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MERCURY UPTAKE AND TRANSLOCATION BY INDIGENOUS PLANTS

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ABSTRACT
Contaminated soil can be remediated by various methods. Phytoremediation is an on-site remediation approach that employs plants to remove inorganic pollutants and immiscible soil contents. The remediation potentials of four native plant species namely *Tridax procumbens, Ruellia tuberosa, Dodonaea viscosa* and *Azadirachta indica* from Hg-contaminated soils were studied in pot culture experiment. Mercury (Hg) content in plant roots and shoots were analyzed at 20, 40 and 60th days of the study period. According to BCF & TF values, *Tridax procumbens* showed increasing BCF values ranges from 0.163 to 0.228, a higher ability in Hg uptake and translocation to roots than shoots. As per TF values *Ruellia tuberosa, Dodonaea viscosa* and *Azadirachta indica* has shown ≥1, indicates translocation of mercury from the plant roots to the aerial parts. Hg contamination caused a significant reduction in vegetative growth parameters and photosynthetic pigments, whereas proline and MDA contents in plants parts increased with the increasing days of contamination.
Keywords: Mercury, native plants, phytoremediation, proline and MDA.

INTRODUCTION

Mercury is a one of the major environmental pollutants that is derived from numerous (natural and anthropogenic) sources and has a huge international impact because of its toxicity, complex chemo dynamics within the surroundings and tendency for bio magnification in ecosystems.[1-3] Mercury has been in use since from the last 2500 years due to its unique physical and chemical properties.[4] Mercury production since from the beginning of the industrialization has been estimated at 0.64 million metric tons globally.[5] Karunasagar[6] reported mercury pollution in water, sediments and fish samples of Kodia Lake in Tamil Nadu which was caused by the thermometer factory due to Hg emissions and waste. Ram[7] determined Hg pollution in water and sediments of Ulhas Estuary in Maharashtra due to indiscriminate discharge of industrial effluents of chlor-alkali plants. Krishnamoorthy and Nambi[8] evaluated T-Hg concentrations in the vertical soil profile in Thane Creek, Mumbai, to estimate the effects from a large number of major chemical industries. Hg contaminations of sediments pose threats to ecosystems and humans health not only due to the toxicity of inorganic Hg itself but also because of its potential to be converted to methyl mercury (MeHg). Methyl mercury is a more potent neurotoxin than inorganic forms of Hg[9] and symptoms of mercury (methyl mercury) poisoning include blindness, instantaneous neurological damages,[10] chromosome damages, paralysis, particularly irritability, insanity and birth defects. Well known example of acute mercury poisoning is the "Minamata disease" which causes loss of balance, mental disturbance, sight and hearing difficulty in swallowing, speech, and degeneration of brain.[11]

Soil, act as the most important resource of mercury, once it is released into the environment and can act as a mercury deposition record.[12,13] Mercury concentrations in soil ranged from 0.01 to 0.2 mg/kg, [14] but these concentrations are significantly higher in soils affected by Hg mining. Currently available technologies for the remediation of heavy metal contaminated soils relies heavily on 'dig-and-dump' or

replacing the affected soil, immobilization or extraction by physicochemical techniques would be costly and is often suitable only for small areas.[15,16] It has been estimated that it would cost 40,000 to 70,000 US$ to remove each pound of mercury from the environment,[17] hence there is an urgent need for the development of alternative remediation strategies. Consequently, the cost-effective technology, in-situ application of phytoremediation is a pleasing technique as it offers partial decontamination, site restoration, maintenance of the physical structure of soils and the biological activity and is potentially cheap, there is the possibility of bio-recovery of metals.[18, 19] Some of the unique properties of several plant species make them ideal for the remediation of contaminated soil and water.[20, 21, 22, 23] Numerous plant species have been identified for remediation of heavy metals. This research intended to evaluate the possibility of achieving soil cleanup using native plants widely grown in the local environment and the least studied metal with this native plant has been mercury. Native plants are superior at cleaning soil, because they are adapted to the climate, have deeper roots and require less maintenance. Most of the hyperaccumulators are native plants.[24]

The objectives of the present study were (i) To investigate Hg uptake and transport from soil to plant parts by studying distribution and accumulation of Hg in the roots and shoots of four native plants (ii) To study the effects of mercury on plants physiological and morphological changes.

EXPERIMENTAL

Experimental Setup

Plant seeds were collected from the botanical garden, Yogi Vemana University, Kadapa, dried and preserved in zip lock polythene covers. Pre-germinated seedlings were transferred into polythene (Plastic) pots containing approximately 5 kg of soil and allowed to stabilize for two months. All the experiment was carried out in the controlled greenhouse (N 14^o 19' 33.4'', E 078^o 46' 38.4'') condition to support the plant growth and survival at the initial phase of phytoremediation. The experimental plants were spiked with 10 ml of 5 mg/L $HgCl_2$ solution to each pot for alternative days whereas control plants were irrigated with normal tap water. Four replicates were maintained for control and treatment. The plants were carefully removed from soil 20, 40, 60 days after planting and analyzed the following parameters.

