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REMEDIATION OF ^{241}AM USING *VETIVERIA ZIZANOIDES* L. NASH: INFLUENCE OF CHELATING AGENTS

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ABSTRACT

The potential of *Vetiveria zizanoides,* a plant used for environmental conservation was tested for remediation of radionuclide americium (^{241}Am) from solution and soil. Plant could remediate high levels of ^{241}Am (62%) from solutions, remediation of radionuclides from soil was limited. Addition of chelating agents- citric acid (CA) and diethylenetriaminepentaacetic acid (DTPA) enhanced phytoremediation of ^{241}Am from soil. Translocation of ^{241}Am to the shoot biomass showed an enhancement in the presence of chelating agents in the solution as well as soil, whereas DTPA promoted higher translocation of radionuclides than citric acid. The present studies have shown that *V. zizanoides* is a suitable candidate for remediation of ^{241}Am and addition of DTPA enhanced translocation of radionuclide to shoots.

Key words: *Vetiveria zizanoides*, americium, phytoremediation, chelators

1. INTRODUCTION

Proliferation of nuclear power industry, nuclear weapon testing, dismantling of existing nuclear weapons under the arms reduction agreement and occasional accidents such as Chernobyl accident in 1986 and Fukushima Daiichi nuclear plant in Japan in 2011 have contributed to an enhancement in the level of radionuclides in the environment, which needs effective methods of remediation. Although various physical and chemical methods have been employed for remediation of radionuclides, they are not practical effective when concentrations of radionuclides are low and areas/volumes involved are large. Phytoremediation – the use of plants for remediation of toxic metals and radionuclides has been recognized as an aesthetically pleasing, low cost *in situ* successful method, which can be used for remediation of low levels of toxic pollutants from the environment (McGrath et al. 2002; Arthur et al. 2005). ^{241}Am has a half-life of 433 years, poses a threat to all living organisms including humans and needs effective remediation (IAEA, 1973). Due to high toxicity, low mobility in soil and long half life of ^{241}Am, it is desirable to develop methods which will enhance their mobility and help in translocation to the shoot biomass of plants. Vetiver grass (*Vetiveria zizanoides*), due to its unique morphological and physiological characteristics has been widely documented for its effectiveness to prevent soil erosion and sediment control. (Srivastava et al, 2008). There are a few reports on the use of *V. zizanoides* for the remediation of metals and organic pollutants (Paquin et al, 2002; Chantachon et al, 2004; Chen et al, 2004; Boonyapookana et al, 2005). We have earlier reported that vetiver grass could remediate ^{137}Cs (β, γ emittor) and ^{90}Sr (β emittor) from spiked solutions as well as low level nuclear waste (Singh et al, 2008). The present study was performed to investigate the potential of vetiver grass for remediation and translocation of alpha-emitting actinide element- americium from solutions and soil. Chelators are known to enhance desorption of metals from soil, thus facilitating their uptake by plant roots. Effect of different chelators like citric acid (CA) and diethylenetriaminepentaacetic acid (DTPA) on uptake of ^{241}Am and their translocation to shoots are also presented here.

2. MATERIALS AND METHODS

2.1 Plant Material

Vetiver grass (*Vetiveria zizanoides* L. Nash) multiplied by axillary bud development on MS medium (Murashige and Skoog 1962) supplemented with 2 mgL^{-1} benzyladenine (BA) and 0.1 mgL^{-1} indole acetic acid (IAA) were transferred to liquid MS medium in test tubes supplemented with 1 mgL^{-1} α-naphthalene acetic acid (NAA) without a raft for rooting (Eapen 2007). One month old *in vitro* plantlets having well developed roots were further transferred to Hoagland's liquid medium and plants were incubated at 25ºC under white fluorescent light (12.2 µM photon ms^{-2} s^{-1}) for photoperiods of 12 h. Six-week-old well developed plants of uniform size were used for the experiments.

2.2 Remediation of ^{241}Am from hydroponics

2.2.1 Experimental Set up

Vetiveria zizanoides plants (20 cm. long) were incubated with roots immersed in 20 ml of distilled water spiked with ^{241}Am (100 Bq mL^{-1}) in 100 ml test tubes for a period of 30 d along with one set of control under controlled conditions. Plants were allowed to grow for 30 d in the spiked solution prior to harvesting. One set each of ^{241}Am was also set up without plants to ensure if any loss of radioactivity is there due to adsorption on the glass surface if any. Samples (0.1 ml) were drawn out from each solution at different time intervals (0, 1, 5, 10, 15, 20, 25 and 30 d) and analyzed for radioactivity using ZnS (Ag) scintillation detector to estimate the uptake of radionuclides in the plant tissues.

