EFFECT OF EDTA ON ARSENIC PHYTOEXTRACTION BY *ARUNDO DONAX L.*

Nosheen Mirza, Arshid Pervez*, Qaisar Mahmood*†, Mohammad Maroof Shah* & Umar Farooq***

ABSTRACT

Ligand assisted metal uptake by plants is recent trend in environmental clean-up. Arundo donax L. has been demonstrated as a suitable bioresource for the phytoextraction of arsenic recently. A. donax L. plants were grown in arsenic contaminated soil with doses (5 and 10 mg kg^{-1}) of Ethylene Diamine Tetra Acetic Acid (EDTA) to investigate chelator-assisted phytoextraction. The arsenic treatments included control (no metal) and five doses of arsenic, i.e., 50, 100, 300, 600 and 1000 μg kg^{-1}. The arsenic concentrations linearly increased in parts of plant with the increasing arsenic and EDTA in growth medium. Ligand addition also resulted in the increased arsenic accumulation in the shoot over its control plants. EDTA addition @ 5 mg kg^{-1} to the treatment system may effectively increase the arsenic uptake by A. donax without severe growth suppression.

Keywords: *Arsenic, Arundo donax, EDTA, ligands, giant reed, soil toxicity*

1. INTRODUCTION

Arsenic has been detected in almost all geographical regions and circulates in the atmosphere, hydrosphere, lithosphere and biological systems. It exists in a variety of chemical forms possessing variable mobility, bioavailability and toxicity (Su, et al., 2009). Arsenic contamination of water, soil and crops is continuously increasing in many regions of the world (Tripathi, et al., 2007;Verbrugen, et al., 2009). Its long-term exposure causes a variety of diseases, including skin lesions, cancer and diabetes, affecting millions of people around the globe (Raab, et al., 2007; Zhu and Rosen, 2009). Due to its chronic toxic effect on human life, the maximum contaminant level (MCL) of arsenic has been reduced to 10 μg L^{-1} from 50 μg L^{-1} by US Environment Protection Agency (USEPA).

Plants are effective in removing metals from growth medium, and phytoremediation has recently emerged as green technology (Mudgal, et al., 2010; Kausar, et al., 2012). The phenomenon is environment friendly, safe, cost effective and provides the possibility of harvesting the plants for the extraction of absorbed metal. For effective phytoremediation, metal-tolerant plant species capable of accumulating high metal content and fast-growing plant species capable of growth in diverse habitats is recommended (Skinner, et al., 2007). *Arundo donax L.* is a fast growing plant species with an efficient biomass production that may be highly desirable for phytoremediation of metals.

In spite of high arsenic concentrations in soils, only a fraction is found bioavailable for plant uptake, the bulk is commonly found as insoluble and remain unavailable for absorption by hyperaccumulating plants. To increase the mobility and bioavailability of heavy metals, various chelators such as EDTA, Diethylene Trinitrilo Pentaacetic Acid (DTPA), Nitrilo Triacetic Acid (NTA), and citric acid, are used. EDTA has been found as most effective ligand (Benavides, 2007; Islam, et al., 2008; Dan, et al., 2008; Wang, et al., 2009). Therefore, researchers are concentrating their efforts on increasing the solubility and phytoavailability of arsenic, and on the subsequent translocation from the roots to the shoots. In the past decade, chelate-enhanced phytoremediation has received much attention in the scientific community. This technique aims to clean arsenic-polluted soils by solubilizing and allowing it to get accumulated in plants (Mroczek, 2005; Rahman, et al., 2008). The application of chelators like EDTA has been suggested to ameliorate metal toxicity in plants (Dan, et al., 2008). Use of ligands enhances heavy metals solubility (Wu, et al., 2004), uptake, and translocation to aboveground parts (Saifullah, et al., 2009). *A. donax* can grow in different environments with spacious ranges of pH, salinity, drought and trace metals without any symptoms of stresses, and can easily adapt to different ecological conditions and grow in all types of soils (Sabeen, M., et al., 2013). There is still lack of knowledge on the responses of the plant against arsenic in the presence of chelating agent like EDTA.

In the present study, the effect of EDTA along with arsenic was studied at different concentrations in order to investigate the appropriate chelator dose and method of application for chelator assisted phytoextraction of arsenic by *A. donax L.* from contaminated soil.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material was collected from a wetland near Haripur city of Pakistan. The plant collection and growth was accomplished by procedure adopted by Mirza, et al. (2010a).

