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Effect of Oxalic Acid on Cr^{3+} Ion Uptake, Accumulation and Oxidative Stress by Sorrel (*Hibiscus Sabdariffa L.*) Seedlings in Hydroponic Solution

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Abstract

The aim of this study is to investigate the effects of oxalic acid on the uptake, accumulation and oxidative stress of Cr^{3+} ion by sorrel (*Hibiscus sabdariffa L.*) seedlings in hydroponic solution. The growth of 8 weeks old seedlings exposed to 0, 5, 10, 20 and 40 mg/L Cr^{3+} ion and 20.0 mM oxalic acid was monitored in a greenhouse under controlled conditions. The application of oxalic acid for 10 days prior to harvest revealed that Cr^{3+}ion accumulated significantly in the roots ($p < 0.05$) with only a little translocated to the shoots. *Hibiscus sabdariffa L*. accumulated high Cr^{3+} ion concentration (344.44 mg/kg) in the root at a concentration of 40 mg/L.The application of oxalic acid had inhibitory effects on the root and shoot biomass compared with that in the control. Therefore, chelation enhanced Cr^{3+} ion uptake by *Hibiscus sabdariffa L*. The Cr^{3+} ion-induced proline accumulation in shoots was also determined. There is no much difference in proline content of both the chelated and un-chelated treatment, however, the proline content decreased slightly with increasing Cr^{3+}ion in the nutrients solution. Free proline is known to accumulate in plants under heavy metals exposure and is considered to be involved in stress resistance.

Keywords: Hydroponic, chelation, sorrel, greenhouse, oxalic acid, phytoremediation, chromium

1. Introduction

Chromium exists in several oxidation states but the most stable and common forms are chromium (III) and chromium (VI) species. However, a high concentration of chromium is highly toxic to plants [1]. Chromium toxicity in plants depends on its valence state. Chromium (VI) which is highly mobile is considered the most toxic form of chromium. It is associated with oxygen as chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$) or oxyanions. Chromium (VI) ion is rapidly reduced to Cr^{3+} ion in the presence of organic matters and in reducing environments. Chromium (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments [2]. The strong effect of chromium contamination in the physiology of plants depends on the oxidation state of the metal, which is responsible for its mobilization, uptake and toxicity in the plant system. Chromium is taken up by plants through carriers of essential ions such as sulphates. Symptoms of chromium toxicity in plants include decrease in seed germination, reduction of growth, decrease of yield, inhibition of enzymatic activities, impairment of photosynthesis, nutrient and oxidative imbalances, and mutagenesis. Recently there is huge

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interest in the use of phytoremediation for the remediation of soils, sediments and water contaminated by heavy metals [3]. Phytoremediation of chromium pollution can be achieved by extraction of the metal from polluted soils into harvestable plant tissues by the accumulation of the element in the root tissues or by the in situ detoxification of the metal through plant-based chelation, reduction and/or oxidation mechanisms. There is scanty information on research work on the phyto-extraction of chromium from contaminated soils and sediments. Very few plant species such as *Suterafodina, Dicoma niccolifera* and *Leptospermum scoparium* have been reported to accumulate chromium to high concentrations in their tissues [4]. Sorrel (*Hibiscus sabdariffa L.*) belongs to the *Malvaceae* family, and is an annual or biennial plant cultivated in tropical and subtropical regions for its stem fibers, edible calyces, leaves and seeds. The leaves are used for making soup, while the succulent calyces are used for making syrups, jelly, jam, and alcoholic drink. Recently the possibility of its use for phytoremediation is being given attention, because as a plant with small requirements, *Hibiscus sabdariffa L.* may be encountered in many ecosystems.

The objectives of this research work are; to study the uptake and accumulation potential of Cr^{3+} ion by *Hibiscus sabdariffa L.* seedlings under Cr^{3+} ion toxicity in the presence and absence of oxalic acid and whether *Hibiscus sabdariffa L.* can be used as a phytoremediator.

2. Materials and Methods

2.1. Source of *Hibiscus sabdariffa L.* Seeds

Hibiscus sabdariffa L. seeds were obtained from International Institute of Tropical Agriculture (IITA) station, Tarauni, Kano with coordinates latitude $11^0$58'49" N, longitude $008^0$33'26.5" E and altitude 492.5 m above sea level.

