CHAPTER 2

REVIEW OF LITERATURE

2.1. Heavy metal pollution: sources and its effect

Contamination of soil through the heavy metal causes major environmental problems. The process of excavation, removal of contaminated soil and subsequent deposition involved in the process of cleaning up the environment. Besides the natural activities human activities are also responsible to produce heavy metals as side effects. Heavy metals are required by the plants for the normal growth and metabolic functions but the excess amount can cause disturbances in the same processes, they become toxic for the organisms at the higher concentration (Marschner, 1995). Metals are present in the Earth's crust at the various levels. Industrialization and agricultural practices lead to elevates the release of metals into the ecosystem, which cause serious environmental problems which ultimately posing a threat to human health (Lantzy and Mackenzie, 1979; Ross, 1994). The heavy metals become toxic at higher concentration because they cause oxidative damage along with the other metals the redox active transition metals can produce free radicals which can cause oxidative damage (Cho and Park, 2000).

By replacing the other essential metals in pigments and enzymes, cause disturbance in the function of the molecules heavy metal cause toxicity (Rivetta *et al.*, 1997). Several methods are used to clean up the environment from these contaminants.

2.2. Heavy metal toxicity

The low and high dosage of heavy metals revealed the fact of adverse effects on physiological activities of plants, means stimulation of reaction by plants to low heavy metal stress (Cheng, 2003). The visual evidence of toxicity is the reduction in growth of the plant (Sharma and Dubey, 2007), including chlorosis in leaf, necrosis, loss of turgour, decrease in germination rate, senescence, even lead to death of the plant (Carier *et al*., 2003). The toxicity by heavy metals triggers a wide range of physiological and biochemical changes (Dubey, 2011; Villiers *et al*., 2011).

They inhibit the biosynthesis of chlorophyll by inhibiting the aminolevulinic acid dehydratase and protochlorophyllide reductase (Ouzounidou*et al*., 1995). The accumulation of heavy metaldecreased transplantation rate (Bazzaz*et al*., 1974a,b), inhibit the Hill reaction, electron transport system (Tripathy and Mohanty, 1980), increased the activity of chlorophyll (Drazkiewicz, 1994). It inhibits the PSI and PSII reaction centers, results in the inhibition in the evolution of oxygen, NADPH reduction and photo phosphorylation (Krupa and Baszynski, 1995).The primary target of heavy metal toxicity may be the enzymatic phases of photosynthesis, which can slow down the reaction of light phase either by down regulation or by insufficient consumption of ATP, NADPH (Kupper and Baszynski, 1995). Heavy metal inhibits the enzymes of Calvin cycle such as Rubisco, PEP carboxulase, 3-phosphoglyceris acid kinase, NADP-dependent glyceraldehyde-3 phosphate dehydrogenase, ATPase (Prasad and Strzalka, 1999).

Table02. Heavy metal toxicities on plants. Based on (Sunitha *et al.*, 2014).

Metal	Effect	References
Copper	Growth retardation, Chlorosis, Oxidative stress	Stadtman and Oliver, 1991; Lewis *et al*., 2001
Zinc	Retardation in growth, Chlorosis, Senescence	Ebbs and Kochain, 1997
Arsenic	Inhibits photosynthesis, growth, biomass and yield, Death	Carbonell *et al*., 1998.
Cadmium	Chlorosis, Growth inhibition, photosynthetic reduction, reduction in water and nutrient uptake, Brown patches on leaves and root tips, Death	Wojcik and Tukiendorf, 2004
Chromium	Germination alteration, growth inhibition, chlorosis, imbalance in nutrient, inhibition in synthesis of chlorophyll, photosynthesis, yield	Chatterjee and Chatterjee, 2000; Sharma *et al*., 2003; Scoccianti *et al*., 2006
Cobalt	Growth retardation, Decreased chlorophyll and catalase activity, reduced transpiration rate	Chatterjee and Chatterjee, 2000; Li *et al*., 2009
Lead	Retardation in growth, morphology, photosynthesis, inhibition of enzymatic activities, water imbalance, oxidative stress	Sharma and Dubey, 2005; Reddy *et al*., 2005
Nickel	Chlorosis, Necrosis, Disorder of cell membrane function, Imbalance in water and nutrient uptake	Gajewska *et al*., 2006
Mercury	Alteration of water flow, interference in mitochondrial activity, disruptions of cellular and biomembrane lipids, Oxidative damage	Zhou *et al*., 2007

Toxic heavy metals get in food through the roots and can deposited in the edible portion of the plants and can easily accumulate in the food chain. Contaminated medium like soil, water are major environmental threat. These problems can be overcome by an emerging new technology Phytoremediation. Phytoremediation is a term where the green vascular plants, including trees, grasses are used to remove, destroy or sequester hazardous contaminants from the environment. This term includes all plant influenced biological, chemical and physical process that aid them to contain, degrade or eliminate metals, pesticides, explosives, crude oil and various other contaminants from the mediums and soil (Lasat *et al*., 1996; Salt *et al*., 1998; LeDuc and terry, 2005).

2.3. Hyper accumulating plants

The term hyperaccumulatorcriterion is applied to many heavy metals like Co, Cu, Cd, Pb, Zn etc (Baker *et al*., 2000). It defines the capacity of plant that being in their natural habitat they can accumulate the metal even at higher concentration (Tangahu *et al*., 2011). The hyperaccumulator plants have the ability to accumulate the heavy metals even at higher concentration; it may be 100-1000 times higher than that of non hyperaacumulator plants. Hyperaccumulators should thrive in toxic environment which require little maintenance and produce high biomass. Hyperaccumulator plants usually have a shoot to root metal concentration ratio of >1, whereas non hyperaccumulator plants generally have higher metal concentration in roots compare to shoots (Shen *et al*., 1997).

