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Synergistic Removal of Cr (VI) by *Eichornia crassipes* in Conjunction with Bacterial Strains

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Abstract: Two chromium resistant bacterial strains i.e. *Bacillus* sp. S6 and *Staphylococcus* sp. CrM-3 were used in conjunction with *Eichornia crassipes* to study the effect of chromate removal from chromium contaminated waste waters. Bacterial strains resulted in reduced uptake of chromate into inoculated plants, which was 17.24 and 11.29% with S6 and CrM-3 respectively, at an initial Cr (VI) concentration of 100 $\mu\text{g ml}^{-1}$ as compared to non-inoculated control plants. Different heavy metals (CuSO_4 , NiSO_4 , MnCl_2 and $\text{Pb}(\text{NO}_3)_2$) at a concentration of 100 $\mu\text{g ml}^{-1}$ were added in chromate-supplemented solution to check their effects on chromate uptake by the plants. At different chromate concentrations the uptake of Cr (VI) into the plants was 28.73, 5.38, 7.15, 12.29 and 15.35% less at an initial Cr (VI) concentration of 100, 300, 500, 1000 and 2000 $\mu\text{g ml}^{-1}$ in comparison to metal free condition. Cr (VI) uptake by the *Eichornia crassipes* was occurred at different pH tested, but maximum uptake was observed at pH 5. Nevertheless the bacterial strains caused some decrease in chromate uptake into the plants but the combined effect of plants and bacterial strains conduce more removal of Cr (VI) from the solution.

Key words: Synergistic removal, *Eichornia crassipes*, chromium, heavy metals, resistant

Introduction

Heavy metals pollution represents a serious threat for the survival of living biota and the physico-chemical nature of the environment. Water is a natural and preferred sink for the contamination and its pollution becomes an important concern for human health. Different methods were devised for the wastewater treatment such as biological as well as chemical reduction of most toxic forms of heavy metals. Biological wastewater treatment particularly relays on microorganisms and plants. Phytoremediation of heavy metals by non-food crops could be a reliable source in controlling the heavy metals contents of agriculture soils and industrial wastes (Rossi *et al.*, 1999).

Chromium is an industrially important metal and is considered to be quite toxic to human via oral exposure, causing kidney injury and lung cancer (Gibb *et al.*, 2000; Ewis *et al.*, 2001; Proctor *et al.*, 2002). It enters into the water through electroplating factories, leather tanneries and textile manufacturing facilities. Chromium occurs in oxidation states ranges from Cr^{+2} to Cr^{+6} but Cr^{+3} and Cr^{+6} are most abundant forms and are of biological significance. Cr (III) is less toxic and less mobile while Cr (VI) is most toxic (Zhang *et al.*, 2001), mutagenic (O'Brien *et al.*, 2001) and carcinogenic (Cabrera *et al.*, 2001). It is necessary to convert this toxic Cr (VI) to less mobile Cr (III). Several bacterial strains are now known that reduced toxic hexavalent chromium in to trivalent chromium (Puzon *et al.*, 2002; Ganguli and Tripathi, 2002). Besides these some plants species accumulate hexavalent chromium in

their tissues thus helps to remediate soils and wastewater (Srivastava *et al.*, 1999; Simon *et al.*, 2001). Plant assisted-bioremediation, is an emerging field, in which plants roots in conjunction with their rhizospheric microorganisms are used to remediate polluted environment. Some water plants have great capacity to accumulate heavy metals from the polluted water. Considering the high chromate accumulating capability of *Eichornia crassipes* plants, the present work was initiated to evaluate the effects of bacterial strains in conjunction with *Eichornia crassipes* at different pH and heavy metals concentration on the removal of Cr (VI) from aqueous solution.

Materials and Methods

Plant material: *Eichornia crassipes* plants were collected from fresh water pound near G.T. road, Kamokee, Lahore, that was not previously exposed to contaminated environment. In laboratory prior to the experiment, plants were maintained in a growth chamber and were supplied with nutrient solution (Hewitt, 1963) at a temperature of $25\pm 2^\circ\text{C}$ with a 12 h photoperiod (10 Klux). Equal sized plants (12-15 cm) were selected for the experiment to ensure the accuracy of the experimental results.

Bacterial strains: Two chromium resistant bacterial strains i.e. *Bacillus* sp. S6 and *Staphylococcus* sp. CrM-3 previously isolated from chromium contaminated soil and water, respectively, were used in this study (unpublished results). Both strains were able to tolerate high level of chromate i.e. up to 50 mg ml^{-1} of K_2CrO_4 on nutrient agar

and 25 mg ml⁻¹ in nutrient broth. These strains also have high capacity to accumulate and reduced Cr (VI). Generally bacterial strains were cultured in nutrient broth (Gerhardt *et al.*, 2000).