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2.2 Arsenic Treatment

The experiment was laid out in randomized complete block design (RCBD) with three replicates. The treatments included control (no metal or EDTA) and five doses of arsenic, i.e., 50, 100, 300, 600 and 1000 μg kg^{-1}. Another two sets included similar arsenic treatments but in addition to that two doses of EDTA @ 5 and 10 mg kg^{-1}. The EDTA dose selection, method of application and handling was according to Dan, et al. (2008). The physicochemical properties of the soil were: loamy soil, with pH and organic matter content of 6.23 and 0.93%, respectively.

2.3 Phytoextraction Ability

The phytoextraction ability of *A. donax L.* plants was assessed using both the bioaccumulation factor (BF) and the translocation factor (TF) as follows:

$$BF = \frac{[As]\ plants}{[As]\ solution} \qquad (1)$$

$$TF = \frac{[As]\ Shoot}{[As]\ Root} \qquad (2)$$

2.4 Arsenic Analysis

Arsenic in the soil and plant parts was determined through colorimetric method using UV-Spectrophotometer at 840 nm (Bigham, 1996).

2.5 Biomass Production / Yield Data

Various growth parameters of *A. donax L.* studied under arsenic stress included plant height; number of leaves per plant; number of nodes per plant; average root length; and toxicity symptoms prior to and after the arsenic treatments. The average values of these parameters before treatments have been presented in Table-1.

3. RESULTS

3.1 Arsenic Content in Plant Parts and Soil

The results of the various arsenic concentrations determined in the soil and different plant parts under various arsenic and EDTA treatments are presented in Figures 1 to 3.

The total arsenic concentrations in various plant parts had linear relationship with the increasing applied arsenic concentrations (Figure-1). However, the arsenic accumulation under EDTA addition was significantly higher than those without EDTA (Figures 2&3). The maximum arsenic accumulation occurred in the leaves at highest arsenic treatment (1000 μg kg^{-1}) compared to other organs. Decrease in the soil arsenic concentration was pronounced for all applied arsenic treatments of 10 mg kg^{-1} EDTA (Figure-3). Significant difference was noted for arsenic accumulation in various plant parts of *A. donax L.* at 5 mg kg^{-1} of EDTA compared to control. Except for the arsenic treatment of 600 μg kg^{-1} with 5 mg kg^{-1} EDTA, the order of arsenic accumulation, at various applied arsenic and EDTA concentrations was:

Soil < Root < Leaf

3.2 Bioconcentration Factors

The bioaccumulation factor (BF) and translocation factors (TF) for control and treated plants are shown in Figure-4. A marked difference in the BF and TF values was observed for the arsenic treatment with and without chelator addition. For all treatments, the maximum BF value was observed for the arsenic dose of 1000 μg kg^{-1} supplied, i.e., 3.1 without chelator addition, and TF value was 2.1. BF values for same arsenic treatment with 5 and 10 mg kg^{-1} were 8.1 and 19.3, respectively (Figure-4). The TF values for arsenic treatments in the presence of ligand were around 1 for all treatments. For both the EDTA

Table-1: Average Values of Various Morphological Parameters Before Arsenic Treatments

Parameter	Values
Plant Height	18±1 cm
Average root length per plant	15±2 cm
Dry weight of plant parts	25±5 g kg^{-1}
No. of nodes per plant	4±2
No. of tillers per treatment	2±1
No. of leaves per plant	4±1

