CHAPTER 1

INTRODUCTION

1.1. Tea

Tea is an aromatic beverage; an evergreen shrub belongs to family Theaceae, indigenous to China and some parts of India. They are found in tropical, subtropical and temperate regions. Climate and geography are the key factors in determining both where the tea can be grown and how the tea grown in a particular region tastes. The tea plants are highly adaptable and can be grown in a broad range of conditions. The tea plants can be handle a light frost and even snow. It can grow from subtropical climates to tropical climates but generally require a fair amount of humidity and rainfall during the growing season. Although it can grow in hot tropical climates, if they are sufficient humid, the highest quantity teas mostly come from subtropical climates with some seasonality.

1.1.1. History of Tea

India is the second largest producer of tea in world after China. A renowned tea, such as Assam and Darjeeling also grow exclusively in India. The state Assam is the world's largest tea growing region lying on the either side of Brahmaputra River. This part of India experiences high precipitation, during the monsoon period as much as 10-12 inches of rain per day. The day time rises to about 40ºC creating greenhouse like conditions of extreme humidity and heat. The tea plant is grown in the lowlands in Assam unlike Darjeeling and Nilgiris which are grown in the highlands.Tea is considered a beneficial drink as it contains many essential nutrients. Tea is the oldest, most popular, non-alcoholic caffeine containing beverage, in the world. Tea helps in normalizing blood pressure, lipid depressing activity, prevention of heart diseases (Ambadekar *et al*., 2012).

1.1.2. Climatic conditions

The optimum conditionsfor the growth of tea plants are as follows, they require temperature of 5-25ºC, humidity of 80-90%, annual rainfall of 1500-2000 mm. deep well drained and acidic soil conditions are favorable for the growth of tea (Musgrave, 2002).In India they are highly found in north east region, Assam. Moreover, they are prominently grown in West and South part of the country.

Tea plants are prone to the attack of many diseases which can be controlled by the treatment of pesticides. Tea is particularly highly prone to fungal attack (biotic stress) by several types of fungi (Singh, 2005). To overcome the problem of fungal attack many fungicides, pesticides are used. The fungicides are categorized under abiotic stress because they are mainly constituents of heavy metals. Fungi can cause serious damage in agriculture resulting in critical losses of yield, quality and profit. Growth and productivity of plants largely depends on their adaptability to abiotic stress (Upadhyaya *et al*., 2011). Adaptation is necessary for plants to escape in unfavorable conditions for their growth and development. The main sources of heavy metals in plants are the growth media, nutreints, soil along with the pesticides and fertilizers.

1.2. Heavy metal

Heavy metals are the natural components of earth, as a parental material. Some lighter metals and metalloids are toxic and for that they are termed as heavy metals with atomic density higher than 4 g/cm^3 (Hawkes, 1997). Most heavy metals have a high atomic number, atomic weight and a specific gravity greater than 5.0 (Ducic & Polle, 2005). The term heavy metal refers to high specific gravity, known as metals. Here the weight is compared to the weight of an equal volume of water. Heavy metals include some metalloids, transition metals, basic metals, lanthanides and actinides. Among the 90 elements 53 are heavy metals (Weast, 1984).

Based on the property of their solubility under physiological conditions, among these 53, 17 heavy metals are important for the ecosystem (Weast, 1984). Some of them are considered as micronutrients such as Fe, Mo, Mn, whereas some like Zn, Cu, Cd, Cr etc. are important in trace amount, but toxic at higher concentration (Reichman, 2002).Heavy metals like Cu, Cd, As, Zn, Mn, Ni, etc. are hazardous pollutant even at very low concentrations (Ross, 1994; Prasad and Strzalka, 2002).

1.2.1. History of Heavy metal

Heavy metals are naturally found on Earth and atmosphere. Heavy metal becomes concentrated as a result of human activities. Due to rapid urbanization and industrialization like mining, smelting, fuel production, vehicle emissions, fertilizers, paints, power transmission, radioactive wastes, etc. have resulted in elevated emission of toxic heavy metals entering the biosphere (Kabata-Pendias, 2001; Adriano, 2002). All these activities cause pollution in the atmosphere. Pollution is the introduction of contaminants into the natural environment. The pesticides, insecticides are used for the controlling the pests and insects, are the other major reason for the contamination of soil through heavy metals which directly or indirectly effect the quality and quantity of the plants as stress. Both the biotic and abiotic stresses can be defined as the adverse effect on plants and other living organisms. A common consequence of both the biotic and abiotic stress is the production of reactive oxygen species, at some stage of exposure of stress (Polle and Rennenberg, 1993).

As a trace elements some heavy metals are require to maintain the metabolism of human body, whereas at the higher concentrations they can be hazardous. In the terrestrial system the prime sources of heavy metals are earth crust, atmosphere. Volcanoes and continental dust are the natural sources of heavy metals. Now a day's human activities are effecting the environment with a greater extent of emission of heavy metals through coal mining, combustion of fuels, fertilizers etc. (Angelone and Bini, 1992).

