REVIEW
OF
LITERATURE

**Heavy Metal And Plant Growth**

Phytotoxic effects of heavy metal ions have been widely reported. Heavy metal toxicity causes multiple direct and indirect effects on plant growth and alters many physiological functions (Woolhouse, 1983). Wheat plants were grown on Cu treated plots, plant growth significantly decreased when it was compared with untreated control plots (Lanaras et al. 1993). Cu decreased the plant height (Kahle 1993; Arduini et al., 1994; Ouzounidou 1994, 1995a, 1995b). Higher concentration of Cu adversely affected the plant growth and productivity of lentil. Root growth was stunted, chlorosis was noted in interveinal area of leaves (Khan and Khan 1994). Mozaffari et al., (1996)
reported that high copper content produced toxicity symptoms in citrus. Both rooting and shooting were affected, but root formation was more inhibited. This might be due to accumulation of Cu in roots and less translocation of Cu to shoots.

Increasing concentration of Cu led to increased Cu contents in the shoot of rice plants shoot growth was inhibited (Fernando and Fernando 1998). Copper inhibited the root, stem and leaf growth of rice (Lindon and Henriques 1998). The germination of Black Binweed was not affected in laboratory test with soil from a Cu polluted site but the biomass of plant decreased with increasing Cu concentration (Kjaer et al., 1998). Increasing concentration of Cu concomitantly reduced the growth of *Lemna minor* (Teisseire et al., 1998). In sand culture experiment, safflower was grown with varying levels of Cu ranging from 0.001 to 10μML⁻¹. Total dry matter and seed yield were maximum at 1μML⁻¹ Cu supplies. Growth depression was observed at excess (2 and 10μML⁻¹) Cu. There was 50% decrease in seed yield (Pandey and Sharma 1999).

Chatterjee and Chatterjee (2000) reported the phytotoxicity of Cu in cauliflower. Bruus et al., (2000) studied the toxicity of Cu to Black Binweed. The accumulation pattern for root and shoot of Binweed indicated that mainly root accumulated excessive Cu. Cu effects on growth follows the pattern of Cu accumulation in shoots. Jiang et al. (2000) reported that excess Cu damaged the root growth of *Helianthus annuus* L. The graded concentrations i.e. 8, 16, 32, 96, and 125μM CuSO₄ lowered the dry weight of root and shoot of *Vicia faba* cultivated in pots. Higher concentration of Cu significantly lowered the biomass of root and shoot. Root was affected more than shoot (Roy et al., 2001). The free Cu activity in Cu enriched peat soils was correlated to the severity of phytotoxicity as measured by several
indicators in maize, including leaf chlorosis, root stunting and reduced shoot growth and Fe concentration. This Cu activity measurement is more directly related to phytotoxic effects than other soil tests (Mc Bride 2001).

Bean was grown in refined sand at levels of Cu ranging from 0.0065 to 6.5 mg l\(^{-1}\). High Cu concentration (6.5 mg l\(^{-1}\)) reduced the growth of plants, young leaves turned chlorotic later changing to bleached and papery (Khurana and Chatterjee 2001). 22 days old *Cucumis sativus* seedlings were treated with 10 µg Cu/g for 5 days. Young expanding leaves showed a reduction in leaf area, while mature leaves exhibited a significant decline in photosynthesis. The growth reduction was more likely due to a reduction in whole plant leaf area. Relative growth rate and leaf area ratio of stressed plants were significantly below that of control plants while no significant decrease of net assimilation rate was observed for Cu-stressed plants (Vinit-Dunand *et al.*, 2002).

The five ruderal plant species namely *Poa annua* L., *Dactylis glomerata* L., *Senecio vulgaris* L., *Hypochoeris radicata* L., and *Andryala integrifolia* L. were grown on Cu (0-400mg/kg) contaminated soil. The plant survival, growth, reproduction were noted. High concentration of Cu in the soil resulted in low survival, low total biomass, delay in flowering and fruiting, and low seed set. The effects differed among species. The high soil Cu concentrations had contrasting effects on patterns of resource allocation (Brun *et al.*, 2003). Jiang *et al.*, (2004) studied the growth response of *Elsholtzia splendens* to Cu. The results indicate that plant exhibited high tolerance to Cu toxicity in the soils. This species was able to grow even with high Cu concentration i.e. 1000 mg kg\(^{-1}\).
The acute toxicities of Cu to important crop plants *Sorghum bicolor*, *Cucumis sativus*, *Triticum aestivum* and *Zea mays* were compared. The concentration of metal in the soil reduced the growth of shoots and roots. The root growth was more sensitive to the toxicity end point than shoot growth (Youn-Joo 2005). Xiong Ting (2005) reported the Cu toxicity and its bioaccumulation in Chinese cabbage. Sheldon and Menzies (2005) reported the copper toxicity on Rhodes grass. Cu damaged plant roots with the symptoms ranging from disruption of the root cuticle and reduced root hair proliferation, to severe deformation of root structure. A reduction in root growth was observed at an external Cu concentration more than 1µM in solution culture. Lower concentration did not cause much effect on dry weight of root and shoot but higher concentration significantly reduced root dry weight. Similarly higher concentration caused significant reduction in plant height, root length etc.

36 days old rice varieties viz. Pusa Basmati and Pusa Sharbati were treated with 0.1 and 0.2 mM CuSO₄. After 9 days of metal supply at 0.2mM Cu, growth of rice was depressed and young leaves developed interviernal chlorosis. Later the effects were intensified and irregular brown necrotic spots developed on the affected leaves. With increase in age the affected leaves were completely bleached. Excess Cu also reduced the rice biomass. In both the genotypes, the accumulation of Cu was higher in roots, than in leaves, more in Pusa Sharbati than in Pusa Basmati. Rice genotype Pusa Sharbati was more sensitive to Cu toxicity (Dube *et al.*, 2005). Pot soil experiments showed that Cu is highly toxic to rice. Rice grain yields decreased exponentially and significantly with the increase of soil Cu levels. Root was more sensitive to soil Cu toxicity than other parts of rice plant at relatively lower Cu levels (less than 300-500 mg kg⁻¹) but the growth of
whole rice plant was severely inhibited at soil Cu levels i.e. 300-500 mg kg\(^{-1}\) (Jiakuan et al., 2006).

Munzuroglu and Gur (2000) reported the effects of heavy metals on pollen germination and pollen tube growth of apple. Higher concentration of CuSO\(_4\) lowered the yield of wheat (Singh 1996). The biomass as well as pod and seed yields of bean were decreased significantly at high Cu concentration 0.065 mg (Khurana and Chatterjee 2001).

The level of Co in the roots of white bean builds up considerably in plants whose growth was inhibited by excess Co (Rauser 1978). When *Phaseolus vulgaris* seedlings were exposed to excess Co, callose accumulated on the sieve plates in the phloem of affected seedlings, but this was apparently not sufficient to account for the observed effect (Peterson and Rauser 1979, Rauser and Samarakoon 1980). Austenfield (1979) studied the phytotoxicity of Co on *Phaseolus vulgaris*. Higher concentration of Co decreased the growth of plants (Pancaro et al. 1984, Osma and Esmat 1989, Saradhi and Saradhi 1991). Robson and Snowball (1987) reported that Co caused adverse effects on growth of plants. Root growth in soybean was affected more than shoot growth. It might be due to high accumulation of Co in the roots, particularly in root hairs (Werner et al., 1985).

Co also reduced extensibility of the cell wall (Fry 1986, Pandolfini et al., 1992). Kamenova et al., (1993) reported the adverse effect of Co on maize plant. Application of 2mg kg\(^{-1}\) Co increased the green foliage and dry matter yield of cluster bean, but 4mg kg\(^{-1}\) decreased it (Singh and Singh 1994). Co 200 and 400ppm decreased the seed germination, plant growth and biomass of chickpea (Khan et al., 1996). The presence of Co (0, 5, 15 & 30 mgL\(^{-1}\)) in nutrient solution reduced tomato leaf production and elongation, especially
at higher treatments (Moreno et al., 1997). Excess concentration of Co produced visible symptoms of toxicity in *Phaseolus aureus* (Tewari et al., 2002).

Benavides et al., (2005) reviewed the work done in relation to Cd toxicity in plants. Cd toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The effect of Cd on vegetative growth and development is reviewed by Balsberg and Pahlsson (1989) and Das et al., (1997). Physiological aspects of Cd and toxic effects of Cd on higher plants are discussed by Seregin and Ivanov (2001). The graded concentrations of Cd nitrate (2, 4 and 6 mM) gradually decreased the root and stem growth of lentil plants raised on sand (Luiji et al., 1981). The root dry weight of tomato was decreased by high concentration of Cd, shoot dry weight was decreasing with increasing Cd concentration (Someni et al., 1987). 3μM Cd applied for 48h decreased the water content of expanding bean leaves (Poschenrieder et al., 1989). Barcelo and Poschenrieder (1990) reviewed the work done on plant-water relations as affected by heavy metal stress. Metal toxicity causes multiple direct and indirect effects in plants, which concern practically all physiological functions. As heavy metals adversely affect root growth, water balance is disturbed. Cd reduced the root elongation of maize plant (Rascio et al., 1993). Growth of tomato was affected negatively by 10 or 30mgCd L⁻¹, root, stem length and fresh weight were decreasing with each increment of Cd added to the nutrient solution (Moral et al., 1994).

Higher concentration of Cd lowered the shoot length of lentil. Root shoot dry weight was also suppressed with increasing Cd concentration. The number and dry weight of leaves were also reduced (Beri and Setia 1995). The dry matter of sugar beet shoot was progressively declined with increasing
concentration of CdCl$_2$ from $10^{-4}$, $10^{-2}$ and 1mM (Kevresan et al., 1998). Cd toxicity in terms of chlorosis and necrosis were not found after 35 days of Cd application to sunflower (Simon, 1998). 1 mg Cd did not cause any effect on root tip and length but roots were thick with fewer branches and less root hair treated with 10mg Cd Kg$^{-1}$. 1mg Cd Kg$^{-1}$ did not affect the fresh weight and dry weight of plants. 10mg Cd decreased inflorescence weight (Simon et al., 1996).

Rice production in China was decreased when paddy fields irrigated with wastewater from smelters and coal mine. The human disease whose symptoms were similar to that of Itai-Itai in Japan (Wu et al., 1981, Jia 1992) was found (Yanai et al.,1998). 25 mg Cd kg$^{-1}$ soil decreased the number and yield of *Chamomolla recutita* grown in pot (Grejtopvsky et al., 1998). In *Brassica juncea* Cd exposure decreased the transpiration rate and leaf expansion without affecting on photosynthesis (Haag-Kerwer et al., 1999).

The graded doses of CdCl$_2$ i.e. 0.1, 0.5 and 1.0 mM in earthen pots inhibited the overall growth and yield of cumin without chlorosis and necrosis. The toxic effects were dose dependant. Root of younger plant was more sensitive to Cd (Vyas and Vediya 1999). *Lepidium sativum* is an important medicinal plant. Seeds are medicinally useful. The seeds of plant were sown in plastic bags containing 0.0, 200 and 400 ppm CdSO$_4$ separately. At regular interval growth was noted. Cd inhibited the vegetative growth of plant, 400ppm was more effective. Senescence was early in Cd treated plants. Root study was selected as parameter for studying the Cd toxicity and Cd tolerance in *Lepidium* (Vyas et al., 2000). 4 to 40μM Cd application for 7days in hydroponic culture of pea gradually decreased the plant biomass (Dixit et al., 2001).
Brassica napus was cultivated on a reconstituted soil contaminated with Cd (100mg Cd kg\textsuperscript{1} dry soil). The chlorosis was very sharp. Leaf chlorosis was attributed to a marked decrease in the chloroplast density caused by a reduction in the number of chloroplasts per cell and a change in cell size, suggesting that Cd interfered with chloroplast replication and cell division (Baryla et al., 2001). Pea plants were grown with CdCl\textsubscript{2} (0-50µM). The growth of root and leaves as well as rate of transpiration and photosynthesis were significantly inhibited (Sandalio et al., 2001). The presence of Cd as CdCl\textsubscript{2} (10-100µM) in the nutrient solution resulted in a strong inhibition of root growth and to a lesser extent shoot weight of Arabidopsis thaliana. This strong reduction in the root growth and fresh weight was linked to a large accumulation of Cd in root tissue (Perfuse-Barbeoch et al., 2002).

More than 0.125 mM Cd significantly inhibited the growth of pepper cultivars although different sensitivities to Cd ions were observed among the cultivars (Leon et al., 2002). White lupin plants were grown hydroponically with different Cd concentrations in the nutrient solution (0, 18 and 45 µM). A significant decrease in both root and shoot dry weight of 35 days old plants was found when plants were grown on 45 µM Cd (Zomoza et al., 2002). The graded concentrations of CdCl\textsubscript{2} (10-100 µM) in pot gradually lowered root stem elongation, leaf number, fresh weight and dry weight of root, stem and leaf of cumin. Reproductive growth was affected. Cd also decreased the harvest index value (Vediya and Vyas 2002a).