2.2.2 Effect of chelators on ^{241}Am uptake in solution

In order to study the effect of chelators on uptake of ^{241}Am by *V. zizanoides*, CA and DTPA (50 µg mL^{-1}) were added individually to solution supplemented with ^{241}Am. Samples (0.1 ml) were withdrawn after 0, 1, 5, 10, 15, 20, 25 and 30 d and analyzed for radioactivity.

2.3 Remediation of ^{241}Am from soil

2.3.1 Experimental set up

V. zizanoides were transferred to soil (collected from Trombay garden pH = 7.6) spiked with either ^{241}Am (100 Bq g^{-1}) in polypropylene containers along with one set of control and grown under controlled conditions. Plants were allowed to grow for 30 d in the spiked soil prior to harvesting. One set of soil supplemented with ^{241}Am were also set up without plants to check the possibility of any loss of radioactivity. Soil and plant samples were analyzed after 30 d.

2.3.2 Effect of chelators on uptake of ^{241}Am from soil

To study the effect of chelators on uptake of radionuclides, CA and DTPA (50 µg g^{-1}) were added to soil supplemented with ^{241}Am.

2.4 Translocation studies

At the end of each experiment (hydroponics as well as soil), harvested plants were thoroughly washed with distilled water, separated into root and shoot, blotted dry and dried in an oven at 60^{o} C for 48 h. The dried plant tissues were digested in HNO_3: $HClO_4$ (5:1, v:v) and analysed for radioactivity as described above. Translocation index (TI) was calculated for ^{241}Am and also after addition of chelators.

TI= [(radionuclide content of the shoot) / (radionuclide content of the whole plant)] x 100)

2.5 Sample Analysis

Analysis of radioactivity in the hydroponic samples was carried out by plancheting known volume of the samples, drying under IR lamp and planchets were fired to fix the radioactivity on it. Subsequently, the activity was determined using a scintillation counter. For alpha activity determination, alpha counter was calibrated with standard plutonium source prior to the estimation of samples. Accuracy of alpha counter was estimated using standard sources and was found to be 5% expressed in terms of percentage error. Coefficient of variance was found to be in the order of 1.5 - 2%, indicating the reproducible nature of the work. In case of soil samples, prior to analysis, 1 g of soil was digested in HNO_3: $HClO_4$ (5:1, v:v) and radioactivity was estimated as described above. All the experiments were performed in triplicates, repeated twice and standard error (S.E.) calculated.

3. RESULTS

When the efficiency of *V. zizanoides* for ^{241}Am remediation from solution was studied, it was found that plants could remediate ^{241}Am from spiked solution with a maximum remediation of 63.7% after 25 d. In the presence of CA and DTPA, plants were able to remediate 52.1% and 30.1% of ^{241}Am respectively after 30 d of revelation period (Fig. 1). No significant variation was observed in ^{241}Am solution without plant (negative control). Addition of CA and DTPA to solution spiked with ^{241}Am reduced the plant's ability to remediate ^{241}Am from solutions. Distribution of toxic elements in different plant parts is an important parameter when phytoremediation strategies are designed. When *V. zizanoides* plants were exposed to ^{241}Am, activity was mostly found in roots, with low levels of translocation to shoots (Fig. 2). However in the presence of chelators, higher TI was observed- 23.2% for CA and 74.9% for DTPA addition respectively. Addition of CA and DTPA enhanced the translocation of ^{241}Am to the shoots. Maximum translocation was observed in the presence of DTPA, TI being 65% for ^{241}Am (Table 1). In case of ^{241}Am, low activity was translocated to shoots showing TI of almost 9% in the absence of any chelator. When DTPA was supplied to solution, TI was found to be 8 times higher in case of ^{241}Am, when compared to without chelators. When DTPA was added to the ^{241}Am solution, it enhanced the translocation of the actinide to the shoot. When *V. zizanoides* plants were grown in soil spiked with ^{241}Am, they could remediate only low levels of actinide (5% of Am) after 30 days (Figs. 3). However, remediation capacity of the plant showed an enhancement in the presence of chelators. In case of ^{241}Am, plants were able to remediate 14% and 35% of the total supplied activity after 30 days on addition of CA and DTPA respectively, which is almost 2 and 4 times of the non-chelated soil. Addition of chelating agents to soil helped in the translocation of ^{241}Am to shoots (Figs 4).

