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Accumulation, tolerance and impact of aluminium, copper and zinc on growth and nitrate reductase activity of *Ceratophyllum demersum* (Hornwort)

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Abstract: *Ceratophyllum demersum (hornwort) was subjected to toxic concentrations of Al (3 and 9 mg l^{-1}), Zn (3 and 9 mg l^{-1}) and Cu (2.5 and 7 mg l^{-1}) in culture solutions for 15 days. The higher dose of Al enhanced the chlorophyll content significantly ($p<0.05$) in the first 6 days of treatment while other treatments caused marked reductions. Nitrate reductase activity (NRA) was significantly reduced ($p<0.05$) by Al, Cu and Zn toxicity and ceased completely in plants treated with Cu by the 6th day of treatment. Dry biomass and relative growth rate were reduced significantly ($p<0.05$) by metal treatment. Tolerance index of the plant was low for Cu (21.62 and 13.43% at low and high doses, respectively) and moderate for Zn (63.74 and 54.85%) and Al (72.83 and 68.79%). Accumulation of Al, Zn and Cu was threefold at higher doses compared with the lower doses but the bioconcentration factors (BCF) were very low indicating that this plant is not a hyper accumulator of these metals.*

Key words: *Al, Zn Cu, Tolerance index, Chlorophyll, Growth, Nitrate reductase activity*
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Introduction

Copper (Cu) and zinc (Zn) are micronutrient elements essential for plant growth while aluminium (Al) is beneficial for the growth of a few plants, such as rice (Howeler and Cadavid, 1976) and tropical legumes (Andrew *et al.*, 1973). Excess Cu, Zn and Al cause numerous toxic effects in plants. All three metals have been shown to cause reduction in chlorophyll content and the rate of photosynthesis while Al and Cu inhibit respiration in some plants (Sarkunan *et al.*, 1984; Fernandes and Hendriques, 1991; Doncheva, 2001; Lim *et al.*, 2006).

Toxicity thresholds of plants have been shown to be highly variable. A number of plants have been shown to have the ability to accumulate metals in their shoots and show an exceptionally high tolerance to them while showing phytotoxic effects to others (Pandey, 2006; Akinola and Ekiyoyo, 2006). *Thlaspi caerulescens* is a hyperaccumulator of cadmium (Cd) and Zn but is very sensitive to toxic concentrations of Cu (Lombi *et al.,* 2001). *T. caerulescens* also has an elevated tolerance to lead (pb) and nickle (Baker *et al.*, 1994). *Catharanthus roseus* accumulates 5-10% Cd but does not accumulate Pb and is unaffected by Pb (Pandey *et al.,* 2007). Maize was used for Pb phytoextraction but is sensitive to Zn toxicity (Huang and Cunningham, 1996; Lombi *et al.*, 2001). Aquatic plants are directly exposed to metal toxicity while a barrier is formed in land plants from the binding of metals, such as Cu, to colloids in soil and sediments (Fernandes and Hendriques, 1991).

Ceratophyllum demersum (Hornwort) is a dicotyledonous plant of the family Ceratophyllaceae. It grows in slow flowing water and thrives in saline habitats. The plant reaches an average length of 50-110 cm and has no roots. The leaves appear in whorls on the main axis and are brittle with rigid and bifurcated spines. Occasionally, flowers develop that are inconspicuous and monoecious. Propagation is mainly through fragmentation (Bursche, 1971).

This study investigates the sensitivity of *Ceratophyllum demersum* to toxic concentration of Cu, Zn and Al through their impact on growth, chlorophyll content and nitrate reductase activity. The tolerance index and bioaccumulation factor of this plant are also determined to assess its phytoremediatory property.