2.1.1. Field Experiment

The field experiment was carried out at the Department of Agronomy farm, Bayero University, Kano with coordinates latitude 8^0 22' to 9^0 25' north and longitude 11^0 57' to 12^0 00' east. The farm falls within the Sudan savanna climatic zone. There are two seasons namely the dry season (harmattan) and the wet (rainy) season. The *Hibiscus sabdariffa L.* seeds were planted and raised for eight (8) weeks. The map of the planting site is shown in Figure 1.

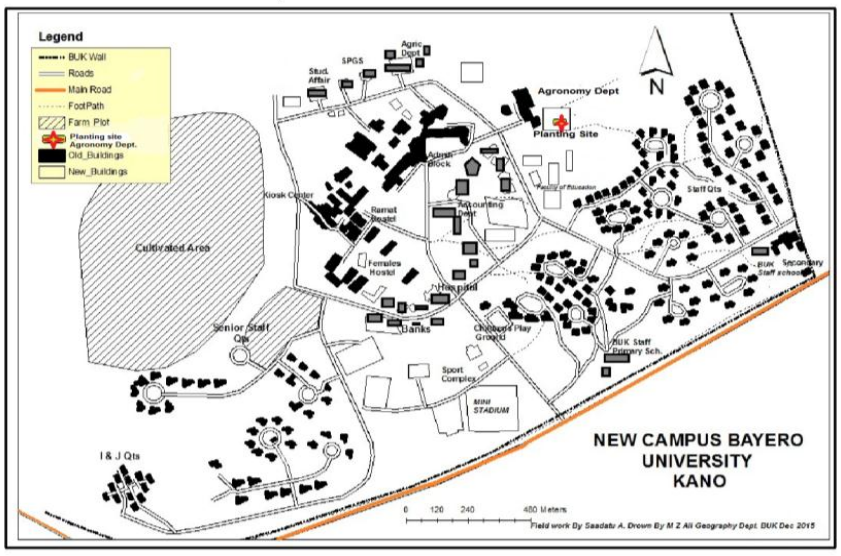

Figure 1. Map of Bayero University New site, Kano Showing the Planting Site (Geography Dept BUK, Dec., 2015)

The mean annual temperature of the areas was estimated to be 32°C. The lowest temperature of between 25°C-28°C is recorded between the months of November-January, while the highest temperature of 36°C-40°C is recorded in the months of March, April and May. The mean annual rainfall of the area is 850 mm, with 4 to 5 months as rainy months (May to September). The mean annual humidity of the area is about 50% with the months of January, February and March recording the lowest humidity values of between 35%-40% while the highest values of 85-95% is recorded in the months of July, August and September.

2.1.2. Growth Conditions and Treatment

Eight weeks old *Hibiscus sabdariffa L.* seedlings were collected from Agronomy Departmental farm, Bayero University,

Kano on the 20th, June, 2014. They were washed with tap water to remove excess soil, and rinsed three times with deionised water before transplanting in hydroponic solution and kept in a greenhouse at 65% constant relative humidity, 16/8 h day/night period under 600 µ mol m^{-2} s^{-1} of light intensity, and day/night temperatures of 38/20°C. Plants were supplied with Hoagland nutrient solution (pH 6.0-6.3) which contained the following nutrients: 1.0 mM KH_2PO_4, 2.0 mM $MgSO_4.4H_2O$, 5.0 mM KNO_3, 5.0 mM $Ca(NO_3)_2.4H_2O$, 4.6 µM H_3BO_3, 0.8 µM $ZnSO_4.7H_2O$, 0.3 µM $CuSO_4.5H_2O$, and 0.1 µM $H_2MoO_4.H_2O$. Iron was supplied as Fe-EDTA (1.8 mM). The treatment consists of three phases in a completely randomized design composed of:

Phase I: Contain only the nutrient solution (hydroponic solution).

Phase II: Contain the nutrient solution and four levels (5, 10, 20 and 40 mg/L) of added Cr^{3+} ion as $CrCl_3.6H_2O$.

Phase III: Contain the nutrient solution and four levels (5, 10, 20 and 40mg/L) of added Cr^{3+} ion as $CrCl_3.6H_2O$ and constant concentration (20.0 mM) of oxalic acid.

Each treatment in triplicates was allowed to stand for 10 days, after which the plants were harvested and subjected to physiological and biochemical analysis.