4. DISCUSSION

^{241}Am contamination of the environment can affect populations inhabiting the contaminated environment. When volumes involved are large and concentrations of radionuclides are low, conventional chemical and physical methods are not suitable and phytoremediation can be used for this purpose. In the present study, *V. zizanoides* plants were found to tolerate supplied activity of ^{241}Am for one month (30 days) and were effective for the remediation of these radionuclides from the spiked solutions. *V. zizanoides* is a monocot plant with high biomass fibrous root system and has the ability to tolerate a wide range of adverse climatic and edaphic conditions which makes it an ideal choice for phytoremediation work. (Antiochia et al, 2007; Singh et al, 2008; Danh et al, 2009). In the present study, most of the ^{241}Am taken up by *V. zizanoides* was found to be retained in the roots after 30 days of exposure in solution as well as soil, possibly because roots are in direct contact with the solutions and could play an important role in the retention of the activity, preventing its excessive accumulation in shoots, thus protecting the shoots from damage due to toxic levels of radionuclides. In normal soil conditions, low levels of remediation of ^{241}Am was observed using *V. zizanoides*. This is because actinides have high affinities for soil particles and are immobilized, with very little uptake by plants under normal conditions. A variety of chelating agents can elute contaminants from soil and facilitate their uptake by plants. DTPA was found to be more efficient for phytoremediation of ^{241}Am compared to CA. Similar findings were also recorded in bush bean plants for ^{241}Am in the presence of DTPA (Hale and Wallace, 1970). DTPA was also shown to increase the uptake of ^{241}Am from the spiked soil in *Paspalum notatum*, *Oryza sativa* and soybean (Adriano et al, 1980). Application of DTPA was shown to enhance the uptake of ^{241}Am by soybean and citrus root grafts (Wallace, 1972). The effect of chelators CA and DTPA on translocation of radionuclides was investigated in the present study using *V. zizanoides*. In hydroponic experiment, activity of ^{241}Am in shoots of treated plants showed an increase with the addition of chelators specially in the presence of DTPA. The same trend was observed in case of soil studies also. Vyas and Mistry, 1983 found similar trends in their americium studies using bean plants. In the present study, transport index (TI) of the plants showed an enhancement in ^{241}Am activity in the presence of the chelators, indicating higher translocation to the aerial parts of the plants. The TI data clearly suggests that DTPA enhanced transport of ^{241}Am in both solutions and soil.

Table 1
Translocation index (TI in %) of Am in V. zizanoides from the solution as well as soil.

Sample	TI in solution (%)	TI in soil (%)
Am	8.75	9.31
Am + CA	23.15	21.43
Am + DTPA	74.92	65