Materials and Methods

Plant cultivation: *Ceratophyllum demersum* was collected from Agbara river, about 34 km from Lagos, Nigeria, with an altitude of 15.4 m, latitude of 6.50^{o} and longitude of 3.11^{o}. Preliminary investigations showed that this plant can tolerate 9 mg l^{-1} Zn and Al but is completely destroyed by the same concentration of Cu. Hence the concentration of Cu in this study was lower than that of Zn and Al. Seven batches of three 500 ml beakers were filled with one fifth strength Hoagland's nutrient solution. Six batches of the nutrient solution were amended with two concentrations of heavy metals: Cu (2.5 and 7 mg l^{-1}), Zn (3 and 9 mg l^{-1}) and Al (3 and 9 mg l^{-1}), while the 7th batch served as the control. 3.5 g segments of the vegetative plant were rinsed with distilled water and introduced into each beaker, (a segment per beaker) in triplicates and these were observed for 15 days. The level of solution was maintained at 500 ml with distilled water. Plant cultures were exposed to 12-13 hr of daylight and an ambient temperature of 20-29^{o}C. At intervals of 3 days, samples were taken for determination of chlorophyll content and nitrate reductase activity.

Another set of samples was prepared with 1 g segments of the vegetative plant but these were left undisturbed for 15 days. Thereafter, the dry biomass was determined after oven drying for 24 hr at 100^{o}C. The relative growth rate (RGR) was determined by the method described by Causten (1994) using the equation:

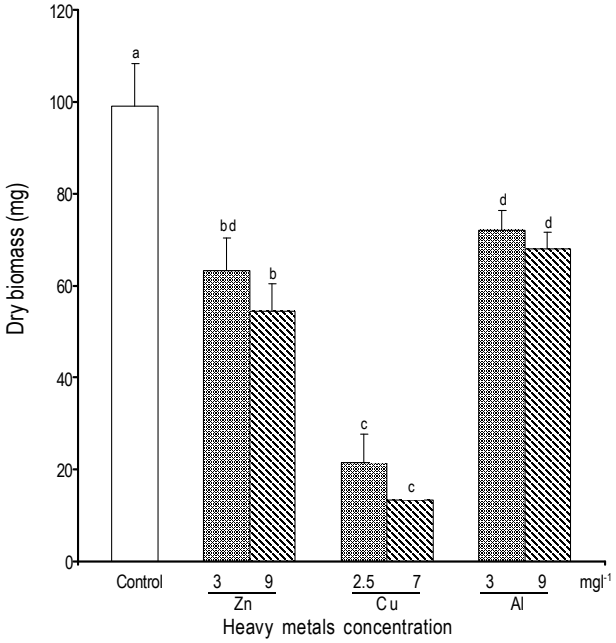

Fig. 1: Effect of toxic levels of Zn, Cu, and Al on the dry biomass of *C. demersum* after 15 days of treatment , Vertical bars with similar letters are not significantly different ($p<0.05$)

$$RGR = \frac{(\log W_2 - \log W_1)\ g\ g^{-1}day^{-1}}{t_2 - t_1}$$

Determination of tolerance index: The tolerance index which is a measure of the tolerance of the plant to Zn, Cu and Al, was determined by comparing the dry biomass of plants subjected to metal treatment with the control using the relationship outlined by Wilkins (1978):

$$\text{Tolerance index} = \frac{\text{Biomass of treated plants}}{\text{Biomass of control plants}} \times 100$$

Estimation of chlorophyll content: The chlorophyll content of vegetative plant segments was determined using the method of Maclachlam and Zalik (1963) as described by Singh and Rao (1981). Extracts were prepared from one gram segments using 15 ml of 80% acetone. These were filtered and the filtrates were centrifuged at 3000 g for 15 min. The optical densities of the extracts were recorded at 645 and 663 nm using spectrophotometer (Corning 258 model).

The amount of chlorophyll a and b (mg g^{-1} fresh weight) was calculated using the formula of Maclachlam and Zalik (1963).

