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Simulation Model for Feasibility Studies on Bioremediation of Uranium Mill Tailings using Hyper Accumulator *Chrysopogon zizanioides*

[1]Shanmugasundaram, H., [1]Sathesh Kumar Annamalai, [1]Kantha Deivi Arunachalam, [2]V.N. Jha, [2]N.K. Sethy, [3]K. Sivasubramaniam, [3]H. Krishnan and [4]M. Pandima Devi

[1]Center for Environmental Nuclear Research, Directorate of Research, SRM University, Kattankulathur, Chennai, Tamil Nadu, India- 603203, India
[2]Health Physics Unit, Bhabha Atomic Research Centre, Po-Jaduguda Mines, East Singhbhum - 832 102, Jharkhand, India
[3]Radiological Safety Division, Indira Gandhi Center for Atomic Research, Kalpakkam -603 102, Tamil Nadu, India
[4]Department of Biotechnology, School of Bioengineering, SRM University, Kattankulathur, Chennai, Tamil Nadu, India- 603203, India

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Corresponding Author:
Kantha Deivi Arunachalam
Center for Environmental Nuclear Research, Directorate of Research, SRM University, Kattankulathur, Chennai, Tamil Nadu, India- 603203, India
Tell: 044-27417144
Fax: 044-27417146
Email:kanthad.arunachalam@gmail.com

Abstract: Contamination of environment by uranium is expected in places where mining of uranium ore and processing, nuclear testing and reactor operations when the control measures are not effective. The daughter radionuclides of the ores, chemical additives and residual uranium are the maincomponents of processed waste from uranium industries. The bioremediation of contaminated areas using plant species or group of the plant may offer a cheap, renewable and promising approach to ensure long-term protection to the environment. In view of this, experiments were carried out to study the uranium immobilization potential of vetiver grass (*Chrysopogon zizanioides* (L.) Nash) under simulated conditions using the complex tailings. The vetiver plants were planted in simulation tanks at Health Physics Unit (HPU), Jaduguda, Jharkhand, India and periodic samplings were carried out to investigate the extent of uranium uptake. The acid aliquot of ashed plant samples, soil and tailings were subjected to solvent extraction followed by UV-Fluorimeter for estimation of uranium. The studies indicated that the plant species could bioremediate up to 49% of the uranium at 90th day of the experiment and the uranium remediation efficiency of vetiver increased with time and uranium was predominantly localized in the roots of the plant.

Keywords: Phytoremediation, Uranium, Jaduguda, Vetiver

Introduction

Contamination of uranium in soil is mainly due to the improper storage (Jones and Serne, 1995; Litaor, 1995) of fission by-products, consequential of nuclear testing reactor operations (Entry *et al*., 1997) and mill tailings of ore mining (Sheppard and Thibault, 1984; Johnson *et al*., 1980). The cost of remediation for radionuclides contamination through conventional technology in Northern America alone is considered to be in the surplus of $200-300 billion (Entry *et al*., 1996). The long-term restoration technology for the process of waste disposal sites of uranium industries includes remediation and reclamation techniques. The phytoremediation technology (using specific plant or plant groups) has been explored as a viable alternative as it offers a cheap, renewable and promising technique to minimize the long-term ecological impact of the waste disposal. Salt *et al*. (1995) has reported that soil contamination by the disposal of mining waste in the tailing ponds lack the permanent soilcover and the uncovered soil is prone to erosion and also the leaching causes environment pollution (Salt *et al*., 1995).

The ideal plant for the removal of radionuclide contaminants should exhibit a maximum efficiency of contaminants removal with rapid growth features, easy handling, low maintenance cost, with less waste disposal requirement (Dushenkov *et al*., 1995; Soudek *et al*., 2004). *Chrysopogon zizanioides* (vetiver) is a native plant of India adapted well to various agro-climatic conditions across the length and breadth of the country. Vetiver has a free adventitious root system enabling to hold soil very well and making it an ideal plant to prevent erosion by wind and water. The plant species is hardy and can survive up to ten years and does not become a weed since it is sessile and vegetatively propagated. During its growth over the years, the plant forms a subsoil mat with its root system and continues to spread laterally forming a plug over the area. The unique capability of this plant to accumulate and tolerate a spectrum of heavy metals ensures its use for phytoremediation. The initial screening demonstrated that vetivercould grow and survive well in the uranium mill tailing environment.

