NBU Journal of Plant Sciences
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Vol. 10, No. 1, March 2016 p. 01-16
ISSN No. 0974-6927

Review Article

Copper toxicity in plants: a review and a case study on tea

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Abstract

Copper in trace amounts is essential for various metabolic processes in the plant such as photosynthesis, carbohydrate distribution, and protein metabolism but at high concentration it causes physiological stress through generation of free radicals that induce the production of reactive oxygen species (ROS) via Haber-Weiss and Fenton reactions. Copper-induced generation of hydrogen peroxide, hydroxyl radicals, or other reactive oxygen species has been directly correlated with the damage to protein and lipids that may lead to reduced growth and even death. Tea (*Camellia sinensis* (L.) O. Kuntze) is an economically important plantation crop in India with round the year productivity. Copper based fungicides are cheap and effective in controlling fungal diseases and are used consistently throughout the year to combat different fungal diseases that pose a major threat to tea production. Excess Cu^{2+} has been found to alter several physiochemical parameters in the tea plants. A more detailed study on mechanisms of Cu^{2+} toxicity at the gene level is warranted.

Key words: Copper, stress, tea, reactive oxygen species, antioxidative enzymes.

Introduction

The role of copper in plants depends greatly on its concentration. Copper in trace amounts is an essential micronutrient for algae and higher plants for its role as a cofactor for metabolic processes like photosynthesis, respiration, carbohydrate distribution, nitrogen fixation, protein metabolism, ethylene perception, oxidative stress reduction, cell expansion and cell-wall lignification. At higher concentrations, copper can induce several negative effects including generation of reactive oxygen species, exchange of essential metal ions from the active sites and visible symptoms such as chlorosis, necrosis and growth inhibition (Marschner, 1995; Prasad, 2004). A well coordinated procedure of uptake, buffering, translocation and storage processes is necessary to uphold essential concentrations of the metal in various tissues and compartments within the narrow physiological limits (Clemens *et al.*, 2002). Copper is transported into the plant cell by COPT family of transporters on the plasma membrane which has been described as a group of highly hydrophobic proteins; all its members contain 3 trans-membrane domains and specific Cu^{2+} binding site rich in methionine and histidine residues at the amino terminus (Kampfenkel *et al.*, 1995; Sancenon *et al.*, 2003; Andres-Colas *et al.*, 2006). Copper homeostasis is maintained inside the cell by copper chaperones which sequester copper to a non reactive form and also interact with other transport proteins for delivering copper to its necessary destinations (Himelblau and Amasino, 2000; Company and Gonzalez-Bosch, 2003; Chu *et al.*, 2005). Two P-type ATPases, PAA1 and PAA2, are required for efficient copper delivery across the plastid envelope and the thylakoid membrane, respectively, in *Arabidopsis* (Shikanai *et al.*, 2003; Abdel-Ghany *et al.*, 2005). Inside the root, Cu^{2+} is said to be strongly accumulated in the cortex and the concentration decreases sharply from the outer to the inner cell layers (Adruini *et al.*, 1996; Ducic and Polle, 2005). Copper is poorly translocated by xylem and thus uptake by shoots is very low (Liao *et al.*, 2000).

The aim of this review is to summarize the toxic effects of Cu^{2+} and focus on the recent developments on the various underlying metabolic changes that bring about such toxic effects. We also focus on tea, which is the most popular drink in the world after water. Tea (*Camella sinensis* (L.) O. Kuntze) is a perennial evergreen plantation

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crop with productivity round the year. The harvest includes tender shoots that are plucked normally at one to three weeks interval. This induces further vegetative growth and ensures continuous supply of green flushes (Burgess and Carr, 1997; Karmakar and Banerjee, 2005). Fungal pathogens such as *Exobasidium vexans* are capable of infecting the pluckable tender leaves thereby warranting a regular spraying of copper fungicides in heavy doses especially during the six month long monsoon period (May-October) when fungal infections assume massive proportions. This causes a buildup of Cu^{2+} in the soil over the years and the concentration of Cu^{2+} can easily overcome the threshold limit for toxicity.