Nitrate reductase activity: Nitrate reductase activity (NRA) of plant segments was done by the method of Stewart *et al.* (1972). Five ml incubation medium comprising 100 ml 0.1M phosphate buffer (pH 7.5), 1.5 g potassium nitrate and 1 ml 4% propan-1-ol, was used to incubate 0.4 g finely cut vegetative plant segments from each replicate, for an hr at a room temperature of 25°C. Then the reaction was stopped by adding 1 ml 1% sulphanilic acid in 2N HCl, followed by 1 ml 0.02% Naphthylethylenediamine dichloride for 20 min for colour development. The absorbance was measured at 540 nm wavelength using a spectrophotometer (Corning 258 model). The concentration of nitrite in the reaction medium was determined by reference to a standard curve prepared using 0-0.8 moles ml^{-1} sodium nitrite ($NaNO_2$). NRA is proportional to the concentration of nitrite in the reaction medium (Ajakaiye, 1987).

Plant metal analysis: The total concentration of heavy metals (Cu, Zn and Al) in the plant was determined at the end of the treatment period (15 days). one gram, dry powdered segments from the vegetative plant was digested with 10 ml concentrated nitric acid and perchloric acid (1:1 v/v) and the digest was made up to 50 ml with distilled water (Jones, 1984). Metal concentration of the digested samples was determined using atomic absorption spectrometry (ALPHA 4 model).

Estimation of bioconcentration factor: The bioconcentration factor (BCF) was calculated by dividing the metal concentration in plant tissue (μg g^{-1}) by the initial concentration (mM) of the metals in the nutrient solution (Zayed *et al*., 1998).

Statistical analysis: The values are expressed as means of three replicates ± SE. The data were subjected to ANOVA. Tests of significance between treatment means at $p<0.05$ were determined by the Duncan's multiple range test.

Results and Discussion

Zinc, copper and aluminium are well known for their toxic effects on plant growth and metabolism but toxicity thresholds are highly variable. *Ceratophyllum demersum* is fairly sensitive to Zn (3 and 9 mg l^{-1}) and Al (3 and 9 mg l^{-1}) but highly sensitive to Cu (2.5 and 7 mg l^{-1}). All concentrations of Cu, Zn and Al caused significant reductions ($p < 0.05$) in the relative growth rate (RGR) of this plant (Table 1). RGR was only positive in the control. Plant biomass was also significantly reduced ($p < 0.05$) in all treated plants (Fig. 1). Cu and Zn toxicity have also been shown to cause reduction in plant growth in pea and wheat (Donocheva *et al.*, 2001; Fernandes and Hendriques, 1991; Ma *et al.,* 2003). Inhibitory effects were most pronounced in *C. demersum* plants treated with Cu and they shed all their leaves by the end of the first week of treatment. Marine plants have been shown to tolerate about 3.5 ppm Cu (Nriagu, 1979) but *C. demersum* is not tolerant to this concentration of copper. Indices of metal tolerance based on plant biomass (Table 1), showed that *C. demersum* was significantly ($p<0.05$) more tolerant to Al than to Zn at similar concentrations (3 and 9 mg l^{-1}) while it had very low tolerance to Cu (21.62 and 13.43% at low and high doses respectively). Another water plant *Pistia stratiotes* has also been reported to show high tolerance to Zn and Cu (Odjegba and Fasidi, 2004).

The chlorophyll content of *C. demersum* was significantly enhanced ($p< 0.05$) in the first six days of treatment with 9 mg l^{-1} Al (Fig. 2). Prolonged exposure of plants to both concentrations of Zn and Al (3 and 9 mg l^{-1}) reduced the chlorophyll content of treated plants significantly ($p<0.05$), suggesting that plants had absorbed both elements to toxic levels. Toxic levels of Zn (0.67-1000 μM)

Table - 1: Relative growth rate, tolerance indices and bioaccumulation factors (BCF) of *C. demersum* subjected to toxic levels of Cu, Zn and Al for 15 days

