

Bioaccumulation of Chromium by Aquatic Hydrophyte *Lemna* sp. and its Associated Rhizosphere Bacteria

Rohit Das¹, Indrani Saha¹, Eshaani Mitra¹, Debadrita Bhowmik¹, Anindita Chakraborty¹, Disha Sengupta¹, Shamayita Roy¹, Sudeshna Shyamchowdhury¹ and Arup Kumar Mitra¹.

Rupa Chakraborty² and Somnath Mukherjee²

¹ P.G. Department of Microbiology, St. Xavier's College, Kolkata – 700 016.

² Division of Environmental Engineering, Department of Civil Engineering, Jadavpur University, Kolkata – 700 032.

Key words: Chromium, Bioremediation, *Lemna*, Bioaccumulation.

Abstract

The release of Chromium enriched effluent is a problem in many water bodies around leather tannery complex in East Kolkata. In the lentic ecosystem of that area the Chromium concentration varies between 5mg/L to 15mg/L, which is highly toxic. A maximum acceptable concentration of 0.05 mg/L (50 µg / L) for Chromium in drinking water has been established on the basis of health considerations. Thus it is necessary to maintain the Chromium concentrations of these water bodies within the acceptable limits. Bioremediation using certain hydrophytic plants can be used successfully to improve the quality of these waters. In this investigation *Lemna* was grown in such Chromium contaminated system in vitro and the plant was found to be tolerant to a maximum Chromium concentration of 60 mg/L. It also showed efficient Chromium uptake with highest efficiency of 91.67% at a concentration of 10 mg/L in 50 hours after which the plants showed partial chlorosis but continued to complete its life cycle. Bacteria isolated from the rhizosphere water of this plant also showed tolerance to a maximum of 130 mg/L of Chromium concentration though its efficiency in removing Chromium remained comparatively lower than the plant at around 65% for all different concentrations up to 50 mg/L in 12 hours. Hence they together can serve as potential bioremediator for Chromium pollution.

Introduction

Heavy metal pollution of water bodies is a cause of major concern due to the toxic effects of the metals on humans and other organisms. The toxicity is due to the ability of the metal ions to bind to protein molecules and prevent replication of DNA and subsequent cell division. The non-essential heavy metals like Cd²⁺, Hg²⁺, Cr²⁺ are released from many industries which is a major cause of such pollution. Activities like metal plating, cooling tower water treatment, hide tanning, wood preservation, production of refractory steel, chromic acid and speciality chemicals production, electroplating cleaning agents, leather processing requires

extensive use of the heavy metal Chromium and leads its subsequent release as effluents in the adjacent waterbodies. Among them the leather processing or the tannery industry causes the highest Chromium pollution in India.

A maximum acceptable concentration of 0.05 mg/L (50 µg / L) for Chromium in drinking water has been established on the basis of health considerations [1]. To avoid health hazards, it is essential to remove these toxic heavy metals from waste water before its disposal [2]. The conventional treatment methods used for this purpose include chemical precipitation, lime coagulation, ion exchange, chemical oxidation, electro dialysis, ultrafiltration and solvent extraction [3]. However, chemical processes are inefficient, energy intensive and prohibitively expensive [4]. Phytoremediation offers an alternative, economical and effective procedure which can be successfully used [5]. There are many plants which can tolerate the presence of Chromium and accumulate them within the plant body in the root, stem and leaves [6]. The metals are converted into less harmful substances within the plants or to their gaseous form which is transpired out [7]. Symbiotic bacteria associated with the rhizosphere of such plants can also uptake Chromium in significant levels. Active research is going on to find suitable plants which can be effectively grown in water-bodies having high Chromium concentrations and thereby help in its removal.

