IMPACT OF HEAVY METALS ON MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS OF *Shorea robusta* PLANT

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Abstract

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A study was conducted to determine the impact of heavy metals (cadmium [Cd], arsenic [As] and lead [Pb]) on the morphological, and biochemical parameters of *Shorea robusta* and to investigate the uptake capacity of this plant for individual heavy metal. The study showed that all three heavy metals had significant adverse impact on most of the plant parameters of *S. robusta*, at all the given concentrations of 1, 5 and 10 mg/l. Maximum reduction in morphological parameters is observed in leaf area (92.67%) followed by shoot length (54%) and, root length (28.78%). Maximum reduction in biochemical parameters is observed in amino acid (75.13%) followed by chlorophyll (68.33%) and, crude protein (35.68%), whereas polyphenol and ascorbic acid showed maximum enhancement of 77.1 and 139.8%, respectively. The accumulation of heavy metals was found to be higher in the root than shoot of *S. robusta* with maximum accumulation being 0.053 mg/g in root for Cd, 0.17 mg/g of As in leaf and, 0.201 mg/g of Pb in soil. Concentration- dependent changes were observed in most of the morphological and biochemical parameters, which may thus serve to determine suitable bio-indicators of heavy metal pollution.

Key words: *Shorea robusta*, biochemical indicator, heavy metals, accumulation, toxicity.

Introduction

Environment Pollution is one of the severe problems faced by the world today. Various efforts have been made for environmental conservation in India, but it still seems to be a formidable task. The term ‘heavy metal’ refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). Heavy metal is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm³ or five times or more, than water (Garbarino et al., 1995; Hawkes, 1997). Toxic metals are biologically magnified through the food chain. They infect the environment by affecting soil properties its fertility, biomass and crop yields, and ultimately human health. Heavy metals are major environmental pollutant, which are originate from activities, mostly industrial wastes (Weisberg et al., 2003). Their accumulation in soil becomes dangerous to all kind of organisms including plants (Gichner et al., 2006). Cadmium, being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient

uptake and homeostasis, is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health (di Toppi, Gabrielli, 1999). Cadmium ranks the highest among all toxic metals in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain (Shah, Dubey, 1998). The presence of excessive amount of Cd in soil commonly results in many stress symptoms in plants, such as reduction growth, especially roots growth, disturbance in mineral nutrition and carbohydrate metabolism (Moya et al., 1993) and may thus reduce biomass production. Lead is one of the most abundant metals in the earth's crust. Exposure to lead in the environmental and occupational setting continues to be a serious health problem (WHO, 1995). Elevated lead in soil may lessen productivity and even a very low concentration can inhibit some vital plant processes, such as seed germination, photosynthesis, mitosis, plant water status, mineral nutrition and enzymatic activities (Patra et al., 2004).

Lead is a potentially toxic heavy metal with characteristic toxic action and is the main source of environmental pollution. It inhibits some metabolic activities in the plants such as the biosynthesis of nitrogenous compounds, carbohydrate metabolism and water absorption (Sharma, Dubey, 2005; Hamid et al., 2010). Pb contamination in soils not only induced changes in soil microorganisms and their activities resulted in soil fertility deterioration but also directly affected the change of physiological indices and, furthermore, resulted in yield decline (Majer et al., 2002). Certain plants absorb these toxic metals and help to clean them up from the soil. These plants are called hyper-accumulators. They plants have been shown to be resistant to heavy metals and are capable of accumulating them into their roots and leaves. Thus, biologically engineered methods are designed to improve the use of plants to reduce the amount of heavy metals in contaminated soil. (Mudgal et al., 2010). The present experiment was undertaken to investigate changes in the level of growth, and biochemical parameters in *S. robusta* treated with Cd, As and Pb and their accumulation in soil and plant in order to contribute to an understanding of *S. robusta* adaptation to environmental stress.

