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Potential of Phytoremediation to clean up uranium-contaminated soil with Acacia species

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ABSTRACT

Pollution by depleted uranium (DU) is considered one of the major problems faced by many countries, where this by-product is considered as a major source of radiotoxic and chemotoxic heavy metal soil pollution. An experiment was designed for uranium uptake from sandy soil treated with different concentration of uranium by using two species of Acacia (*Acacia albida* and *A. nelotica*). Results showed there is a difference in the ability of the Acacia seedlings tested to absorb different concentrations of uranium through their roots. *Acacia nilotica* registered the highest levels of absorption and accumulation of uranium in dry weight of roots in different concentrations (202, 339, 1175, and 1477 µg.g^{-1}) respectively of the concentrations (50, 100, 200, and 500 mgkg^{-1}). Compared to the root of *Acacia albida*, the absorption of uranium was (60, 54, 133, and 526 µg.g$^{-1)}$ in the concentrations of the same samples. The ability of *A. nilotica* is better than that of *A. albida* to uptake uranium from the soil, where 80-90% of the uranium is absorbed by the seedlings, compared to 44-85% in *A. albida*. In the case of low concentrations of uranium (50 and 100 mgkg-1) *A. nilotica* seedlings absorbed about 80-90% of the original concentration, whereast *A. albida* absorbed only 44-70% of the same treatment. In high concentrations (500mgkg-1), we found that the *A. nilotica* uptake of 90% of uranium was higher compared to that of *A. albida* whose average uptake was about 77%. Also, we found a difference between species and treatment in the remainder of the uranium in the soil. The uranium remaining in the soil at the end of the period of uranium application showed a difference between species and treatments. The uptake of uranium by *A. albida* was 14-41% while in *A. nilotica* it it was 58-67%, based upon the concentration in soil solution. In low concentrations (100mg.kg-1) *A. albida* absorbed only 16%, while *A. nilotica* absorbed about 67% from the uranium in soil solution. In high concentrations (2000mg.kg-1) of uranium, 23% was found in *A. albida* and 66% in *A. nilotica*. This shows that *A. nilotica* can uptake uranium from soil solution three times more than *A. albida*.

The biomass results, the shoot height of *A.nilotica* plants decreased with the progression of time during the treatment with high concentrations of uranium, but in low and moderate concentrations (50,100, and 200mg.kg-1) it was less affected than high concentrations (500mg.kg-1). *A. albida* height was reduced when treated with (200mg.kg-1) in all the growth periods, whereas the growth of seedlings gave values less than when treated with the height concentration of uranium (500mg.kg-1) for the two species.

Keywords: Phytoremediation, Depleted Uranium, *Acacia albida*, *A. Nilotica*.

INTRODUCTION

Military wastes are a major cause of uranium soil pollution in the countries that have been caught in wars in recent years, such as Iraq (Jamail 2013, NCCI 2011, Landrigan *et al*. 2004) . This type of pollution originated from the use of ammunition containing depleted uranium ,which is the major waste product of uranium enrichment. DU is the most notorious toxic military contaminant present in Iraq (Zwijnenburg 2013). Uranium is a lithophilic element and, chemically, is considered as the heaviest radioactive element (19.07 g.cm−3 at 25 0C), widely dispersing throughout the earth's crust at the mean content of 2.5 mg kg-1 (Závodská *et a*l.2009). Furthermore, it is a very reactive element, readily combining with most elements to form a variety of complexes (Craft *et al*. 2004) (ATSDR 1999). Also, uranium is considered as a relatively mobile element in the near surface zone with potential migration in the environment, and it may be precipitated by reduction to U(IV), or in the form of uranium minerals (Bleise *et al*., 2003).

Uranium is a toxic metal and an active carcinogen (Alani *et al*. 2011; Polednak and Frome 1981; ATSDR 1999; Fathi *et al*. 2013). We have about 90 mcg. of uranium in our body, and there is some in food and water.

Uranium has low absorption, and fair elimination. The toxicity of uranium usually affects the kidneys (Tasat *et al*. 2012). Total uranium content of the Earth's crust to a depth of 15 - 25 km is approximately 1017 kg, with the oceans containing approximately 1013 kg. Uranium (U) is considered a natural radioactivity heavy metal (WHO 2003). Pollution caused by uranium or depleted uranium is considered the major problem facing many countries as in the case of Iraq. One of the primary causes of this pollution can be directly linked to the effects of military conflicts (Gulf Wars of 1991 and 2003), as well as to different sectors, military industry, remnants of wars or weapons testing. The radiological and chemical properties of DU can be compared to those of natural uranium, in that both possess very similar chemical toxicity (Bleise *et al.* 2003). DU is the decreased portion of U235, while enriched uranium is the increased fraction of U235 (ATSDR 2012).
The understanding of the biogeochemical cycles of uranium, and its movement from soil to vegetation through root uptake, as well as the processes involved in their environmental migration are considered very important for environmental protection and remediation. Many factors influence uranium uptake by a plant from the soil, like uranium concentration in a soil, major ions present in the soil-plant system, effects of rhizosphere processes and soil micro-organisms on bioavailability, with an emphasis on all these factors influencing transport and uptake by roots, and the processes affecting long-term uptake rates (Ehlken and Kirchner 2002). Several species of plants have the ability to absorb uranium, or it may stick to the root surface, while some factors can limit the uptake of uranium by plants (ATSDR 1999) . Uranium in the soil is often toxic to plants, but does not pose a radiological hazard to humans. Uranyl nitrate is an oxidizing and highly toxic compound for organisms and plants, as it contains the isotope U238 of uranium (Vandenhove *et al*. 2006). To prevent the spread of DU in the environment is an emerging challenge to environmental scientists, as they seek to find the best method of uranium remediation, also known as bioremediation (phytoremediation).Uranium is highly soluble and mobile in subsurface environments, and bioremediation of uranium depends largely on reducing its bioavailability in the environment (Ma and Zhai 2012), (Gavrilescu *et al*. 2009). The use of plants to extract U from contaminated soils is an emerging technology (Huang *et al*, 1998).