1.2.2. Mechanism of heavy metal uptake by plants

Every organism has the ability to cope with the availability of both the essential and non-essential elements although they have limitations with their metal tolerance (Ernst *et al*., 1992). Thus plants have to adopt themselves to sustain their lives in the heavy metal contaminated area, lead to acquisition of a wide range of mechanisms involve in metal tolerance (Rauser, 2000). Study at the level of biochemical and molecular mechanisms (Figure 1.1) showed that plants tolerate multiple metal stresses which give us a platform to understand the plasticity of metabolic pathways which may help us in understanding its approach towards genetic engineering (Clemens *et al*., 1999; Xiang *et al*., 2001; Prasad and Strzalka, 2002; Prasad, 2004). With the span of time there has been immense development in the field of science and technology. The global development has raised new challenges, especially in the field of environmental safety and sustainable development. Plants require a range of transition metals as their micronutrients along with macronutrients which plays a vital role in the normal growth and development. But the excess amount can cause adverse action on the growth of the plants. Due to heavy metal pollution the contaminant uptake mechanism by plants has been being explored. The plants act both as accumulators and excluders. The accumulator can biodegrade the contaminant into inert form in their tissues and helps them to survive despite concentrating contaminants (Sinha *et al*., 2004). There are some plant species which actively accumulate metal in their tissues and reflect the level of metal in the soil. Metal excluders can prevent metal from entering their aerial parts or maintain low and constant concentration of metal in soil by restricting the metals in the roots itself. The plant may alter membrane permeability, can change the capacity of metal binding (Prabhat, 2008).

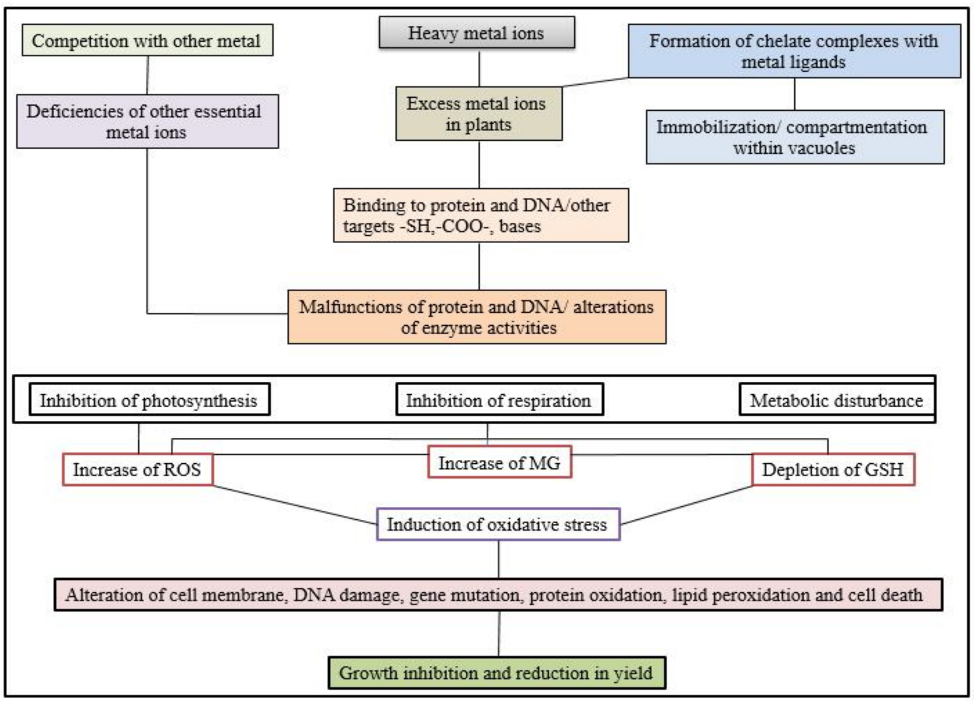

Figure courtesy: International Journal of Advanced Research (Gill M, 2004).

Figure1.1. Possible biochemical and molecular mechanism of heavy metal-mediated ROS induction and damage to the development of higher plants.

Plants can produce chelating agents able to solubilize the metals by changing the pH and redox reactions, through which it can obtain essential micronutrients even when present at low concentration in the media (Tangahu *et al.*, 2011). There are specific metal ion uptake systems in cells especially for essential nutrients that are controlled by transcriptional and post transcriptional levels with specific regulator mechanism (Lasat *et al.*, 1996).

1.2.3. Heavy metal tolerance strategy

A general increase in the level of heavy metal in the environment leads to the pervasive threat to the ecosystem. As heavy metals are essential in trace amount but beyond the threshold level they cause adverse action in plant by inhibiting its growth. Amongst the threat the production of free radicals are the most prominent one. Free radicals are like robbers; snatch the energy from others to satisfy themselves. These free radicals generate reactive oxygen species, ROS (Dietz *et al.*, 1999; Clemens, 2001; Clemens *et al.*,2002; Ducic & Polle, 2005). So by adopting many strategies like exclusions, cell wall binding, chelation and compartmentalization etc., plants cope up with the toxic effect of heavy metals. These mechanisms are known as defensive mechanisms of plants, which are autocatalytic (Choudhury *et al.*, 2006).

ROS are byproducts of metabolic pathways which are produced under stress in cellular compartments (Asada, 2006; Navrot *et al.*, 2007). The accumulation of molecular oxygen (O_2) in earth's atmosphere allows aerobic organisms to use O_2 for cellular respiration. The successive reduction of molecular oxygen into O_2^-, HO and H_2O_2 to yield water, are potentially toxic, because they are relatively reactive compared to O_2. Both in the stressed and unstressed condition ROS are produced by the plants. ROS play an important role in plant's defense system against the attack of pathogens (Alvarez and Lamb, 1997; Bolwell *et al.*, 2002). It helps in developmental stages where it involves in the formation of tracheary element, lignification and other cross linking process in the cell wall (Jacobson, 1996;

Teichmann, 2001; Fath *et al.*, 2002). In some cases ROS act as intermediate signaling molecules, where it can regulate the expression of genes (May *et al.*, 1998, Karpinski *et al.*, 1999, Neill *et al.*, 2002).

