International Journal of Allied Practice, Research and Review
Website: www.ijaprr.com (ISSN 2350-1294)

Capacity of *Azolla pinnata var. imbricata* to Absorb Heavy Metals and Fluorides from the Wastewater of Oil and Petroleum Refining Industry at Vadodara

Punita S. Parikh and *Soma K. Mazumder
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda (MSU), VADODARA, Gujarat, (INDIA).

Abstract-*Azolla pinnata var. imbricata,* a local aquatic macrophyte growing in Vadodara was tested to determine its capacity to absorb heavy metals and fluoride from the two different concentrations (1ml/ml and 0.5ml/ml) of wastewater of Oil and Petroleum refining industry at Vadodara during the post monsoon period. There was found to be significant difference between the initial and final concentrations of the wastewater with respect to metal ions and fluoride after using *Azolla* plant for checking its phytoremediation capacity, but there was found to be no effect of dilution on the absorption capacity of ions by the test plant except for hexavalent chromium and iron. BCF of fluoride, zinc, cadmium, and iron though found to be less than one but BCF of lead, chromium, hexavalent chromium and copper was found to be approximately equivalent to one indicates not only its ability to phytoremedify these metal ions from the multimetal solution but also in pollution monitoring of other heavy metals and fluorides.

Key words*: Hexavalent chromium, Wastewater, Azolla, Fluoride, Phytoremediation*

I. INTRODUCTION

The main sources of heavy metal pollution are mining, milling, petro chemical industries, discharging a variety of toxic metals into the environment. Although the removal of toxic heavy metals from industrial waste waters has been practiced for several decades, the cost effectiveness of the most common physico-chemical processes such as oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, evaporation, ion-exchange and reverse osmosis is limited[33]. The need to remediate these sites has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal. Bioremediation, the use of microorganisms or microbial processes to degrade environmental contaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up of ground water, soils, lagoons, sludges, and process-waste streams. The bioremediation has been proven successful in numerous applications especially treating petroleum contaminated soils [21]. Petrochemical plants generate an aqueous effluent containing various conventional pollutants as well as specific petrochemicals and intermediates.

The effluent of Gujarat Refinery IOC Ltd. at Koyali of Vadodara containing heavy metals and fluorides together with other pollutants though found to be having Pollution Index(PI) less than one after treatment in their plant, since entering the ecosystem through various means may lead to Bioaccumulation, Geoaccumulation and Biomagnifications.

According to the Science News references fluorides and heavy metals are found to be having toxic effects on fishes and human beings even below the permissible limit of CPCB[12]. Fluoride (F) concentration as low as 0.5 mg/l can adversely affect invertebrates and fishes [9]. Even at low ambient concentration fluoride can cause a no. of physiological and biochemical changes in plants without visible sign of injury[22]. Wastewater or effluents can be treated either chemically or biologically or both to lower the load of harmful pollutants and so can be safely reused. Biological treatment is basically bioabsorpotion, which can be done by the mechanisms of phytoremediation. Plants may break down or degrade organic pollutants, or remove and stabilize metal contaminants where using living cells over non- living biomass to remove heavy metals is advantageous.

Azolla pinnata var. imbricata is a local macrophyte growing in Vadodara. *Azolla* spp. are heterosporous free-floating freshwater ferns that live symbiotically with *Anabaena azollae*, a nitrogen-fixing blue-green algae. The genus *Azolla* belongs to the single genus family Azollaceae. Various research findings had proved its ability to absorb metal ions from the media, with single metal solution as well as multimetal solutions and from wastewaters [19],[31]. In the present study *A. pinnata var. imbricata* was used to test its metal and fluoride uptake capacity from the wastewater of Oil and Petroleum refining industry at Vadodara, thereby determining its phytoremediation capacity.

II. MATERIALS AND METHODS

The plant material was collected from the Anand Agricultural University, washed thoroughly and kept in 2/5 N-free Hoagland solution [8], [27] for 5 days before the treatment with wastewater. Plastic pots (15.5cm in diameter and 17cm height) containing 200ml of wastewater in two different concentrations (1ml/ml, 0.50ml/ml) was used as the media for the growth of the plant. One gm of the test material per pot was used as the treatment material and each set was repeated in triplicate and placed randomly in the Botanical garden for 7 days in the post monsoon period.

