WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 9, 1341-1351. Research Article ISSN 2277– 7105

STUDY ON ISOLATION AND IDENTIFICATION OF SOIL FUNGI FOR TOLERANCE TO HEAVY METALS POLLUTANTS

Rajesh Kumar*[1], Krishan Pal[2], Anju Pal[3]

[1]Research Scholar, Dept. of Biotechnology, Shri Venkateshwara University, Gajraula, (UP) – India.

[2]Department of Biotechnology, Shri Venkateshwara University, Uttar Pradesh, India.

[3]Scientist, Medicinal and Aromatic Plants, K.V.K. Haridwar G.B.Pant University of Ag. & Technology, Pantnagar, Uttarakhand, India.

Article Received on
24 June 2015,

Revised on 14 July 2015,
Accepted on 08 Aug 2015

***Correspondence**
For Author
Rajesh Kumar
Research Scholar, Dept. of Biotechnology, Shri Venkateshwara University,
Gajraula, (UP) – India.

ABSTRACT

The modern world is facing today a number of environmental problems – one of the major problems being that of pollution by heavy metals. Due to their mobility in natural water ecosystems as well as their toxicity to higher life forms, heavy metal ions in surface water and ground water supplies are emerging as major inorganic contaminants in the environment. This study was conducted to investigate the tolerance of some resistant fungal strains from soils contaminated with heavy metals. Various fungal strains (**A*spergillus niger, Trichoderma lignorum, Fusarium moniliforme, Sepedonium chrysospermum* and *Botryotrichum atrogriseum***) were isolated from soil samples collected from studied sites which heavy metals like (Cr and Fe) and other pollutants have been emitted in effluents for several years. The present study was undertaken with an aim to obtain from soil some fungal strains which have the potential to tolerate heavy metals. The metals taken for study in the present investigation were: (a) cadmium as cadmium chloride; (b) nickel as nickel chloride; (c) copper as copper sulphate; (d) iron as iron sulphate; and (e) lead as lead nitrate. The approach suggested by Babich and Stotzky (1982) was followed, which is based upon the isolation of tolerant organisms from natural habitats using a nutrient medium in which the pollutant (for example, the metal of interest) has been incorporated. A total of 41 fungal species were isolated from the soil samples in the present investigation. Out of these, 37 species could be identified which belong to eighteen genera. Out of these 37 species, only two species belong

to Zygomycota; one unidentified species belongs to Ascomycota and the remaining 35 were anamorphic fungi (Deuteromycota). The microbial number was remarkably higher in the control soil than contaminated soil samples collected from mining areas. and had the highest concentration in the polluted soils. The minimum inhibition concentrations (MICs) of and showed the highest values against the fungal strains. and were the lowest contaminants in the polluted. The tested resistant strains showed the strongest inhibition.

KEYWORDS: heavy metal, fungi, Pollution, tolerance.

INTRODUCTION

Heavy metal pollution is a serious environmental problem of global concern. Heavy metals are continuously released into the environment due to industrial and techno- logical developments, and contamination of agricultural soil with heavy metals is a major problem at industrial and defense-related sites all over the world.[1] In developing countries industrial or municipal wastewater is mostly used for the irrigation of crops, mainly in peri urban ecosystems, due to its easy availability, disposal problems, and scarcity of fresh water.[2]

The term “heavy metals”, though not strictly defined, is commonly used for those metals which have specific weights more than five grams per cm^3 (Holleman and Wiberg, 1985). The metals with atomic number 23 onwards (except Rb, Sr, Y, Cs, Ba, Fr) are generally referred to as heavy metals. There are about 40 elements which fall into this category. Though a number of heavy metals are essential micronutrients for both plants and animals (Eichenberger, 1986; Wintz *et al.*, 2002), these are potentially toxic at elevated concentrations (Gadd and White, 1989). Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb are believed to be the metals of immediate concern to the mankind specially with respect to pollution caused by them more specifically due to their discharge into the aquatic environment (Son *et al.*, 2004). Even if these metals are present in dilute undetectable quantities, their recalcitrance and consequent persistence in water bodies might ultimately, through biomagnifications, increase their concentrations to such an extent that these begin exhibiting toxic characteristics. Heavy metals, once released into the soil matrix, also find their way into the food web through ground water aquifers (Walton, 1995).