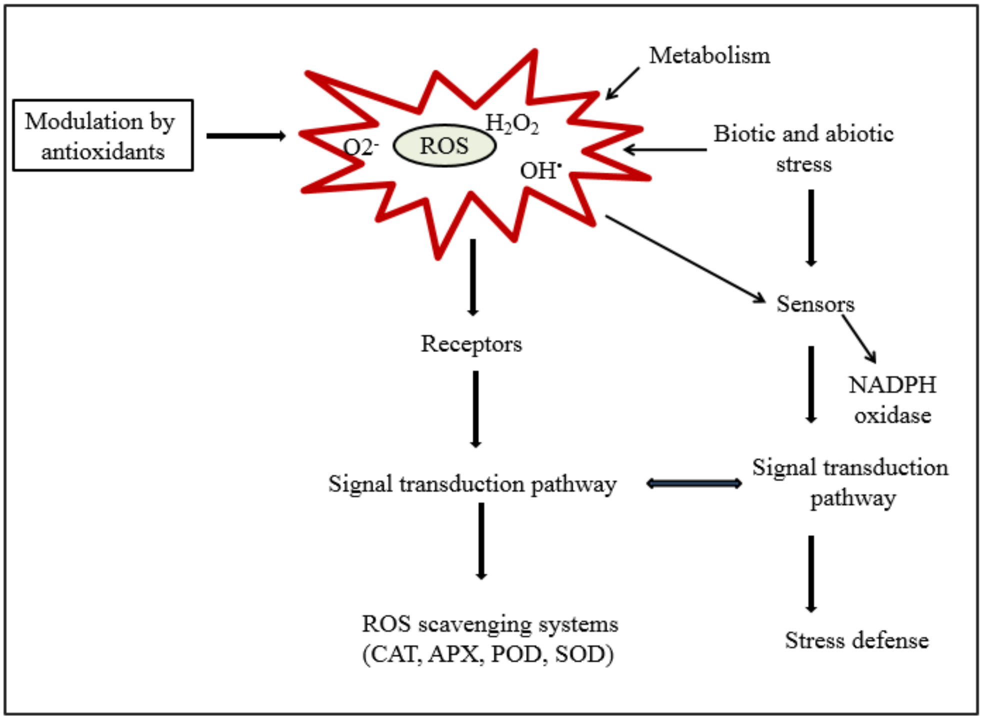

Figure courtesy: Australian Journal of Crop Science (Karuppanapandian et al., 2011).

Figure1.2. Mechanism of Reactive Oxygen Species cycle in Plants.

ROS include superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) singlet oxygen (O_2) all are toxic to plants (Mehta *et al.*, 1992, Luna *et al.*, 1994;Dismukes *et al.*, 2001;Vellosillo *et al.*, 2010). ROS can attack the micromolecules which results in cellular damage ultimately lead to death of plant (Karuppanapandian *et al.*, 2011). Due to generation of active oxygen free radicals, high rate of tissue antioxidants takes place (Luo *et al.*, 1999). The overproduction of ROS by the accumulation of heavy metals and other factors causes oxidative damage to the cell (Karuppanapandian *et al.*, 2006a, b, c, 2009, and 2011). Figure1.2. represents the stimulation of ROS by stress and how the antioxidant defensive mechanism is generating to scavange the overproduction of ROS.

At the lower concentration of ROS production are neutralized by the plants, by the activity of protective enzymes (Foyer *et al.*, 1994). But when the production of ROS is higher, plant evolved a complex antioxidant defense system (Foyer and Halliwell, 1976). ROS are a product of normal cellular metabolism, but due to stress there is a disturbance in the production and elimination of ROS (Apel and Hint, 2004, Vellosillo *et al.*, 2010). From fig 1.3 we can observe the mechanism of ROS production at different concentration in the plants**.**