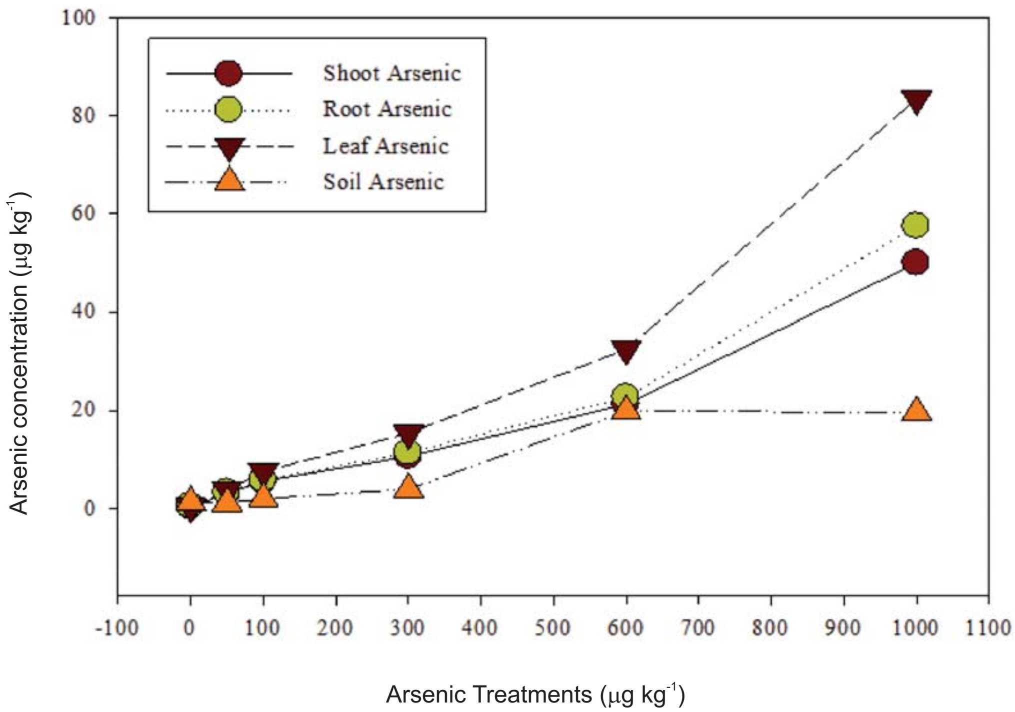

Figure-1: Concentration of Arsenic in Various Parts of Plant Without Chelator Addition

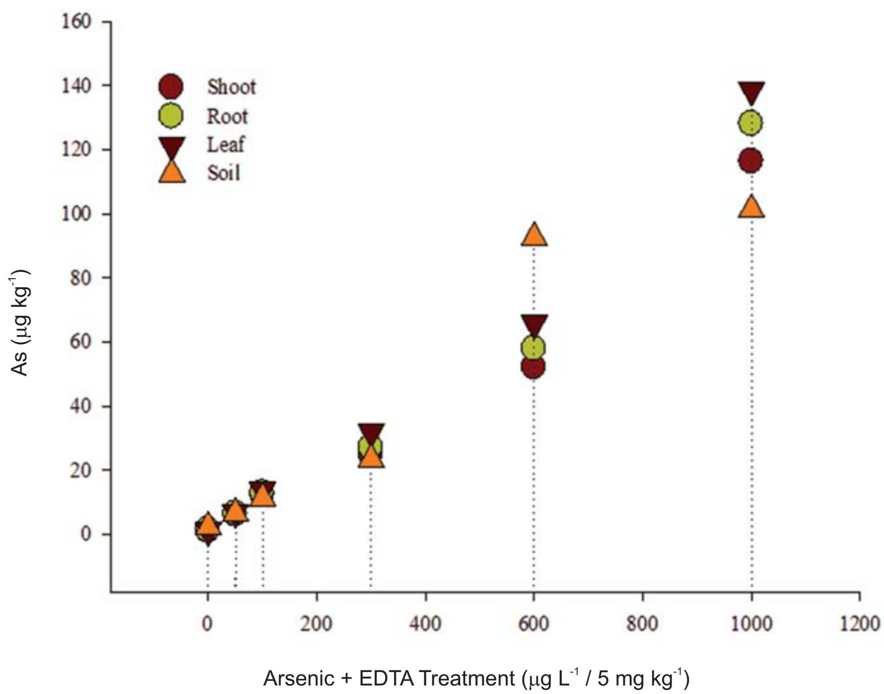

Figure-2: Effect of Applied Arsenic and EDTA @ 5mg kg^{-1} Concentrations on Total Arsenic Concentration in Soil and Plant Parts of *A. donax L.*