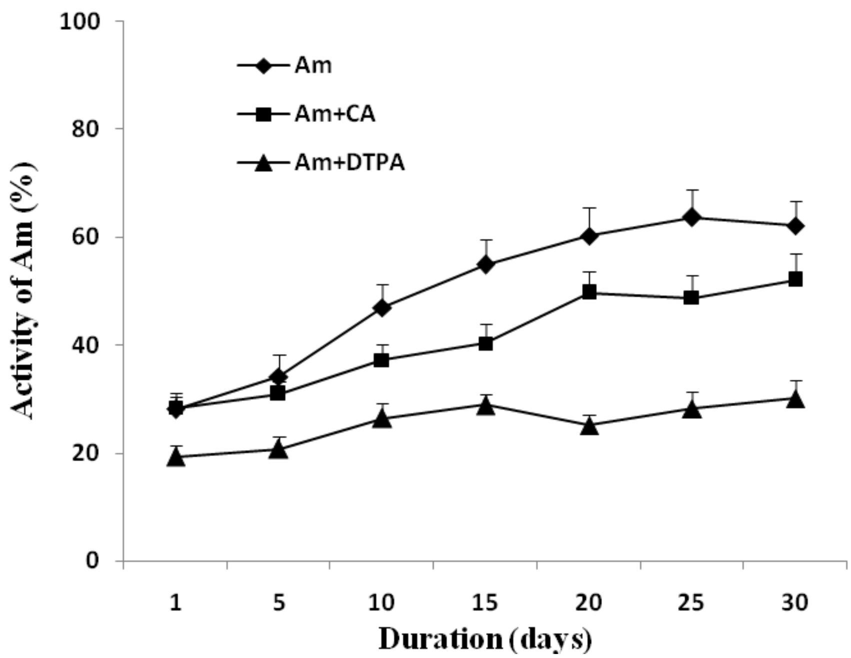

Figure 1
Activity of Am, Am+CA and Am+DTPA in solutions after incubation with V. zizanoides plants for different time periods. The values are mean of three replicates $\pm$ S.E. The initial activity of Am was 100 Bq mL^{-1}.

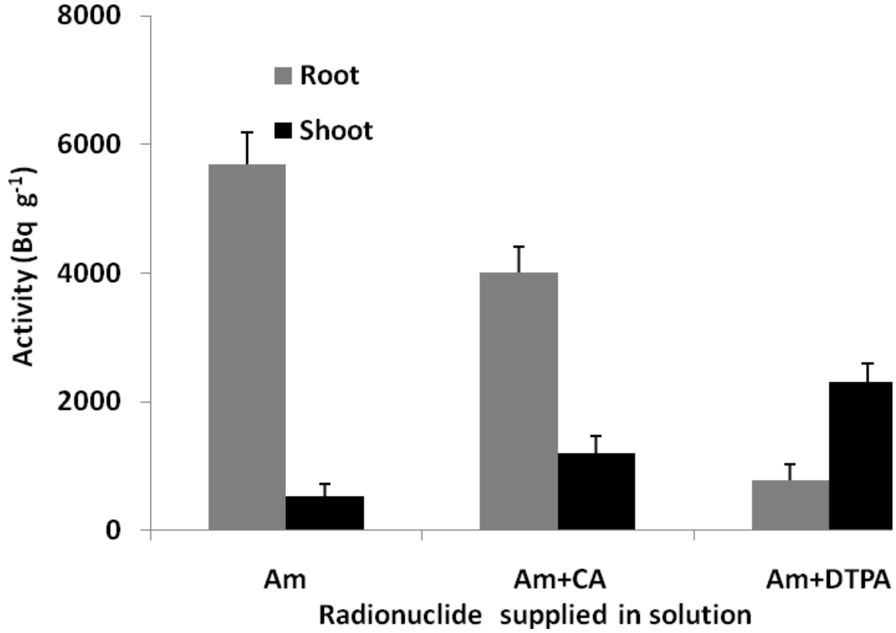

Figure 2

Distribution of Am in roots and shoots of V. zizanoides plants from the solution supplemented with CA or DTPA. The values are mean of three replicates ± S.E. The initial activity of Am was 100 Bq mL^{-1}.