Materials and methods

All the nursery and laboratory studies were carried out at the campus of Forest Research Institute (FRI), Dehradun. In this analysis, seeds were sown directly in the media-filled pots. After germination, the seeds were transferred to the root trainers. The species was placed in the natural condition of central nursery. After 3 weeks, the plants were transferred to polythene bags filled with sand, soil and farmland manure (1:1:1) and irrigated daily. Heavy metal treatment consisted of CdCl_2 , As_2O_3 and $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$, each with three different concentrations of 1 mg/l, 5 mg/l and 10 mg/l. The treatment was given to the plants for 4 months. Three replicates of each plant along with control were taken. After completion of the treatment (4 months), the plants were removed from polythene bags and their parts (root, shoot and leaf) separated. These parts were analysed for morphological and biochemical parameters for heavy metals concentration. Soil samples were also analysed for heavy metal concentration. All the biochemical parameters were estimated by using spectrophotometer (UV 2700).

Leaf area: Leaves at all the positions in the plants were taken. At the end of the experimental period, the leaf area was measured in cm^2 using the leaf area meter Delta-T Device (Buewell Cambridge England) at the end of the experimental period. (Potter et al., 1996).

Root and Shoot length: Root and Shoot length (cm) were measured with the help of metre scale for each individual plant (Dittmer, 1959).

Chlorophyll: Chlorophyll in leaves was estimated by Arnon (1945) method. Fresh leaf samples were homogenised in 80% acetone and optical densities were measured at 645 and 663 nm with spectrophotometer (Spectra scan UV 2700).

Protein: Protein content (mg/g) was estimated by the procedure given by Lowry et al. (1951).

Free Amino Acid: Free amino acid (mg/g) was measured by the ninhydrin method given by Moore and Stein (1948). Absorbance was recorded at 570 nm using a spectrophotometer (Spectrascan UV 2700).

Ascorbic acid estimation: Fresh leaf sample (0.5 g) was homogenised in 20ml of extracting media of 0.5 g oxalic acid and 0.075 g EDTA in 100 ml distilled water. After centrifuging the homogenate for 15 min, 1.0 ml of the homogenate was mixed with 5.0 ml of Dichlorophenol indophenols (20 µg/ml). After shaking well, its optical density was measured at 520 nm (Sadasivam, Balasubramaniam, 1987) and value presented as mg/g.

Polyphenol estimation: Polyphenol estimation was done by the method given by Schandari (1970). About 0.5 g of powdered sample of *S. robusta* leaves were boiled with distilled water for 30 min. Volume of the supernatant was made up to 100 ml by adding distilled water. Folin Denis reagent and sodium carbonate solution were added in known aliquots and absorbance was read at 700 nm after 30 min. Values of polyphenol were presented as %.

Estimation of heavy metals

Air-dried samples (soil and root, shoot leaves of *S. robusta*) were ground and sieved. 0.2 g of plant samples were digested using nitric acid and perchloric acid. Filtrate was analysed with atomic absorption spectrophotometer (AAS) and inductively coupled plasma mass spectrophotometer (ICPMS) as outlined by Wang et al. (1999).

Statistical analysis

The data were analysed using the SPSS program for windows version 15.0. Multiple comparisons and two-way analysis of variance (ANOVA) procedures were used to compare the differences among the treatments. The least significant difference (LSD) tests were performed to determine the significance of treatment means at $P < 0.05$.

Results and discussion

The data incorporated in Table 1 display that the results obtained from the present study showed that after cadmium treatments (1, 5 and 10 mg/l), *S. robusta* plant exhibits the reduction in root length at all concentration over control. At cadmium treatment, maximum decrease of 26.25 % in root length was observed at 10 mg/l treatment, followed by 18.75% at 5 mg/l and 8.75 % at 1 mg/l treatment of heavy metal. The obtained data of shoot length showed that maximum reduction of 18.18 % was observed at 10 mg/l doses and minimum at 5 mg/l (13.63%). For leaf area more reduction was found at 10 mg/l (72.72%) and minimum at 1 mg/l (63.69%).

