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Phytoremediation of arsenic-contaminated soils by *Eucalyptus camaldulensis*, *Terminalia arjuna* and *Salix tetrasperma*

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Summary

Rising levels of arsenic in ground water posing threats to the lives of millions of people residing in the Indus plains, whereas the magnitude of the risk is alarming that needs to control arsenic from leaching down into the ground water becomes essential. The study was designed to assess the potential of three tree seedlings to reclaim the arsenic-affected soils in Pakistan and determining the impact of arsenic on growth parameters and its accumulation in tree seedlings. The experiment conducted at the Botanical Garden, University of the Punjab, Lahore, revealed that *Eucalyptus camaldulensis, Terminalia arjuna* and *Salix tetrasperma* showed varying adaptability to survive under the arsenic stress environment, suggesting them as strong candidates to be exploited for arsenic remediation. Arsenic-treated tree seedlings showed reduced growth in terms of stem height (21.76%), stem diameter (25%), number of branches (22.3%), number of leaves (21%), root length (24.8%), total plant length (25.6%), fresh biomass production (40%) and dry biomass production (49%) as compared to plants grown without arsenic treatment. Arsenic accumulated in all vegetative parts of the seedlings, however maximum arsenic accumulation was recorded in roots of *E. camaldulensis* (37.25 mg kg^{-1}) followed by *S. tetrasperma* (35.76 mg kg^{-1}) and *T. arjuna* (24.13 mg kg^{-1}) when arsenic was applied at a concentration of 4.0 mg L^{-1}. The study has shown that these tree species can be grown on arsenic-contaminated fields to reclaim the soil from arsenic content resulting in its substantial reduction in leaching to the groundwater. The results suggest that *E. camaldulensis* plants accumulated maximum arsenic in its vegetative parts and confirmed it as a tree species with a potential of arsenic phyto-extraction and a good candidate for phytoremediation.

Key words: Arsenic accumulation, metalloid, phytoremediation, reclamation.

Introduction

Arsenic-contaminated soils and water cause environmental and human health problems and the situation is becoming grave with rapid industrialization and disturbance in natural geochemical cycle (MIEKE and RENGEL, 2005; MADHUMITA et al., 2014; PAUL et al., 2015; SINGH et al., 2015). Many areas of the world are affected by arsenic contamination of the underground water (MUKHERJEE et al., 2006; SINGH et al., 2015). Arsenic-contaminated soils and water have been attempted to remove arsenic using a wide range of remediation technologies (UDDIN et al., 2001; MANNING et al., 2002; GAVRILESCU, 2006; TONNI et al., 2006; PAVEL and GAVRILESCU, 2008; SINGH et al., 2015). Generally all these methods are costly, require high technology and by-products from these technologies can be harmful (ALI et al., 2013). Phytoremediation of toxic substances has emerged as a promising, cost-effective, eco-friendly and sustainable approach by using green plants (trees, shrubs, grasses and aquatic plants) to remove and uptake, degrade or isolate toxic substances (heavy metals, metalloids, trace elements, organic and inorganic compounds, and radioactive compounds) from the soils and water (ENSLEY, 2000; MENDEZ and MAIER, 2008; DICKINSON et al., 2009; LUQMAN et al., 2013). In this study, tree seedlings of *E. camaldulensis, T. arjuna* and *S. tetrasperma* were used to reclaim arsenic-contaminated soils. In this study, an attempt has been made to determine the potential of three tree species to uptake and accumulate arsenic from arsenic-contaminated soil in their vegetative parts and to demonstrate their tolerance against arsenic toxicity, so that these trees can be planted on arsenic-contaminated land to remediate the soil through phytoremediation.

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Materials and methods

Raising of seedlings:
Six month old seedlings of *Eucalyptus camaldulensis* DEHNH., Myrtaceae, *Terminalia arjuna* (Roxb.) Wight & Arn., Combretaceae, and *S. tetrasperma*, Salicaceae, were obtained from a nursery of the Forest Department, Government of the Punjab, and were planted in plastic pots (40 cm height × 35 cm internal diameter) containing loamy soil at the Botanical Garden, University of the Punjab, Lahore.