In India, the deepest uranium mining and exploration are carried out at the Jaduguda uranium mines in the state of Jharkhand (Basu *et al*., 2000; Koide, 2004). The Jaduguda mine has been the site of uranium mining and milling for more than four decades (Sethy *et al*., 2011), operating since 1967 providing the basic raw material required to cater the energy requirement of the country (Sarangi, 2003). The uranium mill tailing ponds at Jaduguda and Turamdih receive processed waste from ores mined at the six mine stations at Jharkhand namely Jaduguda, Bagjata, Bhatin and Turamdih, Narwapahar, Banduhurang. If the grade of ore processed is low then a bulk of the waste is anticipated (Laxman Singh *et al*., 2014).

Almost the entire ore mined from these mines comes out as waste after recovery (Lal and Soni, 2010). The tailing of Singhbhum region constitutes some acid-generating properties of pyrite of orestrata; contaminants may dissolve and migrate to the biosphere and hydrosphere. This radioactive element (U) and heavy metals like Mn, Pb, Cu, Fe and Ni present in varying concentrations of the mines waste are found at elevated levels in uranium waste mill tailing ponds (Mishra *et al*., 2009). If toxic heavy metals are mobilized into soil solution and are taken up by plants, then it can be used for decontamination of sites suspected to be affected by tailings dispersal. There are three mill tailing ponds in Jaduguda, Jharkhand namely tailing pond - I, II and III among which the tailing pond II has been filled to its capacity and covered with approximately 30 cm soil cover. The pond - III is currently being utilized by Uranium Corporation of India Ltd. (UCIL) to store the processed ore waste from all the six stations located in the Singhbhum region.

Pang *et al*. (2003) have reported that establishment of soil cover with plants is the most practical and economical method for restoration of the mine effluents and also concluded that vetiver grass is the suitable plant for re-vegetation of the metalliferous mine wastes (Pang *et al*., 2003). Jha *et al*. (2016) have reported that the dissolved uranium in the effluent may affect the biotic and abiotic components of the ecosystem (Jha *et al*., 2016). The plants grown in the mill tailings contains the uranium in the range of 0.02 to 29.03 mg kg^{-1} fresh weight. When the animals grace the plants from this region, it may cause both the radiological and chemical toxicity. To prevent the growth of other native plants and protect the animals from this region the plant *Chrysopogon zizanioides* has been chosen as a phytoremediator for restoration.

The present study is aimed to determine the uranium bioaccumulation using *Chrysopogon zizanioides* through simulation studies representing uranium mill tailing ponds of Jaduguda, whether vetiver plant can be utilized as a suitable phytoremediator for uranium mill tailing ponds. The soil for the simulation model studies was collected from the nearby localities from where the 30 cm soil cover for the uranium mill tailing ponds were taken to be filled to its capacity. The uranium mill tailings for the simulation experiments were obtained from the mill tailing pond - III. The parameters like uranium concentration, uranium transfer factor and uptake of uranium at different time periods by the vetiver plants were evaluated. The results observed from the simulation model studies it can be concluded whether vetiver can be used for phytoremediation of uranium in the field trail at the mill tailing ponds.

Materials and Methods

The present study was carried out to investigate the efficiency of vetiver to remediate uranium from the mill tailings pond by replicating the tailing ponds using simulation model. For this purpose, *Chrysopogon zizanioides* plants were planted in 500-liter tanks filled with 60 cm of soil as a control in five replicates. Another replicates of five tanks containing 30 cm of uranium mill tailings at the bottom topped up with 30 cm of soil to simulate uranium mill tailing ponds were set up as experimental tanks. The simulation set up as shown in Fig. 1 was carried out in Health Physic Unit (HPU), Bhabha Atomic Research Centre (BARC), Jaduguda, Jharkhand, India.