Sampling of the wastewater was done using plastic buckets on the day of the experimental set-up from the outlet channels at Koyali, Vadodara and stored at 4°C, kept under refrigeration for further analysis of physico-chemical parameters methods as prescribed in APHA, 2005 [1], [10]. The samples of wastewater used as the media and test materials were collected and preserved for further testing after the specified period (7days) of treatment.

Digestion and analysis of elements in plant tissue and in the wastewater (before and after the treatment period) was done following the method prescribed in APHA (2005) [1]. Preparation of the test plant materials for elemental analysis was done by wet-digestion method using con. HNO3.The concentrations of metals present in the solutions (for both wastewater and plant tissue) were measured using AAS. The difference between the initial metal concentration and remaining metal concentration in the solution after the treatment period (7days) was taken to be metals bound to the plant[2].

The amount of metal ions per unit of the plant material(biosorbent) was obtained by using the following expression [14]:

$q = [(C0 – C1) V]/M$

where q is the amount of heavy metal onto the unit amount of biosorbents (mg/g) and C0 and C1 are the concentrations of metal ions from the solution (mg/l) before and after biosorption respectively; V is the volume of the aqueous phase and M is the amount of the biosorbent (g) [32].

Percent removal efficiency was calculated by using following formula [15]:

Percent removal efficiency = inlet pollutants- outlet pollutants / inlet pollutants *100

Bioconcentration factor:

The ability of the plant to accumulate metals with respect to the metal concentration in the substrate is known as the bioconcentration factor (BCF). BCF could be calculated as follows[38]:

BCF = Concentration of metal in plant tissue / Initial Concentration of metal in external solution.

Statistical analysis:

Wilcoxson signed ranked test to find out the significant difference between the concentrations of the wastewater before and after the treatment and between the % absorption of metal ions in both the concentrations of wastewater used in the study. Correlation between initial concentration of wastewater and concentration absorbed by plant tissue was done using spss 19.

III. RESULT

Physico - chemical analysis of the wastewater initially and after treatment showed the presence of metal ions [Hexavalent chromium(HCr), Chromium(Cr), Lead(Pb), Zinc(Zn), Nickel (Ni), Copper(Cu), Cadmium(Cd), Iron(Fe)] and fluoride in varying concentrations but within the permissible limit laid down by CPCB (Fig-1). PH, TDS, BOD and COD, EC of the wastewater was found to be 8.21, 19mg/L, 81mg/L, 892mg/L and 1.393dS/m respectively. Temperature and relative humidity ranged from 23°C to 32°C and 56% to 70% respectively during the experimental period**.** Photoperiod received was 12.5hr day and 11.5hr night. BCF of metal ions and fluoride (table-1) though found to be less than one in the pure wastewater but equal to one for hexavalent chromium in the diluted concentration (0.50ml/ml) of wastewater after treatment.There was found to be no significant difference in the percentage absorption of metal ions by the test plant material in both the concentrations of wastewater used at 0.05 level of significance ($p > 0.05$). There was found to be significant difference between the initial and final concentrations of the wastewater with respect to metal ions and fluoride after the treatment with *Azolla* at 0.01 level of significance according to Wilcoxon- singed ranked test ($p=0.004 < 0.01$). Positive correlation between the metal ions and fluoride in the initial solution and the plant tissue absorbed was found at 0.05 level of significance ($p < 0.05$) in both the concentrations of wastewater used. Percentage absorption and per gram absorption by the plant tissue of most of the metal ions was found to be more in the pure wastewater than the diluted concentration of wastewater (0.5ml/ml) except for hexavalent chromium and iron (Fe) (Table-1). Copper, chromium and hexavalent chromium were found to be absorbed more than 50% from the pure wastewater as well as from the diluted concentration(0.5ml/ml) of wastewater whereas lead was found to be absorbed more than 50% from the pure wastewater but less than 50% from the diluted concentrations (0.5ml/ml) of the wastewater. Fluoride, zinc, nickel, cadmium and iron were absorbed in less than 50% from both pure wastewater and its diluted concentration (0.5ml/ml).

IV. DISCUSION

Metal ions are mostly present in nature together with other ions in the soil or in water. Wastewater of most industries are containing an aqueous solution of pollutants in varying concentration. Oil and Petroleum Refining industries also have their effluent treated both chemically as well as biologically before releasing into the outlet channels. So these wastewater generated from them not only contains heavy metals in lower concentrations but also have nutrients necessary for the growth of a plant. Plants can easily grow in them with varying degree of biomass generated in them together with their potential to absorb the pollutant heavy metals helping in further lowering the concentrations of the pollutants. Aquatic vascular plants absorb heavy metals, concentrate them, and play an important role in storing and recycling metals [7],[20],[11],[23],[25]. No generalization, however, is possible due to the fact that different species have different mechanism of absorption, transport, and storage of metals [3], [6],[13].