Lemna is a genus of free-floating aquatic plants from the duckweed family. It grows as a simple free-floating thallus on or just beneath the water surface. Most are small, not exceeding 5 mm in length. The plant grows by vegetative reproduction and can rapidly colonise new water bodies. This aquatic hydrophyte and its rhizosphere bacteria can bioaccumulate many toxic heavy metals including Chromium and it could be used for the bio remediation process [8]. The objectives of this study were (a) to assess the ability of the hydrophyte and its rhizosphere bacteria to tolerate and survive in water contaminated with Chromium; (b) to assess the level of uptake of Chromium by both and thereby determine the feasibility of using them in the bio-remediation process.

Materials and methods

Selection of and Sample collection: The lentic ecosystem surrounding the leather tannery complex in east Kolkata was chosen for studying the extent of chromium pollution and collecting plant and water samples for further study in laboratory. First of all, water samples were collected in plastic bottles which were deionised, distilled and rinsed with 10% nitric acid. Then, the sample of water was filtered using 0.45 micron cellulose acetate filters. Chromium concentrations in these water samples were measured using the DiphenylCarbazide Method (standard methodology according to VARIAN). The hydrophytic plant *Lemna* was collected from water bodies adjacent to the tannery complex. The plant was later grown in the laboratory in Knop's Solution. Water samples from the rhizosphere of *Lemna* plant was also collected for isolating its rhizosphere bacteria.

Propagation of plant and exposure to metals: The plant was washed properly in distilled

water and propagated vegetatively in the laboratory in Knop's solution. Different solutions of varying Chromium concentration was prepared up to 200 mg/L with intervals of 10 mg/L and the plant allowed to grow in it to check its maximum tolerance level. Five different test systems were prepared having Chromium concentrations of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L. 5 grams (dry weight) of plant were measured and then allowed to grow in these test systems to quantify the amount of Chromium uptake by the plants.

Determination of chromium: Water samples were collected at intervals of one hour till 6 hours and also after 28 hours and 50 hours from all the different test systems. The chromium concentrations in these samples were measured using the DiphenylCarbazide (DC) Method to determine the amount of Chromium taken up by the plants.

Isolation of Rhizosphere bacteria: The rhizosphere water was serially diluted and then pour plates were done in Nutrient Agar plates supplemented with varying amounts of Chromium to isolate bacterial samples which can tolerate Chromium in their growth medium.

The bacterial strain isolated was Gram positive rod showing positive result in Methyl red, Voges proskauer, Citrate utilisation, urease production test and starch hydrolysis test but negative result in indole production test.

Chromium uptake by bacteria: Pure cultures of the isolated samples were inoculated in nutrient agar broth in test tubes with chromium concentrations ranging from 10 mg/L to 300 mg/L to determine their highest tolerance level. Four different sets of nutrient agar broth in test tubes with Chromium concentrations of 10 mg/L to 50 mg/L with intervals of 10 mg/L were prepared. The four sets were analysed for Chromium concentrations after 3, 6, 9, 12 hours respectively. First, the test tubes were centrifuged at 1000 rpm for 5 minutes to precipitate out the bacterial cells and then the remaining amount of Chromium in the growth medium was again determined by the standard (DC) method.

Results:

Biosorption of chromium by *Lemna* plant: The ambient air and water temperature were fluctuating during the whole experiment period at around 25°C – 32°C. The *Lemna* plant showed tolerance towards a chromium concentration of up to 90 mg/L. At higher concentrations the plant died within a day and showed insignificant uptake due to the chromium concentrations becoming inhibitory for its growth. A time bound study of chromium removal is represented in Table 1.