The investigation revealed significant reduction ($P < 0.001$) in chlorophyll content for *S. robusta* seedling. A concentration dependent decrease in chlorophyll content over control was observed in the leaves. After Cd treatment maximum reduction of 63.72% was observed at 10 mg/l and minimum at 1 mg/l (25.4%). For protein, higher reduction was found at 10 mg/l (35.68%) and lower at 1 mg/l (15.0%). A gradual decrease in amino acid with increasing concentrations of metals was observed over control ($P < 0.001$). Maximum decrease in amino acid was observed in 10mg/l treatment (64.94%) and minimum in 1mg/l treatment (23.01%). As observed, at 10 mg/l treatment of cadmium, maximum enhancement in ascorbic acid was 98.4% and minimum was 36.5% in 1 mg/l treatment. The results indicated that the increase of polyphenol was dependent on the concentration of heavy metals. A concentration-dependent increase in polyphenol content was observed ($P < 0.001$). After cadmium treatment, the maximum enhancement in polyphenol content was observed in 10 mg/l (40.7%) and minimum was in 5 mg/l (37.2%) (Table 1).

After arsenic treatment the species *S. robusta* exhibits reduction in root length at all concentrations over control (Table 1). Maximum decrease in root length was found at 10 mg/l treatment (25.25%) and slight enhancement of 0.0004% at 1 mg/l treatment. For shoot length, 54% reduction was found in 10 mg/l while increase in shoot length was found in 1

T a b l e 1. Data showing morphological and biochemical changes of *Shorea robusta* seedling plants.

Treatments(mg/l)	Metals		
	Cd	As	Pb
Root length (Cm²)			
Control	40.0 ± 2.887	26.0 ± 2.309	33.0 ± 5.196
1	36.5 ± 2.021	27.0 ± 2.887	39.5 ± 1.443
5	32.5 ± 4.330	23.5 ± 1.443	23.5 ± 2.598
10	29.5 ± 2.598	19.5 ± 0.289	27.5 ± 1.443
Shoot length (Cm²)			
Control	44.0 ± 4.041	25.0 ± 0.577	42.3 ± 7.263
1	37.0 ± 5.196	27.0 ± 5.774	36.00 ± 3.464
5	38.0 ± 4.041	26.0 ± 5.774	29.500 ± 1.443
10	36.0 ± 9.815	11.5 ± 0.289	26.50 ± 2.598
Leaf area (Cm²)			
Control	2361.37 ± 95.79	1449.32 ± 220.20	1525.0 ± 176.09
1	857.18 ± 270.25	1082.60 ± 110.10	942.50 ± 07.22
5	852.50 ± 59.06	106.44 ± 20.48	1433.60 ± 0.17
10	644.12 ± 0.030	305.22 ± 18.26	1308.72 ± 188.9
Total chlorophyll (mg/g)			
Control	0.295 ± 0.003	0.295 ± 0.003	0.300± 0.006
1	0.220 ± 0.006	0.230 ± 0.006	0.243 ± 0.009
5	0.167 ± 0.017	0.180 ± 0.006	0.170 ± 0.006
10	0.107 ± 0.007	0.140 ± 0.012	0.095 ± 0.003
Protein (mg/g)			
Control	51.23 ± 0.006	48.33 ± 0.009	32.95 ± 0.006
1	43.52 ± 0.006	40.74 ± 0.006	38.72 ± 0.006
5	42.20 ± 0.006	38.72 ± 0.006	41.32 ± 0.003
10	32.95 ± 0.006	32.34 ± 0.361	46.71 ± 1.943
Total free amino acid (mg/g)			
Control	4.650 ± 0.673	5.430 ± 0.035	5.271 ± 0.001
1	3.580 ± 0.098	3.315 ± 0.001	3.245 ± 0.020
5	2.140 ± 0.017	2.140 ± 0.017	2.335 ± 0.014
10	1.630 ± 0.006	1.350 ± 0.017	1.525 ± 0.009
Ascorbic acid (mg/g)			
Control	2.000 ± 0.104	1.820 ± 0.003	2.000 ± 0.104
1	2.730 ± 0.104	3.185 ± 0.055	4.190 ± 0.104
5	3.915 ± 0.159	2.365 ± 0.107	3.640 ± 0.002
10	3.368 ± 0.579	4.365 ± 0.003	4.360 ± 0.006
Polyphenol (%)			
Control	2.580 ± 0.017	2.050 ± 0.035	2.325 ± 0.009
1	3.590 ± 0.007	3.310 ± 0.003	3.420 ± 0.087
5	3.540 ± 0.017	2.860 ± 0.006	2.900 ± 0.017
10	3.630 ± 0.007	3.630 ± 0.003	3.590 ± 0.006

