Literature Review

2.1. Heavy metal stress

According to Burzynski (1989), treatment of 4 days old cucumber (*Cucumis sativus*) seedlings with $PbCl_2$ and $CdCl_2$ caused a significant increase in the accumulation of heavy metals by plants, especially in the roots. The accumulated Pb initially enhanced the uptake of phosphorous after the plant had been transferred to a nutrient medium (6,24hrs) but after 48 hrs the uptake was dropped to below control level. The plants treated with Cd exhibited a constant nitrate uptake and nitrate reductase activity. It is suggested by the authors that the reason for the decreased nitrate reductase activity lay rather in the lower nitrate uptake than in a direct effect of the heavy metals on the enzyme.

Experiments were carried out by Yamamoto (1990) to determine if Cd was taken up by and distributed within soyabean plant. Unnodulated 23day old soyabean plants were supplied with solution of varying Cd concentrations (0.00 to 0.66) for 4days under continuous intensity (ca.1200 lux) and constant temperature (25^{O}C). As the concentration of the solution was increased, all plant parts showed increased concentration of Cd. In the stems, the response was essentially linear over the entire range of Cd concentrations employed (r^2 =0.991), while in root a linear relationship was found up to 0.43 ppm (r^2=0.983). At all cadmium concentrations used, the roots contained by far the greatest part of the total plant cadmium content, although the roots predominantly decreased to some extent with increasing doses of cadmium. When expressed as concentration ratio which is the ratio of Cd concentration of each plant part to that of external solution, the ratios of 120-160, 14-26 were obtained for roots and stems respectively, over a concentrations range from 0.15-0.43 ppm of added Cd. Addition of Cd to external solution appeared to cause a release of Ca, Mg, Zn, and possibly Mo into the solution but not of Fe, Cu and B.

The effect of increasing doses of heavy metals (Cd, Cu, Ni, Pb and Zn) applied separately or together on the growth of Italian Ryegrass (*Lolium multiflorum*) and on the content of heavy metals in plants. Among the examined metals only Cu (in a dose of 320 mg/kg soil) and Zn (in a dose of 320 mg /kg soil) caused some disturbances in the development of the rye grass. As a result of these disturbances there occurred a decrease in the yield of the first cut caused by Cu, and a decrease of the mass of roots caused by Cu and Zn. The toxicity of Cu and Zn considerably increased when all five investigated heavy metals were applied together. An effect of interaction of heavy metals on their absorption by plants was visible as regard as Cd, Ni, Pb and Zn. The contents of Cd, Ni, and Zn and Pb increased in ryegrass, while the investigated heavy metals did not considerably affect the uptake of Cu by the plant (Gorlach *et al.*,1990).

Wang and Yang (1990) studied the effect of copper pollution on wheat and rice in calcareous soil fertilized with sludge containing copper. Their results were revealed that high concentration of copper in soil affected the growth of the crops and the yields. Rice was more susceptible than wheat, and reduced the yield by about 10% when the soil had been treated with copper by 100 ppm. The absorption and accumulation of copper within the organs following the order of: root>stem>leaf>grain. The copper content in grains of wheat and rice was not higher than 20 ppm. The soil fertilized with sludge, the variation of available copper and soil capacity were also studied. They suggested that 130ppm of copper as critical value and 800ppm as a maximum permissible limit in sludge when it is fertilized to calcareous soil.

The toxicological effect of Cr (III) on some biochemical parameters in pepper both in soil culture and nutrient culture experiments were studied by Zhou *et al.* (1990). According to them heavy metal treatment decreased fresh weight and promoted senescence of pepper plant by decreasing chlorophyll content and activities of super oxide dismutase and catalase activity as well as increasing iron content and peroxidase activities over control values.

The single and combined effect of metals like lead, cadmium, nickel and UV and radiation on protein content and protein synthesis in leaves of barley seedlings was investigated by Bhattarcharya (1991). He reported that the treatment produced an increase in protein synthesis' and total soluble protein content. Combined treatments produced changes depending on the stages of irradiation, metal content of the substratum and storage period of seeds after irradiation.

Leita *et al.* (1991) studied the distribution of cadmium and induced Cd – binding protein in roots, stems, and leaves of *Phaseolus vulgaris*. Roots, stems and leaves of *Phaseolus vulgaris* L.(cv.Rubino PF 1H) grown in Hogland's solution supplemented with 1,2 and 2.5 mM $Cd(NO_3)$ were analysed. The distribution of cadmium in plant tissues showed that total cadmium in roots exceeded by about one and two order of magnitude total cadmium of stems and leaves, repectively. Results showed that most of the cadmium can be extractable by complexing with EDTA from the apoplast of root, stem and leaf. Water extractable cadmium present in ionic form in intracellular spaces as Cd^{2+}. Gel filtration of tissue extract showed the total cadmium present as free metal as ion in extracts of leaves 83.4%, whereas 56.6% and 48.7% was found in stem and roots extracts respectively. The remaining part of the total cadmium was associated with protein fractions. One type of cadmium protein fraction of about 10KDa molecular weight (K_{av} 0.54) was present in roots, stems and leaves , binding 24.1%, 43.4% and 16.6% of total cadmium respectively. A second protein fraction with apparent molecular weight >30KDa was present only in roots, binding 27.2% of total root cadmium. this results was confirmed by SDS-PAGE electrophoresis, showing a cadmium induced protein bands common to leaves , stems and roots with an apparent molecular weight 9.2KDa, which can be interpreted as phytochelatins, and an intensively stained cadmium induced band , present only on root extracts of about 42KDa apparent molecular mass .

The metal content of *Juncus acutus* (Juncaceae) seedling using Pb $(NO)_3$, $CuSO_4$ and $CdCl_2$ were studied by Stefani *et al.* (1991). They observed that the germination was not affected by any of these metals, though the initial growth was strongly inhibited by $Pb(NO_3)_2$ concentration from 0.12 X 10^{-5} M. Results showed that at all the tested concentration of $CuSO_4$ root was mostly affected than shoots and they also fail to develop. Moreover, the accumulation of Cd in the seedling was higher than that of Pb and Cu.

Moustakas *et al.*(1994) conducted a field experiment to evaluate the effects of Cu and Pb on photosynthesis and growth characteristics of oats. The plants grown on the site elevated levels of Cu-Pb were reduced in height and biomass, comparerd to control plants , and appeared chlorotic while the accumulations of both Cu and Pb in the above ground parts were in the range considered to be phytotoxic. Cu and Pb led to pronounced reduction (47%) of chlorophyll (Chl) (a+b) content, accompanied by proportional changes in ribulose 1, 3-bisphosphate carboxylase /oxygenase (RuBPCO) activity. Hence, Cu and Pb effects did not result in the destruction of photosynthetic apparatus but in its coordinate reduction. Growth at the heavy metal contaminated site resulted in a decreased (7%) quantum yield of photochemistry in photosystem 2(PS 2), as given by the ratio F-v/F-m measured in dark adapted leaves in the field.The half rise time (t-1/2) from the initial (F-O) to maximal (F-m) Chi flurescence was increased, suggesting that the amount of active pigments associated with the photochemical apparatus decreased and that the functional Chl antennae size of the photosynthetic apparatus was smaller compared to the control plants . Although, Cu and Pb affected the photosynthetic apparatus in multiple ways, the prevailing effect was that on RuBPCO activity, which in turn must have limited the overall photosynthetic activity.

Seven-day old seedlings of *Vigna catjang* Endl. were treated with distilled water or 10^{-5} M $PbCl_2$ or 10^{-5} $CdCl_2$ or (10^{-4} M $PbCl_2$+ 5 times 10^{-5} reduced glutathione (GSH) or [10^{-5} M $PbCl_2$ +5 times 10^{-5} buthionine sulfoximine (BSO) or (10^{-5} M $CdCl_2$+ 5 times 10^{-5} M GSH) or (10^{-5} $CdCl_2$+ 5 times 10^{-5}M BSO) for 6 days under open air conditions in a net house (Bhattacharyya and Choudhuri ,1995). They observed that the heavy metal treated plants showed significant decline in biomass, leaf area, root length, root metabolic activity, relative water content, pigment and protein content and there was a significant rise in MDA, alpha- NH^2, proline content and electrical conductivity of leaf leachate. In all the cases Cd^{2+} was more effective than Pb^{2+}. Treatment with GSH showed different degrees of recovery of stress induced damages whereas BSO treatment augmented the stress-induced damages. Author suggested that the possible involvement of phytochelatin like substances in the mitigation of metal induced damages.

Six rice genotypes *viz.*, Mahsuri, Pankaj, IET 66, TTB 101-14, Biraj and Khoram were taken by Baruah and Bharatnath (1996) and grown in sand in a green house with different levels of Fe in nutrient solutions *viz* control (2 ppm), 100 ppm and 200 ppm to observe the change in growth, ion uptake and metabolism of rice (*Oryza sativa* L.) seedlings at excess level of iron. Leaf yellowing was observed in the seedlings when grown at higher Fe concentration in the medium. Pankaj and TTB 101-14 maintained higher leaf chlorophyll along with higher total soluble protein and nitrate reductase activity in the leaves at 100 and 200 ppm Fe. Higher Fe concentration in the medium exerted an inhibitory effect on the contents of macro and micro nutrients in different genotypes. However, Pankaj and TTB 101-114 had relatively higher N, K, Mn and Zn content when grown in higher Fe level. They concluded that Pankaj and TTB 101-114 are suitable for growing under higher toxic concentration of iron.

Six year field study was conducted by Gigliotti *et al.*(1996) to evaluate heavy metal accumulation in top 20cm of a clay-loam calcareous soil amended with urban waste compost and to determine heavy metal uptake and distribution in corn plants grown in the soil, compared with untreated soils. Compared with untreated soils, amended soils showed a significant increase only in Cu, Zn, Pb and in the last 2 years Cr concentrations. They concluded that, corn plants grown on the amended soil showed a general increase in metal uptake, which was about three times greater for Pb and two times greater for the other heavy metals than in plants grown in untreated soil. At times, the diluting effect resulting from enhanced growth rates of the plants with compost application resulted in lower concentrations in the plants grown on treated plots. Cr and Pb were less mobile in the corn plant and were accumulated only in root tissues. The trace amount of Pb was found in the stalks in the last 3 years of experiment. The limited mobility of Pb was confirmed in a contemporary hydroponic greenhouse experiment. The values of the plant plant/soil transfer coefficients were within the lower range reported in the literature, indicating that in the soil studied (which contained 14% $CaCO_3$) there was limited transfer of heavy metal ions from the soil to the corn plants. They concluded that the long-term application of large amounts of urban waste compost to $CaCO_3$ –containing soils does not necessarily cause medium-term problems to the plants, animal or human health.

The effect of nickel toxicity in *Hyptis suaveolens* (L.).Poit and *Helianthus annus* L. was observed by Pillay *et. al.* (1996). Ni treatment of the plants resulted in an increase in Ni content of the leaves causing a disruption in their metabolism. The concentration of soluble nitrogen and protein decreased, wheareas reducing sugar and starch contents were markedly higher than the control in both the plants. Catalase activity decreased while peroxidase and polyphenol oxidase activity was increased .In sunflower reduction in catalase activity was 5 times and the increase in peroxidase and polyphenol oxidase was 4 and 8 fold respectively. Decrease in P content was correlated with increased in the activity of acid phosphatase and ATP ase.

Mishra and Chowdhury (1997) conducted a study on seeds of rice (*Oryza sativa* L.cv.IR-36 and Ratna) subjected to heavy metal (Pb^{2+} and Hg^{2+}) stress .They observed inhibition in germination percentage , shoot and root length and in their fresh and dry mass after 7 days . Hg^{2+} was more effective than Pb^{2+} in inhibiting germination and IR-36 was more tolerant than Ratna to these heavy metals. Both Pb^{2+} and Hg^{2+} inhibited the starch hydrolysis due to inhibition of L-amylase. When the embryos were treated with these heavy metals and grown *in vitro*, 2% sucrose in the medium could overcome the inhibitory effect of Pb^{2+} on embryo and while same, could not erase the inhibitory effect of Hg^{2+} on embryo growth significantly. Thus, Hg^{2+} was shown to be potentially more lethal than Pb^{2+} in inhibiting germination of rice seeds as Pb^{2+} inhibited the germination of the seeds and seedling growth by impairing the hydrolysis of endosperm starch without significantly affecting the embryo, while Hg^{2+} inhibited the same by damaging the embryo itself.

Cadmium and copper uptake and distribution as well as their effects on growth and lipid composition in 17 day old tomato seedlings (*Lycopersicon esculantum* Mill.cv.63/5 F1) grown in culture solution supplied with two concentrations of Cd or Cu (0, 5 and 50mu-M) were investigated by Ouariti *et al.* (1997). The accumulation of these metals considerably higher in roots than in primary leaves and accumulation of metals increased with external metal concentration. Biomass production of the growing roots and primary leaves was strongly depressed at high metal levels. Significant decrease in lipid classes and changes in fatty acid composition were recorded in heavy metal-stressed plants in comparison with controls. Glycolipid contents were decreased more in leaves than in roots by Cd- treatment, but Cu decreased both to similar extents in both organs. These metals reduced the phospholipid and neutral lipid contents more in roots than in leaves. Heavy metal treatment induced an alteration in the fatty acid desaturation processes, as it was observed that in almost all lipid classes the proportion of palmitic acid (16:0) increased, and that of linoleic (18:2) or linolenic (18:3) acid decreased. Moreover, the accumulation of palmitate (16:0) rather than stearate (18:0) indicated an alteration in the ratio of products from the fatty acid synthase. Cu was found to be the most unfavourable for plant growth and lipid metabolism.

Massive accumulation of proline in the leaves of *Silene vulgaris* in responses to copper, cadmium and zinc was reported by Schat *et al.* (1997). It was established from their results that, metal induced proline accumulation depends on the development of the metal induced H_2O_2 deficit in the leaves.

Shah and Dubey (1997) studied the effect of cadmium on protein, amino acid and protease, amino peptidase and carboxy peptidase in rice seedlings. Extractable proteins, free amino acids and the activities of the enzymes protease, leucine aminopeptidase and carboxypeptidase were determined in seedlings of two rice culltivers , Roma and Jaya, raised in cadmium nitrate [$Cd(NO_3)_2$] containing medium. With 500 µM $Cd(NO_3)_2$, the protein level was increased by 1.7 to 3.0 times in roots and 0.23 to 1.8 times in shoots of 20 days grown seedlings. Also 15 days old plants contained 0.2 to 0.4 times higher amino acid level in roots 0.4 to 0.8 times higher in shoots compared to non stressed seedlings. Cd^{2+}treatments significantly reduced protease activity in roots and shoots. In vitro activity of protease was inhibited markedly at concentration in excess of 100µM $Cd^{2+}$and leucine aminopeptidase and carboxylase activities were inhibited by 48 to 68% respectively in roots, whereas in shoots the activity increased by 36% to 47% with 500µM Cd^{2+}concentration.

Keltjens and Van Beusichem (1998) studied the copper and cadmium uptake and induction of phyto-chelatins (PC) synthesis in hydroponically grown maize and wheat plants exposed to these metals. They observed a close positive relationship between the concentrations of cadmium and PC in the plant shoot material. A decreased shoot concentration of cadmium after addition of copper, due to metal competition at common root absorption sites, coincided with lower PC levels. Differences in metal uptake and xylary metal transport among the two plant species were reflected in corresponding difference in PC concentration. According to the authors, the use of biomarkers such as phyto-chelatins, non-protein thiols specially induced in plants upon exposure to heavy metals, can be diagnostics criteria in heavy metal research and practice.

To elucidate the role of proline in plant responses to heavy metal stress, Sharma *et al.* (1998) studied the effect of proline on Cd-induced and Zn induced inhibition of glucose-6-phosphate dehydrogenase (G-6- PDH; EC 1.1.1.49) and nitrate reductase (NR; EC 1.6.6.2) *in vitro*. Proline appeared to protect both enzymes against Zn and though less effectively, against Cd. Measurements with a Cd 2+ specific electrode strongly suggested that this protection was based on a reduction of the free metal ion activity in the assay buffer, due to the formation of metal- proline complexes. There were no indications of any significant role for proline-water or proline-protein interactions.

