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Potential of *Vetiveria zizanoides* L. Nash for phytoremediation of plutonium (^{239}Pu): Chelate assisted uptake and translocation

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ABSTRACT

Plants have demonstrated a great potential to remove toxic elements from soils and solutions and been successfully used for phytoremediation of important radionuclides. Uptake potential of vetiver plants (*V. zizanoides*) for the remediation of ^{239}Pu in hydroponic and soil conditions was studied in the present work. High efficiency of *V. zizanoides* for the removal of ^{239}Pu was recorded with 66.2% being removed from the hydroponic solution after 30 days. However, remediation of ^{239}Pu from soil was limited. Remediation of ^{239}Pu from soil was increased with the addition of chelating agents citric acid (CA) and diethylenetriaminepentaacetic acid (DTPA). Accumulation of ^{239}Pu was recorded higher in roots than shoots, however its translocation from roots to shoots increased in the presence of chelators in hydroponic as well as soil conditions. DTPA was found more effective than CA showing higher translocation index (TI). Increase in TI was observed 8 and 6 times in the solution and soil respectively when plants were exposed to ^{239}Pu-DTPA in comparison to only ^{239}Pu. The present study demonstrates that *V. zizanoides* plant is a potential plant for phytoremediation of ^{239}Pu.

1. Introduction

Radioactive contamination of the environment is a global concern. It occurs due to natural processes, global fall-out from nuclear weapon testing, discharge from nuclear installations, disposal of nuclear waste and occasional nuclear accidents *viz.* Chernobyl in 1986 and Fukushima in 2011 and poses serious problem to biological systems (Nagataki and Takamura, 2014; Alkhomashi and Monged, 2015). Therefore, cleanup of radionuclides from contaminated soils and ground water is extremely necessary in order to minimize their impact on the ecosystem. In this context, plant-based clean-up technologies are attractive and sustainable approach for the remediation of contaminated soils and solution (Peuke and Rennenberg, 2005). Plants possess a natural ability to eliminate, detoxify or immobilize environmental contaminants in a growth matrix by means of various biological processes. Phytoremediation has emerged as an alternative feasible option for environmental cleanup when compared to physico-chemical and other biological methods and significantly reduced remedial costs (Singh et al., 2014; Sharma et al., 2015). In addition, this technology can also provide additional benefit to improve soil quality, soil carbon sequestration, soil fertility with inputs of organic matter and phytobiomass for fiber and biofuel production (Mench et al., 2009; Abhilash et al., 2012). Using plants to remove low concentrations of radionuclides from soil *in situ* is expected to be less expensive than mechanical, physical or chemical methods, particularly for treatment of large areas.

Vetiver plant (*Vetiveria zizanioides*) is a subtropical grass having large biomass and a dense root system. Vetiver is xerophytes as well as hydrophyte and is highly tolerant to extreme climatic conditions (Danh et al., 2009). A number of special characteristics make vetiver plant as a prime choice for phytoremediation of heavy metals and organic wastes, thus suitable for phytostabilization, and phytoextraction with the addition of chelating agents.

Over the decade, many researchers have broadly studied the cleaning up of the contamination by vetiver plant (Meagher, 2000; Ondo et al., 2014). Vetiver plant was also used for the remediation of radionuclides ^{90}Sr (β emittor) and ^{137}Cs (β, γ emittor) from spiked solutions as well as low level nuclear waste (Singh et al., 2007), however there is no report on this plant for the phytoremediation of plutonium.

Pu-239 is an isotope of plutonium having half-life of 24,100 years ($t_{1/2}=2.41\times10^4$ years). Plutonium is much more radioactive than the depleted Uranium-238 in the fuel. Plutonium has been a focus of considerable environmental concern in recent years

Abbreviations: CA, citric acid; DTPA, diethylenetriaminepentaacetic acid; TI, translocation index; Pu, plutonium

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because of its toxicity and carcinogenic properties. However, fundamental understanding of the movement of Pu in living plants is lacking.

Chelating agents are known to facilitate the bioavailability by forming neutral complexes (Chiu et al., 2005; Evangelou et al., 2007; Danh et al., 2009; Freitas et al., 2013) and play a significant role in increasing the accumulation efficiency of the plants. A commonly used approach for improving phytoremediation has employed with synthetic chelates (EDTA and DTPA) and naturally occurring organic acids (oxalic, citric, vanillic and gallic acids). Chemical extraction processes with chelating agents have been employed for remediation of soils and ground water polluted with heavy metals (Lim et al., 2004; Zheng et al., 2007). However, until now, there is no report that has focused on the use of *V. zizanoides* for phytoremediation of ^{239}Pu from solutions and soil and its improvement with the use of chelating agents. In the present study, we have focused on the potential of vetiver plant (*V. zizanoides)* for remediation and translocation of alpha-emitting actinide element ^{239}Pu and simultaneously studied the effect of addition of chelating agents citric acid (CA) and diethylenetriaminepentaacetic acid (DTPA) on the uptake and translocation of ^{239}Pu.