The simulation model contains two sets of tanks: (1) Control, (2) Tailings. The control set has replicates of five tanks filled with 60 cm of soil (160 kg). In the tailings set up has replicates of five tanks filled with 30 cm of tailings (110 kg) at the bottom topped up with 30 cm of soil (80 kg) as a soil cover to replicate the conditions present in the tailing ponds where the tailings are covered with 30 cm soil cover. 2.5 liters of tap water from the HPU was utilized to irrigate each tank manually on a daily basis using a watering can.

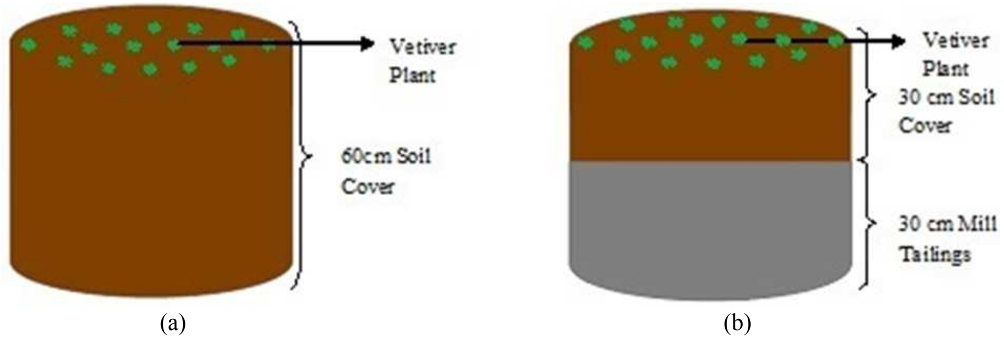

Fig. 1. Cross Section of Simulation tanks set up. (a) Refers to the cross section of Control Tanks, (b) refers to the cross section of Tailings Tanks

Sample Collection

The plant, soil and mill tailing samples were collected during different time intervals on the 0th, 15th, 30th, 60th and 90th day. The plants were rinsed with tap water to remove any soil particles and washed with distilled water, air dried and weighed. The roots and shoots were collected separately. The samples were treated with mild nitric acid to remove completely any soil or undesirable organic particles attached. The soil and tailings samples were collected on the 0th, 60th and 90th day in acid rinsed soil pouch (Mason, 1992). The soil and tailings samples were not collected on the 15th and 30th day as not much variation was expected during the initial period when the plant would be getting acclimatized to the experimental environment. The soil samples were collected from the top 30 cm especially from the root zone of the plant, while the tailings were collected from a depth of 30-60 cm using a soil punch (Mason, 1992).

Physicochemical Analysis

The water, soil and mill tailing samples during this study were analyzed for their physicochemical parameters to ascertain whether the physicochemical changes affect the uranium uptake by the plant as reported by (Shahandeh and Hossner, 2002).

Sample Preparation

The collected plant samples were oven dried until constant weight. The samples were ground for wet digestion. The tailings and soil samples were dried in a hot air oven (Sigma Scientific Instruments, Chennai Model: SSI 100 L) to remove any moisture present in the samples. The oven dried samples were then sieved through a standard sieve of 200 mesh (75 μm) since Gosh *et al*. (1969) reported that all minerals are liberated at 200 mesh fractions to remove pebbles and organic debris.

Uranium Estimation

The estimation of uranium in the plant, soil, mill tailing and water samples was carried out by UV-Fluorometer following the processing of the samples by Sethy *et al*. (2011. The emitted fluorescence intensity of 5,546A° that is unique for uranium was measured using a UV-Fluorimeter (Electronics Corporation of India Limited, Hyderabad Model: FL6224A) with an excitation wavelength 3,650A°. The intensity of fluorescence is directly proportional to the quantity of uranium present in the sample. The standard (1 μg mL^{-1}) and blank were processed simultaneously.