Application of arsenic:
Plants were given different doses of arsenic (0.5, 1.0, 2.0, 4.0 mg L^{-1}) for 18 months. These doses were given to plants once a week in the winter season (October - March) and on alternate days during summer (April - June) and plants were dosed twice a week during the monsoon season (July - September). Sodium arsenite salt ($NaAsO_2$) was used by making stock solutions and given through irrigation water. The level of arsenic in the tap water given to control plants was 5.5 µg L^{-1}. No fertilizer was used in this experiment.

Determination of plant growth and biomass:
The shoot length and diameter (of collar) of arsenic treated and untreated (control) plants were measured monthly during the experiment. At the end of experiment, seedlings were uprooted and thoroughly washed with tap water and rinsed with distilled water. The leaves and number of branches were counted and weighed separately. Dry weight of leaves, shoots and roots was determined after oven drying at 70 ºC for 48 hrs.

Arsenic analysis:
Oven dried plant parts (roots, shoots and leaves) were ground separately using Willey mill (2000 SM, Retsch, Germany) and sieved with mesh (2 mm). One g of plant material was transferred into conical flasks and digested in a solution of HNO_3:$HClO_4$ with 2:1 ratio (v/v) by placing it on a hot plate for about 2 hrs until the plant material became transparent. The concentrations of arsenic were determined by using hydride generation atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan).

Statistical analysis:
The data recorded for each treatment was subjected to the Analysis of Variance (ANOVA) and means of various treatments were compared using Tukey's HSD test at 5% probability level (Steel and Torrie, 1980).

Results

Effects of arsenic on plant growth
Effects of various doses of arsenic on the growth of *E. camaldulensis, S. tetrasperma* and *T. arjuna* plants are shown in Tab. 1.

Stem height (cm)
Stem height varied significantly with increase in arsenic concentration. A reduced value of 272 cm in stem height of *E. camaldulensis* was recorded in T_4 as compared with 331.3 cm in control (T_0) while in the case of *S. tetrasperma*, it was reduced to 92 cm in T_4 compared with 141.3 cm in T_0. In the case of *T. arjuna*, however it was reduced to 166.7 cm in T_4 from 221 cm in T_0. A gradual smooth decreasing trend was observed in stem height (average reduction of 25.8% as compared to control) in all three plant species with the increase in the concentration of arsenic.

Stem diameter (cm)
Stem diameter significantly decreased with the increased concentration of arsenic. A maximum reduction in stem diameter of *E. camaldulensis* was found to be 2.63 cm that was recorded in T_4 compared with 3.8 cm recorded in control (T_0) while in the case of *S. tetrasperma*, it was reduced to 1.4 cm in T_4 down from 1.72 cm in T_0. In case of *T. arjuna* plant, however it was recorded to be 2.65 cm at T_4 down from 3.6 cm at T_0. A gradual smooth decreasing trend in stem diameter was observed with an average reduction of 25% for all three plants species when the concentrations of arsenic were increased.

Number of branches
Similarly, reductions in number of branches were observed with an increase in arsenic concentration. A maximum reduction to the level of 163 (Nos.) in T_4 in number of branches of *E. camaldulensis* was recorded as compared to average 217.3 branches per plant in control (T_0) while in the case of *S. tetrasperma*, it was recorded as 79.8 in T_4 down from 103.7 in T_0. In the case of *T. arjuna* plant, however, it was found to be reduced to 64.7 in T_4 compared with 79.7 in control (T_0). A gradual smooth decreasing trend was observed with an average reduction of 22.27% for all three plants species when the concentration of arsenic was increased.

Number of leaves
Number of leaves was also significantly varied with an increase in arsenic concentration in all species. A maximum reduction in total number of leaves of *E. camaldulensis* plant was recorded to be 449.7 in T_4 as compared with 610.7 in control (T_0) while in the case of *S. tetrasperma*, the number was reduced to 348 in T_4 as compared with 430 in T_0. In the case of *T. arjuna*, however it was reduced to 408.7 in T_4 down from 510.7 in T_0. A gradual smooth decreasing trend was observed with an average reduction of 21.76% for all three plants species as the doses of arsenic were increased.

Tab. 1: Effects of arsenic application on plant growth parameters.

