CHAPTER-2

REVIEW OF LITERATURE
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Heavy metals in general term means those metallic elements with a density of 5 or more (Passow et al. 1961). The classical definition of a heavy metal is that it is a metal precipitable in acid solution by H₂S. Most heavy metals occur naturally in soils as trace elements. The biota require some of these elements (zinc, copper, chromium, cobalt, magnesium etc.) as essential micronutrients; but may be sensitive to their higher concentrations (Farooqui et al. 1991). The release of heavy metals in biologically available forms, as a result of human activity, may damage or alter both natural and man made ecosystems.

The most obvious sources of metals pollution is the process extraction and purification: mining, smelting and refining, use of metal containing agricultural sprays or soil amendments such as soil ameliorants, pesticides, fertilizers etc., industrial effluents, coal or oil-fired electricity generating stations, automobile combustions, municipal incinerators and sewage sludge.

The most serious concern about heavy metal is that unlike many other pollutants they are not biodegradable and hence once they accumulate, they remain as such and their proportion increase in course of time.

Klein (1972) investigated 264 surface soil samples from industrial, agricultural and residential areas for heavy metals like Hg, As, Cu, Co, Cr, Fe, Ni, Pb, and Zn. and observed that compared to others, samples from industrial areas contained considerably higher amount of these metals. Rao (1979) reported high concentration of Pb, Hg, Cd, and Cu in soil of the industrial areas Mumbai. However, most alarming is the fact that water and particularly the sediment of Ganga river which is considered as holy river and held in
high esteem by Hindus all over the world, contain considerably higher concentration of heavy metals like, Zn, As and Cr particularly in the middle stretch of the river compared to the upper stretch. Saikia et al. (1988) and Singh et al. (1992) recorded the similar concentration of some heavy metals in sediment as follows: Zn, (60.8 - 88.4 mg gm\(^{-1}\)), As (9.4-18.4 mg gm\(^{-1}\)) and Cr (8.0-14.8 mg gm\(^{-1}\)) in middle stretch of the Ganga river. It is evident that on either side of the middle stretch of the Ganga river there are scores of industries and urban centre, whereas the upper hilly stretch is devoid of industries and urban centres except few.

Many heavy metals contaminate plants, enter food chain, often posing health problem. Studies on the ready-to-eat foods for infants reveal that many of these products are contaminated by heavy metals. Cadmium levels in some of these products were found to be between 7.8 to 2.8 ppb (Parts Part Billion), creating of an average monthly intake of 0.04 mg in the first month of life of infants. (Schulte - Loebbert et al. 1978). Mercury and cadmium became notorious in the recent past as a result of Minamata and Itai-itai diseases respectively in Japan.

2.1 HEAVY METAL TOLERANCE AND HYPERACCUMULATION BY PLANTS:

Excessive metal concentration in contaminated soils can result in decrease soil microbial activity, soil fertility and yield losses. (McGrath et al. 1995). Removal of heavy metal contamination from soil is not an easy task. Existing physical or chemical methods of soil clean-up are expensive, and often result in destruction of soil structure and fertility. Bioaccumulation through specialised plant species may provide an effective and \textit{in situ} way of removing heavy metals from contaminated soils. (Baker et al. 1994a, Mc Grath et al. 1993).
Two basic strategies exist by which plants respond to elevated concentration of heavy metals in the environment: exclusion mechanisms, whereby plants avoid excessive uptake and transport of metals, and accumulation and sequestration mechanisms, whereby large amount of metals are taken up and transported to the plant shoots (Baker 1981). Plants capable of accumulating more than 100 times larger concentration of metals than normal plants have been termed hyperaccumulators (Brooks et al. 1977). *Thlaspi caerulescens* has been identified as Zn and Cd hyperaccumulator (Reeves and Brook, 1983). Baker et al. (1994b) reported that the natural population of *T. caerulescens* in the UK contained up to 21,000 mg Zn kg\(^{-1}\) and 160 mg Cd kg\(^{-1}\) dry matter in the shoots. The ability to hyperaccumulate heavy metals from soils is one of the most important criteria in the selection of plant species for phytoremediation purpose. The phenomenon of metal hyperaccumulation by plant whilst being of considerable interest academically may also be exploitable economically in the clean-up of metal contaminated soils.