Biomass and Water Content

Control and mercury spiked plants were carefully removed from the soil and washed with distilled water. Below and above ground parts were separated, weighed and dried in an oven at 110^o C for 2 hours for measuring dry weight.[25] Dried plant samples were ground into a fine powder and stored in a polythene bags for mercury analysis. Each individual plant height was measured during the experiment. The relative water content (WC %) also calculated as per the formula[26]:

$$WC\ \% = (FW - DW)/\ FW \times 100 \quad (1)$$

Photosynthetic Pigment Analysis

Chlorophyll a, Chlorophyll b and β-carotenes content were determined according to the method by Lichtenthaler.[27] 1 g of fresh matured leaves were washed with deionized water, and homogenized in a pre-chilled mortar with 20 ml of 80% acetone solution. The homogenated solution was centrifuged at 10,000 rpm for 10 mins. The extraction was repeated until the residue become colorless. The final volume was made up to 100 ml with 80% acetone. The absorbance of the extract was measured at 470, 645 and 663 nm respectively.

Proline Content Assay

0.5g of fresh leaves was homogenized in 10 ml of 3% w/v sulfosalicyclic acid and filtered through Whatman's No.1 filter paper. Then 2 ml of filtrate was mixed with 2 ml of acid-ninhydrin, 2 ml of glacial acetic acid and the mixture was heated at 100^{o}C for 1 hr in a water bath, and the reaction was stopped in an ice bath. The reaction mixture was extracted with 4 ml toluene and the chromophore-containing

toluene was aspirated after which the absorbance was recorded at 520 nm. Proline concentration was calculated using a calibration curve and expressed as µmol proline g^{-1} FW.[28]

Malondialdehyde Assay

Lipid peroxidation was estimated by measuring MDA content. 1 g of fresh leaves had been homogenized with the mixture of 10 ml of 0.25 % thiobarbituric acid (TBA) in 10 % trichloroacetic acid (TCA). The mixture was heated at 95° C for 30 min and then quickly cooled in an ice bath. Then it was centrifuged at 10,000 rpm for 10 min and the absorbance was measured at 532 nm, for correction of non-specific turbidity was also measured at 600 nm. The MDA content was calculated according to its extinction coefficient of 155 $mM^{-1}$and expressed as µmol g^{-1}FW.[29]

Sample Preparation and Mercury Analysis

Soil samples (0.5 g) were treated with 12 ml of Aqua regia (HNO_3**:** HCl, 1:3) solution. The mixtures were heated at a low temperature initially for 1 hour, and then 20 ml of 2 % HNO_3 was added. Then the mixture was digested at high temperature for 30 min. The sample was diluted with 25 ml of 2 % HNO_3 and filtered with Whatman No.42 filter paper. The filtrate was analyzed by ICP-OES.[30]

Dried plant samples (0.5 g) were digested with 5ml of diacid (HNO_3**:**$HClO_4$). The mixture was heated at a low temperature for 1 hour, and then 3 ml of diacid was added and heated at high temperature until the mixture remains 2 ml. The sample was diluted with 50 ml of 2% HNO_3 and filtered with Whatman No.42 filter paper. The filtrate was analyzed by ICP-OES.[31]

Bio-Concentration Factor

The ability of plant to accumulate metals from available soil source can be estimated by BCF (Bio-concentration factor), were defined as the ratio of metal concentration in roots to that in soil.[32]

$$BCF = [metal]\ root / [metal]\ soil \quad (2)$$

Translocation Factor

Translocation factor: The ability of plant to accumulate metals from the roots to the aerial parts of the plants measured using the TF (Translocation factor), were defined as the ratio of metal concentration in the shoots to the roots.[32]

$$TF = [metal]\ shoots / [metal]\ roots \quad (3)$$

RESULTS AND DISCUSSION

Soil pH ranges from 8.98 ± 0.06 to 9.7 ± 0.02. Soil pH plays a predominant role in the accumulation of heavy metals. The increase in pH with an increase in treatment period was attributed to the 'alkalizing' effect of soils.[25]The Electrical conductivity at initial was found to be low at 20th day followed by a 40th day and drastically increased with the increase of time period 60th day; this may be because of the increased concentrations of mercury salts ($HgCl_2$) in the soil. Similar results have been reported in uranium mine tailing remediation by native plant species.[25] The mobility, bioavailability, ecological and toxicological effects of mercury are strongly dependent on its chemical speciation.[33]The organic matter, Phosphate and nitrate content were also analyzed which support the plant growth and helps in mobility of metals.

Effect of Hg on Physiological Parameters

The physiological responses of the plants like shoot growth rate, biomass (fresh and dry weight) were recorded during the experiment Table-1. *Tridax procumbens* and *Dodonaea viscosa* showed a decrease in shoot length where as *Ruellia tuberosa* and *Azadirachta indica* showed a minor difference than that of the control plant at 60th day. Similarly, the reduction of biomass due to the impact of Hg was also recorded Table-2, when the plants are exposed to Hg results in a significant reduction of fresh and dry biomass.

