Full Length Research Article

IN VITRO BIOACCUMULATION METABOLIC STUDIES OF HEAVY METALS BY AQUATIC DUCK WEED *LEMNA POLYRRHIZA* L. (LEMNACEAE)

*1Abubacker, M. N. and 2Sathya, C.

1PG & Research Department of Biotechnology, National College, Tiruchirappalli 620 001, Tamil Nadu, India
2Department of Botany, Seethalakshmi Ramaswami College, Tiruchirappalli 620 002, Tamil Nadu, India

ABSTRACT

In vitro experiments on chromium, copper, lead and zinc heavy metals bioaccumulation using duck weed *Lemna polyrrhiza* L. (Lemnaceae) was conducted with 5, 10 and 20 mg/100 ml concentrations for a period of 20 days. The SEM-EDX results revealed the bioaccumulation of lead as high as 20.91% followed by copper 9.71%, zinc 5.66% and chromium 1.86%. There was a change in the *pH* of the medium, the bioaccumulation of metals were confirmed by FTIR analysis involved in the binding characteristics of alcoholic, phenolic, amino and amides groups in the biomass. Unique metabolic studies of heavy metal sorption have been predicted using GC-MS analysis.

INTRODUCTION

The danger of accumulation of heavy metal in land and aquatic ecosystem caused due to human impact resulting in nutrient imbalance, productivity loss in the ecosystem. Therefore heavy metals such as As, Cd, Co, Cr, Cu, Se, Ni and Zn are accumulated and assimilated by green plants and transferred within food chains by biomagnification process (Alkorta *et al*., 2004). Related researches on bioaccumulation of essential and non-essential metals by aquatic plants (Singh and Ghosh, 2005; Peng *et al*., 2008). This property of bioaccumulation was found useful in monitoring the water bodies (Vajpayee *et al*., 1995; Whitton and Kelley, 1995). From water, the aquatic plants have the ability to accumulate heavy metals which are essential for their growth and development. These metals include Cu, Fe, Mn, Ni and Zn. Certain aquatic plants also have the ability to accumulate heavy metals which have no known biological function. However, excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate elevated levels of heavy metals and accumulate

them in very high concentration have evolved both independently and together in number of different plant species (Cheng, 2003). The emphasis of most studies gradually shifted towards the use of aquatic plants as monitors for heavy metal water pollution. In recent years, there has been an ever-increasing interest in the study of metal accumulating plants for environmental remediation application, termed as phytoremediation. One method of phytoremediation is phytoextraction which uses metal accumulating plants to remove pollutants from contaminated sites by concentrating in the harvestable form from the plant (Zhuang *et al*., 2007). Phytoremediation of metals is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove toxic metals, from soil and water (Chen and Cutright, 2002; Huang *et al*., 2011). In the present study the aquatic duck weed *L. polyrrhiza* L. (Lemnaceae) was subjected to heavy metal concentrations in *in vitro* conditions for bioaccumulation metabolic studies was carried out through SEM-EDX, FTIR and GC-MS analyses.

MATERIALS AND METHODS

Plant sample collection: *Lemna polyrrhiza* L. (Lemnaceae), the aquatic duck weed employed in this study was collected

*Corresponding author: Abubacker, M. N.
PG & Research Department of Biotechnology, National College, Tiruchirappalli 620 001, Tamil Nadu, India

from a polluted water body in Tiruchirappalli, Tamil Nadu, India. The plant is frond like flat thick leaf, flat above slightly convex below, in which several rootlets arise from the lower surface of the leaves (Gamble, 2008). The plants were acclimatized for 5 days in tap water in 250 ml flask and then subjected to *in vitro* studies.

***In vitro* experimental design**: After acclimatization, the plants were tested in *in vitro* condition for 3 different concentration of chromium (Potassium dichromate, Merck), copper (Copper-II) sulphate, Himedia), lead (lead acetate, Merck), and zinc (zinc sulphate, Himedia) at 5, 10, and 20 mg/100 ml respectively for 20 days as experimental time. Triplicate batch tests were conducted in Petridishes. Desired heavy metal concentrations were added in each Petridish from prepared stock solution. All the Petridishes were exposed to normal sunlight for detention time of 20 days. The Petridishes were shaken gently at regular interval for uniform distribution of metals in aqueous medium. The bioaccumulation of Cr, Cu, Pb and Zn were tested for *pH*, SEM-EDX, FTIR, GC-MS studies for metabolic studies. The statistical analysis was carried out by using Statistical Package SPSS16 version One-way ANOVA, Post Hoc = Tukey Alpha, significant at 0.01 level.