Heavy metal pollution can originate from natural as well as anthropogenic sources. Activities like mining and smelting operations as also agriculture have led to the contamination of extensive areas all over the world (Smith *et al*., 1996; Shallari *et al*., 1998; Zantopoulos *et al*.,

1999; Herawati *et al*., 2000). Heavy metals are commonly used in many industries; for example, those concerned with the manufacturing of pesticides, batteries, alloys, electroplated metal parts, dyes, steel, electrical transformers etc. These generate aqueous effluents containing metal pollutants the concentration of which must be reduced to the levels which do not cause much harm to the environment (Joseph, 1995; Lovely and Covas, 1997). Small scale and medium scale industries in India usually suffer from low budget and, therefore, are the major sources of pollution because of two reasons: (a) use of obsolete technologies in manufacturing processes; (b) lack of financial ability to invest on conventional waste water treatment systems which are quite expensive (Horsfall *et al.*, 2003). The conventional techniques for the removal of metals from industrial effluents include precipitation, ion-exchange and electrolytic techniques (Blanco *et al.*, 1999).

Chemical precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. However, a large amount of sludge containing toxic compounds is produced during the process. In ion-exchange process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. It has two major disadvantages: (i) high cost, and (ii) partial removal of certain ions. In electrolysis, with the help of semi-permeable ion-selective membranes, the heavy metals are separated out. Application of electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. But, it results in the formation of metal hydroxides which clog the membrane.

In the 1990s, a new scientific area of biosorption has come into limelight that had an objective of recovering heavy metals. It is based on the metal-binding capacities of various biological materials. Several studies have revealed that non-living and living biomass can be used effectively for removing metals from the environment (Lujan *et al.*, 1994; Sood *et al.*, 1994; Mofa, 1995; Gardea-Torresdey *et al.*, 1996; Ingole and Bhole, 2000; Horsfall *et al.*, 2003; Mahvi *et al.*, 2005). A number of workers in the past had advocated the use of microorganisms for accumulating heavy metals and radionuclides from their external environment (Gadd and Griffiths, 1978; Macaskie and Dean, 1989, 1990; Volesky, 1990; Birch and Bachofen, 1990; Avery *et al.*, 1991; Gadd, 1992).

The term ‘biotraps’ has been coined (Crusberg *et al.*, 2004) for the organisms (living or dead) or component of an organism which can alter the form of, or bind with the toxic metal or metal ion allowing its removal and recovery from various streams, or rendering it

harmless.The toxicity of heavy metals, ability of fungi to serve as biotraps for heavy metals and biosorb them has attracted the attention of a number of workers and this aspect has been the subject of several reviews including that by Volesky and Holan (1995), Blackwell *et al.* (1995) , Kapoor and Viraraghavan (1997) , Dua *et al.*(2002), Srivastava *et al.* (2002), Eisler (2003), Ramteke (2005), Bellion *et al.* (2006), Fomina *et al.* (2005, 2006) and Duruibe *et al.* (2007).

MATERIALS AND METHODS

Collection of soil samples

The soil samples for the present study will be collected from the fields situated near Ghaziabad. Five samples were collected from different sites. The upper layer of soil will be removed with the help of a trowel to remove extraneous litter/organic matter. Soil samples will be then taken out with the help of a trowel and were collected in fresh sterile polythene bags. These will be brought to the laboratory where all the five samples were mixed to obtain one composite sample. These will be stored in a refrigerator for further studies.

