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Potential of *Vigna unguiculata* as a Phytoremediation Plant in the Remediation of Zn from Contaminated Soil

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Abstract

Population explosion in the last decades together with global industrialization has caused heavy-metal contamination of air, water and soil, resulting in diverse incurable effects on humans and on the stability of the ecosystem. Non-biodegradable heavy-metals can remain in the ecosystem and the threat associated with their bioaccumulation in food chains represents one of the major environmental and health problems of present day society. Several studies were carried out to understand the ecological effects of the heavy-metal Zn in soil-plant systems. Plants often have a zinc uptake that their systems cannot handle, due to the accumulation of zinc in soils. Of the several Zn toxicity symptoms, fatal are yield reduction, stunted growth, chlorosis, reduced chlorophyll synthesis and chloroplast degradation. *Vigna unguiculata* is an herbaceous, annual plant in the pea family Fabaceae. In the present study, an experiment was performed to evaluate the Zn phytoextracting ability of *V. unguiculata* under *in vitro* condition. We establish that *V. unguiculata* can uptake a considerable amount of the heavy-metal zinc and this phytoextraction property can be utilized in long run for the cleanup of zinc contaminated soil. To the best of our knowledge, this is the first report of Zn phytoextraction ability of *V. unguiculata*.

Keywords

Heavy-Metal Toxicity; Phytoremediation; Proline; Atomic Absorption; *Vigna unguiculata*

1. Introduction

Population explosion in the last few decades together with global industrialization has caused heavy metal con-

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tamination of air, water and soil, resulting in diverse incurable effects on humans and also on the stability of the ecosystem. Heavy metals due to their non-biodegradable property can remain in the ecosystem for a long time. Heavy metal toxicity and the threat associated with their bioaccumulation in food chains represent one of the major environmental and health problems of our present day society.

Several studies has been carried out to understand the ecological effects of the heavy metal Zn in soil-plant systems, which indicated that plant yield decreases due to a toxic effect of Zn on the ecosystem [1]-[4]. Plants often have a zinc uptake that their systems cannot handle, due to the accumulation of zinc in soils. Elevated levels of Zn in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of Zn is the major cause of Zn toxicity in plants [5] [6]. Zn toxicity symptoms include reduced yields and stunted growth, Fe-deficiency-induced chlorosis through reductions in chlorophyll synthesis and chloroplast degradation, and interference with P, Mg and Mn uptake [5]-[9]. In humans, reports of Zn-toxicity have been mostly in response to food poisoning incidents. Effects of long-term, excessive zinc intakes (ranging from 150 mg/day to 1 - 2 g/day) include nausea and vomiting, hypochromic anaemia, leukopenia, epigastric pain, abdominal cramps, and diarrhea [10] [11].

The current state-of-the-art technology for the clean-up of toxic metal-contaminated soils includes physical remediation, chemical remediation and phytoremediation [12]-[14]. The limitations of first two methods are high cost, destruction of soil structures and had an adverse effect on biological life. The development of phytoremediation technique is being driven primarily by the high cost of many other remediation methods, as well as the desire to use green, sustainable process [15]. Phytoremediation is environmental friendly method which utilizes the uptake ability of plants for the removal of heavy metals from the soil-water environment. Phytoremediation is basically an assemblage of four different techniques, namely, rhizofiltration, which employ plants to clean various aquatic environments; phytostabilization, where plants are used to stabilize contaminated soil; phytovolatilization, where plants extract specific metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants extract metals from soil and translocate them to the shoots where they accumulate [16]. The two terms, phytoremediation and phytoextraction, are sometimes used as synonyms; however, phytoremediation is a concept while phytoextraction is a definite cleanup technology [16].

Vigna unguiculata is a herbaceous, annual plant in the pea family Fabaceae. The domestication of *vigna unguiculata* was originated in West Africa. It is one of the most widely used legumes in the semiarid tropics including Asia, Africa, southern Europe and Central and South America. Hundreds of experiments have been conducted on this legume to understand its morphology, physiology, as well as to understand the effect of different stresses on this legume. However, only a scanty number of experiments have been carried out to study the Zn phytoextracting ability of *V. unguiculata* [17]. In the present study, an experiment was performed to evaluate the Zn phytoextracting ability of *V. unguiculata* under *in vitro* condition. We establish that *V. unguiculata* can uptake a considerable amount of the heavy metal zinc and this phytoextraction property can be utilized in long run for the cleanup of zinc contaminated soil. To the best of our knowledge, this is the first report on the study of the Zn phytoextraction ability of *V. unguiculata*.