In the present study the test plant material was found to absorb copper, chromium and hexavalnt chromium more than 70% and lead above 50% from the pure wastewater. Dilution of the wastewater was not found to increase the percentage absorption of metal ions except hexavalent chromium which was absorbed totally from the diluted wastewater after treatment. Hexavalent chromium, chromium and fluoride have an anagonistic effect on the uptake of other metal ions by competing with their carrier proteins or by some other way of interactions [34], [28]. Lead, copper and cadmium was also found to be having an interfering effect on each other in their absorption capacity by plants [5]. Zinc, Nickel, and Iron are absorbed below 40% from pure wastewater by the test plant whereas from the diluted wastewater (0.5ml/ml) it was 0%, 0% and 35% respectively. 4.6% of Fluoride was absorbed from the pure wastewater whereas non from the diluted wastewater (0.5ml/ml). Non essential elements generally may compets with the absorption of essential elements as they generally do not have any specific carriers or mechanism of absorption by the plants or not yet investigated.[34], [24]. According to Das et al. (1997) Cd was reported to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants [16], [30]. Since lead was absorbed more than 45-50% from the medium it may inhibit the activity of enzymes at cellular level by reacting with their sulfhydril groups, cause water imbalance, and alterations in membrane permeability, disturbs mineral nutrition and thus may affect the uptake of other ions [35], [30]. However no synergistic/antagonistic effect was noted for the multiple metal experiments, in terms of metal removal for lead and nickel [4]. Though the concentration of Zinc in the initial concentration of pure wastewater was more than copper but still copper was found to be absorbed of about 88% and 80% from the pure and diluted wastewater respectively whereas according to Ebbs and Kochian (1997) [17] Zn rich media have an antagonistic effect on copper. Heavy metal uptake is not linear in response to the increasing concentrations. As far as the growing environment is concerned the increase in pH, i.e., the environment becoming more alkaline, and decrease in Eh (redox potential), i.e., the environment becoming more reducing, result in decrease in availability of heavy metals, or metals in general to plants [29], [30]. The absorption capacity of a plant thus is not only the outcome of the combination of metal ions present in the solution or media together with other influencing factors like PH, BOD, COD, TDS, EC etc. but also the type of the plant growing in it [37]. The concentration of chromium and iron are high in comparision to other metal ions in the medium which might be the main interacting factor affecting the absorption of other ions.Thus interaction and chelation are two most important reactions which influence significantly the uptake of ions by plants from any medium. The percentage absorption of metal ions was found to be in the order of Cu> HCr> Cr> Pb> Ni> Cd> Zn> Fe> F in pure wastewater (1ml/ml) whereas it was in the order of HCr> Cu> Cr> Pb> Fe> Cd> Ni=Zn= F in the diluted medium (0.5ml/ml). No significant difference in the % absorption in the different concentrations of the wastewater shows no effect of dilution on the absorption capacity of ions by the test plant. Strong correlation between the metal ions in the solution and the plant tissue absorbed shows its bioindicating capacity of metal ions. BCF of lead, chromium, hexavalent chromium and copper indicates its ability to phytoremedify these metal ions from the multimetal solution [26]. Thus *Azolla pinnata var. imbricata* have the capacity to phytoremedify the wastewater from Oil and Petroleum Refining Industry at vadodara as *Azolla* was also reported to be capable of phytoremedifying different types of metal ions from wastewater and effluents from various sources [36],[18]. Further studies needed to be done to investigate the effectiveness of its phytoremediation capacity of the wastewater from Oil and Petroleum Refining Industry at Vadodara in different seasons of the year.

V. CONCLUSION

Significant difference between the initial and final concentrations of the wastewater with respect to metal ions and fluorides after the treatment with *Azolla pinnata var. imbricata* and BCF of lead, chromium, hexavalent, chromium and copper being found to be approximately equivalent to one indicates its ability to phytoremedify these metal ions from the multimetal solution as well as pollution monitoring of heavy metals and fluorides.

VI. ACKNOWDGEMENT

We are thankfull to the staff of GPCB for their valuable guidance in the analysis of wastewater.