Cr (VI) uptake assay: Experiments were conducted by exposing the *Eichornia crassipes* plants to the aqueous solution containing different concentration i.e., 100, 300, 500, 1000 and 2000 µg ml⁻¹ of K₂CrO₄ in 250 ml beakers for 48 h. Inoculum was given from freshly prepared overnight cultures. One ml of bacterial suspension containing 10⁸ cfu ml⁻¹ was added in aqueous solutions at a ratio of 1:40 (v/v). As regards the bacterial inoculations, there were four treatments i.e., control (without inoculum), S6, CrM-3 and S6+CrM-3 (mixed). Plants were placed in a growth chamber for 12 h photoperiod with light intensity of 10 Klux. After 48 h of exposure plants were harvested and washed with distilled water twice to remove the loosely bound or attached chromium to the roots and aerial parts. Harvested plants were oven dried at 80°C for 24 h. For determination of chromium contents, one-gram of dry plant material of each treatment was processed for wet digestion (Humphries, 1956). The amount of Cr (VI) left in the solution and loosely bound or attached to plant roots was also determined.

Effects of heavy metals: In order to analyze the effect of different heavy metals on the chromate uptake by the *Eichornia crassipes* plants in the presence of bacterial strains, another experiment was designed. Different heavy metals (CuSO₄, NiSO₄, MnCl₂ and Pb (NO₃)₂) each at a concentration of 100 µg ml⁻¹ were added in chromate-supplemented solution. Plants were exposed to these solutions for 48 h. Bacterial inoculum was given as describe above. Plants were harvested, oven dried at 80°C for 24 h and total chromium content were determined as described above.

Effects of pH: The removal of Cr (VI) by *Eichornia crassipes* was tested in the range of pH 3 to 11, under both inoculated and controls conditions. The effects of different pH were observed at an initial chromate concentration of 500 µg ml⁻¹.

Bacterial Cr (VI) reduction: Experiments were also conducted to study the bacterial chromate reduction. At the start of experiment 100 µl sample from each treatment was taken and amount of Cr (VI) was determined. Plants were grown in these solutions (100, 300, 500, 1000 and 2000 µg ml⁻¹ of K₂CrO₄) with and without bacterial strains. At the end of experiment, total amount of Cr (VI) left in solution was also determined.

The experiments were performed in triplicate and the samples were analyzed in triplicate.

Results

Cr uptake: Under controlled conditions (without bacterial inoculation) the uptake of Cr (VI) in the plants was concentrations dependent. As the initial concentration was increased, the amount of chromate uptake also increased. It was observed that *Eichornia crassipes* could easily tolerate Cr (VI) at a concentration of 2000 µg ml⁻¹ and no visible symptoms of Cr toxicity were observed after 48 h of exposure. In the presence of bacterial strains, the amount of chromate uptake into inoculated plants was less i.e., 17.24 and 11.29% reduction with S6 and CrM-3 respectively, at an initial Cr (VI) concentration of 100 µg ml⁻¹ as compared to non-inoculated control plants (Fig. 1). This can be attributed to the ability of these strains to reduce Cr (VI) into Cr (III). Trivalent chromium is less permeable and less available to the plants root. In this way bacterial strains could limit the entry of chromate into the plants. At the completion of experiment (after 48 h) amount of Cr (VI) was more in those plants that were grown in the absence of bacterial strains. Both bacterial strains used in this study reduced significant amount of Cr (VI) after 48 h in the presence of *Eichornia crassipes*. In case where plants were exposed to the mixed culture bacterial inoculation, maximum reduction of Cr (VI) into Cr (III) occurs which ultimately resulted in reduced uptake by the plants in comparison to monoculture inoculation (Fig. 1).

Effects of heavy metals on Cr uptake: Uptake of chromate by *Eichornia crassipes* in the presence of different heavy metals at concentration of 100 µg ml⁻¹ was also investigated (Fig. 2). At different chromate concentrations the uptake of Cr (VI) into the plants was 28.73, 5.38, 7.15, 12.29 and 15.35% less at an initial Cr (VI) concentration of 100, 300, 500, 1000 and 2000 µg ml⁻¹ (Fig. 2) in comparison to metal free condition (Fig. 1). Maximum retardation of Cr (VI) uptake in the presence of different heavy metals was observed at lower concentration especially at 100 µg ml⁻¹ of K₂CrO₄. In case where bacterial inoculum was applied, the retardation effect was less and 9.67, 13.25, 13.18, 4.09 and 5.23% decreases in this parameters were observed at 100, 300, 500, 1000 and 2000 µg ml⁻¹ initial Cr (VI) concentration in the presence of strain S6. This might be attributed to the chelation effect or uptake of heavy metals by the strains, thus lowering the effect of metals on chromate uptake into the plants.

