⁺ in Jute mallow grown in Unchelated (treatment without citric acid) and chelated (treatment with citric acid (5mM)).

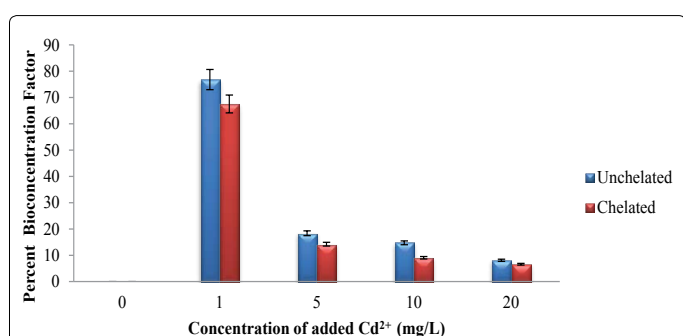


Figure 3: Bioconcentration Factor (BCF) of Cd²⁺ in Jute mallow grown in Unchelated (treatment without citric acid) and Chelated (treatment with citric acid (5mM)).

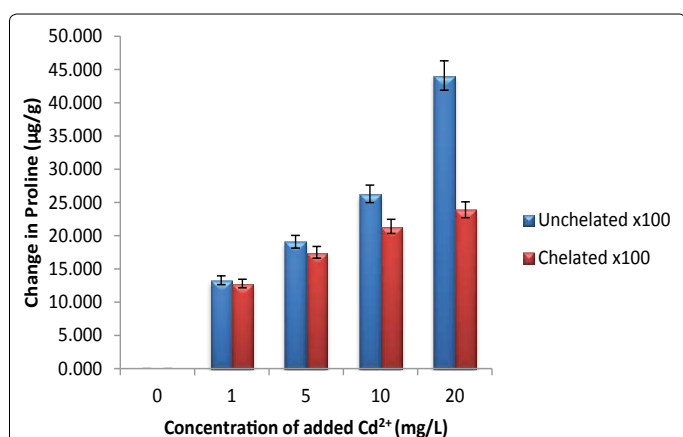


Figure 4: Proline accumulation in shoot of Jute mallow (*Corchorus olitorius*) seedlings.

Interestingly, application of CA at (5mM) increased Cd²⁺ concentration and uptake by the plants as compared to the control, but decreased Cd²⁺ concentration and uptake by the plants as compared to the Cd treated plants (Figure 1). Reduced uptake might be due to the reason that Cd is highly soluble and bioavailable, whereas citric acid might transform the exchangeable Cd to relatively stable organic and residual forms. The distribution of metals with plant tissues is an important property that can act as an indirect indicator of detoxification mechanism. In present study, Cd²⁺ accumulation were lower, less than 500 mg/kg with the highest Cd²⁺ 20 mg/L, level in the nutrient solution in the whole Jute mallow plants.

Cadmium concentration in shoots of *B. napus L.* has been reported up to 555 mg/kg dry weight only on exposure for fifteen days and about 240 mg/kg dry weight only on exposure for ten days in solution [14].

Cadmium concentration in plants varies with type, genotype and duration of Cd exposure to plants as of *B. napus L.* [18] and *B. juncea* [5]. It has been reported that plant may have no limitation of Cd uptake until Cd caused significant damage to plants [19-21]. Upper threshold of Cd accumulation in Jute mallow has not been shown and further study is required in order to investigate the limitation of Cd accumulation in plants. In present study, duration of plant exposure to Cd was short, ten days and CA application enhanced antioxidant enzyme activity and gas exchange parameters which may increase Cd in the shoots and roots. Moreover, Jute mallow may accumulate relatively high Cd in shoots and roots after a long term exposure, judging from the growth characteristics being exhibited by the plants. However, further research is required to evaluate, the Jute mallow ability to uptake Cd²⁺ from the soil especially with CA application and its utilization as a hyperaccumulator.

Conclusion

In conclusion, the results indicated that Cd²⁺ supply significantly depressed plant growth and biomass. Cd²⁺ accumulated more in roots than the shoots. Addition of 5mM CA alleviated Cd²⁺ toxicity by reducing oxidative stress, through CA chelating property and increased antioxidant capacity when compared with Cd²⁺ treatment alone. Jute mallow (*Corchorus olitorius*) proved to be an effective accumulator for cadmium, in the range 0 to 20 mg/L. The concentration of citric acid (5mM) did not show any advantages for phytoextraction of cadmium. The results can be beneficial to the society in the monitoring of areas contaminated by heavy metals as a result of mining and industrial activities.

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