RESULTS

Studies on bioaccumulation of heavy metals such as Cr, Cu, pb and Zn was conducted for a period of 20 days at 5, 10 and 20 mg/l 100 ml concentrations using *L. polyrrhiza* aquatic weed (Fig. 1).
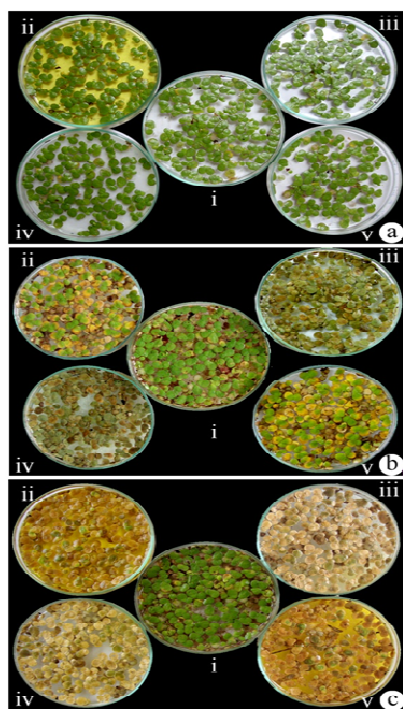
a) Initial stage of bioaccumulation (Day 1)
b) Second stage of bioaccumulation (Day 10)
c) Third stage of bioaccumulation (Day 20)

i) Control (water)., ii) Chromium., iii) Copper., iv) Lead., v) Zinc (20mg/100ml)

Figure 1. Bioaccumulation of heavy metals by lemna polyrrhiza L. (Lemnaceas)

pH: The *pH* of each sample was measured by using *pH* meter, *pH* reduction was noticed in all the samples (Cr, Cu, Pb and Zn) from the initial stage to the 20th day of experimental time

(Table 1). The maximum *pH* 7.80 was noticed in 20 mg/100 ml concentration of chromium at the initial stage was reduced to 7.20 on the 20th day of bioaccumulation as against 6.90 for control. For copper the maximum pH 7.60 was recorded at the initial stage was reduced to 7.10 on the 20th day. For lead the maximum *pH* 7.70 was reduced to 7.30 and for zinc the maximum *pH* 7.90 was reduced to 7.10 on the 20th day of bioaccumulation.

Table 1. *pH* reduction during bioaccumulation process in *Lemna polyrrhiza*

Heavy metals	No. of days treated		
	1	10	20
Chromium (20 mg/100 ml)	7.80 ± 0.11	7.30 ± 0.05	7.20 ± 0.11
Copper (20 mg/100 ml)	7.60 ± 0.26	7.20 ± 0.20	7.10 ± 0.20
Lead (20 mg/100 ml)	7.70 ± 0.15	7.50 ± 0.15	7.30 ± 0.10
Zinc (20 mg/100 ml)	7.90 ± 0.20	7.30 ± 0.15	7.10 ± 0.15
Water (Control)	6.90 ± 0.20	6.90 ± 0.11	6.90 ± 0.15

One-way ANOVA - mean difference is significant at 0.01 level

SEM-EDX analysis: Scanning Electron Microscopy equipped with Energy Dispersive X-ray (SEM-EDX) analysis consisting 3.5 nm and 2.5 nm resolution for tungsten filament (La B6) and EDX detector resolution 130 eV (TESCON, Czechoslovakia) (Jamari *et al.*, 2014) was performed to determine the cellular and sub-cellular bioaccumulation of heavy metals in *L. polyrrhiza* biomass. The bioaccumulation results revealed 20.91% for Pb, 9.71% for Cu, 5.66% for Zn and 1.86% for Cr. In control sample these metals are not detected (Table 2).