Bakonyi et al., (2003) reported that Cd (maximum rate of 270 mg kg\textsuperscript{-1}) decreased the biomass of wheat, sunflower, sorrel, barley and rape. The presence of Cd 500mg kg\textsuperscript{-1} in pot soil suppressed the growth of sunflower and it was due to inhibition of root growth (Chandragupta and Gopalsingh...
The addition of Cd (10 and 20 mg kg\(^{-1}\)) to soil inhibited soybean growth. Root was most sensitive. Inhibitory effect was increasing with increasing Cd concentration. The weight ratio of soybean root/leaf decreased as the Cd concentration increased. Cd content in plant was in the range of roots > stems > seeds, it was one of the reasons for severe effect on root systems (Chen et al., 2003 a). Liquid culture and pot experiment was carried out to study physiological mechanism of plant root exposure to Cd. The germination rate and growth of roots of carrot and radish were inhibited at the concentration of 20mgCd L\(^{-1}\). The inhibition increased with increasing concentration of Cd in liquid culture and in the pot experiment (Chen et al., 2003b).

Bhanderi et al., (2004) reported the effects of CdCl\(_2\) on growth of medicinal plants *Cassia tora*, *Cassia occidentalis* and *Plantago ovata*. The vegetative and reproductive growth was reduced by Cd application to the soil. The root elongation was most sensitive process. They suggested root bioassay as a technique for assessment of Cd toxicity in medicinal plants. The graded concentrations of Cd gradually lowered the mulberry plant growth (Wang et al., 2004). The two varieties of pea and their hybrid were treated with graded concentrations of CdCl\(_2\) in pot experiment using river sand as nutrient medium. Low doses of Cd increased biomass of root and above ground parts whereas higher doses decreased shoot biomass of both the species. Cd decreased both root and shoot and above ground biomass in the hybrid. Hybrid was affected much more than its parents (Dewan and Dhingra, 2004). Vyas (2004) reviewed the work done on response of medicinal plants to stress environment. Cd is considered as stress for plants. Cd as a soil pollutant suppressed the overall growth of vasaka. Primary metabolites in the leaves were also decreased. The total alkaloid, vassacine in the leaf of vasaka
was lowered by Cd. The health of the plants decide the medicinal value of the plant i.e. better health more herbal value and vice versa.

Cd stress decreased the growth and caused over production of phytochelatin in root of wheat (Sun et al., 2005). The treatment of soybean seedlings with 9mg kg\(^{-1}\) Cd induced a slight growth inhibition in roots, stems and leaves and a significant desiccation of cotyledons and leaves (Drazic and Mihailovic 2005).

Sorghum was grown in nutrient solutions with 0, 0.1, 1 and 10 mg Cd dm\(^{-3}\), in absence and presence of organic matter (32 mg Cd dm\(^{-3}\)), for various periods up to 20 days. A decrease in sorghum biomass was observed at 10 mg Cd but lower concentration enhanced it without any visual toxicity symptoms. The presence of organic matter further increased the biomass production. Cd was mainly retained in the sorghum roots, as usually found in tolerant plants. The presence of organic matter decreased the Cd uptake but promoted the translocation of Cd to shoot. Thus it may pose a risk to human health because it enhanced the uptake of Fe, both in the presence and absence of organic matter (Pinto et al., 2004). Colocassia esculentum plants were grown in pots containing different concentrations of Cd. Cd effects were analyzed for dry matter content. Cd depressed dry matter, fresh weight and total matter content. Plant accumulated large portion of heavy metal in root followed by stem and leaf (Patel et al., 2005).

One month old miscanthus plants were exposed to 0.75, 1.25, 2.25 and 3 mgL\(^{-1}\) Cd for 36 days. 0.75 mgL\(^{-1}\) caused about 50% decrease in biomass whereas 2.25 mgL\(^{-1}\) and higher concentration completely inhibited growth and restricted the Cd translocation to the shoot. The Cd concentration in different organs was in the order roots>rhizome>culms>leaves. The highest
Cd concentration was found in the plants grown with 3 mgL\(^{-1}\) Cd. Concentration of Cd was less in aerial parts than in the hypogeal parts (Arduini et al., 2006).

The dry biomass of both roots and shoots was significantly reduced in Cd-treated plants compared to the control plants (Sanita di Toppi and Gabbrielli 1999; Baryla et al., 2001; Mysliwa et al., 2004). High heavy metal concentrations in the majority of cases inhibited plant growth, especially root growth (Karataglis et al., 1991).

Pollen germination in five herb species was inhibited by Cd concentration of 2.51 µg ml\(^{-1}\) and higher, and tube growth was inhibited at concentrations of 1.58 µg ml\(^{-1}\) and higher. But at low concentration pollen tube growth was stimulated. Cd may adversely affect plant reproduction by inhibiting pollen germination and tube growth (Xiong and Peng, 2001). Pollen germination and tube growth of pea varieties were decreased with increasing Cd concentration (Rajkumar et al., 2000).

Cd adversely affected reproductive development. Cd inhibited the growth of wheat plant (McMahon and Anderson, 1998). Cd reduced the wheat and soybean yield (Haghiri, 1972) and sorghum yield (Mehla et al., 1989). Bramley and Barrow (1994) reported similar results for the yield of medicago, santiago, capeweed and subterranean clover. The presence of Cd in nutrient medium decreased the yield of tomato (Moral et al., 1994). Fresh weight of fruit was not affected. Beri and Setia (1995) reported that Cd delayed flowering of lentil plant. Cd suppressed the number of flowers, number of pods, and dry weight of pod. Number of seed per pod also decreased with increasing concentration of Cd. Similarly partitioning coefficient and harvest index were lowered with increasing concentration of...
Cd. The suppression in reproductive structure was reported in pea with Cd (Setia et al., 1989). There was a negative correlation between water extractable Cd in the soil and head length of *Setari glauca*. This correlation suggests seed head length as a biomarker for soluble Cd in contaminated soil (Kosma et al., 2004). Populations of metallophyte *Thlaspi caerulescens* originating from metal mines at Belgium were cultivated hydroponically with 5 to 500μM Cd. The plants could tolerate 500μM Cd in the solution showing minor visible symptoms of toxicity but with a 32% decrease in fresh weight (Wojcik et al., 2004).

During last two decades, it has been well documented that compared to other metals, mercury and its compounds are more toxic for seed germination and plant growth. According to Mishra and Choudhuri (1998), Bonifacio and Montano (1998) Hg has the highest toxicity potential in plants. Patra and Sharma (2000) reviewed the work done on Hg toxicity in plants. Varshney (1990) studied the effects of Hg on *Vigna radiata* cv. MG-46. Seeds were sown in polythene bags having soil amended with 1X10^{-4}M (31.87mg^{-1}) Hg acetate. After 30 days from emergence under amended soil condition, the root and stem growth and leaf area was inhibited up to 66%, 68% and 32% of control respectively. Hg treatment completely inhibited flowering in lentil plants grown in sand culture. The reduced vegetative growth ultimately affected the reproductive development and hence the yield of the plant. Hg treatment completely inhibited the floral evocation. This reduction also led to reduce harvest index (Beri and Setia 1995).

Fenugreek var. local was raised in earthen pots with different concentrations (50, 100, 150, 200 and 250mg Kg^{-1} of soil) of HgSO₄. The metal toxicity was evaluated by noting the various growth parameters i.e. root length, stem length, leaf number and fresh weight and dry weight of root, stem and leaf of
60 days old plants. The growth was stunted (Vyas et al., 1997). Tripathi and Tripathi, (1999) reported that HgCl₂ (1, 5 and 10 mg/l) adversely affected root shoot length, leaf area and biomass of Albizia lebbek. The graded concentrations of HgCl₂ and CdCl₂ as a soil pollutant lowered the growth of Ashwagandha. Root was the target organ. The growth was inhibited without visible symptoms. Before using a root as herbal drug it must be analyzed for heavy metal like Cd and Hg (Trivedi et al., 2001). HgCl₂ (0.1, 0.5 and 1.0mM) added to the soil retarded the root elongation, shoot elongation, leaf number, fresh weight and dry weight of root, stem, and leaf of cumin var. Guj-l. Inhibitory effects on all growth parameters were positively related with HgCl₂ concentration. The fruit number, fresh weight and dry weight were severely reduced. Root was the most sensitive organ to Hg, Hg effects on cumin may be evaluated with the help of root study (Vediya and Vyas 2002b).

Sardar varieties of guava were grown in field conditions. The first spray of Bordeaux mixture was given when the fruits were of areca nut size and four subsequent sprays at 20 days interval were given. The higher concentrations were toxic than their lower doses. Bordeaux mixture caused heavy russetting. Russetted fruits failed to attain normal size and thus reduced the quality of fruits to a greater extent (Gaikwad and Nimabalkar 2002).

Foliar or root application of 0-10.9 ppm Cd in once a week for 12 weeks was given to the plants. Cd toxicity was more and symptoms were more severe in foliar treated plants than that in root treated plants. Cd decreased dry matter of whole plant, shoot and root in foliar treated plants. The dry matter was decreased with increasing Cd concentration (Salim et al., 1992). Salim et al., (1993) reported the effect of root and foliar treatment with Cd on growth of radish plants. Radish seedlings after 2 weeks of transplantation were treated
via the soil or by foliar application with three different concentrations of Cd. Cd was inhibitory to growth. Foliar application was more harmful to plants than soil treatment. Shoots tended to accumulate Cd. Radish plants treated with solution containing more than 1mg Cd L\(^{-1}\) posed a health hazard to human. Three days old seedlings of maize were exposed to 10,100 and 250 μM CdNO\(_3\). 21 days old potted plants of mungbean were sprayed with 40, 60, 80 and 100μM concentration of CdSO\(_4\). Control set was sprayed with DW, spraying was done once a week; dry weight of 56 days old plants was observed. All the concentrations of CdSO\(_4\) showed inhibitory effect on shoot length, leaf area and length of pods as compared to control. The effect was significant in older plants and it was co-related with concentration (Keshan and Mukherji 1994). Salim et al., (1995) reported that Cd had remarkable effects on the growth of pepper plants. Foliar plants were affected more than root treated plants, the least growth inhibition and Cd uptake was found in plants grown in soils with organic matter content.

Pasquale et al., (1995) investigated the effects of high levels of Cd in soil and in the atmosphere on Coriandrum sativum plants. Plants grown in contaminated soil (0, 10 or 100 ppm Cd) showed a significant reduction in the length of the stems and roots and number of inflorescences, yellowing and ultra structural alterations of the leaves and significant decreases in the contents of essential oil components in the fruits. Similar effects were observed in plants exposed to simulated atmospheric pollution (plants sprayed with CdCl\(_2\) solutions containing 0, 10 or 100 mg Cd L\(^{-1}\)).

Dill is one of the medicinal plant. Dill var. MH Local were raised in the field and plants were sprayed with DW, 200, 400, 600, 800 and 1000ppm of HgCl\(_2\) after 40 days of growth. The foliar spray was given at the interval of 15 days and that up to 85 days. Control, DW sprayed and Hg sprayed plants
were studied for growth. Vegetative and reproductive growth was remarkably inhibited by Hg. DW spray increased overall growth (Vyas et al., 2001).

Adverse effects of Ni, Cu and Co have been reported on growth characters, biomass, yield etc. of celery, tomato and lentil plants both in ambient and artificial treatment conditions (Bisessar et al., 1983; Khan et al., 1987 and 1988). Reductions in shoot growth of lentil plants by Ni, Cu and Co in the artificial treatment have been observed (Khan et al., 1987; 1988). The sub-lethal, toxic and lethal concentrations were different for inducing the toxic effects of heavy metals on growth and metabolism of fenugreek. The radical growth and root elongation of fenugreek were drastically reduced with the increasing concentrations of Pb, Cd, and Hg. The toxic effects were more pronounced in earlier than in later growth (Madhavi and Charyulu 1998).

*Portulaca oleracea* is a medicinal plant. The two heavy metals Se and Hg affected both root and shoot development. They completely arrested shoot development at all concentrations. However, they produced concentration-dependant changes in the development of root, which ranged from their complete inhibition to variation in their initiation time, number and length. Hg was more toxic than Se (Thangavel et al., 1999). Vyas et al., (2001) reported that CuSO₄ and CdSO₄ (200 mg and 400 mg each kg⁻¹ soil) retarded the over all growth of Isabgol. Cd was more toxic than Cu. The mucilage content was not much affected but yield was poor. Vyas (2002) discussed the heavy metal toxicity and tolerance in spice i.e. cumin, coriander, mustard, dill and ajwain also medicinally useful crops. Cd and Hg as a soil pollutant inhibited the vegetative and reproductive growth of spice crops. Root elongation study of very young plants can predict toxic effects of heavy metals. Heavy metal uptake study may not be the only criteria for evaluating
the heavy metal toxicity, the effects can be known through simple physiological parameters like root elongation and leaf biomass i.e. biotest. Root elongation method was selected as a good parameter for determining the effects of heavy metals on plants (Wilkins 1978, Brown and Martin 1981, Baker *et al.*, 1986, Ernst *et al.*, 1992, Brej 1998).