Till date there are many plant species have been identified as metal hyperaccumulator which is an evolutionary response where <0.2% are angiosperms (Brooks, 1998; Baker *et al*., 2000). Table 03 representing some of the hyperaccumulator plants which have the ability to accumulate the metals at higher level without any toxicity.

Table03. Some heavy metal hyper accumulating plants. Based on (Sunitha et al., 2014).

Plant	Metal	References
Astragalus racemosus	Selenium	Beath *et al*., 1937
Ipomea alpine	Copper	Baker and Walker, 1989
Berkheya coddii	Nickel	Robinson *et al*., 1997
***Phragmites australis, Zea mays* L. Cv Ganga 5**	Chromium	Sharma *et al*., 2003
Pteris vittata	Arsenic	Dong, 2005
Sesbania drummondi, Sedum alfredii	Cadmium	Israr and Sahi, 2006; Jin *et al*., 2008
Sedum alfredii	Zinc	Jin and Liu, 2009
Phytolacca americana	Manganese	Pollard *et al*., 2009

2.4. Heavy metal toxicity in the environment

2.4.1. Deficiency of copper

Cu is an essential microelement require for growth. There are different symptoms has been observed due to deficiency of Cu in plants, starts from tips of new leaves extended up to leaf margins. Leaves were twisted show chlorosis, necrosis (Marschner, 1995). Cu deficiency reduce photosystem I (PSI) electron transport due to decreased formation of plastocyanin (Baszynski *et al*., 1978; Shikanai *et al*., 2003). Decrease in photosystem II (PSII) activity was also observed in Cu-deficient chloroplasts (Droppa *et al*., 1987; Henriques, 1989). Cu-deficient plants show disintegration of the thylakoid membranes of chloroplasts (Baszynski *et al*., 1978; Henriques, 1989). It decrease pigment content, reduced plastoquinone synthesis (Barón *et al*., 1992).

2.4.2. Toxicity of copper

Higher concentration of Cu causes adverse action by inhibiting the growth and by interfering the cellular mechanisms involved in the plants (Marschner, 1995; Prasad and Strzalka, 1999). Cu could involve either in the destruction of the oxygen-evolving complex polypeptide composition or the interaction with ions necessary for proper functioning of the complex such as Mn, Ca and Cl (KruppaandBaszynski, 1995). Structure and the composition of the thylakoid membranes can be altered by Cu, may lead to the decrease in the photochemical activity (Baszynski *et al*., 1988, Ouzounidou *et al*., 1992, Lidon *et al*., 1993).At high concentration Cu can become extremely toxic by causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth (van Assche and Clijsters, 1990; Marschner, 1995).

At the cellular level, toxicity may result from *i)* binding to sulfhydryl groups in proteins, thereby inhibiting enzyme activity or protein function; *ii)* induction of a deficiency of other essential ions; *iii)* impaired cell transport processes; *iv)* oxidative damage (van Assche and Clijsters, 1990; Meharg, 1994). Excess of Cu can generates oxidative stress leads to the production of Reactive oxygen species (Stadtman and Oliver, 1991). The generation of ROS brings changes in the content some components of the antioxidative pathways (*i.e.*, ascorbate peroxidase (APX), superoxide dismutases (SODs), guiacol peroxidase) (De Vos *et al*., 1992; Luna *et al*., 1994; Stohs and Bagchi, 1995; Gupta *et al*., 1999; Wang *et al*., 2004). Thus, for healthy plant growth and development Cu must be acquired by the plants which should be distributed and compartmentalized within different tissues.

2.4.3. Effect of Copper on growth of plant:

Copper is an important component for plant, but the excess amount of Cu plays a cytotoxic role which induces stress and causes injury (Lewis *et al*., 2001).In cell it causes problems by inhibiting the plant growth by impairing important cellular processes like photosynthetic electron transport (Demirevska *et al*., 2004). Being the character of both an essential cofactor and a toxic element, Cu involves a complex network of metal trafficking pathways. This prevents accumulation of the metal in the freely reactive form (metal detoxification pathways) and ensure proper delivery of this element to target metalloproteins. Cu^{2+} and Cu^{+} are the transition metals can catalyze the production of highly toxic hydroxyl radicals by redox cycling, by damaging DNA, lipids, proteins and other biomolecules (Halliwell and Gutteridge, 1984).

Both deficient and excess concentration of Cu can cause disorders in plant growth and development by adversely affecting the physiological process in plants. Thus, for healthy plant growth and development Cu must be acquired from the soil under normal conditions, transported throughout the plant, distributed and compartmentalized within different tissues and its content carefully regulated within different cells and organelles. Cu is essential for human health too but prolonged exposure can cause hazard to health. The interaction of metals with physiological and biochemical pathways within the plants the growth retards with the reduction in biomass.

Accumulation of excess Cu as well as other heavy metals inhibits the growth elongation (Maksymiec *et al*., 1994, 1995, Maksymiec and Baszynski, 1996b). Cu toxicity affected the growth of *Alyssum montanum* (Ouzounidou, 1994) Cd on Cucumber (Moreno-Caselles *et al*., 2000) and *Brassica juncea* (Singh and Tewari, 2003). Cu in combination with Cd has affected adversely the germination, seedling length number of lateral roots was observed in *Solanum melongena* (Neelima and Reddy, 2002). Root viability reduced under heavy metal stress. On the accumulation of Cu, Zn, Pb and Cd the root vitality of *Stylosanthes guianensis* was reduced where the absorption of inorganic nutrients was prevented led to chlorosis which significantly affected the growth (Shu *et al*., 1997).