Values expressed are means ±standard deviation of three separate experiments.

mg/l and 5 mg/l doses as compared to the control value. Maximum reduction in leaf area (compared to control) was found in 5 mg/l treatment (92.67%) and lower in 1mg/l (25.3%).

Species seedling when treated with arsenic treatment exhibited reduction in total chlorophyll content at all concentration over control (Table 1). After treatment more reduction was observed in 10 mg/l (52.54%) and less in 1 mg/l (22.03%). Arsenic exhibited reduction in protein content at all concentration, and maximum reduction of 33.09% was observed in 10 mg/l doses and minimum in 1 mg/l (15.70%) doses. Reduction in free amino acid content was found in 10 mg/l treatment that was 75.13% and minimum in 1 mg/l (38.95%). It was observed that in ascorbic acid, maximum enhancement was found in 10 mg/l (139.8%) and minimum was in 1 mg/l (75%). A concentration-dependent increase in polyphenol content was observed. Higher reduction (77.1%) was observed at 10 mg/l dose, and the reduction was lower at 1 mg/l (61.46%) treatments. Species treated with Pb exhibited reduction in root length at 1 mg/l and 5 mg/l concentration over control while at 10 mg/l treatment increase in root length was observed that was 28.78%. For shoot length, maximum reduction was found in 10 mg/l (37.35%) and minimum in 1 mg/l treatment (14.82%). The percent reduction in leaf area was higher at 1 mg/l (38.19%) treatment.

Species seedling when supplied with lead condition exhibited reduction in total chlorophyll content at all concentration over control. As observed, maximum reduction was observed in 10 mg/l (68.33%) treatment and minimum in 1 mg/l (19%). The species revealed maximum loss of protein content when supplied with lead treatments. Enhancement of 41.76% was observed in 10mg/l treatment. For amino acid, maximum reduction was observed in 10 mg/l (71.06%) and minimum in 1 mg/l (38.43%). The enhancement in ascorbic acid was found in different concentration of heavy metals, maximum enhancement was found in 10 mg/l (118%) treatments and minimum enhancement was in 5mg/l treatments that was 82%. After lead treatment, 10 mg/l (54.4%) exhibited maximum enhancement in polyphenol content and minimum in 5 mg/l (24.7%) (Table 1).

Correlation coefficient calculated (Table 3) for morphological and biochemical parameters, metals and treatment showed that the correlation between amino acid and chlorophyll ($r^2 = 0.927$, $P < 0.001$) and leaf area with root length ($r^2 = 0.418$, $P < 0.05$), chlorophyll ($r^2 = 0.509$, $P < 0.05$) and protein ($r^2 = 0.556$, $P < 0.05$) were significantly and positively correlated. Significant negative correlation was found between polyphenol and chlorophyll ($r^2 = -0.746$, $P < 0.001$) and amino acid ($r^2 = -0.782$, $P < 0.001$) and ascorbic acid ($r^2 = -0.774$, $P < 0.05$). Ascorbic acid showed negative correlation with chlorophyll ($r^2 = -0.704$, $P < 0.01$) and amino acid ($r^2 = -0.747$, $P < 0.01$) and positive correlation with treatment ($r^2 = 0.640$, $P < 0.01$). Amino acid negatively correlated with treatment ($r^2 = -0.873$, $P < 0.001$) but revealed significant positive correlation between chlorophyll ($r^2 = 0.927$, $P < 0.001$) and leaf area ($r^2 = 0.584$, $P < 0.05$). The given results indicate that in the positive correlation if one variable increases another will also increase, and in negative correlation if one variable will increase and another will decrease.