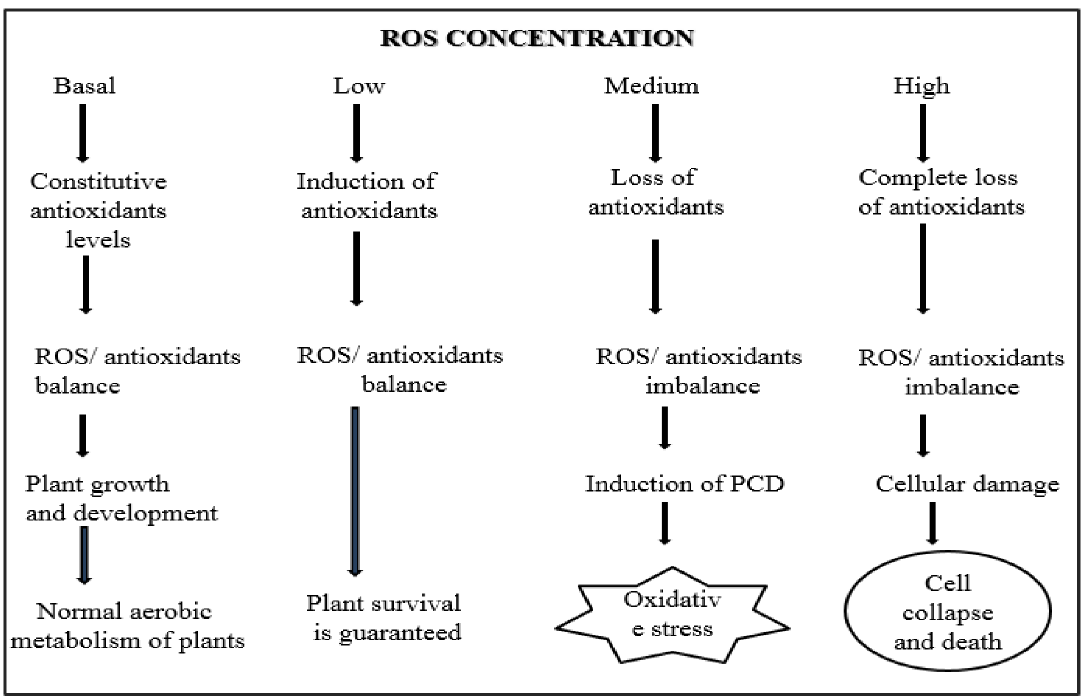

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Figure1.3. Schematic representation of the cellular concentration of reactive oxygen species (ROS) and their effects in plants.

1.2.4. Antioxidant Enzymes

A sequence of detoxification steps is often required to avoid the conversion of one reactive species into a second, more harmful one. The control of oxidant levels are achieved by antioxidant systems. Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) are the important plant protective antioxidant enzymatic system, plays a vital role in neutralizing the free radicals of ROS by making the balance in ROS production to keep the plant free from injury (Foyer and Noctor, 2005; Navrot *et al*., 2007).

1.2.4.1. Superoxide dismutase

Superoxide dismutase (SOD) is metalloenzymes discovered by McCord and Fridovich (1969) that convert O_2 to H_2O_2 in all aerobic organisms as well as some anaerobes (Hassan, 1989). The most notable example is the conversion of O_2 to H_2O_2 by SOD; an insufficiency in the next step, the H_2O_2 detoxification, would lead to H_2O_2 accumulation, inactivation of SOD, and formation of OH radicals. This also implies that multistep defense systems, if tilted out of balance, may collapse and get out of control.

Based on the metal cofactor by the enzyme SODs can be classified into as follows: irons SOD (Fe SOD) are in chloroplasts, Manganese SOD (Mn SOD) in mitochondria and peroxisomes and copper- zinc SOD (Cu, Zn SOD) in different compartments of cell. Specialized functions by the SODs are due to the location of enzymes and upstream sequences in sequence of genome (Alscher *et al*., 2002).

1.2.4.2. Catalase

CAT is a heme containing enzymatic antioxidant that catalyses the dismutation of H_2O_2 into H_2O and O_2. During the oxidative stress, CAT remove the generation of H_2O_2 in peroxisomes by oxidases (Vellosillo *et al*., 2010).CAT is highly sensitive to light, may be due to the presence of heme group, which absorbs light or may be the inactivation of H_2O_2 (Karuppanapandian, 2011). The stress causes reduction in the activity of CAT (Karuppanapandian, 2006; Karuppanapandian and Manoharan, 2008).

1.2.4.3. Ascorbate Peroxidase

APX uses molecules of ascorbate as a donor of H_2O_2 to reduce H_2O_2 to H_2O, with the generation of monodehydroascorbate (MDHA) (Asada, 2000). MDHA can be directly reduced to ascorbate within the cell. The generation of O_2^- can be trapped and converted to H_2O_2 scavanged by APX (Asada 2000, 2006). SOD and APX enzymes exist in soluble and thylakoid- bound forms in chloroplasts (Asada 2000, 2006).

1.2.4.4. Peroxidase

Peroxidase can be matabolised by the cation of peroxidases like GPX, present in the cell and CAT in the peroxisomes (Bowler, 1992; Azevedo *et al*., 1998).

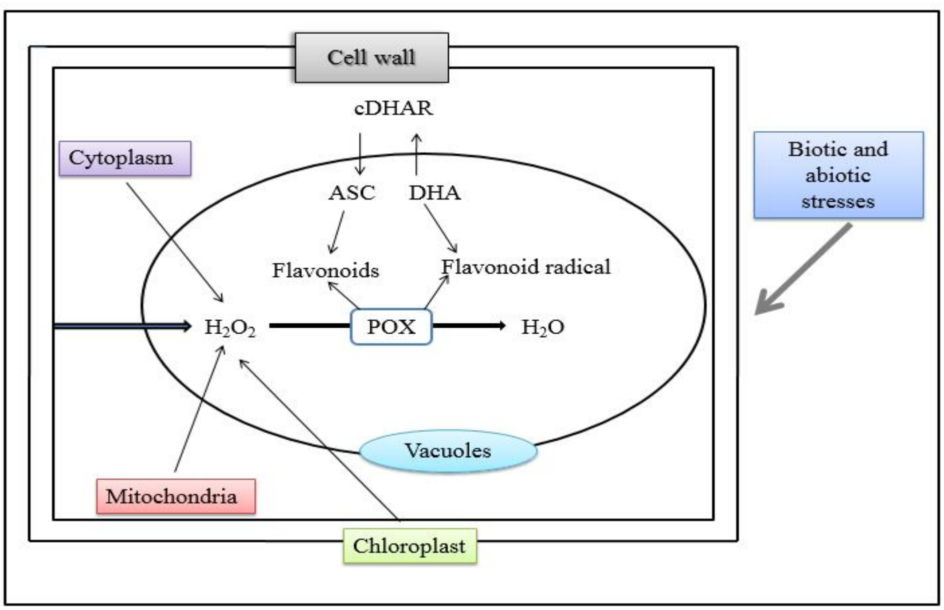

Figure1.4. Detoxification mechanism against H_2O_2 produced in cell compartment during stress and development.