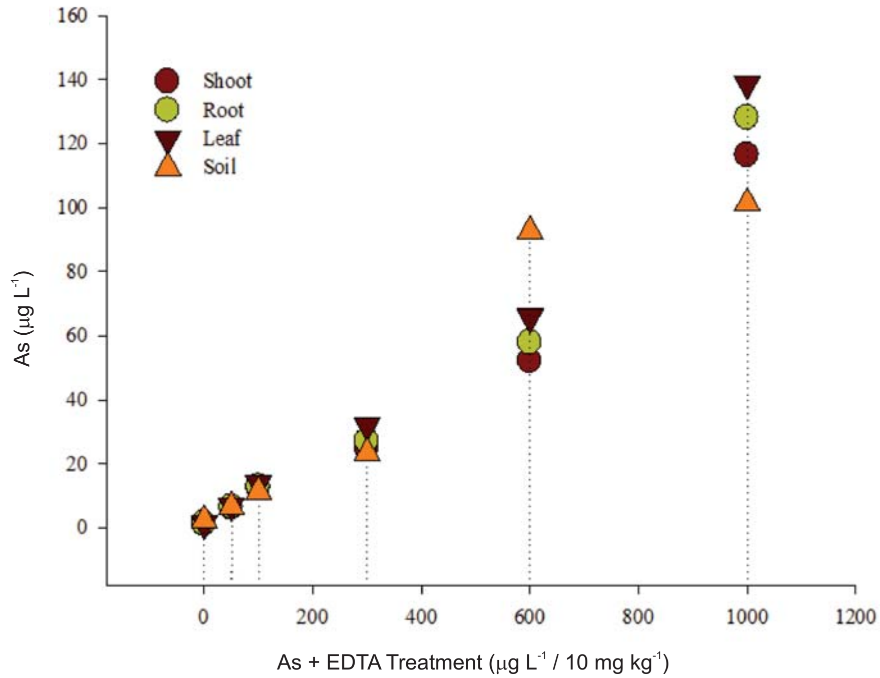

Figure-3: Effect of Applied Arsenic and EDTA @ 10 mg kg^{-1} Concentrations on Total Arsenic Concentration in Soil and Plant Parts of *A. donax L.*

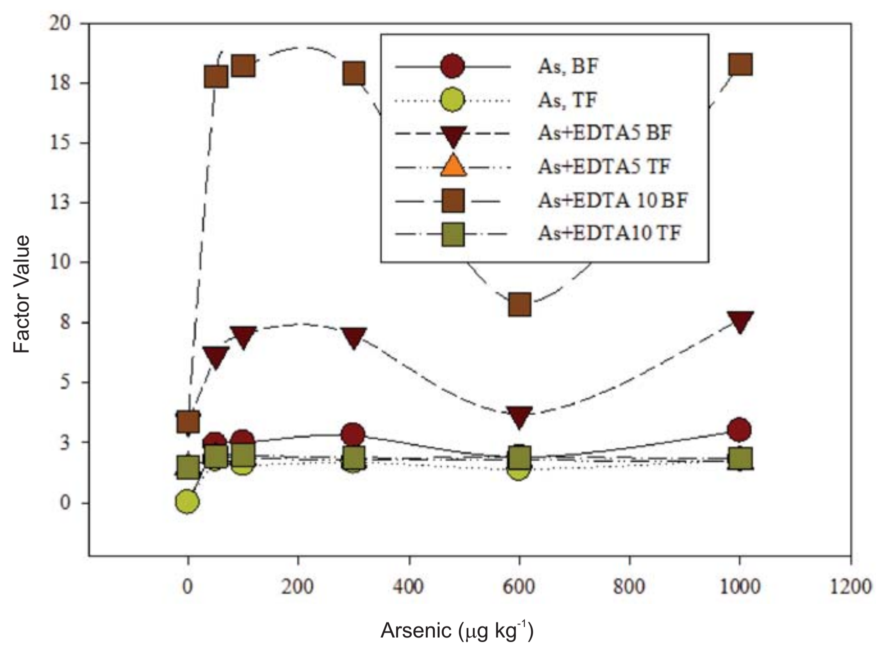

Figure-4: Relative Total Arsenic Bioaccumulation and Translocation Factors of *A. donax L.* at Various Arsenic and EDTA Concentrations in Soil

