CHAPTER II:
REVIEW OF LITERATURE
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Heavy metal contamination in the environment and their effects on biotic factors with special reference to plants

With the rapid growth in urbanization and industrialization, heavy metal pollution in the environment is also increasing which further reaches the plants, animals and human beings through the foodchain. Heavy metals have been detected in different parts of India including North east India in river sediments (Singh et al., 2005), in ground water (Chakraborti et al., 2008; Dutta, 2013), in dam and lake (Anu et al., 2011), in drinking water (Chakrabarty and Sharma, 2011), along the river basins (Manoj et al., 2012), and in soil (Shaikh and Bhosle, 2013; Tripathi and Mishra, 2012). In an attempt to find out the extent of arsenic contamination in groundwater of the Imphal West district, Manipur, India, a study was conducted with 30 study sites in pre and post-monsoon seasons. The results revealed that 23.3% of the groundwater samples contained arsenic more than the Indian standard of 50 g L\(^{-1}\) and 73.3% exceeded the WHO permissible limit of 10 g L\(^{-1}\) that might be an alarming concern in the use of groundwater for drinking and other domestic purposes (Singh et al., 2012).

Heavy metals affect human health causing allergic dermatitis, haematological alterations, kidney damage, neurological and reproductive disorders, and cancer (Das et al., 2008; Mudgal et al., 2010; Mishra et al., 2010; Cantone et al., 2011; Pizent et al., 2012). In livestock, metal toxicity causes weight loss, mouth ulceration, reduced milk yield, and visual and gastrointestinal tract disorder (Raikwar et al., 2008).

Studies showed that besides the adverse effects on animals and humans, heavy metals also produce harmful effects indifferent plant seedlings. In a pot study conducted on wheat plant for 90 days, significant reduction in plant growth and grain yield were observed due to toxic
effects of Cd, Cu, Ni, Zn, Pb and Cr (Athar and Ahmad, 2002). The application of Cu, Ni, and Hg on tobacco plants reduced the rate of germination and on the other hand, Hg, Cd and Ni reduced the rate of pollen tube length (Tuna et al., 2002). In another investigation with Arabidopsis thaliana, Hg$^{2+}$, Pb$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ were observed to reduce seedling growth, while Cd$^{2+}$ inhibited both seed germination and seedling growth (Li et al., 2005). Brassica juncea L. exhibited a decline in growth, chlorophyll and carotenoids contents when treated with different concentrations of Cd and Pb (John et al., 2009). Heavy metals such as Cu, Co, Mn, Pb, and Zn were found to reduce the nitrate reductase activities, total chlorophyll and protein content of Azolla pinnata plants after one month exposure (Pillai, 2009). Treatment of safflower seeds for 25 days with a mixture of Cd, Cu, Ni and Zn decreased germination rate, shoot fresh and dry weight, and root and shoot length (Houshmandfar and Moraghebi, 2011). Long-term application of Cu fungicides on Czech hop (Humulus lupulus L. variety Agnus) resulted in an initial increase in photosynthetic rate that was reduced after 10-14 days (Krofta et al., 2012). Coccinia indica, Mentha viridis and Trigonella foenum-graecum seedlings exposed to 100, 300 and 500 ppm of Ni and Pb exhibited substantial reduction in germination and growth of the plants in a 30 days exposure (Srinivas et al., 2013).

**Importance of Ipomoea aquatica**

A study on nutritional composition of water spinach (Ipomoea aquatica Forsk.) leaves showed that these had high moisture content (72.83±0.20%), crude lipid (11.00±0.50%), crude fibre (17.67±0.35%) and available carbohydrates (54.20±0.68%), low crude protein content (6.30±0.27%), and an energy value of 300.94±5.31 kcal/100g. Analysis of mineral content revealed that the plant leaves served as a good source of K (5458.33±954.70 mg/100g), Mn (2.14±0.22 mg/100g) and Fe (210.30±2.47 mg/100g) for human consumption, while Mg
(301.64±2.69 mg/100g) content in this plant would be beneficial for consumption especially by adult females and children (Umar et al., 2007). The plant was found to have high moisture content (51.36%), high carbohydrate content (42.18%), and relatively high iron, magnesium, calcium, vitamin A, B₁, C and K. Phytochemical screening test revealed high concentrations of alkaloids, reducing sugar, soluble carbohydrates, and flavonoids. The nutritional and phytochemical analysis revealed that the plant material was a good source of nutrients and also had promising pharmacological and therapeutic potential (Igwenyi et al., 2011).