Exposure of cabbage plants to excess (500μM) of Co and Cd in sand culture led to increased accumulation of the metals, inhibition of growth and induction of visible symptoms of metal toxicity. In addition to chlorosis Co treated plants exhibited reddish purple coloration along leaf margins. While Cd treated plants developed purple coloration along leaf margins. At equimolar concentration, inhibition of growth was more severe with excess Cd (Pandey and Sharma 2002). Athar and Ahmad (2002) studied the effects of heavy metals on growth and metal uptake by wheat. The pot culture experiment was conducted to investigate the toxic effects of certain heavy metals on growth and grain yield of wheat. The results revealed that the heavy metals brought about significant reduction in both parameters, Cd being most toxic followed by Cu, Ni, Zn, Pb and Cr. The growth of *Nicotiana glauca* was inhibited on Cu, Cd contaminated soil but no other obvious stress symptoms were apparent. The long-term experiments under controlled conditions are planned to study the mechanism of heavy metal tolerance and its accumulation in *N. glauca* (Barazani *et al.*, 2004). Youn-Joo *et al.*, (2004) studied the Cu and Cd toxicity in *Cucumis sativus*. The toxicity end point was plant growth, which was measured as root length and shoot length after 5 days exposure. Uptake of Cu and Cd was noted in plants.

**Heavy Metal And Photosynthetic Pigments**

The loss of pigments during environmental stress is a highly visible indicator of such events as diseases, industrial pollution, mineral deficiency, mineral
toxicity, water deficiency, high temperature etc. (Hendry et al., 1987, Brown et al., 1991). Chlorophyll determination is considered as one of the most important parameters of environmental assessment. Chlorophyll is a general indicator of environmental condition with universal applicability from algae to green higher plants with a clear-cut response (Dubey, 1991). Changes in chlorophyll-carotenoid ratio are sensitive indicator of oxidative damage (Hendry and Price 1993). Heavy metals interfere with biochemical reaction of plants and induced physiological disorders like reductions in leaf chlorophyll (Khan et al. 1988). Nover (1989) discussed the plant responses to heavy metal stress. Amongst them, Pb, Ag, Hg, Cu, Cd, Zn, Co and Mn are highly toxic. Heavy metals damage the photosynthetic apparatus, impair the uptake or leakage of nutrients, change the cell ultrastructure and ultimately reduce the plant growth. The decrease in photosynthetic activity in metal treated plants may be due to combined effects of various factors like decreased chlorophyll content, decreased stomatal conductance etc (Bishnoi et al., 1993).

Baszynski et al., (1988) reported a slow rise in the Chl/Car ratio observed in spinach and oat plants treated with Cu. They suggested that it was due to strong inhibition of the synthesis of carotenoids. The increased chl/car ratio after two or four days treatment is due to more accumulation of chlorophylls than the carotenoids, whereas an increase in the ratio after 8 days of treatment is due to stronger inhibition of carotenoid synthesis than that of chlorophylls. Cu ranging from 20 to 80mM in growth medium did not exert a marked reduction in chlorophyll ‘a’ in third leaf of three year old coconut seedlings. Chlorophyll ‘b’ was significantly reduced. Carotenoids level also showed a decline although to a much lesser extent than chlorophylls (Shivshankar et al., 1989). The ratio of chlorophyll ‘a’ to chlorophyll ‘b’ was increased in Cu treated seedlings. Chlorophyll ‘a’ and chlorophyll ‘b’ levels
were reduced in Cd treated coconut seedlings. The ratio of chlorophyll ‘a’ to ‘b’ was also higher in metal treated seedlings. Chlorophyll ‘b’ was reduced to greater extent than chlorophyll ‘a’. The chlorosis observed in the presence of toxic levels of metals could be attributed to the metal induced deficiency of Mg. Excess Cu reduced the chlorophyll ‘a’ and ‘b’ in rice genotypes and Cu toxicity effects closely resembled iron deficiency, which might be due to displacement of iron by Cu (Lanaras et al., 1993). Cu suppressed the leaf pigment of lentil (Khan and Khan, 1994). More than 0.65 mg 1⁻¹ in refined sand decreased the concentration of chlorophyll ‘a’ and ‘b’. Plants grown in presence of high levels of Cu normally reduced the biomass and show chlorotic symptoms. A lower content of chlorophyll and alterations of chloroplast structure was found (Baszynski et al., 1988, Lindon and Henriques 1991, 1993, Ciscato et al., 1997, Patsikka et al., 1998, Quaartacci et al., 2000). Determination of chlorophyll content was considered as one of the method of Cu toxicity test in Lemna minor (Teisseire et al., 1998).

Excess Cu altered the structure of chlorophylls (Hill et al., 2000). Excess Cu decreased chlorophyll content in spinach leaves. The Cu toxicity was developed as chlorosis of young leaf from apex to base. Chlorosis of leaves intensified into brown necrotic spots (Nautiyal and Chatterjee, 2002). Prolonged exposure to Cu damaged photosynthetic systems, altered chlorophyll ratios and carotenoids pigments in oak seedlings. Leaf chlorosis was reported (Wisniewski and Dickinson, 2003). The gradual increase in Cu concentration in growth media gradually decreased the photosynthetic pigments and CO₂ assimilation in Salvinia minima. The inhibitory effect on growth was due to the detrimental effect on photosynthetic pigments and CO₂ assimilation (Al- Hamdani et al., 2004). Application of 0.1 and 0.2 mM CuSO₄ reduced the chlorophyll ‘a’, ‘b’ and total chlorophyll content in rice variety Pusa Sharbati and Pusa Basmati. The effect was sharper in Pusa
Sharbati. The effect was related with Cu concentration. Excess Cu reduced total and active iron in rice (Dube et al., 2005).

Co reduced the chlorophyll in lentil (Khan et al. 1988). 200 and 400 ppm of CoCl₂ reduced the chlorophyll content in leaves of chickpea. The significant reduction in chlorophyll ‘a’ was noted. Chlorophyll ‘b’ was not affected much (Khan et al. 1996). Co concentrations higher than 25 µg/g decreased chlorophyll ‘a’ and chlorophyll ‘b’ in barley (Aery and Jagetiya, 1998). Excess concentrations of Co decreased the concentrations of chlorophylls and carotenoids and increased carotenoids and chlorophyll ratio in Phaseolus aureus (Tewari et al., 2002).

Long term exposure of whole plant to Cd may affect chlorophyll synthesis, thus chloroplast development in young leaves is affected and photosynthesis is inhibited (Stobart et al., 1985). Chlorophyll and carotenoid contents in cotyledonary leaves of 7 days old okra varieties were decreased by Cd. The effect was increased with concentration (Shrivastava and Singh 1986). Naguib et al., (1986) reported decreased chlorophyll ‘a’ and chlorophyll ‘b’ contents in wheat and rice seedlings in presence of Cd and observed symptoms of iron deficiency. They attributed this decrease in chlorophyll content due to decrease in the Fe uptake by the root resulting in inhibition in formation of chloroplast through inhibition of protein synthesis. Distruption of chlorophyll synthesis and plastid ultrastructure was greater in young trifoliate leaves than in primary leaves of bush bean plants treated with Cd (Barcelo et al., 1988).

reported the phytotoxic effect of Cd on chlorophyll degradation in mung bean seedlings decreased. Bishnoi et al., (1993) reported a decrease in chlorophyll content of Cd treated wheat plants. The decrease was more in 4 days treated plants. Cd showed an adverse effect on chlorophyll content in *Triticum aestivum* (Kalita et al., 1993). The pigment content in the first leaf of *Brassica* plant was decreased after 5 days of treatment. Decrease was more with higher concentration (Mishra et al., 1994).

Cd caused decline in chlorophyll and carotenoids contents in *Vigna* and *Hydrilla*. Chlorophyll 'a' and 'b' decreased consistently although decline in chlorophyll 'a' was pronounced and induced senescence under Cd stress. The depression of pigment by Cd might be either due to inhibition in synthesis or due to acceleration in its degradation (Bhattacharya and Choudhuri 1994). 10^-5 M CdCl₂ treatment decreased the chlorophyll and carotenoids in 7 day old *Vigna catjung* seedlings (Bhattacharyya et al., 1995). Cd lowered the chlorophyll and carotenoids in maize leaves (Prasad 1995). 5 μM Cd decreased the Chl a/b ratio in sunflower (Gadallah, 1995). Cd decreased the chlorophyll content in runnerbean plants (Skorzynska and Bazynski 1997). 1.7μM Cd in the nutrient solution significantly decreased the concentration of chlorophyll in the 4th leaf of young maize plants (Lagrieffoul et al., 1998). Cd treatment did not affect chlorophyll ‘a’ significantly, where as chlorophyll 'b' and total chlorophyll were affected at higher concentrations in leaf of *Solanum melongena* plants (Mehindirata et al., 1999).

The rate of photosynthesis, chlorophyll content, activities of photosystem I and II and photosynthetic enzymes in pea seedlings were decreasing after 6 and 12 days of Cd treatment. On extending the period of exposure to 12 days, the rate of photosynthesis and activities of enzymes showed a further decline (Chugh and Sawhney, 1999). Oncel et al., (2000) reported that Cd
reduced the chlorophyll 'a' and chlorophyll 'b' in wheat seedlings. 0, 0.05, 2 or 5 μM Cd decreased the vitality index and photosynthesis in sunflower plants (Pankovi et al., 2000). Cd stress reduced the chlorophyll content in leaves of barley seedlings (Hegedus et al., 2001). Graded concentrations (5, 10, 15, 25 and 50 (μg/g soil) of CdCl₂ decreased chlorophyll ‘a’, chlorophyll ‘b’, carotenoids in the leaf of Cajanus cajan. The significant results were found in early stages of plant development, photosynthetic rate was also lowered in Cd treated leaves (Khudsar et al., 2001). Baryla et al., (2001) reported that in Brassica napus at low concentration (5 μM) Cd in soil reduced growth, chlorophyll content, the photochemical quantum yield of photosynthesis and led to stomatal closure. CdCl₂ decreased the chlorophyll content of pea leaves. 50μM CdCl₂ reduced the chloroplast structure (Sandalio et al., 2001).

Cd decreased the chlorophyll content and was concomitant with decrease in the activities of the Fe-enzymes-catalase and peroxidase, suggesting reduced availability of Fe for chlorophyll-heme biosynthesis in cabbage Cd decreased chlorophyll content (Pandey and Sharma, 2002). Cd decreased the chlorophyll content in Lemna (Panda and Khan, 2002). 500 μM Co and Cd exposure in sand culture decreased the chlorophyll content in cabbage. The metal effect was in the range of Cd>Co (Pandey and Sharma 2002). Cd decreased the chlorophyll content in pot grown sunflower plants (Chandragupta and Gopalsingh 2003). The decrease in Chl/Car ratio was observed in bean exposed to Cu (Maksymiec and Baszynski, 1996). Ceratophyllum demersum L. was exposed to 2.5, 5.0, 7.5 and 10.0 μM Cd for eight days. Chl a, chl b, total chl and carotenoid contents decreased with increased Cd treatment after 6 and 8 days. Hormosis (increased pigment content over the control) was noticed for 5μM and 2.5 μM treatments for 2 and 4 days, thereafter decreased in further concentrations. Chl a/b ratio
increased over the control at 2.5 and up to 5 μM after 2 and 4 days of treatment respectively and decreased in further concentrations. An increase in chl/car ratio over their corresponding control was found up to 7.5 μM at all treatments durations. The differential effect of treatment concentration and duration for different pigments showed significant differences only between concentrations, whereas no significant difference was observed between treatment durations by two-way ANOVA (Kumar and Prasad, 2004).

The chlorophyll content was decreased in the leaves of soybean treated with Cd (Drazic and Mihailovic 2005). Exposure of pea seedlings to Cd (0.01, 0.1 and 1 mM) for 2h reduced the efficiency of photosynthesis (Balakhnina et al., 2005). Cd lowered the chlorophyll content in *Colocassia esculentum* (Patel et al., 2005).