Isolation of fungi

Serial dilution plate method (Waksman, 1927) will be followed to isolate fungi from the soil samples. 20 g of the sample were placed in 200 ml of sterile water and stirred for fifteen minutes using a magnetic stirrer to get a stock solution (1:10 dilution). 10 ml of this solution were immediately transferred to a conical flask containing 90 ml of sterile distilled water to get a suspension of 1:100 dilution. This suspension will be used for the preparation of further serial dilutions (1:1000 and 1:10,000).The suspension of 1:10 dilution will be discarded. From the suspension of each of the remaining three dilutions (1:100, 1:1000, 1:10,000), 1ml aliquots will be transferred to each of a set of three Petri dishes followed by the addition of approximately 20 ml of sterilised and cooled (45°C) Czapek's Dox Agar medium (Raper and Thom, 1949) with 30 ppm of rose bengal and 30mg of streptomycin. The composition of medium is as under:

Composition of Czapek-Dox Agar Medium (Raper and Thom, 1949):

Sodium nitrate ($NaNO_3$)	2.0 g
Potassium phosphate (K_2HPO_4 or KH_2PO_4)	1.0 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5 g
Potassium chloride (KCl)	0.5 g
Ferrous sulphate ($FeSO_4.7H_2O$)	0.01 g

Sucrose	30.0 g
Agar-Agar	15.0 g
Water	1000.0 ml
Rose bengal	0.03 g

This medium will be served as control and is designated as N (Normal). In addition to the normal medium, media amended with different concentrations (40 ppm, 200 ppm and 400 ppm) of each the five heavy metals *i.e.*, Pb (as lead nitrate), Cu (as copper sulphate), Fe (as iron sulphate), Cd (as cadmium chloride) and Ni (as nickel chloride) were also prepared.

For each type of amended medium, a set of nine Petri dishes were used and processed as done with the normal medium.

In this way, 36 Petri dishes were used for each metal *i.e.*, three dilutions × four types of media (control + amended with 40 ppm, 200 ppm and 400 ppm) × three replicates. These Petri dishes containing the media and the inocula were incubated at 25 □ 1°C for 6 to 8 days.

MAINTENANCE OF CULTURES

The pure cultures will be maintained on the slants of PDA Medium (Riker and Riker, 1936). The composition of which is given below and will be stored in a refrigerator.

Composition of Potato Dextrose Agar :

Agar-Agar	20.0 g
Potato (Peeled)	200.0 g
Dextrose	20.0 g
Distilled water	1000.0 ml
pH	6.2

Statistical Analysis

The experiments were set up with three replicates. Analysis of variance was performed by using statistical software to compare resistance to metal among individual isolates.

RESULTS AND DISCUSSION

As a result, fifteen types of amended media were prepared: (a) M_1: Medium + 40 ppm $Pb(NO_3)_2$; (b) M_2: Medium + 200 ppm $Pb(NO_3)_2$; (c) M_3: Medium + 400 ppm $Pb(NO_3)_2$; (d) M_4: Medium + 40 ppm $CuSO_4$; (e) M_5: Medium + 200 ppm $CuSO_4$; (f) M_6:

Medium + 400 ppm $CuSO_4$; (g) M_7: Medium + 40 ppm $FeSO_4$; (h) M_8: Medium + 200ppm $FeSO_4$; (i) M_9: Medium + 400 ppm $FeSO_4$; (j) M_{10}: Medium + 40 ppm $CdCl_2$; (k) M_{11}: Medium + 200ppm $CdCl_2$; (l) M_{12}: Medium + 400 ppm $CdCl_2$; (m) M_{13}: Medium + 40 ppm $NiCl_2$; (n) M_{14}: Medium + 200 ppm $NiCl_2$; (o) M_{15}: Medium + 400 ppm $NiCl_2$. The Petri dishes containing the media and the inocula were incubated at 25±1°C for 6-8 days and the fungi growing in the Petri dishes were incubated, maintained in axenic cultures and were identified with the help of standard monographs. The axenic cultures were maintained on the slants of PDA medium.