Jemal *et al.* (1998) reported that peper plants (*Capsicum annuum*), like many other plant species, respond when stressed with cadmium chloride by the synthesis of phytochelatins [(Glu-Cys)$_n$ Gly] (PCs) and desglycyl phytochelatins [(Glu-Cys)$_n$] , where n=2-4 . Higher molecular weight PCs with a chain length longer than four have also been detected;

Chugh and Sawhney (1999) conducted an experiment to determine the effect of cadmium in one month old pea seedlings (*Pisum sativum* L.cv. Bonneville).Seedlings were raised in sand culture and provided with nutrient solution containing 0, 2.5, 5, 7.5 and 10 mM Cd. The effect on various aspects of photosynthesis was investigated after 6 and 12 days of treatment. The rate of photosynthesis, chlorophyll content, activities of photosystem I (PS I) and II PS (II) and a few photosynthetic enzymes *viz.* ribulose -1, 5- bisphosphate carboxylase (EC 4.1.3.9), NADP – glyceraldehydes - 3phosphate dehydrogenase (EC 1.2.1.13), fructose- 1, 6- biphosphatase (EC 3.1.3.11) and NADP – malate dehydrogenase (EC 1.1.1.82) declined progressively with the increasing concentration of applied Cd . As compared to the other parameters such as dm^{-2} leaf area, mg $^{-1}$ fresh weight (FW) and mg^{-1} cholorophyll (chl), the rate of photosynthesis showed maximum decline on per plant basis. The rate of photosynthesis and activities of enzymes showed a further decline on extending the period of exposure to 12 days. Cd had a more pronounced effect on the activity of PS II during the initial stages; however, extending the exposure for 12 days, the function of PS I also affected equally. Addition of Cd to the chloroplasts isolated from untreated plants impaired the functioning of PS II without any discernible effect on that of PS I. In presence of 0.1 mM Cd in the reaction mixture, the activity of PS II was inhibited by 46% whereas that of PS I remained unaffected. Activity of photosynthetic enzymes showed far greater inhibition 12 days after treatment compared to the effect on the rate of photosynthesis (mg^{-1} chl basis) and on photosystems.

Furting *et al.* (1999) carried out an experiment on reed plants fed with heavy metal concentrations of 100 μM $Cu^{2+}$and 10 mM Fe^{2+} under hypoxia. They found 1mg g^{-1} dry weight (DW) Cu^{2+} and 8 mg g^{-1} DW Fe^{2+} in roots of the plants when fed with heavy metal concentrations of 100 μM Cu^{2+} and 10 mM Fe^{2+} under hypoxia. Roots seemed to act as a kind of filter since the amount in rhizomes were only 0.06 mg Cu^{2+} g^{-1}DW and 2 mg Fe^{2+} DW . Increased contents of both the ions reduced post hypoxic respiration capacity by 40 – 50% and also the sum of adenylates (ATP , ADP , AMP) by the same order of magnitude , although the energy charge values remained above 0.85 in Cu^{2+} and 0.79 in Fe^{2+} treatments . The energy metabolism of the rhizomes was not affected. When roots were fed with 40 μM Cu^{2+} and 1mM Fe^{2+} respectively Cu and Fe contents of roots as well as rhizomes were high enough to induce oxidative stress . Authors concluded that increased, but environmentally attainable, amounts of copper and reduced iron ions disturb root energy metabolism, and root functioning and development .Latent injuries, based on oxidative stress, may be harmful for roots and rhizomes under long term exposure.

Cadmium sulphate ($3CdSO_4$, $8H_2O$) was applied as a foliar spray to mung bean at the concentration of 40, 60, 80and 150μM at weekly intervals. The activity of ammonia assimilating enzymes viz, glutamate dehydrogenase, and glutamine synthase and glutamate oxoglutarate amino trasferase were inhibited to variable extents in the root nodules. Of the enzymes, GDH and GOGAT registered maximum decline. The fine structure of chloroplasts in Cd treated plants was degenerated similar to the senescing leaves. The principal symptomps of Cd action were the presence of osmiophilic plastoglobuli and a disorganization of the lamellar structures, mainly grana stacks. In Cd treated nodules, the peribacteroid membranes around the bacteroids seen to degenerate. Probably considerable amount of Poly-B hydroxyl but β-hydroxybutyrate granules, inside the bacteroids renders the root nodules ineffective (Keshan, 1999)

Heavy metal accumulation in field receiving fly ash from a thermal power plant and subsequent uptake in the different parts of the crop plants growing in the respective field were investigated by Barman *et al.* (1999). The metal content (Cd, Cu, Zn, Fe, Ni, Cr and Pb) in the soil samples are higher than that in control soil and lower than that in background value. In case of Cd, Zn and Pb the concentration is either below or within the critical concentration. In the edible parts of the plant Cu, Zn and Pb concentration are within the recommended permissible limits, whereas Cd, Cr and Ni show a little higher concentration. Overall the results showed that the heterogeneous accumulation of metals in plants varies from species to species and also within the different parts of the same palnt.

Toppi *et al.* (1999) reported that production of stress ethylene in carrot plants was highly stimulated by 1mM Cd. Plants pretreated with buthionine sulfoximine (BSO) and cell suspensions produced phytochelatins, and no lipid peroxidation was detected. In cell cultures, the *in vitro* activity of phytochelatin synthase was assayed in the presence of Cd and glutathione: the first product (PC_2) was detected in less than 30min. Absence of ethylene (after treatment with aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, or use of ethylene traps) caused both a decrease in the phytochelatin synthase activity of cell suspensions and a strong lowering in the Cd-induced SH groups in plants. However, 1-aminocyclopropane-1-carboxylic acid (ACC) supply did not increase either phytochelatin synthase activity or total SH level.

Leopold *et al.* (1999) investigated the induction and heavy metal binding properties of phytochelatins in heavy metal tolerant (*Silene vulgaris*) and sensitive (tomato) cell cultures, in water cultures of these plants and in *Silene vulgaris* grown on a medieval copper mining dump. Application of heavy metals to cell suspension cultures and whole plants of *Silene vulgaris* and tomato induced the formation of heavy metal –phytochelatin –complexes with Cu and Cd and the binding of Zn and Pb to lower molecular weight substances. The binding of heavy metal ions to phytochelatins seemed to play only a transient role in the heavy metal detoxifications, because the Cd and Cu complexes disappeared in the roots of water cultures of *Silene vulgaris* between 7 and 14 days after heavy metal exposition.*Silene vulgaris* grown under natural conditions on a mining dump synthesized low molecular weight heavy metal binding compounds and no complexes of heavy metal ions to phytochelatins. The free heavy metal ions were not detectable in the extracts of all investigated plants and cell cultures. Author suggested that the induction of phytochelatins is a general answer of higher plants to heavy metal exposition, but some of the heavy metal ions are able to form stable complexes with phytochelatins. They also suggested that the investigation of tolerant plants from the copper mining dump showed the phytochelatins are not responsible for the development of the heavy metal tolerant phenotypes.

Lipid peroxidation in relation to senescence of detached rice leaves caused by excess copper was investigated by Cher (1999). Excess copper, which was found to promote senescence, increased the level of lipid peroxidation but not the level of H_2O_2. Catalase and glutathione reductase activities were reduced by excess copper. Super oxide dismutase and ascorbate peroxidase activities did not seem affected by excess copper. Free radical scavengers inhibited excess copper-promoted senescence and the lipid peroxidation, suggesting that, lipid peroxidation induced by excess copper is mediated through free radicals.

Panda and Patra (2000) studied the relationship between the toxicity of Cr (III) ions and the oxidative reactions in plant cells in wheat seedlings. Leaves from 7 and 9 days old wheat seedlings were incubated in various concentrations of Cr (III) ions containing solutions for 24 hr in light. Cholrophyll and carotenoid breakdown and increases in membrane permeability and lipid peroxidation was noticed at higher concentrations of Cr (III) ions. Free radical scavengers such as mannitol and sodium benzoate prevented the increase in senescence parameters. Mannitol and sodium benzoate both were effective for the leaves of both ages. Catalase activity was increased in Cr (III) ions in younger leaves while the activity was decreased in older ones. Peroxidase activity decreased with increasing Cr (III) ion concentration. Superoxide dismutase activity was increased slightly in both the leaves at lower Cr (III) level while it decreased at higher concentrations. Free radical scavengers protected these enzymes against inactivation. Their results indicate an excess Cr (III) mediated oxidative reactions in light, which accelerated the leaf senescence.

[redacted]

Peralta *et al.*(2000) have reported in preliminary studies that alfalfa plants (*Medicago sativa*) can grow in some heavy metal contaminated soils. Based on it they studied the individual effects of several doses of Cd (II), Cr (VI), Cu (II), Ni (II) and Zn (II) on the growth of live alfalfa plants using solid media. They used the doses of 0, 5, 10, 20 and 40 ppm for the study. The seed germination and the growth of the plant was affected significantly by Cd (II) and Cr (VI) at 10 ppm , as well as by Cu(II) and Ni (II) at 20 ppm and higher concentrations ($P < 1\%$) . Zn (II) did not affect seed germination. The roots of the plants exposed to 5 ppm dose of Cd (II) , and 5 and 10 ppm dose of Cr(VI) , Cu(II) , Ni(II) and Zn(II) grew more than the roots of the control treatment by more than 30%. The shoot size was reduced by 16% as compared to control when it was exposed to 5 ppm of Cd (II). Whereas, Cr (VI), Cu (II), Ni (II) and Zn (II) increased the shoot size by 14.0%, 60.0 %, 36.0% and 7.7% respectively, only Zn (II) promoted the shoot growth at the doses of 20 and 40 ppm.

Yadav and Srivastava (2000) carried out an experiment to evaluate the cadmium effect on foliar epidermal traits, five molar concentrations of cadmium chloride (10^3 M - 10^{-7} M) were used for treating the seeds/seedlings of four commercially important crops (*Carthamus tinctorius* cv. Tara, *Cicer arietinum* cv. K-468, *Hordeum vulgare* cv.Jyoti and *Setaria italic acv.* Saket-4). It was observed that $CdCl_2$ treatment could increase /decrease the epidermal cells peer unit area and stomata per unit area of stomatal pore. Cadmium chloride treatment could also induce several types of stomatal anomalies like stomata with unequal guard cells /subsidiary cells, stomata with more or less than one guard cells / subsidiary cells, disintegration of guard cells /subsidiary cells. It was observed that *Cicer arietinum* has shown less susceptibility to cadmium as compared to the other crops.

The influence of cadmium on growth and development of *Vicia faba* L., broad bean was assed in pot cultures with cadmium iodide (CdI_2) in different concentrations ranging from 15 to 500 mg per kg of soil. There was a decline in plant height and total dry weight. Root size was decreased most significantly with a corresponding reduction in the frequency of root nodules. Total soluble protein in leaf, stem and root suffered a pronounced loss with increasing concentration of cadmium. Chlorophyll a was the most sensitive pigment followed by chlorophyll b and carotenoids. Nitrate reductase activity too was adversely affected. Cadmium contamination induced abnormalities in stomata and trichomes (Bhatnagar *et al.* 2000)

Schiizendiibel *et al.* (2001) reported that unspecific peroxidase in pine roots were not inhibited by Cadmium, but increased slowly with the time pattern clearly distinct from that observed for the constituents of the SOD-ascorbate- glutathione pathway. Its activities were elevated in root tips which showed increased concentrations of phenolics and lignification in response to Cadmium.

Prasad *et al.*(2001) conducted an experiment to find out effect of heavy metals in aquatic plants .Several physiological responses of aquatic vascular plants like *Lemna trisulca* L.were investigated when they were exposed to the elevated concentrations of cadmium (up to 10mM) and copper (up to 50μM) . They found that Lemna fronds were able to accumulate the two heavy metals but copper treated plants showed toxic symptoms at concentrations 1000-fold lower in comparison to cadmium. *Lemna trisulca* could tolerate elevated levels of cadmium i.e. up to 10mM, without significant changes in photosynthetic pigment concentration. On the other hand copper concentrations 25 and 50 μM showed the significant pigment degradation. Total gas exchange and net photosynthesis were affected by Cd in *Lemna* fronds. On the contrary, the inhibition of total gas exchanges and net photosynthesis caused by Cu (2-50μM) correlated with Chl a and carotenoid concentrations decrease as well as with the decay of fluroscence from PS-II- . An increasing impact of respiration was observed in total oxygen exchange when the *Lemna* plants were treated with increasing concentrations of Cd (up to 5mM) and with Cu (2-50μM). In SDS PAGE analysis in Cd trated fronds , a dose-dependent accumulation of two polypeptides with apperant molecular weight 18 and 10 kDa , respectively as well as the appearance of two smaller polypeptides apparent molecular weights 8 and 7 kDa) was observed but in Cu-treated fronds neither accumulation of existing proteins nor any extra proteins were appeared .

Monni *et al.* (2001) measured chlorophyll, organic (citric and malic acids) and abscisic acid (ABA) contents and stem water potential on *Empetrum nigrum* L. (crowberry) to indicate the possible physiological effects of heavy metal deposition on the plant. The leaves and stems of *E. nigrum* were collected at distances of 0.5 and 8 km from the Cu-Ni smelter at Harjavalta, South- West Finland. The results showed all the investigated parameters were clearly affected by heavy metal emissions. Chlorophyll content in leaves as well as organic acid contents in the leaves and stems was lower close to the emission source.. On the contrary, ABA contents in stems and leaves in general, were higher in plants growing 0.5 km from the pollution source. The results showed that the plant exposed close to the smelter the stem water potential was less negative during the day but more negative during the night. These results suggest that smelter emissions have a negative effect on the ecophysiology of *E.nigrum* even though it is considered to be a tolerant species to heavy metals.

Diaz *et al.*(2001) reported that pepper (*Capsicium annum* L.) plants growing in a nutrient solution with excess copper , showed an increase in shikimate dehydrogenase (SKDH, EC 1.1.1.25) and peroxidase(EC 1.11.1.7) activities in the hypocotyl. Peroxidase activity was also induced in roots, but the SKDH activity per organ was depleted rather than enhanced. Cu stress caused stunting in the plants, reflected by a decrease in the fresh weight of all the organs. The induction of both enzymatic activities was associated with the accumulation of soluble phenolics and lignin was observed in hypocotyls. The two SKDH isozymes present in the control hypocotyls (SKDH-3 and SKDH-4) increased in a similar proportion after Cu stress. In case of peroxidases, two new isozymes (PRX-A2 and PRX-A4) were detected in Cu stressed hypocotyls, and the other two isoperoxidases, PRX-B and PRX-A3, were enhanced ten and three times respectively, with respect to control. The application of the chelator EDTA was able to counteract all the stress effect of the metal stated above.

In a study by Hegedus *et al.* (2001) with green and greening barley seedlings which represent two different stages of development to evaluate the effects of cadmium stress induced alterations in the activities of several representatives of the enzymatic antioxidant defense system such as guiacol peroxidase (POD) , catalase (CAT) and ascorbate peroxidase (APX) . Although the roots were the main site of cadmium accumulation, 1.5- 3% of cadmium was translocated into leaves and causes the oxidative damage which was indicated by the reduced chlorophyll content and increased malondialdehyde content of the leaves. The APX activity was increased without any increase in the activity of POD in the roots of both types of seedlings exposed to various cadmium concentrations. In leaves, however, elevated level of POD and APX was observed. In roots of green seedlings at high concentration of cadmium the APX activity was reduced on the fourth day of culture but no inhibition was found in the POD activity. CAT activity in leaf which mainly represented the peroxisomal enzyme activity did not showed any changes under cadmium stress. The results showed that at both developmental stages barley seedlings exhibit a well-defined activity of the enzymatic antioxidant system, which operates differentially in roots and shoots subjected to the heavy metal stress.

The combined effect of Copper and Cadmium adversely affected the germination, seedling length and number of lateral roots in *Solanum melongena*, reported by Neelima and Reddy (2002).

The effects of various nickel levels (0.05, 0.5, 5.0 and 50μM) on photosynthesis and chlorophyll content in rapeseed (Brassica *campestris* var. Toria PT 303) was examined by Pratibha and Rathore (2002). The studies revealed that while low levels of nickel (upto0.5μM) enhance the photosynthetic rates, however high levels of 5μM had reverse effect. A decrease in the total chlorophyll content with increasing concentrations of nickel was also observed in the rapeseed plants.

The influence of Cd on pepper plant was also studied by Leon *et al.* (2002) .They investigated the effect of growing five different cultivars of pepper plants (*Capsicum annum* L.) with $CdCl_2$ concentrations ranging from 0.125 to 0.5 mM on the different physiological parameters , and antioxidative enzymatic activities of leaves. On the basis of growth parameters they found that pepper plants were relatively tolerant to Cd, although metal concentrations higher than 0.125 mM produced a significant inhibition of growth and net photosynthesis, and water use efficiency. Different responses to the Cd^{++} stress were observed among cultivars, Abdera being the most resistant to cadmium stress, and Mondo and Herminio the most sensitive cultivars. The increase in activity of glutathione reductase and guaiacol peroxidase of most cultivars was observed in the cadmium concentrations of 0.5 mM, while catalase (CAT) and superoxide dismutase (SOD) were slightly depressed at that concentration. The analysis of SOD activity pattern by native-PAGE showed the presence of four SODs in most cultivars which were identified as Mn-SOD, Fe-SOD, CuZn-SOD I and CuZn –SOD II. However, the CuZn-SOD s were absent in the Cd sensitive cv. Herminio. The growth of pepper plants with 0.5mM Cd inhibited the activity of CuZn-SODs in all cultivars, whereas, the activity of Mn-and Fe-SOD was enhanced. The activity of NADPH – dehydrogenase (glucose -6-P-dehydrogenase, 6 – phosphogluconate dehydrogenase, NADP –isocitrate dehydrogenase and malic enzyme) showed a Cd dependent enhancement in most cultivars, the highest increase being in the tolerant cv.Abdera. The results suggest that in pepper plants the tolerance to Cd toxicity is more dependent on the availability of NADPH than its antioxidant capacity.