The observed reduction in the shoot growth rate, biomass and morphological symptoms may be the culmination of the toxic impact of mercury on the plant metabolism and mineral uptake. Several studies demonstrated that heavy metals can function as stressor, causing some physiological constraints that decreases the plant vigor and inhibit plant growth.[34-36]

Table-1: Effect of Hg on Shoot Growth Rate (cms)

Plant	Control	20th day	Control	40thday	Control	60th day
Tridax procumbens	62.15 ± 2.75	57.8 ± 1.4	75.2 ± 2.8	62.05 ± 6.85	77.05 ± 2.45	67.8 ± 5.3
Ruellia tuberosa	33.5 ± 3.3	27.35 ± 1.7	38.62 ± 5.12	34.4 ± 2.5	55.85 ± 0.45	54.25 ± 2.15
Dodonaea viscosa	33.75 ±13.45	31.15 ± 2.65	46.8 ± 7.4	43.05 ± 2.65	57.8 ± 7.5	51.95 ±2.65
Azadirachta indica	47.60 ± 2.04	46.42 ± 1.78	53.72 ± 1.6	51.17 ± 1.81	63.65 ± 0.85	60.2 ± 7

Table-2: Effect of Hg on Plant Biomass

Plant		Fresh weight (grams)			Dry weight (grams)		
		Root	Stem	Leaf	Root	Stem	Leaf
Tridax procumbens	Control	26.32± 2.12	33.2 ± 3.06	42.59 ± 3.31	5.34 ± 0.90	8.55 ± 1.31	6.42 ±1.18
	20	12.18 ±5.67	21.26 ± 0.04	32.11 ± 4.97	2.02 ± 0.47	4.16 ± 0.35	3.48 ± 1.4
	40	17.4 ± 2.2	33.09 ± 0.87	48.78 ± 4.15	2.31 ± 0.18	8.23 ± 0.09	8.85 ± 0.22
	60	25.97 ± 0.31	32.59 ± 2.70	42.52 ± 5.91	3.38 ± 0.08	7.65 ± 0.125	6.03 ± 0.73
Ruellia tuberosa	Control	28.37 ± 2.53	10.59 ± 2.33	15.67 ± 2.28	6.65 ± 0.49	2.64 ± 0.47	2.65 ± 0.93
	20	13.81 ± 2.75	6.21 ± 0.22	12.47 ± 0.78	5.68 ± 0.52	1.26 ± 0.29	1.96 ± 0.25
	40	15.75 ± 2.69	6.66 ± 0.15	13.61 ± 1.54	3.5 ± 0.27	1.42 ± 0.04	2.28 ± 0.27
	60	26.37 ± 2.53	11.20 ± 1.89	17.93 ± 2.28	6.01 ± 1.15	2.9 ± 0.53	3.55 ± 0.93
Dodonaea viscosa	Control	7.97 ± 0.093	11.27 ± 1.21	23.73 ± 0.19	2.85 ± 0.31	5.89 ± 0.75	2.63 ± 0.53
	20	2.48 ± 0.72	2.53 ± 0.05	9.21 ± 0.96	0.97 ± 0.07	0.67 ± 0.02	1.02 ± 0.23
	40	5.28 ± 0.34	5.06 ± 0.1	34.14 ± 0.98	1.36 ± 0.16	1.3 ± 0.06	4.7 ± 0.16
	60	7.51 ± 0.15	9.14 ± 0.45	21.51 ± 3.07	2.54 ± 0.12	3.83 ± 0.41	1.28 ± 0.7
Azadirachta indica	Control	42.98 ± 3	25.12 ± 1.3	26.37 ± 3.48	18.34 ± 1.5	9.07 ± 0.31	5.04 ± 0.57
	20	15.39 ± 0.86	6.30 ± 1.89	11.02 ± 2.09	3.45 ± 1.13	2.03 ± 0.73	2.32 ± 0.52
	40	20.2 ± 1.54	12.69 ± 2.15	17.27 ± 2.41	7.57 ± 1.40	5.65 ± 0.99	4.05 ± 0.07
	60	33.88 ± 4.92	23.57 ± 1.29	26.46 ± 1.66	15.48 ± 1.36	7.49 ± 0.63	5 ± 0.02

Effect of Hg on Photosynthetic Pigments

The effects of Hg concentrations on chlorophyll a, b and carotenoids pigments were estimated. Mercury concentration in soil significantly reduced the total chlorophyll, chlorophyll a, chlorophyll b and carotenoids content. The effect mercury on photosynthetic pigments with increasing days of growth was shown in Table-3. The observed results showed that a significant decrease in Chl.a, Chl.b and carotenoids were found in *Tridax procumbens (*58.92%, 65.85% and 40% respectively), *Ruellia tuberosa (*74.24 %, 87.23% and 63.75% respectively), *Dodonaea viscosa (97.05 %*, 22.16% and 56.95% respectively) and *Azadirachta indica* (84.02 %, Chl. b 91% and 85.79% respectively) with increasing days of mercury exposure. The heavy metal ions showing toxicity in plants[37] were observed through leaves.[38] This result is considered due to the fact that mercuric chloride served as a strong inhibitor of chlorophyll accumulation. Likewise, chlorophyll content of Pistacia lentiscus and Tamarix gallica grew in hydroponic culture was observed with increasing mercury concentration.[39] Reduction of chlorophyll synthesis caused by mercury

stress due to decreased uptake of photosynthetic elements (Mn & Fe) and damage of the photosynthetic apparatus .[40]

Proline

The plant exposed to heavy metals seems to induce accumulation of free proline.[41, 42] Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles.[43] The data regarding proline content was increased in all plants up to 40 days and then decreased at 60th day of contamination (Fig.-1). Highest proline content was recorded in *Dodonaea viscosa* (7.*63*µ moles/g) followed by Azadirachta *indica* (7.38µ moles/g), *Tridax procumbens* (6.16 µ moles/g) and lowest in *Ruellia tuberosa* (5.51µ moles/g). Varun[44] have been reported the increased proline accumulation in *Abutilon indicum* is due to increased Cd concentration in soil. In the present study, proline content was increased due to mercury stress. It shows that proline content increases the tolerance of plants through biochemical mechanisms such as osmoregulation, protection of enzyme denaturation and synthesis of protein to mercury stress.