There was significant increase in Cu concentration in leaves blades, shoots and roots in rice plants with the application of $CuCl_2$ (Sudo *et al*., 2008). It was found to be toxic on exposure of above 45 µM Cu exposure (Sudo *et al*., 2008).The Cu content on tea plants was observed from 9.1 to 11.5 mg/Kg (Gebretsadik and Chandravanshi, 2010).In some tea samples it was observed that the accumulation of Cu was below the PFA limit of 150 µg/g (Sushama *et al*., 2011). The exposure of Cr on tea plants did not showed the symptoms of diseases until 6 months. The roots suffered mostly. The leaves were observed with brown spots, number of new stem leaves was less the rate of growth was slowed down. Most of the leaves became withered. The growth of tea was inhibited by high concentration of stress. At higher concentration the inhibition was high. At 600µM the inhibition was strongest (Tang *et al*., 2012). Rice leaves The accumulation of Cr content in tea plants was found in order of stubble> root> leave> stem>branch, the accumulation in stubble was 5-7 times than that of root, 49-149 times of branch, 35-90 times of stem, 11-31 times of leaves, and 59-126 times of new stem. The content of Cr on tea plants was positive correlated with concentration of Cr (Tang *et al*., 2012)**.**The variance for the essential mineral content was found to be a significant <0.05 on Na, P, Mg, Cu, Zn and Mn contents whereas non-significant P>0.05 was observed for K and Fe contents on tea leaves. Cu contents of tea leaves at different withering hours showed highest concentration (12.6 mg/Kg) at 24 h while the lowest (10.2 mg/Kg) at 23 h. There were biochemical changes during withering. The leaves started loosening of moisture immediately after the start of the process. It was reported that tea contained Cu contents ranged from8-28 mg/Kg (Jabeen *et al*., 2015).

According to the regulations imposed upon tea in Japan (100 mg/kg) and the United States (150 mg/kg) (Ning *et al*., 2011), the determined levels of Cu in the tea samples were all below the limits. The effect of some micronutrients like Pb, Cd, Cr, Ni was observed in some Chinese tea varieties, where the contents of Cu were 17.01-63.07 mg/kg was observed in green tea, 7.73-20.49 mg/kg in oolong tea, 22.61-33.45 mg/kg in black tea, 21.06-31.35 mg/kg in Pu'er tea, and 19.21e30.35 mg/kg in jasmine tea. The lowest Cu content was found in oolong tea, while the highest content was found in green tea. Green tea was observed with higher Cu level than the other types of tea, and oolong tea had the lowest Cu level on average.The Cu levels in the green tea from India and Japan ranged from 23.1 mg/kg to 36.5 mg/kg (Zhong *et al*., 2015).Therefore, the maximal content of Cu in tea is limited in some regulations.

2.4.4. Effect on photosynthetic pigment

Photosynthesis is one of the most important processes. Heavy metal causes effects on photosynthetic apparatus. Prolonged exposure of heavy metals cause reduction in growth of leaf decreased photosynthetic pigments and disturbed the ultrastructure of chloroplast (van Assche and Clijsters, 1990, Sheoran and Singh, 1993). Carotenoid is also an important pigment which can play a vital role in photosynthesis, is also an essential component of photosynthetic membranes in plants, algae and cyanobacteria (Demig-adams *et al*., 1996).Decrease in chlorophyll and carotenoid was observed in response to drought stress in tea (Upadhyaya *et al*., 2008). Damage in chlorophyll pigments decrease efficiency of photosynthesis in plants which lead to reduction in growth of tea plants. A positive relationship was established between transpiration efficiency and leaf chlorophyll concentration in plants (Sheshshayee *et al*., 2006). Metals like Cd, Cu induce decrease in dry mass of leaf, antioxidant enzymes showed differential activities in response to Cu and Cd (Upadhyaya and Panda, 2013).

Drought stress induces oxidative damage in tea plant affects antioxidative systems by altering different physiological and biochemical processes (Upadhyaya and Panda, 2004a). Application of $CuCl_2$ on rice affected the photosynthetic and transpiration rate at 130µM (Sudo *et al.*, 2008).Total chlorophyll content was found to be decreased significantly with the increase in the concentration of Cu in both the TS-520 and TS-462 cultivars, where TS-520 was recorded with higher decrease (Saha *et al*., 2012).There was a negative was established between the content of chlorophyll a, chl b, total chlorophyll and the concentration of Cr in tea plants. The composition of chl was changed, the ratio of chl a/b decreased with the increase in concentration of Cr. The degree of damage of chl varied with different concentration of Cr (Tang *et al*., 2012). Drought stress changes in nutrient status of tea plant and findings reveled that drought stress caused reduction in mineral content of leaf which varies in different cultivars (Upadhyaya and Panda, 2013).

2.4.5. Metal toxicity induce Oxidative damage

Cu is constituents of many enzymes and proteins so they are essential for normal growth and development of plant. Now a day's agent Cu has been used in agriculture as an antifungal but the extensive released of Cu by human activities like application of pesticides into the environment cause pollution. But the elevated amount of the same can cause toxicity by inhibiting the growth of plant. The symptoms of toxicity are results of the presence of excessive amounts of heavy metals which can cause a range of interactions at the cellular or molecular level (Hall, 2001).