Table 1: Concentration (in mg/L) of chromium removed from the solution after bioabsorption by *Lemna* plant:

Time	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour	28 hour	50 hour	percentage uptake
Initial Chromium Conc. (in mg/L)									
10	4	5	6	6	7	8	8	9.5	91.67%
20	5.8	8	8.5	9.6	9.7	10.5	13	15	75%
30	4	7	7.2	7.5	8	9	11	16	53%
40	4	6	7	7.9	9	10	12	13	32%
50	3	6	10	10	11	11	11	12	24%

Uptake of chromium by Rhizosphere bacteria: The rhizosphere bacteria of this plant showed a higher tolerance level till 130 ppm. A time bound study of the bacterial uptake of chromium is represented in Table 2

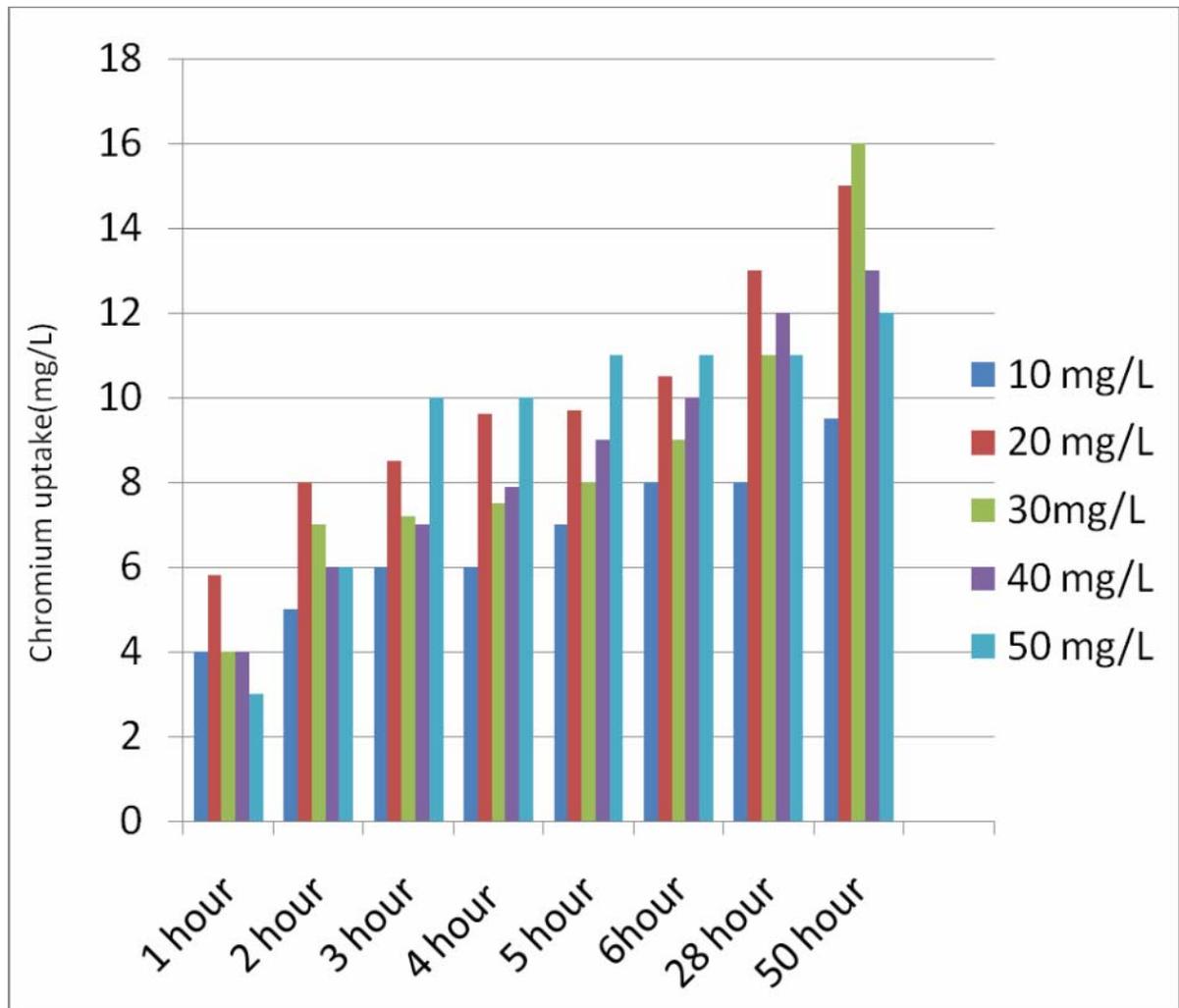
Table 2: Concentration (in mg/L) of Chromium removed from the solution after bioabsorption by rhizosphere bacteria associated with *Lemna* plant:

Time	3 hrs	6hrs	9 hrs	12 hrs	percentage uptake
Initial Chromium concentration (in mg/L)					
10 ppm	3	4	5	7	70%
20 ppm	7	7.8	11.1	13.1	65.5%
30 ppm	14	14.8	17.6	20	66%
40 ppm	9	21	25	26	65%
50 ppm	25	28	31	33	66%

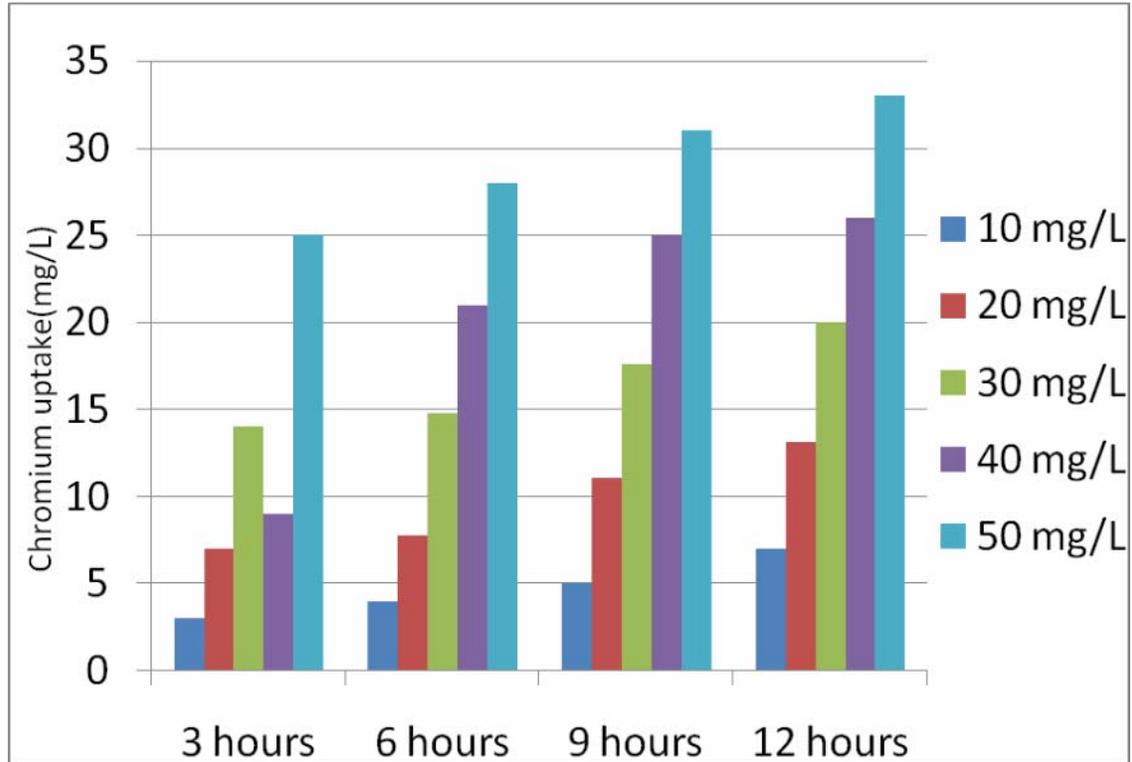
Discussion:

