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Detection of Uranium Contamination in Acacia Cell Sap by Capillary Zone Electrophoresis (CZE) Technique

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Abstract : The study was carried out to detect uranium level in the cell sap of acacia plant cells, for this purpose callus cultures of *Acacia albida* was used as well as plants. Cell saps from both callus and plant leaf were taken using Micro capillary syringe and detected using Capillary zone electrophoresis technique. It was shown that using citrate buffer of 3.0pH help in detecting uranium accumulated in the cells. Prospective calculation for the level of uranium uptake showed that 1.64mM is the level of uranium in the leaf cells that had been grown on soil with addition of 500 $mg.kg^{-1}$ uranyl nitrate for 3 months, while for callus which grown on MS medium with addition of 500 mg/l uranyl nitrate for the same time (3 months), uptake of uranium reached 0.8 mM. The comparison between TXRF analysis of uranium accumulated in plant tissues and CZE efficiency in detecting uranium level in cell sap of both leaves and callus cells, it was shown that both techniques prove that *A.albida* plants can accumulate uranium with a level double as that taken by callus cells.
Keywords : Capillary Zone Electrophoresis, *Acacia albida*, Uranium

Introduction

The impact of environment pollution should be of scientific concern especially the radiation contamination , in order to minimize the threat of environment pollution. (de Matos *et al*. 2001). Metal contamination found within food grown in Iraq exceeds current WHO levels in some of the regions and there is serious concern about the presence of depleted uranium in Iraq. The Iraqi environment, in some regions, is heavily polluted by various toxic metals and radioactive pollution especially in the soil, water and air (Fathi, *et al.* 2013) . The pollution pathways have had serious impacts on the food chains and subsequently on human health, largely through plant uptake into edible food crops (Shatalov *et al*. 2011). Uranium is a toxic metal, also a reproductive toxicant (Craft *et al*. 2004), (Hindin *et al.,*2005). Soil contaminated with uranium possess a long-term radiation hazard to human health through exposure via the food-chain and other pathways. So, any mechanism for reducing the environmental exposure to depleted uranium will greatly reduce the risk of human exposure (Fathi *et al*. 2014).
Endeavors in capillary electrophoresis began as early as the late 1800's , but in 1930, Arnes Tiselius first showed the capability of electrophoresis to separate proteins in free solutions(Sam. 1992), (Petersen and Mohammed 2001). Capillary Zone Electrophoresis (CZE) is the most commonly analytical used technique to detect trace organic and minerals in a free mixture solutions, which can be separated ions into its individual components quickly and easily, also provides high resolution separation (Tomos and Sharrock, 2001) (Stuart and Sweedler, 2003). The separation is based on the differences in electrophoretic mobility, molecule charge, the viscosity of the solvent and the atom radius.(Sam 1992), (Kevin 1995). Capillary electrophoresis" describes also as a family of related techniques in which separations are carried out in a narrow bore capillary due to an electric field applied between its ends (Tagliaro *et al*. 1996). It is suitable for chemical, biochemical and pharmaceutical fields. (Kuhn and Hoffstetter-Kuhn 1993; Camilleri, 1993). The separation could be carried out using various types of samples, ions, isotopes, toxins and proteins. In capillary zone electrophoresis (CZE), ions are separated according to their charge to-size ratio and because of the differences in their electrophoretic mobility (Rovio 2012). Capillary electrophoresis has already been used in many areas, such as pharmaceuticals ,food and beverages, environmental and clinical analysis (Wasielewska, *et al.* 2014). It is both the simplest and most efficient separation technique available for the analysis of both large and small molecules (Loken *et al*., 1990; Beckers and Bocek, 2000). Capillary zone electrophoresis(CZE) performed in buffer-filled capillaries, normally from 25 to 100 pm in internal diameter (Mosher *et al*. 1992; Landers 1997).
Uranium measurements (from various sources) using CZE were very limited, may be because of the complexicity of uranium which had the ability to interact with other compounds to form various complexes (Mishra, *et al.* 2011). One of the main advantages of using CZE for determination of Uranium is the possibility of formation of anionic complexes (Sladkov 2008).
Uranium translocation among tissues of Indian mustard *Brassica juncea* and this is recommended as a potential species for phytoextraction for U-contaminated soil due to its high U accumulation of aboveground biomass (a.c. 2200 lg per plant) (Peichun Chang *et al.* 2005). Stojanović *et al.* (2009) studied the accumulation of Uranium in

plant tissue shoots and roots of corn—maize (*Zea mays*).
The aim of this study for the first time is to detect uranium level in the cell sap of acacia callus cultures as well as plant using CZE technique to evaluate using Acacia trees in phytoremediation programs in the polluted soils .