Treatments	Species	Stem Height (cm)	Stem Diameter (cm)	No. of Branches	No. of Leaves	Root Length (cm)	Total Length (cm)
T_0	*E. camaldulensis*	331.3±3.41 a	3.8±0.09 a	217.3±2.22 a	610.7±3.5 a	165.3±1.45 a	496.7±4.77 a
	S. tetrasperma	141.3±2.82 def	1.7±0.33 d	103.7±1.15 d	430.0±8.71 fgh	149.2±3.33 ab	290.5±3.21 def
	T. arjuna	221.0±4.50 bc	3.6±0.33 ab	79.7±0.88 ef	510.7±2.19 bc	134.0±2.50 ab	355.3±4.07 bcde
T_1	*E. camaldulensis*	292.0±4.51 a	3.3±0.33 abc	193.7±2.70 b	564.0±15.10 ab	155.0±3.04 ab	447.0±2.52 ab
	S. tetrasperma	130.3±2.71 def	1.6±0.08 d	90.0±1.48 de	400.3±1.04 ghij	132.3±1.86 ab	262.6±2.70 ef
	T. arjuna	189.5±2.88 cd	3.0±0.10 abc	78.0±1.51 ef	489.7±2.19 cde	117.0±1.76 ab	306.5±2.15 cdef
T_2	*E. camaldulensis*	283.0±3.04 ab	3.0±0.16 abc	182.0 ±2.86 bc	502.0±7.51 cd	147.1±1.50 b	380.1±4.02 bcd
	S. tetrasperma	120.2±1.00 ef	1.5±0.33 d	86.0±1.67 de	381.0±2.08 hij	121.0±1.15 ab	241.2±2.33 ff
	T. arjuna	178.0±1.53 cde	2.8±0.11 bc	72.3±0.23 ef	460.0±3.51 cdef	110.2±1.00 ab	288.2±1.88 def
T_3	*E. camaldulensis*	276.0±1.86 ab	2.9±0.12 bc	171.0±1.15 c	485.0±10.54 cde	134.0±1.15 ab	410.0±2.85 abc
	S. tetrasperma	95.3±1.86 f	1.5±0.10 bc	79.8±0.58 ef	362.0±0.44 ij	116.0±3.86 ab	211.3±2.69 f
	T. arjuna	170.0±2.61 cde	2.7±0.09 c	64.7±0.67 f	437.7±1.45 efg	102.0±0.00 ab	272.0±3.93 ef
T_4	*E. camaldulensis*	272.0±3.30 ab	2.6±0.33 c	163.0±2.52 c	449.7±1.76 defg	130.3±0.88 ab	402.2±2.89 abc
	S. tetrasperma	92.0±0.88 f	1.4±0.07 d	79.8±1.76 ef	348.0±0.20 j	111.1±1.00 ab	203.1±3.17 f
	T. arjuna	166.7±1.67 cde	2.7±0.10 c	64.7±0.58 f	408.7±1.76 fghi	97.0±0.58 b	263.7±2.37 f

T_0 = Tap water (control); T_1 = 0.5 mg L^{-1} arsenic; T_2 = 1.0 mg L^{-1} arsenic; T_3 = 2.0 mg L^{-1} arsenic; T_4 = 4.0 mg L^{-1} arsenic. Values with different letters are significantly different from each other at $p \leq 0.05$.

Root length (cm)

Variation in root length was observed with change in arsenic doses in all three species. A maximum reduction in root length of *E. camaldulensis* was recorded to be 130.3 cm in T_4 as compared with 165.3 cm in T_0, while in the case of *S. tetrasperma* it was reduced to 111.1 cm in T_4 down from 149.2 cm in T_0. In the case of *T. arjuna*, however, the root length was observed as reduced to 97 cm in T_4 in comparison to 134 in control (T_0). A gradual smooth decreasing trend was observed for all three plant species with increasing concentration of arsenic having an average reduction of 24.8%.

Total plant length (cm)

Total plant length varied significantly with an increase in arsenic doses. A maximum reduction in total plant length of *E. camaldulensis* was observed to be in T_4 due to the highest dose of arsenic whereas it was recorded to be 402.2 cm as compared with 496.7 cm at control (T_0) while in the case of *S. tetrasperma* it reduced to 203.1 cm in T_4 as compared with 290.5 cm in T_0. In the case of *T. arjuna*, however, it was reduced to 263.7 cm down from 355.3 cm in control (T_0). A gradual smooth decreasing trend was observed for all three plants species with increasing concentration of arsenic having an average reduction of 25.6%.