Table 2. Bioaccumulation of heavy metals by *L. polyrrhiza* by SEM-EDX analysis

Metals	Control	Cr %	Cu %	Pb %	Zn %
Chromium	–	1.86	–	–	–
Copper	–	–	9.71	–	–
Lead	–	–	–	20.91	–
Zinc	–	–	–	–	5.66

Fourier transform infrared (FTIR) spectroscopy analysis

FTIR spectroscopy (BRUKER, Model: ALPHA) was used to detect vibration frequency change in *L. polyrrhiza* biomass before and after the heavy metal bioaccumulation. The spectra were collected by with the range 4000-400 cm^{-1} using ethanol as mulling agent. The background obtained from the scan of ethanol was automatically subtracted from the sample spectra (Figure 2). The FTIR spectrum of *L. polyrrhiza* control plant showed the wave length at 3405.30 cm^{-1} for –O–H stretching for alcoholic and phenolic compound, the Cr bioaccumulated samples showed the shift at the 3357.14 and wave length for –NH$_2$ in primary amides with NH$_2$ antisym stretch, 3239.89 cm^{-1} for amines and amides. Cu bioaccumulated samples revealed the shift at 3640.45 and 3579.62 cm^{-1} for –OH stretch and –NH$_2$ in aromatic amines, primary amines for NH stretch at 3390.01 cm^{-1} –OH in alcohols and phenols with OH stretch. The Pb mediated bioaccumulation material showed the shift in the wave length 3463.92, 3433.62, 3403.82 cm^{-1} for –NH$_2$ in aromatic amines, primary amines for–NH stretch for –NH$_2$ in primary amides with NH stretch in solids and also in polypeptides and protein (amines and amides) for 3365.01 cm^{-1}, whereas Zn bioaccumulated plant samples revealed the shift in the wavelength at 3465.89 and 3429.40 cm^{-1} for –NH$_2$ in aromatic amines, primary amines with NH stretch. The

wavelength 2362.97 and 2337.04 cm^{-1} for –PH in phosphines with P–H stretch in the control plant and 2076.82 cm^{-1} for displayed –NH$_3$ stretching of amino acid (Fig.2).
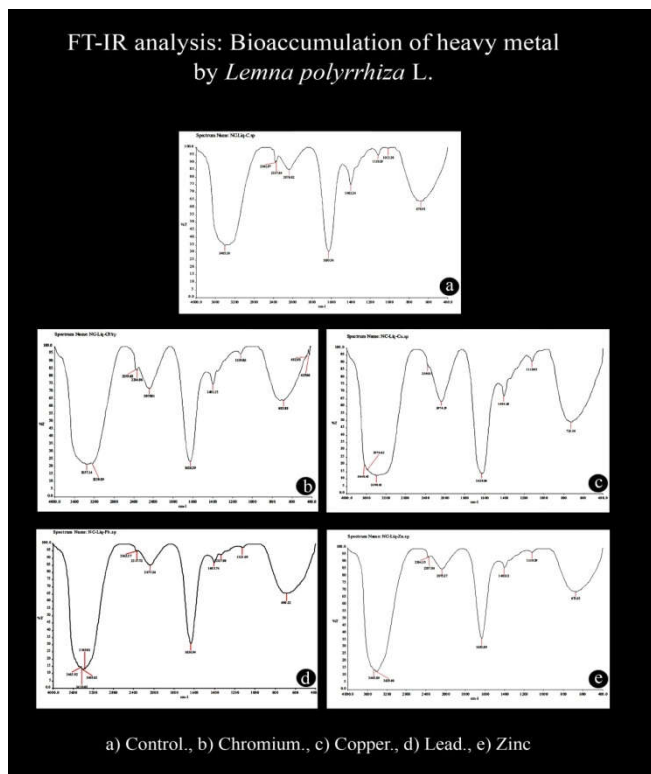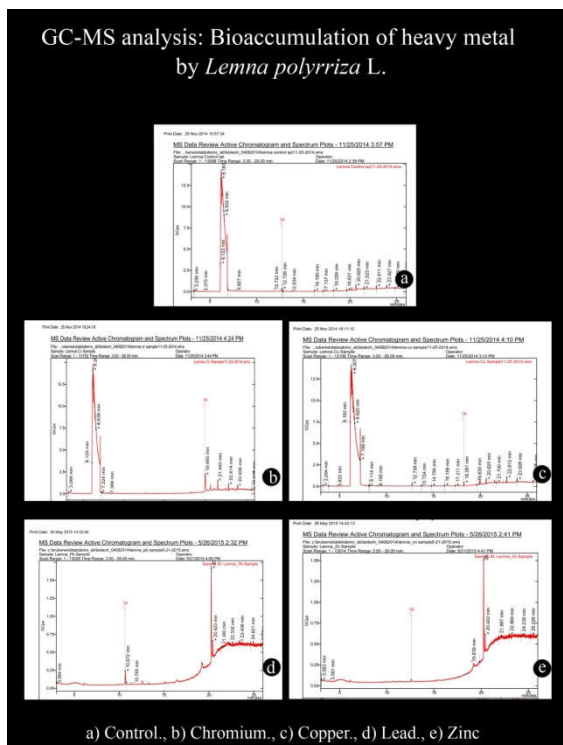

Figure 2.