Quality Control

Thequality control was carried out simultaneously by analyzing the National Bureau of Standards (NBS or now NIST) certified pure U_3O_8 solution which was obtained from Sigma-Aldrich, the USA and Reference Materials from Natural Resource Canada-Canada Centre for Energy and Mineral Technology (CANMET), Till-1 and Till-3. Till refers to the soil based reference material with provisional concentration provided. The determination of uranium and the processing of the reference material were carried out following the same protocol as that of actual samples and included in Table 1. In each case, triplicate samples were analyzed and the results presented using descriptive statistics. The percentage of recovery obtained in the standard reference material was high which lead us to conclude that aforementioned protocol could be followed for uranium estimation.

BioConcentration Factor (BCF)

The bioconcentration factor for the terrestrial plants is the ratio of uranium concentration in the plant (expressed in μg/kg dry weight) to that of soil (mg/kg dry weight). The factors like physiochemical conditions of the environment, the chemical form of radionuclides and individual species characteristics govern the uncertainty in the bio-concentration factor. The methodology used to determine the bio-concentration factor is also an important limiting factor (NCRP, 1992).

Table 1. Quality control analysis of reference materials for uranium

Reference material	Unit	Certified concentration	Observed concentration	% Recovery
Till 1	µg/g	2.20	1.72±0.4	78.18
Till 3	µg/g	2.10	1.64±0.6	78.10
U_3O_8 Standard	mg/ml	1.00	0.95±0.04	95.00

Phytoremediation Efficiency

The phytoremediation process was characterized by determining the phytoremediation efficiency and capacity using the following Equation 1:

$$Phytoremediaiton\,Efficiency(\%) = \left(\frac{C0 - Cf}{C0}\right) \times 100 \quad (1)$$

where, *C*0 is the initial concentration of uranium in the tailings (mg/kg dry weight) and *Cf* the final concentration (mg/kg dry weight) of uranium in the tailings.

Statistical Analysis

The degree of variance was calculated using two-way ANOVA without replication to determine if there was any statistical difference between the samples.

Results

Determination of Uranium in Tap Water

The plants were regularly watered utilizing the tap water from Health Physics Unit, Jaduguda. The concentration of uranium in the tap water used for the experiments was analyzed and the uranium concentration range varied from 15.75±1 on the 0th day to 17.65±0.02 on the 90th day (µg/l) with the mean concentration of 16.7±0.51 (µg/l) as shown in Table 2.

The Texture and Particle size Analysis of Soil and Mill Tailing Samples

The texture and particle size of the soil and mill tailing samples collected on the 0th day and 90th day indicates that most of the particles present in soil and the tailings are above 150 microns and only 0.02% of the soil samples on the 0th day and 7.02% of the tailings samples are between 75 and 53 microns. From our analysis, as shown in Table 3 the soil samples are predominantly clayey with 92.8% on the 0th day and 82.01% on the 90th day. The tailing samples were shown to have a sandy texture with 64.8% on the 0th day and 65.2% on the 90th day.

Determination of Uranium in Soil and Mill Tailings

As shown in Table 4, on the 0th day the initial uranium concentration in the soil used for the study was 0.67±0.02 mg kg^{-1} (dry weight) while the uranium concentration the mill tailings of tailing pond-III was 9.34±2.26 mg kg^{-1} (dry weight). The concentration of uranium in the tap water used for irrigating the plants during the entire duration of the study was 16.7±0.51 µg L^{-1}. The pH of the tailings and soil was pH 6.77 and 7.04 respectively which was lower than the pH of the tap water used for watering the plants which were 7.73. The tailings contained 0.90% total organic carbon while the soil contained 1.35%. The electrical conductivity measured at 25°C was found to be 1,795 µmhos/cm for tailings and 972 µmhos/cm for soil and 435 µmhos/cm for the tap water. This might be due to the tailings being the byproduct of the ore processing industry and a culmination of various chemicals used during the ore processing the electrical conductivity was significantly higher than the soil and water of Jaduguda.