Effect of arsenic doses on biomass production of plants

The results of the study regarding effects of varying concentrations of arsenic application on biomass production of *E. camaldulensis, T. arjuna* and *S. tetrasperma* are shown in Tab. 2.

Leaf fresh biomass (g)

Increase in arsenic contents resulted in reduction of leaf fresh biomass. With an increase in arsenic doses, a reduced value of 201 g in leaf fresh biomass of *E. camaldulensis* was recorded in T_4 as compared with 300.8 g in control (T_0) while in the case of *S. tetrasperma*, it reduced to 28.3 g in T_4 compared with 49.5 g in T_0. In the case of *T. arjuna*, however, it was reduced to 61 g in T_4 from 125.3 g at T_0.

Stem fresh biomass (g)

Increase in arsenic contents resulted in reduction of stem fresh biomass. A 955.7 g in stem fresh biomass of *E. camaldulensis* was recorded in T_4 as compared with 1221.7 g in control (T_0) while in the case of *S. tetrasperma*, it reduced to 55 g in T_4 compared with 195 g in T_0. In the case of *T. arjuna*, however, it was reduced to 225 g in T_4 from 372 g at T_0.

Root fresh biomass (g)

Similarly, root fresh biomass also varied with arsenic increase. In response to arsenic doses, a reduced value of 457.8 g in root fresh biomass of *E. camaldulensis* was recorded in T_4 as compared with 555.6 g in control (T_0) while in the case of *S. tetrasperma*, it reduced to 65 g in T_4 compared with 164.2 g in T_0. In the case of *T. arjuna*, however, it was reduced to 146 g in T_4 from 256.6 g at T_0.

Leaf dry biomass (g)

Increase in arsenic contents resulted in reduction of leaf dry biomass. In response to arsenic doses, a reduced value of 105 g in leaf dry biomass of *E. camaldulensis* was recorded in T_4 as compared with 160.5 g in control (T_0) while in the case of *S. tetrasperma*, it reduced to 14 g at T_4 compared with 29 g in T_0. In the case of *T. arjuna*, however, it was reduced to 27 g in T_4 from 62.5 g in T_0.

Stem dry biomass (g)

Increase in arsenic contents resulted in reduction of stem dry biomass. A reduced value of 506.2 g in stem dry biomass of *E. camaldulensis*

Tab. 2: Effects of arsenic doses on biomass production of tree seedlings of *E. camaldulensis, S. tetrasperma* and *T. arjuna*.