GPX prefers aromatic electron donors such as guaiacol and pyrogallol (Asada, 1997). GPX is a heme containing protein; oxidize substrates at the expense of H_2O_2. Amongst the antioxidants GPX considered as one of the key antioxidants, as it involves both of its extra and intracellular forms participate in breakdown of H_2O_2 (Karuppanapandian *et al.*, 2011). The action of GPX depends on plant species and stress condition. Catalases doesnot require any substrate, like peroxidases for the conversion of $H_2O_2$to H_2O and molecular oxygen. The equilibrium of SOD, APX and CAT is essential to determine the steady state level of O_2^- and H_2O_2. Figure 1.4. demonstarates how the antioxidants are involved in detoxification of H_2O_2.

1.2.5. Malondialdehyde

Malondialdehyde (MDA) is the final product, whose content in plants is an indicator of lipid peroxidation (Cheng, 2003). MDA is considered a measure of lipid peroxidation status (Choudhury *et al.*, 2006). MDA is a cytotoxic product which produces free radicals and as a result tissue damage occurs (Ohkawa *et al.*, 1979). The production of O^{2-} determines the content of lipid peroxidation (Choudhury *et al.*, 2006). MDA is a short life time radical that not only reduces disproportionates to ascorbate by GSH-dependent glutathione reductase but also can reduce to ascorbate by non-enzymatic reaction by monodehydroascorbate reductase (Sakihama *et al.*, 2002).

1.2.6. Phenols

Phenols are the secondary metabolites abundantly found in plants (Jung *et al.*, 2003). The antioxidative properties of phenolics arise from their reactivity as electron donor can fight against the ROS (Jung *et al.*, 2003). Phenols are divided into several different groups, characterized by number of carbon atoms in conjunction with structure of the basic phenolic skeleton (Chaudiere *et al.*, 1999, Rice-Evans *et al.*, 1997). Phenolic compounds acting as antioxidants and function as terminators of free radicals and as chelators of redox active metal ions. ROS are not only produced as toxic by-products but also act as defense response during stress conditions. Figure 1.4 demonstrates the biosynthesisleading to formation of main groups of phenolic compounds**.** ROS act as environmental stress indicators and leads to the activation of defense mechanism in plant. The flavonoids are able to alter the kinetics of peroxidation by modifying the lipid packing model (Arora *et al*., 2000). They stabilizesmembranes by decreasing fluidity of membrane as a result it hinder the diffusion of ROS (Arora *et al*., 2000, Blokhina *et al*., 2003). Phenolic compound has a great tendency to chelate metals, so they can act as an antioxidant (Michalak, 2006).

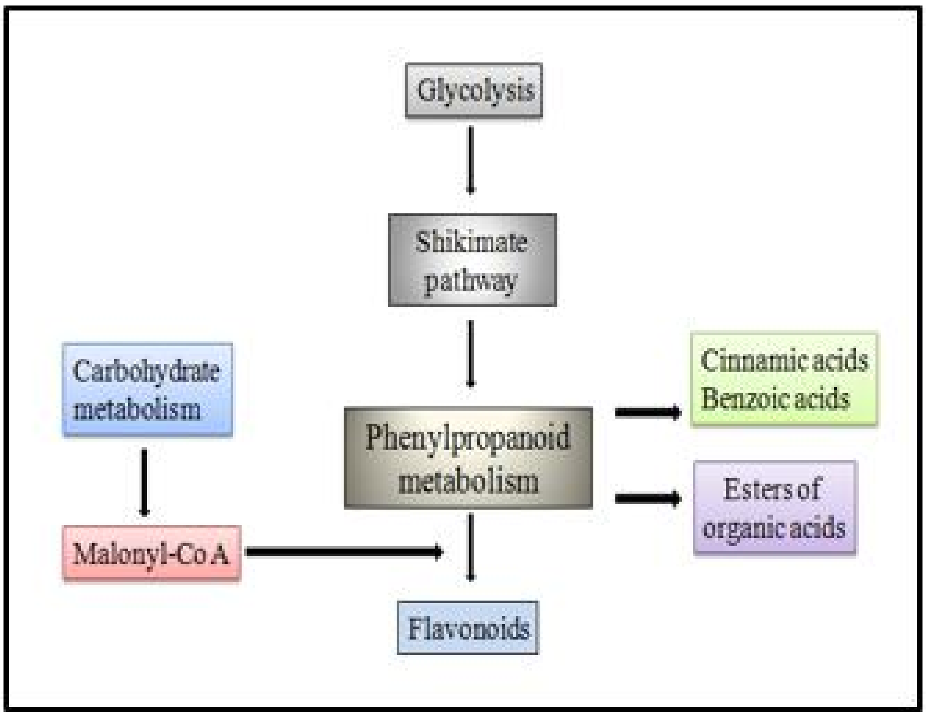

Figure courtesy: Polish 1. Environ. Stud. (Michalak A, 2006)

Figure1.5. Biosynthesis pathways leading to formation of main groups of phenolic compounds.

1.3. Copper

Copper is a chemical element with symbol Cu and atomic number 29. It is a ductile metal with very high thermal and electrical conductivity. Cu is essential to all the living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome. In molluscs and Crustacea Cu is a constituent of the blood pigment hemocyanin which is replaced by the iron complexes hemoglobin in fish and other vertebrates.