Phytoremediation of uranium (U) contaminated soil has been hampered by a lack of information relating U speciation to plant uptake and the aims of the present study is to remove Uranium from soil for the safety of the environment. In the Laboratory of Environment Centre Wales (ECW), School of Environment, Natural Resources and Geography/ College of Natural Sciences/ Bangor University, United Kingdom, some studies have been done to examine Acacia plants for Phytoremediation of Uranium.

MATERIAL AND METHODS

The objective of this study was to determine the uptake of uranium from soil, as well as to study the translocation of uranium in plants, and to understand its uptake and accumulation in Acacia seedlings (*Acacia albida and A. nelotica*) from soil contaminated with uranium, and whether different contents of uranium (U) in the substrate affect its concentration in plants and their biomass. This study was also designed in order to investigate the uptake of uranium from sandy soil by *Acacia albida and A. nilotica* seedlings, as well as to study the effects of Uranium on the growth of Acacia seedlings under laboratory conditions (growth cabinet), with a special focus on several subsets including types of phytoremediation of uranium such as phytostabilization, influencing factors (such as plant species, uranium concentration, soil properties, and uranium accumulation by different parts of plants (shoot and root separately), as well as the remaining of uranium pollution in the soil after soil remediation. The soil in which the experiments have been conducted was washed sand.

A pot experiment was conducted with three-month-old Acacia seedlings to remove depleted uranium from soil under laboratory conditions as model crops. 500g of washed sand were taken from each pot for the different treatments, and four replicates were taken for each treatment. An extra set of pots which contained no added uranium were also taken which served as control group. Seeds of two Acacia species were imported from Pakistan and stored at 3-5 0C then germinated for one month. After that, they were transplanted in individual plastic pots (12 cm diameter), and the pots were filled with 500 g of dry sand, placed in a growth chamber. The growth cabinet experiment was supplemented with 16 h of light and 8h of darkness, 25- 30 ℃ day-night regime with 70% humidity. Plant height was measured throughout the growing period on 0 DAP, 7 DAP, 14 DAP, and 21 DAP (days after application of Uranium). Uranium concentration in plant and soil was measured at the end of the application. During the three week exposure period, plants were monitored for uranium uptake and biometric parameters. Three-month-old seedlings (plants approx. 10 cm high) were exposed to different concentrations of Uranium under the form of Uranyl nitrate solutions (UO2(NO3)2), (0.0,50,100,200, and 500 mgkg-1 soil). These

solutions were prepared from a stock solution of 20g-1 of UO2(NO3)2) and the nutrient solution used was ½ strength of Hoagland's solution.

Biomass: The test plants for biomass production were harvested after 21 days of uranium application. Roots and shoots were dried at 80 ◦C for 24 hr, and then weighed separately. The effects of both uranium concentrations for both species of Acacia were expressed in terms of tolerance index (TI) equations (1) (Baker *et al.* 1994). In addition, the production of dry mass was expressed as Grade Growth inhibition (GGI) equations (2), and evaluated through comparison of dry matter production of metal treated and control plant tissues (Leita *et al*. 1993) (Jagetiya and Purhohit 2006).Ione mobility calculated by the equations (4)

$$\text{Tolerance Index} = \frac{\text{Mean biomass of plant species with U treatment}}{\text{Mean biomass of control plant species}} * 100 \quad (1)$$

$$\text{Grade Growth Inhibition} = \frac{\text{Dry mass of control plants} - \text{dry mass of U treated plants}}{\text{Dry mass plants}} * 100 \quad (2)$$

Concentration ratios (CR) equations (3) between plant and soil were calculated separately for shoots and roots, and all results were corrected relative to the dry matter content (Roivainen et al. 2011):

$$CRt,p = [U]p \ [U]^{-1}_{total} \quad (3)$$

[U]p is the concentration ((mg.kg-1(DW)) of uranium in plant parts p (p=root and fine root, and shoot including stem and leaf).

$$CRm,p = [U]p \ [U]^{-1}_{mobile} \quad (4)$$

RESULTS

Uranium uptake in sandy soil by Acacia sp. Results of uranium uptake by Acacia species in sandy soil showed a highly significant ($p < 0.001$) difference in the ability of the Acacia seedlings (*Acacia albida* and *Acacia nilotica*) to absorb different concentrations of uranium in root and no significant effects in shoots between two species. *Acacia nilotica* registered the highest absorption of uranium in dry weight roots in different concentrations (202, 339, 1175, and 1477 µg.g-1) respectively of the concentrations 50, 100, 200, and500 mgkg-1). Compared to the root of *Acacia albida*, the absorption of uranium was (60, 54, 133, and 526 µg.g-1) in the concentrations of the same samples(figure 1). Also, obtained results showed that when uranium was added to soil, concentration of U in plants increased. The effects between species and concentration in root absorption are highly significant ($p < 0.001$) ($F = 13.199$) and ($F = 7.622$) (figure 1,2,3 and 4).