Table-3: Effect of Hg on Biochemical Responses of Plants

Plant		Chlorophyll a	Chlorophyll b	Chlorophyll total	Carotenoids
Tridax procumbens	Control	1.3 ± 0.02	0.59 ± 0.036	1.9 ± 0.05	5.21 ± 0.12
	20	0.61 ± 0.03	0.25 ± 0.019	0.87 ± 0.04	2.60 ± 0.12
	Control	1.11 ± 0.09	0.69 ± 0.11	1.8 ± 0.123	4.45 ± 0.22
	40	0.71 ± 0.028	0.44 ± 0.049	1.15 ± 0.039	3.23 ± 0.06
	Control	1.12 ± 0.08	0.41 ± 0.01	1.15 ± 0.07	3.19 ± 0.08
	60	0.66 ± 0.009	0.27 ± 0.019	0.93 ± 0.02	3.19 ± 0.08
Ruellia tuberosa	Control	2.06 ± 0.017	0.54±0.001	2.65±0.01	7.89 ± 0.041
	20	1.61±0.008	0.49 ± 0.05	2.15±0.01	6.18±0.023
	Control	1.22 ± 0.04	0.49 ± 0.05	1.71±0.001	4.76 ± 0.050
	40	1.10 ± 0.01	0.38 ± 0.042	1.48±0.056	4.18 ± 0.055
	Control	1.32 ± .009	0.47 ± 0.022	1.79 ± 0.025	4.58 ± 0.172
	60	0.98 ± 0.03	0.41 ± 0.044	1.40 ± 0.010	2.92 ± 0.038
Dodonaea viscosa	Control	1.47 ± 0.115	1.1 ± 0.3	2.58 ± 0.07	4.45 ± 0.038
	20	0.87 ± 0.009	0.75 ± 0.003	1.62 ± 0.07	2.99 ± 0.038
	Control	1.54 ± 0.041	0.48 ± 0.019	2 ± 0.033	4.78 ± 0.04
	40	1.35 ± 0.028	0.466 ± 0.14	1.84± 0.031	4.70 ± 0.059
	Control	1.36 ± 0.04	1.66 ± 0.011	3.03 ± 0.04	7.69 ± 0.36
	60	1.32 ± 0.02	0.398 ± .003	1.72 ± 0.022	4.38 ± 0.09
Azadirachta indica	Control	1.89 ± 0.16	1.04 ± 0.06	2.92 ± 0.09	9.34 ± 0.06
	20	1.56 ± 0.22	0.95 ± 0.067	2.51 ± 0.16	8.72 ± 0.09
	Control	1.71 ± 0.01	0.775 ± 0.02	2.48 ± 0.01	8.04 ± 0.12
	40	1.54 ± 0.08	0.72 ± 0.009	2.26 ± 0.06	6.55 ± 0.84
	Control	1.44 ± 0.109	0.94 ± 0.05	2.38 ± 0.07	7.32 ± 0.19
	60	1.21 ± 0.13	0.86 ± 0.057	2.08 ± 0.06	6.28 ± 0.30

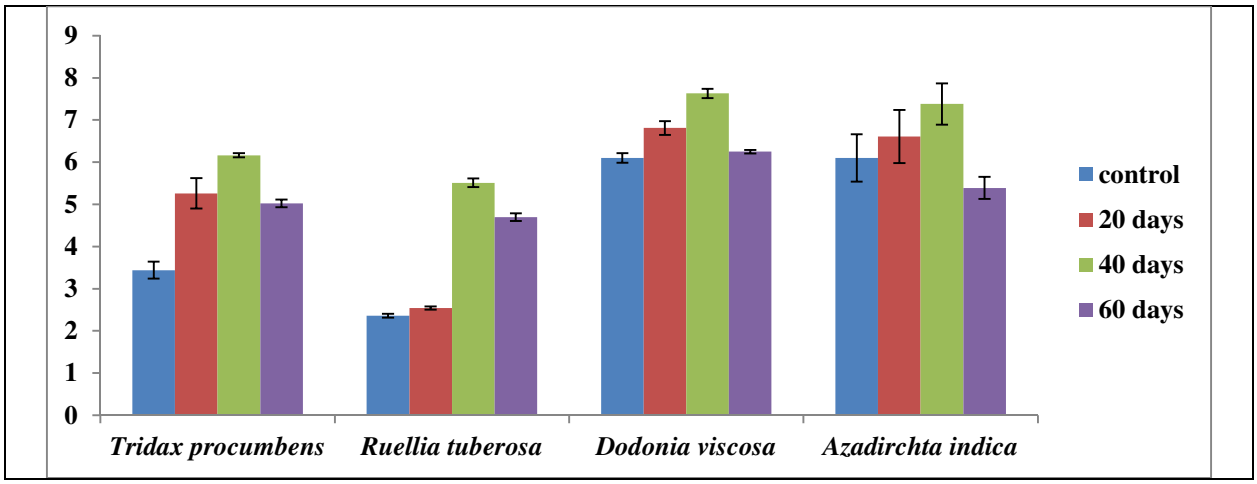


Fig.- 1: Effect of Hg on Proline Content (µ moles/g)

Malondialdehyde

Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation. It is also an indicator of free radical production and consequent tissue damage.[45] Oxidative stress due to the existence of the toxic metals can be demonstrated by MDA content. As proline, MDA content was also increased up to the 40th day of contamination and then decreased at 60th day (Figure-2). Highest MDA content was recorded in *Tridax procumbens* as 3.09 µmoles/g and lowest in *Dodonaea viscosa as* 2.43 µmoles/g. *Ruellia tuberosa* showed 2.88 µmoles/g and *Azadirachta indica recorded* 2.49 µmoles/g to MDA content. These results are similar to the work studied by Amira[26] in phytoremediation of Pb & Cd by native tree species.