Cu exists as Cu^{2+} and Cu^{+} in normal conditions, can regulate proteins and also participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, metabolism of cell wall and hormone signaling (Marschner, 1995; Raven *et al.*, 1999). Cu ions act as cofactors in many enzymes such as Cu/Zn superoxide dismutase (SOD). Copper is an essential metal required for normal growth and development of plants (Gang *et al*., 2013). The average content of Cu in plant tissue is 10 $\mu g.g^{-1}$ dry weight (Baker and Senef, 1995). Copper participates in number of physiological processes and is an essential cofactor for many metalloproteins. Cu ions are essential components of a variety of enzymes, transcription factors and other proteins. As a whole Cu act as an essential micronutrient for normal growth and development; in its absence plants develop specific deficiency symptoms by affecting young leaves and reproductive organs. As copper has redox property, it can inherent toxicity too.Copper is a vital component of electron-transfer reactionsmediated by proteins such as superoxide dismutase, cytochrome oxidase and plastocyanin (Clemens, 2001). However, can be toxic and inhibit growth of plants when present at excessive levels (Reichman, 2002; Hall, 2002).

Copper is a redox active heavy metal, are directly involved in redox reaction in cells and result in the formation of O^{2-} with the production of H_2O_2 and OH via Haber-Weiss and Fenton reactions (Schützendübel and Polle, 2002).Though it is an important component for plant, but the excess amount of copper in cells causes problems by inhibiting the plant growth by impairing important cellular processes like photosynthetic electron transport (Demirevska *et al*., 2004). The excess of Cu plays a cytotoxic role which induces stress and causes injury (Lewis *et al*., 2001).

1.3.1. Effect of Copper

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.

1.4. Chelating Agent

Plants like all other living organisms evolved mechanisms which control and respond the uptake and accumulation of essential and nonessential heavy metals, which is exhibited by an interaction between a genotype and its environment (Macnair *et al.*, 2000). Potential mechanisms are developed by the plants at the cellular level which deals with the detoxification and impairing tolerance to heavy metal stress (Sunitha *et al.*, 2014). Immobilization, plasmamembrane exclusion, uptake restriction, transport of metals, chelation and sequestration of heavy metals by ligands are the adaptive steps of the mechanism which are evolved by the plants (Cobbett, 2000; Clemens 2006; Sharma and Diezt, 2009). In Plants Phytochelatins and metallothioneins are the prominent chelators.

1.4.1. Phytochelatins

Phytochelatins are the enzymatically synthesized cysteine rich peptides. PCs form a family of structureGly; (γ-Glu-Cys) $_n$-Gly, where n is in the range of 2-11. PCs are generally found in many varieties 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 terminal Serine, Iso-PC, [iso-(PC)-Glu] with C terminal Glutamic acid, and Des-Gly PC [des-(Gly-PC)] respectively (Zenk, 1996).

Structurally PCs are related to glutathione (GSH; γ-GluCysGly). There are different pathways of phytochelatin synthesis, leads to the detoxification of heavy metals (Steffens, 1990). Synthesis of phytochelatins induced by the entry of metal ion into the cell (Maitani *et al.*, 1996). Along with the detoxification of heavy metals they plays a vital role in homeostasis of essential metal ion (Thumann *et al.*, 1991). The role of heavy metal detoxification by phytochelatins revealed the activation of phytochelatin synthase in a broad spectrum (Howe and Merchant, 1992). GSH deficient mutants of *Schizosaccharomyces* *pombe* and *Arabidopsis* are deficient in Pc too and hypersensitive of Cd (Cobbett and Goldsbrough, 2002). By the action of Phytochelatins, metals can be complexed, inactivated and transformed into physiologically tolerable (Zenk et al., 1996; Baranowska-Morek, 2003).

The synthesis of Phytochelatins consists of two distinct steps: (i) formation of γ-GlutamyleCysteine concomitant with the cleavage of glycine from GSH, (ii) transfer of γ-EC unit from the enzyme to acceptor molecule.

(i) γ-EC-Gly + PC-----------------> γ-EC-PCS + Gly

(ii) γ-EC-PCS + (γ-EC)n-Gly -----------------> (γ-EC)n+1-Gly + PCS

Crystal structure revealed that Phytochelatins belong to papain family of cysteine protease and is dimer; the catalytic actin involved a trial of cys-70, His-183 and Asp-201 in prokaryotic Phytochelatins, which is equivalent to cys-56, His-162, and Asp-180 in eukaryotic Phytochelatins (Vivares *et al*., 2005). GSH as an acceptor should bind in a putative site close enough to the first GSH binding site to allow the synthesis of PC (Vivares *et al*., 2005; Rea, 2006).

1.4.1.1. Phytochelatin synthase gene

Phytochelatin synthase catalyzing the biosynthesis of PCs from GSH was characterized (Grill *et al*., 1989). PCS enzymes were characterized from *Silene cucubalus*, molecular mass of 100 kDa, pH 7.9, and temp. 35ºC (Grill *et al*., 1989). The genes involved in the formation of PCs are phytochelatin synthases *(PCS)* which is glutamylcysteine transpeptidases. The PC synthase gene was first identified in *Arabidopsis* (Cobbet and Goldsbrough, 2002). *PCS* gene were constitutively expressed in plants (Howden *et al.*, 1995, Grill *et al*., 1989). *PCS* genes were identified in *Arabidopsis thaliana, Triticum aestivum*

(Clemens *et al*., 1999). The genetic, molecular and biochemical data demonstrates that *PCS* gene encode the PC synthesis. The application of molecular biology and genetic engineering has provided us the molecular level to improve the phytoremediation capability of plants (Yang *et al*., 2005). Many authors have revealed the fact that the overexpressing *PCS* genes alleviates the tolerance to heavy metals. Many genes encoding PCs have been cloned from different organisms like, Os*PCS*1, Ta*PCS*1, At*PCS*1 and Ce*PCS*1 from rice, wheat, Arabidopsis and Caenorhabditis elegans to enhance tolerance against heavy metals (Vatamaniuk *et al*., 1999; Clemens *et al*., 1999).

