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Enhancing the transfer of ^{238}U and ^{226}Ra from soils to *Brassica juncea*

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Abstract. In order to test the suitability of using *Brassica juncea* for the remediation by phytoextraction of soils contaminated with ^{238}U and ^{226}Ra, the transfer process to the plant was studied. A soil with high natural uranium mineralization was used for the study. When the soil was not manipulated, the transfer factor (TF) was 0.24 ± 0.02 and 1.5 ± 0.3 for ^{238}U and ^{226}Ra, respectively. These low values, especially for ^{238}U, are due to the radionuclides being associated in the soils with fractions unavailable to the plant. For the possible use of phytoextraction for remediation of contaminated soils it is therefore necessary to enhance the availability of the radionuclides in the soil. In this sense, soil pH is a major factor influencing the availability of these elements in the soil for plant uptake through their speciation. In this work, the effect of pH on the TF of ^{238}U and ^{226}Ra was studied. Also, citrate was used to attempt to enhance the bioavailability of the two radionuclides in the soils, and therefore their uptake by *Brassica juncea* and translocation to the shoots.

1. INTRODUCTION

Naturally occurring radionuclides are present in many natural resources. Human activities that exploit these resources may lead to enhanced concentrations of radionuclides or enhanced potential exposure. Such activities include, for example, the mining and processing of ores, the combustion of fossil fuels etc. The lack of suitable management of these wastes can produce the spread of radioactive contaminants over great extensions of soils and surface and groundwaters [1].

The use of plants to clean up soils and waters contaminated by radionuclides (phytoremediation) has been a subject of burgeoning interest over the last ten years [2–5]. These technologies are especially useful for the decontamination of soils and waters where there are low levels of widespread contaminants. In these cases, where conventional remediation techniques (soil excavation and burial, soil washing, etc.) can be too costly, phytoremediation can be a very suitable alternative. Some authors [6] have estimated that total system costs for some phytoremediation applications will be 50 to 80% lower than alternatives. In particular, phytoextraction refers to the use of terrestrial plants to transport and concentrate metals from the soil into the harvestable parts of roots and above-ground shoots [7, 8].

The selection of an appropriate plant species for decontamination is a crucial step in successful decontamination. From the literature, *Brassica juncea* [4, 9] is a promising candidate for phytoremediation programs.

In this work we studied the suitability of *Brassica juncea* for removing ^{238}U and ^{226}Ra from contaminated soils. The half-lives of ^{238}U and ^{226}Ra are long enough to guarantee long term exposure and the presence of many other radioactive descendants: ^{238}U heads the $4n + 2$ natural radioactive series, and ^{226}Ra, belonging to the same radioactive series, is the parent of an important sub-chain that contains ^{222}Rn. These radionuclides are therefore the major nuclides responsible for the doses to humans from natural radionuclides (through ^{222}Rn and ^{210}Pb). The ^{238}U and ^{226}Ra radionuclides normally present low bioavailability and therefore relatively low mobility (principally due to lixiviation or run-off by surface waters). However, the ^{222}Rn, produced by ^{226}Ra, escapes from the surface soil and, once in the atmosphere, can cause high dose levels to humans by inhalation [10].

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The low bioavilability of ^{238}U and ^{226}Ra in soils can be an impediment to the optimization of the phytoextraction technique. In this sense, it is well known the importance of the pH on element speciation in solution and therefore on the plant uptake process [4, 11, 12]. The use of an organic acid or chelating agents has been used to enhance the bioavailability of heavy metals such as Cd, Cr, Cu, Ni, Zn, etc., obtaining goods results [13, 14] with chelating agents such as ethylene-diamine-tetraacetic acid (EDTA) and citric acid. For natural uranium a few studies have been performed, obtaining good results by using citric acid [9]. However, ^{226}Ra has been less widely studied in the context of phytoremediacion, although numerous studies have been devoted to the determination of its transfer from other environmental compartments (water, soil, or sediment), with particular attention being paid to the soil-to-plant transfer of ^{226}Ra [15, and references therein].

Here, our main objective was to study the influence of pH on the uptake process of ^{238}U and ^{226}Ra by *Brassica Juncea* in order to optimize the phytoextraction process. The effect of the presence of citrate on the bioavailability and therefore on the uptake of these radionuclides by *Brassica Juncea* was also studied.

2. MATERIALS AND METHODS

2.1 Experimental design

2.1.1 Soil and plant samples

Soil samples were collected from a rehabilitated uranium mine in the Extremadura region (south-west Spain). The soils were collected from the upper 10 cm after having removed the root mat. Due to the soils having a natural uranium mineralization, the activity concentrations of ^{238}U and ^{226}Ra are sufficiently high for this type of study to be carried out. The speciation in these soils corresponds to their natural origin (with the highest percentage associated with more resistant phases). The soils are of granitic type with a slightly acid (pH $\approx$ 5), and granulometrically can be classified as loamy sands.