Parameters	Control	Metal concentration (mg l^{-1})					
		Zn		Cu		Al	
		3	9	2.5	7	3	9
Relative growth rate ($X10^{-2}$ g g^{-1} day^{-1})	0.67^{a}	-2.35^{ab}	-3.38^{c}	-9.82^{d}	-12.69^{e}	-1.48^{f}	-1.79^{g}
Tolerance index (%)		63.64^{ae}	54.54^{ab}	20.67^{cd}	13.33^{d}	72.72^{e}	68.00^{e}
Bioconcentration factor (BCF)		104.00	138.07	202.13	200.00	171.43	221.05

Means with similar superscripts on the horizontal axis have no significant difference ($p<0.05$)

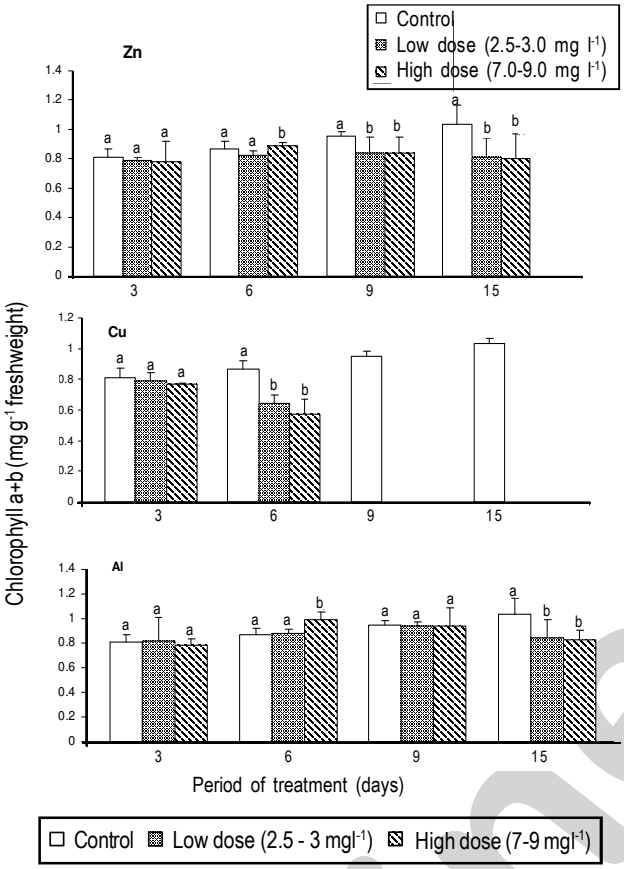

Fig. 2: Effect of toxic levels of Zn, Cu, and Al on the chlorophyll content of *C. demersum*. Vertical bars with similar letters at each period of treatment are not significantly different ($p<0.05$)