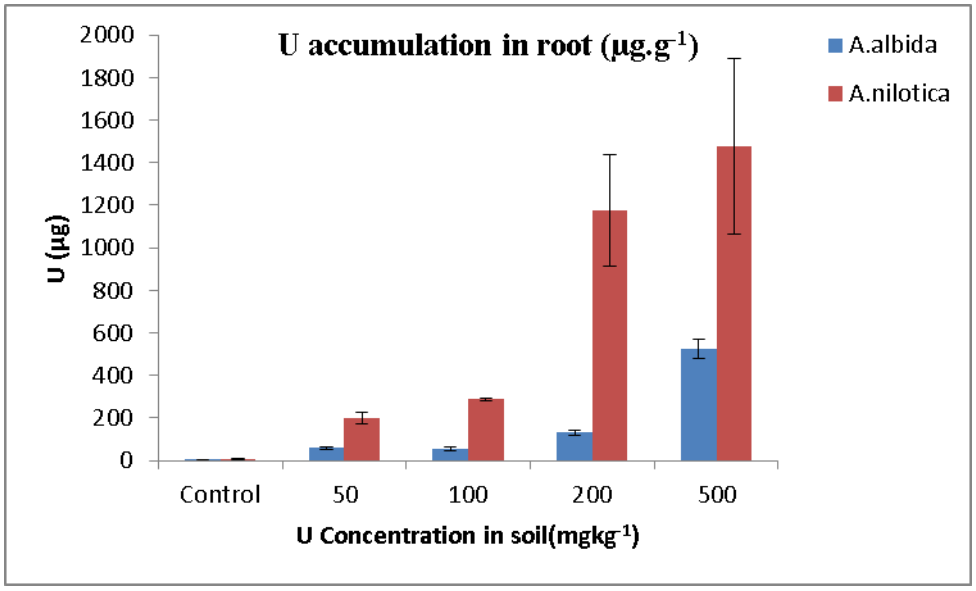

Figure 1: Uranium accumulations in the root of *A. albida* and *A. nilotica* (µg.g^{-1})

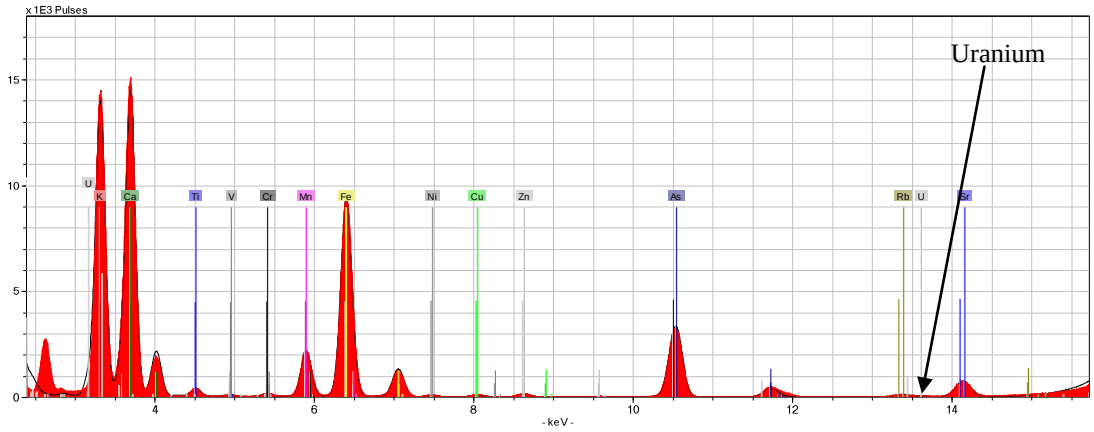

Figure 2: TXRF Chromatogram of *A. nilotica* root sample control ((0 treatment(control))

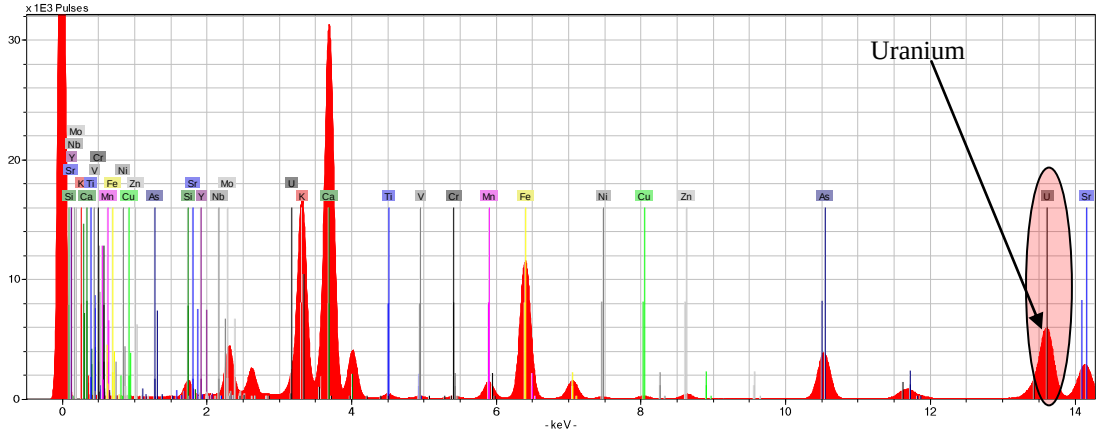

Figure 3: TXRF Chromatogram of *A. nilotica* root sample treated with uranium concentration 100mg.kg^{-1}