Binding of metals to sulphydryl groups in proteins causes inhibition activity or disruption of structure, results in toxicity to the plants (van Assche and Clijsters. 1990). This toxicity stimulate the generation of free radicals and reactive oxygen species (Dietz *et al*., 1999; Dat *et al*., 2000). Cu induced the generation of hydrogen peroxide, hydroxyl radicals, or ROS which are directly related to the damage of proteins and lipids that may lead to death of plant (Dat *et al*., 2000). The generation of ROS and their products in the cell causes peroxidation of lipid membrane which is one of the most damaging effects.

Plants have a range of potential mechanisms that may help them in detoxification of metals and thus evolved the tolerance races that can survive on metalliferous soil. There are some evidences that tolerant species show enhanced avoidance and homeostatic mechanisms to prevent the onset of stress (Dietz *et al*., 1999; Dat *et al*., 2000; Arora *et al*., 2002). The tolerance ability of plants may be defined as the mechanism plants to survive in metalliferous soil that is toxic to other plants (Hall, 2002). The study of genetic basis which deals with adaptive metal tolerance where it governed by a small number of major genes along with some minor genes (Macnair, 1993; Schat *et al*., 2000).

The ROS include superoxide anion (OH), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), alkoxyl radical (RO), organic hydroperoxide (ROOH), singlet oxygen ($.O_2$) etc cause oxidative damage (Fleschin *et al*., 2000). Oxidative stress is an imperfect condition that reflects an imbalance between the generation of free radicals and the ability of the plants to detoxify the harmful effects through neutralizing by the antioxidants (Shaw *et al*., 2004). However, plant has scavenging system for ROS which prevent or reduce the injury caused by the generation of ROS (Sudo *et al*., 2008). SOD, CAT, APX etc. are ROS scavenging enzymes which change their activities in response to Cu exposure in excess amount (Luna *et al*., 1994; Lombardi and Sebastiani, 2005). The toxicity caused by heavy metal can be reduced by chelating of heavy metal ligands or effluxes of heavy metal ions (Clemens, 2001; Hall, 2002).

For centuries in India and China, tea has been used medicinally. Polyphenols act as potential indicators of drought tolerance in *Camelia sinensis* (Cheruiyot *et al*., 2007). The effect of stress on plants depends on dose, duration of stress exposure and developmental stages of plants. Difference in inherent antioxidant potentialities of tea is responsible for the variation in responses to various abiotic and biotic stresses by the different varieties of tea plant (Upadhyaya and Panda, 2004b; 2013). The plant responses to stress as an individual cell, induced changes in the expression of gene which participate in the generation of hormones (Upadhyaya and Panda, 2013).

Various osmosensors and signal transduction helps in interpreting the drought stress activates stress responsive genes which are responsible for detoxification of stress. The generation of ROS and inactivation of antioxidant systems due to drought stress resulting in oxidative damage in the different varieties of tea. Among the clones of the tea plant, TV-1 showed less decrease in antioxidants with higher activities of POX, CAT, GR, phenolic contents revealed the fact of better drought tolerant potential. Whereas TV-29 and S_3A_3 were drought sensitive clones (Upadhyaya *et al*., 2008).

Cu as a redox metal can interfere with various physiological processes which indicate its toxicity in plants (Baryla *et al*., 2000). Exposure of Cu resulted in the accumulation of lipid peroxidation in tea leaves. The level of TBARS increased with the increase in Cu concentration and exposure time in TS-520 and TS-462 up to 7 days beyond which the rate of increase declined (Saha *et al*., 2012). Among the cultivars TS-520 and TS-462, there was significant difference in the lipid peroxidation in response to Cu where TS-520 was found to be more Cu-sensitive (Saha *et al*., 2012). Concentration of Cu lead to the increase in peroxidase activity in both the cultivars. In TS-520 the POD activity increased at the lower concentration of Cu but decline subsequently at concentration higher than 400 µM. On the other hand the cultivar TS-462 showed an increase in the POD activity with increase in the Cu concentration except at the highest concentration of 700 µM there was a sharp decline in POD activity was observed. Two new isozymes POD1 and POD2 were induced in the leaves of Cu treated tea plants (Saha *et al*., 2012). There was an increased content of phenolic compounds in both the cultivars at Cu concentration below 400µM. at higher concentration, the activity either changed insignificantly or decreased (Saha *et al.*, 2012).

The SOD isozymes are metalloenzymes which utilize metal cofactors like Cu, Zn, Fe and Mn for activity (Alscher *et al.*, 2002). TS-462 cultivar was recorded with the higher increase in SOD activity during the first 7 days, however, the prolonged exposure and above 400 µM concentration of Cu lead to the activity level off. In TS-520, above 400 µM there was decrease in SOD activity but at the highest concentration of 700 µM after 10 days of exposure the SOD activity returned almost to the original level from where it has been started (Saha *et al.*, 2012).

Table04. Summary of potential mechanisms involve in the detoxification of and tolerance to specific metals. Based on (Hall JL, 2002)

Mechanisms	Metal	Key reference
Mycorrhizas	Zn, Cu, Cd	Jentschke and Godbold (2000)
Cell wall, exudates, plasma membrane	Various, including Ni, Al	Salt *et al.*, (2000); Ma *et al* (1997)
Phytochelatins	Cd	Cobbett (2000)
Metallothioneins	Cu	Murphy and Taiz (1995)
Vacuolar compartmentation	Zn	Van der Zaal *et al.*, (1999)

A sharp increase in APX activity was observed at concentrations 400 µM to 600 µM at the 10th day of its exposure in TS-462. A fourfold increase in the more sensitive cultivar, TS-

520 was observed but the APX activity declined at concentration higher than 400 µM (Saha *et al*., 2012). An insignificant increase in CAT activity was observed in both the tea cultivars. Moreover Cat activity remained unaltered or was marginally increased in response to oxidative damage induced by Cu stress (Saha *et al*., 2012).