2.1.4 Proline Content Determination

Proline content of the shoots was estimated in which 0.5 g of fresh leaf was homogenized in 10.0 mL of 3% aqueous sulphosalicylic acid [5]. 2.0 mL of the filtrate was then mixed with 2.0 mL of acid-ninhydrin and 2.0 mL of glacial acetic acid and heated at 100°C for 60 min. The reaction was terminated in an ice bath, 4.0 mL of toluene was added to the mixture and contents of tubes were stirred for 20 s. Absorbance of the pink red upper phase was recorded at 520 nm against toluene blank using a spectrophotometer (BUCK SCIENTIFIC VGP 210). A proline concentration was determined from a standard curve and calculated as a dry weight basis using Equation 1.

$$Proline\{\ \mu g\ /\ g\ dry\ weight\ (DW)\} = (\mu g/ml\ proline\ x\ vol.\ of\ toluene\ x\ volume\ of\ SS\ acid)/\ (g/DW\ x\ 115.5) \quad 1$$

Ninhynhidrin solution contained: 2.5% w/v ninhydrin, in solution of acetic acid, H_2O and orthophosphoric acid (CH_3COOH: H_2O: H_3PO_4, 6:3:1 v/v).

2.2. Spectrophotometric Determination of Cr^{3+} ion in Roots and Shoots of Harvested *Hibiscus sabdariffa L.* Seedlings

After 10 days exposure, the *Hibiscus sabdariffa L.* seedlings were harvested and washed with tap water, followed by 1% HNO_3 and rinsed with deionised water [6]. The roots and shoots were separated and oven dried at 80°C for 48 hr. They were ground with wooden mortar and pestle to a fine powder. A dried porcelain crucible was ignited on a hot electric plate for 5 min. 2.0 g of shoot and 0.5 g of root were weighed into the crucible and gently heated on a hot electric plate until the smoking ceased. It was then transferred and dried to constant weight in a muffle furnace at 550^0C for 4 hr. The ash was cooled in a dessicator, dissolved in 0.1 M HNO_3, filtered into 50 mL volumetric flask and made to the mark. The Cr^{3+} ion content in the roots and shoots was analyzed using atomic Absorption Spectrophotometer (BUCK SCIENTIFIC VGP 210) at 359.7 nm. The concentration of Cr^{3+} ion was reported as mg/g dry weight.

2.3. Statistical Analysis

All data were treated using Excel 2010 program for window and significance test were performed using One-way ANOVA at 95% confidence level. Data were expressed as mean followed by SD. Statistical significance was assumed at $p < 0.05$.

3. Results and Discussions

3.1. Uptake and Accumulation of Cr^{3+} Ion by *Hibiscus sabdariffa L.* Seedlings

Changes in root and shoot Cr^{3+} ion concentrations were determined relative to their respective controls as follows; change in root concentration of Cr^{3+} ion (ΔRtCr) = (root concentration of Cr^{3+} ion of a particular treatment) – (root concentration of Cr^{3+} ion in control). Change in shoot concentration of Cr^{3+} ion (Δ ShCr) = (shoot concentration of Cr^{3+} of a particular treatment) – (shoot concentration of Cr^{3+} ion in control). Figure 2 shows the changes in the root and shoot Cr^{3+} ion accumulation against the concentration of added Cr^{3+} ion in nutrient solution.

The root and shoot concentrations of Cr^{3+} ion in plant grown in chelated and unchelated treatment were significantly higher than the control ($p < 0.05$).They increased with concentration of added Cr^{3+} ion in the nutrient solution. Plants accumulate chromium mainly in the root [7]. The accumulation of chromium in the roots is independent of chromium species applied. The reason for the poor translocation of chromium from root to shoot is that plants have no specific transport system for Cr^{3+}ion. Chromium uptake occurs only through passive transport [6]. The increase in the plant uptake of Cr^{3+} ion is proportional to the concentrations of Cr^{3+} ion in solution.

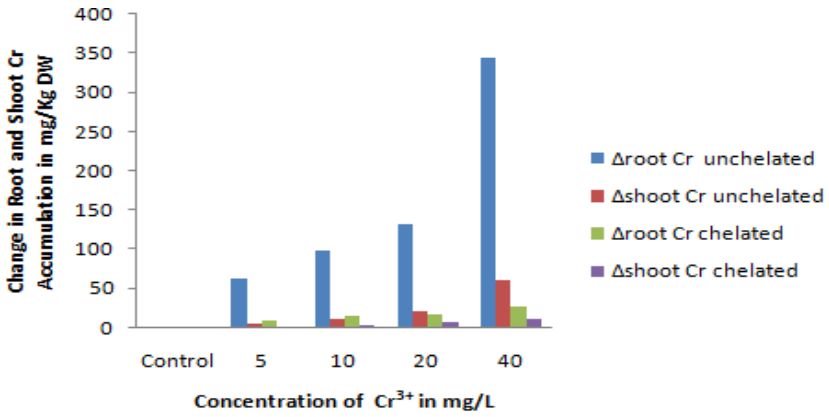

Figure 2. Cr^{3+} Ion Uptake in Roots and Shoot of *Hibiscus sabdariffa* L. Seedlings in Chelated and Unchelated Treatments of the Same Cr^{3+} Ion Concentrations