There is report that green remediation employs plants native to metalliferous soils with a capacity greater than normal level in the plant dry matter exhibiting high concentration of potentially phytotoxic metals such as Zn, Ni, Cd, Pb, Cu in their above ground biomass (McGrath et al. 1994). The criterion for hyperaccumulation varies for different metals and represents a concentration in above ground dry matter greatly in excess of normal or physiological levels (Baker and Brooks 1989). Persson (1948) used Mosses as indicator of areas with higher copper content. Sphagnum has been found to be a good accumulator and hence indicator for metals like Zn, Pb and Cd (Little and Martin, 1974; Parakarines and Tolonen 1976). Suckchroon (1979) reported *Ceratophyllum demersum* as an excellent indicator for mercury. The term rhizofiltration has been used to mean metal accumulation by root system from polluted effluents (Duskenkov et al. 1995). Salt et al. (1995) found that hydroponically grown sunflower can dramatically reduce the levels of Cr, Mn, Cd, Ni
and Cu from water. Samashekhar (1997) studied the heavy metal content in the sediment of Cauvery river and ability of four submerged plants *Hydrilla verticillata*, *Vallisnaria spiralis*, *Ceratophyllum demersum*, and *Potamogeton crispus* to accumulate heavy metals. All four species exhibit accumulation of Cu, Pb, Zn, Ni, Co, Cd and Cr to considerable extent and *Hydrilla verticillata* was found to be best with accumulation of Pb (57.5 μg/gm) and Cd (50μg/gm) against a sediment content of 10.1 μg/gm and 0.4 μg/gm respectively. Satyakala and Jamil (1997) found that *Pistia stratiotes* can accumulate Cu and Cd considerably even when the concentration of the metal in water was as high as 100 ppm. They also found that compared to shoot, root accumulate more metal. Patra and Panigrahi (1994) experimented with aquatic plants *Hyrilla* and *Pistia* on effluents of chloroalkali industry containing residual mercury and found that both the plants are efficient in accumulating mercury which is dependent upon the period of exposure. Garg and Chandra (1994) reported that *Wolffia globosa* which is quite common in the aquatic bodies can accumulate considerable amount of chromium. They also found that metal accumulation is high when concentration of metal in water is low. Sood *et al.* (1994) working with *Marselia minuta*, *Hydrilla verticillata* and *Nymphaea stellata* observed that of the three metals, viz. Pb, Hg and Cd, cadmium was always accumulated in much higher proportion than the other two. It is well established from the various reported results that many aquatic plants have ability to tolerate the impact the heavy metal and at same time they can also accumulate them in significantly higher amount.

Some species of *Allyssum* and *Thlaspi* (*Brassicaceae*) can accumulate up to 3% Zn, 0.5% Pb and 0.1% Cd (Parry 1994). *Allyssum* species particularly *A. lesbiacim* and *A. murale* apart from hyperaccumulating Ni, can also accumulate high amount of Zn and to a certain extent Cd, Cr and Cu. Extensive screening by Salt *et al.* (1994) has shown that mustard (*Brassia juncea*) is an excellent hyperaccumulator of Pb. Moreover, it has ability to
tolerate and accumulate Cd, Cr, Ni, Zn and Cu. Hardiman et al. (1984) reported that Phaseolus vulgaris can tolerate and accumulate Cd, Pb and Cu and maximum accumulation occur in root. It was observed that accumulation of Cd and Pb increased with increase in concentration of the metals in soil, whereas Cu accumulation remain constant, despite increase of the metal in soil.

Jawarker and Shende (1986) working with Hordeum vulgare observed that when Pb and Cd were applied in combination, in addition to their application separately; the adverse affect on growth, development and yield were more severe than the impact of either metal applied singly. Cadmium was found to accumulate in considerable amount in grain, apart from root and shoot.

Lead is a difficult target metal for phytoextraction, because it is strongly inclined by soil organic matter and soil minerals. Relatively few plants have been recorded to hyperaccumulate lead; Thlaspi rotundifolium reported to contain 130-8200 μg/g (Reeves and Brooks 1983). Much of the lead accumulated in plants was found in roots (Huang and Cunnigham 1996). Kumar et al. (1994) screened Brassica species for lead and found a range from 1416 to 18,812 μg/g dry matter in the shoots, with highest in B. juncea.

Semu et al. (1988) working on the impact of mercury on wheat found that concentration of mercury in shoot and leaves was more than other organ and increased with increasing concentration of mercury in soil. However, the grains were found to contain very little mercury and the content of mercury in grain did not increase despite increasing the concentration of the metal in soil.