On the other hand CAT activity was reported at significantly higher levels in Cu stressed *Prunus cerasifera* plantlets (Lombardi and Sebastiani, 2005). The investigation on TS-520 and TS-462 tea cultivars showed that excess Cu ions caused significant increase in APX activity along with SOD and POD activities which are consequences of excess accumulation of H_2O_2 in tea leaves. Whereas CAT was markedly immobilized for protection against damage caused by oxidative stress. This may be due to the excess accumulation of H_2O_2 inactivated CAT (Wang *et al*., 2004). Exposure of Cu directly to the rice roots caused significant increase in the accumulation of Cu concentration in the leaf blades, shoots and roots (Sudo *et al*., 2008). At 130µM the photosynthetic and transpiration rate were significantly affected.

The increase content of oxygen in tea plants on exposure of Cr at different concentration declined the SOD, POD and CAT activity and showed a negative correlation coefficient of -0.81, -0.96, -0.96 respectively. It was observed that the MDA content was increased with the increase in Cr concentration and showed a significant negative correlation and the correlation coefficient was 0.99 (Tang *et al.*, 2012).

2.5. Phytochelatins and Phytochelatin synthase

Some heavy metals like Cu and Zn are essential micronutrients for the normal growth and other physiological activities via the action of Cu- and Zn-dependent enzymes. Both the essential and nonessential heavy metals are highly reactive and can be toxic to living cells. Thus plants have evolved a series of mechanisms including chelation and sequestration of heavy metals by ligands to control and respond the uptake and accumulation of metals. The two are the best characterized metal binding ligands in plants are Phytochelatins (PCs) and metallothioneins (MTs) (Rauser, 1999; Cobbet and Goldsbrough, 2002).

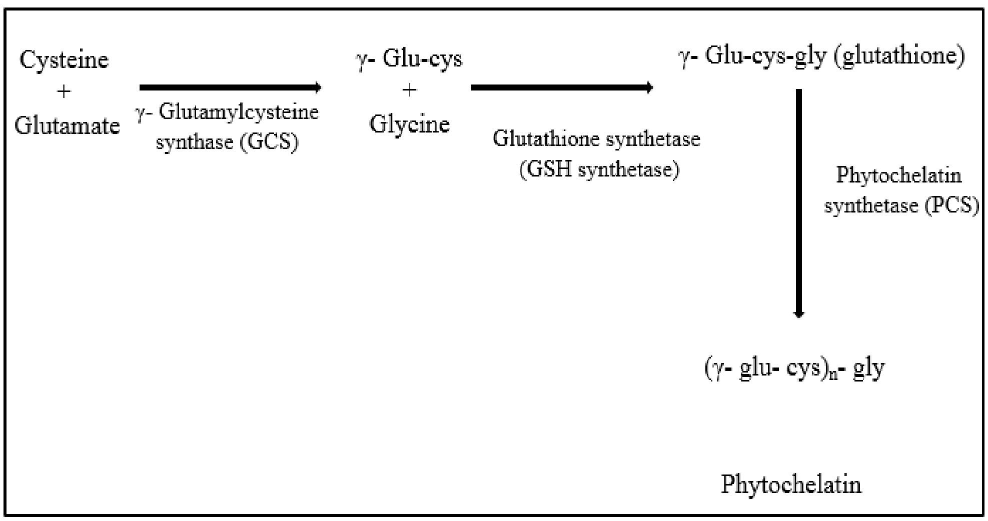

Figure courtesy: Plant Cell Biotech and Mol. Bio. (Sunitha et al., 2014).

Figure2.1. Biosynthesis of phytochelatins in plant systems.

PCs form a family of metal complexing peptides have general structure Gly; (γ-Glu-Cys)$_n$-Gly, where n is in the range of 2-11. PCs are generally found in many variety of plants and in microorganisms too (Gekeler *et al.*, 1988, 1989). PC falls in to five main classes containing different C- terminal residues. These are Canonicals Phytochelatins,(γ-Glu-Cys)$_n$-Gly with C terminal Glycein, [iso-(PC)-ß-alanine] with C terminal ß-alanine, Hydroxymethyl-PC[iso-(PC)-Serine]with C terminalSerine, Iso-PC,[iso-(PC)-Glu] with C terminal Glutamic acid, and Des-Gly PC [des-(Gly-PC)] respectively (Zenk, 1996). Table06 shows Induction of PCs in various plants in response to different metals.

The genes for PC synthase have now been identified in Arabidopsis and yeast (Clemens *et al*., 1999; Vatamaniuk *et al*., 1999). PCs are structurally related to glutathione (GSH; γ-Glu-CysGly), and number of physiological, biochemical and genetic studies revealed that GSH is the substrate for the biosynthesis of PC (Tomaszewska *et al*., 1996; Vatamaniuk *et al*., 2000).