2. Materials and Methods

2.1. Plant Material and Treatments

Seeds of *Vigna unguiculata* var. *unguiculata* were surface sterilized in 0.1% $HgCl_2$ solution and washed with distilled water prior to germination. Equal numbers of seeds were germinated in experimental pots in triplicates containing half strength Hoagland solution as a nutrient media at controlled temperatures. The basic composition in the growth medium includes N, K, Ca, P, S, Mg, B, Fe, Mn, Zn, Cu, and Mo. Five days old seedlings were stressed with the heavy metal Zn at the concentration of 250 µM and 500 µM, respectively. The stated metal concentrations were added from the stock solutions of analytical grade heavy metal salts ($ZnSO_{4.7}H_2O$) prepared in distilled water. Plants grown on medium without heavy metals constituted the control. Experiments lasted for 15 days, during which period the plants were harvested at 3rd, 6th, 9th, 12th and 15th day after the initiation of treatment. After harvesting, half of the sample amount was used for determining morphological parameters and proline accumulation and the other half was used to study the Zn uptake. To study the metal uptake, each plant was separated into leaf portions and combined root-stem portions and metal uptake was measured from each portion separately.

2.2. Growth Parameters

Two parameters were measured; plant height and total fresh weight. These parameters were measured at 3rd, 6th, 9th, 12th and 15th day, after initiation of the treatment. Plant heights were measured with a metre tape from the root tip to the apex of the plant. The fresh weights were obtained by uprooting the plant from medium and weighed on a weighing balance immediately after harvest to avoid water loss.

2.3. Proline Estimation

Proline content was determined following the method of Bates [18]. One gm of tissue was homogenized in 10 ml of sulfosalicylic acid (3%) and centrifuged at 4000 rpm for 10 mins. About 2 ml of extract supernatant was taken in a test tube and to it 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent was added. The reaction mixture was boiled in water bath at 100˚C for 30 minutes. After cooling the reaction mixture, 4 ml of toluene was added and vortex for 30 seconds. The upper phase containing proline was measured with spectrophotometer at 520 nm using toluene as a blank. Proline content (µmol g-1 fr. wt.) was quantified using the ninhydrin acid reagent method by using L-proline as a standard.

2.4. Zinc Accumulation

Harvested plants were thoroughly washed in distilled water. Leaves and combined root-stem were separated and oven dried at 80˚C for 24 hours. Oven dried samples were grounded into fine powder and kept in a mixture of percholoric acid and nitric acid (1:5) for overnight. Next day, samples were digested in water bath at 80˚C for 1 hour, filtered and the clear filtrate was taken. The volume of filtrate was increased to 15 ml with distilled water. Total Zn concentration in the samples was then determined by hydride generation atomic absorption spectrophotometer (AA 6800, Shimadzu). Zn from $ZnSO_4$ was used as for calibration.

3. Results and Discussion

3.1. Statistical Analysis of the Data

Each of the three experiments for measurement of growth parameters (plant height and total fresh weight), estimation of proline content and estimation of Zn accumulation were done in triplicates. From individual experiments, the obtained data were statistically analyzed by calculating the standard deviation and error bars based on the standard deviations.

3.2. Phytotoxic Effects of Zn on Plant Height and Plant Weight

Figure 1 and **Figure 2** shows the variation of plant heights and plant weights in treated and control plants at different Zn concentrations, respectively. With enhancing external Zn concentrations, the lengths of the treated seedlings were decreased in comparison to the controls. Maximum decrease (26.8%) was recorded for 15 days old seedlings at 500 µM Zn stress (**Figure 1**). There are reports that Zn stress causes multiple direct and indirect effects on plant growth by disrupting metabolic processes [19]-[21]. Zn accumulation in cell wall components, especially pectic substances and hemicelluloses, reduces the frequency of mitosis with a direct consequence in the decrease of plant height [22]. In the present study, the reduced height and weakly developed root systems of the *V. unguiculata* Zn-treated seedlings compare to the controls, at increasing Zn concentration were due to such effects of the heavy metal Zn on the growing seedlings. Similar results were observed while considering total plant weight. Total weight of the treated seedlings with respect to the controls decreased with enhancing external Zn concentration. Maximum decrease (16.7%) was observed in 15 day old seedlings under 500 µM Zn stress (**Figure 2**). Earlier investigations showed that the heavy metals chromium, cadmium, manganese, lead and nickel [17] [23]-[25] reduces the leaf area; consequently total weight of the plant reduces. In this study, the reduced weight of the Zn- treated seedlings can be attributed to either a reduction in the number of cells or due to reduction in cell size. Zn-treated plants spent more energy for their survival in the hostile environment, which otherwise would be available for their overall growth processes, resulting in a reduced height and weight. However, we found that the Zn-treated plants, with the reduced height and weakly developed root systems, withstand the Zn stress and the overall vigor of the treated plants was not that affected compare to the control plants, indicating that *V. unguiculata* plants can accumulate the heavy metal Zn.