Their water holding/field capacity is about 16%. After the sampling, the soil samples were oven-dried at 80 °C to constant weight, and sieved at a pore size of 2 mm. Physical (texture), chemical (major element concentrations), and radiological (^{238}U and ^{226}Ra activity concentrations) analyses were performed in order to characterize the soil.

Seeds of *Brassica juncea* (Indian mustard) were obtained from the Regional Plant Introduction Station, Ames, Iowa. The seeds were pre-treated with an aqueous solution of $CaCl_2$ (10 mM) in continuous agitation for two hours. Then they were transferred to a container with filter paper as substrate, watered with distilled water, and incubated for two days in a Selecta HotCold600 growth chamber till the roots presented a length of about 2 cm. The germinating seedlings were then transferred to 1 L pots with 0.5 kg of soil previously brought to field capacity.

2.1.2 The pH and citrate experiments

In order to evaluate the influence of pH on the transfer process, seedlings of *Brassica juncea* were grown in pots with different pH values. Firstly, the seedlings were grown for four weeks without any amendment. The field capacity was maintained throughout the experiment by weighing daily and adding de-ionised water when required.

After that, the soil pH adjustment was performed by adding HCl or KOH to raise or lower the pH with respect to the control soil, respectively. The seedlings were allowed to grow for three days more before harvest.

In each pot, nine seedlings were grown. Five replicate pots were considered for each pH value. Twelve pH values were considered in the range 3–10.

The effect of citrate on the assimilation and translocation of ^{238}U and ^{226}Ra by *Brassica juncea* was also studied. After growing the seedlings for four weeks, the soils were amended with tri-sodium citrate

up to a final concentration of 25 m mol $\cdot$ kg^{-1} dry weight. The influence of pH on the citrate effect was also studied. Three pH values were selected: 4, 5, and 8. Although the citrate was added in salt form, the degradation of this organic compound produced a rise in pH and the formation of carbonates. The final pH of the soil three days after the citrate addition rose up to two units higher than the pH of the control soil (pH $\approx$ 8). HCl was used to fix the final pH values to 4, 5, and 8. Three replicates were considered for each pH value.

2.2 Radiochemical method and measurement techniques

The activity concentrations of the uranium and radium isotopes in soils and plants were determined by alpha-spectrometry with PIPS semiconductor detectors of 450 mm^2 active area, housed in NIM spectrometers (Canberra, Mod. 7401VR), coupled to low-noise preamplifiers, amplifiers, and a multi-channel analyser.

The sample digestion prior to the radiochemical assay was performed by acid digestion under pressure in a microwave oven (Millestone Mod. Ethos 900). For soils, the attack was performed using HF and HNO_3 (3 : 6 mL) as reagents, and for plants, the reagents used were HNO_3 and H_2O_2 (6 : 1 mL).

For uranium isotopes, the radiochemical method followed until the preparation of the high-resolution alpha sources was based on the chemical separation by tri-n-butyl phosphate (TBP) [16]. Finally, the sample was electrodeposited [17]. The method used for the determination of radium was based on chemical purification by precipitation of $PbSO_4/BaSO_4$, and the subsequent source preparation by microprecipitation of $Ba(Ra)SO_4$ [18]. The radiotracers used for the determination of the radiochemical yields of uranium and radium were ^{232}U and ^{225}Ra, respectively, added at the start of the radiochemical procedure.

3. RESULTS AND DISCUSSION

Although in each experiment the activity concentration of three radionuclides of natural uranium (^{234}U, ^{235}U, and ^{238}U) was analyzed, the results to be show concern ^{238}U. In our analyses the activity ratio between ^{238}U and ^{234}U was always in agreement with the natural equilibrium, so that any conclusion obtained from the fate of ^{238}U can be equally applied to ^{234}U. The third isotope of uranium, ^{235}U, is considerably less important in the context of this study because it appears in the natural isotopic mixture only as a minor contributor whether in mass units or in activity.

The transfer factor (TF) defined as the ratio of the activity concentration in plant (Bq $\cdot$ kg^{-1}) (only above-ground fraction was considered) to the activity concentration in the substrate (Bq $\cdot$ kg^{-1}) [19] was used to evaluate the assimilation and translocation process. A log-normal distribution was considered for the TF data.

The different TF values were compared by Student's t-test using as null hypothesis the "identical mean" of the populations and considering a 95% confidence level.

The activity concentration (Bq $\cdot$ kg^{-1}) in the soil sample was: 366 ± 34 and 227 ± 62, for ^{238}U and ^{226}Ra, respectively. These results correspond to the mean value and standard deviation of ten aliquots of soil.

The TF values of seedlings of *Brassica juncea* after growing for five weeks in this soil without amendment were: 0.24 ± 0.02 and 1.5 ± 0.3, for ^{238}U and ^{226}Ra, respectively. These values are higher that those found for pasture grass growing in the area where the analysed soil was collected [20], but are too low for remediation purposes. It is therefore necessary to enhance the bioavailability of ^{238}U and ^{226}Ra in this soil in order to improve the assimilation by *Brassica juncea*. The soil pH is a major factor influencing the availability of elements in soil for plant uptake [21]. The experiments designed in order to evaluate the effect of pH on the ^{238}U and ^{226}Ra TF values showed that the behaviour is different for the two radionuclides.