Materials and Methods

A sequent of experiments conducted in 2010 and 2011, in the growth chamber of the Environment centre Wales (ECW), laboratory School of Environment, Natural Resources and Geography/ College of Natural Sciences/Bangor University United Kingdom.
Seeds of *Acacia albida* were imported from Pakistan and stored at 3-5 ^{0}C then germinated for one month after that transplanted in individual plastic pots (12 cm diameter) with 500g of soil, pots placed in growth chamber supplemented with a 16 h light and 8 h dark , 25- 30°C day-night regime with 70% humidity . Plants of two – months age (Fig 1 A) were exposed to different concentrations of Uranium as Uranyl nitrate solutions ($UO_2(NO_3)_2$) 100,500 and 1000 mg.kg-1 soil, with ½ strength of Hoagland solution used as nutrient solution (Fathi *et a*l. 2014).
Callus cultures:
To initiate callus sterilized seedlings of 25-30 days old were excised into 1-1.5 cm for stem explants and cultured on MS (Murashige & Skoog,1962) which supplied with 0.5mg.l-1 of each NAA and and BA ,and addition of 500 mg/l Uranium (as Uranyl Nitrate $UO_2(NO_3)_2$) (Al-Salih *et al*. 2013).callus cultures of 3 months age (Fig 1B) were used for sampling with capillary Zone Electrophoresis.

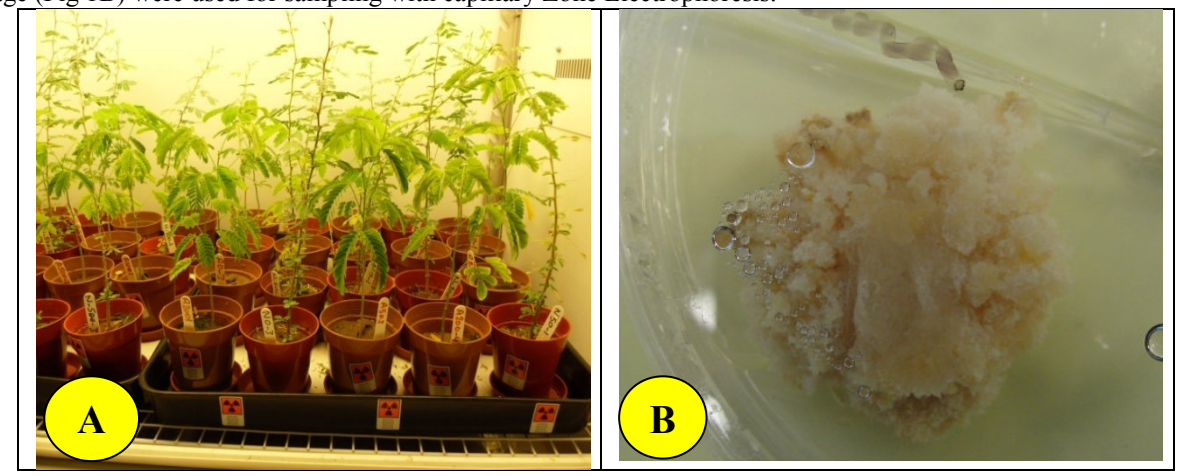

Figure 1 : *Acacia albida*: A- plants treated with500 mg/l Uranyl Nitrate grown in the growth chamber, B- callus culture grown on MS with 0.5mg.l-1 of each NAA and BA and addition of 500 mg/l Uranyl Nitrate.
Uranium detection with TXRF :
Shoots from plants of three months age which grown on soil with addition of 100 ,500 and 1000 mg/l Uranium (as Uranyl Nitrate, 2 g of leaves were cut into small pieces , cell saps were collected by freezing and thowing ,. 10 µl of arsenic was added as standard. 5 µl of the treated cell sap was applied directly into a polished carrier made of quartz or acrylic glass, and dried on heat plate (80 ^{0}C). The disc was then placed in the TXRF for analysis, uranium and other elements contained in plants by quantitative analysis Total Reflection X-ray Fluorescence spectrometer (Bruker,S2 PICOFOX , USA), which is optimally suited for trace element analysis . with the same steps 2 g of callus cultures of the same age (3 months) grown on MS solidified medium with addition of 0.5mg.l-1 of each NAA and BA and 100, 500 and 1000 mg/l Uranyl Nitrate, were also exposed to freezing and thawing to obtain the cell saps which were then prepared to TXRF analysis .
Capillary Zone Electrophoresis:
Vacuolar samples were taken from the upper epidermis of the leaf of three-month age plant according to the method mentioned by (Bazzanella *et al.* 1998). The same method was used also to take vacuolar samples from callus cells (Fig 2). Cell sap is the cytoplasm jellylike material outside the cell nucleus in which the organelles are located, while Protoplasm is the extraction of cell contents (cell sap sampling) and the analysis of extracted cell sap through it.
The experiments were carried out using CE system ,this system was equipped with a lambda 1000 UV detector (Bischoff ,Leonberg, Germany) and a high voltage power supply type HCN 6 M-30000 from FUG (Rosenheim, Germany). Untreated fused silica capillaries (Chromatographie Service, Langerwehe, Germany) of 75-µm I.D. x 360 µm O.D. were used. The length of the capillary was 70-100 cm on the injection side 2 cm of the polimide coating was removed and the outer diameter of the capillary tip was reduced to 150 µm by manual grinding on a fast rotating corundum plate. Microcapillaries were used for sampling and for measuring identical aliquots of samples. Capillaries for cell sap sampling were 1.0 mm O.D. ×860 µm I.D. (Clark Electromedical Instruments,

