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Uranium and Manganese Sequestration by Selected Plants Raised in Uranium Mine Tailings

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***Abstract*— Uranium mine tailings are found to contain toxic metal contaminants like Uranium and Manganese are in elevated concentrations. As a part on phytoremediation study, four plant species viz., *Sacchurum spontaneum*, *Typha latifolia*, and *Cyprus compressus*, screened based on their potential for phytoremediation, were tested under control conditions for sequestration of trace metals and radionuclides from tailings of uranium mines. Intensive pot culture experiments with these four plant species confirmed the earlier findings of significant metal accumulation character which is useful in developing site specific phytoremediation applications. The study revealed that while *Sacchurum spontaneum* is found to be more suitable for U sequestration, *Pteris vittata* is suited the most for Mn as well as U.**

Keywords*— Bioremediation; Nuclear wastes; Phytoremediation; Radionuclide; Tailing pond; Trace metals; Uranium

I. INTRODUCTION

Almost the entire mined ore in uranium mining comes out as waste after recovery [1]. The uranium ore is processed at a mill and converted to Sodium diuranate -yellowcake through acid leaching, which is 80% rich in uranium oxide U_3O_8. This is further processed to make fuel rods for nuclear reactors on enrichment. In the process of mining to milling, leaching and final production of yellow cake, it generates large quantity of hazardous waste called Uranium tailings[15] that are disposed of in vast area of land called tailing pond adjoining the mill[13].In underground mines, wastes generated from the mine are used as the filling material for the void created by excavation of ore. Whereas, the coarser fraction is used for filling, the finer fraction is disposed off in tailings ponds. Though major portion of uranium present in the ore is extracted, a fraction though quantitatively small, remains unextracted and is finally discharged with the tailings. The supernatant water, after decantation and treatment in the Effluent Treatment Plant(ETP),is released into local stream. The release of radionuclides and heavy metals from Uranium mine beneficiation, waste sites and their subsequent mobility in the environment is a subject of intense public concern[10]. Release of metals without proper treatment into soil, water and air systems possess a significant threat to public health and ecosystem because of its persistence, biomagnification and accumulation in food chain. Natural radionuclides and trace metals are present in soil in varying concentrations, some of these are found in elevated concentrations in uranium waste tailings[1].No level of radiation exposure above background radiation can be deemed 'safe'[12] because of the presence of half-life of various radionuclides ranging from 14 billion years to 3.1minutes. Heavy metals and their precursors in such tailings constitute a long term hazard[5]. Vandenhove studied feasibility of phytoextraction to clean up low-level uranium-contaminated soil[18].Earlier work of the authors [14]evaluated the quantity of uranium (U) and other selected trace metals (Sr, Cs, Cr and Hg),their mobilization in water and soil,level of metal accumulation in plant species collected from the tailing pond sites of uranium mines and rate of metal transfer from soil to plant species (Transfer factor) which confirmed the results of earlier workers[2].The study[14] revealed higher concentration of eight elements (Al, U, Mn, V, Fe, Ni, Cu and Zn) in tailing soils. Vanadium (101 ppm), U(69 ppm) and Mn(5466 ppm) were found to be the predominant contaminants, whereas, the threshold limits of these elements are 10,3and 510 ppm respectively. The study screened 26 plant species found abundantly in experimental areas for their potential abilities to accumulate and remediate the contaminated soils. Among the 26 species, *Sacchurum spontaneum*, *Pteris vittata*, *Cyprus compressus*, *Lantana camara Typha latifolia*, *Ricinus communis*, *Alstonia scholaris*, *Buchanania lanzan* and *Cassia alata* were found to be playing active role in accumulation of radionuclides and trace metals. Further evaluation and considering various other factors of plant suitability for phytoremediation, four plant species viz;*Sacchurum spontaneum*(Al =54 ppm, Mn= 31 ppm,U= 8ppm, Cr =16ppm), *Typha latifolia*(Cr =2ppm,U =3ppm, Mn= 68 ppm, Sr= 2 ppm, Pb =3 ppm), *Pteris vittata*(Mn= 211 ppm, Pb= 4ppm, U= 4ppm) and *Cyprus compressus(*U= 2ppm, Mn =76 ppm) are found to hold good potential for phytoremediation of chief contaminants like trace metals(Mn) and radionuclides(Uranium).In order to verify the results of this earlier field evaluation, the present study was taken up under controlled conditions with the objectives of, (i) confirming and comparing the metal accumulation potential of the selected plants , (ii) to evaluate the response of the plants to various levels of concentration of the contaminant in the medium and (iii) to find out optimum time of sequestration of metals by the selected plants for developing phytoremediation solutions for the contaminants. Results pertaining only to the chief contaminants of Uranium(U) and Manganese (Mn) are discussed here.