Mukherji and Nag (1977) and Nag et al., (1981) reported that Hg treatment depressed chlorophyll development and Hill reaction in the leaves of rice seedlings with a concomitant increase in the activities of chlorophyllase and few oxidizing enzymes. Hg, one of the heavy metal toxicants is discharged into the environment from the release of industrial wastes and has been reported to inhibit several vital physiological processes, including chlorophyll biosynthesis (Prasad and Prasad 1987a, 1987b, Puranik et al., 1990). Decrease in chlorophyll level may be due to higher activity of chlorophyllase as reported by De et al., (1985), Keshan and Mukheiji (1992) and Keshan et al., (1993).

The inhibition of chlorophyll biosynthesis by Hg can have a negative effect on the productivity of plants growing in Hg polluted sites. Changes in the pigment content of mulberry plants exposed to solid waste of chlor-alkali industry was reported (Mohapatra et al., 1990). Drastic but significant
decline in pigment contents in exposed plants was observed at higher concentration of the solid waste and prolonged exposure periods also caused similar effect. Under amended soil conditions ($10^{-4}$ Hg acetate) chlorophyll ‘a’ and chlorophyll ‘b’ content in *Vigna radiata* showed significant inhibition up to 60% and 53% of control respectively (Varshney 1990).

The aquatic plants, *Pistia* and *Hydrilla* were exposed to 500 mg/l of the solid waste of a chlor-alkali industry in an aquarium under controlled conditions. Drastic depletion in chlorophyll content was marked in the exposed plant leaves when compared to control leaves. A positive and significant increase in residual Hg level was observed in the exposed plants with an increase in exposure period (Patra and Panigrahi, 1994).

Thomas and Singh (1995) reported that Hg treatment to cucumber inhibited chlorophyll accumulation in a concentration dependant manner, the inhibition at the higher concentration being severe. The inhibition of carotenoid accumulation by Hg was even more severe than that of chlorophyll. 1, 5 and 10 mg HgCl$_2$/l decreased the chlorophyll ‘a’ and ‘b’ in *Albizia lebbek*. Inhibitory effects were gradually increasing with increasing concentration (Tripathi and Tripathi, 1999). The chlorophyll content in the leaves of tomato seedlings was lowered by Hg (Cho and Park 2000).

Pb and Cd (10$\mu$M) inhibited the growth of cucumber plants, chlorophyll content of Cd treated plants was very low. Cd caused more than 50 % inhibition of the photosynthetic activity (Fodor et al., 1996). Chlorophyll ‘a’, chlorophyll ‘b’, carotenoids and xanthophylls in fenugreek were decreased with the increasing concentration of Pb, Cd, and Hg (Madhavi and Charyulu, 1998). Excess Cu and Cd inhibited growth and pigment accumulation and
also decreased the efficiency of some photosynthetic processes in *Arabidopsis* plants. (Maksymiec and Krupa, 2002).

Irrigation with higher concentration of Cu and Cd decreased the total chlorophyll content in leaves of *Typha* plants. The ratio of chlorophyll ‘a’ to chlorophyll ‘b’ was also decreased and it may be due to chlorophyll hydrolysis in metal treated plants (Manios *et al.*, 2003). Photosynthetic pigment may be considered as an indicator of heavy metal stress in the *Avicennia marina* (Macfarlane and Burchett, 2001).

The role of carotenoid is 2-fold, (1) they help in photosynthesis (2) they protect light harvest pigments against photochemical damage caused by reactive oxygen species (Woodall *et al.*, 1997). Krinsky (1989) described the antioxidative function of carotenoid. Cd and Zn decreased the chlorophyll content in *Hydrilla*, decrease was correlated with concentration of heavy metals. Cd was more effective than Zn (Arunachalam *et al.*, 1996).

**Heavy Metal And Plant Metabolism**

Phytotoxicity is a consequence of the interference of metals with metabolic processes in the plants (Van Assche *et al.*, 1980). The toxicity of heavy metals is mainly attributed to their ability of binding enzymes, resulting in the alteration of their catalytic functions and inactivation (Van Assche and Clijsters, 1990). Heavy metals caused several toxic effects on plants such as metabolic disturbances by altering essential biochemical reactions (Krupa *et al.*, 1993). Several studies have been conducted on the effect of heavy metals on plant growth and metabolism (Dubey and Passarakli 1995, Dubey 1997). At supraoptimal concentration heavy metal inhibits various metabolic processes in plants, thus retarding their growth and development (Iqbal and Khudsar 2000). The activation or modification of plant metabolism and
induction of enzyme in heavy metal treated plants are considered as general biochemical stress defense responses. Due to such changes the adequate functioning of metabolic pathways and rapid repair of damaged structures are possible (Verkleij and Schat 1990, Prasad 1999, Sanita di Toppi and Gabrielli 1999, Hall 2002, Cho et al., 2003). Murthy and Rajagopal (2001) discussed the alteration in enzyme activities of plants under heavy metal ion stress.

**Carbohydrate Metabolism**

One of the major biochemical lesions arising from the action of toxic compounds is the disturbance of carbohydrate metabolism. Since carbohydrate catabolism provides energy for other metabolic processes, deviations from the normal metabolic pattern might be expected to result in impairment of respiratory chain reactions (Jerneiov et al., 1978). Ernst et al., (1990) reported that excess Cu interferes with cellular metabolism and altered several physiological processes by inhibiting certain enzymes activities in *Silene vulgaris*. Heavy metal decreased the sugar content in agricultural crops (Hemalatha et al., 1997).

Maize var. Ganga safed-2 seedlings were raised in pots using sterilized sand and 50, 100, 150, 200, 250 and 300mg CuCl₂/Kg sand. The uppermost leaf of 7 days old seedlings was analysed for amylases, invertase, total and reducing sugars. Both the enzymatic activities were lowered. Sugars were accumulated under higher concentration of CuCl₂ (Vyas and Nagoor 1993). Nonreducing sugars were decreased while reducing sugars were increased in leaf of safflower grown in Cu contaminated soil (Pandey and Sharma 1999). The reducing sugar and nonreducing sugar in leaves and seeds of bean plants was lowered by excess Cu as a soil pollutant (Khurana and Chatterjee 2001). 22 days old *Cucumis sativus* seedlings were treated with 10 ug Cu for 5
days, sucrose and starch content rose in young and mature leaves (Vinit-Dunand et al., 2002).

When sugarbeet was cultivated in the presence of Cd$^{2+}$, the growth of the plant was retarded due to a decreased carbohydrate formation following an inhibited photosynthesis and CO$_2$ assimilation (Greger and Bertell 1992). Carbon metabolism in leaves of Cd treated wheat seedlings was inhibited (Malik et al., 1992a). Maize plants were raised under sewage water. The soil was treated with 5, 10, 25, and 50mg CdCl$_2$/Kg soil. Sugars (total and reducing) were estimated from 40 days old plants. Reducing, nonreducing and total sugar content of maize decreased with increasing level of Cd and Zn. Mean total sugars decreased from 13.86 to 5.89 percent from 0 to 50mg Cd Kg$^{-1}$. The total sugars decreased from 10.48 to 8.64 percent. Similarly reducing sugars decreased from 1.31 to 0.74 percent with Cd. A sharp reduction in sugar content occurred at 25mg Cd kg$^{-1}$ soil which coincides with reduction in yield (Narwal and Singh 1993).

The reducing sugar in *Salvinia* was decreasing with increasing Cd concentration, lower doses of Cd increased total sugar and nonreducing sugar but higher doses decreased it (Kumari and Mishra, 1995). Sugars were accumulated in Cd treated wheat cultivars Kalyansona and C-306. accumulation was more in Kalyansona (Yadav and Yadav 1995). Total soluble sugars and starch contents of soybean plants reduced with increasing Cd concentration in different media (Ghorbanii et al., 1999). Bhattacharyya and Choudhuri (1994) studied the effects of Pb and Cd on soluble, insoluble carbohydrates, total carbohydrates and amylase activity in leaves of *Vigna* and *Hydrilla* plants in solution culture for six days under normal conditions. Insoluble and total carbohydrates declined in all the cases, the soluble carbohydrate declined in *Vigna* but increased in *Hydrilla*. The amylase
activity was more in metal treated plants, though increase was more in *Hydrilla* than in *Vigna*. Low doses of Cd (2.5 mM) did not affect soluble sugar in the seeds of two pea parents but induced a significant increase at higher concentration; starch content was decreased (Dewan and Dhingra, 2004).

In embryo of mungbean, total sugar and reducing sugar were greatly reduced by HgCl₂. In cotyledon total sugars were progressively increased under increasing Hg levels (Nag *et al.*, 1989). Carbohydrate level increased in one day old maize seedlings soaked in Pb and Hg but decreased in 5 and 7 days old seedlings (Kalimuthu and Sivasubramanian, 1990). A gradual increase in HgCl₂ concentration gradually decreased the soluble sugar in *Albizia lebbek* (Tripathi and Tripathi, 1999). Nagoor and Vyas (1997) reported the changes in α-amylase and β-amylase, invertase activities and reducing and nonreducing sugar contents in the embryo and endosperm of wheat seedlings grown with and without CuCl₂, CdCl₂ and HgCl₂. Heavy metal altered the carbohydrate metabolism in wheat seedlings. All heavy metals lowered the invertase activity in embryo but stimulated it in the endosperm. The content of reducing sugar and nonreducing sugar were more in the embryo and endosperm of heavy metal treated wheat seedlings.

**Protein Metabolism**

Free proline accumulation has been observed in response to a wide range of abiotic and biotic stress in plants. It is considered to be one of the first metabolic responses to stress, in many cases acts as an osmolyte (Hare and Cress 1997).

Higher concentration of Cu decreased the protein in the leaf of safflower grown on Cu contaminated soil (Pandey and Sharma 1999). Nagoor and Vyas (1999) reported the effects of graded doses of CuCl$_2$ on protein metabolism in wheat seedlings. Protease activity in embryo was enhanced by CuCl$_2$, in endosperm significant increase was found upto 72h of germination, and then the activity was decreased. Protein content was higher in CuCl$_2$ treated embryo, lower in endosperm. Lower concentration of CuCl$_2$ reduced the amino acid content in embryo axis, whereas higher concentration enhanced it. Accumulation of amino acids also occurred in metal treated endosperm. Khurana and Chatterjee (2001) reported that excess Cu in the refined sand decreased the seed protein content of bean.

Barley was grown in cobalt-amended soils. Total soluble proteins increased with increasing concentration of Co and a significant positive correlation was found to exist between metal content of the soil and that of different plant parts (Aery and Jagetiya 1998).

Cd increased the soluble protein in soybean leaf (Lee et al., 1976). Hirt et al., (1989) observed an increased protein synthesis with Cd concentrations 100-150µM in growth medium. The soluble protein content was increased in the leaf of 7 days old barley seedlings treated with CdCl$_2$ (0-50ppm). The increase was proportionate to the concentration of the applied metal. The rise in the soluble protein content can originate from the particulate fraction or from de novo synthesis (Bhattacharya 1991). The protein content was
decreased in 14 days old maize seedlings treated with 10, 100 and 250μM Cd nitrate (Ferretti et al., 1993). Soluble protein in leaf of Cd treated *Brassica juncea* seedlings was slightly increased, where the concentrations of Cd were 0.1 and 2mM respectively (Mishra et al., 1994). Crude enzyme extract was prepared from water-imbibed seeds of *Cicer* and *Triticum*. When such extract was subjected to heavy metals i.e. Cd, Pb, Hg, Ni, and Zn, protease activity was inhibited (Kumar and Banerji 1992). The protease activity was more in the leaf of Cd and Pb treated *Vigna* and *Hydrilla*. The protein content was decreased while free amino acid content was increased. The decline in protein with corresponding rise in protease activity due to heavy metal stress suggests the promotion of catabolic activities. Thus imposition of heavy metal stress may induce early senescence (Bhattacharyya and Choudhuri 1994). Free amino acids were decreased in wheat var Kalyansona treated with Cd but unchanged in var C-306. Soluble protein/seedling was decreased in both the varieties but a greater extent in Kalyansona (Yadav and Yadav 1995).

Protein and free amino acids were increased significantly in Cd treated 4C days old maize plants (Narwal and Singh 1993). Heavy metals reduced soluble protein in agricultural crops (Hemalatha et al., 1997). Protein metabolism in term of protease activity, protein content, amino acid content and proline content were affected in embryo and cotyledon of cowpea var. Pusa Falgunti seedlings grown in CdCl₂ and HgCl₂ (Nagoor 1997). Brune and Dietz (1995) reported that heavy metal toxicity caused an increase in protein content of leaves and roots, the effect being most pronounced in the presence of Cd. Protein synthesis can be disturbed at many levels by a variety of mechanisms either by affecting the nucleic acid metabolism or structure, or in the protein forming-system itself. Toxic agents act directly or:
ribosomes, RNA, enzymes or co-enzymes may also have a drastic influence on protein synthesis.