Results indicate that heavy metals affect the morphological and biochemical parameters of *Shorea robusta* leaves with increasing concentration of metals. Lead and cadmium toxicity have become important due to their constant increase in the environment (Muhamad et al., 2008). The results obtained from this study indicate that excess of heavy metal causes a va-

T a b l e 2. Accumulations of heavy metal in soil, root, shoot and leaf of *Shorea robusta* seedling plants.

<i>Shorea robusta</i>			
Soil (mg/g)	Cd	As	Pb
Control	ND	ND	ND
1mg/l	0.0092 ± 0.001	0.0111± 0.0001	0.0165 ± 0.001
5mg/l	0.0218 ± 0.0003	0.0339 ± 0.0012	0.1136 ± 0.041
10mg/l	0.0367 ± 0.0084	0.066 ± 0.0001	0.201 ± 0.088
Root (mg/g)			
Control	ND	ND	ND
1mg/l	0.0098 ± 0.0001	0.022 ± 0.001	0.014 ± 0.0001
5mg/l	0.023 ± 0.0007	0.076 ± 0.001	0.065 ± 0.029
10mg/l	0.053 ± 0.014	0.056 ± 0.0001	0.035 ± 0.0024
Shoot (mg/g)			
Control	ND	ND	ND
1mg/l	0.0084 ± 0.00024	0.024 ± 0.002	0.014 ± 0.0001
5mg/l	0.0019 ± 0.0001	0.078 ± 0.0002	0.055 ± 0.002
10mg/l	0.0044 ± 0.0011	0.141 ± 0.0002	0.0254 ± 0.0043
Leaf (mg/g)			
Control	ND	ND	ND
1mg/l	0.0082 ± 0.00016	0.031 ± 0.0003	0.014 ± 0.0004
5mg/l	0.0011 ± 0.00035	0.090 ± 0.01	0.089 ± 0.001
10mg/l	0.0018 ± 0.00013	0.178 ± 0.001	0.038 ± 0.005

Values expressed are means ±standard deviation of three separate experiments.

T a b l e 3. Correlation coefficient ‘r’ between morphological and biochemical parameters, metals and treatment of *S. robusta*.

	Leaf area	Root length	Shoot length	Chloro-phyll	Protein	Amino acid	Ascorbic acid	Polyphenol
Metal	0.080	-0.207	-0.099	0.029	-0.176	0.027	0.233	-0.210
Treatment	-0.473*	0.013	-0.082	-0.933***	-0.374	-0.873***	0.640**	0.615**
Leaf area	1.00	0.418*	0.294	0.509*	0.556*	0.584*	-0.357	-0.490
Root length	0.418*	1.00	0.426*	0.010	0.297	0.083	-0.160	0.046
Shoot ength	0.294	0.426*	1.00	0.052	-0.051	0.100	-0.088	0.044
Chlorophyll	0.509*	0.010	0.052	1.00	0.300	0.927***	-0.704**	-0.746***
Protein	0.556*	0.297	-0.051	0.300	1.00	0.349	-0.267	-0.296
Amino acid	0.584*	0.083	0.100	0.927***	0.349	1.00	-0.747***	-0.782***
Ascorbic acid	-0.357	-0.160	-0.088	-0.704**	-0.267	-0.747***	1.00	0.774***
Polyphenol	-0.490	0.046	0.044	-0.746***	-0.296	-0.782***	0.774***	1.00

*- significance at 0.05, **- significance at 0.01, ***- significance at 0.001

riety of toxicity symptoms in plants, such as reduced growth. According to Eun et al. (2000) the reduction in plant growth during stress is due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress. The effect of treatments was more

pronounced in case of root length. Roots are more prone to heavy metal toxicity relative to shoots (Oncel et al., 2000).