V. REFERENCES

[1] APHA, AWWA, WEF. (2005). In: Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Environment Federation, 21 Ed. Washington, D.C.

[2] Aisien, F. A., Faleye, O. and Aisien, E. T. (2010). Phytoremediation of Heavy Metals in Aqueous Solutions. *Leonardo Journal of Sciences*,17 (July-December): 37-46.

[3] Antonovies, J., A. D. Bradshaw & R. D. Turner. (1971). Heavy metal tolerance in plants. Adv. Ecol. Res. 7: 2-85.

[4]Axtell, R. N., Sternberg, P.K.S., Claussen, K. (2003). Lead and Nickel removal using *Microspora* and *Lemna minor*, Biosource *Technology*, 89 (1): 41-48.

[5] AL-Subu, M. M.,Salim, R.,Douleh, A. and Atallah, A. (1993). Combined effects of Cadmium, Lead and Copper on the growth and on metal uptake of broad beans, carrots, radishes and marrow vegetables. *Rev.Int. Contam.Ambien*t. 9 (l): 1-9. www.journals.unam.mx/index.php/rica/article/download/29852/27757

[6] Baudo, R. & P. G. Varini. (1976). Copper, manganese and chromium concentrations in five macrophytes from the delta of River Toce (northern Italy). *Mem. Ist. Ital. Idrobiol.* 33: 305-324.
--, G. Galanti, P. Guilizzoni & P. G. Varini. (1981). Relationships between heavy metals and aquatic organisms in the Lake Mezzola hydrographic system (northern Italy). 4. Metal concentrations in six submerged aquatic macrophytes. *Mem. Ist. Ital. Idrobiol*. 39: 203-225.

[7] Boyed, C. E. (1970). Vascular aquatic plants for mineral nutrient removal from polluted waters. Econ. Bot. 24: 95-103.

[8] Chris, A., Luxmisha, G., Masih, J. and Abraham, G.,(2011). Growth, photosynthetic pigments and antioxidant responses of ,Azolla filiculoides to monocrotophos toxicity,J. Chem. Pharm. Res., 3(3):381-388. http://jocpr.com/vol3-iss3-2011/JCPR-2011-3-3-381-388.pdf

[9] Carmargo, J. A., (2003). Fluoride toxicity to aquatic organisms: a review, *Chemosphere*, 50 (3): 251-64. www.ncbi.nlm.nih.gov/pubmed/12656244

[10] Cano-Rodriguez, C.T., Rao-Morales, G., Amaya-Chavez, A., Valdes-Arias, R.A., Barrera-Diaz, C.E. and Balderas-Hernandez, P. (2014).Tolerance of *Myriophyllumaquaticum* to exposure of industrial wastewater pre-treatment with electro coagulation and their efficiency in the removal of pollutants. *Journal of environmental Biology*, 35 (special issue): 127-136.

[11] Cowgill, U. M. (1974). Hydrogeochemistry of Linsley Pond, North Bradford, Connecticut, Ill. The chemical composition of the aquatic macrophytes. Arch. Hydrobiol., Suppl. 45:1-119.

[12] CPCB report. (2008). SCHEDULE – VI, The Environment (Protection) Rules, 1986, CPCB, pp 545- 551. http://cpcb.nic.in/GeneralStandards.pdf

[13] Chandra, P., Kulshreshtha, K.(2004).Chromium accumulation and toxicity in aquatic vascular plants, The Botanical Review, *New York Botanical Garden*, 70 (3).

[14] Demirbas, E., Kobya, M., Sender, E., Ozkan, T. (2004). Adsorption kinetics for the removal of Cr (VI) from aqueous solutions on the activated carbons prepared from agricultural wastes, *Water SA*, 30: 533 –539.

[15] Deval, C.G., Mane, A.V. Joshi, N.P. and Saratale, G.D. (2012). Phytoremediation potential of aquatic macrophyte *Azolla caroliniana* with references to zinc plating effluent. *Emir. J. Food Agric*. 24 (3): 208-223.http://ejfa.info/

[16] Das .P, Samantaray S., Rout G.R. (1997). Studies on cadmium toxicity in plants: a review. *Environ Pollut*, 98:29–36.

[17] Ebbs, S.D., Kochian, L.V. (1997) Toxicity of zinc and copper to Brassica species: implications for phytoremediation. *J Environ Qual*., 26:776–781.