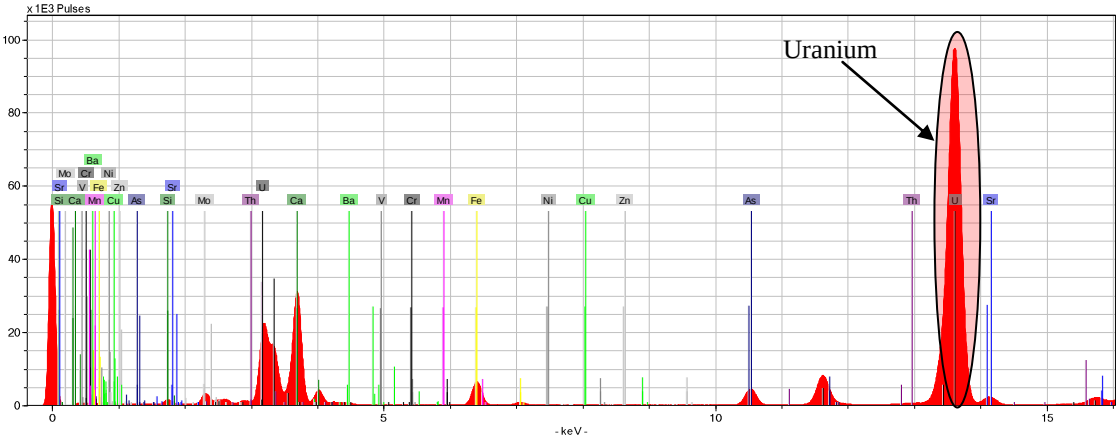

Figure 4: TXRF Chromatogram of *A. nilotica* root sample treated with uranium concentration 2000 mg.kg^{-1}

The accumulation of uranium in the shoots found less than its accumulation in roots in both species with highly significant ($p<0.001$) between species, treatments and interaction of species with treatments. Figure 5 showed the accumulation of uranium in the shoot of *A. nilotica*, which were 16, 28, 50, and101 µg.g^{-1} of dry weight in the different concentrations of uranyl nitrite in soil 50,100,200 and 500 mg.kg^{-1} respectively, while in the shoots

of *A. albida* were 26, 31, 42 , and 112 µg.g^{-1} in the same treatments. In general the quantity of uranium uptake and its accumulation in roots and shoots depend on the concentration of uranium in surrounding soil solutions.(Figure 5).

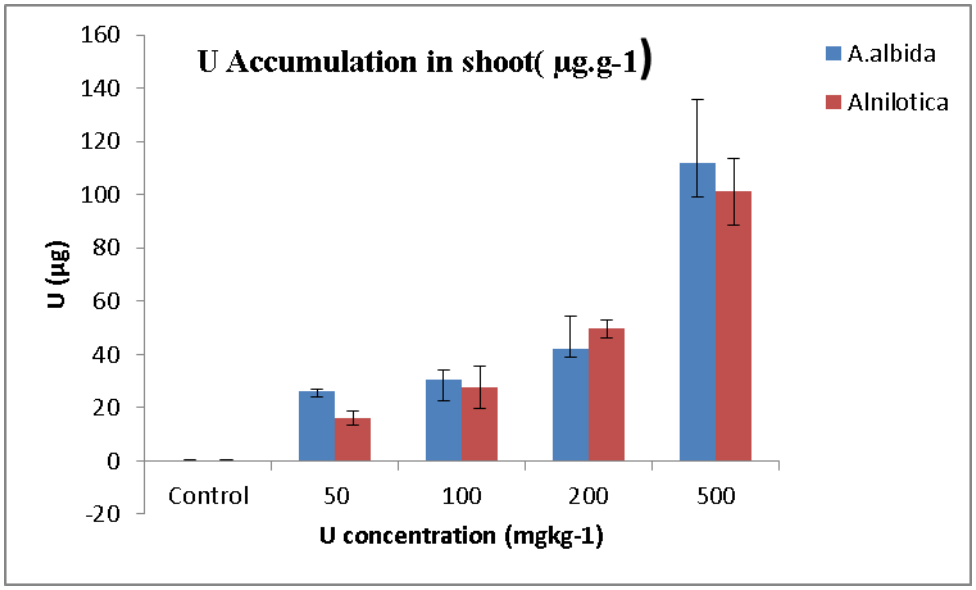

Figure 5: Uranium accumulation in *A. albida* and *A. nilotica* shoot (µg.g^{-1})

At the end of the treatment (21days) of uranium uptake by Acacia seedlings, the uranium remaining in the soil is explained by the following figure (6) which shows that there is a significant difference $p<0.05$ between species but highly significant in the treatment of both species. The ability of *A. nilotica* is better than that of *A. albida* to uptake uranium from the soil, where 80-90% of the uranium was absorbed by seedlings, while in *A. albida* only 44-85% of the uranium was absorbed. In low concentrations of uranium (50 and 100 mgkg$^{-1)}$ *A. nilotica* seedlings absorbed about 80- 90% of the original concentration, while *A. albida* absorbed only 44-70% of the same treatment. In high concentrations (500mgkg-1), we found the uptake of *A. nilotica* was 90% of uranium followed by *A. albida* with an average uptake of about 77.5%. Also, we found a highly significant difference ($p<0.001$) between species and treatment in the remainder of the uranium in the soil (figures 7 and 8).