Seedlings of two rice var i.e. Ratna and Jaya were treated with 100 μM or 500 μM Cd(NO₃)₂ in pots. Seedlings were uprooted at 5 days intervals up to 20 days of growth. The protein, amino acids and protease activity were determined in seedlings. With 500 μM CdNO₃, the protein level increased by 1.7 to 3.0 times more in roots and 0.23 to 1.8 times higher in shoots at 20 days of growth. Concentration of amino acids was also higher in roots and shoots. More accumulation was found in shoot. Cd treatment significantly decreased protease activity in root and shoot. The higher amount of protein in Cd treated seedlings may be due to synthesis of protein under Cd stress (Shah and Dubey 1997b). Brune et al., (1995) reported that heavy metal toxicity caused an increase in protein content in leaves and roots.

Cd decreased soluble protein content in leaves of sugarbeet plants treated with 10⁻⁴, 10⁻² and 1mM CdCl₂ (Kevresan et al., 1998). The accumulation of amino acids under stress condition may help in stabilization of enzymes (Jolivet et al., 1982) and also protect the cellular constituents against free-radical induced damage (Smirnoff and Cumbes, 1989). Accumulation of amino acids may be due to their increased synthesis under stressful conditions (Chen and Kao 1995). The increase of free amino acids is involved in defense mechanism and tolerance adaptation against heavy metals (Vanaja et al., 2000). Vediya and Vyas (2001) reported that 10-50 ppm CdCl₂ as a soil pollutant decreased the protein content, protease activity and invertase activity in cumin leaves. The graded concentrations of Cd gradually lowered the total soluble protein content in root tip of barley seedlings (Liu et al., 2005). Proline was accumulated in root and leaf of wheat seedlings treated with CdCl₂. Accumulation of free proline was more
at higher Cd concentration. Proline plays a role in water balance maintenance. It may give protection against hydroxyl radicals produced in Cd treated seedlings (Panda 2001). Cd increased the concentration of proline in the leaves of barley seedlings (Guo et al., 2004). Proline concentration increased significantly in nodules and roots of soybean plants treated with moderate i.e. 50µM and high 200µM doses of Cd, 200µM Cd was more effective than 50µM (Balestrasse et al., 2005).

Lower dose of Hg stimulated protease activity in *Pisum sativum* (Sharma 1983). Maize seeds were soaked in Pb and Hg for 24h in different concentrations i.e. 20, 50, 100 and 200µg/ml and germinated in paper towel at room temperature. The amounts of protein, free amino acids, carbohydrates and RNA were measured on 1st, 5th, and 7th day of germination. Protein content decreased in all seedlings at all the employed concentrations of Pb and Hg. In 5 day old seedlings, Hg slightly increased the amino acid level at all concentrations (Kalimuthu and Sivasubramanian 1990).

Nagoor and Vyas (1998) studied the effects of Cd and Hg (50 to 300µg/ml) on protein metabolism in wheat seedlings. Cd and Hg stimulated protease activity in embryo upto 72h of germination then it was suppressed. Hg was more effective, in endosperm both metals stimulated the activity upto 48h then it was decreased. Protein content was more in embryo treated with lower concentration. Higher concentrations of Cd and all the concentrations of Hg decreased the protein content. In endosperm protein content was less. Total amino acid and proline were significantly accumulated in endosperm. Accumulation of proline in endosperm can be considered as biochemical marker for heavy metal stress. A concentration dependant decrease in soluble
protein content from HgCl₂ treated Albizia lebbek was reported (Tripathi and Tripathi, 1999).

The effects of Hg, Cd and Ni (1µg/ml) on nodulation and nitrogen fixation in Cicer arietinum inoculated with rhizobium were studied. The metals significantly reduced protein, free amino acid, and total nitrogen contents, RNA and DNA content, total hexose, and specific activity of acid phosphatase and alkaline phosphatase. Addition of metals severely reduced the plant yield (Pal 1996). Cd treatment of pepper plants increased the synthesis of two 10-kDa proteins, which differ in their amino acid composition and are absent in untreated plants. The function and role of these two proteins is still unknown, but they might also be involved in defense mechanisms against heavy metals (Jemal et al., 1998).

Maize seeds were germinated in DW and 50-300µg CuCl₂/CdCl₂ or HgCl₂/ml Protease activity, protein content, amino acids and proline were analysed in the embryo and endosperm at 48, 72, 96 and 120h of germination. Lower concentrations of Cu generally stimulated protease activity. Cd and Hg generally decreased the protease activity in embryo and endosperm. Total amino acids and proline accumulation were generally increased by heavy metals (Nagoor 1999).

Tamas et al., (1997) reported that stress proteins were accumulated in intracellular spaces of barley leaves subjected to heavy metals. Quariti et al., (1997) reported that Cd and Cu induced changes in membrane function via membrane proteins in tomato. 10 days old mustard seedlings of 10 genotypes were treated with different levels of CdCl₂ (0.0, 0.5, 1.0, 1.5 and 2.0mM) in nutrient solution. The protease activity, protein content and amino acid pool were studied after 24, 48 and 72 h of treatment. The protease activity was
enhanced in all genotypes. The enhancement was dose and time dependent. The similar effect was found for amino acid pool. Soluble protein was increased at 24 and 48 h of treatment but declined after 72 h treatment. There was a positive relation between protease activity and soluble amino acid pool. The patterns of changes were similar for all the varieties but the intensity of effects were different with different varieties. Thus genotype differences with reference to leaf senescence and leaf protein metabolism in *Brassica juncea* genotypes were observed (Qadir et al., 2003).

Cu and Cd decreased the protein content in the wheat grown on heavy metal contaminated soil. The decrease was related with concentration of heavy metal in the soil (Athar and Ahmad 2002). Proline was accumulated in the leaves of cabbage exposed to 500 μM Co and Cd (Pandey and Sharma 2002). Shaw and Rout (2002) studied the HgCl₂ and CdCl₂ induced changes in proline content of seedlings of *Phaseolus aureus* and *Triticum aestivum*. Hg was more effective than Cd.

Plants exposed to heavy metals accumulate an array of metabolites, some to high millimolar concentrations. This review deals with N-containing metabolites frequently preferentially synthesized under heavy metal stress such as Cd, Cu, Ni and Zn. Special focus is given to proline, but certain other amino acids and oligopeptides, as well as betaine, polyamines, and nicotianamine are also addressed. Particularly for proline a large body of data suggests significant beneficial functions under metal stress. In general, the molecules have three major functions, namely metal binding antioxidant, defense and signaling (Shanti and Dietz, 2006).

**Antioxidative Enzymes And IAA Oxidase Activity**

The antioxidative enzymes are important components in preventing the
oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes is generally increased in plants when exposed to stressful conditions (Alien, 1995). Agarwala et al., (1961) reported the suppression of catalase and peroxidase activities in barley seedlings exposed to heavy metals. Agarwala and Kumar (1962) reported similar results for sunflower plants in sand culture experiment. Mukherji and Dasgupta (1972) found increased activities of catalase, peroxidase and IAA oxidase in lettuce seedlings treated with toxic concentrations of Cu. Coombes et al. (1976) reported that Cu stress increased IAA oxidase activity in barley. Excess Cu inhibits growth and metabolism due to many physiological constraints by inducing the synthesis of large number of enzymes like catalases and peroxidases (Lin and Wu 1990). Mechanism of growth inhibition and biomass loss due to increased peroxidase activity in plant tissues at excess Cu was recorded in Phaseolus vulgaris (Jolaunda et al., 1996). Its role in induction of an efficient defense mechanism and development of tolerance has been described in many other higher plants against Cu stress (Matasuda et al., 1994, Foyer et al., 1997).

Peroxidase activity significantly increased in leaf and root of maize seedlings grown in a nutrient solution containing various Cu concentrations. The increase was related with Cu content. Measurement of peroxidase activity might be used as a biomarker to assess the phytotoxicity of maize grown on copper-contaminated substrata (Mocquot et al., 1996). Cu increased the peroxidation in wheat (Karataglis et al., 1991), in tomato (Mazhoudi et al., 1997). Peroxidase plays an important role in reducing oxidative stress by catalyzing the reduction of H₂O₂ (Weckx and Clijsters 1996). Luna et al., (1994) reported that excess Cu caused oxidative damage in oat leaves. Cu toxicity activates superoxide dismutase and ascorbate peroxidase in sunflower and bean seedlings (Cuypers et al., 1999, Garcia et al., 1999).
Devi and Prasad (1998) reported the Cu toxicity in Ceratophyllum with the help of antioxidant enzyme and antioxidant. Increase in Cu from 0.001 to 2μM Cu increased the polyphenol oxidase activity in safflower (Pandey and Sharma 1999). Nautiyal et al., (1999) reported that excess Cu decreased the polyphenol oxidase activity in rice. Cu increased the peroxidase activity in rice root. H₂O₂ was also increased. Exogenous application of H₂O₂ inhibited root growth. The root growth inhibition caused by copper was associated with H₂O₂ dependent peroxidase-catalyzed formation of cross-linking among cell wall polymers (Chen et al., 2000).

Excess Cu enhanced the peroxidase activity in both root and shoot of Vicia faba (Roy et al., 2001). The root and shoot growth was inhibited. The inhibitory effect on biomass was related with higher peroxidase. The peroxidase reduced the extensibility and lignification of cell wall. It suggests a peroxidase regulatory mechanism of plant growth in Vicia faba. Excess Cu reduced the activity of polyphenol oxidase and enhanced that of peroxidase and acid phosphatase with accumulation of phenols in bean (Khurana and Chatterjee 2001). According to Macfarlane and Burchett (2001) peroxidase activity may be considered as indicator of heavy metal stress in Avicennia marina. Peroxidase activity and polyphenol oxidase activity were stimulated in spinach cv. Banarasi plants treated with Cu (100μM) in sand culture experiment and it might result in peroxidative damage of the thylakoïc membranes or lowered auxin content in tissues inhibiting the growth of plants (Nautiyal and Chatterjee 2002).

Demirevska-Kepova et al., (2004) reported the biochemical changes in barley plant after excessive supply of Cu. Excess Cu affected non-protein SH groups. Oxidative stress under Cu toxicity was most probably the
consequences of depletion in low-molecular antioxidants as a result of their involvement in detoxification processes and disbalance in antioxidative enzymes. Excess Cu reduced the catalase, acid phosphatase and polyphenol oxidase activities but increased the activity of peoxidase in leaves of rice varieties Pusa Basmati and Pusa Sharbati (Dube et al., 2005).

The presence of excess Cu can cause oxidative stress in plants and subsequently increase the antioxidant responses due to increased production of highly toxic oxygen free radicals. Excess Cu in plants led to oxidative stress inducing changes in the activity and some components of the antioxidative pathways (Luna et al., 1994, Gupta et al., 1999, Drazkiewicz et al., 2003, Wang et al., 2004). Gaetke and Chow (2003) discussed the Cu toxicity, oxidative stress and antioxidant nutrients.

Tewari et al., (2002) reported that excess Co caused a marked increase in the activities of anti-oxidative enzymes in Phaseolus aureus. Peroxidase activity was increased. The increase in concentration of Co decreased the H₂O₂ and it was due to increased activity of antioxidative enzymes viz, peroxidase and ascorbate peroxidase. The Co toxicity was correlated with generation of ROS at higher i.e.200-400 μM Co supplied to Phaseolus aureus.

Cd has been shown to affect various aspects of metabolism in different plant systems (Chen and Kao 1995, Shah and Dubey 1997b). Peroxidase activity was increased in seedlings of wheat var Kalyansona and C-306 treated with Cd. Effect was more in Kalyansona (Yadav and Yadav 1995). Cd stimulated free radical production by imposing oxidative stress (Foyer et al., 1997, Schiekler and Caspi 1999). Oxidative damage suggests a correlation between metal treatment and oxidative stress. Treatment of Lemma with graded concentrations of CdCl₂ increased the total peroxides content. Membrane
damage was also observed in Cd treated plant. Plant exposure to heavy metal stress results in the production of toxic oxygen species such as O·, OH, RO, H₂O₂ (Asada, 1994, Weckex and Clijsters, 1996, Alia et al., 1997, Behera et al., 1999). Increase in H₂O₂ might result in to hydroxyl radical formation and it may cause lipid peroxidation. Lipid peroxidation will result in loss of membrane integrity (Luan et al., 1994, Gallego et al., 1996, Chaoui et al., 1997, Behera et al., 1999, Prasad et al., 1999).

IAA metabolism was down regulated by toxic metal (Mishra et al., 1994). IAA oxidase activity in mustard (Brassica juncea L. cv. RH-30) was increased with increase in Cd concentration from 0.1 to 2.0 mM. The enzyme activity increased two fold at high concentration (2 mM), the auxin imbalance is inevitable under stress condition due to manifold increase in IAA oxidase activity in the leaf (Singh et al., 2000). The reduction in growth of metal treated plant was due to the loss in antioxidant capacity and auxin content (Chaoui and El Ferjani 2005). Gallego et al., (1996) reported that Cd decreased the activity of superoxide dismutase, catalase and ascorbate peroxidase in sunflower plants. Lagriffoul et al., (1998) reported that lower concentration of Cd increased the peroxidase activity in the leaf but not in root of maize. This concentration does not cause any appreciable effect on the growth parameters; the measurement of peroxidase activity may be included as early biomarkers in a plant bioassay to assess the phytotoxicity of Cd contaminated soils on biomass.