Perfus–Barbeoch *et al.* (2002) investigated the Cd^{2+} toxicity effects on plant water loss, gas exchanges and stomatal behavior in *Arabidopsis thaliana* L. Effects of 1week Cd^{2+}application in hydroponic condition ($CdCl_2$ 10-100 μM) were analyzed . No significant effects on the plant water relationship and carbon assimilation was observed at a10μM Cd^{2+}concentration. At higher concentrations, a Cd^{2+}dependent decrease in leaf conductance and CO_2 uptake was observed despite the photosynthetic apparatus appeared not to be affected as proved by fluorescence measurements. In epidermal strip bioassays, nanomolar Cd^{2+}concentrations reduced stomatal opening under light in *A.thaliana* , *Vicia faba* and *Commelina communis* .5mM ABA application limited the root to shoot translocation of cadmium. The cadmium induced stomatal closure was likely ABA independent, since a 5 day water treatment with 50 μM Cd^{2+}did not affect the plant relative water content. A similar cadmium induced stomatal closure was observed in the ABA insensitive mutant abi 1-1, which showed a higher transpiration rate than the wild type but did not accumulate more cadmium as cadmium uptake is not dependent only on the transpiration flow. Application of putative calcium channels inhibitors suppressed the inhibitory effects of cadmium in epidermal strip experiments, suggesting that cadmium can enter the guard cell trough calcium channels. Patch-clamp studies with *V.faba* guard cell protoplast showed that plasma membrane K^+channels were insensitive to external Cd^{2+} application whereas Ca^{2+} channels were found permeable to Cd^{2+}. In conclusion, they proposed that Cd^{2+} affects guard cell regulation in an ABA independent manner by entering the cytosol via Ca^{2+} channels.

Different clones of *Salix viminalis* with different resistances to Cd , Cu, and Zn were cultivated hydroponically in the presence of 7μmol L^{-1} Cd, 3 μmol L^{-1} Cu and 70 μmol L^{-1} Zn for 20 days. The clones were then compared with regard to the concentrations of free radicals, estimated by measuring thiobarbituric acid –reactive material (TBA-rm) and glutathione (GSH). The enzymatic activities of the aspartate peroxidase (APX), guaiacol peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) were also analyzed. Salicylic acid was also measured since it is known to be involved in antioxidative activities. Some differences in sensitive and resistant clones could be observed .The SOD activity was higher in untreated resistant clones compared with the sensitive clones. However, under metal treatment, the SOD activity was similar. Moreover, TBA-rm was higher in shoots of the resistant clones compared to the sensitive ones, while the opposite was found in roots (Landberg and Greger, 2002)

Fediuc and Erdei (2002) studied the capability of common reed and cattail (*Phragmites australis* (Cav.) Trin.ex Steud. and *Typha latifolia* L.) to accumulate and translocate Cd^{2+}at the level of thiol metabolism and antioxidant enzyme activity. Cadmium treatment was applied as a concentration series between 0.1 and 100 μmol L^{-1} for 40 and 100 days for reed and cattail, respectively. Plants samples were taken for assays and analysis in 2 -4 weeks intervals. They reported that most of the Cd^{2+}taken up was retained in the roots in both species. *Typha* accumulated more cadmium in shoots than *Phragmites*.The increasing accumulation of cadmium in *Typha* had a positive correlation with the increase of thiol content while in *Phragmites* glutathione reductase, catalase and peroxidase activities were increased. Author concluded that different defence strategies operate in the two plants under cadmium stress. In *Typha*, this strategy relies more on thiol induction and metal binding leading to the heavy metal avoidance, while in *Phragmites* increased antioxidant enzyme activities and thus based on scavenging of active oxygen species.

Shu *et al*. (2002) reported that both Fankou and Leachang lead /zinc (Pb/Zn) mine tailings located at Guangdong Province contained high levels of total and DTPA extractable Pb, Zn and Cu. Different populations of the two grasses growing on the tailings were *Paspalum distichum* and *Cynodon dactylon.* Tillers of these populations including those from an uncontaminated area were subjected to the following concentrations: 5, 10, 20, and 30mg L^{-1} Zn, or 0.25, 0.50, 1 and 2 mg L^{-1} Cu for 14 days, respectively. The dominant species colonized naturally on the tailings. Pb, Zn and Cu accumulation and tolerance of tolerance index (TI) and EC_{50} (the concentrations of metals in solutions which reduce 50% of normal root growth) were calculated. The results suggested that both grass populations showed a greater tolerance to the three metals than those growing in the uncontaminated area, which indicate the co-tolerant ecotypes have evolved in the two grasses. Among the two grass populations *P. distichum* collected from Fankou tailing had the highest tolerance to Cu while Lechang population had highest tolerance to Pb and Zn among the tested populations. The tolerance levels of *P. distichum* had a better growth performance than *C. dactylon* when they were grown together on the tailing sites. The tolerant populations of these species would serve as potential candidates for re-vegetation of wastelands contaminated with Pb, Zn and Cu.

The inhibition of plant growth and induction of visible symptomps of metal toxicity led to increased accumulation of the metals was reported by Pandey and Sharma (2002) in cabbage plants to excess (500 µM) of Co^{2+} , Ni^{2+} and Cd^{2+} in sand culture . In addition to chlorosis, Co^{2+} treated plants exhibited reddish purple coloration along the leaf margins, Ni^{2+} treated plants showed the black spots near the leaf margins and the plants treated with Cd^{2+} developed purple coloration along the leaf margins. At the equimolar concentration, inhibition of growth was most severe with excess Cd^{2+} and induction of visible symptoms was most severe with the application of excess Ni^{2+}. The uptake of Fe and its translocation to leaves decreased due to the exposure of excess concentrations of heavy metals. Exposure to each Co^{2+}, Ni^{2+} and Cd^{2+} decreased the chlorophyll content ($Ni^{2+} > Cd^{2+} > Co^{2+}$), concomitant with decrease in the activities of the Fe enzymes –catalase and peroxidase, suggesting reduced availability of Fe for chlorophyll – heme biosynthesis. Each Co^{2+}, Ni^{2+} and Cd^{2+} exposure developed water stress by decreasing water potential and transpiration rate, associated with increase in diffusive resistance showing the water stress. The enhanced accumulation of proline in the leaves was further substained when the plants exposed to Co^{2+}, Ni^{2+} and Cd^{2+} .

Shrivastava *et al.* (2002) conducted a study to elucidate the heavy metal status in various morphological components of normal green and chlorotic plants of sugarcane variety CoLk8102 which indicated that chlorosis induced significant variation in Pb and Cr contents. Morpho-physiological components did not differ significantly with respect to any of the heavy metals. There was a marginal increase in transport index of heavy metals in chlorotic plants. Although chlorosis induced significant variation in Pb and Cr contents, on overall basis the partitioning of heavy metals followed a trend similar to that of partitioning of dry matter. The lead content was somewhat higher in the chlorotic plants as compared to the normal green plants on per unit plant weight basis.

Zornoza *et al.* (2002) studied cadmium stress in nodulated white lupin. White lupin plants (*Lupinus albus* L., cv.Multolupa) were grown hydroponically on perlite with different Cd concentrations in the nutrient solution (μM): 0, 18 and 45. Changes in growth, nodulation, nutrient concentrations, nutrient uptake and distribution, Cd bound to cell wall and some Cd stress indicators were studied in roots and shoots (leaves plus stem) of 35-d-old plants as a result of Cd uptake. A significant decrease in both root and shoot dry weight was found only when plants were grown on 45 μM Cd. Whereas nodulation and total N content decreased for both Cd levels. White lupin plant retained up to 88% of total Cd in the roots, showing some capacity of Cd translocation to the upper parts, but an important fraction of Cd was bound to cell wall. Cadmium addition reduced P, K, Fe, Mn and Zn concentrations in the shoot and Mn in the root. Despite the reduction in Mn concentration, Mn level in Cd-treated plants remained within normality, probably because white lupines a Mn accumulator. In Cd treated plants, the level of lipid peroxidation remained unmodified, whereas total thiols showed a pronounced increase in roots. Results obtained suggest that white lupin has developed several strategies of defense against Cd, as a high retention of Cd by cell walls and complexion by thiol groups, both contributing to diminish the level of free Cd . Moreover, the high Mn content of white lupin could contribute to the prevention of Cd damage on photosynthesis, since, chlorosis was not found.

Barman Roy and Bera (2002) studied the individual and combined effect of mercury and manganese on phenol and proline content in leaf and stem of mungbean seedlings. The mungbean (*Vigna radiata* L.Wilczek) cv. Pusa Baisakhi seedlings were raised in individual (0, 1, 10, 100 and 1000 ppm) and combined solutions (1:1, 10:1, 1:10 ppm Hg: Mn) of mercury and manganese for 6 days. Phenol and proline were found to accumulate in leaves in response to treatments with heavy metals. The magnitude of accumulation was correlated with concentration of metals. However, a reverse trend was noticed in stem for phenol. Accumulation of phenol in response to heavy metal treatment was organ specific and occurred at higher rate in plant parts, which faced the stress mostly. However, accumulationof proline helped the plant to survive stress situation. In combined solutions, amelioration of mercurial toxicity by manganese was recorded.

The effect of aluminium on the growth and physiology of *Acacia nilotica* seedlings, a fast –growing tree legume was studied by Malathi *et al.*(2002) The toxic effect of aluminium in acid soils poses a major threat to plant species. The short term effects of Al in hydroponically grown *A.nilotica* seedlings were studied for 30 days. Al was supplemented to the nutrient solution in the form of AlK $(SO_4)_2$. Chlorophyll content showed a significantly decreasing trend with increasing Al. Stomatal conductance showed a similar trend as did nitrogen and phosphorous contents. Protein contents were correlated to that of nitrogen levels. The result showed that, the seedlings can not tolerate Al levels above 100 ppm.

It was reported the repeated use of copper (Cu) to control vine downy mildew, caused by *Plasmopara viticola* , is mainly responsible for the heavy increase of Cu concentration in the upper layers of vineyard soils. Brun *et al.*(2003) created an artificial soil gradient with Cu enrichments ranging from 0-400 mg kg-1for the determination of the effects of elevated soil Cu on the development of plant. On this gradient, five plant species commonly found in vineyards in southern France (*Poa annua* L., *Dactylis glomerata* L., *Senecio vulgaris* L., *Hypochoeris radicata* L., and *Andryala integriflolia* L.,), were quantified for survival, growth and reproduction throughout one flowering season. They reported that the high concentrations of Cu in the soil resulted in low survival, low plant biomass, delay in flowering and fruiting, and low seed set. The effects differed among speices. Moreover, high soil Cu concentrations had contrasting effects on patterns of resource allocation depending on the plant speices.

Singh and Tiwari (2003) reported that excess of cadmium induced changes in oxidative scenario and water status of plants viz. total water content, specific water content, water saturations deficit(WSD) and transpiration of *Brassica juncea* grown in soil pot culture. Although lower and marginal level of excess cadmium (100 and 250 ppm) improved growth but higher levels (500 ppm) caused significant suppression of growth. Proline accumulation, an indicator of water stress, occurred at higher level of cadmium. Gradual increase in activities of certain antioxidative enzymes such as catalase and peroxidase along with increased lipid peroxidation were also observed. The excess levels of cadmium also decrease the concentration of soluble protein and chlorophylls and increase the ratio of chlorophyll a/b.

Sottnikova *et al.*(2003) studied the growth parameters of six fast growing trees which showed that the roots are more sensitive to Cd treatment than shoots. Cd treatment suppressed rooting and root growth (length and biomass production) and its development in all tested species. *Salix cinerea, Salix alba, Populus cv. Robusta* were more tolerant root system to Cd stress than the root system of the other studied species. *Salix* species showed significantly reduced shoot growth parameters unlike *Populus* species, which were not affected by Cd treatment.

Survival and behavior of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] under different conditions of heavy metal concentrations were studied by Soltan and Rashed (2003). Plants were grown in different media (distilled water, Nile water, waste water and different concentrations of heavy metals) and visual changes were also observed in plants during each experiment. The heavy metal concentrations (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn), pH and conductivity of the media were measured before, during and at the termination of the experiments. The effects of different media on metal accumulation by the plants were analyzed with the plant samples after termination of the experiments. Their results showed that water hyacinth can survive in a mixture of heavy metal concentrations up to 3mg L^{-1} and in 100 mg Pb L^{-1} solutions, whereas higher concentrations of metals as mixture and 100 mg Cd L^{-1} led to rapid fading of the plants. Water hyacinth decrease the pH of the growth media as it has exhibited a deprotonation reaction during the uptake of the metal ions .The results indicated that the water hyacinth plays an outstanding role as a heavy metal decontaminator; but its role as a pollutant by releasing metal ions into the aquatic environment was also noted. They suggested that elevated pH and ammonia concentrations, along with the low dissolved oxygen content in the microenvironment around the root hairs, are the main factors for the rapid wilting of the plants growing in Kima drain wastewater.

The effect of Cd on dry matter accumulation and grain yield of different rice cultivars ,the differences among rice cultivars and genotypes in Cd uptake and translocation, the interactions between Cd and five mineral nutrients Fe, Zn, Cu and Mg in response of the uptake and translocation in rice plant were investigated by Liu *et al.*(2003). They conducted a pot experiment on 20 rice cultivars of different genotypes and origins by adding 100 mg kg^{-1} of cadmium (Cd) to the soil. The results showed that the effect of Cd on rice growth and development were variety dependent; some cultivars were strongly tolerant to soil Cd stress, where others were very sensitive to the heavy metal. Differences were also observed among the cultivars for Cd uptake and distribution in rice plants, but the differences were not necessarily related to rice genotypes. Cd concentrations fell rapidly from roots to brown rice along rice plants, so the concentrations of Cd were very low in brown rice compared with other parts of the rice plants. The effects of Cd on the concentrations of the mineral nutrient in the roots and, leaves were mostly significant, however the results varied with metal elements, rice plant organs and growing stages. Under soil Cd stress, the variations in of the grain and straw yield of the cultivars were not correlated with the changes of any mineral nutrient in the rice plant. The regression analysis showed that for their concentrations in roots and leaves, significant positive correlation between Cd and Fe, Cd and Zn, Cd and Cu existed, but no significant correlation between Cd and Mg and the relationship between Cd and Mn varied with the organs of the rice plants. The results revealed that the rice cultivars differed greatly in the growth and development to Cd and in absorption and translocation of different metals like Cd, Fe, Zn, Cu, Mn and Mg. The interactions of Cd and Fe, Zn, Cu are synergetic in uptake and translocation from root to shoot by rice plant.

Stolt *et al.* (2003) studied the PC accumulation in 12 days old seedlings of two cultivars of spring bread wheat (*Triticum aestivum*) and two spring durum wheat cultivars (*Triticum turgidum*) with different degree of Cd accumulation in the grains.

Shoots and roots were analyzed for dry weight, Cd and PC accumulation. The results showed neither significant differences between the species or the varieties in the growth responses to Cd, nor the distributions of PC chain length or PC isoforms. At 1 µM external Cd, durum wheat had a higher total Cd uptake than bread wheat, though; the shoot-to-root Cd concentration ratio was higher in bread wheat. The results when comparing varieties within a species, the high grain Cd accumulators showed the lower rates of root Cd accumulation shoot Cd accumulation and root PC accumulations, but higher shoot-to-root Cd concentrations ratios. Intraspecific variation in grain Cd accumulation is apparently not only explained by differential Cd accumulation but rather by a differential plant-internal Cd allocation pattern. However, the higher average grain Cd accumulation in the durum wheat than the bread wheat is associated with a higher total Cd accumulation in the plant, rather than with differential plant internal Cd allocations. The root internal PC chain length distributions and PC-thiol-to Cd molar ratios did not differ significantly between species or varieties, suggesting that differential grain Cd accumulation is not due to is not due to differential PC based Cd sequestration in the roots.