2. Material and methods

2.1. Plant Material and tissue culture

V. zizanoides L. Nash shoot tips were collected as explant from Experimental Field Station, Bhabha Atomic Research Centre, Trombay, Mumbai. Explants were initially washed thoroughly under tap water for 10 min and subsequently by disinfected with 70% ethyl alcohol for 3 min than immersed in aqueous solution of 0.1% (w/v) $HgCl_2$ for 3 min and finally washed several times with sterilized distilled water. Surface sterilized explants were aseptically transferred on Murashige and Skoog's (MS) (1962) medium supplemented with 2 mg L^{-1} 6-benzylaminopurine (BAP) and 0.1 mg L^{-1} indole acetic acid (IAA) and 3% sucrose. The medium was adjusted to pH 5.8 by 0.1 N NaOH or HCl before adding 0.8% agar and autoclaved at 121 °C at 103.42 kPa for 20 min Cultures were regularly sub-cultured after three weeks on the same medium constituents and maintained under similar culture conditions. After several subcultures, *in vitro* plantlets were transferred to Hoagland's liquid medium. Six week old well developed plants of uniform size were used for the experiments.

2.2. Remediation of ^{239}Pu from hydroponics

2.2.1. Experimental Set up

Plantlets of *V. zizanoides* were incubated in 20 mL of distilled water spiked with ^{239}Pu along with one set of control under controlled conditions. The initial ^{239}Pu activity was 100 Bq mL^{-1} (total Pu activity =2000 Bq). Plants were allowed to grow for 30 days in the spiked solution prior to harvesting. One set each of ^{239}Pu was also set up without plants to ensure if any loss of radioactivity is there. Experiments were performed in a completely randomized block design in controlled conditions at 25 ± 1 °C for 16 hr light provided by cool white fluorescent tubes to deliver 40 μmol m^{-2} s^{-1} light intensity. Samples (0.1 mL) were drawn out from each solution at different time intervals (0, 1, 5, 10, 15, 20, 25 and 30 days) 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 ^{239}Pu uptake in solution

CA and DTPA (50 μg mL^{-1}) were added individually to solution supplemented with ^{239}Pu to study the effect of chelators on ^{239}Pu uptake by *V. zizanoides*, Samples (0.1 mL) were withdrawn after 0, 1, 5, 10, 15, 20, 25 and 30 days and analyzed for radioactivity.

2.3. Remediation of ^{239}Pu from soil

2.3.1. Experimental set up

Uncontaminated garden soil (GS) was used for the experiment. The garden soil was finely grounded and passed through 2 mm sieve to get a uniform size, before filling up in polypropylene containers (25 cm in diameter). Physiochemical characteristics of soil were: texture, clay loam; pH, 7.7; electrical conductivity of soil extract, 2.9 dS m^{-1}, organic matter, 1.3%, Salinity, 0.2%, Na, 61 mg $L^{-1,}$ K, 14 mg L^{-1}. Initial soil Fe, Cr, Zn, Mn and Cu concentrations (μg g^{-1} dw) were 254, 1.2, 7.18, 63 and 22.4 respectively.

Plants of *V. zizanoides* were transferred to soil spiked with ^{239}Pu (100 Bq g^{-1}) in polypropylene containers along with one set of control and grown for 30 days under controlled conditions (same as hydroponics). One set of soil supplemented with ^{239}Pu was also set up without plants were to check the possibility of any loss of radioactivity. The tap water was provided to keep the pots moist and avoid leaking from the pots. Plants were watered every alternate day and it was ensured that no water comes out of the pot. Further, petridishes were kept below each pot to collect the leachate if any. Soil and plant samples were analyzed after 30 days.

2.3.2. Effect of chelators on uptake of ^{239}Pu from soil

CA and DTPA (50 μg g^{-1}) were added to soil supplemented with ^{239}Pu to study the effect of chelators on uptake of radionuclides.

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, and dried in an oven at 60 °C for 48 h. Dried plant tissues were digested in HNO_3:$HClO_4$ (5:1, v/v) and analyzed for radioactivity. The concentrations of HNO_3 and $HClO_4$ were 65% and 60% respectively. Translocation index (TI) was calculated for ^{239}Pu and also after addition of chelators by following formula:

TI=[(^{239}Pu content of the shoot)/(^{239}Pu content of the whole plant)] × 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 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 the analysis, 1 g of soil was digested in HNO_3:$HClO_4$ (5:1, v/v) and radioactivity was estimated as described above.

2.6. Statistical analyses

The whole experiment was set up in the randomized block design. All the experiments were conducted with three replicates and repeated at least twice. Statistical analysis was done using Microcal TM Origin Pro 6.1. To confirm the variability of data and