Many works has been done in the way of knowing the role of PCs in detoxifying the heavy metals. The range of biochemical and genetic evidences has supported in detoxification of Cd. Isolation of series of Cd sensitive mutants of Arabidopsis have a range of variation in accumulation of PCs, which correlated with the degree of sensitivity to Cd (Howden *et al*., 1995a, b). Treatment of Cd and Cu in *Arabidopsis* resulted in the increase transcription of genes for glutathione synthesis and lead to the detoxification of the metals by PCs (Xiang and Oliver, 1998). The rapid induction of PC biosynthesis helped in the accumulation of Cd in *Brassica juncea,* where the PC content was sufficient enough to chelate all the Cd which protects photosynthesis but failed in preventing the declination in transcription rate (Haag-Kerwer *et al*., 1999). Both the Cu sensitive and Cu tolerant ecotypes of *Silene vulgaris* were exposed to Cu giving either no and 50% of growth inhibition which showed equal synthesis of PC in root tips (Schat and Kalff, 1992). In the presence of y-glutamyl cysteinyl synthase the exposure of Cu in *Mimulus guttatus* cause reduction in growth of root (Hall, 2001).

Table05. Induction of PCs in various plants in response to different metals.

Plant species	Metal	PCs	Reference
Marchantia polymorpha	Zn	PC2, PC3	Gekeler *et al*., 1989
Nicotiana rustica	Cd	Cadmium binding peptides	Vogeli-Lange and Wagner, 1990
Thalassiosira weissflogii	Cd, Pb, Cu and Ni	PC2-PC4	Ahner *et al*., 1995
Zea mays	Cd	PC2-PC4, hPC2-hPC3	Rauser and Meuwly, 1995
Rubia tinctorium	Ag, Cd, Cu, Ga, Hg, Ni, Zn etc	Various PC species induced in different concentrations by different metals	Maitani *et al*., 1996
Lycopersicon esculentum	Cd, Ag, Au, Cu, Fe, Hg, Ni, Pb and Zn	PC2-PC5	Chen *et al*., 1997
Vallisneria spiralis	Pb, Hg	PC2-PC3	Gupta *et al*., 1999
Oryza sativa	Cu and Cd	PC2-PC3	Yan *et al*., 2000
Mucor racemosus, Articulospora tetracladia	Cd, Cu, Zn	PC2-PC3	Miersch *et al*., 2001
Vicia faba	Pb	PC2-PC4	Piechalak *et al*., 2002
Pheodactylum tricornutum	Cd and Pb	PC2-PC6	Scarano and Morelli, 2003
Cuscuta reflexa	Cd	PC3-PC4	Srivastava *et al*., 2004
Thlaspi caerylescens	Cd	PC2-PC4	Wojcik *et al*., 2005
***Nostoc* spp PCC 7120**	Cd	PC2	Tsuji *et al*., 2005

PCs can possibly play a vital role in Cu tolerance (Salt *et al*., 1989).It was concluded that Cu tolerance in *S. vulgaris* does not rely on the differential production of PC.

Thus the role of PCs in tolerance of Cu remains to be resolved. Evidences for detoxifying Cd by PCs are strong. PCs plays role in detoxifying Cd in *S. vulgaris* (Dietz *et al.*, 1999; Cobbett, 2000). The Cu responsive genes in rice showed some notable features with both up and down-regulation of genes (Sudo *et al.*, 2008). The number of defense and stress response genes were greatly outnumbered the down-regulated genes. The up regulated genes in response to Cu confirm its role as an elicitor of abiotic stress (Graham, 1980).

Table06. Characterization of Phytochelatin Synthase Gene in Plants

PCS gene characterized	**Plant**	**Reference**
***AtPCS*l**	*Arabidopsis thaliana*	Vatamaniuk *et al*. (1999)
***TaPCS*l**	*Triticum aestivum*	Clemens *et al*. (1999)
***SpPCS*l**	*Schizosaccharomyces pombe*	Ha *et al*. (1999)
***CePCS*l**	*Caenorhabditis elegans*	Vatamaniuk *et al*. (2001)
***AtPCS*2**	*Arabidopsis thaliana*	Cazale and Clemens (2001)
***GmhPCS*l**	*Glycine max*	Oven *et al*. (2002)
***TjPCS*l**	*Nostoc sp*. PCC7120	Tsuji *et al*. (2004)
***PvPCS*l**	*Pteris vittata*	Dong *et al*., (2005)
Alr 0975 (Phytochelatin Synthase like protein)	*Anabaena variabilis* ATCC 23413	Clemens *et al*. (2006)

Table07. Transgenic plants overexpressing phytochelatin synthase *PCS* gene in relation to heavy metals.

Transgenic	Gene transformed	Effect	Bioremediator metal	reference
Brassica juncea	Gsh1 encoding γ-glutamylcysteine synthetase	Increase in Cd tolerance level by transgenic seedlings.	Cd	Zhu *et al*., 1999
Brassica juncea	Gsh2 encoding γ-glutamylcysteine synthetase	Increase in Cd tolerance level by transgenic seedlings than wild type.	Cd	Zhu *et al*., 1999
Poplar	Gsh1 encoding γ-glutamylcysteine synthetase	Enhanced tolerance of Cd and S in shoots	Cd	Arisi *et al*., 2000
Brassica juncea	Adenosine triphosphate sylfurylase (APS1) or γ-glutamylcysteine synthetase	More level of Cd , Znaccumulated by ECS and GS transgenic plants than control	Cu, Cd, Zn, Pb	Bennet *et al*., 2003
Arabidopsis thaliana	AtPCS1 encoding phytochelatin synthase	Transgenic line showed 12-25 fold higher accumulation of AtPCS1 mRNA. Transgenics were hypersensitive to Cd and Zn, due to reduced availability of	Cd	Lee *et al*., 2003