Pande et al. (1992) studied the impact of mercury from solid waste deposit of chloro-alkali plant on barely and found that root accumulated considerably high amount of the metal, whereas in the shoot accumulation was low. In this study, mercury was found to
accumulate in grain in significant amount, but mercury content in grain did not increase with increasing of the metal in soil.

Wadge and Hutton (1986) grew barley and cabbage in green house in soil amended with refuge incinerator fly ash containing significantly elevated levels of Cd, Pb and Fe. It was found that both the crop preferentially absorbed more Cd than the other two metals, particularly cabbage grown on 20% ash amended soil contained 146 times more Cd than control. Khan and Frankland (1983) working on phytotoxicity of Cd and Pb found that Cd accumulated in shoot and leaves in higher amount whereas Pb mostly accumulated in the root. The significance of these observation was that absorbed Cd readily translocated to shoot system whereas Pb translocation was poor and hence mostly confined to root. Some 24 copper hyper accumulator species have been recorded under family as diverse as the Cyperaceae, Lamiaceae, Poaceae, Scrophulariaceae (Baker and Brooks 1989).

Banuelos and Schrale (1989) reported that Brassica juncea contained the highest concentration (2500 μg) selenium, being present more in the tops rather than the roots of the plants. Homer et al. (1991) made a comparative study between Ni hyperaccumulator Allyssum troodi and five other Allyssum species and non-accumulator Aurinia saxatilis to know the accumulation, pattern of Ni, Co and Cu in different organs. They reported that in case of A. troodi, Ni mostly accumulated in leaves and stems. (8000-10,000 μg gm⁻¹) whereas in root it was much lower (2000 μg gm⁻¹). In A. saxatilis Ni content in stem and leaves was only 380 μg mm⁻¹ and in root it was even lower. A. troodi accumulated maximum Co in leaves (2325 μg mm⁻¹), which is 10 times higher than non-accumulator species. In stem and root the Co content was slightly lower. It can be made a view that nickel tend to accumulate in stem and leaves whereas the absorbing organ i.e. root system retain only a small part of the metal and translocate major part to aerial parts. Behura et al.
(1989) studied the response of five aromatic Cymbopogon species viz., Palmarrosa, Jamrosa, Citronella, Lemongrass and Cymbopogon pendulus to chromite amended soil and found that the natural hybrid Jamrosa has maximum tolerance to chromite amended soil. In another study Behura et al. (1988) demonstrated that when chromite overburden soil was amended with organic manure at the ratio of 1:1, 2:1 and even 3:1; the adverse affects of chromite overburden soil were effectively neutralised.

It is almost clear that, there are differences between a metal tolerant or resistant plant and a hyperaccumulator. Tolerant is due to avoidance of absorption or mechanism to neutralise the metal once it is absorbed or a combination of both. A tolerant species may or may not be a hyperaccumulator but a hyperaccumulator is always a tolerant one. A tolerant but non accumulator agricultural plant will be of great help for agriculture in future in polluted crop field. The hyperaccumulator can be used to clean up polluted soil and aquatic bodies by using them as metal scavangers. Hyperaccumulators are already finding commercial application in environmental clean up, but some technical problems find its way. Hyperaccumulator during cleaning environment, accumulates the metals in their body, thus creating toxic bio-mass. The solution is that, such metal ladden bio-mass can be sent to smelter to extract the metal for recycle but this is not yet economically viable. The practical alternative is to dump them in special dumping site.

2.2 FACTORS AFFECTING METAL UPTAKE BY PLANT:

Bioavailability of metal is influenced by a number of factors such as chemical form of the metal, pH, presence of other metal, chelating agents, type of soil etc. Mere presence of metal in soil water does not imply their easy uptake by plants.

The most promising method of increasing plant lead uptake found to date seems to be mobilizing lead from the soil particle by chelates. Huang and Cunningham (1996) applied
0.2% HEDTA (EDTA as acid) to a sandy loam soil containing 2500μg/g total lead and found that lead concentrations in the shoots increased by a factor of 265 after week, at which time the plants had died. One of the nutritional problems encountered by plants on lead polluted soils is the lacking of phosphate, which is due to formation of insoluble lead phosphate. The lack of phosphate can be overcome by application of phosphate to the aerial parts of the plant (Huang et al. 1997). Huang and Cunningham (1996) stated that the increase of lead in plant tissues after application of chelates was related to the amount of lead in soil solution. The quest of yield and plant metal concentration has been reported in case of zinc which are key variables in the efficiency of phytoextraction process (Chaney et al. 1997; McGrath and Dunham 1997).