The uranium concentration in the soilon the 90th day of the simulation studies was 3.12±0.78 mg kg^{-1} (dry weight) as shown in Table 5, while the uranium concentration of the mill tailings from tailing experimental setuptank was 4.75±0.75 mg kg^{-1} (dry weight). The uranium concentration in the soil has also been influenced by the concentration of uranium present in the tap water used for irrigating the plants during the experimental period. The pH of the tailings and soil was pH 7.04 and 6.45 respectively. The tailings contained 1.01% total organic carbon while the soil contained 1.15%. The electrical conductivity measured at 25°C was 1,576 µmhos/cm for tailings and 652 µmhos/cm for soil. From the two-way analysis of variance test (Table 6), we can say with 95% confidence that there significant difference between the uranium concentration with respect to sampling days as $p = 1.5E\text{-}07 \leq 0.05$ ($F = 199.31 > 4.46 = F$ crit) and since the $p = 0.06 > 0.05$ ($F = 3.83 < 3.94 = F$ crit) we can say with 95% confidence that there is no significant difference between the uranium concentration with respect to the five replicates on each sample collection day.

Determination of Uranium in the Plants

The vetiver plant samples were taken for uranium analysis from both experimental mill tailing tanks and control tanks. Figure 2 shows the condition of the plants on the 90th day when compared to the 0th day. The concentration of the uranium in the plants was analyzed separately for roots and shoots of the plants. From Fig. 3 it was observed that the concentration of uranium increased from 2.3±0.79 µg kg^{-1} on the 15th day to 30±11 µg kg^{-1} on the 90th day in the root of the control set up. While in the shoot system; on the 15th day, the uranium concentration increased from

2.3±0.79 μg kg^{-1} to 20±10 μg kg^{-1} on the 90th day. The uranium concentrations of 16.7±0.5 μg L^{-1} in the tap water (Table 2) used for irrigation has influenced the uranium concentration in the soil (Table 5) thereby exposing the plants to take up additional uranium from the soil. It was observed that the roots from the tailings tanks set up have taken up to 80±10 μg kg^{-1} of uranium on the 90th day compared to the control tank roots which has taken up only 30±11 μg kg^{-1}. The concentration of uranium in the shoots system from the tailings tanks set up showed 40±4 μg kg^{-1} of uranium on the 90th day compared to the control tank showed which has taken up only 20±11 μg kg^{-1}.

Table 2. Uranium concentration in the tap water used for irrigation of vetiver plants in the simulation model studies

	Uranium Concentration (μg/l)		
Sample code	0th day	90th day	Mean ± SD
Tap water	15.75±1	17.65±0.02	16.7±0.51

Note: The uranium concentrations represented is Mean ± SD of triplicates

Table 3. Particle size and texture of soil and tailing samples

	Result (%)			
	Soil		Tailings	
Test parameters	0th Day	90th Day	0th Day	90th Day
Texture:				
Clay	92.80	82.01	33.70	34.20
Sand	7.07	17.50	64.80	65.20
Silt	0.20	0.60	0.60	0.70
Particle Size (μm):				
Material Size above 150	38.30	45.30	46.20	46.50
Materials between 150 and 125	10.40	14.60	14.50	17.60
Materials between 125 and 105	23.60	21.90	11.80	21.40
Materials between 105 and 90	25.30	7.08	5.20	3.40
Materials between 90 and 75	2.40	23.08	13.90	26.02
Materials between 75 and 53	0.02	2.50	7.02	2.50

Table 4. Physiochemical characteristics and concentration of uranium in water, soil and mill tailings on the 0th day of the simulation studies

	Physiochemical characteristics		
Sample	pH	Total organic carbon (%)	Uranium concentration
Water (n = 3)	7.73	-	16.7±0.51 μg L^{-1}
Soil (n = 5)	7.04	1.35	0.67±0.02 mg kg^{-1} dry weight
Tailings (n = 5)	6.77	0.90	9.34±2.26 mg kg^{-1} dry weight

Note: The uranium concentrations represented is Mean ± SD of five replicates for soil and tailings and of three replicates for water samples

Table 5. Physiochemical characteristics and concentration of uranium in soil and mill tailings on the 90th day of the simulation studies