Alkiyumi et al. (2012) showed the protective action of I. aquatica extract against thioacetamide (TAA)-induced hepatotoxicity. Sprague-Dawley (SD) rats were orally fed with I. aquatica (250 and 500 mg/kg) for two months along with TAA (i.p. injection 200 mg/kg three times a week for two months). The treated animals showed lower level of hepatic enzyme markers in TAA-induced serum (albumin, bilirubin, prothrombin, protein, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase). Further, there was a reversal in the superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels with both low and high dose of plant extract as compared to hepatotoxic group.

I. aquatica also finds use in traditional medicine. Boiled extract of tender shoot of I. aquatica is used against thirst in diabetic patients by Meitei-pangal community in Manipur, India (Khan and Yadava, 2010). This plant is also used as an emetic in case of opium and arsenic poisoning. The buds are used in ringworm treatment (Swapna et al., 2011).

As far as phytoaccumulation potential was concerned, I. aquatica was found to accumulate different heavy metals in its plant parts. Göthberg et al. (2002) showed the accumulation potential of Hg, Pb and Cd by this plant cultivated in the greater Bangkok region of Thailand. In this study, concentrations of Pb and Cd in I. aquatica were found to be relatively low
compared to Hg and so not a direct threat to human health when consumed. On the contrary, Hg was higher in leaves than in stems and might cause possible harmful effect to children and foetus. In another study this plant showed over 74% removal of added Cr (VI) up to 28ppm of Cr used without any visible toxicity symptoms (Weerasinghe et al., 2008).

In hydroponic studies three plant species, viz., arum (Colocasia antiquorum L.), radish (Raphanus sativus L.), and water spinach (Ipomoea aquatica) were selected to study the potential of cadmium accumulation. Cd concentrations in different plants increased with increase in metal concentrations. Arum and water spinach showed greater accumulation of Cd in root portion of the plants while in case of radish, greater accumulation of Cd was observed in leaf tissues (Kashem et al., 2008). I. aquatica was also found to be an efficient tool of chromium (VI) removal than fluoride in industrial wastewater (Gandhi et al., 2013).

**Cu contamination and its effects on biochemical and antioxidant properties of plants**

In an attempt to study the heavy metal contamination in the vegetables, Abelmoschus esculentus L., Beta vulgaris L. and Brassica oleracea L. were collected from both market sites and production sites of India and were tested for Cu, Cd, Zn and Pb contamination. Vegetables from market sites were found to accumulate heavy metals more than that of the production sites which might be because of the transportation and marketing systems of vegetables. The mean concentration of Cu in B. oleracea and that of Zn and Cd in both B. vulgaris and B. oleracea from the market sites were above the PFA (Prevention of Food Adulteration) standard. On the other hand Zn concentration was above the PFA standard in cauliflower while Cd concentration in vegetables tested exceeded the EU (European Commission) standard in both the production and market sites (Sharma et al., 2009).
The toxic and beneficiary effects of Cu upon the growth rate were measured in greengram (Vigna radiata (L.) Wilczek). Based on the exposure duration of 45 days after sowing, a reduction in the growth, dry matter production and nutrient content at higher Cu concentrations of 100, 150, 200 and 250 mg kg\(^{-1}\) were noted. While those greengram plants exposed to 50 mg kg\(^{-1}\) of Cu showed increase in the overall growth, dry matter yield and nutrient content (Manivasagaperumal et al., 2011).

Cu stress upon antioxidative enzyme activities in the roots of rice seedlings (Oryza sativa) was studied. It was found that the activities of ascorbate peroxidase, superoxide dismutase, glutathione reductase and peroxidase were enhanced but that of catalase activities was not affected when treated with 30 µM of CuSO\(_4\) after 5 days of treatment (Chen et al., 2000).