Cadmium and zinc have been known to differ not only in differences in their Zn/Cd specificity in accumulation, but also in relation to their response to the concentration in soil (McGrath et al. 1993).

Root soil interactions in the rhizosphere have important influences on the availability or solubility of many elements such as Zn, Fe, Mn, Cu and P (Marschner et al. 1987). Acidification of the rhizosphere and lower the pH in the rhizosphere soils presumably increases the extractability of Zn. (McGrath et al. 1997). The changes in the rhizosphere pH were more likely to show an excess uptake of cations over anions by hyperaccumulator (Haynes 1990). Changes in pH and redox potential were studied in the rhizosphere soil of nickel hyperaccumulator Alyssum murale and Raphanus sativus. Differences in rhizosphere pH and reducing activity were found between the lateral and the main root of both species, but the pH changes in the rhizosphere were similar in both species. Changes in pH were associated with the relative uptakes of cations and anions. The concentrations of heavy metals in the growth medium did not have any effects on the rhizosphere pH. (Bernal et al. 1994). Kuboi et al. (1987) made an important study to see cadmium
tolerance of plants of five families viz. Brassicaceae, Cucurbitaceae, Poaceae, Leguminosae, and Solanaceae grown in soil containing 0-700 ppm of cadmium at different pH level. It was found that irrespective of the pH level, plants of Brassicaceae family had maximum tolerance whereas plants of Leguminosae had minimum tolerance. Jackson and Alloway (1991) working on cadmium uptake by lettuce and cabbage found that cadmium uptake was maximum at low pH and minimum at high pH. Similar observation was recorded by Wood et al. (1984) who observed that aluminium toxicity in *Trifolium repens* was severe at low pH of 4.5, but when the pH level was raised to 6.0 or more through application of calcium salt, the toxicity was markedly overcome. It appears that in general under acidic condition of soil the uptake of metals by root is more and uptake rate fall with increase in pH level.

Homer et al. (1991) experimented with nickel hyperaccumulator *Allyssum troodi* and non accumulator *Aurinia saxatilis* to know the synergistic interactions of cobalt and nickel and found that when plants were grown in the media containing equal concentration of Co and Ni, Ni level considerably dropped than the level when the plants were grown in Ni only exhibiting uptake of Co by suppressing Ni. Hardiman et al. (1984) showed that in bush bean (*Phaseolus vulgaris*) uptake of heavy metals like cadmium and lead was associated with a decrease in the level of Zn in plant organ. They also made different observation in aquatic plant *Azolla pinnata* and *Lemna minor*, when both the plants were exposed to equal concentration of zinc and lead, the content of lead dropped to 25.44% of the level when lead was used singly showing the presence of zinc suppressed the uptake of lead.

There is report that chelating agents also affects the uptake by root. Xian (1989) experimented with five well defined forms of cadmium, lead and zinc namely exchangeable, bound to carbonate, bound to Fe-Mn oxide, bound to organic matter and residual and studied their uptake by cabbage reporting uptake as maximum when the
metals are in exchangeable form followed by carbonate form. Willaert and Verloo (1988) showed that when nickel was added to soil as nickel EDTA complex it resulted in very high mobility and uptake by the test plant Spinach even at high pH. It was reported that nickel complex mainly remained in negatively charged and enhanced the uptake of the metal much more than when nickel was added as Ni^{2+} ion. Salt et al. (1995) demonstrated that associating cadmium with chelating agent EDTA increased the uptake of cadmium by the test plant mustard, more than five times than cadmium was applied alone.

Soil types are considered as another important factor in the rate of uptake of metal. Jawarkar and Shende (1986) showed that in case of soil with high clay content, higher cation exchange capacity and presence of calcium carbonate immobilise considerable amount of lead and cadmium. The experiment revealed that barley plants grown in clay soil amended with lead and cadmium showed lesser phytotoxicity and uptake of metals. Bijerre and Schierup (1985) reported the uptake of metals like cadmium, lead, zinc, copper, manganese and iron in three different soil types namely loam, sandy and organic soil by oat. It was found that, uptake of all metals were highest in sandy loam soil followed by sandy and organic soil. It was also found that out of six metals studied, cadmium uptake was maximum in all the three soil types and was found in all parts of the plant. The study also revealed that lead and copper were preferentially bound by organics and oxides. Humic acid, which is generally found in varying concentration in organic soil is known to suppress cadmium uptake as shown in maize and snap beans (Tyler and Mebride 1982). However, humic acid had no effect on cadmium translocation within plant. The study also revealed that increased in Ca^{2+} ion had no apparent effect on cadmium uptake by roots, but it reduced the translocation of cadmium from root to shoot. Similar results were obtained by Cabrera et al. (1988) working with cadmium toxicity on barley.
2.3 BIOCHEMICAL AFFECTS OF HEAVY METAL POLLUTANTS:

Heavy metals in general have adverse affect on plant, which may be of various levels including the death of plant. Bhattacharjee and Mukherjee (1996) experimented with seeds of two rice cultivars (Ratna and Hamilton), which differed in sensitivity towards salinity. These when treated with PbCl$_2$ and CdCl$_2$ continuously showed a relatively higher electrolyte leakage compared to control. Heavy metal induced membrane damage was reported to be the affect of magnitude of stress as evidenced by injury index data, greater leakage of ε-amino nitrogen, soluble carbohydrate content and malondialdehyde level. In another experiment the same author found reduced activities of peroxidase, catalase and increased hydrogen peroxide level in two rice cultivars, Ratna and Hamilton when exposed to lead and cadmium. They also noted that activity of superoxide dismutase also declined.

A number of studies suggest that heavy metals reduce the activity of nitrate reductase, a key enzyme for nitrogen assimilation in plants. Kumar et al. ('1993) showed that in *Sessamum indicum* exposed to lead significantly inhibit both *in vitro* and *in vivo* nitrate reductase activity which was found to be co-related with the concentration of lead in nutrient solution and accumulation in plant. Mathuchelian (1991) working with *Cycas circinalis*, a tropical gymnosperm found that heavy metal inhibit the activity of nitrate reductase in leaf, irrespective of concentration. There was drastic inhibition of the enzyme activity within short term exposure and it was suggested that metals exert their toxicity through reduced NADPH supply and might be due to competition for binding sites. Growth of germinating pea seed (*Pisum sativum*) was significantly inhibited by as low as 0.25 mM cadmium and the elongation of the radicle was affected more severely than that of plumule. Mercury significantly reduced *in vitro* nitrate reductase activity in both primary and secondary leaves in maize and the degree of inhibition was more with
increasing concentration of mercury (Pandey and Srivastava, 1994). The inhibition was so severe that above 10 mM of Hg$^{2+}$ no enzyme activity was detected. However, in root the enzyme activity was unaffected at low concentration of 0.1 and 10 mM but at higher concentration there was inhibition. Interestingly, presence of calcium and magnesium could considerably reduce the degree of inhibition indicating that calcium and magnesium can protect the enzyme from toxic affect of mercury. Kumar and Arciaswamy (1994) reported that zinc reduced nitrate reductase activity in *Sorghum bicolor* and was observed at concentration of 50μm and above and relationship between reduction in nitrate reductase activity and zinc concentration was found to be simple linear and negative.

Divalent metals ions like Zn, Cd, Hg, Cu and Pb considerably inhibited the activity of ATPase in plasma membrane of maize (Kennedy, Gonsalves 1989) Mukherji and Floyd (1991) reported that in case of soyabean exposed to aluminium caused reduction in nitrogenase activity, apart from reduction in nodule formation. Gangopadhyay and Santra (1996) also found similar reduction in nitrogenase activity in the aquatic plant *Azolla pinnata* exposed to heavy metals. Siegal (1974) observed that heavy metals caused a decline in enzyme activity by forming metal protein complexes, but at the same time induced or activated some new enzymes. Mukherjee and Mukherji (1991) studied the impact of cadmium on peroxidase isozymes in mung-bean and found that cadmium caused alteration in the zymogram pattern. It was also reported that in case of rice seedling treated with cadmium, there was considerable reduction in the activity of cell wall hydrolysing enzymes. Nashikka and Chakraborti (1994) studied the impact of heavy metals on calalase and peroxidase enzymes in some crop plants and observed that exposure to heavy metals invariably caused a reduction in the activity of both catalase and peroxidase. They suggested that the activities of catalase and peroxidase enzymes can be used as an indicator of heavy metal pollution in soil and subsequent stress situation in plants. Nag et
al. (1981) experimenting on the impact of mercury and zinc on rice seedling found that both the heavy metals caused the reduction in chlorophyllase activity with a significant decrease in chlorophyll content.