	Physiochemical characteristics			
Sample	pH	Total organic carbon (%)	Electrical conductivity (μmhos/cm)	Uranium concentration (mg/kg dry weight)
Soil (n = 5)	6.45	1.15	652	3.12±0.78
Tailings (n = 5)	7.04	1.01	1576	4.75±0.75

Note: The uranium concentrations represented is Mean ± SD of five replicates

Table 6. Two-way ANOVA without replication for uranium variation in the tailings samples

Source of variation	SS	df	MS	F	P-value	F crit
Within five replicates	2.56	4	0.64	3.83	0.06	3.94
Day of collection	66.55	2	33.28	199.31	1.5E-07	4.46
Error	1.34	8	0.17			

Fig. 2. A and B represents the status of the plants on the 0th day i.e. on the day the experiment was started, C and D represents the status of the plants on the 90th day i.e., the last day of the simulation experiment. The condition of the plants in the control set of tanks can be viewed from A and C, while that of the tailings set of tanks can be viewed from B and D. The insert E shows the lush growth of the vetiver plants on the 90th day, in the insert the tanks on the right are the tailings tank and the tanks in the left are the control tanks

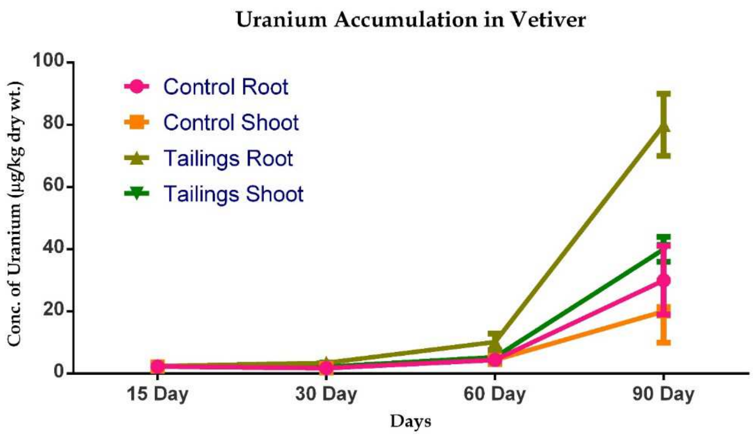

Fig. 3. Uranium Accumulation in Roots and Shoots of *Chrysopogon zizanioides* during the duration of the simulation study; Note: The uranium concentrations represented is Mean ± SD of five replicates

Table 7. Bioaccumulation factor of uranium from soil to root and shoot of *Chrysopogan zizanoides*

	60th Day	90th Day
Control soil to plant root	0.0020	0.010
Control soil to plant shoot	0.0008	0.006
Tailings through soil to plant root	0.0040	0.030
Tailings through soil to plant shoot	0.0020	0.010

Table 8. Phytoremediation efficiency of uranium by vetiver from mill tailings

Days of Collection	Phytoremediation efficiency (%)
60th day	46.36
90th day	49.14

From the results, it is evident that part of the uranium taken up by the root system has been transported to the shoot system and part of the uranium has been predominantly immobilized in the root system. The bioaccumulation factor of uranium in vetiver (*Chrysopogon zizanioides*) was calculated for soil to the root and shoot of the plant. It was observed that uranium predominately accumulated in the roots of the plant. The bioaccumulation factor for the root and shoot for both the control and the tailings set of tanks are shown in Table 7 and it can be concluded that the concentration of uranium increases with exposure time, so bioaccumulation factor is time dependent.

The phytoremediation efficiency of the vetiver in relation with uranium was time dependent i.e., with anincrease in the exposure time the phytoremediation efficiency of the plant was also found to increase (as shown in Table 8).

Discussion

There is no significant variation in the uranium content of the soil among the five replicates of the control tanks. The concentration of uranium in the mill tailings from below the root zone of the plant showed reduced concentration of uranium which can be attributed to the remediation capabilities of the plant. The increase in the soil uranium concentration from 0.67±0.02 to 3.12±0.78 mg kg^{-1} (dry weight) can be attributed to the uranium concentration of 16.7±0.51 µg L^{-1} in the tap water used to irrigate the plants during the study which is the reason for the increased uranium taken up by the plant.