[18] El-All, A.B.D., Azza, M. A., Aref, M. E. and Hassanein, M. A. H. (2011), Bioaccumulation of heavy metals by the water fern *Azolla pinnata, Egypt. J. Agric. Res*., 89 (4): 1261- 1276.

[19] Forni, C., Chen, J., Tancioni, L., GrillliCaiola, M. (2001).Evaluation of the fern Azolla for the growth, Nitrogen and Phosphorus removal from wastewater.*Wat. Res.*, 35 (6): 1592-1598.

[20] Frazier, J. M. (1972). Current status of knowledge of the biological effects of heavy metals in the Chesapeake Bay. *Chesapeake Sci*., 13 (suppl.): 149-153.

[21] Plaza, G., Nalecz-Jawecki,G., Ulfig, K., Robin, Brigmon L. (2005). Assessment of genotoxic activity of petroleum hydrocarbon-bioremediated soil. *Ecotoxicology and Environmental Safety*, 62:415-420.

[22] Gautam, R., Bhardwaj, N. (2010).Bioaccumulation of fluoride in different plant parts of *Hordeumvulgare* (Barley) var. RD- 2683 from irrigation water, *Research Report Fluoride*, 43 (1): 57-60.www.fluorideresearch.org/431a/files/FJ2010_v43_n1_p057-060.pdf

[23] Gommes, R. & Muntau. H. (1975). La Distribution de quelques metaux lourds (Zn, Cu, Cr, N[I.sub.1] Mn, Co) duns la zone littorale des bassins and et de Pallanza du lac Mageur. *Mem. Ist. Ital. Idrobiol*. 32: 245-259.
-- & --. (1981). Determination in situ des vitesses d'absorption de mentaux lourd par les macrophytes du lago Maggiore. *Mem. Ist. Ital. Idrobio*l., 38: 347-377.

[24] Kieling-Rubio, M. A., Droste, A., Windisch, P. G. (2010). Germination and sporophytic development of *Regnellidium diphyllum* Lindman (Marsileaceae) in the presence of hexavalent chromium, *Braz. J. Biol*., 70, no.4 (Suppl.): 1149-1153. http://dx.doi.org/10.1590/S151969842010000600003

[25]Low, K. S. & Lee, C. K. (1981). Copper, zinc, nickel and chromium uptake by Kankong air (Ipomoea aquatica Forsk). *Pertanika* 4: 16-20.

[26] Lorestani, B., Cheraghi, M., and Yousefi,N. (2011). Phytoremediation Potential of Native Plants Growing on a Heavy Metals Contaminated Soil of Copper mine in Iran. *World Academy of Science, Engineering and Technology*. 5: 05-24.

[27]Ling-Peng, D., Xin-Jiao, D., Hai-Hu, M. (2012). Antioxidative and Chelating Properties of Anthocyanins in *Azolla imbricate* Induced by Cadmium, *Pol. J. Environ. Stud.* 21(4): 837-844. http://www.pjoes.com/pdf/21.4/Pol.J.Environ.Stud.Vol.21.No.4.837-844.pdf.

[28] Machado, M. D., Soares, E. V. and Soares, H. M.V.M.(2011). Impact of fluorides on the removal of heavy metals from an electroplating effluent using a flocculent brewer's yeast strain of Saccharomyces cerevisiae. *Chemical Speciation & Bioavailability*, 23 (4): 237-242.

[29] Misra S.G., Mani, D. (1991). In: Soil pollution. Ashish Publishing House, Punjabi Bagh.

[30]Nagajyoti, C.P., Lee, D. K., Sreekanth, M. V. T. (2010). Heavy metals, occurrence and toxicity for plants: a review, *Environmental Chemistry Letters*, 8(3): 199- 216.

[31] Pabby, A., Prasanna, R. and Singh, P.K. (2004). Biological Significance of Azolla and its Utilization in Agriculture, *Pro. Indian Natl. Sci. Acad*., BTO(3):299-333. http://www.new.dli.ernet.in/rawdataupload/upload/insa/INSA_1/2000c954-299.pdf

[32] Padmapriya, G. and Murugesan, A.G. (2012). Phytoremediation of various heavy metals (Cu, Pb and Hg) from aqueous solution using water hyacinth and its toxicity on plants. *International Journal of Environmental Biology*, 2 (3): 97-103. http://www.urpjournals.com, http://urpjournals.com/tocjnls/13_12v2i2_11.pdf

[33] Rajasulochana, P., Dhamotharan, R., Murugesan, S. and Rama Chandra Murthy, A. (2009), Bioremediation of Oil Refinery Effluent By Using Scenedesmus Obliquus, *Journal of American Science* , 5(4):17-22.

