The 4th International scientific Conference on Nanotechnology& Advanced Materials &Their Applications (ICNAMA 2013)3-4 Nov, 2013

Uptake of Zinc Nanoparticles by Prosopisfarcta L.Plants Callus Cultures

Rana Tariq Yahya
College of Science, University of Mosul/ Mosul
Email:biology19802007@yahoo.com
Dr. Hanaa Saeed Al-Salih
College of Science, University of Mosul/ Mosul

ABSTRACT

The current study showed the ability of Prosopisfarcta L. plants to uptake and accumulate pollution caused metals present in the environment, and their role on the phytoremediation by indication of Zinc oxide nanoparticles uptake at various concentrations.

For this purpose Zinc oxide nanoparticles of 120 nm were used with concentrations of 1.0, 10, 50, 100µg ml^{-1}.

Resultsof this study referred to the formation of *Prosopisfarcta*plants stems callus on MS modified medium supplemented with 1.0 mg L^{-1} NAA (Naphthalene acetic acid) and 4.0 mg L^{-1} TDZ (Thidizurone). It was clear from the results that callus cultures of *Prosopisfarcta* had the ability to uptake and accumulate nanoparticles of Zinc oxide.Scanning electron microscope (SEM) photography showed that 100µg $ml^{-1}$of Zinc oxide nanoparticles was the higher to be accumulated by callus tissues ,photographs showed a high density of these particles on the surface of the cells. In the same time a linear increasing of callus fresh weight with the increasing of Zinc oxide nanoparticles concentration was recorded. The study revealed that Prosopis seedlings had the ability to uptake Zinc oxide at 50 µg ml^{-1}more than the other concentrations.

استقطاب الدقائق النانوية لأوكسيد الزنك بواسطة مزارع كالس نباتات الخرنوب.Prosopisfarcta L

الخلاصة

اظهرت الدراسة الحالية قدرة نباتات الخرنوب.Prosopisfarcta L على استقطاب وتجميع العناصر الملوثة المتواجدة في البيئة ودورها في المعالجة النباتية بدلالة استقطابها عنصر الزنك المستخدم بهيئة دقائق نانوية لاوكسيد الزنك.

لتحقيق هذا الهدف استخدم اوكسيد الزنك النانوي بدقائق بحجم 120نانوميتر وبتراكيز مختلفة هي 100, 50, 10,1.0 مايكروغرام مل$^{-1}$.

اشارت نتائج الدراسة الى تكوين مزارع كالس سيقان نباتات الخرنوب على وسط MS المحور والمزود باضافة1.0 ملغملتر$^{-1}$NAA (Naphthalene acetic acid) و4.0 ملغم لتر$^{-1}$TDZ (Thidizurone). وبدا واضحا من النتائج قدرة نسيج كالسالخرنوبعلى استقطاب وتراكم الدقائق النانوية لاوكسيد الزنك. وبينت نتائج فحص عينات الكالس باستخدام المجهر الالكتروني

الماسحScanning Electron Microscope (SEM) ان التركيز 100مايكروغرام مل$^{-1}$ كان الاكثر تراكما على اسطح خلايا الكالسوبكثافة عالية. وبذات الوقت لوحظ زيادة طردية للوزن الطري للكالس مع زيادة تراكيز اوكسيد الزنكالنانوي. وبصورة مماثلة اوضحت الدراسة قدرة بادرات نباتات الخرنوب على استقطاب اوكسيد الزنك بتركيز 50مايكروغرام مل$^{-1}$ اكثر من باقي التراكيز المستخدمة .

INTRODUCTION

Nano particles refer to materials and components which have at least one dimension in the size range 1-100 nanometer (1). Combined with nanotechnology, Zinc oxide nanoparticles can be prepared, which possess some unique characters, such as small particle size and large area surface. Zinc oxide nanoparticles have selective toxicity and are generally regarded as a safe reagent to human and animals (2).Zinc oxide nanoparticles have their own importance due to their vast area of applications, e.g., gas sensor, chemical sensor, bio-sensor, cosmetics, storage, optical and electrical devices, window materials for displays, solar cells, and drug-delivery and in agriculture (3,4).