PCS expressing cells accumulates more Cd tolerance compared to control. Expression of *PCS* mediates Cd tolerance in yeast mutants that are either deficient in vacuolar acidification or impaired in the biogenesis of vacuolar (Clemens *et al*., 1999). Metal resistance produced by the induction of PCS is lost upon exposure to an inhibitor of glutathione biosynthesis (Clemens *et al*., 1999). Different groups of overexpressed PCS enhance the tolerance of heavy metals (Gasic and Korban, 2007; Lee *et al*., 2003, Li *et al*., 2003; Liu *et al*., 2011).

1.4.2. Metallothioneins

The thiols group of MTs cysteine residue helps them to bind with the heavy metals (Hamer, 1986). Metallothionein proteins are the low molecular weight (4-8 kDa), cysteine rich, metal binding proteins, are the products of mRNA translation (Kagi, 1993). MT proteins and genes are found in almost all plants and animals (Cobbet and Goldsbrough, 2002). MTs contain two metal binding, cysteine rich domains which gives dumbbell confirmation to metallothioneins. Figure 1.6 demonstrates the cellular mechanism of metal detoxification in plant system.

The functions of MTs are as follows:

(i) Maintaining the homeostasis of essential transition heavy metals.

(ii) Sequestration of toxic heavy metals

(iii)Curing the plants from damage caused by intracellular oxidation (Gasic and Korban, 2007).

(iv) MT can act as scavengers of reactive oxygen species (Akashi *et al*., 2004).

(v) It can also regulate cell growth, proliferation, activity of metalloenzymes and factors of transcription (Palmiter, 1998; Haq *et al*., 2003).

(vi) It involve in the metabolism of metallodrugs, response to stress, involvement in the inflammation and cell apoptosis and act as possible redox sensors (Vasak and Hasler, 2000; Fabisiak *et al*., 2002).

1.4.2.1. Classes of Metallothioneins

With the help of marcaptide bonds, the large number of cysteine residues in MTs are able to bind with metals (Cobbet and Goldsbrough, 2002). The diversity of MTs in plants can be subdivided into three classes on the basis of the arrangement of Cys residues (Zhou *et al*., 2006).

Class I MT proteins are characterized by two Cys- rich domains. These are separated by central cysteine residues which display the cysteine arrangement patterns similar to the mammalian counterparts but contain long spacer regions (Vallee, 1991; Coyle *et al*., 2002). Class II MT proteins contain cysteine residues which are grouped into three cysteine rich domains generally separated by 10 to 15 residues (Lane *et al*., 1987; Zhou *et al*., 2005). Class III MT proteins are consists of Phytochelatins which are enzymatically synthesized peptides with a poly (g- Glu-Cys)-glycine structure (Cobbett and Godsbrough, 2002).