Pangbourne, reading, UK) to a tip diameter of about 1 µm using a vertical pipette puller Model 700C (David Kopf Instruments, Tujunga, CA, USA).

Droplets of samples taken with volumes of nl-range was produced using an ultra-micro pump (world Precision Instruments, Berlin, Germany), equipped with a modified micro syringe (ILS, Stützerbach, Germany) with luer connected glass microcapillary. Sample injection was carried out by moving the end of the separation of capillary with a micromanipulator (Leitz, Wetzlar, Germany) towards the sample droplet. All operations were observed with stereo-scopic microscope (Fig. 3) For determination of cations, an electrolyte consisting of 5 mM imidazole and 2 mMof 18-crown-6 (Merck, Darmstadt, Germany) was used, the pH was adjusted to 4.5 for Uranium determination electrolyte of Citrate buffer 1 mM pH 3.0 was used.

A micro capillary with an electrolytes were dissolved in tip diameter of 1 mm is filled with water-saturated silicon oil. Using a micromanipulator, the microcapillary is inserted into the vacuole of a plant cell. Due to the cell turgor, vacuolar sap immediately enters the tip of the microcapillary. The microcapillary is then rapidly removed from the cell since the collapsing turgor will draw water from the surrounding tissue into the cell osmotically, which can lead to sample dilution. After sampling the tip is immediately immersed in a polyethylene petri dish filled with paraffin oil in order to protect the sample from evaporation. The sample droplet, which calculation of the subsample volume and the solute has a volume of about 10 to 60 pl, is ejected onto the bottom of the petri dish, which contains several 5-nl (Fig. 3) .

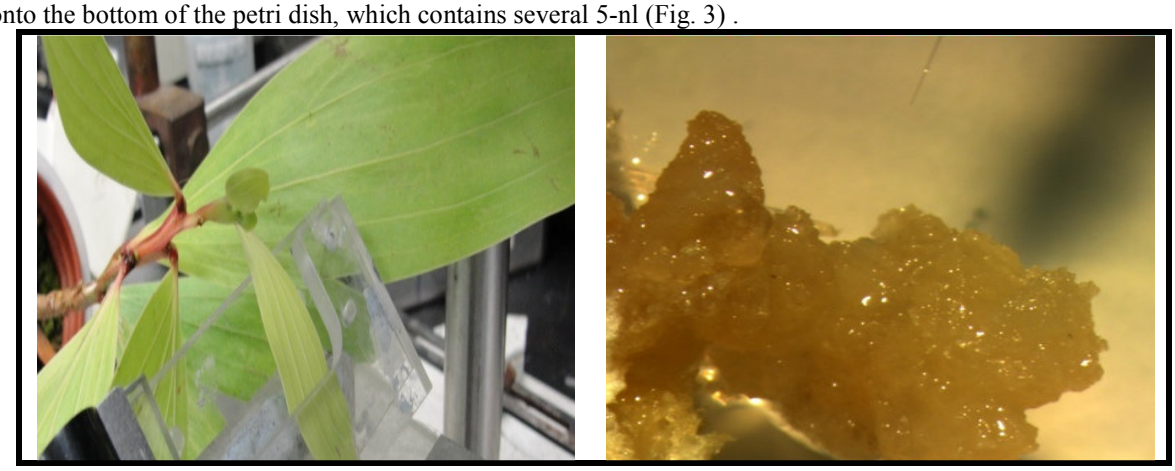

Figure 2 : Samples of leaf and callus used in the microcapillary tests .