Cd enhanced the catalase, SOD, ascorbate peroxidase in leaves and roots but variations were found in the intensity of the effects (Dixit et al., 2001). CdCl₂ decreased the catalase, SOD and guaiicol peroxidase activity in pea leaves. There was a concentration dependant situation in leaves, characterized by an accumulation of lipid peroxides and oxidized proteins as
a result of the inhibition of the antioxidant systems which suggests that Cd may induce leaf senescence (Sandalio et al., 2001). Hegedus et al., (2001) reported the effect of Cd stress on antioxidant defense system such as guiaicol peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) in green and greening barley seedlings. Most of the Cd accumulated in the root but 1.5- 3% of Cd was translocated to the leaves and it induced oxidative damage, which was indicated by the reduced chlorophyll and increased malondialdehyde of the leaves. Ascorbate peroxidase activity was enhanced but guiacol peroxidase activity was not affected in root. While in leaves guiacol peroxidase activity and ascorbate peroxidase were increased. They suggested that a well-defined activity of the enzymetic antioxidant system, operates differentially in roots and shoots subjected to Cd stress.

Iannelli et al., (2002) reported that Cd increased the SOD, ascorbate peroxidase, glutathion reductase and catalase in leaves and roots of *Phragmites*. Increased activities of antioxidative enzymes in Cd treated plants, suggests that metal tolerance in this plant might be associated with efficiency of this mechanism. Peroxidase activity was decreased in the leaves of cabbage plants exposed to 500 μM Co and Cd (Pandey and Sharma 2002). 0.5 mM Cd increased guaicol peroxidase in most of the pepper cultivars but decreased the catalase and SOD (Leon et al., 2002). Four barley genotypes were subjected to 1 and 5μM Cd in green house. SOD, peroxidase and catalase were stimulated. Peroxidase activity was higher in tolerant variety. Cd-stress induced a concentration and genotype dependent oxidative stress response in barley leaves. Mainly SOD and peroxidase may be attributed to the genotypic difference in Cd tolerance i.e. higher activity indicates Cd tolerance, higher the activity more the tolerance (Wu et al., 2003). Cd altered the catalase, ascorbate, peroxidase and guiacol peroxidase in leaves of wheat (Milone et al., 2003). Romero-Puertas et al., (2004)
reported the accumulation of H$_2$O$_2$ in Cd treated pea leaves. The Cd induced production of reactive oxygen species; could be attributed to the phytotoxic effect of Cd. Peroxidase activity was higher in Cd treated barley seedlings (Guo et al., 2004).

Cd treatment increased the peroxidase activity in 10 genotypes of pea. Cd sensitivity to genotypes was correlated with relative increase in peroxidase activity. Cd was found in root and leaf but amount was different and this difference has no correlation with Cd tolerance and peroxidase activity in Cd treated plants (Mettally et al., 2005). The activity of peroxidase in leaves of pea increased even at low concentration (0.01 mM) of Cd. The stimulation of the activity of antioxidant enzymes is a pathway for pea plant adaptation to toxic effect of Cd (Balakhnina et al., 2005). Cd increased the antioxidant enzyme, catalase and peroxidase in Colocassia esculentum (Patel et al., 2005).


Peroxidase activity was higher in Hg treated Phaseolus seedlings. In plants, peroxidase protect cell against harmful concentrations of H$_2$O$_2$ (Castillo et al., 1992). Hg increased the peroxidase activity but decreased the SOD and catalase in 15d old seedlings of rice var Ratna and IR 36, the Ratna var was
more susceptible than IR 36 to Hg (Mishra and Choudhuri 1996). Polyphenol was significantly increased in leaves of *Albizia lebbek* grown with HgCl₂ contaminated soil (Tripathi and Tripathi 1999).

Excess Hg increased the H₂O₂ content and antioxidant enzyme activities such as SOD, catalase and peroxidase in 30 days old tomato seedlings. The phytotoxic effects of Hg in tomato seedlings may be achieved by an enhanced production of active oxygen species (AOS) and subsequent lipid peroxidation (Cho and Park 2000). Peroxidase and IAA oxidase were enhanced in Hg treated *Phaseolus* seedlings. Hg inhibited growth. There was an inverse relationship between growth and IAA and peroxidase activity (Parmar and Chanda 2004). IAA oxidase plays a role in the regulation of cellular IAA (Parmar and Chanda 2004).

*Phaseolus aureus* was exposed to HgCl₂ and Cd(NO₃)₂ either at the germination stage in concentration 0.5, 5 and 25 mM for 48 and 96 h, or at the seedling stage (5th day of germination) in concentration 0.5, 5 and 20 mM for 6, 24 and 48 h. Both root and leaf tissues of the plants treated at the germination stage showed enhanced lipid peroxidation and activities of the antioxidative enzymes (catalase, guaiacol peroxidase and ascorbate peroxidase), except the catalase in leaf in 25 mM Cd treatment (Shaw 1995). Determination of peroxidase activity was considered as one of the damage criteria in heavy metal treated plants (Gritsan et al., 1995). Blinda et al., (1997) reported that there was an enhancement of peroxidase activity in apoplastic extracts of barley shoots under heavy metal stress. Lycholat and Bilchuk (1998) reported the changes in peroxidase activity, polyphenol oxidase activity and catalase activity in *Festuca rubra* available in Cd and Cu polluted area. Higher amount of Cd increased the peroxidase activity but Cu decreased it, Cu increased the polyphenol oxidase activity. The revealed
changes of enzyme activity catalyzing oxidation-reduction are the compensatory mechanism improving the resistance of plant organism to negative action of metals. The peroxidase activity in *Elymus repens* available in Cu, Zn, Pb, Cd and Ni contaminated area was more than control. Catalase activity was also markedly higher, but only in the most contaminated populations (Brej 1998). Peroxidase activity was inhibited when crude enzyme extracts of water imbibed seeds of *Cicer* and *Triticum* were subjected to Cd, Hg, Pb, Ni, and Zn (Kumar and Banerji 1992). Peroxidase regulates the conversion of ACC to ETH (Gasper et al., 1991) and peroxidase protects the plant cell against oxidative damage (Mehlhorn 1990).

Cd(NO₃)₂ or CuSO₄ metals increased lipoperoxidation in the leaves. 20µM did not affect the growth. Peroxidases used as a stress marker. No change in the antioxidant capacities was observed in plants treated with 20 µM of Cu. But at this dose, Cd reduced growth could be associated to elevation in the activities of IAA oxidase and lignifying peroxides. 100 µM Cu also increased peroxidase activity (Chaoui and El- Ferjani 2005).

Activity of various antioxidant enzymes is known to increase under stress (Sairam et al., 1997). A positive relationship between cell antioxidant activity and the levels of protein and pigments has been reported in various plant species (Leipner et al., 1999). Levels of antioxidant enzymes decide the level of oxidative stress experienced by cells/plants (Foyer 1993, Foyer et al., 1994). Lower level of antioxidant enzymes increased oxidative stress and these may cause lower levels of pigments. Low content of carotenoids is also associated with oxidative stress (Laxman and Srivastava 2000 a, 2000 b). Heavy metal stress accumulates H₂O₂. It acts as a signaling molecule and initiates secondary reactions such as lignification (Schutzendubel et al., 2001 and Schutzendubel and Polle 2002). Metal induced lignification may cause
cell wall strengthening and tissue ageing as evidenced by decrease of growth. Tahlil et al., (1999) reported the qualitative and quantitative peroxidase changes in Cucurbita pepo cultivars stressed with heavy metals. Results indicate that peroxidase activity could be used as a biomarker for Cd, Cu and Zn stress.

**Phenolic Substances**

Phenols were considered as one of the growth regulators in plants (Wain and Taylor 1965). Soluble and insoluble phenolics were accumulated in Phaseolus vulgaris plants exposed to Cd (Furher 1982). The synthesis and transformation of phenol compounds were increased in skoch pine seedlings grown on CuSO₄ contaminated soil (Fuksman et al., 1998). The phenol content in bean leaves was lowered by Cu and lowering was correlated with concentration (Khurana and Chatterjee 2001). The level of total soluble phenolic was significantly altered in seedlings of two wheat var grown with high Cd content i.e. 50 mg/l and above (Oncel et al., 2000). The influences of various Cd concentrations on metabolism of wheat seedlings were studied. Varying concentrations of Cd altered the levels of major biochemical constituents such as the protein, free amino acids, starch and soluble sugars. The growth was reduced. The Cd toxicity at structural and functional level was established (Shukla et al., 2003).

An investigation was conducted to study the differential action of heavy metal such as Ni, Cr and Hg on biochemical and chemical parameters of Albizia lebbek. Significant reduction (P< 0.001) in chlorophyll, protein, carbohydrate and sugar in leaves was observed which was positively and significantly correlated with leaf area, root-shoot length and biomass of plant. Polyphenol, proline, ascorbic acid and nitrate reductase activity of leaves were significantly (P< 0.001) increased over control and negatively
correlated with root-shoot length, leaf area and biomass of plant, showing stress on plants. These concentration dependent changes were observed in most of the parameters. Hence the physiological and biochemical traits may serve to determine suitable bio-indicators of heavy metal pollution and to select protection species (Tripathi and Tripathi, 1999).

200ppm (moderate) and 600ppm (severe) of CuCl$_2$, CdCl$_2$ and HgCl$_2$ lowered the invertase activity, protease activity, polyphenol oxidase activity in mustard and fenugreek seedlings. IAA oxidase activity was promoted. Heavy metal lowered the level of primary metabolites i.e. reducing sugar, nonreducing sugar, protein and total amino acid. The heavy metal effect was in the order of Hg>Cd>Cu. The biochemical changes were sharp in mustard than in fenugreek. Peroxidase was suggested as marker of heavy metal sensitivity of crop (Vyas and Trivedi, 2004). Bhandari and Vyas (2005) also reported that increase in concentration of Cd lowered the a-amylase, β-amylase activity, invertase activity, protease, polyphenol oxidase, peroxidase and IAA oxidase activities of *Cassia tora* seedlings. The similar results were found for reducing sugar, nonreducing sugar, protein, total amino acid, phenol content and proline. Phenol was accumulated in bean seeds (Khurana and Chatterjee 2001).

**Heavy Metal Uptake**

The distribution and deposition of heavy metals have been studied in different plant species (Greger, 1999). Wheeller and Power (1995) discussed the plant uptake and plant toxicity of various ions in wheat. The leaf tissue concentration of Cu increased with increase in Cu supply to pot grown safflower plant (Pandey and Sharma 1999). *Vicia faba* plants were grown with various levels of Cu. The accumulation of Cu increased with increasing concentration in nutrient medium and duration of treatment. The biomass
production was decreasing with increasing Cu concentration and growth period (Roy et al., 2001). In Cu excess situation, Cu ions are strongly absorbed and accumulated by plant roots, but not by top tissues (Kabata-Pendias and Pendias 1999, McBride 2001).

Jiang et al., (2004) reported the concentration dependent uptake of Cu at an early growth stage by the Elsholtzia splendens plant. Cu and Cd uptake by wheat grain was reported in pot culture experiment. The metal uptake was directly related to the applied heavy metal concentration in the soil (Ather and Ahmad 2002). Youn Joo (2005) reported that Cu was accumulated in Sorghum bicolor, Cucumis sativus, Triticum aestivum and Zea mays. Accumulation was concentration dependant. The amount of accumulation was different in different species. The differences in the toxicities of Cu in plant species should be taken into account in biomonitoring and ecological risk assessment.

Ategerai et al., (1992) studied the total and extractable Co in soil and the vegetables. Samples of soils and edible vegetable were taken from 15 different sites, corresponding to four agricultural areas exposed to different degrees of environmental pollution, high industrial and traffic, high industrial and urban and low industrial and urban pollution. Statistically significant correlations were observed between the total and extractable cobalt of soil and of reets and bulbs.