Effects of pH on Cr uptake: Cr (VI) uptake by the *Eichornia crassipes* was occurred at different pH tested,

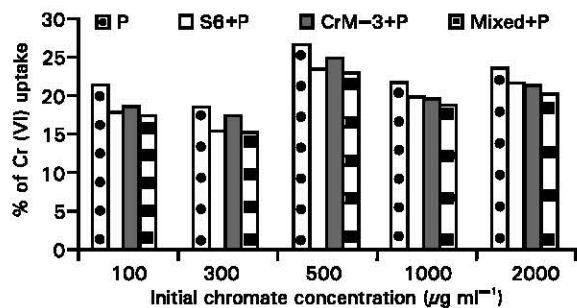


Fig. 1: Uptake of Cr (VI) by *Eichornia crassipes* at different concentrations of K_2CrO_4 in the presence of Bacterial strains after 48 h of exposure. Symbols: P, *Eichornia crassipes*; Mixed, S6+CrM-3

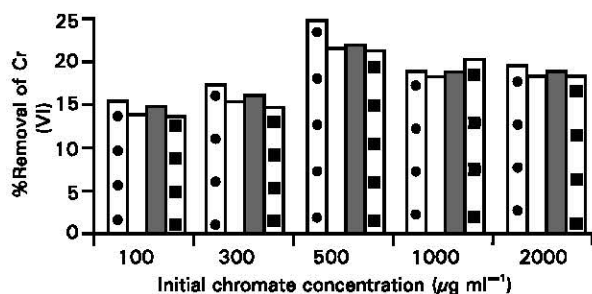


Fig. 2: Effect of different heavy metals on the uptake of Cr (VI) by plants roots after 48 h in the presence of bacterial strains

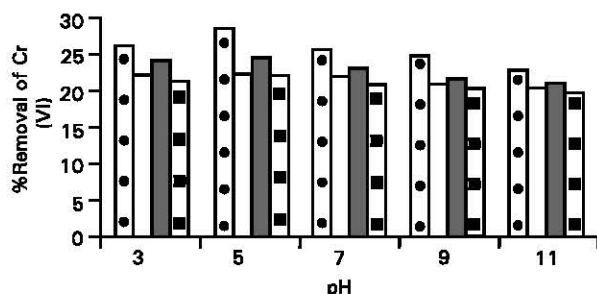


Fig. 3: Effect of different pH's on the uptake of Cr (VI) by the *Eichornia crassipes*

but maximum uptake was observed at pH 5 (Fig. 3). Under alkaline pH the uptake of Cr (VI) was rendered and 22.38% less chromate uptake was observed at pH 11 in comparison to pH 5 (Fig. 3). Uptake percentage of Cr (VI) was more at acidic to neutral pH, but as we increase the pH from 5 onward it resulted in decreased uptake capability of plants. At different pH, bacterial inoculations, however, did not show a significant influence on the Cr (VI) removal by *Eichornia crassipes*.

Effects of bacterial strains on Cr uptake: In contrast to control, experiment in the presence of bacterial strains i.e. *Bacillus* sp. S6 and *Staphylococcus* sp. CrM-3 resulted in

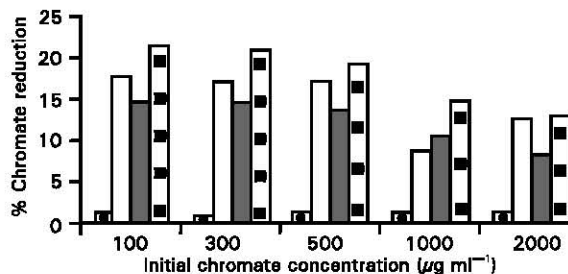


Fig. 4: %Reduction of Cr (VI) Removal from aqueous solution by the combined action of *Eichornia crassipes* plants at different concentrations of K_2CrO_4 after 48 h