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.). Common heavy metal contaminants are: Cd, Cr, Cu, Hg, Pb, and Zn. Contamination, however, resulted from industrial activities, such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuelproduction, fertilizer and pesticide application, and generation of municipal waste (5).Heavy metals, such as Zinc (Zn) and copper (Cu) are required in trace amounts by higher plants to complete their life. In extended concentrations, however, all are toxic (6). Zn is found to be involved in many cellular functions such as protein metabolism, photosynthetic carbon metabolism andindole acetic acid metabolism, yetits higher concentrations cause toxicity(7).Phytoremediation is a promising technology using plants to remove contaminations as heavy metals and radioactive elements from the environment ,it is a generic term for several ways in which plants can be used to clean up contaminated soil and water (8). According to the concept of phytoremediation and the facilities provided by nanotechnology ,this study included the use of ***Prosopisfarcta*** plantsfor the treatment with Zinc nanoparticles as a heavy metal that causes problems in soil ,and to be sure of which concentration is the most one to uptake and accumulate in this plant.

MATERIALS AND METHODS

Callus initiation from seedling hypocotyl stems

Seeds of Prosopisfarcta L. provided from mature fruits of plants grown naturally nearly in Hay Al-Arabi / Mousl. Were sterilized by soaking in ethyl alcohol 96% for two minutes, followed by 1:2 (V:V) sodium hypochlorite (NaOCl): water with stirring for five minutes, then rinsed thoroughly in sterilized distilled water three times (9). Sterilized seeds were placed on MSO (10) medium solidified with 0.8% agar and supplemented with 3% sucrose, pH adjusted to 5.8 before autoclaving. Cultures maintained in culture room at 25±2 ºC in the dark. The seedlings produced after seven days of culture transferred to light condition with 16 hour light daily at 1500 lux.

Forcallus initiation, 2.0cm ofseedling hypocotyl stems excised and placed on MS* medium(10) (MS* = MS medium modified by increasing KNO_3 to 2000 mg L^{-1}

, Thiamine-HCl to 0.5 mg L^{-1}, Pyrodoxine-HCl to 1.0 mgL^{-1}**)** and supplemented with 1.0 mg L^{-1} NAA (Naphthalene acetic acid) and 4.0 mg L^{-1} TDZ(Thidizurone)(11).The callus aggregates formed were then subcultured on the same medium after 30 days for maintaining callus growth.

Preparetion of Zinc oxide nanoparticles solution

1.0 gm of Zinc oxide (Zinc oxide *ReagentPlus*®, powder, <120 nm particle size, 99.9% , Sigma- Aldrich, UK) was dissolved in 1.0 liter of distilled water as stock solution, then other concentration of 1.0, 10, 50, 100 µg ml^{-1} were prepared.

Culture of *Prosopisfarcta* seeds and callus on MS medium supplemented with the concentration of Zinc oxide nanoparticles

1.0gm of callus was transferred to MS* modified(MS* = MS medium containing 1.0 mg L^{-1} NAA and 4.0 mg L^{-1} TDZ and supplemented with Zinc oxide nanoparticles at 1.0, 10, 50, 100 µg ml^{-1} each of them alone.

Whereas, the seeds were cultured on MSO supplemented with 50 µg ml^{-1} of Zincoxide nanoparticles only.

Determination of callus fresh weight

Callus fresh weight determinate after 30 days of cultured with different concentration of Zinc oxide by calculation the difference between the weight of flasks with culture and when it is with medium (12).

Preparation of samples (tissues) for the microscopic photography

Seedling segments and callus tissues of Prosopisfarcta which treated with Zinc oxidenanoparticles were dried at 80ºC using oven (Gallenkamp oven BS Model, England) for 18 hours (13). Scanning Electron Microscope (SEM) (VEGA/ TESCAN, Czech Republic) in Nanotechnology Center, University of Technology, Baghdad, Iraq was used to detectZinc oxide nanoparticles in *Prosopisfarcta* tissues.