II. MATERIALS AND METHODS

A. *Selection of plant species*

Based on the results of the earlier study and taking into consideration the following parameters viz., (i)Accumulator of metals and radionuclide, (ii) Perennial, (iii) Shallow rooted, (iv)Easy to adapt, grow and harvest, (v) Non-browsable, four plant species were selected for pot culture experiments.

1. *Typha latifolia*
2. *Sacchurum spontaneum*
3. *Pteris vittata*
4. *Cyprus compressus*

B. *Collection of soil media*

Tailing soil was collected from the active tailing pond at UCIL Uranium mines, Jaduguda, Jharkhand State, India. Garden soil was collected locally from the experimental site at Hyderabad.

C. *Experimental design*

The experiment was laid out in completely randomized design with following three treatments.

T1= control standard garden soil(no tailings)
T2= 50% tailing soil and 50% standard garden soil
T3= 100% tailing soil

Each treatment was replicated five times and each replication consisted of 12 seedlings, one each in 300 CC root trainers, uniformly watered and shade effect avoided.

D. *Preparation of sample solution*

1. **Soil:** Soil samples were crushed, mixed thoroughly and air-dried for 5 to 6 days.Then dried in hot air oven for 24 hours at 65^{o}C and finally ground in to fine powder to pass through 2 mm sieve.

2. **Plant:** Harvested samples are cleaned with tap water to remove any contaminates(on site). The root and shoot parts of individual plant species were separated, weighed (fresh weight) and placed in separate paper bags[16]. In laboratory the samples were cleaned again with distilled water, air dried and kept in hot air oven at 65º C for 2 days, and dry weight were taken. After taking dry weight, the samples were ground into fine powder to pass through a 2 mm sieve with Wiley mill followed by coffee grinder (Kenstar mixer grinder MG 0411),which was carefully cleaned between samples [11]. Metal analysis was carried out at the beginning of the experiment and thereafter at 30 days interval as per schedule given below.

D0–Metal analysis prior to experiment
D1–Metal analysis after 30 days
D2–Metal analysis after 60 days
D3–Metal analysis after 90 days
D4–Metal analysis after 120 days

E. *Laboratory analysis*

All soil metals were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICPMS). Approximately 0.5 g of the plant samples were weighed accurately and transferred to a Teflon container. 5 ml of 65% HNO_3 and 1 ml 30% H_2O_2 added. After microwave/hotplate digestion cycle, digested samples were made up to 25 ml with de ionized water (Yasemin *et al.,* 2007).

F. *Statistical analysis*

All statistical analyses were performed using software SPSS, version 17.0. Multivariate general linear model was used to test mean difference among plant species and among media for accumulation of U and Mn in plant parts. Where statistically significant difference was found among means, Fisher's LSD post hoc test was done to determine the best treatment. Paired T-test was used to find out statistical significance of mean difference between initial (beginning of experiment) and final concentrations (end of experiment) of metals in soil as well as in plant parts.

III. RESULTS AND DISCUSSION

The results of analysis of variance for U and Mn uptake in plant parts at completion of experiment are presented in Table 1.