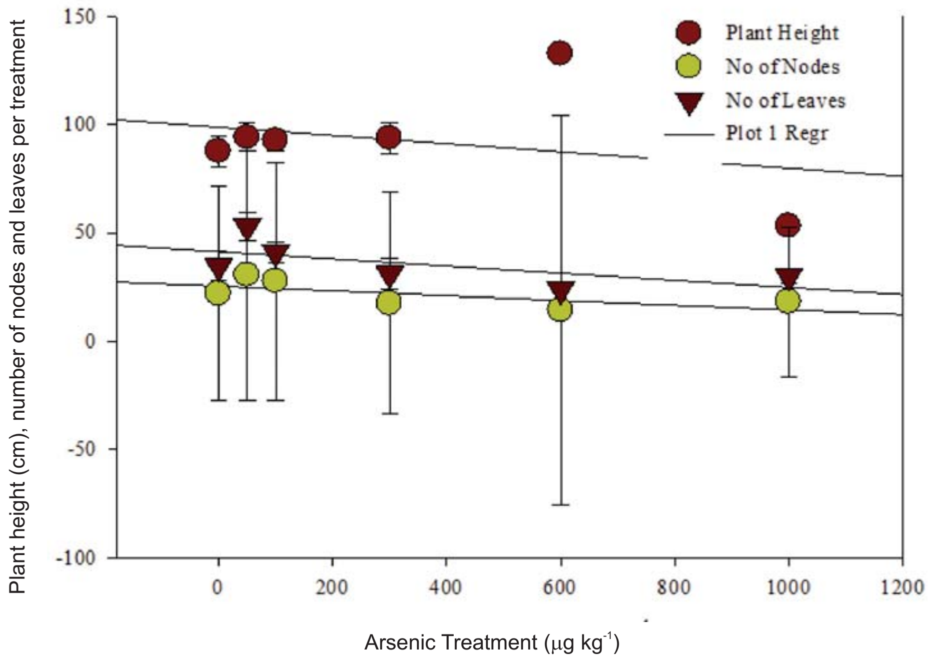

Figure-5: Effect of Applied Arsenic Treatments on Plant Height, Number of Nodes and Leaves After 21 Days of Growth in Soil

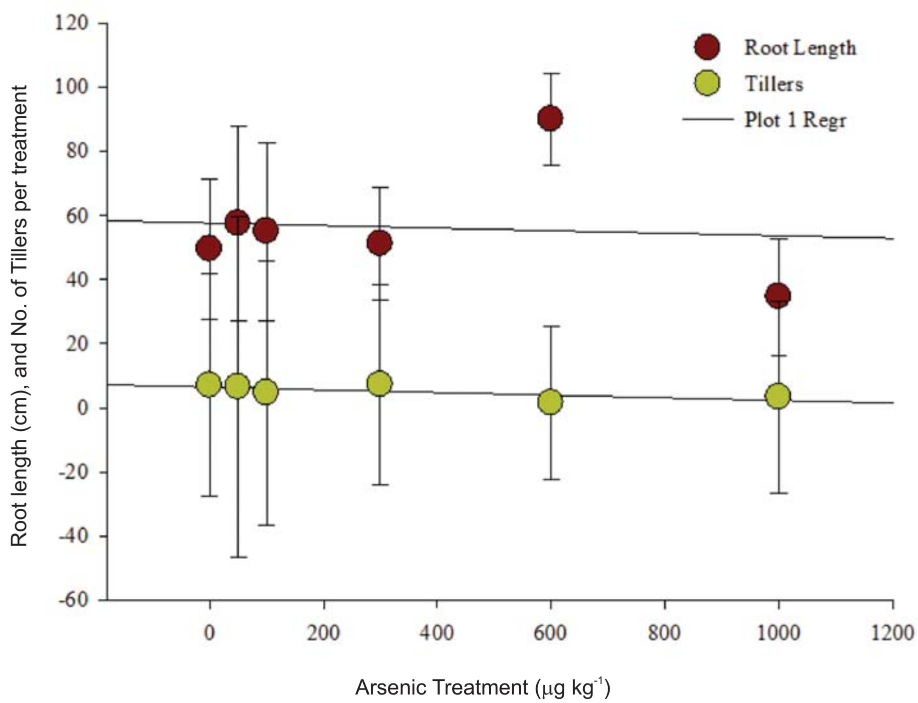

Figure-6: Effect of Applied Arsenic Treatments on Root Length and Number of Tillers After 21 Days of Growth in Soil