Cu at exceeded level can be toxic to plants leading to alterations in the normal functioning of the plants. Nicholls and Mal (2003) assessed the effect of Cu and Pb on the growth and survival of Lythrum salicaria plants. The plant was exposed to high (2000 ppm) and low (1000 ppm) concentrations of Cu and Pb separately and again with high (1000 ppm Pb + 1000 ppm Cu) and low (500 ppm Pb + 500 ppm Cu) concentrations of lead and copper mixture. There was complete withering and death of the above ground parts in all the metal treated plants and shoot length of some of the surviving plants were found to be significantly lower than that of the control plants on a weekly based measurements. The dry mass of roots and living shoots at harvest after 55 days of treatment was significantly greater in control when compared to treatment plants.

Brassica juncea plants showed an enhanced anti-oxidative enzyme activities and more active lipid peroxidation but depletion of glutathione in roots and shoots when treated with various concentrations of Cu (50 - 200 µM) for 48 h. Increase in the activities of ascorbate
peroxidase and superoxide dismutase with increasing Cu concentrations was observed in eight day old *B. juncea* seedlings in both shoots (leaves) and roots. However, catalase activities increased in leaves with increasing Cu concentrations like the other antioxidant enzyme activities but remained unchanged in case of root tissues (Devi and Prasad, 2005).

A study on cucumber plants showed an unbalanced nutrient uptake and upward translocation of Ca, K and Mg ions thus inhibiting leaf expansion under Cu stress (Alaoui-Sossé *et al.*, 2004). Cu toxicity resulted in the disruption of root cuticle, and reduced hair proliferation in *Chloris gayana* Knuth. (Sheldon and Menzies, 2005).

The effects of Cu on ascorbic acid content in bean (*Phaseolus vulgaris* L.) seedlings along with other biochemical changes were investigated. The study showed that the bean plants treated with different concentrations of CuCl$_2$ at 0.1, 0.2 and 0.3 mM showed decreasing trend in total chlorophyll content in primary leaves. An increase in the ascorbic content along with chlorophyll $a/b$ ratio, retinol, $\alpha$-tocopherol, and proline content occurred in the primary leaves with increase in Cu concentrations after 10 day of exposure (Zengin and Munzuroğlu, 2005).

Under experimental condition, mosses *Thuidium delicatulum* (L.) Mitt., *Thuidium sparsifolium* (Mitt.) Jaeg. and leafy liverwort *Ptychanthus striatus* (Lehm. & Linderb.) were exposed to Cu, Zn and Pb to study their impact on chlorophyll content of mosses and liverworts. Cu treatment at the concentrations range of $10^{-10}$ to $10^{-2}$ M showed an inhibitory effect on chlorophyll $a$, chlorophyll $b$ and total chlorophyll content in both mosses and liverwort. While mosses exposed to Zn and Pb at the concentration range of $10^{-10}$ to $10^{-2}$ M showed an insignificant decrease in chlorophyll content, a significant decrease in chlorophyll content was observed in the leafy liverwort (Shakya *et al.*, 2008).
The cytogenetical effects of Cu comprising chromosomal stickiness, and broken nuclei, when treated with $10^{-3}$ M Cu in the root meristem cells of *Allium sativum* L were observed. There was progressive decline in mitotic index with either increase in copper concentration used ($10^{-5}$, $10^{-4}$, $10^{-3}$ M Cu) or exposure time, thereby reflecting the decrease in the frequency of cell division due to Cu treatment. Significant ultrastructural changes with disruption of nuclear material and disintegration of organelles leading to death of some $10^{-4}$ M copper treated cells occurred after 36 - 72 h. Cytochemical tests also revealed that root cells exposed to $10^{-4}$ M Cu showed an increase in the number of cysteine-rich protein in the vesicles and cytoplasm from 4 h to 72 h of exposure (Liu et al., 2009).

When two months old *Atriplex halimus* grown in hydroponic conditions were treated with different concentrations of CuSO$_4$ (50, 500, 1000 and 2000 µM), a progressive decline in chlorophyll $a$ and $b$ content was observed with increasing Cu concentrations. Protein content was decreased to 21% after 6h treatment at 500 µM but after 24 h and 48 h an increase of 13% and 155% was observed in 500 µM of Cu treatment, suggesting that synthesis of proteins might be a way to cope with the excess of copper in *A. halimus* plants (Brahim and Mohamed, 2011).