The influence of cadmium (Cd^{2+}) on the wheat plant (*Triticumt aestivum* L.) was also studied by Shukla *et al.*(2003) . Cd^{2+}accumulation and distribution were analyzed in 3weeks old seedlings grown in nutrient medium containing varying concentrations of Cd^{2+} (control, 0.25, 0.50, 1.0, 2.5 and 5.0 mg L^{-1}). The effect of varying Cd^{2+} concentrations was studied in detail up to 21 days on biomass productivity, plant growth, protein, amino acids, photosynthetic pigments, starch, soluble sugars and essential nutrient uptake to explore the level up to which the plant can withstand the stress of heavy metal. Plants showed symptoms of heavy metal toxicity as observed by various morphological parameters which were recorded with the growth of the plants when treated with 0.5, 1.0, 2.5, and 5.0 mg L^{-1} of Cd^{2+} major biochemical constituents which plays a major role in plant metabolism such as chlorophyll,protein, free amino acid, starch and soluble sugars levels were altered by the heavy metal . The root, shoot-leaf length and the biomass progressively decreased with increasing Cd^{2+}concentration in the nutrient medium. The Cd^{2+}uptake and accumulation was found to be maximum during the initial growth period ,it also interfere with the nutrients uptake, especially Ca^{2+}, Mg^{2+}, K^{+} , Fe^{2+} ,Zn^{2+} ,Mn^{2+} from the growth medium.

Panda and Khan (2003) observed that higher concentrations of Zn and Cr decreased the pigment content in senescing rice leaves with the increasing duration of excision. Accumulation of osmolyte proline was noticed with increasing metal concentrations .Lipid peroxidation measured in terms of thiobarbituric acid reactive substance content increased in senescing leaves concurrently in total peroxide content. Ascorbate content showed higher in under heavy metal treatment, whereas the activities of catalase, guaiacol peroxidase and superoxide dismutase decreased with the increase in the period of excision and metal concentration. The results showed acceleration senescence in rice under heavy metal toxicity stress.

Parida *et al.* (2003) conducted an experiment using an alkaline sandy loam equilibrated with the graded levels of Ni (0, 10, 20, 30, 40, 50,60,70,80, 90,100,150, 200,250 and 300 mg kg^{-1} soil) to investigate the Ni accumulation pattern and its influence on growth and micronutrient distribution in fenugreek plants. They reported that green and dry matter yields of fenugreek increased slightly upto 20 gm Ni kg^{-1} soil but decreased significantly with the application $\geq$ 40mg Ni kg $^{-1}$ soil. The characteristics toxicity symptoms of interveinal chlorosis of the crops were observed in the the pots receiving $\geq$ 40mg Ni kg $^{-1}$ soil. The increasing rates of Ni application increased consistently the total Ni content in the plant tissues. The roots accumulated much higher amount of this element compared to the shoots. The Fe content in the plants showed an increase whereas that of Cu and Zn experienced a decrease content with the rise in the applied Ni.

Skorzynska-Polit *et al.* (2003) studied the changes in the content of reactive oxygen species (ROS) and the activities of the antioxidant system in leaves of *Arabidopsis thaliana* (L.) Heynth exposed to Cd^{2+}. Mature plants growing in the nutrient solutions were treated with Cd^{2+} at different concentrations (0, 5, 25, 50, 100 muM). An increase of O^{2-} content in leaves was observed at the concentrations of 5, 25, and 50 muM Cd^{2+}. A strong accumulation of H_2O_2 was found only at the lowest Cd^{2+} concentration. The content of OH was high at 50 and 100muM Cd^{2+}. The Cd^{2+} treated plants always showed the higher superoxide dismutase (SOD) activity than in control plants. Catalase (CAT) activity decreased with the increasing Cd^{2+} concentration in the nutrient solution. Guaiacol peroxidase (POX) activity was particularly high at the lowest and highest Cd^{2+} concentrations and ascorbate peroxidase (APX) activity additionally at 50muM Cd^{2+}.Strong reduction in ascorbate (AA) content and enhanced activity of monodehydraye ascorbate reductase (MDHAR) were observed at 25 muM Cd^{2+}. Glutathione reductase (GR) activity was always higher than in the control but decreased when the concentration of Cd^{2+} increased. However, it was accompanied by gradual content increase of SH groups.

The effects of copper and lead applied in the form of chloride salts on root, shoot, and leaf growth of the bean (*Phaseolus vulgaris* L.) was studied by Zengin and Munzuroglu (2004). It was observed that both heavy metals significantly prevented the growth of root, shoot and leaves of seedling. A parallel relation was observed between an increase in the concentration of the heavy metal salt and rate of inhibition of root, shoot and leaf growth. Extension of the exposure time to heavy metals of seedling led to greater decrease of root, shoot and leaf growth. It was determined copper had highest toxic effect than lead.

Panda and Choudhury (2004) investigated the changes in nitrate reductase (NR) activity and subsequent induction of oxidative stress in *Polytrichum* under chromium toxicity. It was observed that exposure of Cr at different concentrations for 24 hr and 48 hr reduced the NR activity in moss cells with significant inhibition after 48 hr at 100mM of Cr. Reduction in total chlorophyll content was observed in moss cells after Cr treatment. High accumulation of Cr was seen after 24 hr and 48 hr. Cr prompted the malondialdehyde (MDA) production. Increase in MDA content was followed by activation of antioxidant enzymes like catalase (CAT), guaiacol peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD). Increasing trend of all the enzymes was seen after 24 hr and 48 hr of Cr application. Increase in CAT, GR or SOD was highly significant with the increasing concentration and the duration of the metal treatment. The GPX activity was decreased after 48 hr of Cr exposure.

The changes in fresh weight, total protein contents , concentration of cadmium , and glutathione content in maize kernels cultivated for 5 days at three different Cd concentrations (0, 10 , 100 μmol L^{-1} $CdCl_2$) was studied by Klejdus *et al.* (2004) . Maize kernels exposed to the highest cadmium concentration (100 μmol L^{-1}) germinated formerly and much better. A rapid increase of the fresh weight probably relates with more intensive uptake of water in order to decrease the concentration of cadmium. An intensive preservation of homeostasis of Cd^{2+} ions in the germinating plants by defending mechanisms might explain the differences of uptake rate of cadmium .The defending mechanisms might be triggered by the all studied concentrations of heavy metals at the time of exposure by linear increase of GSH content.

The effect of Cd, Pb, Al, or Cu on *Corchorus olitorius* plant was studied by Mazen (2004). When the plants were treated by 5μg cm^{-1} of Cd, Pb, Al or Cu in hydroponic culture for 6 days, they accumulated 190, 150, 350 and 325 μg g^{-1} (dm) of these metals in the leaves. The sharp rise in amino acid content in the leaf tissues were noted when the plants were exposed to the tested metals; however, the magnitude of accumulation was different from one metal to another. Presence of sulphur in the growth medium significantly increased uptake of Cd and Pb; and cysteine (cyst) was more effective than K_2SO_4. Similarly, addition of salicylic acid (SA) in the growth medium significantly enhanced the ability of *Corychorus olitorius* plant to accumulate all the metals. The plant growth was significantly reduced by the treatment of all the metals except Cu and added cys, K_2SO_4 or SA alleviated the growth retarding effect of these metals.

The potential accumulation of Cd (II), Cr (VI) and Cu (II) in *Convolvulus arvensis* L. using an agar based medium was studied by Torresday *et al.* (2004). The result showed that shoots of the plants demonstrated the capability of accumulate more than 3800mg Cr, 1500 mg of Cd, and 560 mg of Cu kg^{-1} of dry tissues, when the plant was exposed to 20 mg L^{-1} of these heavy metals .This study and the field data reported previously showed that *C.arvensis* is a suitable candidate for the phytoremediation of Cd (II), Cr (VI) and Cu (II) contaminated soils. Furthermore, the concentration of Cr determined in the dry leaf tissue (2100 mg kg^{-1}) indicates that the plant could be considered as a potential Cr-hyperaccumulator.

Backor *et al.* (2004) determined copper uptake, potassium efflux and free proline accumulation in copper enriched liquid cultures of wild- type *Trebouxia erici* as well as in copper tolerant strain. They found that, the highest intracellular copper uptake from 2mM Cu media occurred within 4 hr in both strains, but significantly less accumulated in tolerant species by using inductively coupled plasma atomic emission spectrometry. The copper tolerant strain exhibited significantly more intracellular proline and less potassium efflux than the wild strain. By 24 hr differences between strains in intracellular Cu diminished, as concentrations in both strains reached their highest level. Proline accumulation was decreased significantly at the same time. High copper concentration in agar media after 2weeks of cultivation, showed decreased in growth, pigment content, chlorophyll a degradation and chlorophyll a fluorescence in wild –type of *T.erici*. Proline alleviated the toxic effects of Cu in both strains, but markedly so in case of the tolerant strain.

Alfalfa plants grown in soil at different stages were exposed to separate batches of Cr (VI) at 100 mgL^{-1} and Cd (II), Cu (II), Ni (II), or Zn (II) at 500 mgL^{-1} (Videa *et al.* 2004). Four days after germination, all metals, except Zn (II), had lethal effects on the seedlings. Furthermore, when the heavy metals were applied 16 days after germination, Cr (VI) and Ni (II) still had lethal effects on the seedlings and Cd (II) and Cu (II) destroyed more than 50% of the plant populations. While approximately 90% of the plants exposed to Cd (II), Cu (II) and Zn (II) were able to grow without apparent negative effects on 20 days after germination, but Cr (VI) and Ni (II) still showed lethal effects. The heavy metal concentration in shoot dry tissues was 1209 mg kg^{-1} for Cd, 887 mg kg^{-1} for Cu and 645 mg kg^{-1} for Zn. The results demonstrated that the tolerance of alfalfa plants to Cd, Cu and Zn was positively correlated with the age of the plants. From these results possibility of using alfalfa plants, via transplant, to clean up soils was opened, where the concentration of Cd, Cu or Zn is high enough to avoid the alfalfa seed germination.

The concentrations of lead, zinc, copper, and cadmium accumulated by 12 emergent rooted wetland plant species including different populations of *Leersia hexandra, Juncus effuses* and *Equisetum ramosisti* were investigated in field conditions of China by Deng *et al.* (2004). The results showed that metal accumulation by wetland plants differed among species, populations and tissues. Metal accumulation by wetland plants were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. Population grown in substrata with the elevated level of metal concentration contained significantly a higher metal concentration in plants. They observed that some plant species /populations could accumulate relatively higher metal concentrations (far above toxic concentration to plants) in their shoots, which indicated the internal detoxification of metal tolerance mechanism(s) were also included. The metal accumulation of wetland plants was dependent on some factors like metal concentrations, pH and the nutrient status in the substrata. Mostly concentrations of Pb and Cu in both aboveground and underground tissues of the plants were significantly positively related to their total and /or DTPA –extractable fractions in substrata while negatively to soil N and P respectively.

Several complementary studies were carried out by Viard *et al.* (2004) to asses the contamination induced by traffic at the vicinity of a highway (A31, France), on two sites, with different profiles and traffic intensity. Concentrations of zinc, lead and cadmium were measured by atomic absorption spectrophotometry in deposits, roadside soil and autochthonous plants (Graminaceae) gathered at the vicinity of the highway (1-320 m). According to the results obtained for different compartments, the highway induces a contamination on the surrounding environment, up to 320 m, but the maximum contamination observed between 5 and 20 m, the concentrations measured in plants at the vicinity of the highway were 2.1.mg Pb kg^{-1} DW, 0.06 mg Cd kg^{-1} DW, 62 mg Zn kg^{-1} DW and the concentrations measured in snails were 21.3 mg Pb kg^{-1} DW, 5.7 mg Cd kg^{-1} DW ,510.8 mg Zn kg^{-1} DW . The levels decreased with increasing distance from the highway. The study revealed that, among the three metals lead indicated the best metal to evaluate road transport contamination.

Barazani *et al.* (2004) conducted an experiment in which the ability of *Allium schoenprasum* L. (chives) to accumulate and tolerate cadmium in aqueous Hogland medium at 50μM and 250 μM were tested under continuous growth or several successive harvests of shoots. After 28 days of continuous growth, chives accumulated the heavy metal up to 0.2% and 0.5% of its dry weight, when grown in 50 μM and 250 μM, respectively. The leaves were harvested in every 16 days; there were no obvious stress symptoms after six harvests during a period of 96 days at 50 μM Cd, whereas, at 250 μM, after 64 days and four harvests, inhibition of growth occurred. A total of 1.2g kg^{-1} DW and 2.4 kg^{-1} DW was accumulated in the leaves, respectively in each treatment. Total SH compound concentration in leaf was found significantly higher by 3 and 7.4 times in plants treated with Cd at 50 μM and 250 μM in comparison to the control, respectively, while no difference in the concentration of glutathione (GSH +GSSG) were found. Thus it is assumed that sulpher containing compounds, yet unknown, are involved in defensive mechanisms against heavy metals in chives. The results pointed to chives phytoremediation potential, but also on the potential risk in accumulation of heavy metals in commonly edible plants.

Ajasa *et al.* (2004) conducted an experiment in which the concentration levels (ppm) of selected toxic metals (Fe, Mn, Cu, Pb and Zn) and macronutrients (Na, K, Mg and Ca), along with P, were estimated in some of the important herbal plants of the southwest part of Nigeria. The atomic absorption spectrophotometer was used for the estimation of heavy metals on 10 plant species collected from different locations within Ogbomoso. The plants used for the study were *Anacardium occidentale, Azadirachta indica, Butyrospermum paradoxum, Mangifera indica, Morinda lucida, Ocimum canum, Solanum erianthum, Solanum torvum, Zingiber officinale and Hyptis suaveolens*. The metal contents in the samples were found at different levels. The highest mean levels (ppm) of Zn (35.1± 0.01) and Cu (24.4 ±0.01) were found in *Hyptis suaveolens* while those of Mn (685± 0.02) and Ca (51340±21) were found in *Morinda lucida.* Their results also showed that *Ocimum canum* had the highest amount of K (36600± 350), P (3700± 35) and Fe (241± 0.05). *Anacardium occidentale,* had the highest concentration of Na (613± 0.60) while *Azadirachta indica* had the highest mean concentrations of Pb (0.49±0.03) and Mg (5630± 12).

Cucumis sativus (cucumber) was tested to assess an ecotoxicity in soils contaminated by heavy metals copper, cadmium and lead separately and in combinations. The growth of the plant was the toxicity endpoint, measured as shoot and root lengths after 5 days exposure. Sum of toxic unit (TU) at 50% inhibition for the mixture ($EC50_{mix}$) was calculated from the dose (TU-based) response relationships by the Trimmed Spearman-Karber method. Binary metal combinations of Cu+Cd, Cu+Pb, and Cd+Pb produced all three types of interactions; concentration additive ($EC50_{mix}$=1TU), synergistic ($EC50_{mix}$<1 TU), and antagonistic ($EC50_{mix}$>1 TU), responses. Ternary combination of Cu+Cd+ Pb produced an antagonistic response for the growth of *Cucumis sativus*. Bioaccumlations of the heavy metals like Cu, Cd and Pb were observed in *Cucumis sativus*, the accumulation of one metal was influenced by the presence of other metals in metal mixtures. In general, antagonistic and /or synergistic responses reflected bioaccumulation patterns in some binary combinations, but the patterns in mixtures were not always consistent with toxicity data. The study indicated that TU approach appears to be a good model to estimate the combined effect of metals in plant systems, and mixture toxicity may be closely-related to the bioaccumulation pattern within plants (An *et al.* ,2004).

Cadmium extraction potential and degree of resistance to Cd stress was determined in ten *B.juncea* cultivars (V_1- V_{10}) commonly grown in India by Quadir *et al.*(2004). Ten days old seedlings of *B.juncea* cultivars were exposed to various levels of cadmium chloride (0.0 – 2.0 mM) for 72 hr in hydroponics culture and the leaf samples were analyzed at 24, 48 and 72 hr after treatment (HAT) for the changes in the rate of lipid peroxidation, plant length, biomass accumulation, cadmium accumulation and activities of catalase (CAT 1.11.1.6) ,superoxide dismutase (SOD 1.15.1.1), ascorbate peroxidase (APX 1.11.1.11) and glutathione reductase(GR 1.6.4.2) along with ascorbate (Asc) and glutathione contents. A reduction in the plant length, biomass accumulation, CAT activity and ascorbate content was noted in all the cultivars, however, a significant increase in lipid peroxidation rate, Cd accumulation, activities of APX, GR, SOD and glutathione content was observed in *B.juncea* cv. Pusa Jai Kisan (V_5) showed the least increase in the lipid peroxidation rate but accumulated higher levels of biomass, Cd and glutathione contents among the studied cultivars. The results indicate that, cv. Pusa Jai Kisan possesses a better Cd sequestering and antioxidant system. They suggested that high increase in the levels of glutathione indicates its possible incorporation in synthesis of the phytochelatins and metallothioneins to sequester Cd and to combat Cd stress.