The presence of Co was noted in pea grown with Co. The uptake was increasing with increasing Co in the medium (Tewari et al., 2002). Cd is accumulated by many cereals, potatoes, vegetables and fruits and that human take up at least 70% of the Cd which originates from plant food (Wagner, 1993). Chizzola (1998) reported the presence of Cd in Chamomilla recutita,
*Hypericum perforatum* and sunflowers grown on Cd contaminated soil. The accumulation of Cd in all the plants was more than German guide value of 0.5 mg/kg. 4 week old pepper plants were treated with graded doses of CdCl$_2$ (1 and 150μM). After 7 days Cd uptake was studied. Cd was accumulated and accumulation was correlated with concentration. The accumulation was maximum in root but low in stem and leaf. The level of metal accumulation was similar in stem and leaves (Jemal *et al*., 1998). Sunflower plant was raised in plastic pots, 21 days old plants were treated with CdSO$_4$. Cd was accumulated in root, shoot, leaf and head, significant accumulation was found in roots (Simon, 1998). Shah *et al.* (2001) reported that the rice plants grown in Cd contaminated sand absorbed Cd and its concentration was increased in rice shoot with parallel increase in Cd concentration in the growth medium. Dixit *et al.* (2001) reported that exposure of pea plants to Cd resulted in the accumulation of Cd in root, stem and leaf. The accumulation was in order of root > shoot. Presence of Cd in hydroponic culture resulted into accumulation of Cd in root and shoot of white lupin. Accumulation was more in root than in shoot (Zomoza *et al*., 2002).

Cd was accumulated in the wheat seedlings treated with Cd (Shukla *et al*., 2003). Cd was accumulated in root and shoot of maize seedlings treated with graded concentrations of Cd. Accumulation was more in root than in shoot and accumulation was directly related with concentration of Cd in the media (Sepehr *et al*., 2003).

Lettuce was grown with CdCl$_2$ and phosphate fertilizers. The transfer of added Cd was higher for CdCl$_2$ than for fertilizers. Of the amount of the Cd added from the fertilizers an average of 1% or less was accumulated in the harvested lettuce tissue. Applications of the fertilizers at high rates could result in increased Cd accumulation in the soil over time (Huang *et al*., 2003).
2004). Cd was accumulated in the root and shoot of *Thlaspi caerulescens* cultivated hydroponically with 5 to 500µM Cd. Accumulation was more in root than in shoot. Phytochelatin accumulation was not detected in these plants growing in Cd contaminated environment or in a green house with Cd contamination. Thus naturally selected tolerance in *Thlaspi caerulescens* is not associated with phytochelatin synthesis (Wojcik *et al.*, 2004). Chan and Hale (2004) reported that durum wheat accumulates Cd from the soil. Accumulation depends upon cultivars. Moreover accumulation was also different at different stages. Cd accumulation was also reported in grain and it is a function of total shoot accumulation. Phosphate fertilizers were a source of Cd. *Colocassia esculentus* plant accumulated larger portion of the heavy metal in the roots followed by stem and leaf (Patel *et al.* 2005).

The concentration of Hg in the leaves of *Rosmarinus officinalis* growing at 7 sites on Mt. Amiata were compared with those of the soil and air, and control samples obtained from the Pisa area (low soil concentrations of Hg). There were no significant differences in the concentrations of Hg in the air at all sites, except at one on Mt. Amiata, which was close to a geothermal power plant at Piancastagnaio. No relationship was observed between the concentration of Hg in the air, and the concentration of Hg in the leaves. However, a significant relationship was observed between the concentration of Hg in the soil and the concentration of Hg in the leaves, indicating that the Hg accumulation rate increased with increasing metal concentration in the soil (Barghigiani and Ristori 1995).

Plants can contain heavy metals from their presence in the soil (including contamination of the plants material with soil), water or air (McLaughlin, 1999). High levels of toxic metals can occur in medicinal preparations when they are used as active ingredients, as in the case of Pb and Hg in some Chinese, Mexican and Indian medicines (Levitt, 1984; Chan *et al.*, 1993) or
Plant extracts for medicinal value have gained considerable importance in alternative medicine in recent times. A large number of Indian (Ayurvedic), Chinese, Tibetan and local traditional medicines containing plant extracts have an increasing demand all over the world. Although, slow in action compared to modern chemotherapeutic agents, these medicines are popular on account of long term effectiveness against many chronic disorders. They are also considered safe, relatively free from side effects and problems of overdose. However, there is an inherent health risk associated with many of these medicines, viz. presence of contaminating heavy metals. Presence of toxic metals in plant extracts can be attributed to (i) accumulation in plants through metal-contaminated soils, (II) contamination during processing in industries and (III) leaching of metal from metallic containers during storage (McGregor et al. 1996).

In a recent sample survey carried out in India, over three thousand samples of a variety of plant extracts were analyzed for their toxic metal content. It was observed that – 20% of the samples tested contained heavy metals such as lead, cadmium and chromium in concentrations far exceeding 1 mg kg⁻¹.

Zheljazkov et al., (1995) reported the occurrence of Cd, Pb, Zn, Mn and Cu in Artemisia maritima, Draccocephalum moldavica, Inula helenium, Ruta graveolens and Symphytum officinale grown on heavy metal contaminated soil. Different plant species accumulated different amount of heavy metal and heavy metal concentration in plant was correlated with concentration in the soil. The amount of Cd, Pb, Zn, Mn and Cu were above the critical concentrations for plants. Heavy metal toxic symptoms were not but yield was decreased by 12-21% compared with control. Ewais (1997) reported that Cd, Ni, Pb content was increased in potted wheat plants grown with heavy
metal contaminated soil. The heavy metals were accumulated in roots and shoots. Accumulation was more in roots than in shoots.

Barman et al., (1999) reported accumulation of Cd, Cu, Zn, Fe, Ni, Cr and Pb etc. in vegetables, pulse and wheat cultivated on the soil receiving fly ash from a thermal power plant. The accumulation of metal was varied from species to species and also within the different parts of the same plants. Sharma et al., (1999) reported the presence of heavy metals in agriculture crops irrigated with industrial wastewater. Irrigation with industrial effluents resulted into the accumulation of heavy metals in wheat, mustard and weed (Barman et al., 2000). Donisa et al. (2000) studied the heavy metal contamination of natural soils due to atmospheric transport in the northern part of Eastern Carpathians. The distribution of heavy metals (Pb, Cu, Zn, Mn, Ni, Co, Cr and Cd) was studied along the soil profile and at specific distances from the pollution sources. Seventeen medicinal species were analysed for heavy metals. The highest contents of Cu, Zn, Cd, and Pb among 17 medicinal plants tested in this study were 12.3, 47, 0.65 and 20.3ppm, respectively, which were not higher than the recommended dosage of acceptable daily intake by FAO/WHO (Liu et al., 2000).

Sharma et al., (2001) reported the presence of heavy metals like Zn, Cu, Pb and Cd in cereals and vegetables irrigated with polluted water. The most common distribution pattern of heavy metals in the plant parts was root>shoot>fruit. The other less frequent pattern was fruit>shoot>root. The order of heavy metals in the plant tissues of cereals and vegetables was Zn>Cu>Pb>Cd. Khan et al., (2001) reported the higher amount of Cu, Cd, and Hg in fenugreek collected near the textile industries. The metal uptake was directly related to the applied heavy metal concentration in the soil (Athar and Ahmad 2002).
Liu et al., (2003) reported the correlation between Cd and mineral nutrient in absorption and accumulation in various genotypes in rice under Cd stress. The absorption and accumulation of Cd, Fe, Zn, Mn, Cu and Mg in the roots and leaves of 20 rice cultivars under Cd were investigated with pot experiments. There were significant differences among the rice cultivars in the contents of six mineral elements in both roots and leaves at both heading and ripening periods. Caldas and Machado (2004) reported the content of Cd, Hg and Pb in medicinal herbs (Ginkgo biloba, Centella asiatic, Artichoke, Cascara buckthorn etc.) in Brazil by atomic absorption spectrophotometry. Cd was found in samples of the medicinal herbs at levels up to 0.74 µg/g. The regular use of Cd containing drugs may cause health problem. Study shows the need for a systematic control of toxic metals in plants used as medicines.

Yanqun et al., (2004) reported the accumulation of Pb, Cd, Cu and Zn in 26 samples of 17 plant species collected from lead-zinc mine in China. Different plants have different capacity of Cd and Cu accumulation. Kosma et al. (2004) reported the accumulation of Cd in seeds of Setaria glauca grown on Cd contaminated soil. Cd was found in the root, stem and leaf of Colocassia esculentum grown in Cd contaminated pots. The accumulation was in the order of root>stem>leaf (Patel et al., 2005).

The degree to which higher plants are able to take up Cd depends on its concentration in the soil and its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and concentrations of other elements. Despite of the mobility of metal ions in plants, the metal content is generally greater in roots than in the above ground tissues (Ramos et al., 2005).
The content of Cd in plants decreases in the order: roots > stems > leaves > fruits > seeds (Blum 1997).

Brassica juncea (L.), which has already been recognized as a plant suitable for metal phytoremediation, and of several other plant species (maize, rice and sugar beet) to extract cadmium (Cd) from soils with moderately low levels of Cd contamination. Two of the 56 cultivars of B. juncea were preliminarily screened as high-Cd accumulators using a hydroponic culture solution containing a high level of external Cd (1 mg L\(^{-1}\)). Thereafter, 7 cultivars within 4 plant species (maize, B. juncea [2 cultivars], rice [3 cultivars with different subspecies] and sugar beet) were grown in a hydroponic culture solution containing a low Cd level (0.05 mg Cd L\(^{-1}\)) or in pots filled with 2 types of contaminated soils containing moderately low Cd levels under upland conditions. The 2 soils consisted of a Fluvisol and an Andosol and contained 1.82 and 4.01 mg Cd kg\(^{-1}\) on a dry soil weight basis, respectively, determined using 0.1 mol L\(^{-1}\) HCl-extraction. The results indicated that B. juncea was less able to accumulate Cd in shoots compared with hydroponically cultured rice and sugar beet, and was even less effective when grown in soil culture. Rice and sugar beet displayed a higher accumulation not only of Cd but also of other heavy metals (Cu, Fe, Mn and Zn) in their shoots than B. juncea when they were grown in the tow Cd-contaminated soils. Maize displayed the lowest metal accumulation among the plant species tested. Growing the rice cultivars in both soil types led to the most significant decrease in soil Cd concentration. Rice may be an eligible plant for metal phytoremediation of such soils (Satoru et al., 2006).

**Heavy Metal And Soil**

Among the soil characteristics that determine plant Cd uptake are total and available Cd concentration, pH and organic matter content (Gray et. al., 2002).
1999; McBride, 2002), cation exchange capacity (Lehoczky et al., 2000) clay content and interactions with other elements such as Zn (McKenna et al., 1993) and Cl (Lee et al., 1999). Of these characteristics, pH is usually considered the most important controlling Cd uptake (DEFRA-EA, 2002 a). Sharma et al., (2001) reported the chemical characteristic of crop field soil irrigated with polluted water, well water and well + polluted water. Irrigation with polluted water altered the pH, EC, chloride content and total organic matter content of the soil. Cd interfered with the uptake of Ca, Mg, P, Fe, Zn from the growth medium on which wheat seeds were shown (Shukla et al., 2003).

When heavy metals were present or added to the soil and plants were cultivated on such soil the chemical characteristic of the soil may be changed. Vediya and Patel (2002) studied the Cd content and chemical analysis of the soil samples collected from in and around Modasa, North Gujarat, India. Soil samples collected from Modasa were analyzed for EC, pH, Co$_3$, HCO$_3$, Cl, Ca and Mg. Fertilizer was also analyzed. EC of the soil was increasing with addition of Cd, Co$_3$, HCO$_3$ Cl content were more in the presence of Cd. Ca, Mg and total hardness were also increasing when soil receiving more Cd.

**Mechanism of Metal Toxicity And Tolerance**

Several workers (Iqbal et al., 1991, Shaukat et al., 1999, Al-Yemeni and Al-Helal 2000) reported that inhibition of seed germination at higher concentration of heavy metals is mainly caused by ion toxicity. Ion toxicity is associated with changes in cellular permeability, inhibition of protein activity and / or direct toxicity to the embryo and seedling (Dubey and Dwivedi 1987). The reason for the differential responses of root and shoot to heavy metals is not known but it might be due, in part, to more accumulation
of heavy metals in root than in shoot, or a faster rate of detoxification in the shoot compared to root (AI-Helal 1995, Shaukat et al., 1999). The heavy metal accumulation in the shoot may also lead to increase in phenolic compounds that may be responsible for inhibition of germination and growth. Phenolic acids have been shown to exert dramatic effect on membrane permeability and membrane electrical potential (Glass and Dunlop 1974). Clemens (2001) discussed the molecular mechanisms of plant metal tolerance and homeostatis. 

Growth reduction caused by heavy metals may be linked to a loss of cellular turgor resulting in either a decreasing of mitotic activity and/or an inhibition of cell elongation (Robertson and Meakin, 1980; Powell et al., 1986; Gabbarielli et al., 1990).