This finding is supported by free-ion activity hypothesis which says "the uptake of trace metals by plants is commonly assumed to depend on the free metal-ion activity, rather than the total concentration of dissolved metal" [8]. The enhanced root chromium concentration after the addition of oxalic acid could be explained by the increase in the solubility of Cr^{3+} ion in the solution, which enhanced the mass transfer of Cr^{3+} ion to the plant root surface. The shoot Cr^{3+} ion concentration decrease brought about by the addition of oxalic acid is possibly due to the predominant species of chromium in roots is Cr^{3+} ion.

The translocation factor (TF) is the ratio of the concentration of metal ion in the shoot to its concentration in the root. It measures the ability of the plant species to transfer the metal ion from the root to the shoot [9].TF was calculated from the relation presented in Equation 2.

$$TF = Cr^{3+} \text{ in shoots} / Cr^{3+} \text{ in roots} \qquad 2$$

The dependence of Cr^{3+} ion TF of *Hibiscus sabdariffa L.* seedlings against concentration of added Cr^{3+} ion is shown in Figure 3.

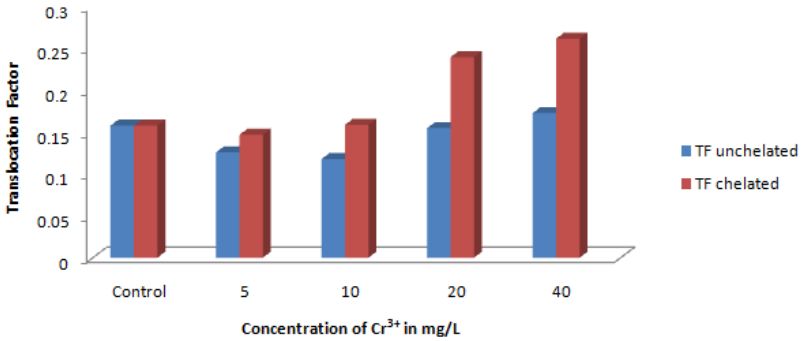

Figure 3. Change in Translocation Factor (TF) of Cr^{3+} ion in *Hibiscus sabdariffa L.* Seedlings in 20 mM Oxalic Acid and Unchelated Treatments of the Same Cr^{3+} Ion Concentrations

The TF of *Hibiscus sabdariffa L.* seedlings in 20 mM oxalic acid and unchelated treatments were ranged from 0.158-0.173 and 0.147-0.262, respectively. This shows that 20 mM oxalic acid enhanced the translocation of Cr^{3+} ion in the concentration range 0-40 mg/L. The bio-concentration factor (BCF) is the ratio of metal concentration in the shoot divided by the metal concentration in the solution [9]. BCF was calculated using Equation 3.

$$BCF = \frac{\text{Chromium content mg/Kg dry plant tissue}}{\text{Chromium content mg/L nutrient solution}} \qquad 3$$

The value was used in this work to explain the uptake of Cr^{3+} ion by *Hibiscus sabdariffa L.* seedlings from the solution to its root. The BCF is a better indicator in the metal accumulating ability of a plant as it takes into account the trace element concentration in the substrate [10].

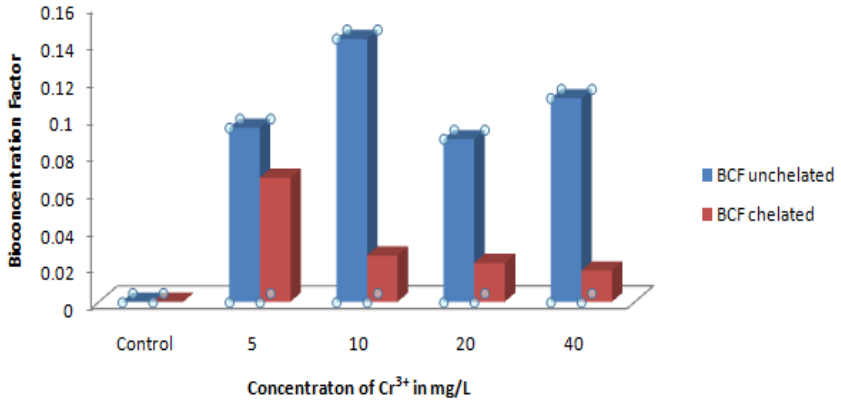

Figure 4. Bioaccumulation Factor of Cr^{3+} Ion in *Hibiscus sabdariffa* L. Seedlings in 20 mM Oxalic Acid and Unchelated Treatments of the Same Cr^{3+} Ion Concentrations