Treatments	Species	Fresh Weight (g)			Oven Dry Weight (g)		
		Leaf	Stem	Root	Leaf	Stem	Root
T_0	*E. camaldulensis*	300.8 ± 3.23 a	1221.7 ± 9.99 ab	555.6 ± 2.67 b	160.5 ± 1.49 b	705.2 ± 8.45 a	216.0 ± 2.66 d
	S. tetrasperma	49.5 ± 1.04 fgh	195.0 ± 2.00 b	164.2 ± 2.40 b	29.0 ± 1.76 b	92.3 ± 1.67 fg	80.0 ± 1.36 a
	T. arjuna	125.3 ± 2.14 d	372.0 ± 3.00 b	256.6 ± 3.12 b	62.5 ± 1.51 b	182.5 ± 3.49 c	141.7 ± 1.68 d
T_1	*E. camaldulensis*	255.8 ± 2.73 b	1096.0 ± 8.53 ab	495.8 ± 1.53 b	128.9 ± 5.85 a	604.2 ± 2.19 b	192.5 ± 2.81 b
	S. tetrasperma	41.0 ± 1.53 gh	125.3 ± 1.27 b	104.0 ± 3.22 b	22.0 ± 2.08 b	68.0 ± 1.16 efg	55.0 ± 1.16 ght
	T. arjuna	100.8 ± 1.82 de	297.0 ± 2.06 b	199.0 ± 3.53 b	47.5 ± 2.90 b	151.0 ± 2.08 cd	95.3 ± 1.76 e
T_2	*E. camaldulensis*	235.8 ± 1.15 bc	1076.7 ± 5.33 ab	475.8 ± 2.00 b	119.5 ± 1.20 b	584.2 ± 4.70 b	170.8 ± 2.22 c
	S. tetrasperma	35.0 ± 1.33 gh	101.7 ± 1.76 a	99.0 ± 1.56 b	20.8 ± 0.67 b	55.0 ± 1.53 fg	51.3 ± 1.33 hi
	T. arjuna	81.0 ± 0.22 ef	278.0 ± 3.35 b	177.7 ± 2.40 b	40.0 ± 2.52 b	137.0 ± 2.40 cd	87.0 ± 1.33 ef
T_3	*E. camaldulensis*	222.8± 1.73 bc	1037.7 ± 6.67 a	466.0 ± 3.93 a	112.0 ± 2.00 b	561.5 ± 2.60 b	151.2 ± 2.69 cd
	S. tetrasperma	27.0 ± 0.67 h	75.0 ± 3.51 b	88.0 ± 1.11 b	16.0 ± 1.67 b	45.0 ± 1.53 fg	46.0 ± 0.16 i
	T. arjuna	70.0 ± 1.16 efg	250.0 ± 2.67 b	161.0 ± 2.76 b	35.0 ± 1.76 b	126.0 ± 2.85 cde	75.0 ± 1.40 efgh
T_4	*E. camaldulensis*	201.0 ± 1.00 c	955.7 ± 5.33 ab	457.8 ± 1.16 b	105.0 ± 1.00 b	506.2 ± 1.16 b	132.8 ± 1.20 cd
	S. tetrasperma	28.3 ± 1.76 h	55.0 ± 3.06 b	65.0 ± 2.19 b	14.0 ± 2.03 b	40.0 ± 1.53 g	30.0 ± 0.73 i
	T. arjuna	61.0 ± 1.76 fgh	255.0 ± 3.06 b	146.0 ± 2.0 b	27.0 ± 2.00 b	115.0 ± 2.52 def	63.0 ± 1.73 efg

T_0 = Tap water (control); T_1 = 0.5 mg L^{-1} arsenic; T_2 = 1.0 mg L^{-1} arsenic; T_3 = 2.0 mg L^{-1} arsenic; T_4 = 4.0 mg L^{-1} arsenic. Values with different letters are significantly different from each other at $p \leq 0.05$.

was recorded in T_4 as compared with 705.2 g in control (T_0) while in the case of *S. tetrasperma*, it reduced to 40 g in T_4 compared with 92.3 g in T_0. In the case of *T. arjuna*, however, it was reduced to 115 g in T_4 from 182.5 g at T_0.

Root dry biomass (g)

Increase in arsenic contents resulted in reduction of root dry biomass. In response to arsenic doses, a reduced value of 132.8 g in root dry biomass of *E. camaldulensis* was recorded in T_4 as compared with 216 g in control (T_0) while in the case of *S. tetrasperma*, it reduced to 30 g in T_4 compared with 80 g in T_0. In the case of *T. arjuna*, however, it was reduced to 63 g in T_4 from 141.7 g in T_0.

Accumulation of arsenic by tree seedlings

Effects of arsenic doses on the uptake and accumulation of arsenic in roots, shoots, leaves is shown in Tab. 3.

Accumulation of arsenic in roots

Arsenic accumulation tends to increase in roots with the higher dose of arsenic in all tested plant species. An increased value of 37.25 mg Kg^{-1} of As in roots of *E. camaldulensis* was recorded in T_4 as compared with 1.83 mg Kg^{-1} in control (T_0) while in the case of *S. tetrasperma*, it increased to 35.76 mg Kg^{-1} in T_4 compared with 1.70 mg Kg^{-1} in T_0. In the case of *T. arjuna*, however, it was increased to 24.13 mg Kg^{-1} in T_4 from 1.98 mg Kg^{-1} at T_0.

Accumulation of arsenic in shoots

Arsenic accumulation tends to increase in shoots with the higher dose of arsenic in all tested plant species. In response to arsenic doses, an increased value of 25.69 mg Kg^{-1} of arsenic in shoots of *E. camaldulensis* was recorded in T_4 as compared with 1.08 mg Kg^{-1} in control (T_0) while in the case of *S. tetrasperma*, it increased to 13.76 mg Kg^{-1} in T_4 compared with 1.03 g in T_0. In the case of *T. arjuna*, however, it was increased to 12.52 mg Kg^{-1} in T_4 from 0.94 mg Kg^{-1} in T_0.