Growth responses were analyzed in *Prunus cerasifera,* a peach root stock after exposure to various copper concentrations, by Lombardi and Sebastiani (2005). The plantlets tolerated Cu concentrations up to 50µM and showed improved Fe uptake unexpectedly under low to moderate concentrations (from 0.1 to 50µM). At 100 µM of Cu, plantlets reduced relative growth rate for both fresh and dry weight and severe browning were developed which progressed to necrosis. CAT and SOD activity levels and the modulation of transcription of catalase and superoxide dismutase genes were analyzed in the plant after exposure to various concentrations of copper. The total catalse and superoxide dismutase and simultaneous induction of gene expression of *Sod* and *Cat* were observed under Cu toxicity. The result demonstrated that, the plant is quite tolerant to the metal and mobilizes catalase and supeeroxide dismutase in order to mitigate Cu-stress damages.

The giant reed (*Arundo donax* L.) plant which grew on the surface soil and irrigated with mixed heavy metal solutions of Cd (II) and Ni (II) were tested to study the impact of these heavy metals on growth and photosynthesis by Papazoglou *et al.* (2005). The tested concentrations were 5, 50, and 100 ppm for each heavy metal against the control and resulted in high cadmium and nickel (DTPA extractable) concentrations in the top zone of the pot soil. The stem height and diameter, no of nodes, fresh and dry weight of leaves, and net photosynthesis (Pn) were examined, the results indicated that the parameters were not affected by the heavy metals and thus indicating that plants tolerate the high concentrations of Cd and Ni. They suggested from the study that, the giant reed plants are very promising energy plants, and they can be cultivated in contaminated soils to provide biomass for energy production purposes.

Plants of two mungbean genotypes MH 85-111 and MH 98-6 were exposed to different levels of cadmium 28 days after sowing. Plants exposed to 3.0 and 4.0 mM Cd^{2+} did not survive and died before entering into the reproductive phase (Kumar and Dhingra, 2005). Cadmium induced reduction in the number of flowers and *in vitro* pollen germination but did not affect the pollen viability. However, it stimulated the growth of the tube. Cadmium although did not affect the pistil length, it decreased number of ovules/pistil. Ovules were morphologically normal and receptive. *In vivo* stylar studies revealed all the ovules were not penetrated by pollen tube and number of unpenetrated proximal ovules was increased by Cd^{2+} and cv. MH 85-111 was affected more adversely than MH 98-6. Cadmium inhibited the number of pods, seeds, seed weight / plant and 100 seed weight, inhibition being more in MH 85-111 than MH 98-6. Cadmium treatment did not affect starch content but increased protein content in physiologically mature seeds. Accumulation of Cd^{2+} was maximum in the roots and least in the seeds. Cadmium accumulation, in general was higher in MH 85-111 than MH 98-6 and stem of MH 85-111 accumulated four times Cd^{2+} than MH 98-6. Seed cadmium however, was comparable in both the genotypes.

Rai *et al.* (2005) studied the effect of different concentration of Cd on *Phyllanthus amarus* Schum. and Thonn.; because *P. amarus* is mostly grown as weed in agricultural and waste lands and is a reputed medicinal plant used in Indian indigenous system of medicine with hepatoprotective, diuretic, stomachic properties and is recently being used for the treatment of hepatitis B. Result showed that, Cd causes significant decrease in fresh and dry weight, length of root and shoot, protein, chlorophyll, caritenoid and and starch content was increased. Moreover, ultra morphological changes were also observed in stomatal opening and wax deposition on both the surfaces of leaves. They noted that, the therapeutically active compounds –phyllanthin and hypophyllanthin, enhanced at certain levels of Cd due to abiotic stress.

In an experiment by Chaoui and Ferjani (2005) 12 days old seedlings of pea were treated for 4 days by 20 and 100μM of $Cd(NO_3)_2$ or $CuSO_4$. The result showed that, in leaves, all treatments caused an increase in the lipoperoxidation product rate but 20 μM of Cu did not affect the growth. Moreover, except for 20 μM of Cu, the activity of unspecific peroxidases, used as stress marker, was enhanced in cell walls of metal-stressed plants. Though no change in antioxidant capacities were observed in plants treated with the same metal concentration. The Cd–reduced growth could be associated to an elevation in the activities of IAA oxidase and of lignifying peroxidases was observed at the same dose. Increase of these latter, with the loss in antioxidant capacities, would be responsible for the growth diminution after exposure to 100 μM of the metal.

An experiment with radish seedlings exposed to 0.25 and 1.0 mM of $CdCl_2$ for 24 hrs was carried out by Vitoria *et al.* (2005). Result exhibited the structural changes of the chloroplasts, mitochondria and nuclei when compared to non-treated control plants. Changes in the organelle shape, an increase in the stroma volume and a deposition of electron-dense material in the double membrane of the chloroplast was observed when the plant was exposed to Cd^{2+} stress. The changes in the chloroplast membranes were not so very drastic; however, a reorganization of the thylakoids and stroma could be detected. In contrast, the breakdown of the nuclear envelope of the plant cells treated with Cd^{2+} was very clear. The accumulation of electron dense granules was also observed in mitochondria. No alterations were observed in the vacuoles of radish seedlings grown at different Cd^{2+} concentrations for the period tested.

In order to understand the difference between Zn, an essential micronutrient and Cd, a non-essential element, Cd-10μM and Zn supplemented (10, 50, 100 and 200 μM) Cd 10 μM treated *Ceratophyllum demersum* L.(Coontail),a free floating freshwater macrophyte was chosen for as study conducted by Aravind and Prasad (2005) Cadmium at 10 μM concentration decreased thiol content , enhanced oxidation of ascorbate (AsA) and glutathione (GSH) to dehydroascorbate (DHA) and glutathione disulfide (GSSG), respectively, a clear indication of oxidative stress. Zinc supplementation to Cd (10 μM) treated plants effectively restored thiols, inhibited oxidation of AsA and GSH maintaining the redox molecules in reduced form. Cd 10 μM slightly induced ascorbated peroxidase (APX, E.C.1.11.1.11) but inhibited monodehydroascorbate reductase (MDHAR, E.C.1.6.5.4), dehydroascorbate reductase (DHAR, E.C. 1.8.5.1) and glutathione reductase (GR, E.C. 1.6.4.2), enzymes of ascorbate-glutathione cycle (AGC).Zn supplementation restored and enhanced the functional activity of all the AGC enzymes (APX, MDHAR, DHARand GR). Glutamylcysteine synthatase (GCS, E.C. 6.3.2.2) was not affected by Cd as well as Zn, but Zn supplements increased glutathione –S-transferase (GST, E.C.2.5.1.18) activity to a greater extent than Cd and simultaneously restored glutathione peroxidase (GSH-PX, E.C.1.11.1.9) activity impaired by Cd toxicity. Zn-alone treatments did not change above investigated parameters. These results clearly indicate the protective role of Zn in modulating the redox status of the plant system through the antioxidant pathway AGC and GSH metabolic enzymes for combating Cd induced oxidative stress.

Smeets *et al.* (2005) did an experiment in which oxidative stress has been shown to be of great importance in the toxicity of several metals. The relationship of cadmium phytotoxicity and antioxidative reaction in bean (*Phaseolus vulgaris* L.) plants was investigated. Eleven days old seedlings were exposed to an environmentally realistic concentration of cadmium (2 μM $CdSO_4$). The antioxidative defence mechanism was significantly activated after 24 hr of cadmium exposure. Some enzymes capable of quenching reactive oxygen species (syringaldazine peroxidase, EC 1.11.1.7; guaiacol peroxidase, EC 1.11.1.7) as well as enzymes important in the reduction of NAD $(P)^+$ (isocitrate dehydrogenase , EC 1.1.1.42: malic enzyme, (EC 1.1.1.40) were significantly elevated by cadmium exposure. Furthermore, the ascorbatte-glutathione cycle appeared to be a very important mechanism against cadmium –induced oxidative stress. In leaves, significant increase of ascorbate peroxidase (EC 1.11.1.11) and glutathione reductase (EC 1.6.4.2) and significant changes in the ascorbate and glutathione pool were observed. Morphological and other biochemical parameters (lipid peroxidation) were significantly enhanced 48 hr after the start of the cadmium exposure. At the end of the experiment (72h after the start of the metal treatment), even visual effects, such as chlorosis, were observed. The result showed that, cadmium, like other metals, induces cellular redox disequilibrium suggesting that an environmentally realistic concentration of cadmium can cause oxidative stress.

Treatment of rape seedlings with increasing $CdCl_2$ concentrations in the culture medium resulted in a cadmium accumulation within plant tissues, which increased with external metal doses; such accumulation was more important in roots than in leaves reported by Youseef *et al.* (2005). Biomass production was severely inhibited, even at low cadmium concentration. They also reported that the metallic ion seemed to affect selectively chloroplastic membranes due to an inhibition of polyunsaturated fatty acid biosynthesis. Moreover, a lipid peroxidation occurred due to the spectacular increase of malondialdehyde (MDA) content observed in cadmium treated leaves.

Soyabean seedlings treated with 6 mg kg^{-1} Cd during 72 hr induced a slight growth inhibition in roots, stems and leaves as observed by Drazic and Mihailovic (2005). A significant desiccation of cotyledons and leaves with a decrease in chlorophyll content in leaves were observed. Application of salicylic acid (SA) applied simultaneously at the concentrations of 10^{-6}, 10^{-5} and 10^{-4} M significantly alleviates the effect of Cd. Cd and SA act synergistically on K content inducing its effect on the significant decrease in roots. Under the influence of Cd, Fe content was decreased in roots and increased in leaves, while SA removes the effect. Magnesium content was substantially decreased in root and stem under the influence of Cd, SA attenuated the the effect of Cd only in roots, while in leaves it induces a significant increase of the content of this element. Cd uptake was not decreased by SA, but changes its distribution in plant organs depending on the concentration of added Cd. The result indicated that, the influence of SA on the alleviation of toxic effects of Cd was indirect, through a development of general antistress response of the seedlings which includes also the regulation of K and Mg distribution.

Panda and Choudhury (2005) reported the toxicity of chromium in plants. Chromium is known to be a toxic metal that can cause the severe damage to plants and animals. Chromium induced oxidative stress involves induction of lipid peroxidation in plant that cause the severe damage to cell membranes. Oxidative stress induced by chromium initiates the degradation of photosynthetic pigments causing decline in growth. High chromium concentration can disturb the chloroplast ultra structure thereby disturbing the photosynthetic process. Like Cu and Fe, Cr is also a redox metal and its redox behavior exceeds that of other metals like Co, Fe, Zn, Ni etc. The redox behavior can thus be attributed to the direct involvement of chromium in inducing oxidative stress in plants. Cr can affect the antioxidant metabolism in plants. Antioxidant enzymes like SOD, CAT, POX and GR are found to be susceptible to Cr resulting in a decline in their catalytic activities. However, both metallothioneins and organic acids are important in plants as components of tolerance mechanisms and are also involved in detoxification of this toxic metal.

Singh and Agarwal (2005) reported the effect of different heavy metal salts on the growth, yield and metal accumulation pattern of wheat (*Triticum aestivum*) cv. HD

2285. The studies revealed that, application of heavy metals in soil before sowing caused varying extent of reduction in yields of wheat. Mercury caused maximum reduction in biological as well as economic yields followed by copper, lead and cadmium, while zinc did not affect the growth and grain yield of wheat markedly. The number of spikes/pot and grains / spike were reduced, while 1000grain weight increased significantly by the application of copper, lead, and cadmium in soil. The heavy metal stress, however did not affect the harvest index of wheat plants. The content of all the tested metals increased both in straw and grain by their application in the soil, but their accumulation was much higher in vegetative shoots (straw) than in reproductive shoot (grain). However, zinc registered higher content in grain than in straw of wheat plants. The content of metals in wheat shoots was in the order of Zn > Cu > Cd > Pb. The larger proportion of both essential (Cu) and toxic metals (Pb and Cd) absorbed by wheat plants thus remained in straw and small proportion of the same only transported to edible part (grains).

Metwally *et al.*(2005) carried out an experiment to evaluate the correlation between selected biochemical responses to toxic C;d and the degree of Cd sensitivity in a set of pea (*Pisum sativum* L.) genotypes. Ten genotypes were analyzed that differ in their growth response to Cd when expressed as root or shoot tolerance indices (T/s). Concentrations of non protein thiols (NPTs) and malondialdehyde (MDA) , activity of chitinase ,peroxidase (POX), and catalase significantly increased in all genotypes of pea treated with Cd; Cd sensitivity genotypes was correlated with relative increase in MDA concentrations as well as activities of chitinase and POX , suggesting similar Cd stress effects. Activities of ascorbate peroxidase (APX) decreased, but concentrations of glutathione (GSH) increased in the less Cd-sensitive genotypes. Differences in root leaf contents of Cd revealed no change with TI, metabolic parameters, and enzyme activities in Cd treated plants, respectively, except that, shoot Cd concentration positively correlated with shoot chitinase activity. Toxic Cd levels inhibited the uptake of nutrient elements such as P, K, S, Ca, Zn, Mn, and B by plants in an organ and genotype specific manner. Cd sensitivity was significantly correlated with decreased root Zn concentrations. The results showed that both the similarities, as well as distinct features, in Cd toxicity expression in genotypes of one species, suggesting that independent and multi-factorial reactions modulate Cd sensitivity on the low- tolerance level of plants. The study illustrates the biochemical basis of earlier detected genotypic variation in Cd response.

Ranieri *et al.* (2005) indicated that, the bread wheat (*Triticum aestivum* L.) cv. Albimonte can be defined as "shoot cadmium excluder" – by comparing the cadmium (Cd) content in leaves and roots and by calculating the shoot –to –root cadmium concentration ratio. Furthermore, they evaluated whether the excess Cd exposure could generate oxidative stress in leaves and roots of this cv., in terms of hydrogen peroxide (H_2O_2) accumulation , NAD(P)H oxidation rate , and variations in reduced glutathione (GSH) content and peroxidase (POD, EC 1.11.1.7) activity. Finally they surveyed possible quali-quantitative differences in thiol –peptide compound pattern between roots and leaves, in order to verify whether phytochelatins (PCs) and related thiol-peptides could contribute in limiting Cd –induced oxidative stress. Unambiguous characterization of PCs and related forms present in the root samples was obtained by electrospray ionization mass spectrometry (ESI-MS) and ESI –tandem MS (ESI-MS/MS). The result revealed that, in leaves the stress generated by the low accumulation of Cd (due to a moderate translocation in plant) seems to be counteracted by the antioxidant response and by the PC biosynthesis. On the contrary, in roots, in spite of the elevated presence of PCs and related thiol-peptide –compounds, the excess of Cd causes a decline in the antioxidant protection of the organ, with the consequent generation of considerable amounts of H_2O_2, which is a direct agent of the oxidative stress.

The influence of two heavy metal salts lead and cadmium ($Pb^{2+}$and Cd^{2+}) on plants, including plant and root size, plant genome stability as well as global genome expression were analyzed by Kovalchuk *et al.*(2005). Metal uptake was measured and showed that, there was a significantly higher incorporation of Cd than of Pb, 0.6 and 0.15uM g^{-1} of dry weight respectively. The analysis of the root length and plant size showed a dose dependent decrease in plants exposed to Cd. In the contrary, there was little difference in the size of plants exposed to Pb, although there was nearly four-fold increase of the root length. Analysis of the genome stability revealed that Cd lead to a dose dependent increase of homologous recombination whereas Pb had no effect on the same. Analysis of the global genome expression of plants chronically exposed to 50uM of Cd and Pb revealed 65 and 338 up and down regulated genes by Cd and 19 and 76 by Pb, respectively. The result indicated interestingly that, half of the genes that changed their expression in Pb- treated plants also changed their expression in Cd- treated plants. The greater number of genes regulated by Cd reflects generally higher genome instability of plants as well as higher uptake as compared to Pb.

Treatment of different concentrations of both hexavalent and trivalent chromium on *Azolla pinnata* resulted in significant biochemical and oxidative aberrations was investigated by Upadhyay and Panda (2005) .Total peroxide was increased, while lipid peroxidation decreased, as indicated by malondialdehyde formation after 48 hr of treatment. Ascorbate and glutathione contents increased under chromium treatment. Catalase activity was decreased whereas guaiacol peroxidase, glutathione reductase and superoxide dismutase were increased along with the period of metal treatment. These results suggest that, acute chromium toxicity induces oxidative damage in the plant.