TABLE I. ANALYSIS OF VARIANCE (ANOVA) FOR U AND MN UPTAKE BY THE FOUR TEST PLANTS

	U				Mn			
Plant	***Mean***	***SD***	***SE***	***F***	***Mean***	***SD***	***SE***	***F***
Typha latifolia	2.211	1.651	0.426	4.952	27.032	16.558	4.275	39.42
Sacchurum spontaneum	4.775	3.575	0.923		25.309	13.738	3.547	
Pteris vittata	2.235	1.723	0.445		116.751	77.046	19.893	
Cyprus compressus	1.031	0.864	0.223		44.961	29.315	7.569	

Data represent the mean ± SD of 5 replicates. Metal accumulation mean values in ppm. *(parts per million or mg/Kg).* SD=Standard deviation, SE=Standard Error, Significance <0.01

The uptake of both U and Mn was found to be significantly different ($p<0.01$) among the four plant species. Fisher's least significant difference post hoc test revealed highest accumulation of U in *S.spontaneum* and lowest in *C.compressus.* There mean difference between *T. latifolia* and *P.vittata* was not statistically significant as far as U accumulation is concerned (Fig.1).For manganese, *P.vittata* was found to be the highest accumulator. However, the mean difference between *C.compressus* and *T. latifolia, Typha latifolia* and *Sacchurum spontaneum* were not statistically significant as far as Mn uptake is concerned (Fig. 2).These results are in conformity with the earlier reports of the authors{14] as well as other workers [6].The results of analysis of variance for U and Mn uptake from the three potting media are presented in Table 2. Statistically significant difference was found among the three potting media as far as

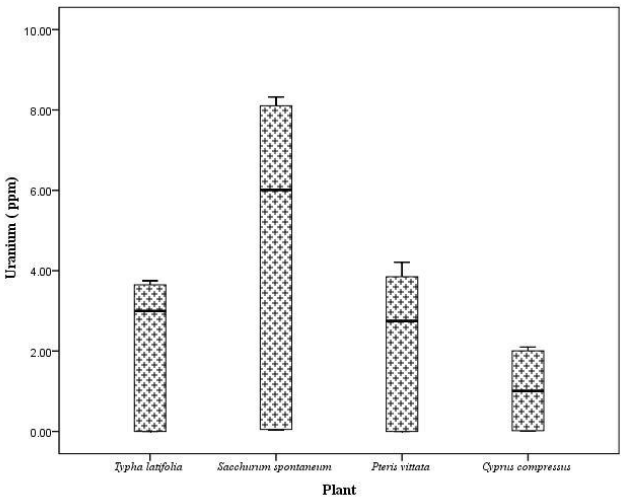

Fig. 1. Uranium accumulation by the four test plants grown in 50% tailing soil + 50% plain garden soil after 120 days. *(Error bars denote the standard error of the mean (n=5).*

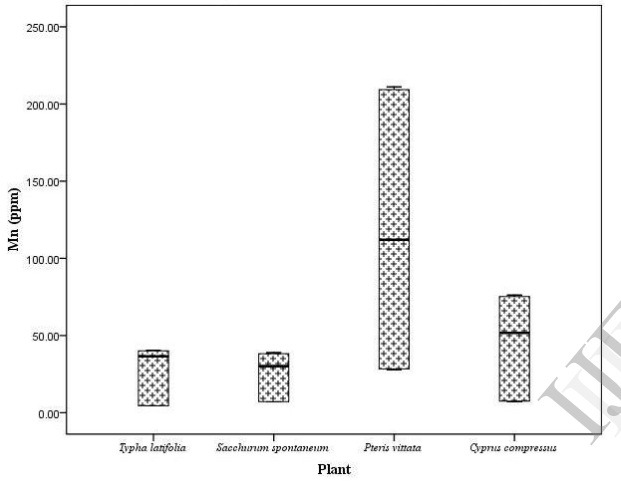

Fig. 2. Mn accumulation by the four test plants grown in 50% tailing soil + 50% plain garden soil after 120 days. *(Error bars denote the standard error of the mean (n=5).*

U and Mn uptake is concerned. Fisher's least significant difference post hoc test revealed highest accumulation of U and Mn from potting media T2 (50% tailing soil and 50% standard garden soil), followed by T3 (100% tailing soil) and T1 (control standard garden soil) (Fig. 3 and 4). The rise in U accumulation in mixed media could be due to other facilitating factors like organic matter, chelating agents or mycorrhiza. This is comparable to the earlier studies of Chen *et al.* [3] & [4],wherein highest accumulation of U was reported in mycorrhiza colonized *Pteris vittata* fronds (9.03 mg kg^{-1}) compared with non-mycorrhizal plants (6.85 mg kg^{-1}). Media amendments like citric acid proved effective, decreasing the soil pH to 5.0 and increasing U accumulation and U uptake by red beet by a factor of 14. [8]. Soil properties determine the tolerance and accumulation of U in plants[9]. Soil type strongly influences the effectiveness of U remediation of soils by plants. A study by Shahandeh and Hossner[17] suggests that plant performance was affected by U contamination rates, especially in calcareous soils.