Plantlets of two different cultivars of *Zea mays* (SC 122 and SC 10) exposed to different concentrations of copper (0.0, 25, 50 and 100 µM) showed depressed shoot length, root length and less number of roots with increasing Cu levels. Decreased chlorophyll and carotenoid content was observed under the highest Cu treatment (100 µM) in both cultivars. However a progressive increase in the level of total soluble sugar was observed with increasing Cu concentrations in both the cultivars but reducing sugars fluctuated with different Cu levels (Amina and Mohamed, 2012).
Exposure to high (600 ppm) and low (150 ppm and 280 ppm) concentrations of Cu was found to damage the physiological and biochemical activities of wheat \textit{(Triticum aestivum L.)} plants in 30 day exposure. Decrease in chlorophyll \textit{a}, chlorophyll \textit{b}, carotenoid, protein, and carbohydrate content was observed with increasing Cu concentrations. However, no significant effect was observed on shoot and root length of the wheat plants at lower concentrations of Cu (150 ppm and 280 ppm) treatments as compared to control plants (Vinod \textit{et al.}, 2012).

\textbf{Ni contamination and its effects on biochemical and antioxidant properties of the plants}

Ni like Cu is also a micronutrient needed by plants in trace amounts but becomes toxic at elevated levels. Considerable research has been conducted to study the micronutrient properties as well as the toxic effects of Ni on plants. When alfalfa (\textit{Medicago sativa}) seeds were exposed to 0, 5, 10, 20 and 40 ppm of different metals Cd (II), Cr (VI), Cu (II), Ni(II) and Zn(II) independently, concentrations of the metals influenced the extent of toxicity to the plant. Plants exposed to 10ppm concentration of Cd (II) and Cr (VI) showed significant effect on seed germination and plant growth, while those exposed to Zn (II) did not show effect on seed germination and also promoted shoot and root growth at all the doses. Plants exposed to Cu (II) and Ni (II) did not show any effect on seed germination upto 10ppm, with root and shoot length also showing increase upto 10ppm even more than the control plants. However, all the growth parameters decreased with increase in Cu and Ni concentrations, thereby revealing their micronutrient properties at lower and toxic properties at higher doses (Peralta \textit{et al.}, 2000).

In an experimental set up, seeds of tomato (\textit{Lycopersicon esculentum L.}) cv. Marito were irrigated with various concentrations (15, 30, 45 and 60 ppm) of nickel sulphate. Increase in Ni concentration improved fruit quality by increasing its length, diameter, weight and dry matter as well as Vitamin C, total soluble solids and total soluble sugar contents (Gad \textit{et al.}, 2007).
Nickel was found to increase the activities of cell wall peroxidase against ferulic acid (FPOX), syringaldazine and phenylalanine ammonia-lyase (PAL). At the same time an increase in the content of lignin was also observed thereby reducing the growth of roots in rice seedlings when treated with 20, 40 and 60 µM concentrations of NiSO$_4$ (Lin and Kao, 2005).

Wheat (Triticum aestivum L.) was exposed to 10 and 200 µM of Ni to study the changes in antioxidative enzymes, proline and chlorophyll contents on 3, 6 and 9 day of exposure. Plants exposed to 10 µM Ni did not show any significant alteration in shoot length, total chlorophyll content, chlorophyll $a/b$ ratio, proline content and antioxidative enzyme activities in all the exposure periods as compared to control plants. However, those plants exposed to 200 µM showed significant reduction in shoot length, chlorophyll $a+b$ content, chlorophyll $a/b$ content, relative water content, superoxide dismutase and catalase activities, accompanied by increase in proline content, and peroxidase and glutathione S-transferase activities (Gajewska et al., 2006). Similar reduction in the activity of catalase with increasing Ni concentrations was observed in Jatropha curcas L. seedlings when treated with 100, 200, 400 and 800 µmol Ni. Activities of other antioxidative enzymes such as superoxide dismutase and peroxidase also decreased at 800 and 400 µmol Ni. On the contrary, phenyl-alanine ammonia-lyase activity increased in all the Ni treatments with the highest activity in 400 µmol nickel (Yan et al., 2008).