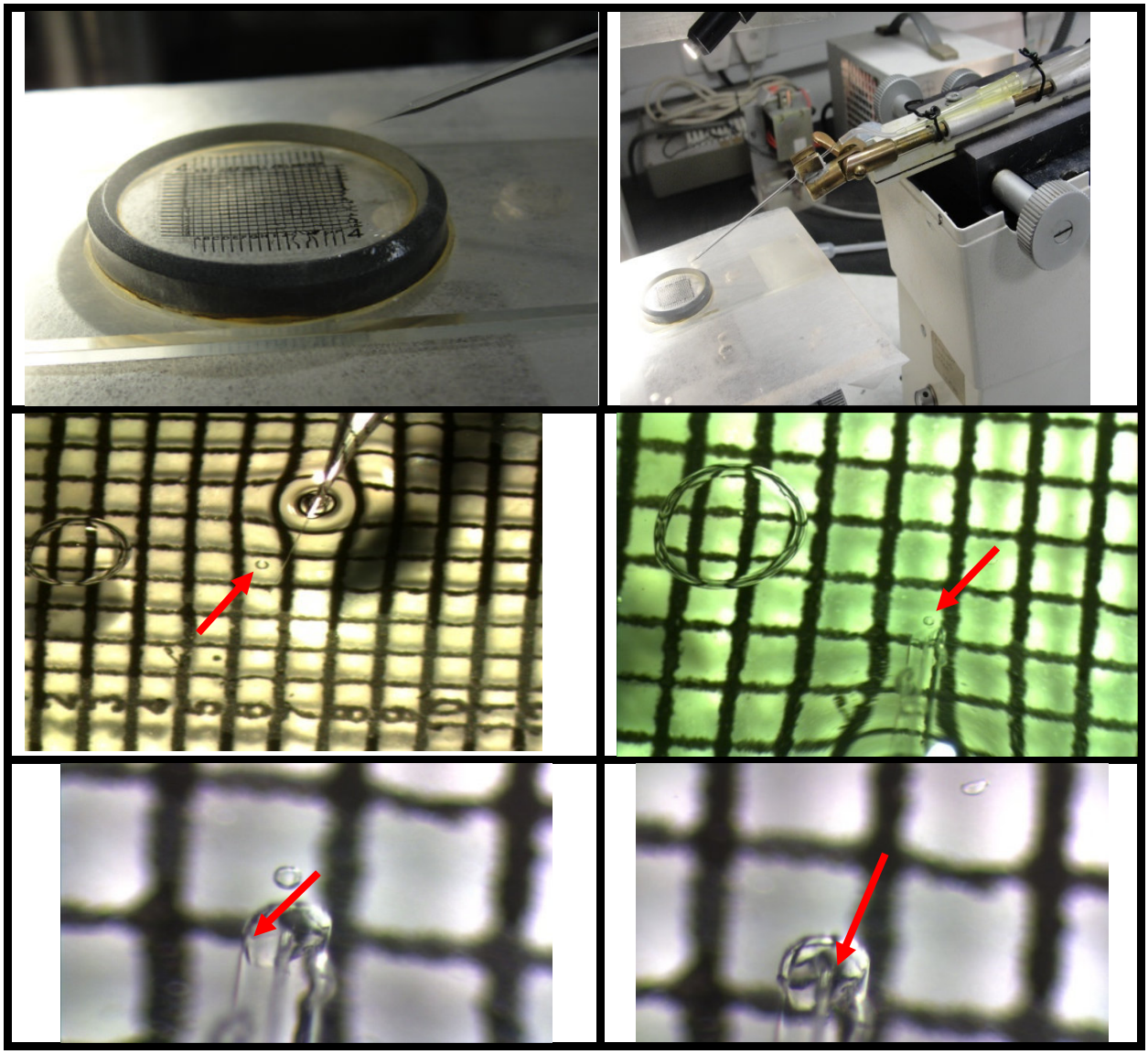

Figure 3: Steps of Droplets of samples taken by Micro syringe

RESULTS:

Uranium detection by TXRF:

Results in figure (4) showed that both callus and shoot cells had the ability to uptake and accumulate uranium, but with different levels. Shoots prove to take uranium from soil with about two times as that taken by callus cells from the nutrient medium, the highest level of accumulation of uranium in callus occurs when shoots and callus cells treated with 500 mg/l uranium, while treatment with 1000 mg/l showed a good accumulation level, but less than 500mg/l.