Bhattacharjee and Mukherjee (1994) studied physiological and biochemical responses of Vignaunguiculata seedlings under the influence of cadmium and lead and observed that with increase of concentration of both the metals, cellular damage was prominent. Pigments like chlorophyll a and b and carotenoids also decreased. The total soluble sugar increased with the increasing concentration of heavy metals but the insoluble sugar decreased. Cadmium was reported more toxic than lead and the root was the most sensitive part compared with stem and leaf. Toxicological affects of leached mercury from chloro-alkali industry on the pigment content in Pistia and Hydrilla was studied reporting drastic depletion in chlorophyll content in the exposed plant leaves while a significant increase in phaeophytin and carotenoid level was marked (Patra and Panigrahi 1994).

Satyakala and Kaiser (1993) reported that the accumulation of Cu and Cr in Eichhornia crassipes altered the oxygen metabolism of chloroplast. A linear increase of these metals was observed in the plants with increasing metal concentration (10 to 50 mg l\(^{-1}\)) in the medium in which the plants were placed. The chlorophyll and protein contents were affected as a result of the bioaccumulation of these metals in leaves. They also studied the effect of heavy metals on Pistiastratioles, P. stratioles with 5-100 ppm of copper and cadmium solution for 72 hours and showed 68% and 63% accumulation of both the copper and cadmium respectively. The accumulation was more in roots than that in the leaves at 100 ppm cadmium concentration. Chlorophyll, sugar and protein content decreased with copper and cadmium treatment, however phenol content and enzyme activities varied differently. De and Mukherjee (1996), studied the effect of mercuric chloride, on metabolism of protein, nucleic acid, proline, hydrogen peroxide and some related enzymes
in tomato seedlings and cultured cells and reported the injurious affect as to be directly related to the concentration.

All these evidences suggest than most of the heavy metals act by interacting with various enzyme systems and in the process alter the level of various biochemical compounds. Keshan and Mukherji (1994) reported than in mung bean following treatment with cadmium sulphate as foliar spray, there was considerable reduction in the level of important biochemical constituents like sugar, nitrogen, amino acids and nucleic acid (DNA and RNA) accompanied by decline in plant growth and yield. A major impact of heavy metals on biochemical level is the damage caused to protein synthesis machinery. Singh and Shrotya (1988) reported that in Eclipta alba cadmium severely reduced the level of soluble protein and RNA and to lesser extent DNA. A concentration of 50 ppm cadmium reduced the level of both RNA and protein to 40% of control. Increase in the level of free amino acid following uptake of nickel had been demonstrated in Allyssum bertolonii (Bick and Deckock 1982). It was also reported that nickel uptake and increase in the level of free amino acid was influenced by seasonal factors, exhibiting highest nickel uptake and corresponding high level of amino acid in February and the same decreased in June and again increased in October. Singh and Shrotlya (1988), observed that zinc at low concentration up to 50 ppm, increased the level of both RNA and protein, while at high concentration of 200 ppm and above caused a significant reduction of both. Similar increase in the level of RNA and protein and metabolic activity by zinc at low concentration was reported by several workers (Srivastava, 1979; Reddy and Rao 1979).

Lead oxide from automobile exhaust was known to reduce considerably the level of protein on Piper bettle grown by the side of highway and petrol pump (Senapati and Mishra 1996).
2.4 IMPACT OF HEAVY METAL AT CELLULAR AND SUB-CELLULAR LEVEL:

Apart from affecting various biochemical activities and physiological processes, heavy metals are also known to exert its effect at cellular and sub-cellular level particularly on mitotoc cell division and cause wide range of chromosomal aberration.

Chaudhuri et al. (1993) studied the effect of cadmium and mercury on *Nigella sativa* and found diverse chromosomal aberration in mitotic cells resulting upon the concentration of the metals. Sengupta and Ghosh (1992) studied with same metals on *Lathyrus sativus* and obtained similar results. Jayprakash et al. (1994) studied the impact of chromium on the mitotic activity in the root tip meristem of *Allium cepa* and observed that chromium acts as prophase poison. Moreover, with increase in concentration there was a proportional drop in mitotic index. Mitotic anomalies in onion root tip cells were also observed by Nag et al. (1992) following treatment with heavy metals like mercury, copper and zinc. Panda et al. (1992) observed both mitotic and meiotic chromosomal anomalies in barley following treatment with mercury. Singh and Singh (1994) studied the toxicity of manganese, with *Allium cepa* and concluded that the metal manganese (MnSO₄) is a severe root length and root number supressor, mild mitotic repressor and fairly strong inductor of chromosomal abnormalities like C-mitosis, chromosome bridge and clumping. Arsenic toxicity on mitotic cell division of *Allium cepa* was reported by Sinha et al. (1996).