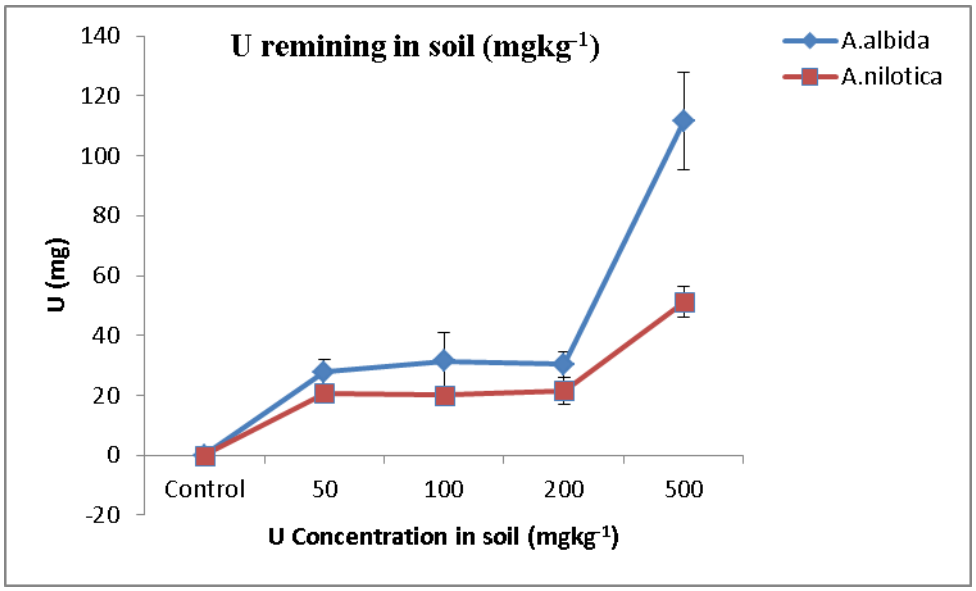

Figure 6: Uranium remaining in the soil after four weeks of uranium application (mgkg^{-1})

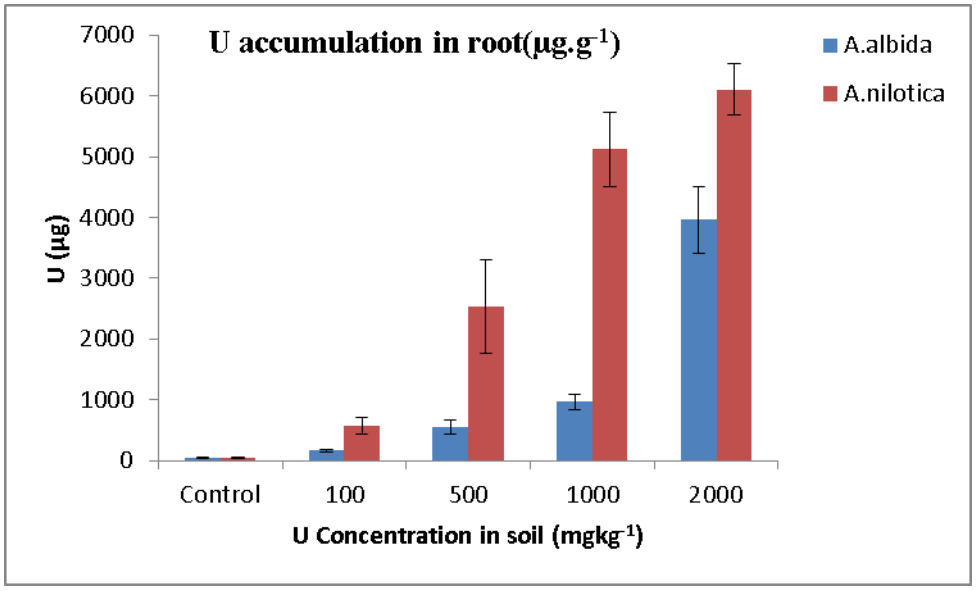

Figure 7: The accumulation of uranium in the roots of *Acacia albida* and *A. nilotica*(µg.g^{1})