The higher impact of heavy metal was observed in the root growth as compared to shoot leading to a reduction in its length and fresh weight (Elloumi et al., 2007). The reduction in root length due to accumulation of metals within the root reduces the rate of mitosis in the meristematic zones of roots, especially by blocking the metaphase in meristematic cells. Therefore, root showed reduction in length as demonstrated by Pant et al. (2011). Root is capable of accumulating significant quantities of this heavy metal and simultaneously restricts its translocation to the shoot. Retarded shoot length due to the presence of the root environment with excess of Pb has been found by Seyyedi (1999). The decrease in shoot length and biomass with increasing concentration of heavy metals may be due to the sensitivity of enzymes of the photosynthetic carbon reduction cycle to cadmium, as reported by De Filippis and Ziegler (1993). Tripathi and Tripathi (1999), reported similar results in an experiment where 1, 5 and 10ppm of Cr, Ni and Hg affected the leaf area and root and shoot length of *Albizia lebbek*. Our results are in agreement with the findings of Tandy et al. (2006), for *Helianthus annuus* growing in nutrient solution with Pb and Guo et al. (2007).

Chlorophyll is essential for photosynthesis and is very sensitive to environmental stress such as heavy metals (Ekmekci et al., 2008). Plant exposed to Pb ions showed a considerable decrease in their dry weight and a decline in the total chlorophyll and thus photosynthetic efficiency (Kambhampati et al., 2005). The plant processes are adversely affected by the increasing Pb levels in soil and even at very low concentration (Patra et al., 2004). The present study shows that cadmium, arsenic and lead are toxic to species as shown by the toxicity symptoms, such as chlorosis, decrease in the biomass and total chlorophyll content, developed in plants. Our results for decrease in chlorophyll content are corroborated with the findings of Siedlecka and Krupa (1996) who have also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. Similar results have been previously reported for sunflower (Zengin, Munzuruglu, 2005), almond (Elloumi et al., 2007), *Casia fistula* (Pandey et al., 2011) and other researchers (Wang et al., 2009; John et al., 2009; Mobin, Khan, 2007). Phetsombat (2006) also reported similar finding in *Salvinia cucullata*. The presence of Cd decreased the content of chlorophyll and carotenoids, and increased non-photochemical quenching in *Brassica napus* as observed by Larsen et al. (1998). A considerable loss in total chlorophyll in the plants treated with heavy metal was confirmed by Tripathi and Tripathi (1998) and Hemalatha et al. (1997).

Proteins are important constituents of the cell that are easily damaged in environmental stress condition. Hence, any change in these compounds can be considered an important indicator of oxidative stress in plants. The results of this study showed varied changes in protein content in leaf after different metal treatments. It has been reported that Cd, As and Pb are able to decrease protein content by inhibiting the uptake of Mg and K ions and promote postranslational modification (Gardea Torresday et al., 2004; Romero-Puertas et al., 2007), decrease in synthesis or increase in protein degradation (Monterio et al., 2009) and the prevention of rubisco activity (Muthuchelian et al., 2001; Siedlecka, Krupa, 1996). Heavy metals reduced soluble proteins in agricultural crops and in this study the effect was much pronounced on forestry tree species as compared to agricultural crops (Hemalatha et al.,

1997). Tripathi and Tripathi (1998) worked on *Albizia lebbek* for Hg, Cr and Ni treatments. The study of protein content coincides with the findings of Singh and Sinha (2005) who also found decrease in protein content in *B. juncea* when grown on various amendments of tannery waste containing heavy metals. The increase in protein under heavy metal stress may be related to induce the synthesis of stress protein such as enzymes involved in Krebs cycle, glutathione and phytochelation biosynthesis and some heat shock proteins (Verma, Dubay, 2003; Mishra et al., 2006).

Amino acids play a central role in plant primary metabolism. Previous studies indicated that decrease in the mitochondrial electron transport activity accompanies increase in proline accumulation under heavy metal stress (Saradhi et al., 1995). The induction of proline accumulation in response to abiotic stress may be due to increase in its de novo synthesis or decreased degradation (Kasai et al., 1998), and the effect of proline on the permeability of membrane (Pesci, Reggiani, 1992). Therefore, the increase of proline can be considered as an indicator of tolerance of heavy metal stress.