Monitoring the level of metal uptake by *Lemna* plant we could arrive at the following observations. *Lemna* plant is capable of absorbing significant amount of chromium from the systems at even very high concentrations. The plant removed as much as 91% Chromium at a concentration of 10 mg/L within 50 hours. Higher Chromium concentrations did not increase the amount of Chromium uptake by any significant amount. Hence the efficiency of removal dropped gradually and the plant could show only a 24% uptake at the highest concentration of 50 mg/L. The plants should gradual chlorosis of the leaves from the margin to the periphery with time, but it continued to complete its life cycle. The chlorosis area was greater with the initial concentrations being higher at any particular time point. The rhizosphere bacteria of this plant though could tolerate higher concentrations of chromium was not so

efficient in its removal. It showed a maximum of only 70% removal at the lowest initial concentration of 10 ppm. But it is significant to note that the percentage of removal remained constant for even higher Chromium concentrations. The following graphs give us a comparative picture of the uptake by the plant and its associated rhizosphere bacteria at different initial starting concentrations.



Graph1: Chromium uptake by *Lemna* plant



Graph2: Chromium uptake by rhizosphere bacteria associated with *Lemna*

Conclusion

Thus we can conclude that *Lemna* plant and its associated rhizosphere bacteria could remove Chromium efficiently from the test systems and hence can be effectively used for bioremediation of the lentic ecosystems polluted with Chromium released as effluent from the leather tannery complex.

Acknowledgements:

We would like to thank our respected Principal, Rev. Dr. J.Felix Raj S.J. and Vice Principal Rev. Fr. Jimmy Keepuram S.J. for the support throughout the entire span of working on the project. We are also grateful to our professors who generously guided us during the experiments.

References

1. F. Sterrett, Drinking water standards, Scavenger, 8, pp. 7-19, 1978.
2. WHO, Water Resource Quality." <http://www.who.int/> (11/17/05), accessed during July, 2011.
3. Rich G., Cherry K., Hazardous Waste Treatment Technologies, Pudvan Publishers, New York, 1987.
4. Vinod, T., "Pollution management in industries", 1989, Environment publications, Karad.
5. House, C. H., Bergmann, B. A., Stomp, A. M., Frederick, D. J. (1999). "Combining constructed wetlands and aquatic and soil filters for reclamation and reuse of water." Ecological Engineering, 12(1-2), pp. 27-38.
6. A. K. De, A. K. Sen, D. P. Modak and S. Jana, Comparative studies on the toxicity of Mercury (II) and Chromium (VI) to aquatic plants and their removal by these plants. In Environmental Management and Planning, A. K. Sen, Ed. Wiley Eastern Limited, New Delhi, 2008, pp.207-218.
7. S.P.B. Kamaludeen and K. Ramaswamy, (2008) Rhizoremediation of metals: harnessing microbial communities. Indian Journal of Microbiology 48 (1): 80-88.
8. MarkinMkandawire and E. GertDudel (2007). Are Lemna spp. effective phytoremediation agents? Bioremediation, Biodiversity and Bioavailability, pp. 56-71.
9. ArindamBasu, Sunil Kumar and SomnathMukherjee, Indian journal of environmental health, 2003, 45: 1,143-150.
10. HosseinEisazadeh, World Applied Sciences Journal, 2008, 3:1, 10-13.
11. Zavoda, J., Cutright, T., Szpak, J., and Fallon, E. (2001). "Uptake, selectivity, and inhibition of hydroponics treatment of contaminants." J Environ Eng., ASCE (127), pp. 502.
12. S. Beszedits. (1988). Chromium removal from Industrial waste waters, in: J.O. Nriagu, E. Nieboer. (Eds.), Chromium in Natural and Human Environment, John Wiley, New York, pp 232-263.
13. Arundhati Pal, Sumana Dutta and A.K. Paul (2005). Reduction of Hexavalent Chromium by cell free extract of *Bacillus sphaericus* 303 isolated from serpentine soil. Current Microbiology 51:327-330.