RESULTS AND DISCUSSION

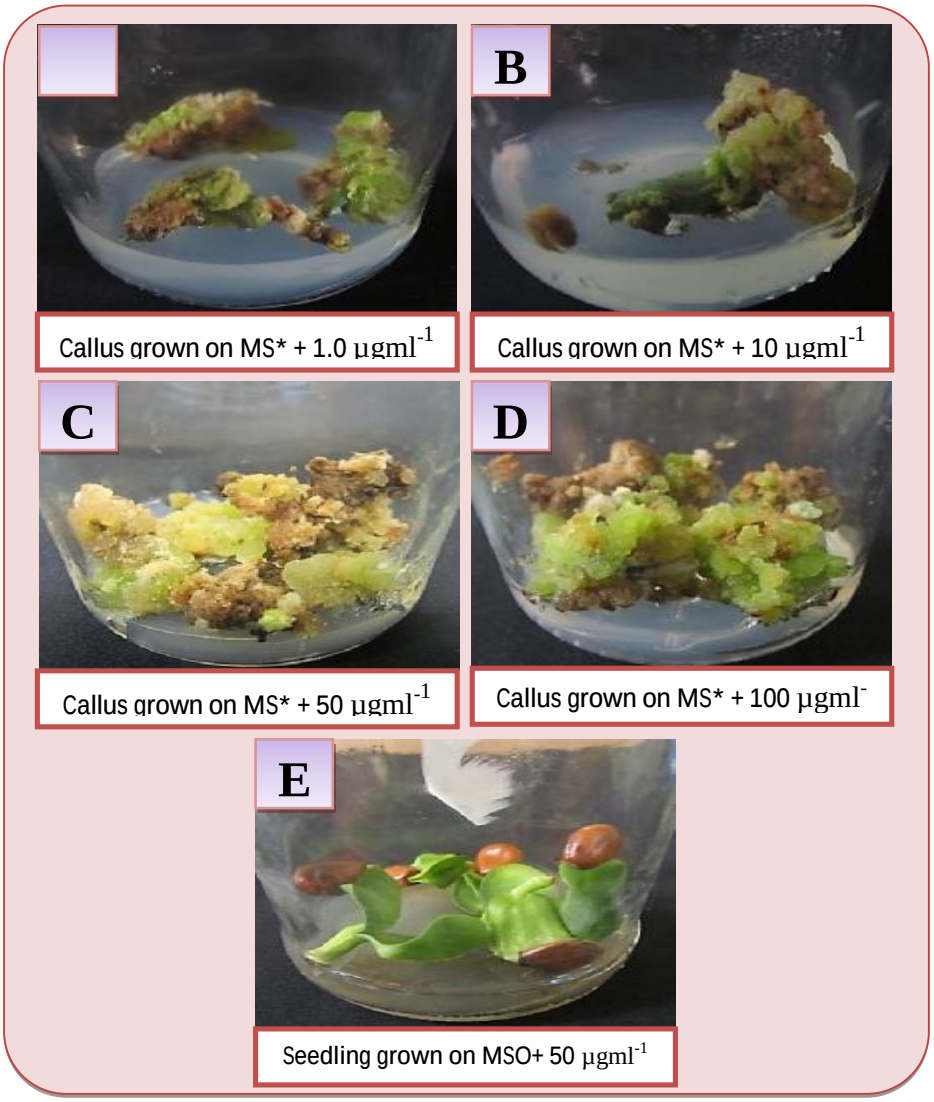
Results in table (1) showed that callus fresh weight of *Prosopisfarcta* varied with the variation of Zinc oxide nanoparticles concentrations.

Table (1) Fresh weight of *Prosopisfarcta* L. callus grown on MS* medium supplemented with different concentrations of Zinc oxide nanoparticles after 30 days of culture.