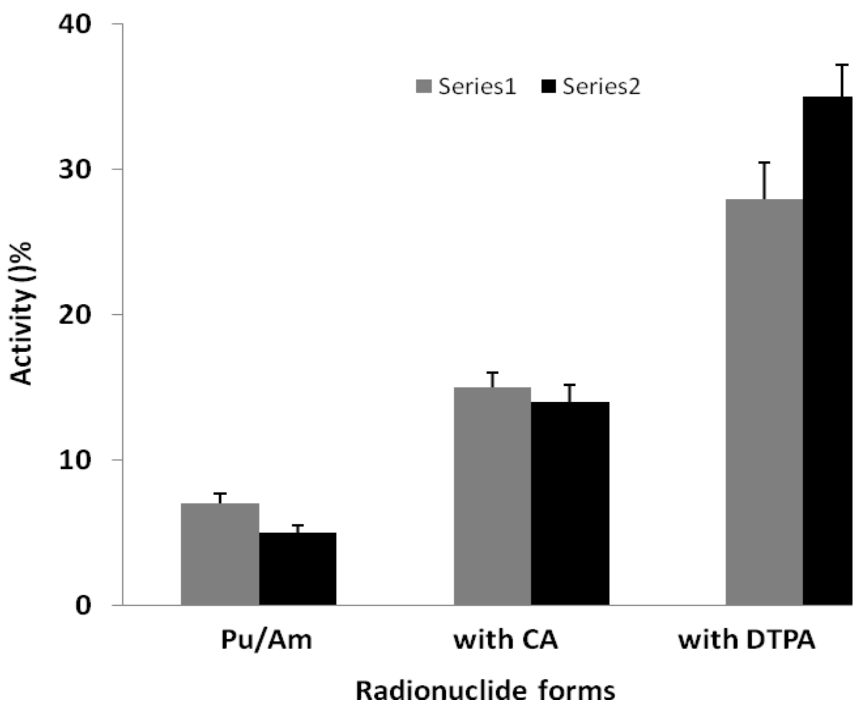

Figure 3

Removal (%) of Am from soil after incubation with V. zizanoides plants (with or without CA and DTPA) after 30 d. The values are mean of three replicates ± S.E. The initial activities of Am were 100 Bq g^{-1}.

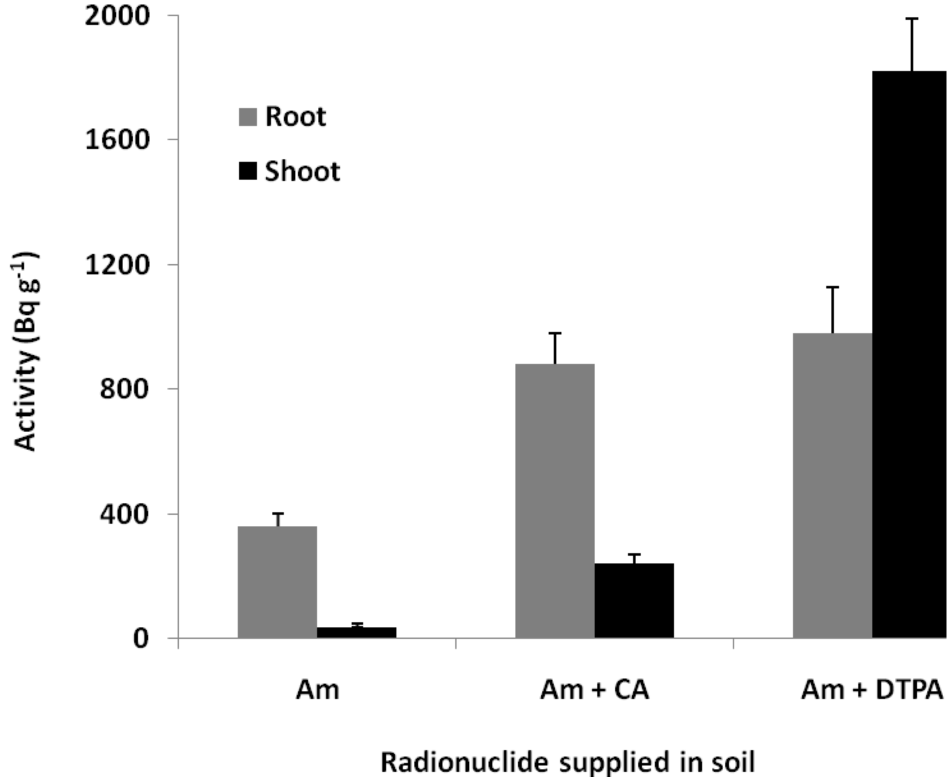

Figure 4

Distribution of Am in roots and shoots of V. zizanoides plants from the soil supplemented with CA or DTPA. The values are mean of three replicates ± S.E. The initial activity of Am was 100 Bq mL^{-1}.

5. CONCLUSION

In the present study, *V. zizanoides* was shown to effectively phytoremediate ^{241}Am from spiked solutions. However, remediation of ^{241}Am was low in the soil. From ^{241}Am spiked soil, plants were able to remediate significant radioactivity in the presence of chelating agents such as CA and DTPA. Translocation of ^{241}Am to shoots was enhanced by the addition of chelators in both solution and soil. Capability of *V. zizanoides* to accumulate high levels of ^{241}Am in the shoots in presence of DTPA makes it possible to cut off the shoots, thus allowing fresh shoots to grow and continue the process of accumulation of ^{241}Am. The present study has shown that *V. zizanoides* is a potential candidate plant for radio-phytoremediation of ^{241}Am - a long lived actinides, which needs remediation and containment.

ABBREVIATIONS

citric acid (CA), diethylenetriaminepentaacetic acid (DTPA), Translocation Index (TI)

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