		GSH		
Arabidopsis thaliana	AtPCS1 encoding phytochelatin synthase	PCs were found in roots, leaves and stem. Transgenic plants showed less accumulation of Cd in roots and enhanced long distance Cd transport into leaves and stems	Cd	Gong *et al*., 2003
Escherichia coli	AtPCS1 encoding phytochelatin synthase	The accumulation of PCs in *in vivo* with decrease in GSH and cellular content.	Cd and As	Sauge-Merle *et al*., 2003
Arabidopsis thaliana	*AtPCS*1	Cd tolerance and accumulation	Cd	Lee *et al*., 2003
Arabidopsis thaliana	AtPCS1 encoding phytochelatin synthase	Transgenic plants showed high resistance to As where it accumulated 20-100 times more biomass on 250 and 300µM As. These transgenic plants significantly synthesized PC2-PC4 and other unidentified thiols specially three thiols designated as a, b, c were hypersensitive to Cd treatment.	Cd and As	Li *et al*., 2004
Brassica juncea	Adenosine	4.3, 2.8 and 2.3 times more Se	Se	Banuelos *et al*., 2005

	triphosphate sylfurylase (APS1) or γ-glutamylcysteine synthetase (γ-ECS, gsh1) or glutathione synthase (GS, gsh2).	was accumulated in plants with gsh1, gsh2 and APS1 respectively with compared to wild type plants		
Tobacco	*AtPCS*1	Cd tolerance and accumulation	Cd	Pompani *et al.*, 2006.
Mustard	*AtPCS*1	As and Cd tolerance	As and Cd	Gasic and Korban, 2007
Tobacco	*AtPCS*1, *CePCS*1	As tolerance and accumulation	As	Wojas *et al.*, 2010.
Poplar	*TaPCS1*	Accumulation of Pb and Zn	Pb and Zn	Couselo *et al*., 2010
Arabidopsis	*AtPCS*1	Enhanced Cd tolerance	Cd	Brunetti *et al*., 2011
Poplar	*PtPCS1*	Higher accumulation of Zn	Zn	Adams *et al*., 2011
Tobacco	*TcPCS1*	Accumulation of Cd	Cd	Liu *et al*., 2011
Rice	*TaPCS1*	Hypersensitivity to Cd	Cd	Wang *et al*., 2012
Arabidopsis	*NnPCS1*	Accumulation of Cd	Cd	Liu *et al*., 2012
Tobacco	*CdPCS*1	As and Cd accumulation	As and Cd	Shukla *et al*., 2013.

2.6. Metallothioneins

Metallothionein proteins are the products of mRNA translation which are characterized as low molecular weight, cysteine rich, and metal-binding protein (Coyle et al., 2002). MTs are polypeptides with gene encoded usually classified into groups. Figure 2.8represent the action of MT in plants. MT genes have now been identified in many plants (Prasad, 1999). In *Arabidopsis* Class 1, Class 2, MT3 and MT4 genes been identified (Goldsbrough, 2000). Other species also contains an extensive MT gene family (Giritch *et al*., 1998). Binding of metals like Cu, Cd and Zn with MTs have been mostly studied (Robinson *et al*., 1993; Goldsborough, 2000). There is evidence for the role of heavy metal, Cu tolerance by induction of MTs in fungi and animals (Hamer, 1986). The role of MTs in detoxification of heavymetals remains to be established (Zhou and Goldsbrough, 1994; Schat *et al*., 2000).

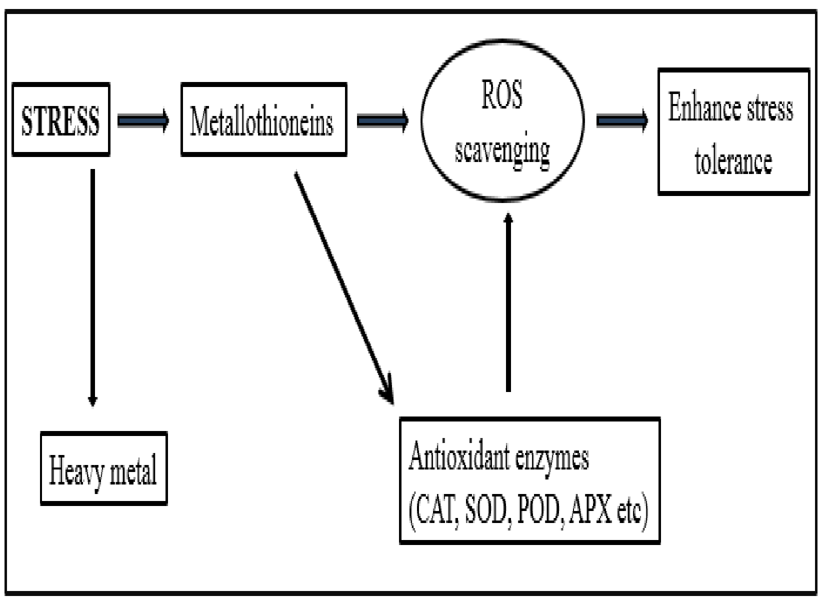

Figure courtesy: Plat Osmic Journal (Jia et al., 2012).

Figure2.2. A model mechanism of MTs action in plants.

It has been reported that the Cu treatment in seedling of Arabidopsis strongly induced MT2 mRNA whereas induced slightlyby Cd and Zn (Zhou and Goldsbrough, 1994). MT1 and MT2 genes from Arabidopsis were expressed in MT deficient mutant of yeast where both the genes complemented the mutation and provided a high level of Cu resistance, expression of MT2RNA increased in a Cu sensitive mutant of Arabidopsis which accumulates Cu at high concentration (van Vliet *et al.*, 1995). The survey of 10 ecotypes of Arabidopsis gave a clear relationship between Cu sensitivity and the expression of MT2 RNA (Murphy and Taiz, 1995a, b). In contrast exposure of Cd on *Brassica juncea* the expression of MT2 was delayed relative to synthesis of PC (Haag-Kerwer *et al*., 1999).