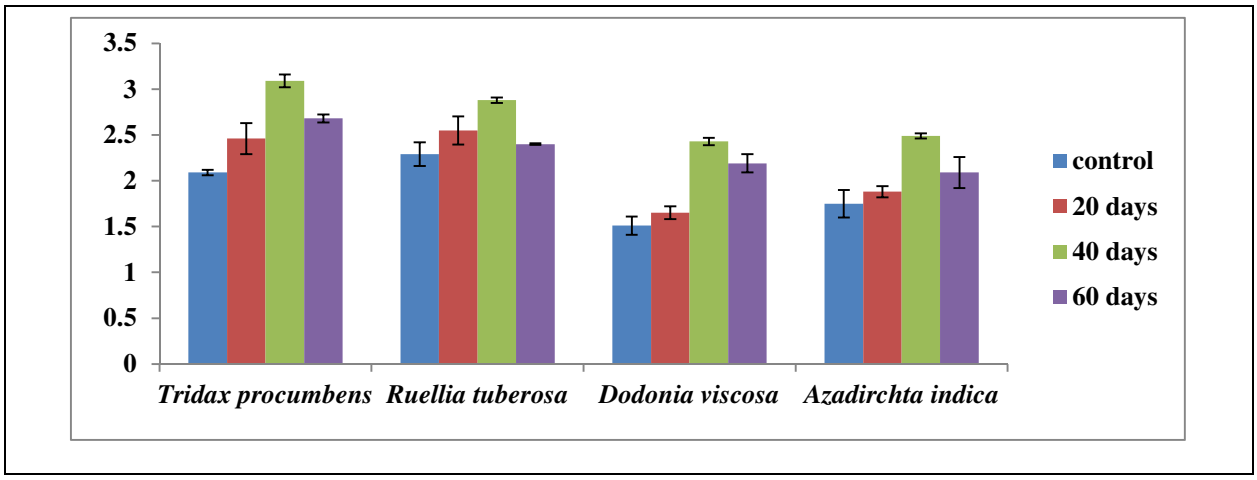
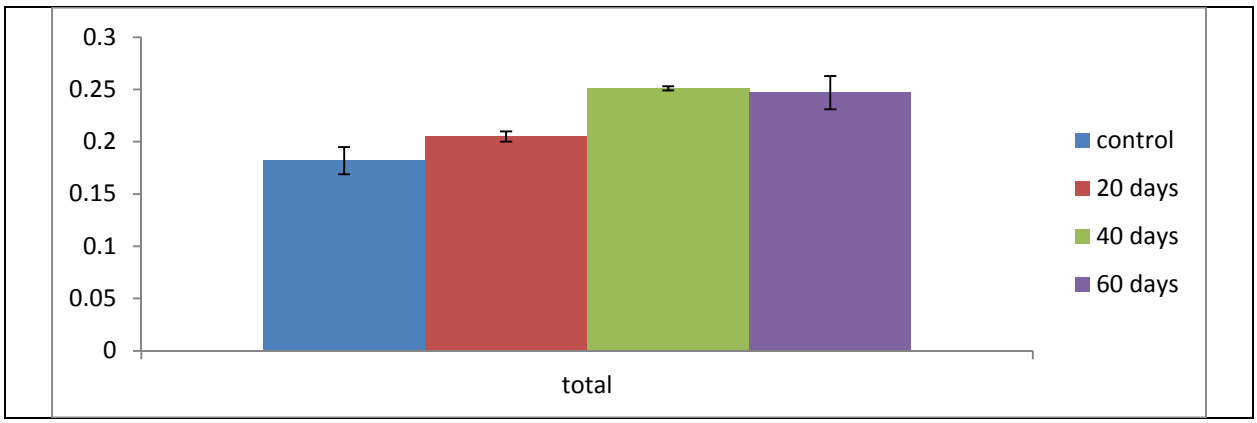


Fig.-2: Effect of Hg on MDA Content (µ moles/g)

Mercury Concentration in Native Plants

Four native plants species were chosen to evaluate the mercury accumulation levels with respect to their shoot and root shown in Figure-3 to Figure-6. In all plants, the root and shoot concentration of mercury was higher in after 60 days of exposure. Comparatively, *Tridax procumbens* accumulates more concentration of mercury in roots (0.190 mg/kg) rather than translocating to aerial parts (0.072 mg/kg) of the plant whereas, *Ruellia tuberosa* showed enhanced translocation of mercury in leaves (0.107 mg/Kg). But no difference in root (0.053 mg/kg) and shoot (0.066 mg/kg) concentration of mercury was found in *Azadirachta indica*, and *Dodonaea viscosa* shows good accumulation in roots (0.113 mg/kg) than shoots (0.071 mg/kg). Similarly, Hg and MeHg were found to concentrate in the *Eichhornia crassipes* with little translocation to the shoots or leaves of the plant.[46] There are several other studies that also show that

plant roots accumulate Hg when they were exposed to Hg-contaminated soils.[47] The uptake of contaminants from the soil by plants occurs primarily through the root system in which the principle mechanisms of preventing contaminant toxicity are found. The root system serves an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth, but also absorbs other non-essential contaminants,[48] because there is a tendency to form a heavy metal complex with inorganic compounds found in the body of organisms.[49] Most plants behave as excluders for Hg, sorting the metal mainly in the root,[50] with the root acting as a barrier to avoid that heavy metal reaching the aerial parts of the plant.[51] Mercury concentration in different wheat tissues was highest in roots, followed by leaves, stalks, shells, and grains.[52]