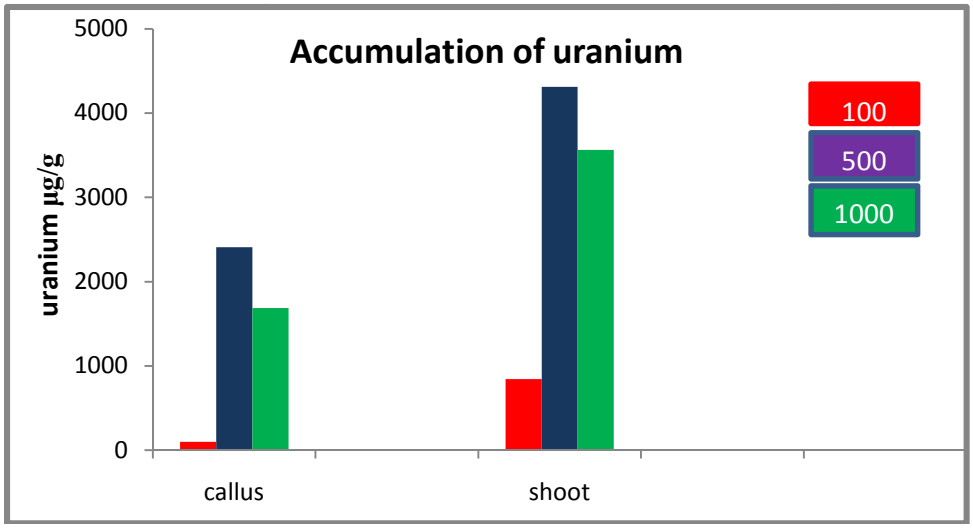

Figure 4: Accumulation of Uranium in callus of shoots and callus tissue of *A. albida* by TXRF.

Uranium detection by CZE:

Results revealed that the using CZE technique analysis is successfully help in identification of uranium uptake by acacia plant and callus cells .

After a series of experiments, using imadazole buffer and Citrate buffer, it was found that using citrate buffer with pH 3.0 was the best to identify uranium (in the form of uranyl nitrate as standard solution). Uranium separated and identified by CZE within a time of 6 min. when many concentrations of the standard solution of uranyl nitrate used (Fig. 5), also micro capillary samples taken from the cell sap of callus cells and the epidermal leaf cells showed that uranium was identified (Fig. 6).

Prospective calculation for the level of uranium uptake showed that 1.64 mM is the level of uranium in the leaf cells that had been grown on soil with addition of 500 mg/l uranyl nitrate for 3 months, while for callus which grown on MS medium with addition of 500 mg/l uranyl nitrate for the same time (3 months) uptake of uranium reached 0.8 mM .these calculations were made depending on the relation between droplets (micro capillary) volume and peak volume (Fig. 7).