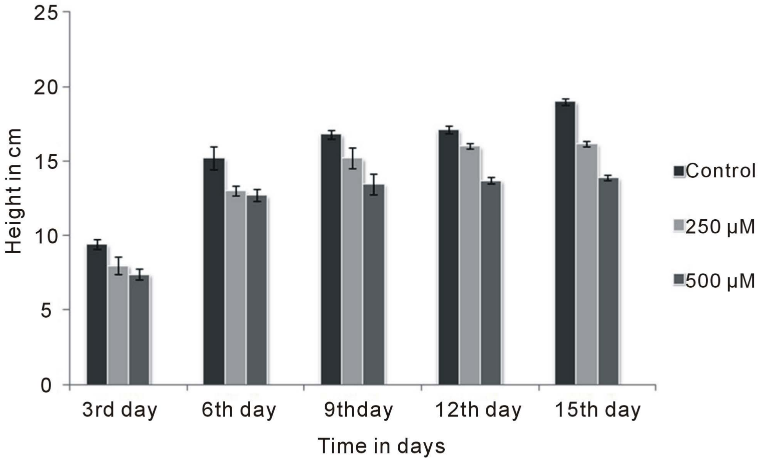

Figure 1. Variation of plant heights in treated and control plants at different Zn concentrations..

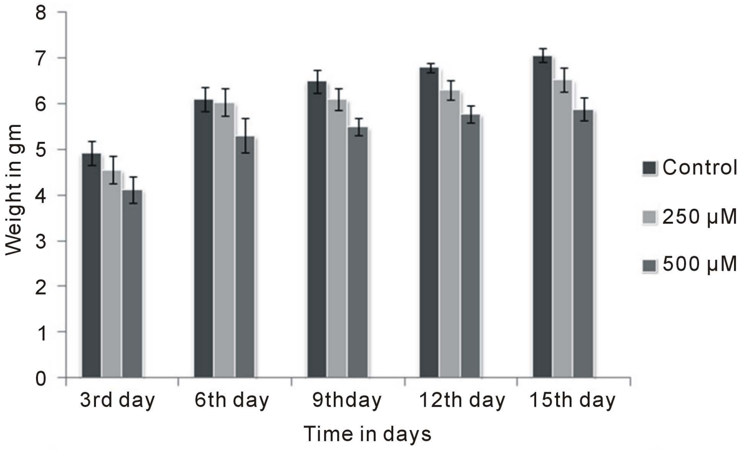

Figure 2. Variation of plant weights in treated and control plants at different Zn concentrations.

3.3. Changes in Proline Content Accompanying Zn Uptake

Results showed that the proline accumulation in treated seedlings increased with increasing Zn concentration and was highest (59%) under 500 µM Zn stress of 15 days old seedlings (**Figure 3**). Accumulation of stress marker proline in treated plants is an indicator of primary defense response to maintain the osmotic pressure in cells. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants and cultivars of many crops [26] [27]. Proline accumulates in many plant species under a broad range of abiotic stresses as water shortage, salinity, extreme temperatures, and heavy metal accumulation [28]-[31] as well as under biotic stresses [32] [33]. At the structural level, proline protects folded protein structures against denaturation by functioning as a hydroxyl radical scavenger and also stabilizes cell membranes by interacting with phospholipids. In the present study, increasing amount of heavy metal Zn concentration in treated seedlings exerted increased amount of stress, which in turn increased the amount of osmoprotectant proline content. Nevertheless, Zn treated plants with increased amount of stress marker proline survived well, indicating that *V. unguiculata* plants can accumulate the heavy metal Zn.

3.4. Effect of Different Treatment of Zn Concentrations on Accumulation of This Heavy Metal

Uptake of Zn by *V. unguiculata* seedlings under two different concentrations of externally added Zn has been conducted in hydroponic medium under *in vitro* condition for a period of 15 days (**Figure 4** & **Figure 5**). The significant increase in the level of Zn in root-stem and leaves was in accordance with the increase in external Zn treatment. Uptake of Zn was found more in root-stem system than leaves of *V. unguiculata* seedlings. The maximum Zn uptake of 409 µg g-1 dry wt in root-stem system was found in 15 day old seedlings under 500 µM Zn stress. Uptake of 369 µg Zn per gm dry wt in leaves was found in 15 day old seedlings under 500 µM Zn stress. Although *V. unguiculata* seedlings accumulated a considerable amount of the heavy metal Zn within the span of 15 days experiment, yet, overall vigor of the seedlings were not that affected with respect to the control plants, indicating that *V. unguiculata* can accumulate the heavy metal Zn in its roots and shoots. However, we did not find and Zn accumulation in the seeds of *V. unguiculata* (unpublished results).