The BCF of *Hibiscus sabdariffa L.* seedlings in 20 mM oxalic acid and unchelated treatments ranged from 0.017-0.067 and 0.094-0.110, respectively. Figure 4 shows that in the BCFs, the bulk concentrations of Cr^{3+} ion remain in the roots. The effect of oxalic acid addition gives a summary of Cr^{3+} ion uptake. Uptake between 20 to 40 mg/L was insignificant ($p < 0.05$) relative to chelated treatments of same concentrations. This shows that 20 mM oxalic acid had no impact on the plants.

A good metal-accumulating plant should have the ability to bioconcentrate the element in its tissue to a BCF > 1000 (%) [11]. The present result showed that the shoot BCF in *Hibiscus sabdariffa L.* seedlings were lower than 1000(%) indicating that the plant is not a hyper accumulator. Values of Cr^{3+} ion BCF and TF in the concentration range 0-40 mg/L are all less than 1.This result is similar to previous findings; eight potential energy crops and *Agropyron cristatum* exposed to Cd and Zn had a BCF and TFs less than 1 [12,13]. The present work showed that the observed increase in the amount of Cr^{3+} ion accumulated by the plants in the presence of oxalic acid is that oxalic acid being a natural organic acid is easily soluble, thereby reducing the complex capacity of the metal. It is pertinent to note that, nature and source of chelator, age of the plant as well as location within the plant in conjunction with the medium, will impact bioavailability as well as affect the selectivity by forming chelator-metal complexes that alters the uptake rate [14, 15].

To assess the extent of stress and damage caused by Cr^{3+} ion uptake, proline was determined in the harvested seedlings using the relation; change in proline content = (proline content of a particular treatment) – (proline content in the control). The variation of Cr^{3+} ion induced proline accumulation in shoots of *Hibiscus sabdariffa L.* seedlings against same concentrations of added Cr^{3+} ion is shown in Figure 5.

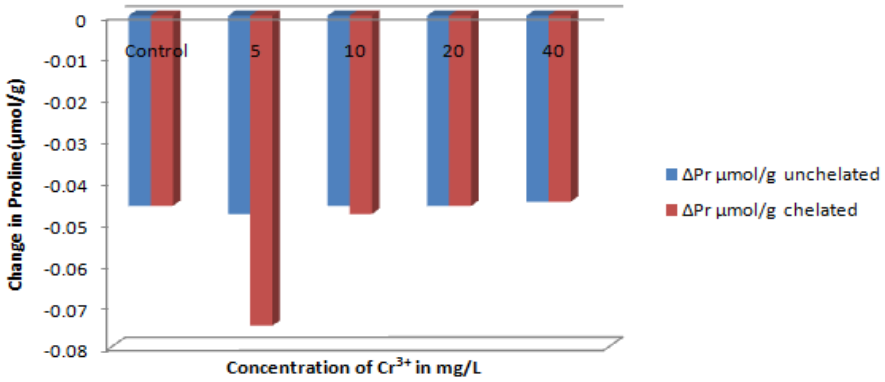

Figure 5. Proline Accumulation in the Shoots of *Hibiscus sabdariffa L.* Seedlings in 20 mM Oxalic Acid and Unchelated Treatments of the Same Cr^{3+} Ion Concentrations

Proline functions as an osmolyte, radical scavenger, electron sink, stabilizer of macromolecules and a cell wall component [16]. There were significant changes ($p < 0.05$) in proline content of seedlings grown in 20 mM oxalic acid and unchelated hydroponics. Proline content decreased marginally with increasing Cr^{3+} ion concentrations ($p < 0.05$). Under a lower stress with Cr^{3+} ion less than 5 mg/L, the contents of proline increased slightly in *Hibiscus sabdariffa L.* seedlings, but decreased under higher concentrations of 20 and 40 mg/L for both the chelated and unchelated treatment of the same

concentration of Cr^{+3} ion. Heavy metal stress increases peroxidase (POD), superoxide dismutase (SOD) and free proline contents of plant as a result of increased production of oxidative stress [17]. However, these may differ from plant to plant. In this study, the proline content decreased as the concentration of Cr^{3+} ion increased in both the chelated and unchelated treatment. Decreased POD activity in leaves of *Hibiscus sabdariffa L.* at higher levels of Cr^{3+} ion stress could be due to the differences in oxidative stress production and its relationship to proline contents.