Sengupta and Ghosh (1996) also observed a striking fall in mitotic indices in *Pisum sativum* following treatment with lead nitrate. The same study also recorded various types of chromosomal aberrations like spindle abnormality, disturbances in cell plate formation, in activation of spindle apparatus with delayed division of centromere leading to polyploidy and aneuploidy.
The results from various fronts envisage that heavy metals apart from exerting the usual toxic effect also act as chemical mutagens too which may lead to deleterious mutation resulting diverse genetical problem. Gupta and Devi (1995) working with aquatic plants, *Salvinia, Azolla* and *Marsilea* exposed to 0.1 ppm of cadmium found that *Salvinia* is a prolific accumulator of cadmium, while *Azolla* is a lesser accumulator. Marsilea was found to be non-accumulator and tolerant as it accumulated very little cadmium even after prolonged exposure. The transmission Electron Microscopy revealed that in case of susceptible species i.e. *Salvinia* and *Azolla* the epidermal cells of leaves collapsed and the plasma membrane converged in case of root which got maximum exposure to metal, the cell organelles became disorganised, particularly in mitochondria, the cristae became sparsed. Another prominent feature was extensive vacuolation in root cells and development of peg like thickening on inner wall of the root cells and root hairs which is considered to be adaptive mechanism to resist metal stress. However, in case of tolerant species of *Marsilea* no such cellular and sub-cellular damage was reported.

There is report that significant loss of pollen viability occured in case of pollens of tomato; pea and pigeon-pea, following treatment with heavy metals viz. mercury and lead in the concentration of 10 and 20 ppm. The magnitude of loss of viability was related to concentration and duration of treatment; compared to mercury, lead had been found to be more toxic. Pollens of tomato were more tolerant, while pollens of pigeon pea were least tolerant (Handique and Baruah 1995). An interesting observation as made by Vaulina et al. (1978) was that mitotic anomalis caused by cadmium in the root tip cells of *Crepis cappellaris* could be greatly reduced by treatment with cystein. Cadmium, apart from chromosomol anomaly also inhibited cytokinesis and it is reported that cadmium ions interact with -SH group, thereby blocking in contractive proteins of cellular spindle.
2.5 IMPACT OF HEAVY METALS ON PHYSIOLOGY, GROWTH AND PRODUCTIVITY:

Growth, development and productivity of plant is the manifestation of biochemical activities within the cells and tissues. Since various heavy metals are known to adversely affect various biochemical activities, it is obvious to expect that they adversely affect the whole plant physiology, growth and development apart from showing morphological stress and symptoms for specific metal toxicity.

Tompkins and Blinn (1976) reported the effect of two mercury salt, viz., mercuric nitrate and mercuric chloride on two algal species viz. *Fragelaria crotonesis* and *Asterionella formosa* and found that the mercury completely inhibited the growth of *F. crotonesis* at 0.1 ppm and that of *A. formosa* at 0.5 ppm. Matson *et al.* (1972) found that mercury inhibited the biosynthesis of lipid, especially galactolipids and chlorophylls in case of fresh water algae *Ankistrodesmus braunii* and *Euglena gracilis*. In case of *Phaseolus aureus*, mercury was fatal at a concentration of 50 ppm whereas *Lens culinaris* could tolerate up to 200 ppm of mercury (Usha and Nathawal 1993).

Morphological manifestation of heavy metals impact had also been studied by several workers. Gangopadhyay and Santra (1996) cultured *Azolla* in nutrient solution containing 5-15 ppm of cadmium and cobalt and observed that *Azolla* turned grey to brown within 1 to 4 days and thalli become fragile, while the control remained green and healthy. Similarly, Satyakala and Jamil (1992) showed that chromium toxicity caused yellowing of leaves due to excessive respiration in aquatic plants *Eichornia crasipes* and *Pistia stratiotes*. Sen and Mandal (1992) reported that copper at a concentration of above 20 ppm caused premature senescence in aquatic plant *Salvinia natans* and hence this can be used for bio-monitoring.
It was reported that relatively low concentration of Pb, Cd, Ni and Al inhibit photosynthesis and respiration in detached sunflower leaves which was found to reduce to 50% of control when tissue concentration was 63 ppm for Al, 96 for Cd, 193 for Pb, and 79 for Ni (Bazzaz et al. 1974). In another study they found that in case of maize and soybean, lead caused decrease in the rate of photosynthesis and transpiration and the degree of inhibition increased with increasing doses of lead. Gupta and Arora (1978) observed an inhibition of photosynthesis in case of *Hyngbya nigra* at a concentration of 0.8 μM of CuSO₄. Copper inhibit electron transportation between the oxidising site of the reaction centre of photosynthesis system II and the electron donating site of 1, 5-diphenylcarbazide (Shioi et al, 1978).