To assess the effects of nickel sulphate on total carbohydrate content along with other physiological aspects, two cultivars of radish Raphanus sativus cv. longipinnatus (white radish) and Raphanus sativus cv. Cherry Belle (red radish) were exposed to different concentrations of Ni (50, 100, 150 and 200 ppm) for a period of 32 days. Decrease in total carbohydrates, total protein, photosynthetic pigments, fresh and dry weights of shoots and roots
in both the cultivars were observed with increasing nickel sulphate concentrations. On the contrary, the activity of catalase, peroxidase and polyphenol oxidase increased with the increase in Ni concentrations, thereby revealing antioxidative properties of these enzymes in removing superoxide radicals, which are harmful to plant cell membranes (Latif, 2010).

Under experimental condition, halophyte *Salicornia brachiata* was exposed to 50, 200, 400 and 800 µM of Ni for 15 days. *S. brachiata* was found to tolerate Ni only upto 400 µM. With the increase in Ni concentrations, shoot length, root length, fresh weight and chlorophyll content decreased progressively as compared to that in control plants (Sharma et al., 2011).

A study was conducted to investigate the effect of Ni on water lettuce (*Pistia stratiotes* L.), which was exposed to 0.01, 0.1, 1.0 and 10 ppm of Ni for 6 days. Plants exposed to 0.01 ppm and 0.1 ppm of Ni did not have altered pigment contents, antioxidative functioning and visible toxicity symptoms. Those exposed to 1.0 ppm and 10 ppm of Ni showed prominent toxicity symptoms including bleaching of leaf margins towards the base, chlorosis in young leaves, browning of root tips and broken off roots. Besides these visible toxicity symptoms; reduction in chlorophyll (*a, b* and total), protein and carotenoids content at 1.0 ppm and activity of catalase at 10 ppm of Ni were observed. However, proline content, peroxidase activity and Ni accumulation increased with the increase in Ni concentrations. The accumulation of Ni was more in root (863 µg g⁻¹ dry weight) than leaves (116.2 µg g⁻¹ dry weight) in 10 ppm dose of Ni after 6 days of treatment (Singh and Pandey, 2011). Reduction in chlorophyll and carotenoid content at high dose (16 mg L⁻¹) of Ni treatment; increase in protein content at lower doses (0.25, 1 and 4 mg L⁻¹); and increase in chlorophyll and carotenoid content at 0.25 and 1 mg L⁻¹ of Ni treatment were observed in *Lemma gibba* during a 72 hour exposure experiment (Doganlar et al., 2012).
The effects of graded nickel concentrations of 1, 3, 5 and 8 mM on growth, pigments and antioxidative defense system in shoot cultures of rare Balkan hyperaccumulating species Alyssum markgrafii was investigated. Reduced fresh and dry weight of A. markgrafii shoots was observed in all nickel treatment plants. Gradual decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content along with ascorbate peroxidase and catalase activities in treated shoots was observed. The amount of malondialdehyde production and soluble peroxidase activities increased with increase Ni concentrations (Stanisavljević et al., 2012).

**Scanning Electron Microscopy (SEM) studies on the effects of heavy metals on plants**

An SEM study on Scirpus lacustris L. treated with 8 µg ml\(^{-1}\) chromium revealed that the roots showed increase in the relative proportion of pith and cortical tissue layers. The number of cortical layers, which was 12 in control root tissue increased to 15 - 18 indicating that the metal accumulated more in the roots after 30 days of exposure (Suseela et al., 2002).

