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Influence of chelating agents on the uptake of ^{239}Pu and ^{241}Am by plants

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Summary The uptake of ^{239}Pu and ^{241}Am from two contrasting soil types, namely, a laterite (Oxisol) and a medium black (Vertisol-Pellustert) and also from nutrient solution as influenced by pre-chelation with synthetic chelating agents, DTPA and EDTA was studied.

Results from soil–plant studies indicated a significant increase in uptake of ^{239}Pu and ^{241}Am by plants from both soil types when added as ^{239}Pu-DTPA, ^{239}Pu-EDTA, ^{241}Am-DTPA or ^{241}Am-EDTA as compared to the treatments where transuranics were added in ionic (nitrate) forms. Concentration ratios (CRs) for Pu and Am in plant shoots increased by factors ranging from 3 to 700 depending on the soil type and the complexing ligand.

Nutrient solution studies also revealed a marked enhancement in uptake as well as translocation of Pu and Am in plants due to supply of chelated transuranics. The shoots CRs in the case of $Pu(NO_3)_4$, Pu-EDTA, and Pu-DTPA were 0.11 ± 0.02, 2.94 ± 0.35 and 4.35 ± 0.41, respectively. The observed values of Am CRs in shoots in the case of $Am(NO_3)_3$, Am-EDTA and Am-DTPA were 1.55 ± 0.18, 1.86 ± 0.25 and 2.85 ± 0.40, respectively. An increase in the values of 'Transport index' by 6 to 50-folds was observed in the case of Am and Pu, respectively, when added as chelates of DTPA in comparison to the nitrate form of the radionuclides. EDTA complexes of the transuranics were observed to be less effective than corresponding DTPA complexes in enhancing the uptake of Pu and Am by plants.

Introduction

The passage of highly radiotoxic transuranic nuclides, ^{239}Pu and ^{241}Am, deposited in the global fallout from nuclear weapon tests or released in controlled or accidental discharges associated with nuclear power industry, through the terrestrial food chain involves their transfer through the soil-plant link of the food chain. Thus studies on the behaviour of Pu and Am in soils and soil-plant systems are of considerable environmental significance. A number of studies on soils of temperate[3,5,6,8–10,12,17,18] as well as from tropical and sub-tropical [20,22,23] regions have shown very limited uptake (CRs ranging from 10^{-3} to 10^{-6}) of the transuranics by a variety of crop plants.

Chelates have been known to make available insoluble cations to plants[14]. Such chelates increase the mobility of cations to roots by replenishing those taken up by plants.

Multidentate chelating agents such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediamine di-O-hydroxy-phenylacetic acid; (EDDHA), and nitrilotriacetic acid (NTA) have, in recent years, been used extensively as a means of supplying micronutrients to plants in intensive agriculture. In addition these chelating agents find widespread applications in various industrial processes and subsequently enter terrestrial as well as aquatic ecosystems through industrial effluents.

Although plant uptake of Pu and Am from soils is limited, the presence of chelating ligands in soils and the possible formation of soluble complexes of Pu and Am with these ligands could enhance the solubility of transuranic radionuclides in soils, and their subsequent transfer to plants[4,26,27]. A marked enhancement in mobility of Pu and Am through three contrasting Indian soil types on leaching with dilute chelating solutions was observed in earlier studies[21]. The present investigation was carried out to examine the influence of prechelation of Pu and Am with DTPA and EDTA on their uptake by bean plants from two different soil types, namely, a laterite (Oxisol) from Ratnagiri, Maharashtra and a medium black (Vertisol-Pellustert) from Jalgaon, Maharashtra, and also by bean plants grown in nutrient solution. In these studies the nitrate form of these radionuclides was used for comparison with the chelated forms.