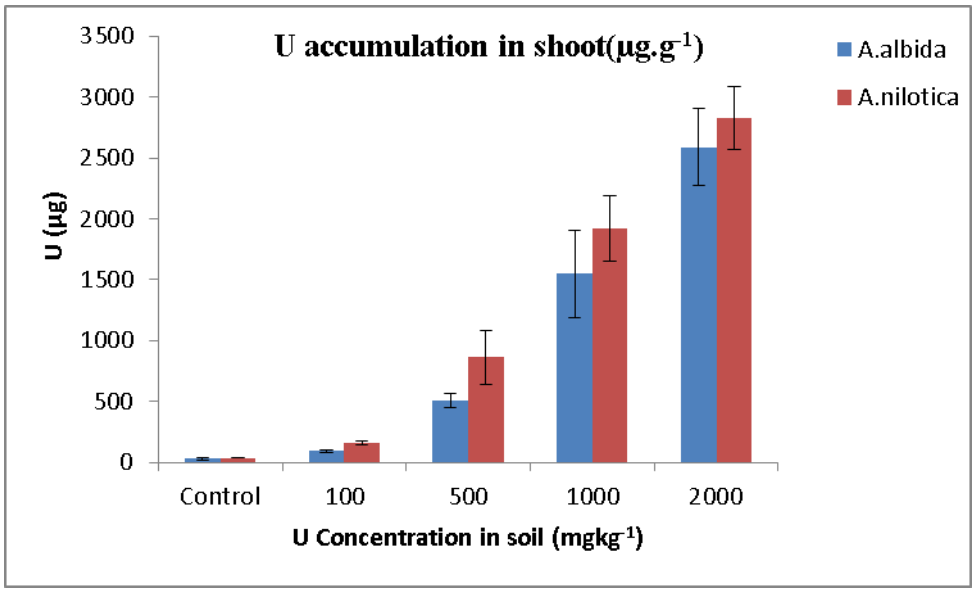

Figure 8: The accumulation of uranium in the shoots of *Acacia albida* and *A. nilotica* (µg.g^{-1})

The uranium remaining in soil at the end of the period of uranium application (3 weeks) was highly significant p<0.001between species and treatments. Figure (9) indicated the quantity of uranium remaining in the soil after plant harvesting. The uptake of uranium by *A. albida* was 14-41% while in *A. nilotica* it was 58-67% upon the concentration in soil solution, in low concentration 100mg.kg^{-1} *A. albida* absorbed only 16%, while *A. nilotica* absorbed about 67% from the uranium in soil solution, and in height concentration 2000mg.kg^{-1} uranium was 23% in *A. albida* and 66% in *A. Nilotica*. This shows that the *A. nilotica* uranium uptake uranium from soil solution is equivalent to three times that of *A. albida*.

Uranium exposure has a highly significant p<0.001 plant growth of different concentrations compared to the control, and in the accumulation of uranium in root, shoot, and uranium remaining in soil at the end of treatment

in both species. The interaction within species and treatments of *A. albida* and *A. nilotica* in the accumulation of uranium in root, shoot and uranium remaining in soil is also highly significant p<0.001.

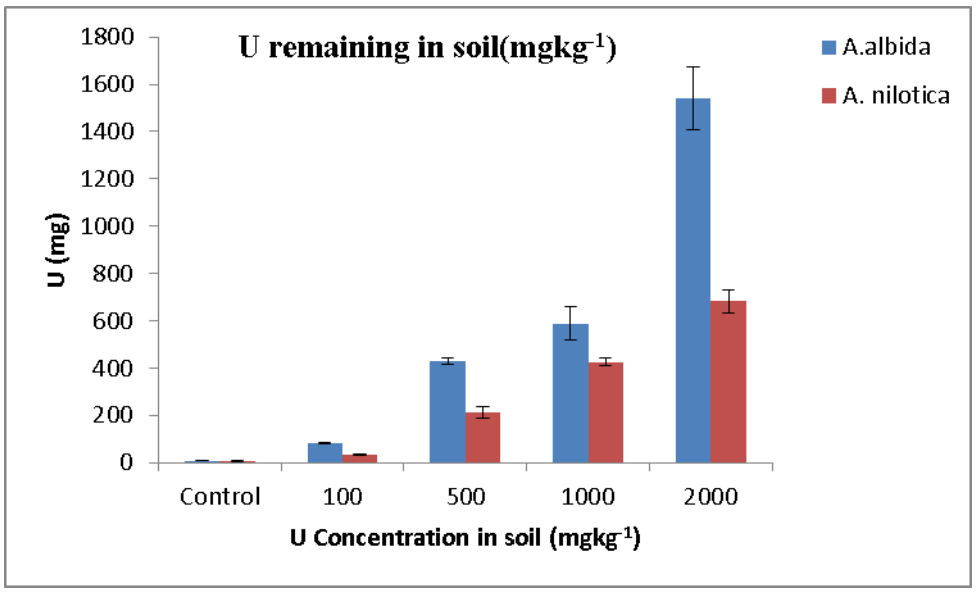

Figure 9: Uranium remaining in soil after plant harvesting ($mg.kg^{-1}$)

From the previous figures (1, 2, 3, 4 ,5 and 6) and table (1), one can distinguish differences between the two species of Acacia studied (*A. Albida* and *A. nilo*tica), and the amount of uranium deposited in the roots and shoots. The percentage of uranium *A. albida* accumulated in roots ranged between 50-66% and in shoots between 33-50% , while in *Acacia nilotica* the percentage was different and ranged between 63.5-73% in roots and 28-36.5 % in shoots. This explains that *A. nilotica* could accumulate higher uranium rates in its roots, up to 2/3 of the whole uranium uptake from soil, but in *Acacia albida* only about ½. This result might indicate that *A. nilotica* is less affected by uranium than *A. albida* on growth and biomass as we see in fig(10).