Scanning Electron Microscopy coupled with Energy dispersive x-ray microanalysis (SEM-EDX) was used to detect the qualitative localization of Ni in some of the Ni-hyperaccumulator species: Euphorbia helenae, Leucocroton linearifolius, L. flavicans, Phyllanthus orbicularis, P. discolor and P. xpallidus collected from Cuban ultramafic soils. The micro-morphological SEM-EDX analysis of leaves showed that the highest Ni concentration was found in the epidermal tissues followed by mesophyll and vascular bundle cells of all the hyperaccumulator species. In case of Leucocroton linearifolis, Ni was also present in the interior of the epidermal cells and in the transition zone between the palisade parenchyma and mesophyll cells of leaf tissues (Berazaín et al., 2007).
A study was conducted to investigate the ultrastructural alterations and localization of Cu in the nodules of white lupin and soyabean plants under the condition of excess copper (192 µM). Energy-dispersive X-ray micronanlysis showed that Cu localization was more in the cell walls of inner cortical cells and infected zone cells as compared to the walls of outer cortical cells in case of white lupin. However, in the soyabean nodules Cu localization was more in the cytoplasm-vacuoles of the inner cortex cells and the infected zone cells than their respective cell walls. Ultrastructural studies on the nodules revealed that white lupin under 192 µM Cu treatment showed deformation of cell walls of the outer cortex zone. Occlusions in the intercellular spaces and those of infected zone showed a number of senescent cells and degeneration and even breakage of peribacteroidal membranes along with increased number of vesicles in the cytosol, which is considered to be a sign of early senescence. The ultrastructural alterations observed in the nodules of soyabean included reduction in the size of sclereid cells and in the volume of the distribution zone cells in the cortex, whereas in the infected zone increased vacuolization, increased electron-dense cytosol, and degradation or separation of bacteroidal membrane were observed (Sánchez-Pardo et al., 2012).

An SEM study on the effect of cadmium toxicity on chickpea (Cicer arietinum L.) growth showed an alteration in the orientation of vascular tissues and other changes in the stomatal complexes. Chickpea was treated with different concentrations of Cd (0.05 - 50 mM) and was used for SEM study after 24 h of treatment. Vascular bundle (phloem and xylem) tissues were enlarged at 0.05 mM treatment, while at 50.0 mM crimped structures of xylem and phloem in the root tissues were observed, indicating impairment of both the conducting tissues with increasing Cd concentrations. In case of leaf tissues control leaves showed normal stomata along with guard cells and the occurrence of stomata was higher than that in the 10 mM Cd
treated leaf tissues. At 50 mM Cd treatment, alteration in stomatal complex was observed and stomatal apertures seemed to be closed (Mondal et al., 2013).

**Studies on the bioaccumulation and uptake of heavy metals on plants**

*Nasturtium officinale* (watercress) were exposed separately to different nominal concentrations of 1, 3, 5 and 7 mg l$^{-1}$ of different metal ion solutions of Cu, Zn and Ni. Cu and Ni were rapidly taken up in the beginning of the experiment i.e. during the 24 h exposure whereas Zn uptake occurred rapidly during 48 h. It was observed that in the given concentration range Cu was accumulated more effectively in comparison to Zn and Ni by *N. officinale* (Kara, 2005).

Chinese cabbage (*Brassica pekinensis* Rupr.) was exposed to different concentrations of Cu, viz., 0.2 and 1.0 mM kg$^{-1}$ dry soil to study the phytotoxic effects and also the bioaccumulation potential of the plants. Increase in chlorophyll content, peroxidase activity and electrolyte leakage were observed in *B. pekinensis* shoots with increasing Cu concentrations. The bioaccumulation of the metal also increased with increase in Cu concentration; plants treated with 0.2 mM kg$^{-1}$ showed shoot Cu concentration of 42.5 mg kg$^{-1}$ and those with 1.0 mM kg$^{-1}$ showed Cu concentration of 119.0 mg kg$^{-1}$ when harvested after 17 days of Cu addition. These values were found to be significantly higher than that of the control (9.9 mg kg$^{-1}$) (Xiong and Wang, 2005).

A study was conducted on Pariyej Community Reserve, Gujarat, India to assess the phytoremediation potential of some selected macrophytes in heavy metal contaminated water and sediments. Seven native aquatic plants; *Ipomoea aquatica* Forsk., *Eichhornia crassipes* (Mart.) Solms, *Typha angustata* Bory & Chaub., *Echinochloa colonum* (L.) Link., *Hydrilla verticillata* (L.f.) Royle, *Nelumbo nucifera* Gaerth. and *Vallisneria spiralis* L. were collected
and chosen for analysis of Cd, Co, Cu, Ni, Pb and Zn contamination. The concentrations of Co and Ni were within the critical range and that of Zn and Cu showed high accumulation rate in the plants revealing severe contamination in the water bodies. The highest mean accumulation of the heavy metals followed the trend of *N. nucifera* (343.54 ppm) > *E. crassipes* (136.46 ppm) > *V. spiralis* (82.42 ppm) > *H. verticillata* (81.89 ppm) > *I. aquatica* (74.81 ppm) > *T. angustata* (70.51 ppm) > *H. verticillata* (0.15 ppm). In this study, the highest amount of Cu concentration was observed in *N. nucifera* (1617.21 ppm) and the lowest (16.32 ppm) in *H. verticillata*. Ni content varied from 4.10 to 28.83 ppm in *V. spiralis* and *E. crassipes*, respectively (Kumar *et al.*, 2008).