A total of 41 fungal species were isolated from the soil samples following the method outlined in the preceding paragraph. Out of these, only two species belong to Zygomycota, thus, confirming the suggestion of some earlier mycologists that there is a paucity of mucorales in the tropical region of India. As many as 35 species belong to Deuteromycota confirming the earlier reports regarding the dominance of Deuteromycota among the soil mycobiota. Even among the Deuteromycota, the Hyphomycetes constituted the major components. The number of Penicillia isolated was much lesser than that of Aspergilli. Thus, the present study also supported the generalization that the Aspergilli are abundant in the warmer regions of the world while the Penicillia are more common in the colder regions.

Five species of fungi *i.e.*, *Aspergillus niger*, *Trichoderma lignorum*, *Botryotrichum atrogriseum*, *Fusarium moniliforme* and *Sepedonium chrysospermum* were selected to study the effect of metals on the ability of fungi to degrade litter. Since a number of studies in the recent past have been conducted in this laboratory with wheat straw as a substrate, it was decided to work out the effect of the metals in question on the decomposition of wheat straw by the selected fungal species. All the five fungal species could cause the decomposition of straw to different extents ranging from 19.38% to 25.66%. Maximum decomposition of 25.66% was caused by *Sepedonium chrysospermum* followed by *Botryotrichum atrogriseum*, *Fusarium moniliforme*, *Aspergillus niger* and *Trichoderma lignorum*. Thus, these fungal species could be arranged as under in the order of decreasing ability to decompose wheat straw: *Sepedonium chrysospermum* > *Botryotrichum atrogriseum* > *Fusarium moniliforme* > *Aspergillus niger* > *Trichoderma lignorum*. Out of the five metals, cadmium was found to have profound inhibitory effect on the decomposition potential of all the five fungal species. The presence of cadmium reduced the decomposition by as much as 36.84% to 54.09%. Maximum reduction in the decomposition potential by cadmium was observed in the case of

Sepedonium chrysospermum followed by *Botryotrichum atrogriseum*, *Fusarium moniliforme*, *Aspergillus niger* and *Trichoderma lignorum*. Nickel was another metal which could cause reduction in decomposition by all the five fungal species investigated for the purpose. However, the degree of inhibition of decomposition by nickel, as also by other metals, was much lesser as compared to that by cadmium. The maximum inhibition of decomposition was in the case of *Sepedonium chrysospermum* followed by *Botryotrichum atrogriseum*, *Trichoderma lignorum*, *Aspergillus niger* and *Fusarium moniliforme*. As far as the adverse effect of metals in relation to decomposition potential of individual fungal species is concerned, these could be arranged as under: (a) *Aspergillus niger*: Cd > Cu > Pb > Fe > Ni; (b) *Botryotrichum atrogriseum*: Cd > Cu > Ni > Pb = Fe; (c) *Fusarium moniliforme*: Cd > Ni >Fe > Cu > Pb; (d) *Sepedonium chrysospermum* : Cd > Cu > Fe > Ni > Pb; (e) *Trichoderma lignorum* : Cd > Ni > Cu > Pb > Fe. Interestingly, maximum reduction in the decomposition potential by all the five metals was observed in *Sepedonium chrysospermum* while maximum decrease in the rate of decomposition was also observed due to metals in the case of this particular fungal species. *Botryotrichum atrogriseum* was closely followed the performance of *Sepedonium chrysospermum*. Thus, it appears that the effect of metals on *in vitro* growth of a fungus is not necessarily a reliable index of its capacity to withstand the metal as far as its activities in the soil especially decomposition is concerned. Stimulation of decomposition in the case of *Trichoderma lignorum* by iron and copper as also in the case of *Fusarium moniliforme* and *Trichoderma lignorum* by lead nitrate indicated that at present it is not possible to provide any generalized statement with respect to the effect of metals on the process of litter decomposition by fungi.