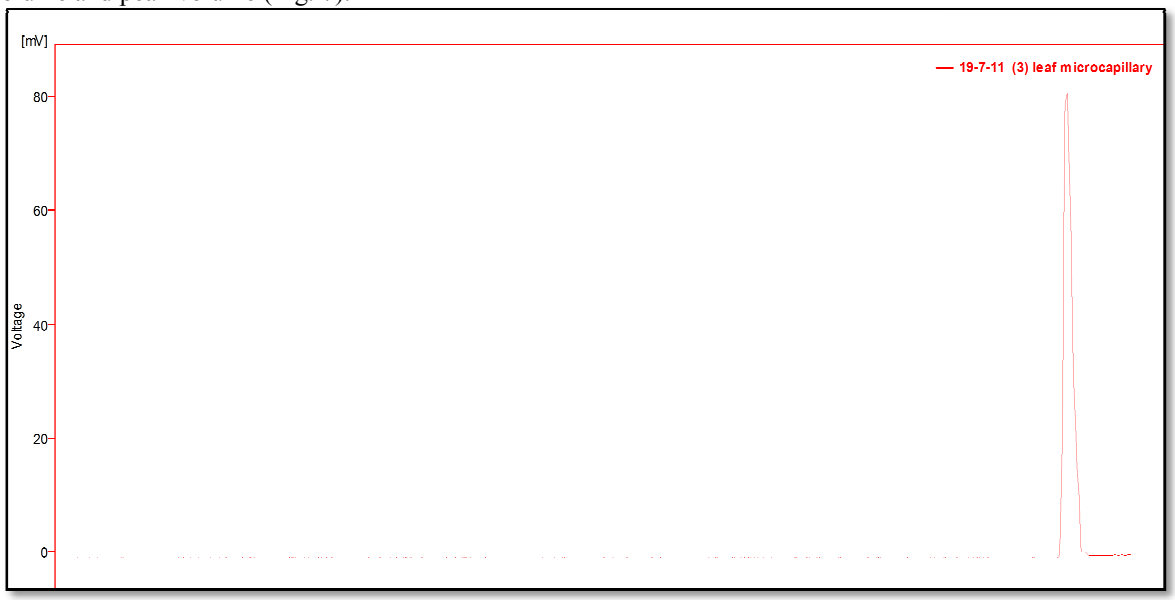

Figure 5: Standard Uranyl nitrate 1mM analyzed by Microcapillary CZE

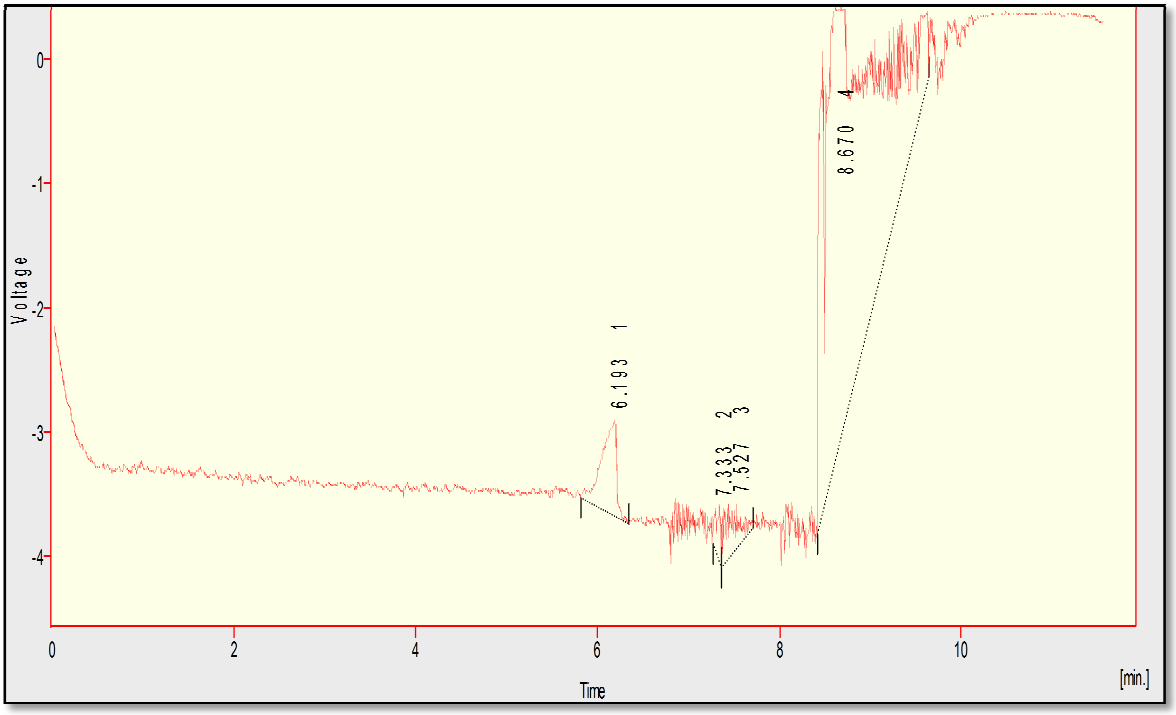

Figure 6: Microcapillary *A. albida* callus grown for three months on MS medium with addition of 0.5mg.l^{-1} of each BA and NAA 500 mg.l^{-1} of uranyl nitrate