Materials and methods

Uptake of Pu and Am supplied in prechelated forms as ^{239}Pu-DTPA, ^{239}Pu-EDTA, ^{241}Am-DTPA and ^{241}Am-EDTA and in the ionic forms as nitrates was studied in two contrasting soil types, namely, a laterite (Oxisol) and a medium black soil (Pellustert). The physicochemical characteristics of the experimental soils are reported in Table 1. Chelates of the radionuclides were prepared in a manner similar to that followed in earlier studies[2]. After maintaining 500 g soil at field capacity moisture status for three days, surface contamination of the soils with 3 μCi of respective radionuclide was carried out. Three replicates were maintained for each treatment. Two days after contamination three red kidney bean (*Phaseolus vulgaris* L.) seeds were sown in each pot. Thinning was carried out one week after germination and one plant in each pot was retained. After four weeks of growth, when plants had completed their vegetative phase, the shoots were harvested and dried to constant weight at 90°C and used for radioassay of the respective radionuclide.

The influence of supply of prechelated forms of the transuranic radionuclides on their plant uptake from nutrient solution was also examined. 5 μCi of each radionuclide as nitrate, DTPA and EDTA chelates were added to separate sets of nutrient culture jars constituting separate treatments. Chelates were prepared in the manner similar to that followed for soil application. Seven-day-old red kidney bean (*Phaseolus vulgaris* L.) seedlings were transferred to culture jars containing 1.5 litres of labelled nutrient solution. The full-strength nutrient solution contained $K^+ - 3.0$, $Ca^{++} - 8.0$, $Mg^{++} - 3.0$, $No_3^- - 10.0$, $SO_4^{--} - 3.0$, and $H_2PO_4^- - 1.0$ meq/litre together with micronutrients Mn, Zn, Cu, B, Mo as inorganic salts and Fe as ferric citrate. The initial pH of the solution was 5.3. Each treatment was replicated four times. When the plants had developed mature pods (6 weeks in labelled solution) they were harvested, dried to constant weight at 90°C and used for radioassay of the respective radionuclide.

Radioassay of ^{239}Pu was carried out by thenoyaltrifluoroacetone (TTA) extraction[16] as reported in our earlier studies[20] with Pu. Radioassay of ^{241}Am in plant samples was carried out through

Table 1. Physicochemical characteristics of the soils used in the investigation

Characteristics	Laterite (Oxisol)	Medium black (Pellustert)
pH (1:2.5)	5.7	8.3
Moisture equivalent (%)	30.0	35.0
Electrical conductivity (mmhos/cm)	0.2	0.2
Cation Exchange Capacity (meq/100 g)	11.48	40.50
Exchangeable calcium (meq/100 g)	3.86	37.96
Exchangeable magnesium (meq/100 g)	1.30	3.26
Free calcium carbonate (%)	1.25	5.75
Organic carbon (%)	0.52	0.39
Available nitrogen – N (kg/ha)	650.0	196.0
Available phosphorus – P (kg/ha)	3.95	5.88
Available potash – K (kg/ha)	153.0	306.0
Coarse sand (%)	13.5	5.1
Fine sand (%)	36.8	20.3
Silt (%)	25.0	35.2
Clay (%)	24.7	39.4
Textural class	Sandy loam	Clay loam
Predominant clay minerals	Kaolinite	Montmorillonite/ Illite

gamma-ray spectrometry using a well-type 7.5 cm × 7.5 cm NaI (Tl) crystal integral-line assembly and a nuclear data 512-Channel pulse-height analyzer attached to an oscilloscope and a computer read-out typewriter. The 60 keV gamma photopeak was used for quantitative estimations of ^{241}Am. For uptake studies, concentration ratios (CRs) were computed as under –

$$CR = \frac{\text{Radionuclide content/g dry tissue}}{\text{Radionuclide content/ml nutrient solution or g dry soil}}$$

Studies described above were conducted in growth chambers; temperature was maintained at 23 ± 2°C with relative humidity at 65 ± 1 per cent and the plants were illuminated for 12 hours at 8000–9000 lux.