All the five fungal species under study exhibited moderate to very good growth on the Hankins and Anagnostakis medium (pH 5.0), thus indicating the ability of five fungal species to produce polygalacturonase. Maximum growth was observed in the case of *Aspergillus niger* followed by *Fusarium moniliforme*, *Trichoderma lignorum*, *Botryotrichum atrogriseum* and *Sepedonium chrysospermum*.

However, only further detailed study on this very aspect could provide a meaningful resolution of the problem.

REFERENCES

1. Atkinson, B. W., Bux, F. and H. C. Kasan Considerations for application of biosorption technology to remediate metal-contaminated industrial effluents. *Water SA., 1998;* 24(2):

129-135.

2. Avery, S. V., Codd, G. A. and G. M. Gadd Caesium accumulation and interactions with other monovalent cations in the cyanobacterium *Synechocystis* PCC 6803. *Journal of General Microbiology., 1991;* 137(40): 5-13.
3. Babich, H. and G. Stotzky Gaseous and heavy metal air pollutants. In "*Experimental Microbial Ecology*" (Eds. Burns, R. G. and J. H. Slater), Blackwell Scientific Publications, London: pp. 631-670.
4. Bellion, M., Courbot, M., Jacob, C., Blaudez, D. and M. Chalot Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. *FEMS Microbiol. Lett.., 2006;* 254:173-181.
5. Birch, L. and R. Bachofen Complexing agents from microorganism. *Experientia., 2006;* 46: 827-834.
6. Blackwell, K. J., Singleton, I. and J. M. Tobin Metal cation uptake by yeast: a review. *Appl. Microbiol. Biotechnol*., 1995; 43: 579-584.
7. Blanco, A. B., Sanz, B., Llama, M. J., and J. L. Serra Biosorption of heavy metals to immobilized *Phormidium laminosum* biomass. *Afr. J. Biotech.., 1999;* 69: 227-240.
8. Bolton, H. and Y. A. Gorby (1995). An overview of the bioremediation by inorganic contaminants. In "*Bioremediation of Inorganics*" (Eds. Hinchee, R. E., Means, J. L. and D. R. Burris). Batelle Press, Ohio: pp. 1-16.
9. Bux, F. and H. C. Kasan Comparison of selected methods for relative assessment of surface charge on waste sludge biomass. *Water SA., 1994;* 20(1): 73-76.
10. Chang, J. S. and J. Hong Biosorption of mercury by the inactivated cells of *Pseudomonas aeruginosa* PU 21 (Rip 64). *Biotechnol. Bioeng*., 1994; 44: 99-100.
11. Crusberg, T. C., Mark, S. S. and A. Dilorio (2004). Biomineralisation of heavy metals. In "*Fungal Biotechnology in Agricultural, Food and Environmental Application"* (Ed. Arora, D. K.) Marcel-Dekker, U. S. A.: pp. 409-417.
12. Dua, M., Singh, A., Sethunathan, N. and A. K. Joshi Biotechnology and Bioremediation : successes and limitations. *Appl. Microbiol. Biotechnol*., 2002; 59: 143-152.
13. Duruibe, J. O., Ogwuegbu, M. O. C. and J. N. Egwurugwu Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences., 2007;* 2: 112- 118.
14. Dushenkov, V., Kumar, P. B. A. N., Motto, H. and I. Raskin Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environ. Sci. Tech., 1995;* 29: 1239-1245.
15. Eichenberger, E. (1986). The interrelationship between essentiality and toxicity of metals

in aquatic ecosystem. In "*Metal Ions in Biological Systems"* (Eds. Siegel, H. and A. Siegel). Marcel Dekker, Inc., New York: pp. 97-100.