The uranium concentration in the plants was found to increase with time and the highest concentration of uranium in the plant parts i.e. the roots and the shoots was observed on the 90th day of the study as depicted in Fig. 3. The sudden rise of the uranium concentration from the 60th to the 90th day was due to the vetiver plant reaching the flowering stage. The ability of the non-native vetiver plant to grow well in the soil of Jaduguda was observed and documented as shown in Fig. 2. The dense growth of vetiver on the 90th day after plantation as illustrated in Fig. 2 indicates that the plant can thrive in the soil conditions of Jaduguda.

The bioaccumulation factor of uranium revealed that uranium is predominately localized in the roots and only minimal amount of uranium is transferred to the shoot. With our observation combined with the fact that vetiver is generally regarded as the last resort of forage for livestock (Greenfield, 2002), there is little chance of uranium being transferred to the environment with the use of vetiver. It was observed from Table 7 that the bioaccumulation factor is time dependent as there is a 500% increase from the 60th day to the 90th day in control soil to plant root and 750% increase from control plant soil to shoot during the same time period, a 750 and 500% increase was also observed on the 90th day in tailings through soil to plant root and shoot respectively when compared to 60th day. This leads us to conclude that the uranium uptake efficiency of the plant and immobilization capacity by root are increased with time.

The removal efficiency of uranium by vetiver plant was 3% higher on the 90th day compared to the 60th day. Previous studies by (Li *et al*., 2011) has indicated that the removal efficiency of bean plants was higher when exposed to lower concentrations of uranium compared to higher concentration and the time required to attain the same removal efficiency of uranium was longer. Shahandeh and Hossner (2002) have stated that the uranium uptake by the plant is affected by the pH, iron and manganese oxides and the texture of the soil (Shahandeh and Hossner, 2002). The average soil pH in our study was 7 while that of the tailings was 6.77. The presence of organic content in the soil also affects the uranium uptake and is inversely proportional to the amount of uranium available for uptake by the plant. The soil was also found to be clayey which aids in the uranium uptake.

A comparison of the previous work on phytoremediation of uranium either in the field or lab conditions showed that vetiver has a moderate phytoremediation effect compared to other plants like *Helianthus annuus*, *Cyperus iria, Parthenocissus quinquefolia, Panicum maximum*, *Juncellus serotinus, Brassica juncea*and *Phragmites australis* (Li *et al*., 2011), (Shahandeh and Hossner, 2002), (Roongtanakiat *et al*., 2010). Similar to our simulation studies in the tanks; in these reports; the analysis were carried out for a maximum period of 90 days, but the work was performed either under laboratory conditions, or samples were taken only once.

Conclusion

The simulation studies carried out under the controlled conditions showed a decrease in uranium concentration from root zones of the plant indicating fixation of uranium by vetiver. This may be due to its ability to immobilize uranium in its roots. Therefore phytoremediation of mill tailings using vetiver provides an encouraging option to fix uranium in contaminated land or mill tailing. The bioaccumulation factor of uranium showed that uranium is predominately localized in the roots. The maximum uptake was observed during the flowering period of the plant. However, the study has to be repeated in field conditionto conclude a more comprehensive picture of the phytoremediation properties of vetiver. Vetiver also serves as a vegetation cover in the mill tailing ponds preventing soil erosion therein preventing leaching of uranium out to the environment from the tailings and minimizing resuspension of tailing dust.

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Author Contribution

H. Shanmugasundaram: Conducted the experiment, analyzed the data and wrote the manuscript.

Sathesh Kumar: Analyzed the data and wrote the manuscript.

Kantha D. Arunachalam: Coordinated the study and wrote the manuscript.

V.N. Jha, K. Sivasubramanian, N.K. Sethy and H. Krishnan: Coordinated the study and analyzed the data.

M. Pandima Devi: Coordinated the study.

Conflict of Interest

We would also declare that there is no conflict of interest among the authors.

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