Zinc oxide nanoparticles µg ml^{-1}	**callus fresh weight (gm)**
1.0	2.09
10	2.5
50	3.4
100	5.7

The best growth of callus determined by fresh weight obtained when callus grown on MS* medium with addition of 100µg ml^{-1} of Zinc oxide nanoparticles Figure (1-D) in which fresh weight of callus reached 5.7 g after 30 days of growth on this medium. The addition of 50µg ml^{-1}also enhanced callus growth to reach 3.4 g in fresh weightafter 30 days. Results represented in Figure 1-C) referred to that callus cultures of Prosopisfarcta have the ability to grow even with the presence of high concentrations of Zinc oxide nanoparticles. Zinc was considered to be as a heavy

metal that is required in trace amounts by higher plants to complete their life (14). Generally results showed that callus seems to be actively grown on MS* medium with addition of 100µg ml^{-1} of Zinc oxide nanoparticles as compared with the other concentrations 1.0, 10µg ml^{-1} Figure (1-A, B).In comparison seedlings of this plant treated with 50 µg ml^{-1}exhibited thickened leaves and shortened stems Figure (1-E).



Figuer (1) Seedlings and callus cultures of Prosopisfarcta L. grown on medium supplemented with different concentrations of Zinc oxide nanoparticles after 30 days of culture.

It was reported that Zn is found to be involved in many cellular functions such as protein metabolism, photosynthetic carbon metabolism and indole acetic acid

metabolism, yet its higher concentrations because toxicity (7).It was also reported that many metals such as Zn, Mn, Ni and Cu are essential micronutrients (15). In commonnanoaccumulator plants, accumulation of these micronutrients doesnot exceed their metabolic needs (<10µgml^{-1}).In contrast, metal hyper accumulator plants can accumulate exceptionally high amounts of metals,in thousands ofµgml^{-1}(16).

SEM examination of seedling samples treated with 50µg ml^{-1}of Zinc oxide nanoparticles showed that there were many particles participated on the surface of the cells compared with control Figure (2-A, B).

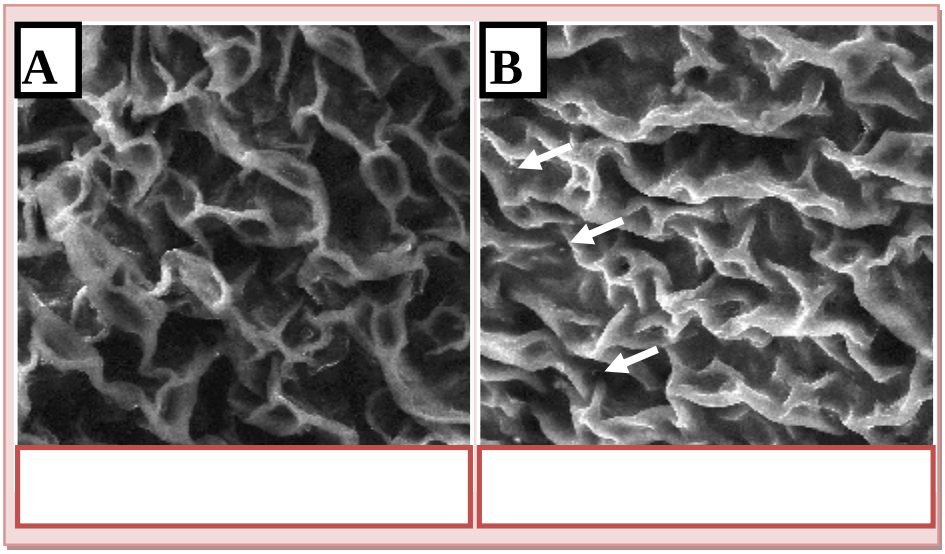


Figure (2) SEMphotograghyat 50 µm of Seedlings cells of Prosopisfarcta Grown on MSO supplemented with 50 µg ml^{-1} of Zinc oxide nanoparticles, arrows referred to the particles of Zinc oxide.

Where as examination of callus cells using SEM showed that callus cells seem to be coated with particles of Zinc oxidenanoparticles which were used in this study. Callus cells treated with 1.0 and 10 µg ml^{-1} of Zinc oxide nanoparticles Figure (3- B, C) were coated on their surfaces with less particles than the cell treated with the higher concentrations 50 and