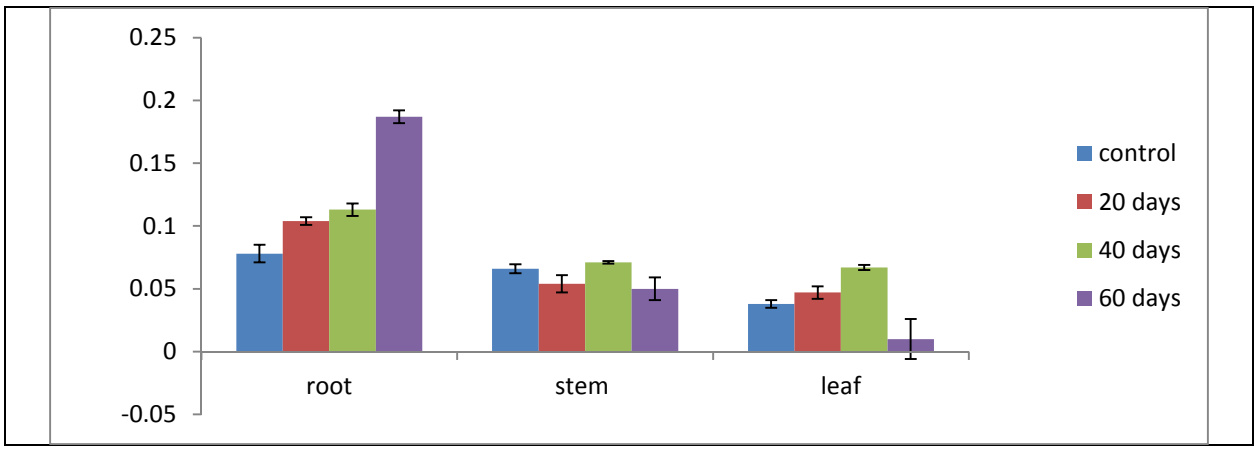
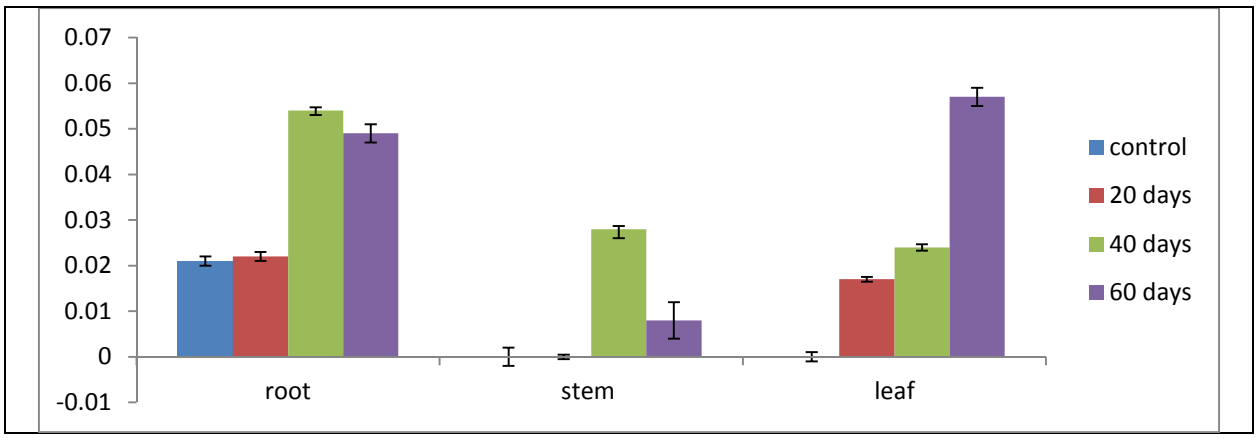