Figure 3.

GC-MS (Bruker 45X-GC44, MS: SCION TQ/SQ) analysis of metabolic pathway of heavy metal sorption in L. polyrrhiza biomass

The GC-MS analysis of *L. polyrrhiza* control plant have shown certain specific compounds like Mannosamine, Amino cyanoacetic acid, 3-Ethyl-2-heptanol, DL-xylose, 3-Mercaptohexanol, Di-ethyl phthalate, 2,2-Dichloroethyl methyl ether, Isophytol, *n*-hexadecanoic acid, whereas chromium bioaccumulated plants have shown Cyclobutane, 1,3-propanediol, tert-butyl dimethylsilyl ether, Formic acid, Hydroxylamine, O-decyl-oxalic acid, Di-methyl phosphine, Oxalic acid, L-glucose, Monoethyl ester, Benzoic acid, Vinyl lauryl ether, d-Mannose and D-Galactose. The copper bioaccumulation resulted in the presence of Oxalic acid, Sulfurous acid, 2-Octylcyclopropene-1-heptanol, Pentadecanoic acid, Monoethyl ester, *n*-Hexadecanoic acid, D-Galactose, Lactose and L-Glucose.

The lead mediated bioaccumulation have shown the biochemical compounds Butanal, Methoxyacetic acid, Triethylene glycol monododecyl ether, Sulfurous acid, Hydroxylamine, Nonadecanoic acid, 1-Monolinoleoylglycerol trimethylsilyl ether, 1-(2-Ethyl-[1-3] dithian-2-yl)-3-methyl-butane-1-ol, 6-Dimethyl (trimethylsilyl) silyloxytetradecane trans-13-octadeconoic acid, Hexadecenoic acid, Ethanesulfonyl chloride, 2,4-Dichloro-phenethylamine, 2-Acetylbenzoic acid, Phthalic acid and Benzoic acid, whereas zinc bioaccumulated biomass of *L. polyrrhiza* revealed the occurrence of 2-Myristynoyl pantetheine, Formamide, Methoxyacetic acid, 4-Cyclopropyl-carbonyloxytridecane, Sulfurous acid, 1-Octadecanesulphonyl chloride, N-(2-Hydroxy-2-methyl-4-phenyl-3-butynyl) threonine, Carbamodithioic acid, Morpholine, 5-Heptenoic acid, 1-Nitro-2-acetamido-1,2,dideoxy-d-mannitol, 3,4-Dichloro-phenethylamine, 2-Chloro-benzene, Diethyl Phthalate and 2-Acetylbenzoic acid (Fig.3).

DISCUSSION

SEM-EDX analysis of *L. polyrrhiza* biomass samples reveals the morphological changes with respect to shape, size, surface texture and pores in the material after accumulation of heavy metals. A clear difference in the surface of control compound to metal-bonded biomass sample was visualized (Giri and Patel, 2012). The FTIR analysis indicates shift in wave length in all bioaccumulated samples. Cr samples showed the shift at 2359.68 and 2334.86 cm^{-1} for –CH$_2$ stretching of alkanes and 2078.81 cm^{-1} for –NH$_3$ stretching of amino acid. For Cu the shift was seen in 2360.11 and 2074.25 cm^{-1} for –CH$_2$ stretching of alkanes. The Pb mediated bioaccumulation have shown the shift at 2362.27 and 2337.72 cm^{-1} for –NH$_3$ stretching of amino acid and 2075.24 cm^{-1} stretching of alkanes. The Zn mediated bioaccumulation revealed –CH$_2$ stretching shift at 2364.35, 2337.84 and at 2075.27 cm^{-1} for –NH$_3$ stretching of amino acid (Harshad Lade *et al*., 2012, 2015; Phugare *et al*., 2011). The bioaccumulation wave length of control plant was at 1630.36 cm^{-1} for –N=N–stretching bonds and 1402.24 cm^{-1} for –OH deformation of alcohol, 1120.29 cm^{-1} for S=O stretching of sulfites, 1031.30 cm^{-1} for –S=O stretching of sulfonic acids, 676.91 cm^{-1} for C–OH alcohol group 452.93 cm^{-1} for C–N–C amines and 425.66 cm^{-1} for NH$_3^+$ in amino acids –NH$_3$ deformation. These wave lengths were shifted to 1628.29 cm^{-1} for NH$_3^+$ in amino acids –NH$_3$ deformation at 1401.15 cm^{-1} for C–N in primary amides with C–N stretch, 1119.86 cm^{-1} C–O–H in secondary or tertiary alcohol with C–O stretch, 685.88 cm^{-1} for C–C–CHO in aldehydes with C–C–CHO bending, 452.93 cm^{-1} naphthalenes, and 425.66 cm^{-1} Cl–C=O in acid chlorides deformation for Cr mediated bioaccumulation. For Cu accumulation process the wave lengths were shifted to 1628.86