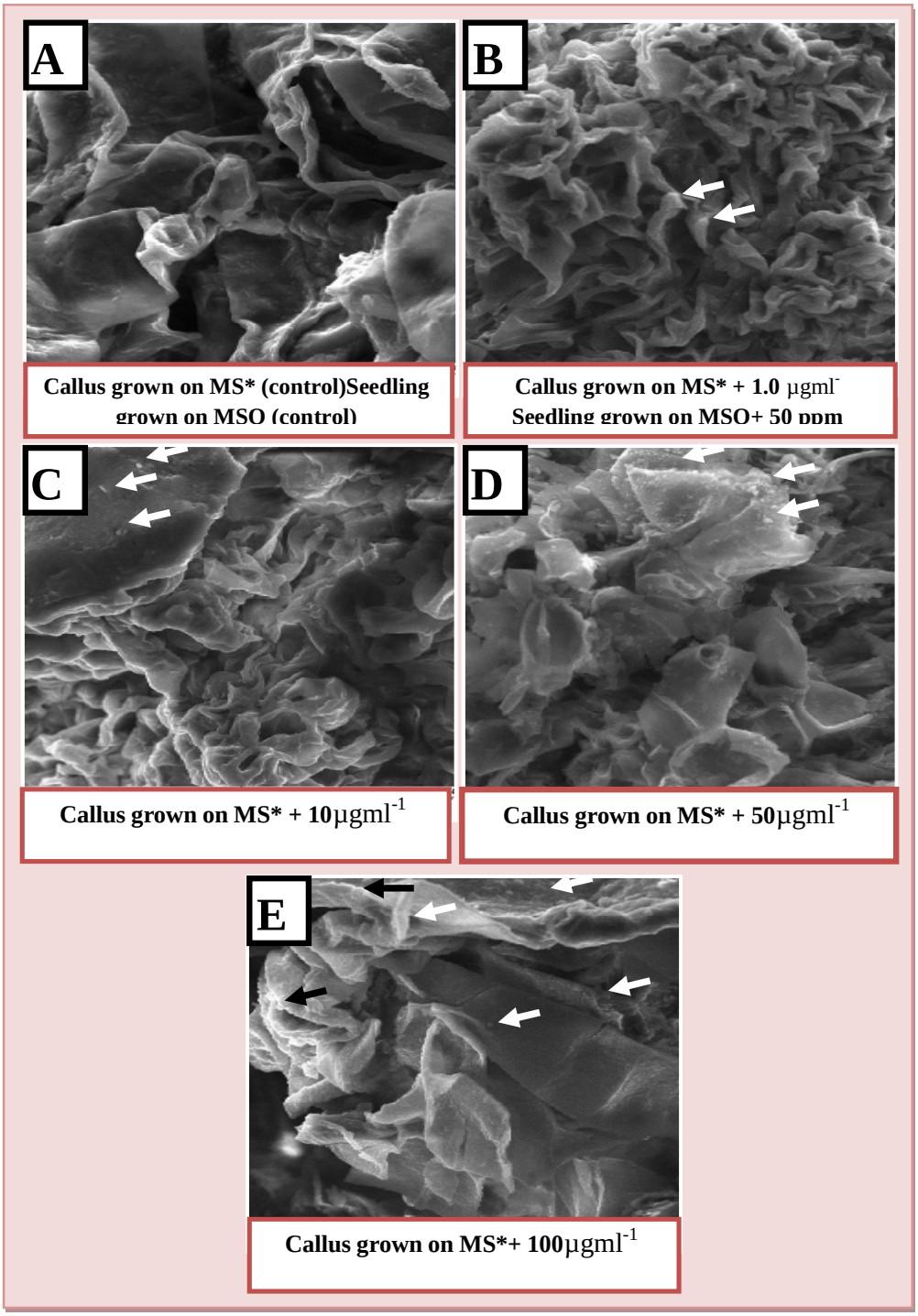


Figure (3) SEM photograghy at 50 µm of callus cells of Prosopisfarcta grown on MS* supplemented with different concentration of Zn_2O nanoparticles.

Zinc oxide. Nanoparticles Arrows indicated to Zinc nanoparticles ccumulated on the surface of the cells.