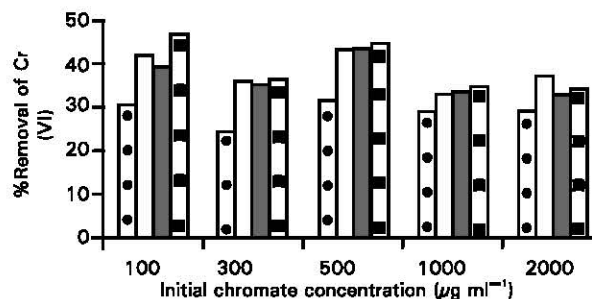


Fig. 5: Total Cr (VI) Removal from aqueous solution by the combined action of *Eichornia crassipes* plants and bacterial strains after 48 h

a decrease in the concentration of chromate accumulated by the plants due to the reduction of more soluble and permeable Cr (VI) to less permeable Cr (III) (Fig. 1). The decrease in chromate uptake at all concentrations tested was less in case of S6 inoculation as compared to CrM-3, because S6 is more efficient chromate reducer as compared to CrM-3 (Fig. 4). In case of mixed culture bacterial inoculation (*Bacillus* sp. + *Staphylococcus* sp.) maximum reduction (21.60% at an initial Cr (VI) concentration of $100 \mu g ml^{-1}$) of Cr (VI) to Cr (III) occurred which resulted in decreased uptake of Cr (VI) in to the plants (Fig. 1). Chromium reduction by the plants and bacterial strains was observed even at high level of Cr (VI) i.e. at $2000 \mu g ml^{-1}$. Maximum chromate reduction was occurred at a concentration of $2000 \mu g ml^{-1}$ in case of mixed bacterial inoculation where 10.33 mg out of 80 mg Cr (VI) was reduced to Cr (III) (Fig. 4).

Nevertheless the bacterial strains caused some decrease in chromate uptake into the plants but the combined effect of plants and bacterial strains conduce more removal of Cr (VI) from the solution. The removal of Cr (VI) from the solution, which was the sum of Cr (VI) accumulated, loosely bound to plant roots and uptake and reduced by the bacterial strains (Fig. 5). At an initial concentration of $100 \mu g ml^{-1}$ (total available chromium 4 mg) maximum Cr (VI) removals i.e. 1.88 mg (47%) was observed in case of mixed strains in comparison to non-inoculated controls

where it was 1.20 mg (30%). In all cases where bacterial inoculum was given significantly more Cr (VI) removal was observed as compared to non-inoculated controls. At an initial chromate concentration of 100 $\mu\text{g ml}^{-1}$ almost 36.17% more Cr (VI) removal was observed with the application of mixed culture bacterial inoculation (*Bacillus* sp. + *Staphylococcus* sp.) when compared with control (Fig. 5).

Discussion

Remediation technologies currently applicable to heavy metal contaminated sites are frequently expensive, environmental invasive and do not make cost-effective uses of existing resources. The focus of much recent work exploit biological (plants and microorganisms) processes to reduce the inherent risk associated with metal-contaminated sites.

Our interest in Cr (VI) accumulation by *Eichornia crassipes* is most likely to increase not only because of its high Cr (VI) accumulation and low cost but also because both component i.e. plants and bacteria support each other growth and activity. From results it is clear that under both controlled and inoculated conditions, amount of Cr (VI) uptake into the plants was concentration dependent i.e., it increases as we increase the initial Cr (VI) concentration of solution. This also shows the widespread adaptability of *Eichornia crassipes* to the nature of effluent and explains the presence of this plant in variety of polluted waters. The removal percentage of Cr (VI) by *Eichornia crassipes* plants reduced with the addition of different heavy metal (CuSO_4 , NiSO_4 , MnCl_2 and $\text{Pb}(\text{NO}_3)_2$) each at a concentration of 100 $\mu\text{g ml}^{-1}$. These effects were more pronounced at lower initial chromate concentration especially at 100 $\mu\text{g ml}^{-1}$ where as at high chromate concentration the interactive inhibition was not pronounced.

Many factors (pH, heavy metals) can affect the uptake of Cr (VI) by the plant roots. Hydrogen ion activity (pH) is probably the most important factor governing metal speciation, solubility, transport and eventual bioavailability of metals in aqueous solution. Experimental results concerning the effects of different pH on the uptake of Cr (VI) into the plants showed that the uptake was favored at lower pHs (Fig. 3). At acidic pH the solubility of Cr (VI) increased. This was significant because the pH range for majority of effluents lies from acidic to neutral.