cm^{-1} for NH$_3^+$ in aminoacids –NH$_3$ deformation, 1404.18 cm^{-1} for C–N in primary amides with C–N stretch, 1119.95 cm^{-1} for C–NH$_2$ in primary aliphatic amines with C–N stretch and 723.36 cm^{-1} for CH=CH in cis disubst alkenes with CH deformation. For Pb bioaccumulation wave lengths were shifted to 1636.96 cm^{-1} for NH$_3^+$ in amino acids, NH$_3$ deformation, 1405.74 cm^{-1}, C–N in primary amides with C–N stretch, 1337.98 cm^{-1}, N=N–O in azoxy compounds with N=N–O sym stretch, 1123.09 cm^{-1}, C–O–C in aliphatic ethers with C–O–C stretch and 690.22 cm^{-1}, Ar–OH in phenols with OH deformation, whereas for Zn bioaccumulation, the shift was noticed at 1633.89 cm^{-1} for NH$_3^+$ in aminoacids, NH$_3$ deformation, 1403.12 cm^{-1}, C–N in primary amides with C–N stretch, 1119.59 cm^{-1} for C–O–C in aliphatic ethers with C–O–C stretch, and 673.63 cm^{-1} in C–OH in alcohols with C–O–H bending (Coates, 1996; Bang *et al.*, 2005; Phugare *et al.* 2011; Harshad Lade *et al.*, 2012, 2015). It is to mention here that the wave lengths 452.93 and 425.66 cm^{-1} for C–N–C in amines with C–N–C bend found in control was totally absent in all the bioaccumulation reactions.

The GC-MS studies of *L. polyrrhiza* control plant biomass showed specific aliphatic, aromatic and thioalcohol, 3-Ethyl-2-heptanol, Diethyl phthalate and 3-Mercaptohexanol respectively, the Cr bioaccumulated biomass revealed the oxidative products of aliphatic, aromatic and thioalcohol such as formic acid, O-decyl-oxalicacid, Oxalic acid and Benzoic acid. In Cu bioaccumulated biomass it was sulfurous acid, *n*-Hexadecanoic acid, Pentadecanoic acid and Oxalic acid. The Pb mediated bioaccumulated biomass revealed the presence of Methoyacetic acid, Nonadecanic acid, Sulfurous acid, Trans-13-octadeconic acid, Hexadecenoic acid, 2-Acetylbenzoic acid, Phthalic acid and Benzoic acid. The Zn bioaccumulated biomass showed the presence of Methoxyacetic acid, Sulfurous acid, 5-Heptenoic acid and 2-Acetylbenzoic acid. The biological mechanism of biosorption of metals by metal-chelating proteins related to metallothioneins as stated by Robinson *et al.* (1993) or by Phytochelatins (Reeves, 2003; Gaikward Rupali and Khan Shahana, 2014) or by acidifying process as stated by Crowley (1991) and Alkorta and Garbisn (2001). From these reports it is to ascertain that the acidifying process was resulted in the bioaccumulation of heavy metals in *L. polyrrhiza* biomass.

Conclusion

The present finding reveals contamination of the aquatic bodies by various pollutants like heavy metals, poly-aromatic hydrocarbons have caused imbalanced in the natural functioning of the aquatic ecosystem. Phytoremediation works best at sites with reducing the pollutant by bioaccumulation on its biomass. SEM-EDX and FTIR analyses confirm the bioaccumulation of heavy metals in the *L. polyrrhiza* biomass. GC-MS analysis revealed the bioconversion pathway of heavy metals in the plant biomass. With this kind of special characteristic feature of this aquatic plant, the plant can be employed easily for cost effective and eco-friendly green technology in pollutant reduction especially the heavy metals from the polluted aquatic ecosystem.