Jawarkar and Shende (1986) found that due to treatment with lead and cadmium, yield of barley get considerably reduced. There are reports that certain hormones can reduce heavy metal toxicity. Nag et al. (1980) reported that IAA and GA₃ can considerably neutralise the toxicity of heavy metals like Hg, Cu, and Zn in wheat and lettuce. It was reported that application of GA₃ can considerably reduce the inhibition of α-amylase synthesis caused by Hg, Cu and Zn. Mukherji and Roy (1978) observed that inhibition of germination and seedling growth by chromium in mung bean can be considerably reversed by GA₃ and simple inorganic salts like KCl, FeCl₂ MnCl₂ as well as by citric acid.

### 2.6 GENETIC BASIS AND MECHANISM OF HEAVY METAL TOLERANCE:

The plants of different families and also different cultivars within a species exhibit differential tolerance indicating that there must be some genetic basis of tolerance and accumulation. McGrath et al. (1996) tested ten species of plants over four seasons showing highest Zn accumulation by *Thlaspi caerulescent*; which also showed similar response for cadmium. However, for cadmium the largest removals (60g Cd/ha in a single
harvest) was reported by *Cardaminopsis halleri*. Another important plant as hyperaccumulator for Ni is *Alyssum murale* (Bernal and McGrath, 1994) and for lead is *Thlaspi rotundifolium* (Reeves and Brooks, 1983). Mackay *et al.* (1990) studied aluminium tolerance of fourteen white clover (*Triblium repens*) cultivars collected from eleven countries exposing to nine different concentrations of aluminium ranging from 2.5 to 750 mg kg$^{-1}$; while seven cultivars were proved to be aluminium susceptible, few were found to be highly tolerant to alluminium. Similar genotypic variation with respect to growth, development, yield and nutrient efficiency ratio (NER) following treatment with aluminium was reported in case of sorghum grown under both field and green house condition (Baligar *et al.* 1989). Aniol (1990) made extensive study of the genetic basis of aluminium tolerance in wheat and reported that aluminium tolerance in wheat is a dominant character governed by several genes.

Resistance to heavy metal impact can be achieved by two ways - one is avoidance, a process whereby metal uptake is avoided or limited; the other is tolerance, in which the root uptake considerable amount of metal but once enter inside plant body the metals can be detoxified. Thuoman (1981) demonstrated that some plant ecotype endemic to heavy metal polluted soils contain heavy metal resistant enzyme like cell wall acid phosphatase which enable them to avoid uptake. Detoxification occurs depending upon metal through chelation, compartmentation or precipitation. Huang *et al.* (1997), reported that most promising method of increasing plant lead uptake from the soil particle using chelates. They tried a wide range of compounds and EDTA proved to be the best desorbing agent. Brokes *et al.* (1981) demonstrated that zinc get chelated to organic acids and accumulated within the vacuole. This was further confirmed by Brune *et al.* (1994) who showed that the intact vacuole isolated from barley leaves contained high levels of zinc. Davis *et al.* (1991) demonstrated that in case of zinc tolerant *Festuca rubra*, vacuolar volume in meristamatic
cells increase following exposure to zinc, showing the compartmentalisation of metal within the vacuole, which are prevented from coming in contact with cellular metabolites and thus tolerance is achieved.

In case of other toxic metals like cadmium, the chelation occurs with a type of metal binding polypeptide, called phytochelatin which are rich in thiol and low molecular weight protein ranging from 1.5 - 4 KDA. Another class of such metal binding protein have size ranging from 8-14 KDA which are reported to be aggregates of phytochelatin (Prasad 1995) and are called metallo thioneins. Salt and Wagner (1993) demonstrated that Cd-metallothionein complex move across the tonoplast and dump the metal in the vacuoles, thus detoxifying the metal. There is report that in case of mustard, exposure to Pb induce synthesis of phytochelatin which detoxify Pb in a manner similar to Cd metallothionein complex (Salt et al. 1995). The genes of metallothionein have been reported from several plant like pea, soyabean, etc. Genes encoding Cd binding protein metallothionein isolated from mammals have been expressed in plants through genetic engineering technique to enhance Cd tolerance (Leftbure et al. 1987; Maiti et al. 1991).