4. Conclusions

Chromium toxicity has a significant effect on root growth and development. The toxicity of chromium depends on the concentration and the medium of uptake. It can be concluded from this study that chromium toxicity behaves differently at different levels of chromium supply. Consequently, Cr^{3+} ion was mainly accumulated in the roots than the shoots, which increased the Cr^{3+} ion immobilization through the root. Chromium concentration in the plants increases with an increase in the concentration of chromium in the media. Cytotoxic symptoms which include chlorosis and necrosis were observed depending on the concentration of Cr^{3+} ion. When *Hibiscus sabdariffa L.* was introduced to Cr^{3+} ion with or without oxalic acid, it proved to be poor phytoremediator. The addition of oxalic acid lessens the effect of chromium toxicity by mitigating oxidative stress through its chelating property compared to chromium treatment only. As a result, this species is recommended for phyto stabilization.

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REFERENCES

[1] Chatterjee J., Chatterjee C., 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. Environ. Pollut., 109, 69-74.

[2] Becquer T., Quantin C., Sicot M., Boudot J. P., 2003. Chromium availability in ultramafic soils from New Caledonia. Sci. Total Environ., 301, 251-261.

[3] Terry N., Banuelos G., 2000. Phytoremediation of contaminated soil and water. Lewis Publishers, New York, pp 389.

[4] Peterson P.J., 1975. In Proceedings of the International Conference on Heavy Metals in the Environment. Ed. T C Hutchinson. 2, 39.University of Toronto, Canada.

[5] Bates I.S., Waldren R.P., Teare I.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil, 39, 205-207.

[6] Wong M.H., Lau W.M., 1985. The effects of applications of phosphate, lime, EDTA, refuse compost and pig manure on Pb contents of crops. Agricultural Wastes, 12, 61-75.

[7] Sundaramoorthy P., Chidambaram A., Ganesh K.S., Unnikannan P., Baskaran L., 2010. Chromium stress in paddy: (i) Nutrient status of paddy under chromium stress; (ii) Phytoremediation of chromium by aquatic and terrestrial weeds. Comptes Rendus Biologies, 333(8), 597-607.

[8] Zayed A., Terry N., 2003. Chromium in the environment. Factors affecting biological remediation. Plant Soil, 249,139-156

[9] Degryse F., Smolders E., Parker D.R., 2006. Metal complexes increase uptake of Zn and Cu by plants. Implications for uptake and deficiency studies in chelator-buffered solutions. Plant and Soil, 289, 171-185.

[10] Ghnaya T., Slama I., Messedi D., Grignon C., Abdelly C., 2007. Cd-induced growth reduction in the halophyte *Sesuvium portulacastrumis* significantly improved by NaCl. J. Plant Res., 20, 309-316.

[11] Rahmani G.N.H., Sternberg S.P.K.., 1999. Bioremoval of lead from water using Lemna minor. Biores. Technol., 70, 225-230

[12] Zayed A., Gowthaman S., Terry N., 1998. Phytoaccumulation of trace elements by wetland plants, Duckweed. J. Environ. Qual., 27, 715-721.

[13] Shi G., Cai Q., 2009. Cadmium tolerance and accumulation in eight potential energy crops. Biotechnol. Adv., 27, 555-561.

[14] Meng L., Guo Q., Mao P., Tian X., 2013. Accumulation and tolerance characteristics of zinc in *Agropyron cristatum* plants exposed to zinc-contaminated soil. Bull. Environ. Contam. Toxicol., 91, 298-301

[15] Salt D.E., Prince RC., Baker A.J.M., Raskin I., Pickering I. J., 1999. Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy. Environ. Sci. Technol., 33, 713-717.

[16] Turgut C., Katie P.M., Cutright T.J. 2004. The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annuus*. Environ. Pollut., 31, 147-154

[17] Sun R.L., Zhou Q.Z., Sun F.H., Jin C.X.., 2007. Antioxidative defense and proline/phytochelation accumulation a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. Environ. Exp. Bot., 60,468-476.