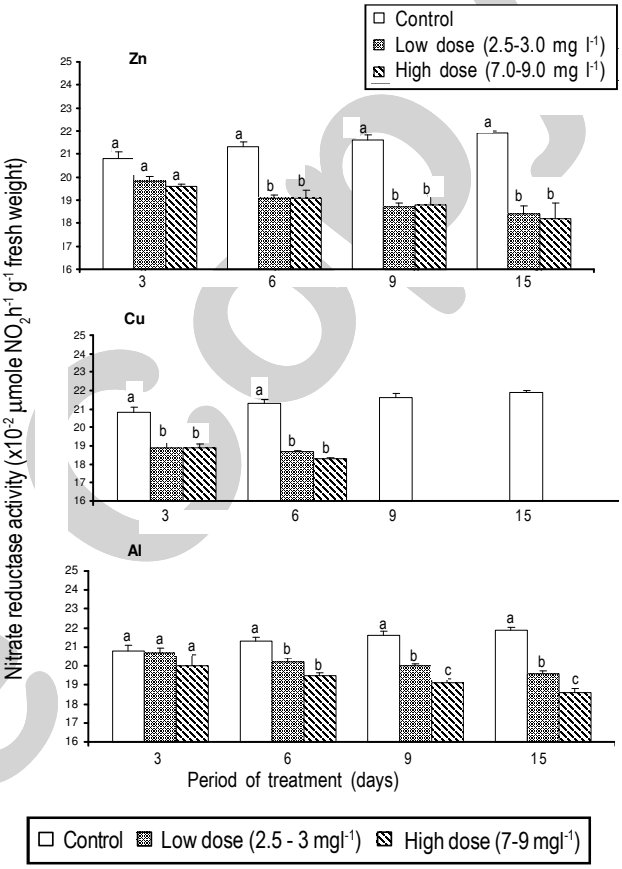

Fig. 3: Effect of toxic levels of Zn (3 and 9 mg l^{-1}), Cu (2.5 and 7 mg l^{-1}) and Al (3 and 9 mg l^{-1}) on the nitrate reductase activity of *C. demersum*. Vertical bars with similar letters at each period of treatment are not significantly different ($p<0.05$)

have also been shown to reduce chlorophyll content in pea plants and induce alterations in the chloroplast structure resulting mainly in decreased granal thylakoids (Doncheva *et al*., 2001). Zn toxicity also causes disintegration of cell organelles (Rout and Premananda, 2003). These would justify the reported reduction in the photosynthetic rate of rye subjected to toxic levels of Zn (2.7 mg g^{-1} in leaves) for 15 days (Monnet *et al*., 2001). Al toxicity has also been shown to reduce the quantity of chlorophyll pigments accompanied by a marked decrease in gross photosynthesis and photosynthetic rate in rice (Sarkunan *et al*., 1984). Cu toxicity caused the most drastic reduction in chlorophyll content and plants shed their leaves after 6 days of treatment. Copper toxicity has been shown to interfere with several aspects of plant biochemistry including pigment synthesis and photosynthesis (Fernandes and Hendriques, 1991). Copper induced inhibition of chlorophyll synthesis probably results from the inhibition of ALA-dehydrogenase, an enzyme in the biosynthetic pathway of porphyrin (Scarponi and Perucci, 1984).

Nitrate reductase activity (NRA) of *C. demersum* was significantly inhibited ($p<0.05$) by the presence of toxic levels of the three metals (Fig. 3). Zn and Cu caused greater inhibition than Al and NRA ceased completely in Cu after 6 days of treatment. Heavy metals have been shown to cause inhibition of nitrogenase activity in legumes (Ibekwe *et al*., 1995) and specifically, Cu inhibits nitrate reductase in *Silene vulgaris* (Larcher, 1980). An efficient N assimilation is said to be favoured by a high rate of CO_2 assimilation (Ferrario *et al.,* 1998). Since toxic levels of Zn, Cu and Al inhibited chlorophyll formation and hence CO_2 assimilation, NRA was equally inhibited.

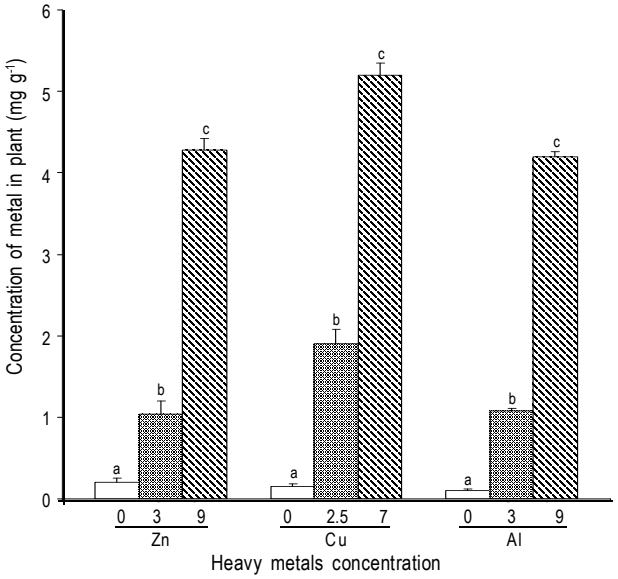

Fig. 4: Accumulation of Zn, Cu and Al by *C. demersum* after 15 days of treatment. Vertical bars with similar letters at each period of treatment are not significantly different ($p<0.05$)