Root growth inhibition has commonly been observed in plants subjected to heavy metal stress (Woolhouse 1983; Eleftheriou and Karataglis, 1989; Barcelo and Poschenrieder, 1990; Pandolfini et al., 1992), but it is difficult to draw a general mechanism about the physiology of heavy metal stress, because metal toxicity in plants results from complex interaction of metal ions with several metabolic processes. Cd disturbs the water balance (Barcelo et al., 1988). Metal toxicity not only affects the length of primary root but also changes the architecture of the entire root system (Breckle, 1989). Toxic levels of Cd enhanced lateral root formation thus root system becomes dense and compact. Root hair density is generally decreased (Breckle, 1989). This morphogenetic change indicates that metal toxicity alters the root hormone balance. The root browning as a consequence of high metal availability has been reported by (Breckle, 1989). The browning may be due to enhanced suberization, which may limit water uptake. Structural changes in hypodermis, epidermis, pericycle and cortical cells may reduce water uptake in membrane water permeability (Barcelo and Poschenried...
The water movement in the plant could be affected by a reduction in the size and number of xylem vessels imposed by the metal toxicity, and also by alterations of hormone balance (Poschenrieder and Barcelo, 1999). The alteration in plasma membrane integrity, through lipid peroxidation, was a primary effect of heavy metal toxicity (Sandman and Boger, 1983; Cakmak and Horst, 1991; Pandolfini et al., 1992; Luan et al., 1994). Lipid peroxidation process can start before the appearance of any visible symptoms of toxicity (De Vos et al., 1989; Van Assche and Clijsters, 1990). Peroxidative damage is mediated by reactive oxygen species (ROS) i.e. superoxide radical (O$_2$\^-), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH\^-). Antioxidant molecules (ascorbic acid, reduced glutathione, tocopherol) and superoxide dismutase, protect the plants against oxidative stress. SOD convert O$_2$\^- to H$_2$O$_2$, then catalase, peroxidase break H$_2$O$_2$. (Cakmak and Horst, 1991; Asada, 1994). Under physiological conditions, plants are well adapted for minimizing damage that may occur from toxic oxygen species. Under environmental stress, the balance between the production of reactive oxygen species and the quenching activity of the antioxidants is upset, thus it results in to oxidative damage (Luan et al., 1994; Weckex and Clijsters, 1996 and 1997). Lipid peroxidation could be due to direct contact of membrane with the metal. The potassium leakage was marked with the enhancement of lipid peroxidation products in roots of *Triticum aestivum* (Pandolfini et al., 1992).

Heavy metal toxicity comprises inactivation of biomolecules by either blocking essential functional groups or by displacement of essential metal ions (Goyer 1997) In addition, autoxidation of redox-active heavy metals and production of reactive oxygen species (ROS) by the Fenton reaction causes cellular injury (Stohs and Bagchi 1995). Clemens (2001) discussed the molecular mechanisms of plant metal tolerance and homeostatis.
The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as (i) uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents; (ii) efflux of heavy metals from cytoplasm to extraplasmatic compartments including vacuoles; (iii) complexation of heavy metal ions inside the cell by various substances, for eg organic acids, amino acids, ferritins, phytochelatins and metallothioneins; (iv) general biochemical stress defense responses such as the induction of antioxidative enzymes and the accumulation of free proline; and (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Verkleij and Schat 1990, Brune et al., 1995, Prasad 1999, Sanita di Toppi and Gabrielli 1999, Hall 2002, Sanita di Toppi et al., 2002, Cho et al., 2003). Plant species significantly differ in tolerance to and uptake of Cd and other heavy metals.

Copper toxicity at cellular level may result from i) binding to sulfhydryl groups in proteins, thereby inhibiting enzyme activity or protein function; ii) induction of a deficiency of other essential ions; iii) impaired cell transport processes; iv) oxidative damage (Van Assche and Clijsters 1990, Mehrag 1994). Mechanism of growth inhibition and biomass loss due to increased peroxidase activity in plant tissue at excess copper was recorded in Phaseolus vulgaris (Jolaunds et al., 1996). Its role in induction of an efficient defense mechanism and development of tolerance has been described in many other higher plants against copper stress (Matasuda et al., 1994, Foyer et al., 1997). Slow translocation of Cu from roots to the shoots may be due the fact that excess Cu interferes with cellular metabolism and alters several physiological processes by inhibiting certain other enzyme activities as reported in Silene vulgaris (Ernst et al., 1990) and other higher
Dube et al., (2005) suggested that decrease in biomass of rice in excess Cu might be due to disturbed nitrogen and carbohydrate metabolism in such conditions. This was also supported by higher accumulation of Cu in various plant parts including roots. These results are somewhat similar to that in sugarcane (Agarwala et al., 1993) and tomato (Liao et al., 2000). The decrease in biomass of rice genotypes might also be due to poor protein synthesis (Marshner 1995). Stimulated peroxidase activity at Cu concentration plays a protective role by scavenging O$_2$ (superoxide dismutase radical) and H$_2$O$_2$ and minimizing catalytic iron in the system (Stohs and Bagchi 1995, Foyer et al., 1997). Various mechanisms for dealing with elevated Cu levels are found in plants (Ernst et al., 1992, Turner 1994, De Knecht et al., 1995, Murphy et al., 1999). Khurana and Chatterje (2001) suggested that Cu lowered the synthetic processes in general and stimulated of catabolic processes, which would automatically promote oxidative and hydrolytic catabolic activities. The reduction in polyphenol oxidase under excess Cu might be due to inhibitory role of Cu in high amounts.

Cu may interfere with biosynthesis of photosynthetic machinery, which modify pigment content and protein and lipid composition of photosynthetic membrane (Maksymeic et al., 1994). Khurana and Chatterje (2001) suggested that the reduction in chlorophyll a and b might be due to less availability of iron in excess Cu conditions. Iron is an important component of precursor of chlorophyll. Weckex and Clijsters (1996) discussed the existence of metabolic mechanism modulating to cope up with excess Cu and development of Cu tolerance. Jonak et al., (2004) reported that the exposure of alfalfa seedlings to excess Cu or Cd ions activated four distinct protein kinase pathways. The principal mechanism of Cu toxicity involves the Fenton reaction, characterized by metal catalysed production of hydroxyl radicals from superoxide and hydrogen peroxide (Elstner et al., 1988, Briat
and Lebrun 1999). Generally, however, Cu is translocated to a very low extent to the shoot, probably because it strongly accumulates in the cell walls of the cortex, where its concentration sharply decreases from the outer to the inner cell layers (Arundini et al., 1996).

It was difficult to isolate the effects of Co on nutrient concentration and distribution on growth responses or toxicity symptoms. In most cases, Co was intermediate in causing the various responses. Palit et al., (1994); discussed about the effects of Co on plants. The mechanisms by which Co affects plants are not yet clearly known. It has been proposed that Co interacts with the uptake of other macro and microelements. Co appears to be toxic when the uptake and/ or action of essential elements such as Ca and Fe are inhibited (Wallace et al., 1971, Agarwala et al., 1977, Terry 1981, Veltrup 1981). However, it appears to be beneficial when the uptake and action of toxic elements such as Cd, Cu, Ni and Zn are inhibited (Wallace and Abou-Zamzam 1989, Dirilgen and Inel 1994). Liu et al., (2001) studied the mechanism of Co toxicity in mung beans. One-week old mung bean seedlings were treated with 0.5 and 5 μM Co. 5 μM Co inhibited the seedling growth and it was associated with chlorosis of younger leaves. Mn content was reduced in the root while Fe content was less leaf. High concentration of Co in the root and leaves disturbed the metabolic processes. The Fe transport to the shoot was strongly inhibited.

Tolerance to heavy metals in plants may be defined as the ability to survive in soils that are toxic to other plants and manifested by an interaction between a genotype and its environment (Macnair et al., 2000). The term is frequently used in the literature in a broader sense to include changes that may occur experimentally in the sensitive response to heavy metals. Tolerance to high concentrations of metals in species and cultivars that grow on metal-polluted soil could be achieved by a range of potential mechanisms.
at the cellular level that might be involved in detoxification. These mechanisms appear to be involved primarily in avoiding the accumulation of toxic concentrations at sensitive sites within the cell preventing the damaging effects rather than developing proteins that can resist the heavy metal effects. Hall (2002) discussed the cellular mechanisms for heavy metal detoxification and tolerance. The biochemical responses of higher plants to toxic doses of heavy metals are very complex and several defense strategies have been suggested. These include complexation of metal ions, reduced influx of metals and enhanced production of antioxidants that detoxify reactive oxidative species produced in response to toxic metals (Van Assche and Clijsters 1990, Radoti et al., 2000, Schutzendubel and Polle 2002). Tolerant plants were able to maintain their constitutive functions by decreasing the rate of Cu-influx, detoxification of the metal through phytochelatin production and quenching of reactive oxygen species (ROS) by superoxide dismutase activity (Hartley-Whitaker et al., 2001).

Generally resistance to heavy metals is achieved either by avoidance, i.e. by protecting the plant from the influence of the stress or by tolerance, i.e. plant survives the effect of internal stress by adopting itself to the toxic concentration of heavy meals through mechanisms (Prasad, 1995). The toxicity and tolerance mechanisms of Cd in plants are discussed at the biochemical and cell physiology level over the last decade (Zenk, 1996; Sanita di Toppi and Gabbrielli, 1999; Rauser, 1999; Kamnev and Leiie, 2000).

Sanita di Toppi and Gabbrielli (1999) reviewed the work on mechanism of response of higher plants to Cd. The dose response relationship indicated that plant response to low and high Cd exposure is a very complex phenomenon indeed: Cd evokes a number of parallel and/or consecutive
events at molecular, physiological and morphological levels. They propose that, above all in responses to acute Cd stress, various mechanisms might operate both in an additive and in a potentiating way. Thus, a holistic and integrated approach seems to be necessary in the study of the responses of higher plants to Cd. This multi-component model, which would call ‘fan-shaped’ response, may occur with the Selyean ‘general adaptation syndrome’ hypothesis. While cadmium detoxification is a complex phenomenon, probably under polygenic control, Cd ‘real’ tolerance found in mine plants or in plant systems artificially grown under long-term selection pressure, exposed to high levels of Cd-seems to be a simpler phenomenon, possibly involving only monogenic / oligogenic control. Tolerance may be associated with detoxification.

SOD and peroxidase could serve as an important components of antioxidant defense mechanisms in rice to combat metal induced oxidative injury (Shah et al., 2001). Increased activities of antioxidative enzyme in Cd treated plants suggest that they have some additive function in the mechanism of metal tolerance in pea plants (Dixit et al., 2001). Schutzendubel and Polle (2002) reviewed the work done on heavy metal induced oxidative stress in plants and role of mychorrhiza to protect the plants against stress. Three different molecular mechanisms of heavy metal toxicity can be distinguished: (a) production of reactive oxygen species by autoxidation and Fenton reaction; this reaction is typical for Fe and Cu, (b) blocking of essential functional groups in biomolecules; this reaction is typical for Cd and Hg, (c) displacement of essential metal ions from biomolecules, common for other heavy metals. Exposure of plants to Cd and Hg resulted in oxidative stress as indicated by lipid peroxidation. Cd also caused a transient depletion of glutathione and inhibition of antioxidative enzymes especially of glutathione reductase. Assessment of antioxidative capacities by
metabolic modeling suggested that the reported diminution of antioxidants was sufficient to cause \( \text{H}_2\text{O}_2 \) accumulation. The depletion of glutathione reductase is critical step in Cd sensitivity.

50\( \mu \text{M} \) CdCl\(_2\) applied to pea leaves degraded the oxidized proteins but enhanced the proteolytic activity. Oxidized stress was mediated by \( \text{H}_2\text{O}_2 \) and proteolytic degradation may be considered as mechanism of Cd toxicity in leaves of pea plants, and they may be involved in the Cd-induced senescence in plants (Romero-Puertas et al., 2002).

Ag, Cd, Pb, Zn, Cu, Ti, Co and Hg exerted nonselective inhibition and root growth was slowed down due to the general toxicity of heavy metals rather than selective inhibition of any particular process or processes (Ivanov et al., 2003). Stohs et al., (2001) and Foyer et al., (1997) explain the oxidative mechanism in the toxicity of metal ions and hydrogen peroxide and glutathione associated mechanism of acclamatory stress tolerance and signaling. Peroxidase plays a protective role by scavenging superoxide radicles and \( \text{H}_2\text{O}_2 \) and minimizing catalytic ion in the systems.

Root is considered as a target organ of Cd toxicity. Root of carrot and radish are economically important. Chen et al., (2003b) studied the physiological mechanism of plant roots exposed to Cd using a liquid culture and a pot experiment with a series of Cd applications. The germination rate and root growth of both plants were inhibited at the concentration 20 mg Cd, and the inhibition was increased with the increasing concentration of Cd. Both in the liquid culture and in the pot experiment, activities of the catalase, peroxidase, polyphenol oxidase, superoxide dismutase were declined in both species treated with Cd. The proline was accumulated and maximum accumulation was found at 20 ppm, but then it declined with increasing