16. Eisler, R. Biorecovery of gold. *Indian Journal of Experimental Biology., 2003;* 41: 967-971.
17. Elankumaran, R., Raj Mohan, B. and M. N. Madhyastha (2003). Biosorption by *Hydrilla verticillata* Casp. and *Salvinia* sp. Eco services Internet.
18. Filipovic–kovacevic, Z., Sipos, L. and F. Briski Biosorption of chromium, copper, nickel and zinc ions onto fungal pellets of *Aspergillus niger* 405 from aqueous solutions. *Food Technol. Biotechnol*., 2000; 38 (3): 211-216.
19. Fomina, M., Burford, E. P. and G. M. Gadd (2005). Toxic metals and fungal communities. In "*The Fungal Community: Its Organization and Role in the Ecosystem"* (Eds. Dighton, J., White, J. F. and P. Oudemans), Boca Raton, FL, USA. pp. 733-758.
20. Fomina, M., Buford, E. P. and G. P. Gadd (2006). Fungal dissolution and transformation of minerals: significance for nutrient and metal mobility. In "*Fungi in Biogeochemical Cycles"* (Ed. Gadd, G. M.), Cambridge University Press, Cambridge, UK. pp. 236 – 266.
21. Gadd, G. M. and A. J. Griffiths Microorganisms and heavy metals toxicity. *Microbial. Ecology., 1978;* 4: 303-317.
22. Gadd, G. M. and C. White (1989). Heavy metal and radionuclide accumulation and toxicity in fungi. In "*Microbe Interactions"* (Eds. Polle, R. K. and G. M. Gadd). IRL Press: pp. 19-38.
23. Gadd, G. M. (1992). Microbial control of heavy metal pollution. In "*Microbial Control of Pollution"* (Eds.Fry, J. C., Gadd, G. M., Herbert, R. A., Jones, C. W. and I. A. Watson-Graik), Cambridge University Press, Cambridge: pp. 216.
24. Gardea-Torresdey, J. L., Tiemann, K. J., Gonzalez, J. H., Henning, J. A. and M. S. Townsend Ability of silica-immobilized *Medicago sativa* (alfalfa) to remove copper ions from solution. *J. Hazard. Material., 1996;* 57: 29-39.
25. Herawati, N., Susuki, S., Hayashi, K., Rivai, I. F. and H. Koyama Cadmium, copper and zinc levels in rice and soil of Japan, Indonesia and China by soil type. *Bull. Environ. Contam. Toxicol., 2000;* 64: 33-39.
26. Holleman, A. F. and F. Wiberg (1985). *Lehrbuch der Anorganischen Chemie*. Walter de Gruyter, Berlin.
27. Horsfall, J. G. (1956). *Principles of Fungicidal Action*. Chronica Botanica. Co., Waltham, Mass.

28. Ingole, N. W. and A. G. Bhole Bio-accumulation of chromium, nickel and zinc by water hyacinth. *Pollution Research., 2000;* 19: 575-583.
29. Joseph, D. E. (1995). *Industrial wastewater treatment* (A guide book). Lewis Publ. CRC Press Inc., New York.
30. Kapoor, A. and T. Viraraghavan (1997). In "*Biosorbents for Metal Ions*" (Eds. Wase, J. and Forster).
31. Kuyucak, N. (1997). Feasibility of biosorbent application. In "*Biosorption of Heavy Metals*" (Eds. Volesky, B.). CRC Press, Boca Raton: 371-378.
32. Lovely, D. R. and J. D. Covas Bioremediation of metal contamination. *Curr. Opin. Biotechnol., 1997;* 8: 285.
33. Lujan, J. R., Damall, D. W., Stark, P. C., Rayson, G. D. and J. L. Gardea-Torresdey Metal ion binding by algae and higher plants tissues: a phenomenological study of solution pH dependance. *Solvent Extr. Ion Exch., 1994;*. 12: 803-816.
34. Macaskie, L. E. and A. C. R. Dean (1989). Microbial metabolism desolubilization, and deposition of heavy metals: metal uptake by immobilized cells and application to the treatment of liquid wastes. In "*Biological waste treatment"* (Ed. Mizrahi, A.), Alan R. Liss Inc., New York: pp. 150-201.
35. Macaskie, L. E. M. and A. C. R. Dean (1990). Metal sequestering biochemicals. In "*Biosorption of heavy metals"* (Ed. Volesky, B.), CRC Press. Boca Raton: Fla. pp. 199-248.
36. Mahvi, A. H., Naghipour, D., Valzi, F. and S. Nazmara Teawaste as an adsorbent for heavy metal removal from industrial waste waters. *Am. J. App. Sci., 2005;* 2: 372-375.
37. Mofa, A. S. Plants proving their worth in toxic metal clear up. *Science., 1995;* 269: 302-305.
38. Ramteke, P. W. (2005). Microbial bioremediation of heavy metals. In "*Biotechnology in Environmental Management*" (Eds. Ghosh,T. K., Chakarbarti, T. and G. Tripathi) A. P. H. Publishing Corporation: pp. 529-561.
39. Raper, K. B. and C. Thom (1949). *A Manual of Penicillia*. Williams and Wilkins Co., Baltimore, Md.
40. Rich, G. and K. Cherry (1987). *Hazardous Waste Treatment Technologies*. Pudvan Publishers, New York.
41. Riker, A. J. and R. S. Riker (1936). *Introduction to Research on Plant Diseases*. John S. Swift Co., St. Louis, Mo.
42. Shallari, S., Schwartz, Z., Hasko, A. and J. L. Moral Heavy metal in soil and plants of