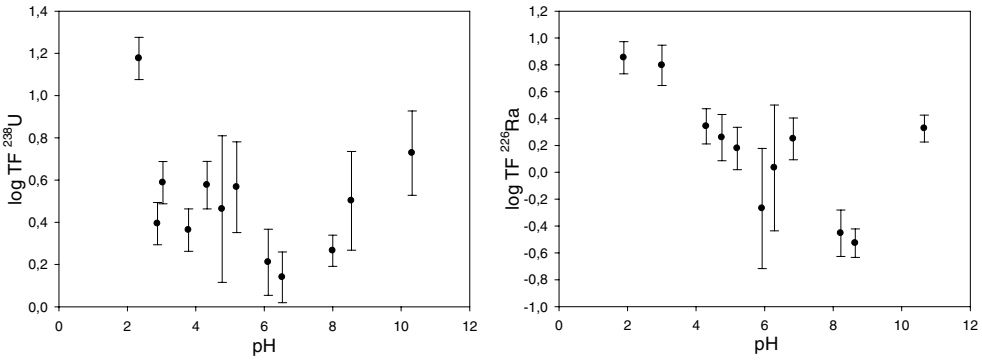

Figure 1. Logarithm of transfer factor for ^{238}U and ^{226}Ra versus pH. Mean values (n = 5) and standard deviations are shown.

In Fig. 1 one can see that for ^{238}U the log TF increases at acidic and basic pH, with the lowest values at pH $\approx$ 6. Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released into the soil solution [21]. This liberation enhances their availability and therefore their assimilation by the plant. On the other hand, with rising pH, there is a tendency for the carbonate concentration to increase which is the most important complexing agent for uranium. Above pH 6, the fraction of uranium forming complexes with carbonates increases, tending to enhance U mobility in the soil and therefore the possible assimilation by the plants [22].

With respect to the behaviour of ^{226}Ra, its log TF shows a clear tendency to increase when the pH decreases. As in the ^{238}U case, under acidic conditions the greater H^+ concentration can displace the Ra-ion from the cation exchange complex. This favours ^{226}Ra uptake by *Brassica juncea*. However, now one does not see the increased TF observed for uranium when the pH rises. This is because radium does not form soluble complexes with carbonates; furthermore, as the pH increases the expected tendency would be to reduce the availability of ^{226}Ra by precipitation (or coprecipitation with Ca(II)) with carbonate and other anions such as sulfate or phosphate.

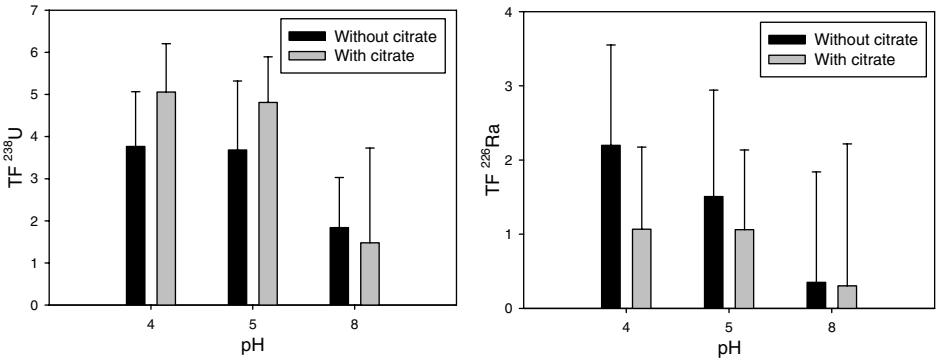

Figure 2. TF for ^{238}U and ^{226}Ra at three pH values, with and without the presence of citrate in the soil. Mean values (n = 5) and standard deviations are shown.

Trying to improve the uptake of ^{238}U and ^{226}Ra by *Brassica juncea*, we performed a second experiment. For this, tri-sodium citrate was added to the soil at a concentration of 25 m mol $\cdot$ kg^{-1} dry weight. The degradation of this organic compound increases the pH and induces the formation

of carbonates. The soil pH three days after the citrate addition reached a value two units higher than the control soil (pH $\approx$ 8). HCl was used to fix the final pH values to 4 and 5.

In Fig. 2 one can see that for ^{238}U the citrate enhanced the TF value for the two acidic pH values, but the differences were not statistically significant. In the case of ^{226}Ra, the opposite was observed. The TF decreases in the presence of citrate in the soil at acidic pH. In this case, the differences observed at pH 4 were statistically significant. It seems that the citrate inhibits the assimilation and translocation of radium by *Brassica juncea*.

One can conclude that the pH is a more important factor in the transfer process than the addition of citrate to the soils. The highest transfers were observed at acidic pH's for both radionuclides studied, but in the case of the uranium isotope another maximum was observed at basic pH (possibly due to the presence of carbonates). Citrate did not increase the TF value to *Brassica juncea* for the radionuclides studied.

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