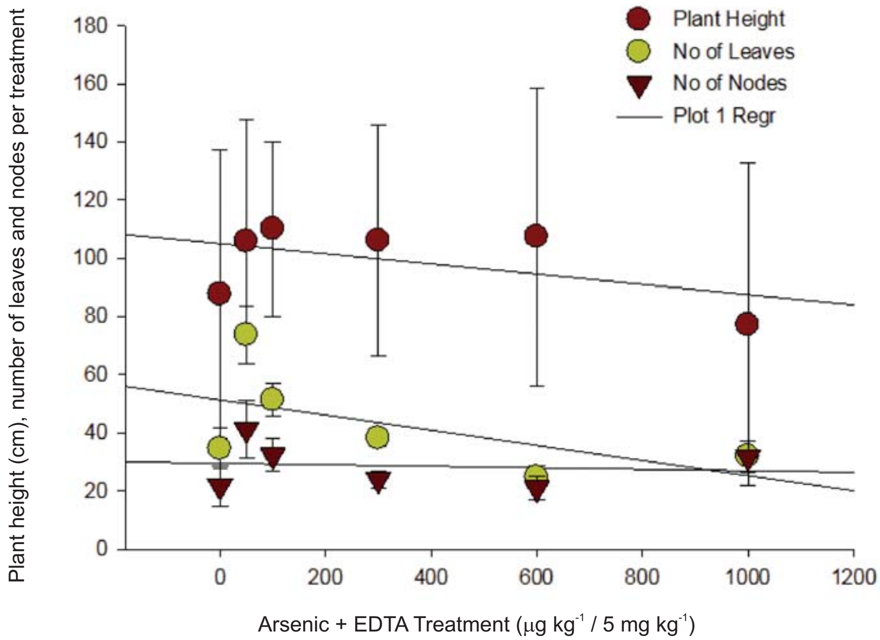

Figure-7: Effect of Applied Arsenic and EDTA @ 5 mg kg^{-1} Concentrations on Plant Height, Number of Nodes and Leaves After 21 Days of Growth in Soil

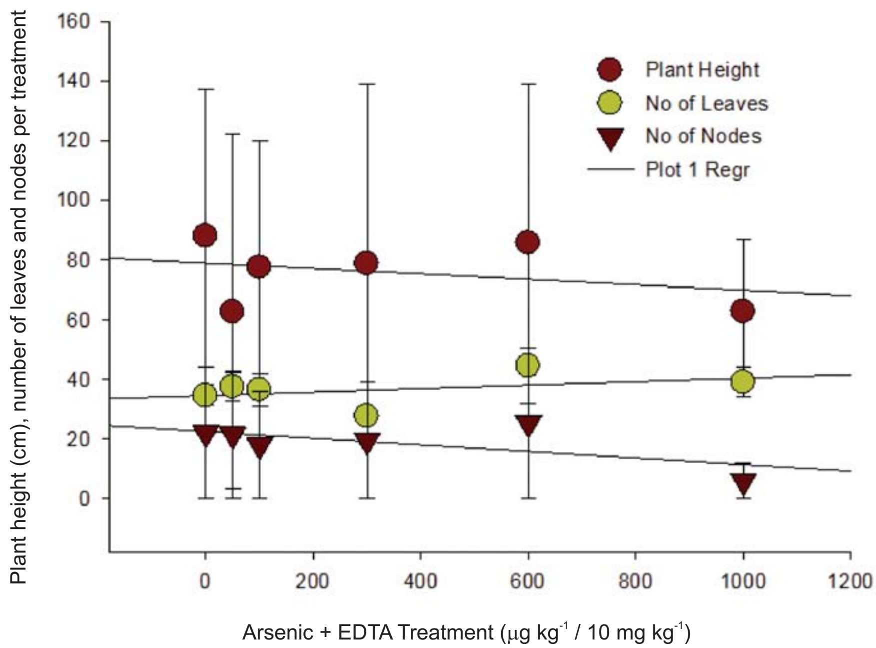

Figure-8: Effect of Applied Arsenic and EDTA @ 10 mg kg^{-1} Concentrations on Plant Height, Number of Nodes and Leaves After 21 Days of Growth in Soil

concentrations (5 and 10 mg kg^{-1}), arsenic BF increased till 300 μg kg^{-1}, decreased at 600 μg kg^{-1} but then was the highest at 1000 μg kg^{-1} (Figure-4). Some toxicity symptoms appeared in plants exposed to 1000 μg kg^{-1} which included an appearance of reddish root tips, red young leaves, and burned necrotic margins of