Acknowledgements

REFERENCES

Alkorta I and Garbisn C 2001. Phytoextraction: A cost effective plant based technology for the removal of metals from the environment. *Biores. Technol.* 77: 229-236.

Alkorta I, Hernandez-Allica J, Becerril J M, Amezaga I, Albizu I and Garbisu C 2004. Recent finding on the phytoremediation of soils contaminated with environmentally toxic heavy metals and mataloids such as zinc, cadmium, lead and arsenic. *Reviews in Environmental Science and Biotechnology* 3: 71-90.

Bang S, Patel M, Lippincott L and Meng X 2005. Removal of arsenic from groundwater by granular titanium oxide adsorbent. *Chemosphere* 60: 389-397.

Chen H, Cutright T J 2002. The interactive effects of chelator, fertilizer and rhizobacteria for enhancing phytoremediation of heavy metal contaminated soil. *J Soils Sediments* 2: 203-210.

Cheng S 2003. Heavy metals in plants and phytoremediation. *Environ Sci Pollut Res* 10: 335.

Coates J P 1996. The interpretation of infrared spectra: Published reference sources. *Applied Spectroscopy Reviews* 31: 179-192.

Crowley D E, Wang Y C, Reid C P P and Szaniszlo P J 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant Soil* 130: 179-198.

Gaikward Rupali S and Khan Shahana J 2014. Role of synthetic chelators in phytoremediation of heavy metals by Indian mustard. *International Journal of Technical Research and Applications* 2: 32-36.

Gamble J S 2008. *Flora of the Presidency of Madras*. Vol. III. Bishen Singh Mahendra Pal Singh Publishers, Dehra Dun. pp. 1110-1111.

Giri A K and Patel R K 2012. Phytoaccumulation potential and toxicity of arsenic ions by *Eichhornia crassipes* in hydroponic system. *Journal of Bioremediation and Biodegradation* 3: 137.

Harshad Lade S, Waghmode T R, Kadam A A, Govindwar S P 2012. Enhanced biodegradation and detoxitaction of disperse azo dye Rubine GPL and textile industry effluent by defined fungal-bacterial consortium. *International Biodeterioration and Biodegradation* 72: 94-107.

Harshad Lade, Sanjay Govindwar and Diby Paul 2015. Low cost biodegradation and detoxification of textile Azo dye C.I. Reactive Blue 172 by *Providencia rettgeri* strain HSL-1. *Journal of Chemistry*. Article ID 894109, 10 pages.

Huang H, Yu N, Wang L, Yang X 2011. The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and Cadmium cocontamination soil. *Bioresour Technol* 102: 11034-11038.

Jamari S, Embong Z and Bakar I 2014. Elemental composition study of heavy metal (Ni, Cu, Zn) in riverbank soil by electrokinetic assisted phytoremediation using XRF and SEM-EDX. *AIP Conference Proceedings* 1584: 221-227.

Peng K, Luo L, Li X and Shen Z 2008. Bioaccumulation of heavy metals by the aquatic plants *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. and their potential use for contamination indicators and in waste water treatment. *Sci Total Envoron* 392: 22-29.

Phugare S S, Kalyani D C, Patil, A V and Jadhar J P 2011. Textile dye degradation by bacterial consortium and

subsequent toxicology analysis of dye and dye metabolites using cytotoxicity, genotoxicity and oxidative stress studies. *Journal of Hazardous Materials* 186: 713-723.

Reeves R D 2003. Tropical hyperaccumulators of metals and the potential for phytoextraction. *Plant Soil* 249: 57-65.

Robinson N J, Tommey A M, Kuske C and Jackson P J 1993. Plant Metallothioneius, *Biochemistry* 295: 1-10.

Singh S P and Ghosh M 2005. A review on phytoremediation of heavy metals and utilization of its by-products. *Applied Ecology and Environmental Research,* 3: 1-18.

Vajpayee P, Rai U N, Sinha S, Tripathi R D and Chandra P 1995. Bioremediation of tannery effluent by aquatic macrophytes. *B Environ Contam Tox* 55: 546-553.

Whitton B A and Kelley M G 1995. Use of algae and other plants for monitoring rivers. *Aust J Ecol* 20: 45-56.

Zhuang P, Yang Q W, Wang H B, Shu W S 2007. Phytoextraction of heavy metals by eight plant species in the field. *Water Air Soil Poll* 184: 235-242.

****