Fig.- 3: *Tridax procumbens*



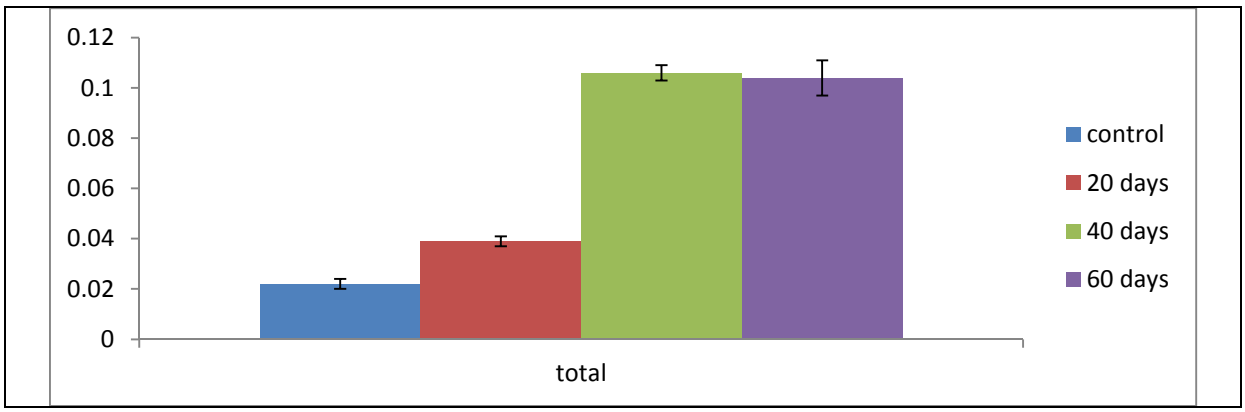
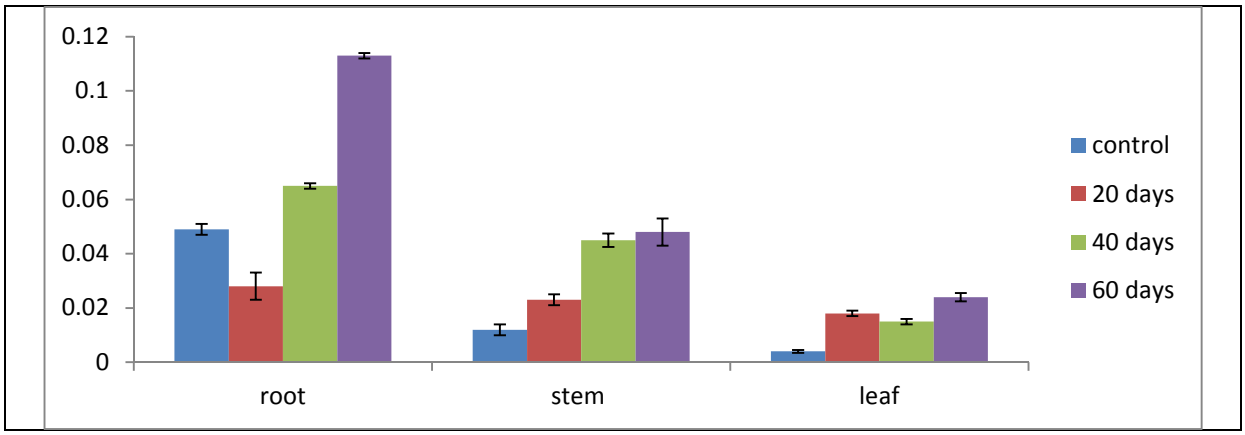


Fig.4- *Ruellia tuberose*



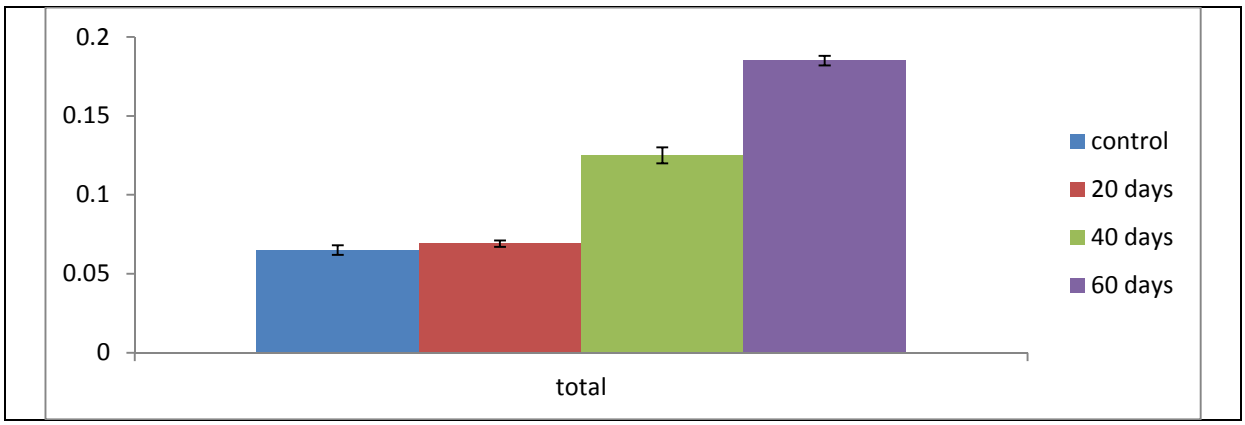


Fig.-5: *Dodonaea viscosa*

Bio Accumulation Index

Results of the present study showed that the values of BCF were <1, which indicates poor translocation of Hg from soil to root in all plant species, although Hg was available to the plants. *Tridax procumbens* showed TF values >1 at earlier days of contamination of 20 & 40th day, whereas TF value <1 at 60th day of contamination (Table 4). As the days of contamination increased, the TF values also increased in *Ruellia tuberosa,* at 60th-day TF value reached to 1.0. *Dodonaea viscosa* showed <1 TF values, and *Azadirachta indica* showed TF values >1 at 20th and 60th day, whereas at the 40th day the TF value was close to 1. TF values >1 indicate the effectiveness of moving the metal elements from the plant roots to the shoots.[53, 54] By the BCF and TF, we can compare the ability of different plants in the uptake of metals

from soils and translocate them to the shoots. This movement depends on plant species, type of metal as well as environmental conditions such as pH, salinity, organic matter, and nutrients respectively.[55, 56] Tolerant plants tend to restrict soil - root and root - shoot transfers, and therefore have much less accumulation in their biomass. Plants exhibiting TF and particularly BCF values less than one are unsuitable for phytoextraction.[57] In the case of *Tridax procumbens* and *Dodonaea viscosa* TF values were >1 and or close to 1 at 20th& 40th day, which indicates Hg is highly transferable during the earlier days of contamination. Zornoza[58] have also found similar results and stated that one of the possible reason to explain the highest Hg translocation occurred during the second month of exposure is that the roots explore a higher volume of soil during this period, promoting better Hg uptake, during later months, the volume of the soil in the container becomes a limiting factor.