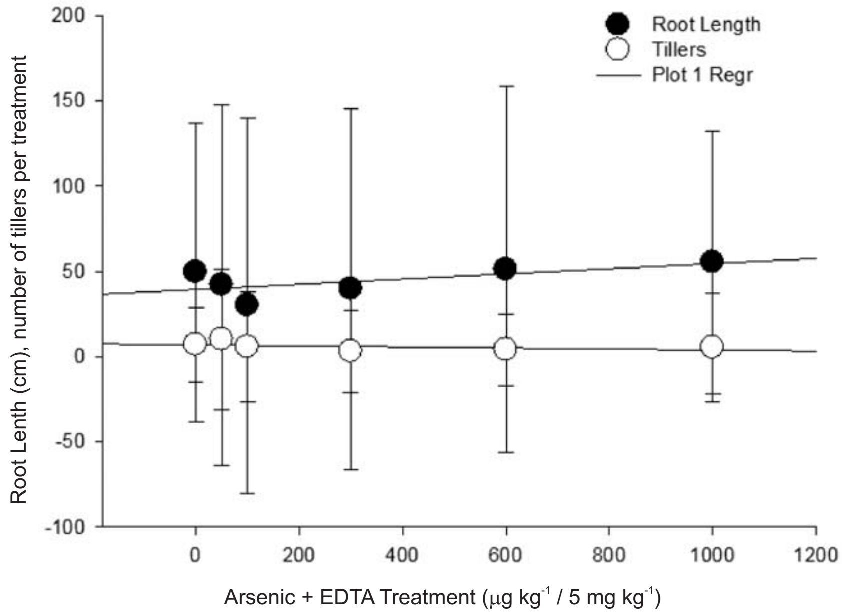

Figure-9: Effect of Applied Arsenic and EDTA @ 5 mg kg^{-1} Concentrations on Root Length and No. of Tillers After 21 Days of Growth in Soil

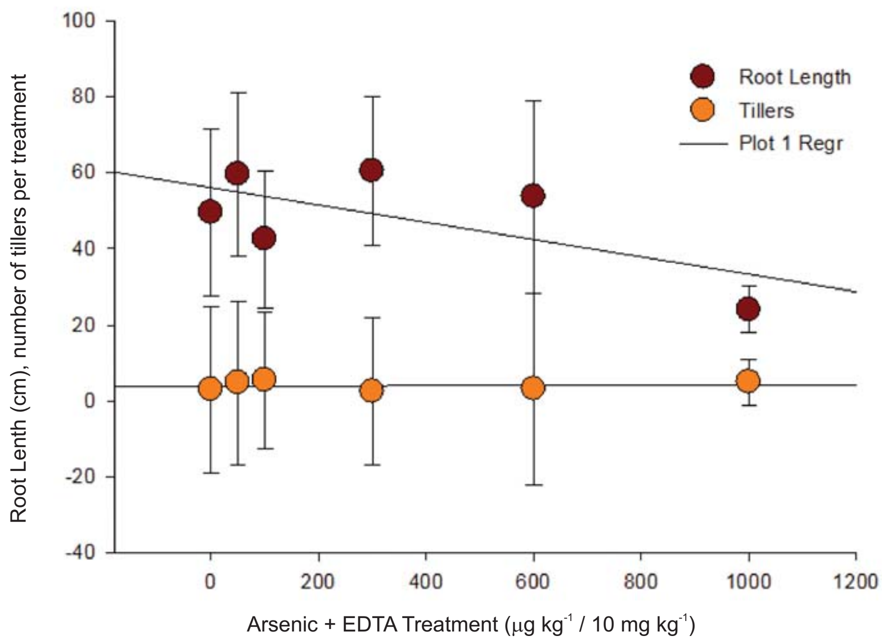

Figure-10: Effect of Applied Arsenic and EDTA @ 10 mg kg^{-1} Concentrations on Root Length and No. of Tillers After 21 Days of Growth in Soil

leaf laminae.

3.3 Growth Performance of *A. donax L*

Growth of the plant in terms of plant height, number of leaves and nodes is presented in Figures 5 to 10. Results show that plant height and root length (cm) are significantly affected at arsenic concentrations of 300 μg kg^{-1} (Figure-5). Almost all growth related parameters increased up to 600 mg kg^{-1} and then they were suppressed at highest supplied arsenic dose in all cases (Figures 5 to 10). In fact, such growth responses were noted for both EDTA concentrations.

As compared to control and 5 mg kg^{-1} EDTA, a pronounced decrease in plant growth and biomass, in terms of plant height, number of leaves, nodes and tillers were observed at 10 mg kg^{-1} EDTA (Figures 7 & 8). Interestingly, significant increase in root length was observed at all arsenic treatments with the addition of 10 mg kg^{-1} EDTA (Figures 9 &10).