The phytoextraction technology, to be fully effective, depends upon several plant characteristics. The two most significant characters include the ability to rapidly accumulate considerable amount of biomass and the ability to accumulate considerable amount of heavy metals within the shoot tissue [34]. It is the combination of both high biomass production and considerable amount of heavy metal accumulation that results in the success of this technology. Present study showed that, *V. unguiculata* being a rapid accumulator of biomass is also efficient at translocating the heavy metal Zn from ground to the harvestable above-ground portions of the plant andcan tolerate 409 µg g-1 dry wt of the heavy metal Zinc. Although, the amount of Zn accumulation by *V. Un-guiculata* does not define it as a hyperaccumulator as per previous report [35] yet its accumulation of a considerable amount of the heavy metal Zn makes it a potential candidate as a phytoremediation plant in the reme-

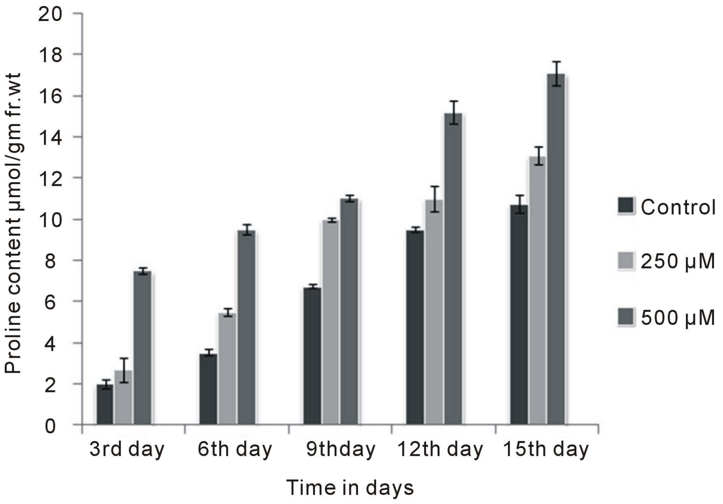

Figure 3. Proline accumulation in treated and control plants at different Zn concentrations.

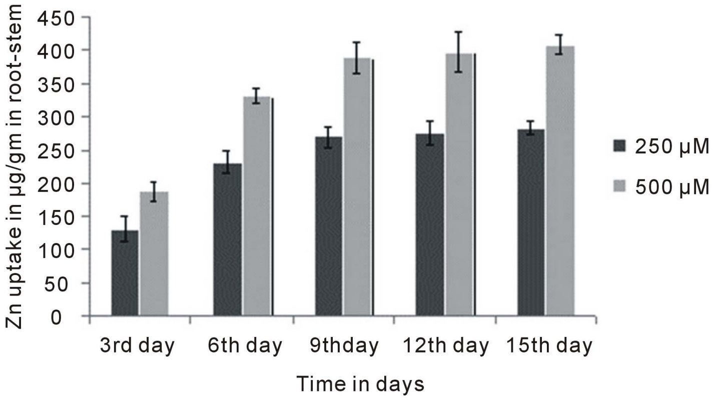

Figure 4. Zn uptake in root-stem portion of treated plants at different Zn concentration.

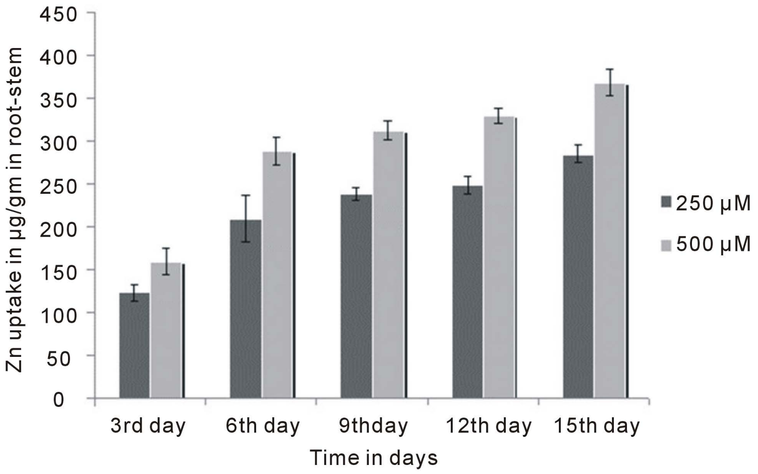

Figure 5. Zn uptake in leaves of treated plants at different Zn concentration.

diation of Zn from contaminated soil. To the best of our knowledge this is the first report on the study of the Zn phytoextraction ability of *V. unguiculata*.

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