100 µg $ml^{-1}$of this element Figure (1-D, E), whereas callus cells grown on MSO, control, were surface free from the particles Figure (3-A). These resultswere considered to be as prove that callus cells of Prosopisfarcta can accumulate high concentration of Zinc oxide nanoparticles without affecting the rate of growth(depending on the concentration).This concept agreed with the essentials of phytoremediation mentioned by (17).So we can concluded that this study succeeded in choosing Prosopisfarctaplants to be a model of plants that could be used in phytoremediation programs to clean the contaminated soils with Zinc as a heavy metals.

REFERENCES

[1].Vaseem, M.; Umar, A. and Hahn, Y. (2010). Zno Nanoparticles: Growth, Properties, and Applications. In Metal Oxide Nanostructures and Their Applications. Edited by Ahmad Umar and Yoon-Bong Hahn, American Scientific Publishers, 5: 1–36.

[2].Reddy KM, Kevin F, Jason B, Cory H, Alex P (2007). Selective toxicity of Zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl. Phys. Lett., 90:1-8.

[3].Wang, Z.L.; Kong, X.Y.; Ding, Y.; Gao, P.; Hughes, W.L.; Yang, R. and Zhang, Y. (2004). Semiconducting and Piezoelectric Oxide Nanostructures induced by Polar Surfaces . Adv. Funct. Mater., 14 :943-956.

[4].Chang, Y.; Zhang, M. ; Xia, L. ; Zhang, J. and Xing, G. (2012).The Toxic Effects and Mechanisms of CuO and Zno Nanoparticles. Materials, 5: 2850-2871.

[5].Karimkhani, N. ; Golchin, A. and Khanmirazei, A. (2012). Cadmium and Zinc accumulation in triticale plant (Triticosecale) in cadmium polluted soil amended with organic matter. Internat. J. Aricult. Res. Rev., 2: 985-990.

[6].Riesen ,O. and Feller ,U.(2005). Redistribution of nickel, cobalt, manganese, Zinc, and cadmium via the phloem in young and maturing wheat. J. of Plant Nutrition 28: 421–43.

[7].Sinhal, V.K. (2007). Phytotoxic and cytogenetic effects of Zn^{2+} and Pb^{2+} in*Viciafaba*. Poll. Res. 26: 417-420.

[8].Meagher, R.B. (2000).Phytoremediation of toxic elemental and organic pollutants. Curr. Opin. Plant Biol.,3:153-162.

[9].Yahya, R. T. and Al-Salih, H. S. (2013). Induction of callus cultures from stems and cotyledonary leaves of ***Prosopsfarcta*** L. by using some plant growth regulators. Accepted in Raf. J. S.

[10].Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant., 15: 473-479.

[11].Al-Anzy, A. A. M. (2013). Conservation of ***rol-genes*** in carrot (***Daucuscarota*** L.) plants regenerated from its transformed tissues by Agrobacterium rhizogenes R1601. Ph.D. Thesis, College of Science, University of Mosul, Iraq.

[12].Al-Taee,R.T.Y.(2005).The effect of some growth regulators in initiation and growth of tissue and cell cultures of Chamomile (MatricariachamomillaL.)and measurement the level of some of their active compounds.,Msc.Thesis, College of Science, University of Mosul, Iraq.

[13].Al-Salih, H.S.;Al-Taee,R.F.; Godbold,D.;Jones,D.(2013).Uptake of Uranium by Callus Cultures of Two Acacia species. Accepted in Rafidain J. of Science 24(1):31-43.
[14].Maruthi Sridhar, B.B.; Han, F.X.; Diehl, S.V.; Monts, D.L. and Su, Y. (2007). Effects of Zn and Cd accumulation on structural and physiological characteristics of barley plants. Braz. J. Plant Physiol., 19: 15-22.
[15].Reeves R.D. and Baker, A.J. M. (1999). Metal-accumulating plants. In Phytoremediation of toxic Metals: Using Plants to Clean up the Environment, eds, I Raskin, BD Ensley, pp 193-229, John Wiley & Sons Inc, New York.
[16].Zhao, F. J.; Lombi, E.; Breedon, T. and McGrath, S. P. (2000).Zinchyperaccumulation and cellular distribution in *Arabidopsis halleri*. Plant, Cell & Environ., 23:507-514.
[17].Raskin, I.; Ensley, B.D. (2000). Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. John Wiley & Sons, Inc., New York. USA.