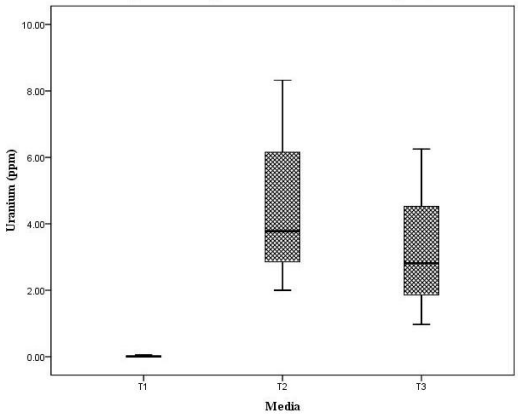

Fig. 3. Comparision of Uranium accumulation by the test plants in 3 different potting media T1 (control ;standard garden soil),T2 (50% tailing soil and 50% standard garden soil), and T3 (100% tailing soil) (after 120 days. *(Error bars denote the standard error of the mean (n=5).*

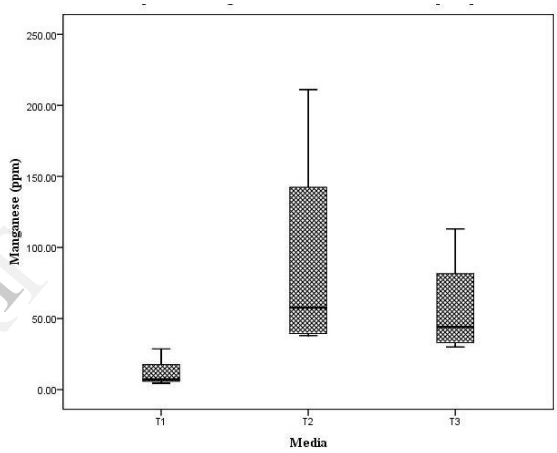

Fig. 4. Comparision of Mn accumulation by the test plants in 3 different potting media T1 (control ;standard garden soil),T2 (50% tailing soil and 50% standard garden soil), and T3 (100% tailing soil) after 120 days. *(Error bars denote the standard error of the mean (n=5).*

On the contrary the present study results suggest that 50% media (T2) supports highest rate of accumulation as compared to the media containing 100% tailings with highest contamination levels. This could be due to availability of more of organic matter and chelating agents[7] & [8]which facilitates metal absorption by plants in the T2 media with 50% tailings. The results of paired t-test for significance of difference between initial (at the beginning of experiment) and final (at the end of experiment) concentrations of U and Mn, both in plant parts and potting media are presented in Table 3. Significant difference was found between initial and final concentrations of U and Mn, in both plant parts and potting media. The pattern of U uptake in *S. spontaneum* and Mn uptake in *P.vittata* from the three potting media is depicted in Fig. 5 and Fig. 6, respectively. Four months seems to be the ideal time for harvesting of *S. spontaneum* and *P.vittata* for recovery of these metals.

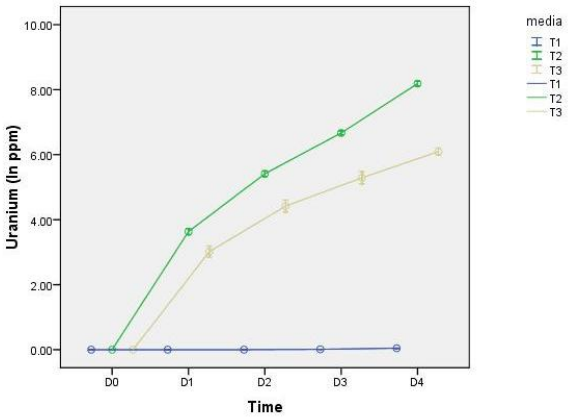

Fig. 5. Trend of U sequestration by *S. spontaneum* in 3 different potting media T1 (control ;standard garden soil),T2 (50% tailing soil and 50% standard garden soil), and T3 (100% tailing soil) (afterD0=initial,D1=30 days,D2=60days,D3=90days and D4= 120 days). Data represent the mean ± SD of 5 replicates.