In a study by Aina *et al.*(2006) rice seedlings were exposed to a range of Cd concentrations (0.1μM, 1 μM, 10 μM, 100 μM and 1mM) for 15 days and a combination of different molecular approaches were used to evidence Cd effects and to asses the plants ability to counteract metal toxicity. Only the highest concentration of Cd (1mM) caused a complete plant growth inhibition, whereas, the lowest concentrations seemed to stimulate the growth. At the genome level, the amplified fragment length polymorphism (AFLP) technique was applied to detect DNA sequence changes in root cells, showing that all the Cd concentrations induced significant DNA polymorphisms in a dose-dependent manner. Data also revealed the absence of preferential mutation sites. Plant responses were analyzed by measuring the levels of glutathione (GSH) and phytochelatins (PCs), the thiol –pepetide involved in heavy metal tolerance mechanisms. Result showed a progressive increase of GSH up to 10μM, of Cd treatment, whereas a significant induction only PC3 was detected in roots of plants exposed to 100 μM of Cd. As suggested by the proteome analysis of root tissues, this last concentration strongly induced the expression of regulatory proteins and some metabolic enzymes. Furthermore, the treatment with 10μM, of Cd induced changes in metabolic enzymes, but it mainly activated defense mechanisms by the induction of transporters and proteins involved in the degradation of oxidatively modified proteins.

Page *et al.*(2006) carried out an experiment on seedlings of wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) radiolabelled for 24 hr with 65 Zn , 109 Cd, 54Mn, and 57Co via one seminal root (wheat) or via the main root (lupin). The plants were grown on rhizoboxes containing soil and the samples were collected throughout the experiment and was analysed afterwards for their radionuclide contents. A strong retention in the labeled part of the root was observed for 57Co in wheat and lupin and for 109 Cd in lupin, while 65 Zn and 54 Mn were transported to the shoot in both plants. 65 Zn was redistributed via the phloem from older to younger leaves, 54 Mn accumulated in the first leaves and no major redistribution within the shoot was observed. 109Cd was present in the shoot of lupin. The redistribution of 65Zn, 109Cd, 54Mn and 57 Co in phloem differed between wheat and lupin. The 65Zn content in the wheat roots appearing after the labeling phase represented 34% of the total content in the plant at the end of the experiment and less than 3% remained in the labeled root, while a high percentage of 65Zn was retained in the originally labeled part of the main root of lupin. The root system of wheat and lupin accumulated smaller quantities of 109 Cd, 54Mn and 57 Co. Nevertheless, heavy metals were found in rhizosphere soil (1-2 mm soil around the roots) and bulk soil (no contact with roots) from both plants. Higher quantities of heavy metals were found in the rhizosphere soil close to the labeled part of the roots. 65Zn was present in large quantities in the rhizosphere soil close to all parts of the root system of wheat. 65Zn, 109Cd, 54Mn, and 57Co were found in the bulk soil for both plants, indicating that, the plant itself might play a role in the redistribution of heavy metals in the soil around its own roots. Phloem-mobile elements may be transported to growing parts of the system and may reach deeper soil layers. The redistribution of heavy metals in the soil may be in vertical and horizontal directions; at least as far as the root system grows.

The effects of different levels of industrial wastes on growth traits and metal accumulation in aerial portions of *Populus* x *euramericana* clone I-214 were evaluated by Giachetti and Sebastiani (2006) .The experiment was started in April 2003. Scions of *Populus* x *euramericana* clone I-214 were grown outdoor near Pisa (Italy), in lysimeters filled with soil naturally present in the land around the experimental site. The climatic parameters were recorded daily throughout the whole experiment and growth relieves were performed during the growing season. The four increasing treatments were applied: soil non- amended, soil amended with 4.8 kg m^{-2} , with 9.6 kg m^{-2} and with 19.2 kg m^{-2} of fresh tannery waste. After six months since the plantation of the scions, aerial portions of every plant were harvested for biomass and metal content analysis. The results indicated that the waste exerted beneficial effects on poplars mainly through a general increase of growth traits and that the nutrient relocation is the mechanisms were involved in modulating the growth rate. The concentration and the amount of the mineral elements were analyzed (N, P, K, Na, Ca, Mg, S, B, Fe, Mn; Cu, Zn, Cr) changed the determinately among treatments, organs and position. They concluded from the study that, phytoremediation strategies of tannery wastes might be possible and sustainable for the polar plantations in soil amended with non-hazardous levels of industrial waste, which maintain total heavy metals concentration close to the background levels.

Labra *et al.* (2006) carried out an experiment to examine the influence of different concentrations of potassium dichromate on the *Zea mays* L. plantlets. A clear effect of chromium on maize plantlets growth and germination of seeds was observed starting from 100-300 ppm up to 1500 ppm. The heavy metal Cr uptake was dependent on the concentration of the metal in the medium. They reported that the metallothioneins, involved in the binding of heavy metal, were measured by capillary electrophoresis (CE) , and showed a dose-response induction . Differential expressions of several proteins were analyzed by two dimensional gel electrophoresis for the protein profile study.Their results showed that, proteins induced by heavy metal exposure are principally involved in oxidative stress tolerance or in other stress pathways. Inductions of proteins were implicated in sugar metabolism. They inferred that the identification of factors involved in plant responses may lead to a better understanding of the mechanisms involved in cell protection and tolerance.

In an investigation by Li-an *et al.* (2006) the growth responses of *Poa pratensis* to the different heavy metal stresses of Cu^{2+}, Zn^{2+}, Cd^{2+},and Pb^{2+} at different concentrations were studied by sand culture. The results showed that, with Cu^{2+}, Zn^{2+},

Cd^{2+} concentrations reaching 100 mg L^{-1}, both the seed germination rates and young – seedling heights of *Poa pratensis* declined to some extent and their decrements increased as the heavy metal concentration increased . Pb^{2+} did not show the significant effect on these two indexes. Cu^{2+} significantly inhibited the root and above ground biomasses and the growth of the root was also inhibited. Cu^{2+}concentrationof 600 mg L^{-1}, the root length was decreased by as high as 96.67%, compared with those in control. With Zn^{2+}, Cd^{2+} and Pb^{2+}concentrations going above 200 mg L^{-1} , the root and the above ground biomasses of the plant appeared to be inhibited , and the inhibitory effects became intensified as the concentration increased. The four heavy metals appeared undifferentiated in the action pattern of chlorophyll, i.e. they enhanced chlorophyll synthesis with their concentrations standing below 200 mg L^{-1} and the chlorophyll content was declined as their concentration continued to increase after reaching 200 mg L^{-1}.

One of the adverse effects of heavy metals on plants is the generation of harmful active oxygen species, leading to oxidative stress (Michalak, 2006). The results obtained by the authorshowed that, during the heavy metal stress phenolic compounds can act as metal chelators and on the other hand phenolics can directly scavenge molecular species of active oxygen. It was concluded from the study that, phenolics, especially flavonoids and phenylopropanoids, are oxidized by peroxidase, and act in H_2O_2 scavenging, phenolic/ASC/POX system.

The efficiencies of neutral salts, strong acids, and chelates for extracting cadmium from three paddy soils were examined by Makino *et al.*(2006). The test results showed that, higher the selectivity of cations of the added neutral salts toward soil adsorption sites, the lower the pH in the extracts and the more soil Cd could be extracted . In addition, soil carbon and nitrogen content and mineral composition were closely associated with the amount of Cd extracted. Calcium chloride and iron (III) chloride were selected as wash chemicals to restore Cd-contaminated paddy soils in situ. They concluded that, washing with calcium chloride led to the formation of Cd chloride complexes, enhancing Cd extraction from the soils. The washing substantially decreased soil levels of exchangeable and acid –soluble Cd, which are the major forms of bioavailable Cd for rice (*Oryza sativa* L.).

Copper (Cu) from various anthropogenic and natural sources plays one of the major heavy metal contaminants in the environment was reported by Xiong *et al.*(2006). Cu induced nitrogen (N) metabolism damage in the popular vegetable Chinese cabbage (*Brassica pekinensis* Rupr.) were studied in aquatic culture experiments with this plant were done. For the aquatic culture experiment two Cu levels [0.3 μ mol L^{-1} (control) and 10.3 μ mol L^{-1}] and two N levels (0.05- and 1 fold Hogland's solution) were used. The results demonstrated the adverse effect of Cu on N metabolism and plant growth. Cu exposure elevated Cu concentration in the roots and shoots. The root length was also shortened and fewer leaves were produced and the biomass was lowered by the Cu exposure. The results were also demonstrated effects of N deficiency on N metabolism and plant growth. N deficiency increased the ratio of root/shoot biomass. In addition, there were interactive effects between Cu exposure and N level on plant biomass and root/shoot ratio. The results suggested that, Cu toxicity to the plant was at least partly due to an influence of N metabolism. The study revealed that, Cu exposure decreased nitrate reductase (NR) activity in the roots and shoots; the total chlorophyll content was also reduced. Treatment increased the total free amino acid content in the leaves and decreased the nitrate contents and NR activity in roots and leaves. In addition the interactive effects between Cu exposure and N level on chlorophyll and nitrate content in the leaves.

Pendergrass and Butcher (2006) conducted an experiment where carrots, lettuce, and tomatoes were cultivated in a greenhouse in control soil and soil with elevated levels of lead and arsenic. The samples were analysed for Pb and As using inductively coupled plasma optical emission spectrometry (ICP-OES). Except for carrot roots grown in the contaminated soil, the concentrations of Pb and As in the plants were below the ICP-OES detection limit. The concentration of Pb in carrot roots was $20\pm11\,\mu g\ g^{-1}$, which represents a bioconcentration factor (BCF) of 0.03.

Yoon *et al.* (2006) investigated whether phytoremediation can be potentially used to remediate metal contaminated sites. They evaluated the potential of 36 plants (17 species) growing on a contaminated site in North Florida. The total metal concentrations of plants and the associated soil samples were analyzed. While total soil Pb, Cu, and Zn concentrations varied from 90 to 4100, 20 to 990, and 195 to 2200 mg

kg^{-1}, those in the plants ranged from 2.0 to 1183, 6.0 to 460 and 17 to 598 mg kg^{-1}, respectively. None, of the plants were suitable for phytoextraction because no hyperaccumulator was identified. The plants with a high bioconcentration factor (BCF, metal concentration ratio of plant roots to soil) and low translocation factor (TF, metal concentration ratio of plant shoots to roots) have the potential for phytostabilization. *Phyla nodiflora* was the most efficient in the accumulation of heavy metals like, Cu and Zn in shoots (TF=12 and 6.3) while, *Gentiana pennelliana* was most suitable for phytostabilization of sites contaminated with Pb, Cu and Zn (BCF=11, 22 and 2.6). Three metal uptakes were highly correlated, whereas translocation of Pb was negatively correlated with Cu and Zn though translocation of Cu and Zn were correlated. From the result they concluded that, native plant species growing on contaminated sites may have the potential for phytoremediation.

In an experiment to study the effect of low levels of heavy metals on plant growth, biomass turnover and reproduction for *Hieracium pilosella*, plants were grown for 12 weeks on substrates with different concentrations of heavy metals obtained by diluting contaminated soils with silica and sand. The result showed that, the more metal-contaminated soil the substrate contained, the lower the leaf production rate and plant mass. The phonological development was also delayed. The flowering phenology was very sensitive to heavy metals. Leaf life span was reduced at the highest and the lowest metal levels, the latter being a result of advanced seed ripening. Even if the effect of low metal levels on plant growth may be small, the delayed and reduced reproduction may have large effects at population, community and ecosystem level, and contribute to rapid evolution of metal tolerance (Ryser and Sauder, 2006).

Stephen *et al.* (2006) conducted a study to evaluate the use of reclaimed lake sediment as a growth media for vegetable production and to estimate whether accumulation of micronutrients and heavy metals in the vegetables would impact human nutrition or health, respectively. Five plant species, bean (*Phaseolus vulgaris* L.), broccoli (*Brassica oleracea* L.), carrot (*Ducus carota* L.), pepper (*Capsicum annum* L.) and tomato (*Lycopersicon esculantum* L.) were grown in pots containing either reclaimed sediment from the Illinois River or a reference soil. Edible and vegetative tissues from the plants were analyzed for 19 elements, including As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se and Zn. Tomato and pepper plants grown in sediment showed significantly greater biomass and yield as compared to plants from the reference soil. The elemental study of the tissues showed that Zn and Mo were only significantly greater in sediment-grown plants on a consistent basis. While significant, Zn concentrations were no more than 3 fold higher than those in plants from the reference soil. The same trend was observed for Mo except for bean tissues, which showed a 10 fold greater concentration in sediment-grown plants. The result revealed that, this reclaimed sediment can be utilized for the production of vegetables intended for human consumption. The result also suggest that, sediment material with similar physicochemical characteristics and elemental concentrations that fall within the pertinent regulatory guidelines should also be a suitable safe medium for vegetable production.

Heavy metal contamination of soil resulting from waste water irrigation is a cause of serious concern. A potential health impact on consuming contaminated product was reported by Sharma *et al.* (2007). They analyzed the impact of waste water irrigation on heavy metal contamination of *Beta vulgaris*, a highly nutritious leafy vegetable that is widely cultivated and consumed in urban India. A field study was conducted at three major sites that were irrigated by either treated or untreated waste water in the suburban areas of Varanasi, India according to normal practice. Samples of irrigation water, soil, and the edible portion of the (*Beta vulgaris* L. var All green H1) were collected monthly during the summer and winter seasons and were analyzed for Cd, Cu, Zn, Pb, Cr, Mn, and Ni. The result showed that, heavy metals in irrigation water were below the internationally recommended (WHO) maximum permissible limit set for agricultural use for all heavy metals except Cd at all the sites. The mean heavy metal concentrations in soil were below the Indian standards for all heavy metals, but the maximum value of Cd recorded during January was higher than the standard value. During summer, in the edible portion of the plant the Cd concentration was higher than the permissible limits of the Indian standards, whereas Pb and Ni concentrations were higher in both summer and winter seasons. The results of linear regression analysis computed to assess the relationship between individual heavy metal concentrations in the vegetable samples and in showed that, Zn in soil had a positive significant relationship with vegetable contamination during winter. Cd, Cu, and Mn concentrations in soil and plant showed a significant positive relationship only during summer. Concentration of Cr and Pb during winter season and Zn and Ni during summer season showed significant negative relationship between soil and plant contamination. From the study they concluded that, the use of treated and untreated waste water for irrigation increased the contamination of Cd, Pb, and Ni in the edible portion of vegetables causing a potential health risk in the long term from this practice. They also noted that, adherence to standards for heavy metal contamination of soil and irrigation water does not ensure safe food.

Gianazza *et al.* (2007) conducted an experiment with seedlings of *Lepidium sativum* (L.). Exposure of the plant to increasing concentrations of Cd resulted in the growth inhibition and the accumulation of proteins in the 10-25 kDa range in cotyledons and hypocotyls of the plantlets. Most of these proteins were also found in extracts of the seeds. Analysis by ESI-MS alter two–dimensional electrophoresis showed that these proteins exibit sequences similar to those of storage proteins from various Cruciferae sp.According to the author the response to metal exposure during germination and initial plantlet elongation thus involves inhibition of both storage protein catabolism and plant protein anabolism. In addition , two of the proteins were present in higher amounts in plantlets exposed to Cd heat-shock, in agreements with literature data , and jasmonate like inducible protein are related to cellular stress and another two (LEAs or late embryogenesis abundant) are involved in embroyogenesis. Changes in protein expression can be detecded by two-dimentional electrophoresis after exposure to heavy metal concentrations lower than those at which morphometric changes become evident. Proteomics of germinating *L.sativum* thus constitutes a very sensitive tool for evaluating environmental pollution.

The accumulation and distribution of arsenic and cadmium by tea plants were studied by Shi *et al.* (2008). The field investigation and pot trial, they found the low mobility of arsenic and cadmium in tea plants. Most arsenic and cadmium absorbed were fixed in feeding roots and only small amount was transported to the above-ground parts. Distribution of arsenic and cadmium, based on their concentrations of unit dry matter, in tea plants grown on un-contaminated soil was in the order: feeding roots>stem> main roots>old leaves>young leaves. When tea plants were grown on polluted soils arsenic and cadmium were transported less to the above-ground parts. The concentration of cadmium in soil significantly and negatively correlated with chlorophyll content, photosynthetic rate, and transpiration rate and biomass production of tea plants.

Insecticide/ Fungicide

Photosynthesis inhibition of soybean leaves by insecticides was studied by Reheem *et al.* (1991). Field grown soybean cv. Williams-82 plants were sprayed with malathion or carbaryl formulations at 30, 60 and 90 days after planting. Net photosynthesis (PN) was measured in the control (water-sprayed) and pesticide-treated plants, 1, 3 and 7 days after treatment, with a LICOR 6200 Portable Photosynthesis System. After the first application the pesticide-treated plants showed a significant reduction (24% with malathion and 20% with carbaryl) in PN. The 60-day spray treatment PN suppression on day 1 and day 3 after treatment was the same as after the first application; but PN reached the same level as that of the water-sprayed control 7 days after treatment. After the 90-day treatment no change in PN was observed with the pesticide-treated plants compared to the control. These data indicate that malathion and carbaryl formulations may exert a detrimental influence on soybean physiology.