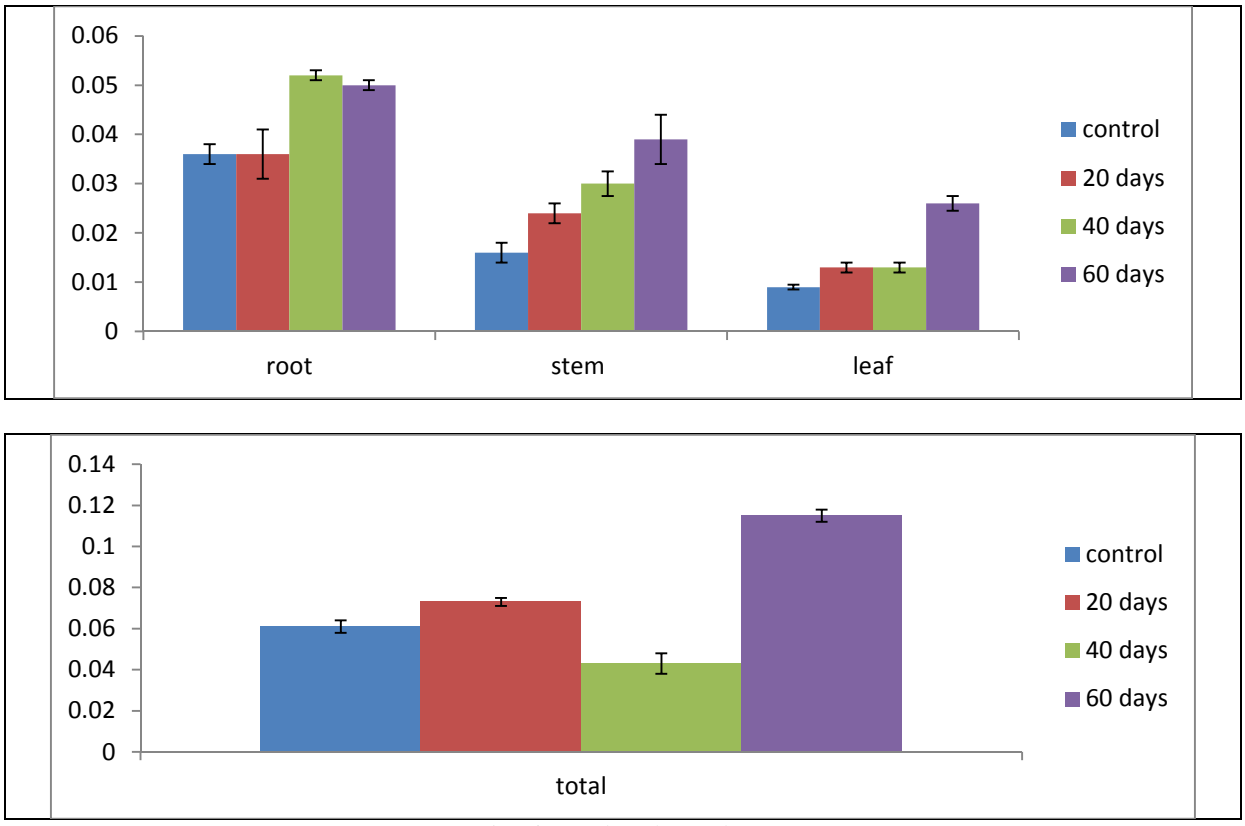


Fig.-6: *Azadirachta indica*

Table-4: Bio Concentration (BCF) and Translocation (TF) Factors

Plant	BCF	TF
Tridax procumbens	3.73	1.37
20th day	0.163	1.09
40th day	0.143	1.3
60th day	0.228	0.37
Ruellia tuberose	1	0.25
20th day	0.034	0.765
40th day	0.074	0.925
60th day	0.073	1.190
Dodonaea viscose	2.47	0.336
20th day	0.050	1.48
40th day	0.124	0.89
60th day	0.154	0.62

Azadirachta indica	1.78	0.68
20^{th} day	0.053	1.12
40^{th} day	0.083	0.98
60^{th} day	0.066	1.31

CONCLUSION

The data obtained from present experiments have shown the possibility of applying native plants namely *Tridax procumbens, Ruellia tuberosa, Dodonaea viscosa* and *Azadirachta indica* in phytoremediation for accumulation potential of Hg from soil to plants parts. The high concentration of Hg was stored in roots instead of being translocated into the shoots at 60^{th} day of contamination in all plants, except *Ruellia tuberosa,* which showed high accumulation at 40^{th} day of contamination. The results also suggested that there was a significant growth inhibition, decrease of biomass and photosynthetic pigments. Proline and MDA content showed positive responses to cope with the Hg-induced stress. Proline accumulation accepted as an indicator of environmental stress and maintaining of the high level of MDA content is one of the important anti-oxidative responses of plants to mitigate the increased oxidative stress.

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