In an experimental setup to study the phytoaccumulation potential of wetland macrophytes, *Hydrilla verticillata* and *Ipomoea aquatica* were treated with 20 ml of 50 mg l\(^{-1}\) of sodium arsenite, cadmium chloride and lead nitrate solutions separately for 24 h. The contaminated solutions after treatment with the macrophytes were used in the culture medium of *Catharanthus roseus*. It was found that pollen germination of *C. roseus* in solution treated with *H. verticillata* with metals As and Cd was quite near to control plants indicating *H. verticillata* to be a good accumulator of As and Cd. On the other hand, *Ipomoea aquatica* was found to be an efficient accumulator of Cd and moderate accumulator of Pb in its plant parts (Ghosh, 2010).

In a study, *Sagittaria sagittifolia* L. and *Potamogeton crispus* L. were used to investigate their Cd, Cu and Pb accumulating potential. The results showed that the roots of *S. sagittifolia* were more sensitive to Cd and Pb than the leaves of *P. crispus*. However, the potential of Cu accumulation was similar in both the plants (Hu *et al.*, 2010).
Azolla caroliniana was exposed to various concentrations of arsenic (5, 10, 20, 40, 80 and 160 mg L\(^{-1}\)) for a period of 30 days to study the uptake of As. Reduction in the growth rate of A. caroliniana was observed at 5 mg L\(^{-1}\) As by 18.14% which became prominent in the higher concentrations of As. Bioconcentration factor of As in roots of A. caroliniana varied from 8.33 to 71.63. The highest As accumulation was observed in 160 mg L\(^{-1}\) As treatment (Moradi et al., 2013).

**Adverse effects of heavy metals on plants**

A study was conducted to assess the effects of Cu, Pb and Al on adventitious bud cultures by using bud of poplar clones (Populus tremula L. × P. alba L.) as initial explants. Cu, Pb and Al were added to the medium as nitrates at the concentrations range of 0.1 - 2.0 mM. In another set, Cu as copper sulphate (0.2 - 1.0 mM) and Al as aluminium sulphate (0.5 - 1.0 mM) were added. Copper either in sulphate or nitrate form was found to be the most toxic metal in this study. Cu at concentrations of 0.25 - 1.0 mM and Pb at 2 mM strongly inhibited shoot development and even increased the level of chlorosis and browning. Again, Cu at 0.5 - 1.0 mM showed complete blockage of root development. However, Al was found to be the least toxic metal showing no significant chlorosis and browning in the culture quality (Bojarczuk, 2004).

A microscopic analysis with novel fluorescent dyes (2',7'-dichlorofluoresceindiacetate that labels peroxides; monochlorobimane that stains reduced glutathione/ homoglutathione; and propidium iodide that marks nuclei of dead cells) were used which allowed recording of minute cellular responses to 0, 3, 10 and 30 µM of Hg or Cd that are visualized in the roots of the alfalfa (Medicago sativa) seedling. After 6 - 24 h exposure to Cd and Hg, oxidative stress and cell necrosis occurred even in 3 µM treatment which further increased with increase in metal concentrations (Ortega-Villasante et al., 2005).
Under experimental conditions, six cultivars of chickpea (Cicer arietinum L.) were supplied with 25, 50 and 100 mg Cd kg\(^{-1}\) soil, and the changes in seedling survival, growth, foliar toxicity symptoms, proline and nitrate reductase activity were assessed on 30 and 60 days after each treatment. The seedling survival percent was found to be the lowest in the highest concentrations of Cd used (100 mg kg\(^{-1}\)) in all the chickpea varieties. Leaf chlorosis and necrosis were observed in all the varieties of Chickpea used which increased with increase in Cd concentrations. There was an increase in the proline content but decrease in nitrate reductase activities in all the chickpea varieties due to Cd toxicity (Faizan et al., 2011)