Both bacterial strains were very efficient in the reduction of hexavalent chromium into trivalent chromium and this activity extenuated in the presence of *Eichornia crassipes* plants. Plants can indirectly enhance the removal of Cr (VI) by releasing exudates, which provide carbon and

energy to sustain microbial population (Cunningham *et al.*, 1996). Much of the reduced chromium (trivalent chromium) settles down as hydroxides and eventually less available to plant roots. In the rhizosphere, bacteria are considered to be the most abundant and active members, which influence the plant growth by different ways. Colonization of the rhizosphere is known to be helpful for bacteria, but their presence is also functional to the plants especially if they produced plant growth promoting substances (IAA) or by enhancing/inhibiting the uptake of toxic metals by the plants. Although the amount of Cr (VI) uptake/removed from aqueous solution by *Eichornia* plants was decreased in the presence of bacterial strains, but the overall removal of Cr (VI) by both the action of *Eichornia crassipes* along with bacterial strains was more as compared to non-inoculated control plants (Fig. 5). These results suggested that the capacity of *Eichornia crassipes* to remove Cr (VI) from solution was found to be significantly accelerated by presence of bacterial strains. The presented bioremediation process is a promising step towards the reclamation of industrial waste waters through the use bacterial strains along with *Eichornia crassipes* plants.

References

- Cabrera, J., S. Burkhardt, D.X. Tan, L.C. Manchester, M. Karbownik and R.J. Reiter, 2001. Autoxidation and Toxicant-Induced Oxidation of Lipid and DNA in Monkey Liver. Reduction of Molecular Damage by latonin. *Pharmacol. Toxicol.*, 225-230.
- Cunningham, S.D., T.A. Anderson, P. Schwab and F.C. Hsu, 1996. Phytoremediation of soils contaminated with organic pollutants. *Adv. Agron.*, 56: 55-114.
- Ewis, A.A., K. Kondo, J. Lee, M. Tsuyuguchi, M. Hashimoto, T. Yokose, K. Mukai, T. Kodama, T. Shinka, Y. Monden and Y. Nakahori, 2001. Occupational cancer genetics: infrequent ras oncogenes point mutations in lung cancer samples from chromate workers. *Am. J. Ind. Med.*, 40: 92-97.
- Ganguli, A. and A.K. Tripathi, 2002. Bioremediation of toxic chromium from electroplating effluents by chromate-reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors. *Appl. Microbiol. Biotechnol.*, 58: 416-420.
- Gerhardt, P., R.G.E. Murry, W.A. Wood and N.R. Kreig, 2000. Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C., 1994.
- Gibb, H.J., S.J.P. Lees, P.F. Pinsky and B.C. Rooney, 2000. Clinical findings of irritation among chromium chemical production workers. *Am. J. Ind. Med.*, 38: 127-131.

- Hewitt, E.J., 1963. Minerals nutrition of plants in culture media. In: Plant Physiology (Steward, F.C., Ed.), Academic Press, New York, pp: 99-137
- Humphries, E.C., 1956. Mineral component and ash analysis. In: Modern Methods of Plant Analysis (Paech, K. and M.Y. Traley, Ed). Springer Verlag, Berlin, Gothigen Heidelberg.
- O'Brien, T., J. Xu and S.R. Patierno, 2001. Effect of glutathionine on chromium-induced DNA cross-linking and DNA polymerase arrest. *Molecular and Cellular Biochemistry*, 222: 173-182.
- Proctor, D.M., J.M. Otani, B.L. Finley, D.J. Paustenbach, J.A. Bland, N. Speizer and E. V. Sargent, 2002. Is hexavalent chromium carcinogenic via ingestion? A weight-of-evidence review. *J. Toxicol. Environ. Health Part A.*, 65: 701-746.
- Puzon, G.J., J.N. Petersen, A.G. Roberts, D.M. Kramer, L. Xun, 2002. A bacterial flavin reductase system reduces chromate to a soluble chromium (III)-NAD⁺ complex. *Biochem. Biophys. Res.*, 294: 76-81.
- Rossi, G., S. Bellicioni, B. Felici and A. Figliolia, 1999. The effect of sewage sludge fertilization on the heavy metal behavior in soil-plant system: a long-term experience. 5th International Conference on the Biogeochemistry of Trace Elements (ICOBTE), pp: 730-731.
- Simon, L., I. SzegvAri and J. Prokisch, 2001. Enhancement of Chromium phytoextraction Capacity of fodder radish with picolinic acid. *Environmental Geochemistry and Health*, 23: 313-316.
- Srivastava, S., S. Prakash and M.M. Srivastava, 1999. Studies on mobilization of chromium with reference to its plant availability - Role of organic acids. *BioMetals*, 12: 201-207.
- Zhang, Z., S.S. Leonard, S. Wang, V. Vallyathan, V. Castranova and X. Shi, 2001. Cr(VI) induces cell growth arrest through hydrogen peroxide. *Molecular and Cellular Biochemistry*, 1/2: 77-83.