Accumulation of arsenic in leaves

Arsenic accumulation tends to increase in leaves with the higher dose of arsenic in all tested plant species. In response to arsenic doses, an increased value of 6.55 mg Kg^{-1} of arsenic in leaves of *E. camaldulensis* was recorded in T_4 as compared with 0.57 mg Kg^{-1} in control (T_0) while in the case of *S. tetrasperma*, it increased to 6.03 mg Kg^{-1} in T_4 compared with 0.57 g in T_0. In the case of *T. arjuna*, however, it was increased to 4.22 mg Kg^{-1} in T_4 from 0.67 mg Kg^{-1} in T_0.

Discussion

An inhibition of plant growth is directly related to the enhanced uptake and accumulation of heavy metals in plants because metal elements adversely affect the plant growth through the generation of reactive oxygen species (ROS) causing constant damage to cellular structures, interfere metabolic process in plants (MARIN et al., 1993; MALLICK et al., 2011). Upon translocation to the shoot, arsenic severely inhibits plant growth by slowing biomass accumulation (FINNEGAN and CHEN, 2012). Results of this study shows that stem height of all three plants species gradually reduced with increasing doses of arsenic and averagely it reduced to 25.8% at 4 mg L^{-1} arsenic dose. Reduction in shoot growth could be attributed to the reduction in chlorophyll contents and activity of photosystem I (PSI) induced by heavy metal stress (SKORZYNSKA-POLIT and BASZYNSKI, 1997). Moreover, accumulation of metal elements to above ground plant part can reduce plant height because of disturbance in cellular

Tab. 3: Arsenic accumulation in leaves, shoots and roots of tree seedlings of *E. camaldulensis, S. tetrasperma* and *T. arjuna*.

Treatments	Species	Arsenic content accumulated in Leaves (mg kg^{-1})	Arsenic content accumulated in Shoot (mg kg^{-1})	Arsenic content accumulated in Root (mg kg^{-1})
T_0	*E. camaldulensis*	0.57 ± 0.33 i	1.08 ± 0.06 j	1.83 ± 0.07 i
	S. tetrasperma	0.57 ± 0.17 i	1.03 ± 0.04 j	1.70 ± 0.09 i
	T. arjuna	0.67 ± 0.06 i	0.94 ± 0.03 j	1.98 ± 0.11 i
T_1	*E. camaldulensis*	2.73 ± 0.33 d	9.65 ± 0.38 f	14.84 ± 0.39 f
	S. tetrasperma	1.87 ± 0.08 h	5.41 ± 0.33 hi	12.82 ± 0.46 g
	T. arjuna	1.73 ± 0.33 h	4.50 ± 0.33 i	10.45 ± 0.33 h
T_2	*E. camaldulensis*	3.31 ± 0.33 c	15.61 ± 0.45 c	20.56 ± 0.67 e
	S. tetrasperma	2.85 ± 0.39 fg	9.78 ± 0.30 g	19.98 ± 0.43 e
	T. arjuna	2.22 ± 0.17 gh	6.85 ± 0.58 h	13.01 ± 0.31 g
T_3	*E. camaldulensis*	5.27 ± 0.33 b	20.94 ± 0.50 b	29.16 ± 0.58 b
	S. tetrasperma	4.50 ± 0.37 e	10.91 ± 0.31 e	27.01± 0.58 c
	T. arjuna	3.22 ± 0.31 f	9.26 ± 0.33 g	19.92 ± 0.46 e
T_4	*E. camaldulensis*	6.55 ± 0.67 a	25.69 ± 0.88 a	37.25 ± 0.61 a
	S. tetrasperma	6.03 ± 0.33 d	13.76 ± 0.30 d	35.76 ± 1.44 a
	T. arjuna	4.22 ± 0.18 e	12.52 ± 0.33 de	24.13 ± 0.33 d