Results and discussion

Soil studies

Plutonium uptake Results presented in Table 2 on the uptake of Pu in the nitrate (ionic) form as well as chelates of DTPA and EDTA (Pu-DTPA and Pu-EDTA), by plants from Oxisol and Pellustert indicated a very significant increase in plant uptake of Pu when supplied in the prechelated forms as compared to the ionic forms. The values of Pu CRs in aerial tissues of bean plants

Table 2. Effect of chelating agents on uptake and concentration ratio (CR*) of ^{239}Pu by bean plants from two different soils. Age of the plants = 4 weeks

Treatments	Uptake** (pCi/g dry wt)	CR $\times 10^{-2}$
Laterite soil (Oxisol)		
$Pu(NO_3)_4$	188.33 ± 7.48	(2.96 ± 0.11)
Pu-DTPA	4435.23 ± 610.48	(69.89 ± 9.62)
Pu-EDTA	224.96 ± 8.99	(3.54 ± 0.14)
Medium black soil (Pellustert)		
$Pu(NO_3)_4$	79.76 ± 8.58	(1.26 ± 0.13)
Pu-DTPA	546.74 ± 46.72	(8.61 ± 0.74)
Pu-EDTA	128.65 ± 19.01	(2.03 ± 0.30)

* $CR = \frac{\text{Pu content/g dry plant}}{\text{Pu content/g dry soil}}$

** Average value ± standard error of the means (N = 3)

grown in Oxisol were $(2.96 \pm 0.11) \times 10^{-2}$, $(69.89 \pm 9.62) \times 10^{-2}$ and $(3.54 \pm 0.14) \times 10^{-2}$ in the treatments $Pu(NO_3)_4$, Pu-DTPA and Pu-EDTA, respectively. The corresponding Pu CR values for the plants grown in Pellustert were $(1.26 \pm 0.13) \times 10^{-2}$, $(8.61 \pm 0.74) \times 10^{-2}$ and $(2.03 \pm 0.30) \times 10^{-2}$ in the case of $Pu(NO_3)_4$, Pu-DTPA and Pu-EDTA, respectively. Thus, with Pu-DTPA treatment, the Pu CRs were increased by a factor of about 25 and 7 over those obtained with $Pu(NO_3)_4$ treatment in the case of Oxisol and Pellustert, respectively. Pu-EDTA also significantly enhanced the uptake of Pu in comparison to that from the nitrate form, but this increase was, however, of a lower magnitude than observed with Pu-DTPA treatment.

There are only two reports[4,15] in the literature on the influence of prechelation on plant uptake of Pu from soils. In the referenced studies where prechelated form of Pu, namely, Pu-DTPA was applied to a sandy soil[15] and arid lands of Hanford site, U.S.A.[4] nearly 1000-fold increase in pea[15] and tumbleweed[4] uptake of Pu was observed with Pu-DTPA application over control (Pu was added in the ionic form). It has also been reported[19] that addition of DTPA and EDDHA to fallout contaminated soils of Nevada Test site, U.S.A. enhanced the uptake of Pu by alfalfa (*Medicago sativa* L.).

Americium uptake Data on the uptake of Am added to soils as ionic (nitrate) as well as chelated (Am-DTPA and Am-EDTA) forms by plants, presented in Table 3, showed a marked increase in plant uptake of Am with prechelated froms of the radionuclide as compared to the nitrate form in both Oxisol as well as Pellustert.

Table 3. Effect of chelating agents on uptake and concentration ratio (CR)* of ^{241}Am by bean plants from two different soils. Age of the plants = 4 weeks

Treatments	Uptake** (pCi/g dry wt)	CR $\times 10^{-2}$
Laterite soil (Oxisol)		
Am $(NO_3)_3$	229.09 ± 20.62	(3.43 ± 0.30)
Am-DTPA	1165.63 ± 315.88	(17.62 ± 4.77)
Am-EDTA	765.64 ± 232.31	(11.47 ± 3.51)
Medium black soil (Pellustert)		
Am $(NO_3)_3$	31.45 ± 4.22	(0.47 ± 0.06)
Am-DTPA	21553 ± 104.91	(325.93 ± 1.58)
Am-EDTA	214.55 ± 9.27	(3.21 ± 0.14)

* $CR = \frac{\text{Am content/g dry plant}}{\text{Am content/g dry soil}}$

** Average value ± standard error of the means (N = 3)

The values of Am CRs ranged from (3.43 to 17.62) $\times 10^{-2}$, and from (0.47 to 325.93) $\times 10^{-2}$ in the case of Oxisol and Pellustert, respectively. There were no significant differences between the two chelated forms (Am-DTPA and Am-EDTA) in respect of Am uptake by plants grown in Oxisol, but significant differences were observed in the case of plants grown in Pellustert. Prechelation of Am with DTPA resulted in an increase in Am uptake by a factor of about 5 and 700 in the case of Oxisol and Pellustert, respectively.