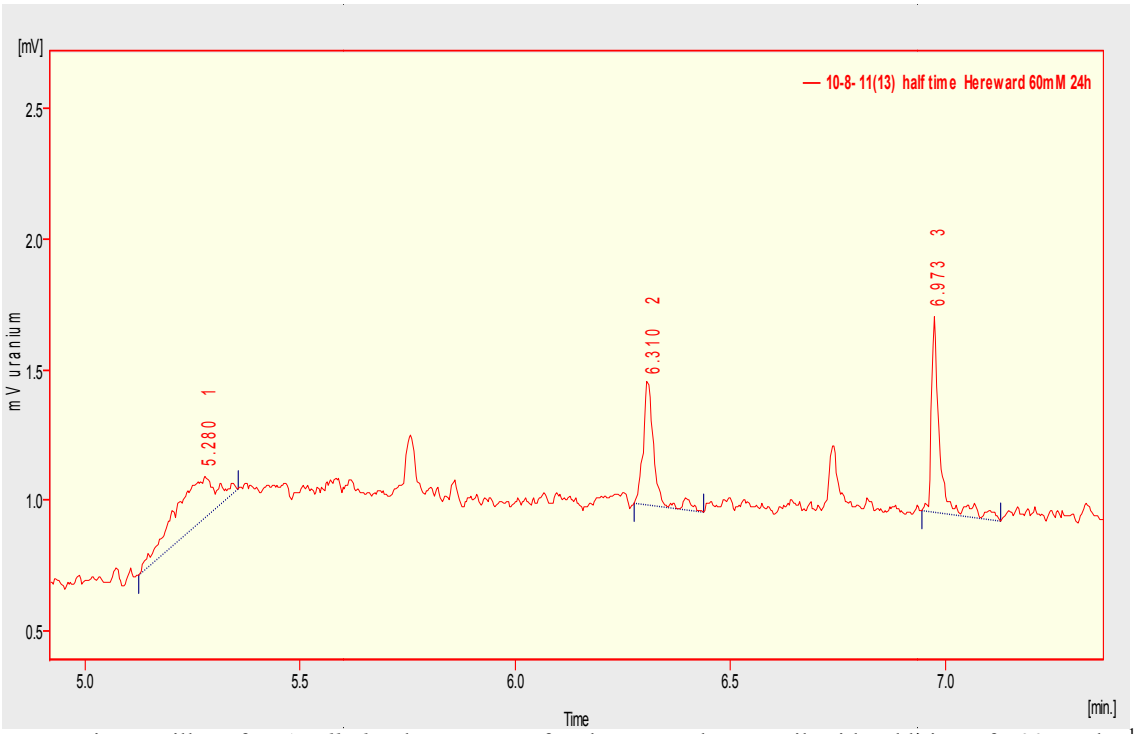

Figure 7: Microcapillary for *A. albida* plant grown for three months on soil with addition of 500 mg.kg^{-1} of uranyl nitrate.

Results of CZE revealed that leaf cells can take and accumulate uranium as it did callus cells of *A. albida*, but the ratio differ, leaf cells can accumulate about 67% which is double the amount of uranium than that accumulated by callus cells that reached to 33% (Fig. 8 and 9), this result agreed with the ratio detected by TXRF (Fig. 4), and it was explained by the same concept, that callus cells depend on passage of nutrients from cells which are directly contact the medium to the other cells .

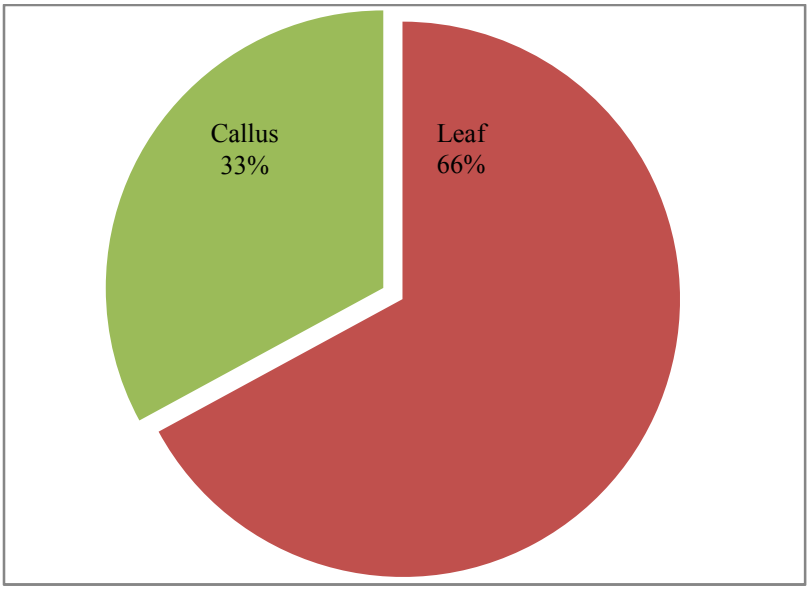

Figure 8: Accumulation of uranium in *A. albida* detected by CZE technique.