Table 1 : The percentage of Uranium in roots and shoots from total uranium absorbed by plants (%)

Uranium Concentration $mg.kg^{-1}$	***Acacia albida***		***Acacia nilotica***	
	U in Root %	**U in Shoot %**	**U in Root %**	**U in Shoot %**
100	66.19	33.81	66.95	33.05
500	51.03	48.97	71.93	28.67
1000	55.71	44.29	72.71	27.28
2000	51.54	48.46	63.48	36.52

Biomass: Uranium added in sand culture of Acacia under growth chamber conditions brought about significant reduction in biomass. Uranium at all levels tested was found to be the most toxic metal for the Acacia and caused the most severe reduction in the dry weight of shoot and root especially in the case of high concentrations. These results are consistent with results. Previous studies have also demonstrated a relatively higher phytotoxicity of Uranium on plants (Sarica 1995), (Singh *et al*. 2005). All shoots and roots of plants have noticeable and gradual stunted growth.

The biomass studied consisted of many figures [plant height (shoot length), fresh and dry weight of shoot and root separately], all these affected highly significant p<0.001 by uranium concentration. In the experiment, the shoots length of *A. albida* and *A. nilotica* seedlings affected by uranium treatments compared with the control. Also the results showed that; *A.nilotica* highly significantly (p<0.001) affected, but less than *A. albida* in all concentration used, it's clear that is reduction in the plant height of *A. albida* when treated with 200 $mg.kg^{-1}$ along all the growth periods , and we found that the growth of seedlings gave values less than the control when treated with the height concentration of uranium(500$mg.kg^{-1}$). In general, the height of *A.nilotica* plants decreased significantly (p<0.05) with time progress, but the results showed that the plant height in low

concentration (50, 100, and 200 mg.kg^{-1}) was less affected than in high concentration (500mg.kg^{-1}) (Figures 11, 12, and 13). We can conclude that uranium exposure can significantly affect plant growth of different concentrations compared to the control.

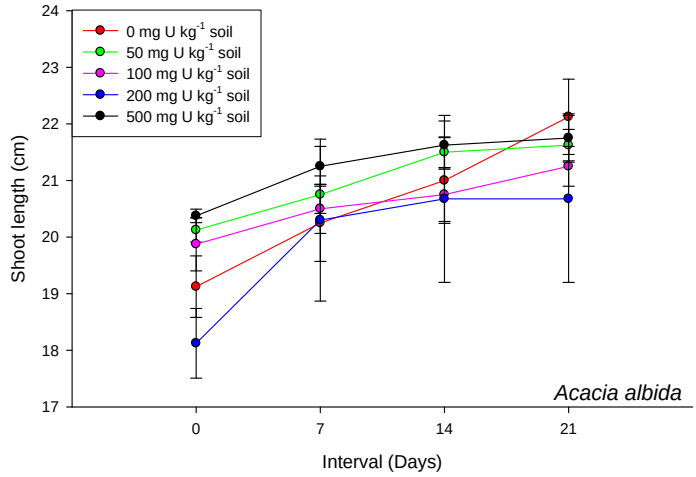

Figure 11: Shoot lengths (cm) as affected by uranium application of *Acacia albida* species

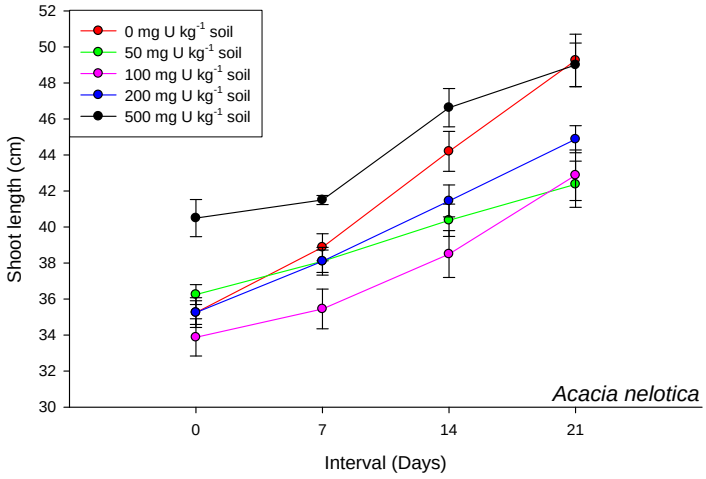

Figure 12: Shoot lengths (cm) as affected by uranium application of *Acacia nilotica* species