Pesticides (Brominal, Cuprosan and Fenvalerate) at 10 and 50 ppm suppressed growth, respiration and nitrogenase activity of*Azotobacter chroococcum, Azospirillum brasilense* and*Azospirillum lipoferum*. The inhibitory effect on respiration of *A. lipoferum* was most pronounced after 3 and 4 days of the pesticide application, studied by Omar and Alla (1992).

Phytotoxic effects of Benzimidazole fungicide on bedding plants were evaluated by . Iersel (1996). Benzimidazoles are effective and widely used fungicides, but they may be phytotoxic. The effects of a single drench application of six benzimidazoles and one acetanilide fungicide on photosynthetic gas exchange, growth, development, and nutrient levels of four species of bedding plants in twenty growth –chambers and four greenhouses were studied. Daily carbon gain and carbon - use efficiency were calculated from continuous crop gas exchange measurements in the growth chambers.

The maximum labeled rate of Benlate DF caused a 7-to 10 day decrease in net photosynthesis and daily carbon gain in transplants of all species. It also caused pronounced interveinal chlorosis and a 2-to 3-day delay in flowering. Growth of Benlate DF –treated plants was reduced more at high (90%) than at low (60% to 80%) relative humidity. Benlate DF had severe effects on 2 week old petunia (*Petunia* x *hybrida*) seedlings in plug flats, reducing photosynthesis 25 % to 57%. Cleary's 3336 WP decreased photosynthesis in some traials Benlate DF reduced photosynthesis within 24 hr, but 3336 WP effects did not become apparent until 1 week after the treatment. This indicates different modes of inhibition. 3336 WP also caused leaf-tip and marginal chlorosis in impatiens (*Impatiens wallerana*). Mertect 340-F was extremely phytotoxic but is not labeled for drench applications (it was included because of its chemical similarities to other benzimidazoles). The only benzimidazole fungicide that did not reduce photosynthesis was Derosal, but it caused slight interveinal chlorosis in some studies with petunia. Leaf Ca levels decreased by Benlate DF and Derosal .Subdue (or metalaxyl), an acetanilide fungicide, did not affect photosynthesis or cause any visual symptoms. The results indicate that some benzimidazole fungicides can cause growth reductions and visual damage in bedding plants.

An experiment was carried out by Sudandara *et al.* (1996) to evaluate the genotoxic effect of an organophosphorous pesticide on *Allium* root meristems in *vivo*. The organophosphorous pesticide malathion not only induces damage to the chromosome but also reduce the frequency of cell division. The root tip cell of *Allium* exposed to malathion when post treated with *Phyllanthus* extract and distilled water could neither restore the normal mitotic index nor bring about reduction in the mitotic irregularities. However, the residual analysis of the treated and post treated cells showed the absence of pesticidal residues.

Fungicide action is generally assumed to be dependent on an antibiotic effect on a target pathogen, although a role for plant defense mechanisms as mediators of fungicide action has not been excluded. It was demonstrated by Molina *et al.* (1998) that in *Arabidopsis*, the innate plant defense mechanism contributes to the effectiveness of fungicides. In NahG and *nim1* (for noninducible immunity) *Arabidopsis* plants which normally exhibit increased susceptibility to pathogens, the fungicides metalaxyl, fosetyl, and $Cu(OH)_2$ are much less active and fail to control *Peronospora parasitica*. However, the effectiveness of these fungicides is not altered in *Arabidopsis* mutants defective in the ethylene or jasmonic acid signal transduction pathways. Application of the systemic acquired resistance activator benzothiadiazole (BTH) in combination with these fungicides results in a synergistic effect on pathogen resistance in wild-type plants and an additive effect in NahG and BTH-unresponsive *nim1* plants. BTH treatment normally induces long-lasting pathogen protection; however, in NahG plants, the protection is transient. These observations suggest that BTH treatment can compensate only partially for an impaired signal transduction pathway and support the idea that pathogen defense mechanisms are under positive feedback control. These observations are strikingly reminiscent of the reduced efficacy of antifungal agents in immunocompromised animals.

Effect of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum*) was studied by Sing and Wright (1999). Two pot experiments were performed to study the effects of three pre-emergence herbicides (terbutryn/ terbuthylazine, trietazine/simazine and prometryn) and a post-emergence herbicide (bentazone) on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum* L.) grown in perlite under nitrogen-free conditions. Decreased nodulation, total nitrogenase activity, net photosynthesis, leaf area, root and shoot dry weight, nitrogen content and seed yield of peas were observed in all pre-emergence herbicide treatment. The effects of herbicides increased with increase in rate of application. Among the herbicides tested, terbutryn/terbuthylazine and trietazine/ simazine had the greatest adverse effects. Pea plant biomass (root plus shoot) was correlated with plant nitrogen content but not total nitrogenase activity. The experiments suggested that the decreased growth of herbicide-treated plants was due to direct effects of the herbicides on peas and not due to indirect effects of the herbicides on rhizobia.

Effect of endosulfan and methylparathion on hydrolytic enzymes in germinating seed of jowar was investigated by Sabale and Misal (2000). A varied response of jowar (*Sorghum bicolor* L.) seeds was recorded under the influence of endosulfan and methylparathion with respect to the level of some hydrolytic enzymes during germination. The result revealed that lower dose of endosulfan (0.05, 0.1% v/v) stimulated alpha amylase, protease, acid phosphatase and alkaline phosphatase activities.Wherease, methylparathion treatment suppressed amylase activity but markedly increased protease level at lower concentrations. Toxic concentrations of both the pesticides shifted the peaks of enzyme activities towards early hours of germination. In general treatment of methylprathion imposed a severe osmotic stress during germination of jowar seeds as compared to endosulfan.

Bisen and Hajra (2000) investigated the persistence and degradation of some insecticides in Darjeeling tea. A field trial was conducted during dry and wet seasons to understand the occurrence of residues, persistence, dissipation rate and half life values of six widely used insecticides at recommended doses / dilutions *viz.* Monocrotophos 36% SL, Malathion 50% EC , Fenvalerate 20% EC , Dimethonate 30% EC , Quinalphos 20% AF and Dicofol 18.5% EC in processed tea. The insecticides were applied as aqueous solutions at the dilution of 1:400 for all the insecticides except Fenvalerate which was applied @ 1: 4000 during dry and wet seasons. The initial deposits (4hrs) of different insecticides except Fenvalerate were found to be higher in dry season than wet season. No residue of monocrotophos after 4 hrs of its application was detected. The residue of malathion, fenvalerate, dimethonate on 5^{th} day after application were found below the permissible maximum residue limit.Wherease, in case of quinalphos it was observed on 7^{th} day after application. The residue of dicofol on 7^{th} day during wet season (0.03 ppm) was found below the tolerance limit. Dissipation followed a first order reaction in all cases and the half life values varied from 0.24 to 2.73 days. The results also indicate that, one round of plucking may be discarded in dry season when quinalphos and dicofol are applied on the tea bushes of Darjeeling.

Gupta and Tripathy (2000) studied the oxidative stress in cucumber (*Cucumis sativus* L) seedlings treated with acifluorfen. Treatment of diphenyl ether herbicide acifluorfen-Na (AF-Na) to intact cucumber (*Cucumis sativus* L cv.Poinsette) seedlings induced over accumulation of protoporphyrin IX in light (75 μmole $m^{-2}s^{-1}$). The extra – plastidic accumulation of protoporphyrin IX during the light exposure disappeared within two hrs transfer of acifluorofen –treated seedlings to darkness. This was due to re-entry of migrated protoporphyrin IX into the plastid and its subsequent conversion to protochlorophyllide. In light, protoporphyrin IX acted as a photosensitizer and caused generation of active oxygen species. The latter caused damage to the cellular membrane lipids that resulted in production of malondialdehyde. Damage to the plastidic membranes resulted in damage to photosystem I and photosystem II reactions. Dark-incubation of herbicide-sprayed plants before their exposure to light enhanced photodynamic damage due to diffusion of the herbicide to the site of action. Compared to control, in treated samples the cation-induced increases in variable fluroscence maximum fluroscence ratio and increase in photosystem II activity was lower due to reduced grana stacking in herbicide-treated and light-exposed plants.

Residues and persistence of chloropyriphos in processed black tea was investigated by Manikandan *et al.* (2001). Field experiments were conducted in wet (September) and dry (February) seasons in 1998 and 1999 at Valparai (TamilNadu , India) to determine the residues of chlorophyriphos in black tea. Residue levels at different harvest intervals, persistence, and dissipation pattern and half-life values were calculated. The initial deposit of chloropyriphos residues on tea leaves was higher in wet season than in dry season. Residues of chloropyriphos dissipated exponentially after spraying during both the seasons and reached below the European Union tolerance limit of 0.1 ppm on 10^{th} day after application during wet season and 12^{th} day after application during dry season. Regression lines drawn for chloropyrifos showed that it followed the first order dissipation. Half –life values varied from 1.62-1.68 days for chloropyrifos and a safety harvest interval of 12 days is suggested.

Kalam and Mukherjee (2001) studied the influence of hexaconazole, carbofuran and ethion on soil microflora and dehydrogenase activities in soil and intact cell. The total microbial count was highly affected (up to 61% at 1000μg level) in presence of hexaconazole and persisted upto 21days. Bacteria were more susceptible than actinomycetes. Carbofuran and ethion were moderately toxic to soil microflora. Inhibitory effects of all the three pesticides gradually decreased after 21days as was evident by increase in total microbial count except carbofuran. GDH activity in soil was also affected initially (up to 14 days) by all the three pesticides (60.3% in hexaconazole at 1000μg level) and inhibition gradually decreased to zero except carbofuran (15-20% toxicity persisted up to 35 days). GDH and LDH activity in presence of hexaconazole was strongly affected in intact cells of some standard culture of bacteria like *Rhizobium* sp.(host *Dolichos* sp., 32.1 and 72.5%) , *Bacillus subtilis* Cohn (86.75 and 76.5%), *Azotobacter* sp. (36.9 and 55.4%) and *B.sphaericus* (67.6% GDH) respectively. Carbofuran inhibited the enzyme activity in B.subtilis (55.55 and 35.3 %) and to some extent in *B.sphaericus*. Ethion moderately inhibited LDH activity in *Rhodococcus* sp. AK1 (17.1 and 33.3%), *Rhizobium* (27.6% LDH), *E.coli* HB 101(34.2% LDH) as evidenced by formazan formation. From the result it might be concluded that among the three pesticides tested hexaconazole strongly inhibited the dehydrogenase system in bacteria including nitrogen fixing bacteria of soil and thus may affect soil fertility. It was concluded that hexaconazole was more toxic than ethion to dehydrogenase enzymes.

Beneficial effects of fungicide seed treatments for soybean cultivars with partial resistance to *Phytophthora sojae* was investigated by Dorrance and Mc Clure (2001). *Phytophthora sojae* is a yield-limiting soybean pathogen in areas where soils remain saturated for long periods of time. *P. sojae* has been successfully managed with single dominant resistance genes (*Rps* genes). The proportion of fields with populations of *P. sojae* capable of causing susceptible interactions with many of the *Rps* genes has increased in number. The fungicides metalaxyl and mefenoxam have been used both as in-furrow and seed treatments to provide protection against damping-off caused by *P. sojae*. To determine the plant age when partial resistance and *Rps* genes are effective against *P. sojae*, author evaluated a greenhouse assay in which soybean seeds were planted and inoculated with a zoospore suspension to compare the disease reaction of soybean seeds and seedlings. Efficacy of different fungicide rates also was evaluated using the cultivar with partial resistance with this inoculation technique. Seeds and seedlings of a cultivar with high levels of partial resistance were susceptible to infection by *P. sojae* while those of a cultivar with an *Rps* gene were resistant. For the cultivar with partial resistance, reductions in percent emergence and the number of damped-off seedlings were significantly higher for plants inoculated at the day of planting compared to inoculations of plants with unifoliates present (5 days after planting). Results also indicated that fungicide seed treatment on cultivars with partial resistance may be beneficial when the environmental conditions that favor *P. sojae* infections occur prior to soybean emergence. This greenhouse assay appears to be useful in examining overall fungicide efficacy; however, it did not detect consistent and quantifiable differences in rates of seed treatment fungicides.

Somara *et al.* (2002) conducted an experiment for the localization of identical organophosphorus pesticide degrading (*opd*) genes on genetically dissimilar indigenous plasmids of soil bacteria : PCR amplification , cloning and sequencing of *opd* gene from ***Flavobacterium balustinum*** . Plasmid borne organophosphorus degrading (*opd*) gene of ***Flavobacterium balustinum*** has been amplified using polymerase chain reaction (PCR) and the resulting PCR product (1.25 kb) was cloned in puc18. Further, a detailed restriction map was determined to PCR product and subcloned as overlapping restriction fragments. The nucleotide sequence was determined for all subclones to obtain complete sequence sequence of of PCR amplified fragment. The sequence showed 98% similarities to *opd* genes cloned from other soil bacteria isolated from diversified geographical regions. The protein sequence predicted from the nucleotide sequence was almost indentical to parathion hydrolase, a triesterase involved in hydrolysis of triester bond found in variety of op-pestisides. The signal sequence of parathion hydrolase contained recently discovered twin arginine transport (*tat*) motif. It appears *tat* motif plays a critical role in membrane targeting of parathion hydrolase.

Effects of systemic fungicides on protein, carbohydrate, amino acids and phenolic contents of susceptible (Mexipak) and resistant (Povan) Varieties of ***Triticum aestivum*** L.was studied by Siddiqui and Ahmed (2002). Application of systemic fungicides caused a significant ($P<0.001$) decrease in total protein and carbohydrate content compared to the control. MexiPak (susceptible) was more adversely affected than Povan (resistant). A substantial increase in total phenol was observed in the two varieties tested. Among the amino acids, proline, methionine, tyrosine and tryptophane were found in appreciable amounts.

Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone were investigated by Wu and Tiedenmann (2002). Two modern fungicides, a strobilurin, azoxystrobin (AZO), and a triazole, epoxiconazole (EPO), applied as foliar spray on spring barley (*Hordeum vulgare* L. cv. Scarlett) 3 days prior to fumigation with injurious doses of ozone

(150–250 ppb; 5 days; 7 h/day) induced a 50–60% protection against ozone injury on leaves. Fungicide treatments of barley plants at growth stage (GS) 32 significantly increased the total leaf soluble protein content. Additionally, activities of the antioxidative enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate-peroxidase (APX) and glutathione reductase (GR) were increased by both fungicides at maximal rates of 16, 75, 51 and 144%, respectively. Guiacol-peroxidase (POX) activity was elevated by 50–110% only in AZO treated plants, while this effect was lacking after treatments with EPO. This coincided with elevated levels of hydrogen peroxide (H_2O_2) only in EPO and not in AZO treated plants. The enhancement of the plant antioxidative system by the two fungicides significantly and considerably reduced the level of superoxide ($O_2^{\cdot -}$) in leaves. Fumigation of barley plants for 4 days with non-injurious ozone doses (120–150 ppb, 7 h/day) markedly and immediately stimulated $O_2^{\cdot -}$ accumulation in leaves, while H_2O_2 was increased only after the third day of fumigation. Therefore, $O_2^{\cdot -}$ itself or as precursor of even more toxic oxyradicals appears to be more indicative for ozone-induced leaf damage than H_2O_2. Ozone also induced significant increases in the activity of antioxidant enzymes (SOD, POX and CAT) after 2 days of fumigation in fungicide untreated plants, while after 4 days of fumigation these enzymes declined to a level lower than in unfumigated plants, due to the oxidative degradation of leaf proteins. This is the first report demonstrating the marked enhancement of plant antioxidative enzymes and the enhanced scavenging of potentially harmful $O_2^{\cdot -}$ by fungicides as a mechanism of protecting plants against noxious oxidative stress from the environment. The antioxidant effect of modern fungicides widely used in intense cereal production in many countries represents an important factor when evaluating potential air pollution effects in agriculture.

Effect of herbicides on growth and development of *Oxalis latifolia* was studied by Pandey and Singh (2003). The efficacy of trifluralin and oxadiazon each at 0.5, 1.0 and 1.5 kg/ha as pre-emergence, as well as glyphosate and 2,4-D each at 0.5 kg/ha applied 30 days after sowing (DAS) and each at 1.0 kg/ha applied 30 and 45 DAS was evaluated on *O. latifolia* in pots under greenhouse condition. Trifluralin at all levels and oxadiazon at 1.5 kg/ha inhibited the formation of bulbils up to 60 days stage. Glyphosate at 0.5 or 1.0 kg/ha applied 30 DAS completely killed the foliage and there was no formation of any leaf, inflorescence and bulbils till 60 days stage, whereas glyphosate at 1.0 kg/ha applied 45 DAS controlled the formation of bulbils and inflorescence up to 105 and 150 days stages, respectively. Glyphosate at 1.0 kg/ha applied 45 DAS proved the most effective in controlling the growth and development of *O. latifolia*. The higher rates of all the herbicides were more effective than the lower rates in reducing the growth and development of *O. latifolia*.