The observed highest values of Pu as well as Am CRs in the case of DTPA chelated radionuclides suggest that when radionuclides were supplied as Pu-DTPA or Am-DTPA, a high degree of solubility in soils was maintained over extended periods resulting in higher uptake of Pu and Am by plants. In other studies from our laboratory[24] on transformations of Pu and Am in major Indian soils it has been observed that when Pu as Pu-DTPA and Am as Am-DTPA was applied to soils, significantly greater quantities of the radionuclides were associated with the 0.1 *M* $MgCl_2$ extractable fractions representing potential biologically available forms over extended periods up to 400 days.

A 30-fold increase in Am uptake by tumbleweed (*Salsolakali* sps.) from arid soils of Hanford site, U.S.A. due to prechelation with DTPA has been reported[4]. In other studies, nearly 1000-fold increase in Am uptake by beans and maize[2] and by rice[1] due to prechelation with DTPA was observed. The influence of application of DTPA and EDDHA *per se* to contaminated Yolo loam soils on the uptake of Am by bush beans and soybeans resulted in an enhanced plant uptake of Am by a factor of 200 to 1000 with the chelate treatments[11,26,27].

Comparison of the present findings on the influence of chelating agents, DTPA and EDTA, on the plant uptake of Pu and Am from soils indicated broadly similar trends in the behaviour of the two transuranic radionuclides. The highest values of CRs in the case of Pu as well as Am were observed in the treatments where both the radionuclides were applied as DTPA chelates. These findings suggest greater stability of Pu-DTPA and Am-DTPA chelates as compared to that of the corresponding EDTA chelates. Literature survey[7,13] also reveals that under identical experimental conditions in solution systems the values obtained for the stability constants of Pu and Am-DTPA chelates range from 10^{24} to 10^{29} which are significantly higher than those obtained for EDTA chelates of Pu and Am where values of stability constants range from 10^{17} to 10^{19}.

It is noteworthy that in quantitative terms, the highest increase, of the order of 25-fold, in plant uptake of Pu wth Pu-DTPA treatment over control [$Pu(NO_3)_4$ treatment] was obtained in the Oxisol. However, for Am the greatest effect due to prechelation with DTPA, amounting to a 700-fold increase in plant uptake of Am with Am-DTPA over control [$Am(NO_3)_3$ treatment], was recorded in the Pellustert. These results would suggest that Pu-DTPA was more stable and remained soluble under conditions of acidic pH, low CEC and predominance of kaolinite clay (Oxisol), whereas, the soil characteristics of alkaline pH, high CEC and predominance of montmorillonite clay (Pellustert) were favourable for greater stability of Am-DTPA chelate.

Nutrient solution studies

Plutonium uptake The uptake of Pu by bean plants grown in nutrient solution as affected by the chemical forms of Pu added to nutrient solution is reported in Table 4. The observed values of Pu CRs for the treatments $Pu(NO_3)_4$, Pu-DTPA and Pu-EDTA were 0.11 ± 0.02, 4.35 ± 0.41 and 2.94 ± 0.35, respectively, in the case of shoots, and the corresponding values of Pu CRs were 247.6 ± 39.1, 173.5 ± 27.6 and 562.9 ± 52.9, respectively, in the case of roots. Data in Table 4 clearly indicate enhanced shoot uptake of Pu where this radionuclide was supplied in the prechelated forms as Pu-DTPA or Pu-EDTA as compared to the ionic forms (as nitrates). The observed values of 'Transport index' were 17.57 ± 1.88, 4.26 ± 0.63 and 0.33 ± 0.03 in the case of Pu-DTPA, Pu-EDTA and $Pu(NO_3)_4$ treatments, respectively. These data clearly suggest that Pu, when added in prechelated forms was translocated to a greater extent to aerial parts of the plants. Although the influence of chelating agents on the plant uptake of heavy metals from soils in extensively studied[25], no previous data are available on the uptake of Pu in prechelated forms by plants from nutrient solution.