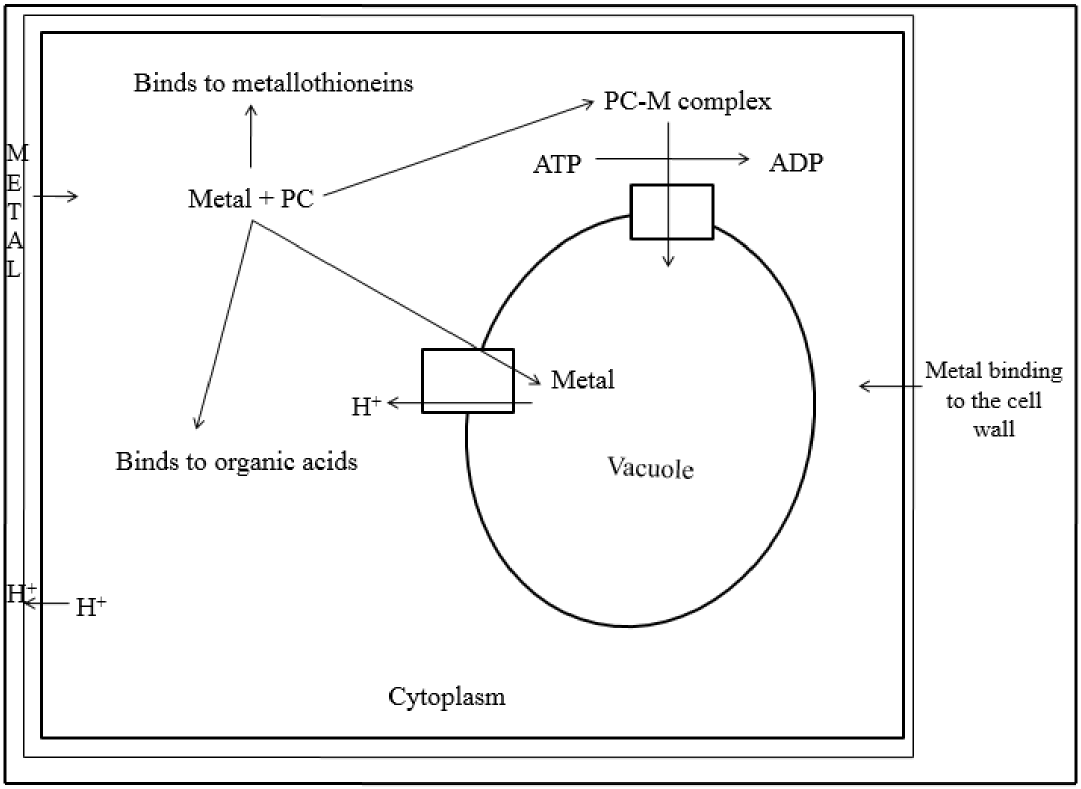

Figure courtesy: Plant Cell Biotech and Mol. Bio. (Sunitha et al., 2014).

Figure1.6. Cellular mechanism of metal detoxification in plant system.

1.4.2.2. Metallothionein genes

Most of the deduced products of MT genes found in both the monocots and dicots belong to class I. Wheat EcMT proteins was the first identified (Lane *et al.*, 1987). After that more than 140 MT sequence have been recorded (Guo *et al*., 2003). The isolated MT genes are categorized into four types based on the sequence of amino acids given in Table no. 01(Cobbet and Goldsbrough, 2002).

Table01. Types of MT genes.

Type		Contain	Domain
Type MTs	1	6-Cys-Xaa-Cys motifs	2, which are separated by 40 amino acids
Type MTs	2	Cys- Cys motifs	2 cysteine rich, separated by 40 amino acids
Type MTs	3	4 Cys residues in N terminal	
Type MTs	4	Cys-Xaa-Cys motifs , additional 8-10 aa.	3, 5or6 coserved cys residue separated by 10-15 aa

In the many organisms, the structure and the regulation of MT genes were studied (Haq *et al*., 2003). Metal ion MT genes are regulated by metal responsive element (MRE) sequence motifs contained d in the promoters are very much essential for response of the heavy metal, which are involved in the development of tissues and physiological reactions (Palmiter, 1998; Coyle *et al.,* 2002; Haq *et al.,* 2003).

Southern blot and cDNA isolation of Arabidopsis and other plant species have indicatedthat MT genes are also pesent as gene families in plants (Giritch *et al.,* 1998; Liu *et al.,* 2002; Guo *et al.,* 2003). The 5'- upstream regions of MT genes have been isolated from several plant species (Chatthai *et al*., 2004; Liu *et al*., 2006). Hormones, cytotoxic agents, heavy metals like Cd, Zn, Hg, and Cu etc. are the factors which induce the biosynthesis of MT (Yang *et al*., 2005, Kagi, 1991). The majority of plant MT genes are identified in angiosperms. Arabidopsis, rice, sugarcane contain MT genes of all four types, indicates the separation of monocots and dicots, majority of flowering plants also contain the four different MT genes (Cobbet and Goldsbrough, 2002). The diversity of plant MT gene family suggests that these may differ not only in the sequence but also in function (Cobbet and Goldsbrough, 2002).

To quantify the mRNA levels, the gene expression were done at different developmental stages under the stress conditions in plants. With respect to developmental stages and stress conditions the MT genes appear to be differentially regulated (Castiglione *et al.*, 2007; Ahn *et al.*, 2012). Many MT genes were isolated and worked out for the study of their role in response to stress which is given in Table no. 04. The external and internal exclusion are responsible for heavy metal tolerance. In external detoxification process, organic acids excreted from plant roots which can form stable metal ligand complexes and can change their mobility and bioavailability. As a result the heavy metal ions will not enter in the plants (Sunitha *et al.*, 2014). In internal heavy metal detoxification, organic acids may chelate the heavy metals in the cytosol, where the ions can be transformed into a nontoxic or less toxic form (Hall, 2002). The expression pattern analysis of genes can help us to reveal their possible biological functions in plants.

1.5. Statement of the problem:

Over the period many pesticides, fertilizers which are highly constituents of heavy metals were used against the pathogen attacks in the plants. They directly or indirectly affected the growth of the plants. Metal excluders plants prevent metal entrance by altering the membrane permeability, change metal binding capacity of cell walls etc.

In the last decade, tremendous developments in molecular biology have been done. The successes of genomics have highly encouraged the studies in molecular genetics. Mainly transcriptomic, to identify functional genes implied in metal tolerance in plants, largely belonging to the metal homeostasis network.Present work has been framed out with a sole objective of study the effect and the expression of gene due to the accumulation of the Cu at different dose levels in the tea (*Camellia sinensis*) plants. The aim of this study was to observe the expression of phytochelatin synthase *PCS*1, and metallothionein genes which are affected directly or indirectly by toxic levels of Cu, some of which may be involved in ameliorating heavy metal, oxygen radical or other stress damage. This was also aimed to study the biochemical, physiological as well as the morphological changes brought to tea plant in response to copper stress.

OBJECTIVES

- To study the effect of heavy metal Copper (through the application of pesticides) in tea plants (in different cultivars).
- Analysis of the morphological and biochemical changes brought about by heavy metal (copper) stress in the tea plants.
- Isolation, characterization and study the expression of the genes (phytochelatin synthase *PCS*1, metallothionein) in the heavy metal stressed tea plants.
- Preparation of cDNA library of the overexpressed phytochelatin synthase (*PCS*1), metallothioneins genes from the stress induced plant for the future aspect.