Accumulation of Zn, Cu and Al by *C. demersum* was 3-fold at the higher doses (Fig.4) and the plant absorbed higher concentration of Cu than Zn and Al. However, to classify a plant as a hyper accumulator, the bioconcentration factor (BCF) is the best indicator (Zayed *et al.,* 1998). BCF measures the ability of a plant to bioconcentrate an element in its tissue taking into account the concentration of that element in the substrate. A good accumulator has a BCF of $\geq$1000. BCF was very low for the three metals (Table 1), indicating that *C. demersum* is not a hyper accumulator of Zn, Cu or Al. Furthermore, this plant failed to meet the other criterion for a good metal accumulator, that is, the possession of well developed root system (Qian *et al.,* 1999). Studies have shown that *Pistia stratiotes* is a good Cu accumulator (Odjegba and Fasidi, 2004).

C. demersum is very sensitive to Cu but is moderately tolerant to Zn and Al toxicity. Toxic effects of these metals include a reduction of growth, chlorophyll content and nitrate reductase activity. It is also not a bioaccumulator of Cu, Zn and Al.

References

Ajakaiye, C.O.: Leaf nitrite, nitrate reductase activity and crude protein in *Corchorus olitorius* L. and *Ceratotheca sesamoides* Esond. *Nig. J. Bot.* **1**, 36-42 (1987).

Akinola, M.O. and T.A. Ekiyoyo: Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Ribila at Odo-nla area of Ikorodu, Lagos state, Nigeria. *J. Environ. Biol.,* **27**, 597-599 (2006).

Andrew, C.S., A.D. Johnson and R.L. Sandland: Effect of aluminium on the growth and chemical composition of some tropical and temperate pasture legumes. *Aust. J. Agric. Res.,* **24**, 325-339 (1973).

Baker, A.J.M., R.D. Reeves and A.S.M. Hajar: Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. and C. Presl (Brassicaceae). *New Phytol.,* **127**, 61-68 (1994).

Bursche, E.M.: A Handbook of Water Plants. Warner Publishers, USA (1971).

Causton, D.R.: Plant growth analysis: A note on the variability of unit leaf rate within a sample. *Annl. Bot.,* **74**, 513-518 (1994).

Doncheva, S., Z. Stoynova and V. Velikova: Influence of succinate on zinc toxicity of pea plants. *J. Plant Nutr.,* **24**, 789-804 (2001).

Fernandes, J.C. and F.S. Henriques: Biochemical, physiological and structural effects of excess copper in plants. *Bot. Rev.,* **57**, 246-273 (1991).

Ferrario, S., M.H. Valadier and C.H. Foyer: Over-expression of nitrate reductase in tobacco delays drought- induced decreases in nitrate reductase activity and mRNA. *Plant Physiol.,* **117**, 239-302 (1998).

Howeler, R.H. and L.F. Cadavid: Screening of rice cultivars for tolerance to Al toxicity in nutrient solutions as compared with a field screening method. *Agron. J.,* **68**, 551-555 (1976).

Huang, J.W. and S.D. Cunningham: Lead phytoextaction: Species variation in lead uptake and translocation. *New Phytol.,* **134**, 75-84 (1996).

Ibekwe, A.M., J.S. Angle, R.L. Chaney and P.V. Berkum: Sewage sludge and heavy metals: Effects on nodulation and nitrogen fixation of legumes. *J. Environ. Qual.,* **24**, 1199-1204 (1995).