[34] Shanker, A. K., Cervantes, C., Loza-Tavera, H., Avudainayagam, S. (2005). Chromium toxicity in plants -Review article, *Environment International*, 31(5): 739-753.

[35] Sharma P., Dubey R.S. (2005). Lead toxicity in plants, *Braz. J. Plant Physiol*., 17:35–52.

[36] Vaseem, H., Banerjee, K.T. (2012). Phytoremediation of the Toxic Effluent Generated During Recovery of Precious Metals from Polymetallic Sea Nodules, *International Journal of Phytoremediation*, 14 (5) : 457- 466.

[37] Yamamoto F., Kozlowski T.T. (1987). Effect of flooding, tilting of stem, and ether application on growth, stem anatomy, and ethylene production of Acer platanoides seedlings, *Scand. J. For Res*., 2:141–156.

[38] Zayed A., Gowthaman S., Terry N. (1998). Phytoaccumulation of trace elements by wetland plants: I. Duckweed, *Environ. Qual*., 27(3): 715-721.

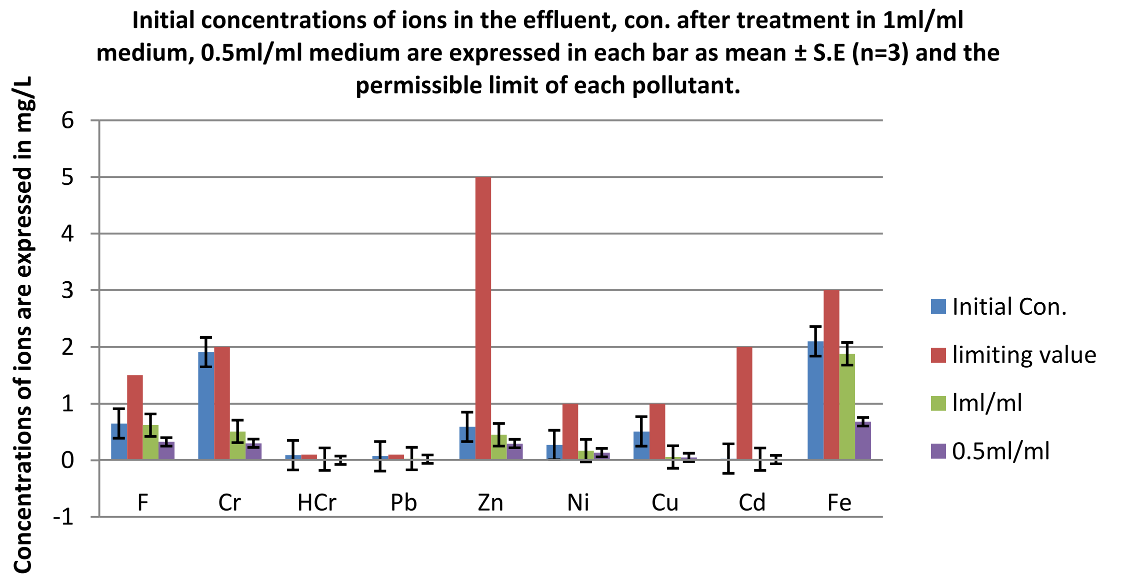

Fig- 1

Table-1:

Ions	% Absorption in 1ml/ml	% Absorption in 0.5ml/ml	BCF in 1ml/ml	BCF in 0..5ml/ml	Absorption in mg/ gm of tissue in 1ml/ml	Absorption in mg/ gm of tissue in 0.5ml/ml
F	4.6153846	0	0.0461538	0	0.0039735	0
Cr	73.298429	68.586387	0.7329843	0.6858639	0.1854305	0.0839744
Hcr	77.777778	100	0.7777778	1	0.0092715	0.0576923
Pb	57.142857	42.857143	0.5714286	0.4285714	0.005298	0.0019231
Zn	23.728814	0	0.2372881	0.2625	0.018543	0.0134615
Ni	37.037037	0	0.3703704	0.0357143	0.013245	0.000641
Cu	88.627451	80.392157	0.8862745	0.8039216	0.0598675	0.0262821
Cd	36.666667	20	0.3666667	0.2	0.001457	0.0003846
Fe	10.47619	35.238095	0.1047619	0.352381	0.0291391	0.0474359