MTs can play a vital role in the repair of plasma membrane (Salt *et al*., 1998), they also can function as antioxidants (Dietz *et al*., 1999). MTs play a vital role in Cu tolerance, homeostasis and long distance transport for sequestration (Guo *et al*, 2003). Compare to other heavy metals rice is more susceptible to Cu toxicity (Chino, 1981).Alfalfa and barley shows high tolerance towards Cu stress whereas rice and potato are less tolerant (Jones, 1998). Exposure of Cd2+ and Cu 2+ bought transcriptome changes in the roots of Cd $^{2+}$ hyper-tolerant metallophyte *Arabidopsis halleri* (Weber *et al*., 2006). Many up-regulated genes were identified on CuSO4 exposure in Cu-tolerant birch clone (Keinanen *et al*., 2007). The modified expression of such genes by Cu stress has provided us a scope to understand the Cu stress response. Sequence of rice genome has the comprehensive identification of Cu stress responsive genes (Sudo *et al*., 2008).

Table08. Transgenic plants overexpressing Metallothionein (MT) genes in relation to heavy metals.Based on (Jia DU *et al.*, 2012).

Gene	Plant	Effect	Reference
Mouse MT	*Ralstonia*	Enhanced Cd immobilizing ability	Valls *et al*., 2000
Mammalian MT	*Tobacco*	Reduced root-shoot Cd transport	Kramer and Chardonnens, 2000
Yeast MT (*CUP*1)	*Brassica*	Accumulation of Cd	Kramer and Chardonnens, 2000
AtMT2a	*Broad bean*	Cd tolerance	Lee *et al*., 2004
CUP1	*Sunflower*	Cd tolerance	Watanabe *et al*., 2005
BjMT2	*Arabidopsis*	Enhancement in tolerance of Cd & Cu	Zhingang *et al*., 2006
TcMT3	*Arabidopsis*	No change in tolerance	Hassinen *et al*., 2009
NnMT2a	*Arabidopsis*	No change in tolerance	Hassinen *et al*., 2009
CcMT1	*Arabidopsis*	Enhancement in tolerance of Cd and Cu	Sekhar *et al*., 2011
NnMT3	*Arabidopsis*	Increased seed germination vigour	Zhou *et al*., 2012
TcMT2a	*Arabidopsis*	Hypersensitivity to Cd and Zn	Schiller *et al*., 2013

Table09. The functions of the isolated MTs genes. Based on (Jia DU *et al.*, 2012).

Species	**Gene**	**Function**	**Reference**
Arabidopsis thaliana	*AtMT* *AtMT*2 *AtMT*2a *AtMT*3 *AtMT*4a	Expression of tissue specific and induction Enhance tolerance to Cd in sensitive yeast Enhance tolerance to Cd in sensitive yeast in Vicia faba guard cells Enhance resistance to Cd in *Vicia faba* in guard cells Enhance Cu and Zn tolerance in A. thaliana	Guo *et al*., 2003 Lee *et al*., 2004 Lee *et al*., 2004 Lee *et al*., 2004 Rodriguez *et al*., 2010
Populus trichocarpas x*deltoid*	*PdtMT1a* *PdtMT1b* *PdtMT2a* *PdtMT2b* *PdtMT3a* *PdtMT3b*	Zn induce expression in root Zn induce expression in root Enhance Cd tolerance in yeast and Zn induced expression in root, leaves and stem Enhance Cd tolerance in yeast and Zn induced expression in root, leaves and stem Enhance Cd tolerance in yeast and Zn induced expression in root, leaves and stem Zn induce expression in leaves	Kohler *et al*., 2004 Kohler *et al*., 2004 Kohler *et al*., 2004. Kohler *et al*., 2004 Kohler *et al*., 2004 Kohler *et al*., 2004

Brassica juncea	*BjMT*2	Enhance resistance to Cd and Cu in E.coli	An *et al.*, 2006
Citrullus lanatus	C*lMT*2	Drought stress induced	Akashi *et al.*, 2004
Oryza satiral	*OsMT*1a *OsMT*2b	Homeostasis of Zn and enhance drought tolerance in transgenic rice Helps in root development and zygotic embryo development of rice	Yang *et al.*, 2009 Yuan *et al.*, 2008
Populus alba L	*MT*1	Induced Zn	Castiglione *et al.*, 2007
Thlaspi caerulescens	*MT2* *MT3*	Contributes to metal adapted phenotype in T. caerulescens Homeostasis function of Cu in T. caerulescens	Hassinen *et al.*, 2009 Roosens *et al.*, 2004
Gossypium hirsutum	*GhMT3a*	Enhances tolerance of biotic stress in transgenic tobacco and yeast	Xue *et al.*, 2009
Avicennia marina	*AmMT2*	Enhances Cd, Zn, Cu, Pb tolerance in transgenic *A. marina*	Hunag and Wang, 2010
Fagopyrum esculentum	*FeMT3*	Enhances drought and oxidative tolerance in buck wheat	Samardzic *et al.*, 2010
Tamarix hispida	*ThMT*3	Enhances Cd, Zn, Cu and NaCl tolerance in transgenic yeast	Yang *et al.*, 2011.