Americium uptake Data on the influence of prechelation of Am with DTPA and EDTA on its uptake by plants grown in nutrient solution are presented in Table 5. The values of Am CRs in the case of shoots for the treatments $Am(NO_3)_3$,

Table 4. Effect of chelating agents on uptake and transport of ^{239}Pu in bean plants grown to maturity in nutrient solution. Duration of growth in labelled nutrient solution = 6 weeks. Total age of the plants = 7 weeks

	Shoots		Roots		
Treatments	Uptake* (nCi/g dry wt)	CR**	Uptake* (nCi/g dry wt)	CR**	Transport index+
$Pu(NO_3)_4$	0.40 ± 0.06	0.11 ± 0.02	924·9 ± 146.6	247.6 ± 39.1	0.33 ± 0.03
Pu-DTPA	16.27 ± 1.54	4.35 ± 0.41	648.2 ± 103.7	173.5 ± 27.6	17.57 ± 1.88
Pu-EDTA	10.57 ± 1.30	2.94 ± 0.35	2103.0 ± 198.5	562.9 ± 52.9	4.26 ± 0.63

* Average value ± standard error of the means (N = 4)

** $\text{CR} = \text{Concentration ratio} = \dfrac{\text{Pu content/g dry plant}}{\text{Pu content/ml nutrient solution}}$

+ $\text{Transport index} = \dfrac{\text{Shoot Pu content}}{\text{Total plant Pu content}} \times 100$

Am-DTPA and Am-EDTA were 1.55 ± 0.18, 2.85 ± 0.40 and 1.86 ± 0.25, respectively, and, in the case of roots the Am CR values were 769.60 ± 40.97, 71.84 ± 16.77 and 784.30 ± 42.42, respectively. The observed value of 'Transport index' in the case of $Am(No_3)_3$ treatment was 4.08 ± 0.09 which was increased to 26.96 ± 5.73 and 5.93 ± 0.74 in the case of Am-DTPA and Am-EDTA treat-

Table 5. Effect of chelating agents on uptake and transport of ^{241}Am in bean plants grown to maturity in nutrient solution. Duration of treatment in labelled nutrient solution = 6 weeks. Total age of the plants = 7 weeks

	Shoots		Roots		
Treatments	Uptake* (nCi/g dry wt)	CR**	Uptake* (nCi/g dry wt)	CR**	Transport index+
$Am(NO_3)_3$	5.32 ± 0.62	1.55 ± 0.18	2634.34 ± 140.11	769.60 ± 40.97	4.08 ± 0.09
Am-DTPA	9.75 ± 1.55	2.85 ± 0.40	245.91 ± 57.35	71.84 ± 16.77	26.96 ± 5.73
Am-EDTA	6.39 ± 0.85	1.86 ± 0.25	2684.68 ± 145.07	784.30 ± 42.42	5.93 ± 0.74

* Average value ± standard error of the means (N = 4)

** $\text{CR} = \text{Concentration ratio} = \dfrac{\text{Am content/g dry plant}}{\text{Am content/ml nutrient solution}}$

+ $\text{Transport index} = \dfrac{\text{Shoot Am content}}{\text{Total plant Am content}} \times 100$

ments, respectively. These data indicate that Am was translocated to aerial organs to a greater extent when supplied in prechelated forms as compared to the ionic form-$Am(NO_3)_3$, in the nutrient solution. Higher values of 'Transport index' when Pu as well as Am were added as complexes of DTPA and EDTA, are suggestive of significant mobility of these chelated forms in the plant systems.

The present results on the influence of chelating agents on uptake of Pu and Am by plants grown in nutrent solutions where interactions with the exchange complex of soil and other soil constituents are absent, confirm the findings of soil-plant studies which have indicated significantly enhanced transfer to plants of these radionuclides when added to soil in prechelated form.

The continued use of chelating agents in intensive agriculture to correct micronutrient deficiencies may result in build-up, if not degraded by soil microbes, of complexing ligands in soils. Although studies on Pu and Am uptake by plants from soils have revealed very limited uptake of these transuranics, the possible formation of complexes with ligands, which can maintain transuranics in soluble plant available forms over extended periods in soil, could markedly influence the quantitative transfer and mobility of these highly radiotoxic nuclides in the soil-plant systems.

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