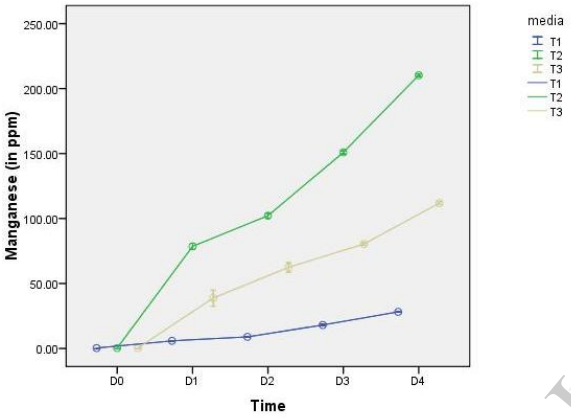

Fig. 6. Trend of Mn sequestration by *P.vittata* in 3 different potting media T1 (control ;standard garden soil),T2 (50% tailing soil and 50% standard garden soil), and T3 (100% tailing soil) (afterD0=initial,D1=30 days,D2=60days,D3=90days and D4= 120 days). Data represent the mean ± SD of 5 replicates.

IV. CONCLUSION

All the four plants accumulated very high quantities of U and Mn. *Sacchurum spontaneum* is best suited for U remediation, whereas *Pteris vittata* is the best accumulator of Mn in the soil mixed with 50% tailings .These two plants are best suited for phytoremediation as they accumulate significant levels of chief contaminants (U and Mn),easy to cultivate and are non-browsable. As a result, there is no danger of the toxic contaminants entering the food chain. The results offer additional information for the use of these plant species as vegetative cover over Uranium mine tailing dumps and for Uranium and Manganese phytoremediation. Finally, it can be concluded that with a proper mix of garden soil to the tailings the above mentioned two plants can be cultivated and their shoots can be harvested at an interval of 120 days and incinerated to effectively remove U and Mn from the contaminated soils/tailings. However, the package of cultural practices specific to the area of cultivation needs to be standardized.

TABLE II. ANALYSIS OF VARIANCE FOR U AND MN UPTAKE FROM THE THREE POTTING MEDIA

Potting media	U				Mn			
	Mean	*SD*	*SE*	*F*	*Mean*	*SD*	*SE*	*F*
T1	0.015	0.019	0.004	98.915	11.793	9.757	2.182	44.932
T2	4.48	2.322	0.519		91.149	72.185	16.141	
T3	3.194	1.881	0.421		57.599	33.138	7.409	

Data represent the mean ± SD of 5 replicates. Metal accumulation mean values in ppm. *(parts per million or mg/Kg).* in 3 different potting media T1 (control ;standard garden soil),T2 (50% tailing soil and 50% standard garden soil), and T3 (100% tailing soil) (after 120 days).SD=Standard deviation, SE=Standard Error, Significance <0.01

TABLE III. RESULTS OF PAIRED T-TEST FOR SIGNIFICANCE OF DIFFERENCE BETWEEN INITIAL AND FINAL (AT THE END OF EXPERIMENT AFTER 120 DAYS) CONCENTRATIONS OF U AND MN, BOTH IN PLANT PARTS AND POTTING MEDIA

Metal	Paired difference			t	Sig. (2tailed)
	Mean	*SD*	*SE*		
U in Plant	2.562	2.632	0.760	3.371**	0.006
Mn in Plant	49.964	59.148	17.074	2.926*	0.014
U in soil	4.098	6.271	1.810	2.263*	0.045
Mn in soil	88.361	134.708	38.887	2.272*	0.044

Data represent the mean ± SD of 5 replicates. Metal accumulation mean values in ppm. *(parts per million or mg/Kg).* in T2 (50% tailing soil and 50% standard garden soil) SD=Standard deviation, SE=Standard Error, * **t** values Significant at 95% and ** at 99% level of significance.

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