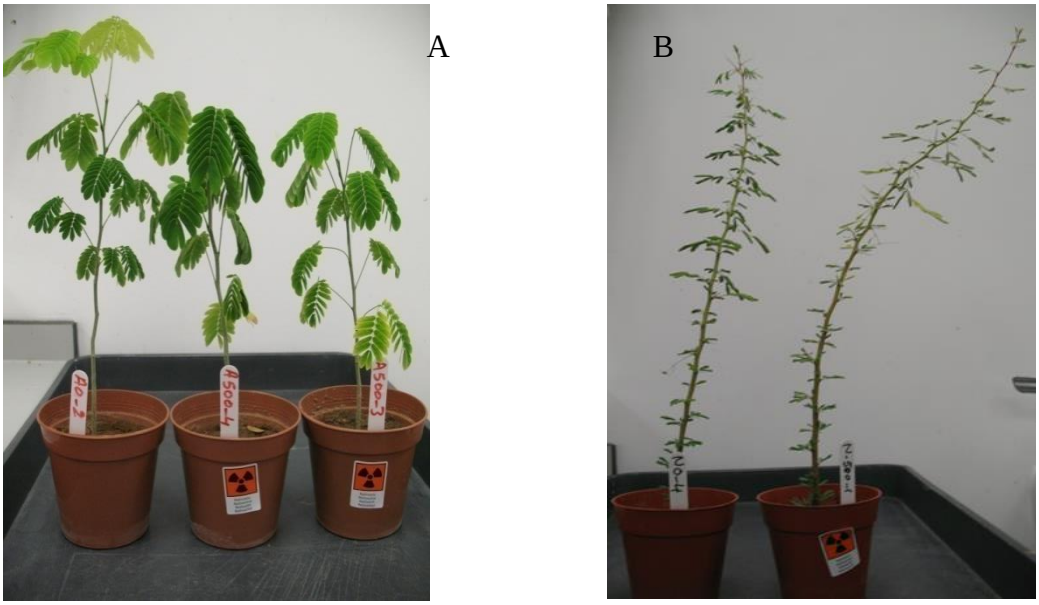

Figure 13: Seedling of *Acacia albida* (A), and *Acacia nilotica* (B) under uranium treatment

Discussion

The goal of this study was to determine the uptake of uranium from soil, as a method of soil remediation of uranium contamination, by a process called (phytoremediation). Secondly, we then studied the translocation of uranium in plants and its effect on Acacia seedlings (*Acacia albida* and *A. Nelotica*). From the results of this work, uranium content is found to vary in different parts (shoots and roots) of Acacia plants grown in soil contaminated with uranium. In soil in which uranium concentration (100-500 $mgkg^{-1}$) is present, *A. nilotica* presented an uptake of 80-90% of uranium, while the *A. albida* average uptake was about 44-77%. This indicates that there is a highly significant difference between species studied. It has been observed that the uranium uptake in plants depends on the nature and age of plants (Singh 2005). Mordtvedt (1996) has mentioned in his study that in all soils that contain available uranium, uranium was found in all plants located in these sites, but lower plants absorb more uranium than higher plants. While uranium is found in higher proportions in the stems and leaves of plants than in cereals and fruit (IRSN 2012). In general, root transfer is considered to be the prevailing pathway for terrestrial plant contamination (Paquet *et al*. 2009). Therefore, there is a strong correlation between the uranium absorbed by plants and the soil's uranium content, where root absorption depends on the same parameters that affect uranium mobility in soil. While the amount of organic matter contained in the soil is crucial, the presence of phosphates, sulfates and carbonates also plays a big role in the amount of uranium absorbed by plants (IRSN 2012). Little physiological information is available on the accumulation of U in plants. Despite this, previous studies by Kochian (2004), Lyubenova and Schröder (2010), McCutcheon and Schnoor (2003) on phytoremediation showed that the hyperaccumulation of heavy metals by plants reveals genes that regulate the amount of metals taken up from the soil by roots and deposited at other locations within the plant. These sites in the plant can be controlled by different genes contributing to the hyperaccumulation trait, and these genes govern processes that can increase the solubility of metals in the soil surrounding the roots as well as the transport proteins that move metals into root cells. From there, the heavy metals enter the plant's vascular system for further transport to other parts of the plant and are ultimately deposited in leaf cells.

Plant growth is found to be affected according to the increase in concentration of uranium in the soil underneath. These findings are consistent with results found from other researchers of different species of plants (Vandenhove 2006) (Shtangeeva1 *et al.* 2006) (Singh *et al*. 2005) (Stojanović *et al*. 2009). For example, the shoot lengths of A. albida and A. nilotica seedlings were affected by uranium treatments compared with the control, but there is a significant difference between the species for the amount of uranium impact. In some

species, uranium exposure did not significantly affect plant growth of different concentrations compared to the control, while others species were highly affected regarding plant growth.

In general , *A. nilotica* can accumulate higher uranium content in the root (2/3 of whole uranium uptake from soil), but for *Acacia albida* the uptake is only about 1/2 . This might indicate that *A. nilotica* is less affected with uranium than *A. albida* on growth and biomass

According to data obtained, it seems that an ideal uranium bioremediation strategy largely depends on a thorough understanding of the genetic, physiological and biochemistry of plants selected, as well as ecological, geochemical features of the selected contaminated site.

Conclusions

We can summarize the results of our study as following:

1. Uranium mobility was observed from the soil to Acacia seedlings, and the uptake of uranium by Acacia seedlings has occurred in laboratory trials.
2. The detection of uranium observed in roots and shoots samples was attributed to the Acacia remediation during the process of uranium treatments.
3. Results of the current study suggest that uranium present in the soil is in metallic or oxides forms, which are basically insoluble and no dissolution of DU had occurred.
4. The movement of uranium from the soil and transfer to plant represent important pathways for the long term of uranium remediation by Acacia species.
5. These results created major challenges to use Acacia trees for soil remediation of uranium or depleted uranium contamination in arid and semiarid regions.

Acknowledgements

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