T_0 = Tap water (control); T_1 = 0.5 mg L^{-1} arsenic; T_2 = 1.0 mg L^{-1} arsenic; T_3 = 2.0 mg L^{-1} arsenic; T_4 = 4.0 mg L^{-1} arsenic. Values with different letters are significantly different from each other at $p \leq 0.05$.

metabolism of shoots (Shanker et al., 2005). A significant reduction in stem growth of pea (*Pisum sativum*) seedlings was also recorded when arsenic was applied at the rate of 5 mg L^{-1} (Castillo-Michel et al., 2007) which is almost similar to this study. Similarly, stem diameter gradually reduced with increasing doses of arsenic and on average it reduced to 25% at 4 mg L^{-1} arsenic dose in case of all three plants species. Leaf growth and total number of leaves are considered a suitable indicator of heavy metal toxicity. Reza et al. (2009) found a decrease in number of leaves per plant with gradual increase of As application to A*maranthus retroflexus* plants. Similarly, a decrease in leaf number was observed in this study, with an average reduction of 22.27% for all three plants species as the doses of arsenic were increased. Root growth is also an important indicator related to the enhanced uptake and accumulation of heavy metals in plants. High concentration of heavy metals retard root respiration leading to reduction in root growth (Menon et al., 2005). Moreover, due to high solute potential in the rhizosphere, nutrient uptake by plants is disturbed causing a decrease in mitotic division of meristematic cells (Odjegba and Fasidi, 2004). Root growth of all three plants species decreased with increasing doses of arsenic with an average reduction of 24.8% at 4 mg L^{-1} dose. Shri et al. (2009) also observed a decrease in root growth of rice seedlings.

The reduction in biomass at high doses of As might be due to the high lipid peroxidation which might have damaged chloroplast, and inhibited chlorophyll and protein contents (Singh et al., 2006; Garg and Singla, 2011). Upon translocation to the shoot, arsenic severely inhibits plant growth by slowing biomass accumulation (Finnegan and Chen, 2012) resulting in biomass reduction. Aydin and Aksoy (2009) applied arsenic on chickpea (*Cicer arietinum* L.) plants and found reduction in fresh and dry biomass production of chickpea plants with increasing arsenic levels.

Arsenic is non-essential for plant growth; therefore, plants have no specific mechanism for its uptake and translocation in plant system. It enters by means of the same uptake transporter as essential nutrients. Higher levels of arsenic in soil interfere with nutrient uptake by plants and their distribution leading to a deficiency of essential nutrients in plants (Mahimairaja et al., 1994). Since roots are the first plant tissue to come in contact with soil and therefore, metal accumulation was more pronounced in roots compared to shoots and leaves (Kuzovkina et al., 2004). Higher accumulation of heavy metals in roots might be due to poor translocation to above ground parts and sequestration in the vacuoles of root cells (Shanker et al., 2005). Metal accumulation in plant tissues was in order of roots>shoot>leaves and the increase was proportional to the external application of metal elements. The variability of metals in various plant tissues may be due to compartmentalization and translocation in the vascular system (Kim et al., 2003). Arsenic in high concentration may hamper with metabolism of plants and adversely affected the uptake of nutrients by competing with plant nutrients for transporters (Meharg and Macnair, 1990; Mokgalaka-Matlala et al., 2008). Cell membranes are vulnerable targets of arsenic stress and damage of membrane is used as an indicator for tolerance to arsenic stress. Arsenic adversely affects the membrane leading to unbalanced nutrient uptake and water content in plant cells (Gadallah, 1999; Garg and Singla, 2011).

Conclusion

In this study the growth of tree seedlings was determined and shown to be affected by the uptake of arsenic as it accumulated in the vegetative parts of the plants grown under arsenic treated media. *E. camaldulensis, T. arjuna* and *S. tetrasperma* have demonstrated adaptability to recover the soil from arsenic with varying magnitude. Although arsenic-treated plants were affected and growth parameters demonstrated reduced number of branches, leaves, reduced root length and low biomass production as compared to plants grown without arsenic treatment. They significantly reduced arsenic from the media. Among the three tree species investigated, *E. camaldulensis* showed the highest tolerance against arsenic toxicity and maximum arsenic accumulation in its vegetative parts. Thus, the study has manifested success in the reclamation of affected soils with contributing environment cleaning and landscaping.

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