Jones, J.B.: Development in the measurement of trace metals constituents in foods. *In:* Analysis of food contaminants (*Ed.*: T. Gilbert). Elsevier Applied Science Pub. pp. 157-206 (1984).

Larcher, W.: Ecological Plant Physiology, 2nd Edn. Springer-Verlag, Berlin (1980).

Lim, C.Y., Y.H. Yoo, M. Sidharthan, W.M. Ma, I.C. Bang, J.M. Kim, K.S. Lee, N.S. Park and H.W. Shin: Effects of copper (1) oxide on growth and biochemical compositions of two marine microalgae. *J. Environ. Biol.,* **27**, 461-466 (2006).

Lombi, E., F.J. Zhao, S.J. Dunham and S.P. McGrath: Phytoremediation of heavy metal- contaminated soils: Natural hyperaccumulation versus chemically enhanced phytoextraction. *J. Environ. Qual.,* **30**, 1919-1926 (2001).

Ma, G.P. Rengasamy and A.J. Rathjen: Phytotoxicity of aluminium to wheat plants in high pH solutions. *Aust. J. Exp. Agric.,* **43**, 497-501 (2003).

Maclachlam, S. and S. Zalik: Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Can. J. Bot.,* **41**, 1053-1062 (1963).

Monnet, F., N. Vaillant, P. Vernay, A. Coudret, H. Sallano and A. Hitmi: Relationship between PS II activity, CO_2 fixation and Zn, Mn and Mg contents of *Lolium perenne* under zinc stress. *J. Plant Physiol.,* **158**, 1137-1144 (2001).

Nriagu, J.O.: The global copper cycle. *In*: Copper in the environment. Part 1: Ecological cycling (*Ed.*: J.O. Nriagu). John Wiley and Sons, New York. pp. 1-17 (1979).

Odjegba, V.I. and I.O. Fasidi: Accumulation of trace elements by *Pistia stratiotes*: Implications for phytoremediation. *Ecotoxicology*, **13**, 637-646 (2004).

Pandey, S.N.: Accumulation of heavy metals (Cd, Cr, Cu, Ni and Zn) in *Raphanus sativus* L. and *Spinacia oleracea* L. plants irrigated with industrial effluent. *J. Environ. Biol.,* **27**, 381-384 (2006).

Pandey, S., K. Gupta and A. K. Mukherjee: Impact of cadmium and lead on *Catharanthus roseus* - A phytoremediation study. *J. Environ. Biol.,* **28**, 655-662 (2007).

Qian, J.H., A. Zayed, Y. L. Zhu, M. Yu and N. Terry: Phytoaccumulation of trace elements by wetland plants; III. Uptake and accumulation of ten trace elements by twelve plant species. *J. Environ. Qual.,* **28**, 1448-1455 (1999).

Rout, G.R. and Das Premananda: Effect of metal toxicity on plant growth and metabolism. I. Zinc. *Agronomie*, **23**, 3-11 (2003).

Sarkunan, V., C.C. Biddappa and S.K. Nayak: Physiology of Al toxicity in rice. *Curr. Sci.,* **53**, 822-824 (1984).

Scarponi, L. and P. Perucci: Effect of some metals and related metal-organic compounds on ALA- dehydratase activity of corn. *Plant Soil,* **79**, 69-75 (1984).

Singh, S. N. and D. N. Rao: Certain responses of wheat plants to cement dust pollution. *Environ. Pollut.* (*Series A*), **24**, 75-81 (1981).

Stewart, G.R., Lee J.A. and T.O. Orebanjo: Nitrogen metabolism of halophytes. Nitrate reductase activity and utilization. *New Phytol.*, **72**, 539-546 (1972).

Wilkins, D.A.: The measurement of tolerance to edaphic factors by means of root growth. *New Phytol.,* **136**, 481-488 (1978).

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