Physiological stress responses of *Vitis vinifera* L. to the fungicides fludioxonil and pyrimethanil was investivated by Saladin *et al.*(2003) The effects of the fungicides fludioxonil and pyrimethanil were evaluated on grapevine leaves using in vitro-grown plantlets, fruiting cuttings, and plants grown in vineyards. *In vitro*, both water content and osmotic potential decreased in treated leaves. Moreover, carbohydrate accumulated, suggesting that plantlets could react to the stress through an active osmoregulation process by uptaking sugars from the medium. Besides, pyrimethanil stimulated the accumulation of proteins, whereas no significant effect was observed using fludioxonil. The cuttings exhibited similar responses than *in vitro* though they appeared to be more tolerant since half of the studied parameters recovered 10 days after treatment. In vineyard, both fungicides modified leaf water content and carbohydrate levels, whereas nitrogenous compounds accumulated transiently. These results suggest that in vineyard-grown plants, a strong sugar translocation from mature leaves to sink organs occurs transiently, as well as a protein synthesis and a stimulation of soil nitrogen uptake.

Water stress and glyphosate treatments to glyphosate-resistant (GR) cotton (*Gossypium hirsutum* L.) can cause abscission of young bolls although the interaction of these factors is not well defined. Studies were conducted by Pline *et al.* (2003) to quantify the effects of water stress and glyphosate treatments on fruit retention, fruit placement, and carbohydrate partitioning in GR and conventional cotton varieties grown in a phytotron environment. Glyphosate-resistant plants treated with glyphosate at the four-leaf stage, postemergence (POST), and at the eight-leaf stage, POST-directed (PDIR), had fewer first-position bolls after 0 and 1 d of water stress than nontreated GR and conventional plants but did not differ after 2 and 3 d of water stress. Glyphosate-treated GR plants reached first bloom 3 to 4 d later than nontreated plants. Five-day-old bolls from plants of one genotype, SG 125RR, treated with glyphosate had lower fructose content than bolls from nontreated plants. Subtending leaf carbohydrates and boll sucrose, glucose, and starch content did not differ after glyphosate treatments. Increasing water stress caused reductions in subtending leaf glucose, sucrose, and starch content, as well as reductions in boll starch and sucrose content. Reductions in boll starch and sucrose content in response to water stress may indicate the potential for abscission. Water stress and glyphosate treatments to GR cotton do not alter carbohydrate profiles in boll or leaf tissues in a like manner. Differences in carbohydrate profiles of young bolls and leaves from glyphosate-treated and water-stressed cotton plants suggest that water stress and glyphosate treatments may promote fruit abscission in different manners.

The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning,remobilization and grain N accumulationin winter wheat cultivars was syudied by Ruske *et al.*(2003). Field experiments were conducted over 3 years to assess the effect of a triazole fungicide programme, and additions of strobilurin fungicides to it, on nitrogen uptake, accumulation and partitioning in a range of winter wheat cultivars. Commensurate with delayed senescence, fungicide programmes, particularly when including strobilurins, improved grain yield through improvements in both crop biomass and harvest index, although the relationship with green area duration of the flag leaf (GFLAD) depended on year and in some cases, cultivar. In all years fungicide treatments significantly increased the amount of nitrogen in the above-ground biomass, the amount of nitrogen in the grain and the nitrogen harvest index. All these effects could be linearly related to the fungicide effect on GFLAD. These relationships occasionally interacted with cultivar but there was no evidence that fungicide mode of action affected the relationship between GFLAD and yield of nitrogen in the grain. Fungicide treatments significantly reduced the amount of soil mineral N at harvest and when severe disease had been controlled, the net remobilization of N from the vegetation to the grain after anthesis.Fungicide maintained the filling of grain with both dry matter and nitrogen. The proportionate accumulation of nitrogen in the grain was later than that of dry matter and this difference was greater when fungicide had been applied. Effects of fungicide on grain protein concentration and its relationship with GFLAD were inconsistent over year and cultivar. There were several instances where grain protein concentration was unaffected despite large (1.5 t/ha) increases in grain yield following fungicide use. Dilution of grain protein concentration following fungicide use, when it did occur, was small compared with what would be predicted by adoption of other yield increasing techniques such as the selection of high yielding cultivars (based on currently available cultivars) or by growing wheat in favourable climates.

The effective durations of pesticide-induced susceptibility of rice to brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), and physiological and biochemical changes in rice plants following pesticide applications, were studied. The effective duration of the herbicide butachlor on the susceptibility of rice variety Zhengdao 2 to BPH exceeded 16 days. The difference in damage rating between rice plants with pesticide treatments and control plants gradually decreased with days after treatment (DAT). There was a significant correlation between damage rating and DAT. The number of rice tillers at 3 - 10 DAT and plant heights at 7-10 DAT declined on Zhengdao 2 with the butachlor treatment. On the other hand, there were no effects of butachlor on damage rating, number of rice tillers and plant height for Xiushui 63. This might be related to tolerance of Xiushui 63 to butachlor. For Zhengdao 2, the fungicide jingganmycin and the insecticide bisultap had a significant influence on BPH damage, number of rice tillers and plant height. In contrast to butachlor, jingganmycin and bisultap had a significant effect on BPH damage to Xiushui 63. However, these two pesticides had no significant effect on the number of rice tillers and plant height of Xiushui 63. In general, the effective duration of butachlor on rice plants was greater than jingganmycin and bisultap. Oxalic acid content and photosynthesis in rice plants declined significantly after jingganmycin and bisultap treatments. These findings are consistent with those of pesticide-susceptibility of rice to BPH. Pesticide-induced susceptibility of rice resistance to BPH counteracts the role of varietal resistance in integrated pest management, (Wu *et al.* 2004).

Effects of fungicide and insecticide mixtures on apple tree canopy photosynthesis, dark respiration and carbon economy were investigated by Untiedt and Blanke (2004). Fungicide/insecticide mixtures were applied at times and doses commonly used in commercial orchard practice. Their effects on photosynthesis and dark respiration were evaluated in two seasons with respect to the potential stress they impose on an apple tree using cv. 'Elstar'. The mixtures included the fungicides mancozeb, flusilazol and dithianon, and the insecticides oxydemeton-methyl or pirimicarb. A new technology was employed to continuously examine photosynthesis, dark respiration and carbon balance of apple trees based on six canopy chambers, which enclosed apple trees under natural conditions in the field, with on-line measurements and continuousanalysis of CO_2 exchange and automated data acquisition.The fungicides mancozeb and flusilazol combined with the insecticide oxydemeton-methyl reduced whole tree canopy CO_2 assimilation mostly at midday and, using hourly means, by an averaged 7.4% on the day of its application. This reduction in whole canopy photosynthesis declined with time, restoring most of the original photosynthetic potential within 3–5% in 3 days, hence, indicating acceptable phytotoxicity. This fungicide/insecticide mixture overproportionally, in relation to the changes in photosynthesis, increased dark respiration by up to 72% in the night after application, thereby drastically affecting the tree's carbon balance in an adverse way.In contrast; the fungicide dithianon combined with the insecticide pirimicarb decreased dark respiration by 15–21% with reductions in canopy photosynthesis in the order of 6–9%. Because the decrease in dark respiration exceeded that in photosynthesis, the apple tree overall gained carbon in a balance.Overall, effects on photosynthesis were smaller than on dark respiration. The effects of the pesticide combinations on photosynthesis are attributed to the CO_2-independent Hill reaction in photosynthesis and to uncoupling the photosynthetic electron flow from phosphorylation, thereby inhibiting energy, viz. ATP formation or its transfer, rendering dissociation of ATP into ADP and P_i.

Two-way effect of pesticides on zeatin riboside content in both rice leaves and roots were studied by Hua Qiu *et al.* (2004). Cytokinins zeatins including zeatins riboside (ZR) play a vital regulation role in growth, development, physiology and biochemistry of rice plant. The effect of four commonly used pesticides in paddy fields on the ZR contents in rice leaves and roots was investigated using Enzyme-Linked Immunosorbent Assays (ELISA). Experimental rice plants were grown under hydroponics culture conditions, and subjected to a foliar spray or root treatment with different concentrations of these pesticides. Zeatin riboside content in rice leaves decreased significantly three days after foliar sprays (3 DAS) with 150 and 300 ppm buprofezin, 30 and 60 ppm imidacloprid, 200 ppm jinganmycin, and 480 ppm triazophos. At 7 DAS a significant reduction occurred irrespective of the pesticide concentration. The ZR content in rice roots did not change so dramatically as in rice leaves. At 3 DAS, it was reduced significantly only in the plants subjected to a foliar spray with 100 ppm jinganmycin or significantly increased in the plants sprayed with 480 ppm triazophos, while at 7 DAS, there were no significant differences in ZR content under all circumstances. When subjected to root treatment with these pesticides, rice plants were extremely sensitive to triazophos and even wilted three days after the treatments (3 DART). Root treatment with 150 ppm buprofezin, 100 ppm jinganmycin, 60 ppm imidacloprid, respectively, caused a significant reduction in ZR contents in rice leaves; however, all treatments except with triazophos did not reduce ZR contents in rice roots significantly. Seven days after foliar sprays with the pesticide mixtures, i.e. triazophos+imidacloprid and triazophos+buprofezin. ZR content significantly reduced in rice leaves but not in roots.

Herbicidal and antioxidant defense responses of transgenic rice plants that overexpressed *Myxococcus xanthus* protoporphyrinogen oxidase gene was studied by Jung and Back (2005). Leaf squares of the wild-type incubated with oxyfluorfen were characterized by nectrotic leaf lesions and increase in conductivity and malonyldialdehyde levels, whereas transgenic lines M4 and M7 did not show any change with up to 100μM oxyfluorfen. The wild -type had decreased F_v/F_m and produced a high level of H_2O_2 at 18 hr after foliar application of oxyfluorfen, whereas transgenic lines M4 and M7 were unaffected. In response to oxyfluorfen, violaxanthin, β-carotene, and chlorophylls (Chls) decreased in wild –type plants, whereas antheraxanthin and zeaxanthin increased. Only a slight decline in Chls was observed in transgenic lines at 48 hr after oxyfluorfen treatment. Noticeable increases of Cu/Zn – superoxide dismutase, peroxidase isozymes 1and 2, and catalase were observed after at 48hr of oxyfluorfen treatment in the wild-type. Non-enzymatic antioxidants appeared to respond faster to oxyfluorfen-induced photodynamic stress than did enzymatic antioxidants. Protective responses for the detoxification of active oxygen species were induced to counteract photodynamic stress in oxyfluorfen-treated, wild type plants.

However, oxyfluorfen-treated, transgenic plants suffered less oxidative stress, confirming increased herbicidal resistance resulted from dual expression of *M.xanthus* Protox in chloroplasts and mitochondria.

Phytotoxicity of copper fungicides viz. Bordeaux mixture , stabilized Bordeaux mixture and copper oxychloride were found phytotoxic to guava fruits , while carbendazim,benomyl and mancozeb were non phytotoxic . Bordeaux mixture was highly phytotoxic and caused heavy russetting, followed by stabilized Bordeaux mixture and then copper oxychloride. The higher concentrations of these fungicides were more toxic than their lower doses. The russetted fruits failed to attain normal size and thus reduced the quality of fruits to a greater extent, Gaikwad and Nimbalkar (2005).

Changes of antioxidants levels in two maize lines following atrazine, a photosynthetic herbicide were studied by Alla and Hassan (2005). Growth and antioxidants levels of shoot of 10-d-old maize lines (*Zea mays* L.Hybrid 351 and Giza 2) differentially responded to atrazine treatment at the recommended field dose (RFD) during the following 20 d. Atrazine significantly reduced shoot fresh and dry weights but significantly accumulated H_2O_2 , lipid peroxides and carbonyl groups in Giza 2 during the whole experiment ; an effect that, prolonged with either elapse of time or increasing the herbicide dose. Mean while , ascorbic acid (AsA) and reduced glutathione (GSH) contents were significantly decreased along with significant inhibitions in activities of superoxide dismutase (SOD; EC 1.15.1.1) , catalase (CAT; EC 1.11.1.6) , ascorbate peroxidase (APX; EC 1.11.1.7) , guaiacol peroxidase (GPX; EC 1.11.1.7) , and glutathione-S-transferase (GST ; EC 2.5.1.18). Similar responses were observed in Hybrid 351 only during the first 12 d, and seemed to be overcome thereafter. These results indicate that an induced oxidative stress in maize following atrazine treatments. Such state appeared to be counterbalanced in Hybrid 351 but continued in Giza 2 concluding Giza 2 as more susceptible to atrazine than Hybrid 351. Therefore, the differential susceptibility of Giza 2 to atrazine is related to deficiency in antioxidant levels.

Effect of Metasystox Application on Cottonseeds Quality was evaluated by Osman *et al.* (2006). Two field experiments were carried out at the Agricultural Farm of the Faculty of Agriculture, University of Khartoum to study the effect of the insecticide Metasystox on cottonseed quality of two local cultivars, Barakat-90 and Barac-67. Three levels of concentrations of this insecticide recommended dose, 1.5 of the recommended dose and 2 fold of the recommended dose were applied on field grown cotton. Oil, protein, phytic acid, and minerals content of cottonseeds were determined. The results showed significant increase in cottonseed oil of Barakat-90 and Barac-67, as influenced by different levels of treatments. Protein content increased significantly in cottonseeds of both cultivars. In contrast, the results of phytic acid, showed no significant difference in Barakat-90 cultivar. However, significant reduction was observed in Barac-67 cultivar. The value of mineral content of both cultivars has no consistent pattern of change.

The effects of glyphosate on protein metabolism, mesophyll cell ultrastructure and nodule ultrastructure and functioning of *Lupinus albus* cv. was investigated by Maria *et al.*(2007). Multolupa inoculated with *Bradyrhizobium* sp. (*Lupinus*) were investigated by them. Young leaves and nodules were especially affected because these organs act as sinks of herbicide. The alterations on nodular and chloroplast ultrasturcture varied depending on herbicide concentration and the time of exposure. After 3 days of 2.5 mM glyphosate application some toxic effects were detected. The most important alterations on nodules were the progressive cellular degradation of plant and bacteroidal cytosol and the rupture of bacteroidal membrane, whilst the peribacteroid membrane of the symbiosomes was preserved. This is the first report on the effect of glyphosate on legume-nodule ultrastructure. Glyphosate inhibited *B*.sp.(*Lupinus*) growth at concentrations higher than 62.5μM . In the mesophyll cells, gradual disorganization of grana and intergrana was observed, loosing the parallel alignment with the chloroplast axis. As in nodules, degradation of membrane systems was observed, with the deformation, and even the rupture, of the tonoplast. These progressive effects were similar to those described in senescence process. The adverse effects produced on infected zone can be due both to a direct effect of the herbicide on microsymbiont and to an indirect effect of glyphostate action on photosynthetic apparatus. Glyphossate produced changes in nodule cytosol and bacteroid proteins content and polypeptide pattern of leaves and nodules. With respect to proteins related to the oxygen diffusion mechanism, a large decrease in leghemoglobin and glycoproteins (recognized by antibodies MAC 236 and MAC 265) content was detected, which suggests that the oxygen diffusion mechanisms were also affected by glyphosate.

Strobilurin fungicides induce changes in photosynthetic gas exchange that do not improve water use efficiency of plants grown under conditions of water stress was studied by Nason *et al.* (2007). The effects of five strobilurin (beta-methoxyacrylate) fungicides and one triazole fungicide on the physiological parameters of well-watered or water-stressed wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and soya (*Glycine max* Merr.) plants were compared. Water use efficiency (WUE) (the ratio of rate of transpiration, E, to net rate of photosynthesis, A_n) of well-watered wheat plants was improved slightly by strobilurin fungicides, but was reduced in water-stressed plants, so there is limited scope for using strobilurins to improve the water status of crops grown under conditions of drought. The different strobilurin fungicides had similar effects on plant physiology but differed in persistence and potency. When applied to whole plants using a spray gun, they reduced the conductance of water through the epidermis (stomatal and cuticular transpiration), g_{sw}, of leaves. Concomitantly, leaves of treated plants had a lower rate of transpiration, E, a lower intercellular carbon dioxide concentration, c_i, and a lower net rate of photosynthesis, A_n, compared with leaves of control plants or plants treated with the triazole. According to the authors The mechanism for the photosynthetic effects is not known, but it is hypothesised that they are caused either by strobilurin fungicides acting directly on ATP production in guard cell mitochondria or by stomata responding to strobilurin-induced changes in mesophyll photosynthesis. The latter may be important since, for leaves of soya plants, the chlorophyll fluorescence parameter F_v/F_m (an indication of the potential quantum efficiency of PSII photochemistry) was reduced by strobilurin fungicides. It is likely that the response of stomata to strobilurin fungicides is complex, and further research is required to elucidate the different biochemical pathways involved.