serpentine and industrial sites of Albania. *Sci. Total Environ., 1998;* 209: 133-142.

43. Smith, C. J., Hopmans, P. and F. J. Cook Accumulation of Cr, Pb, Cu, Ni, Zn and Cd in soil following irrigation with treated urban effluent in Australia. *Environ. Poll., 1996;* 94: 317-323.
44. Son, B. C., Park, K., Song, S. H. and J. Y. Young Selective biosorption of mixed heavy metal ions using polysaccharides. *Korean J. Chem. Eng., 2004;* 21: 1168-1172.
45. Sood, I. S., Sindhu, R. S. and K. K. Sharma Bioaccumulation of heavy metals by some aquatic plants. *Pollut. Res., 1994;* 13(4): 331-343.
46. Srivastava, S. (2000). Metal biorecovery and environmental conservation through soil bacteria. In "*Hazards-Plants and People*" (Eds. Iqbal, M., Srivastava. P. S. and T. S. Siddique) CBS Pub., New Delhi: 348-377.
47. Taylor and Francis, London: 67-85.
48. Volesky, B. (1990). *Biosorption of heavy metals.* CRC Press, Boca Raton.
49. Volesky, B. and Z. R. Holan Biosorption of heavy metals. *Biotechnol Prog*., 1995; 11: 235-250.
50. Volesky, B. and H. A. May-Phillips Biosorption of heavy metals by *Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol., 1995;* 42: 797-806.
51. Waksman, S. A. (1927). *Principles of Soil Microbiology*. Williams and Wilkins, Baltimore Md.
52. Walton, P. H. (1995). Chemistry of waste minimization (Ed. Clarke, J. H.). Blackie Academic and Professional, New York., 1995; 463: 482-483, 489-493.
53. Wintz, H., Fox, T. and C. Vulpe Functional genomics and gene regulation in biometals research. *Biochem. Soc., 2002;* 30: 766-768.
54. Zantopoulos, N., Antoniou, V. and E. Nikolaidis Copper, zinc, cadmium and lead in sheep grazing in North Greece. *Bull. Environ. Contamin. Toxicol., 1999;* 62: 691-699.
55. 1-MINI M., YOUNESI H., BAHRAMIFAR N., AKBARA., LORESTANI Z., GHORBANI F., DANESHI A.,SHARIFZADEH M. Application of response surface methodology for optimization of lead biosorption in anaqueous solution by Aspergillus niger. Hazardous Materials1., 2008; 54: 694.
56. 2-RORA A., KIRAN B., RANI S., RANI A., KAUR B., MITTAL N. Heavy metal accumulation in vegetables irri-gated with water from different sources. Food Chem., 2008; 111: 811.