4. DISCUSSION

Plants absorb toxic metals, they resistant the contamination, translocate and accumulate into their body parts, thus help in cleaning soils and water contamination through phytoremediation (Mudgal, et al., 2010). It is safe, environment friendly, cost effective and provides the possibility of harvesting the plants for the extraction of absorbed contaminants, which cannot be easily biodegraded for recycling (Mirza, et al., 2010a; 2010b). Ligand-assisted metal uptake by plants has only recently been discovered in the remediation industry. EDTA is a commonly used ligand which binds with many metals (Dipu, et al., 2012). The addition of ligands generally proves fruitful in phytoremediation of metals in contaminated soils. Rhizosphere microbes and ligands can enhance metal uptake and accumulation through metal availability in the rhizosphere (Chen and Cutright, 2002). Ligands, especially EDTA, have been widely used in agriculture as an additive in micronutrient fertilizers worldwide (Wallace, et al., 1992).

It was observed that the plant growth was promising when 5 mg kg^{-1} EDTA was added along arsenic treatments. Consequently, tissue arsenic significantly increased; however, the increase was more pronounced at 10 mg kg^{-1} EDTA. The current observations were in disagreement with Wang, et al. (2009) and Dan, et al. (2008).

The addition of chelating agents like EDTA increased the capacity of the plants to absorb the heavy metals; simultaneously, the bioconcentration factor (BCF) was also increased. Wang, et al. (2002) indicated that chelator addition could increase the concentration of available heavy metals in the soil, hence their addition may affect the plant growth, and chelators at higher concentrations could also be toxic to the plants. Considering economic expenditure and potential environmental risks, chelator-assisted phytoextraction might be more suitable for arsenic contaminated soils.

Many researchers (Hall, 2002; Sridokchan, et al., 2005; Verbruggen, et al., 2009; Zhao, et al., 2009) demonstrated that the plants adopt strategy to avoid, build excess metal levels in the cytosol, and thus prevent the appearance of toxicity symptoms. More than one mechanism could be involved in reducing specific metal toxicity with the plant. Tolerance development may also involve plasma membrane, i.e., by stimulating the efflux of metals once having entered into cytosol. Protoplast exhibits a variety of tolerance mechanisms, such as repair of stress damage proteins (heat shock protein), metallothioneins, chelation of metal by organic acids, amino acids or peptides or their compartmentation away from metabolic processes by transporting it to the vacuole. The final step in detoxification is sequestration of arsenic in the vacuoles of root and shoot tissue, and it is believed that, in analogy to yeast, this might be in the form of As(III)–thiol complexes (Dhankher, 2005; Bleeker, et al., 2006) although no evidence of its occurrence in plants has been found till yet. But recent research on hyperaccumulating and tolerant species showed that vacuoles of *P. vittata, P. cretica* and *H. lanatus* contain large concentrations of inorganic As(III) (Ellis, et al., 2006; Pickering, et al., 2006; Tripathi, et al., 2007). Thus, in order to minimize the arsenic load, the reduction of As(V) within the plant, coupled with chelation and sequestration of As(III), may result in increased retention of the arsenic in the aerial plant plants and ultimately exclusion through seasonal leaf drop (Tripathi, et al., 2007). Although, higher arsenic accumulation occurred in plants grown in EDTA concentration of 10 mg kg^{-1}, the appearance of toxicity symptoms along suppressed growth at higher arsenic content suggested that it may be safer to use EDTA at the rate of 5 mg kg^{-1} for effective arsenic uptake and lesser damage to *A. donax L*.

Plant uptake and metabolism of arsenic has been reviewed by Quaghebeur and Rengel (2005) and others (Sridokchan, et al., 2005; Tripathi, et al., 2007; Zhu and Rosen, 2009; Zhao, et al., 2009). In order to understand the bioaccumulation, transformation and translocation behaviors of arsenic within plant system, it is important to understand two inorganic oxidation states of arsenic, arsenite and arsenate. In soil-plant water system, both the states transform and are inter-convertible depending on the redox status of the environment; metabolic pathway is still not fully revealed but arsenite reduction results in arsenate formation (Mark, et al., 2007).

5. CONCLUSIONS

The results show that *A. donax* has ability to tolerate arsenic concentrations (300 to 750 µg kg^{-1}) with both ligand concentrations. The addition of EDTA to the treatment increased plant uptake of arsenic. Although, there is higher arsenic accumulation when 10 mg kg^{-1} EDTA is used, but plants show toxicity symptoms. In view of environmental contamination of EDTA and to avoid metal toxicity to plant, the addition of EDTA @ 5 mg kg^{-1} is recommended.

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