Polyphenol occurs in all plant parts and offers resistance against diseases and pests. In this study, polyphenol content gradually increased in some plants with the increasing concentrations of heavy metals. It has been found that polyphenols concentration can alter with the altered natural environmental conditions, and increases in the tissues surrounding a wound. Similar results were observed by Tripathi and Tripathi (1998) in *Albizia lebbek*. Antioxidants like cysteine, proline, ascorbic acid and nonprotein thiol (sulfhydryl) play an important role in detoxification of toxic metal ions (Singh, Sinha, 2005).

Table 2 shows the concentration of heavy metals in soil and different plant parts (root, shoot, leaf). At 10mg/l treatment, the maximum concentration of cadmium was detected in the root (0.053 ± 0.014 mg/g) followed by soil (0.0367 ± 0.0084 mg/g) and shoot (0.0044 ± 0.0011 mg/g) and least in leaves (0.0018 ± 0.00013 mg/g). At 5mg/l doses, maximum concentration of cadmium was detected in root (0.023 ± 0.001 mg/g) and minimum in leaf (0.0011 ± 0.00003 mg/g). At 1mg/l cadmium doses, maximum concentration was detected in root (0.0098 ± 0.0001 mg/g) and minimum in leaves (0.0082 ± 0.0002 mg/g) (Table 2).

For 10mg/l arsenic doses, the maximum concentration was detected in the leaves (0.178 ± 0.001 mg/g) followed by shoot (0.141 ± 0.00002 mg/g), soil (0.066 ± 0.0001 mg/g) and least in root (0.056 ± 0.0001 mg/g). For 5mg/l arsenic doses, maximum concentration was detected in leaves (0.090 ± 0.0058 mg/g) and minimum in soil (0.0339 ± 0.0012 mg/g). For 1mg/l arsenic doses, maximum concentration was detected in leaves (0.0307 ± 0.0003 mg/g) and minimum in soil (0.011 ± 0.0001 mg/g) (Table 2).

For 10mg/l lead doses, the maximum concentration was detected in the soil (0.201 ± 0.0884 mg/g) followed by leaves (0.038 ± 0.0048 mg/g), root (0.035 ± 0.0024 mg/g) and least in shoot (0.0254 ± 0.0043 mg/g). For 5mg/l lead condition, maximum concentration was detected in soil (0.114 ± 0.041 mg/g) and minimum in shoot (0.055 ± 0.0022 mg/g). For 1mg/l doses, maximum concentration of lead was detected in soil (0.0165 ± 0.0001 mg/g) and minimum in shoot (0.0137 ± 0.00004 mg/g) (Table 2).

The results revealed that under the experimental conditions, the accumulation of heavy metal increased when the exposure time and concentration of metal were increased. The finding of the present study of heavy metal accumulation in soil and different plant parts

coincides with those of Pandey and Tripathi (2010) in *Albizia procera*, when the doses of cadmium, arsenic and lead given to the plant seedlings. Similar results were reported by Umebese and Motajo (2008) in *Ceratophyllum demersum*. At low concentrations, some heavy metals act as micronutrients causing a rapid overall growth of the seedling. However, increasing the concentration of metals above critical level severely inhibits the seedling growth in terms of various morphological and biochemical parameters. From the view of phytoremediation, a good accumulator should have the ability to concentrate the element in the tissue (Rashed, 2010). Once the metals have been concentrated and extracted by the plants, they can be easily removed from the site (Gratao et al., 2005).

Conclusion

In conclusion, our results indicated that the exposure of *S. robusta* to Cd, As and Pb resulted in a decrease in root and shoot length, area and pigment content at higher concentration of heavy metals, and increase of Ascorbic acid and polyphenol was observed at higher concentrations. More As was accumulated more than Cd and Pb in shoot of *S. robusta*. Cd and As was more effective than Pb metals in hampering the plant growth and development.

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