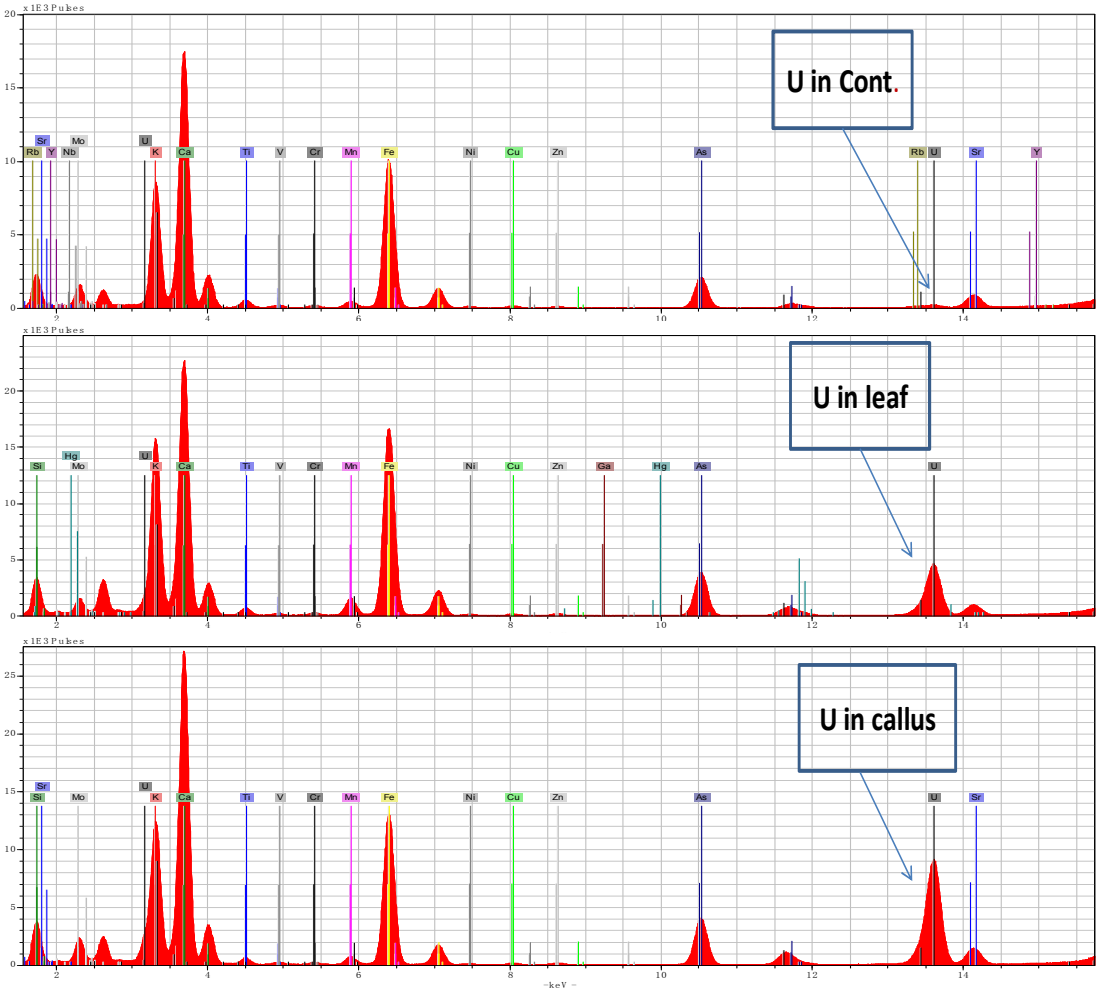

Fig 9: Uranium accumulation in leaf and callus by TXRF analyzer

DISCUSSION

The comparison between TXRF and CZE efficiency in detecting the level of uranium, cell sap of both leaves and callus cells, it was shown that both techniques prove that *A.albida* plants can accumulate uranium with a level double as that taken by callus cells. This result was explained depending on a fact that the plant can get continuous stream of nutrient within its conducting elements, whereas callus cells as it is known can get the nutrients from the medium by indirect uptake from cell to cell (Razdan 2003), also TXRF analyzer measure the uranium accumulation in whole tissue intercellular and intracellular as well as the accumulation on cells wall, while CZE measure only the uranium in cell sap only.

For the assurance of uranium uptake from the soil in case of the acacia plants, and from the nutrient medium in case of callus, for that experiments using CZE analysis carried out which showed that conc. of uranium varied with the variation of its additive level in the soil or in the medium. The behavior of plant organs represent the integral sum of that of their individual cells, analysis of tissue homogenates, is therefore of limited value as it only provides an average of the contribution made by many different types of cell. In recent years techniques designed to look at plant physiology with the resolution of individual cells (Tomos and Sharrock 2001).

Researches on single-cell CE aimed to increase sensitivity for the small quantities of a wider range of analytes to be measured (Stuart and Sweedler 2003). It was mentioned that capillary electrophoresis has advantages that only a small amount of sample required, the analysis time short, as well as that electrolyte preparation is simple (Liu and Sheu 1992).

This study succeeded in uranium identification in leaf and callus cells of *Acacia albida* for the first time using citrate buffer, in detection of uranium more than other buffers, although we use variety of buffers, but in most cases we obtained instable readings, but we making decision to use citrate buffer that playing the major role to get clear separation of the level of uranium in callus and leaf cells. It was mentioned that the separation by CE depends on the differences in electrophoretic mobility within electophoretic media which run in the capillaries (Li 1992).

Several studies mentioned that Humic acid can play a role in detecting uranium using CZE for its feature to produce complex with uranium, this complex can be separated by CZE (Smith and Martell 1976), which helps in using humic acid its existence in soil, water and sediments .in this study we used humic acid but we cannot get a clear separation, for that when we change and use citric buffer pH 3.0 we got efficient separation by CZE, this is consistent with the results found by Pacheco and Havel (2001).

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