Copper in plants

One of the major sites of copper accumulation in plants is the chloroplast. This metal is directly involved as a component of plastocyanin (PC) in the photosynthetic electron transport chain. PC is one of the most abundant proteins of thylakoid lumen (Kieselbach *et al.*, 1998) and is essential for electron transfer between the cytochrome b6f complex and photosystem 1 (Weigel *et al.*, 2003). The metal has a distinct regulatory role in electron transport between the photosystems as the constituent of PC (Maksymiec, 1997). In the chloroplast stroma, Cu/Zn superoxide dismutase (SOD) requires Cu^{2+}, along with Zn, as cofactors to catalyze the dismutation of superoxide radicals (O_2^-) threby forming H_2O_2 and O_2. In *Arabidopsis thaliana*, out of seven identified SOD genes, the most active CSD1 and CSD2 genes both encode a Cu/Zn SOD with CSD1 activity in the cytosol and CSD2 activity in the stroma (Kliebenstein *et al.*, 1998). Polyphenol oxidase is another Cu^{2+} protein found in the thylakoids of some plants, such as spinach (Kieselbach *et al.*, 1998), but not in other species such as *A. thaliana* (Schubert *et al.*, 2002). The enzyme has been proposed to be involved in the photoreduction of O_2 by PS I (Vaughn *et al.*, 1988). Cu^{2+} mediates the activity of several other enzymes such as ascorbate oxidase which catalyses the reduction of O_2 to water. The enzyme contains 8 Cu^{2+} ions which participate in the transfer of electrons in presence of ascorbate, the reducing substrate (Maksymiec, 1997). Other important Cu containing proteins within plant cells include the mitochondrial cytochrome-C oxidase enzyme, the ethylene receptors in the endomembrane system and various apoplastic oxidases (Cohu and Pilon, 2007). Copper is also necessary for amine oxidase function where it catalyses oxidative deamination of polyamines with the simultaneous formation of aldehyde, ammonia and H_2O_2 (Maksymiec, 1997).

Copper as a toxic element

Inspite of the indispensability of copper in plant metabolism, excess copper has strong toxic effects. Copper can be limiting to plant productivity in crops when below 5 $\mu g\ g^{-1}$ dry weight (DW), whereas toxicity is reported above 30 $\mu g\ g^{-1}$ DW (Marschner, 1995). The most common feature of copper toxicity is the decrease in mass of roots. Copper toxicity can be damaging to plant roots, with symptoms ranging from disruption of the root cuticle and reduced root hair proliferation, to severe deformation of root structure (Sheldon and Menzies, 2005; Lequex *et al.*, 2010). Cu^{2+} is toxic to plant cell which lead to plant retardation and leaf chlorosis (Rhoads *et al.*, 1989; Yadav, 2010). High Cu^{2+} concentrations predisposes photosystem II to photoinhibition (Patsikka *et al.*, 2002), causes reduction in chlorophyll content arising from partial destruction of grana and modification of the protein-lipid composition of thylakoid membranes (Lidon and Henriques, 1991; Maksymiec, 1997). Copper toxicity can also results in significant alteration in the concentration of minerals such as Fe, Mg, Ca, Zn, K and Na in both root and shoot (Lidon and Henriques, 1993; Lequex *et al.*, 2010).

Copper is relatively abundant in the earth's crust and better soluble, therefore more mobile than other heavy metals in the surface environment (Flemming and Trevors, 1989). Copper concentration in non-polluted soils range from 10 to 80 ppm Cu^{2+} but soils located near mining areas or metal-processing industries may be contaminated by very large amounts of Cu^{2+} (Hagemeyer, 2004). The bioavailability is determined by the form taken by the metal (ionic, complex

or precipitated) which depends on environmental factors and therefore, varies widely, giving rise to possible conditions of toxicity (Flemming and Trevors, 1989; Greger, 2004). The level of bioavailable copper is increased by human activities which either increases the abundance or causes changes in soil chemistry thus affecting the solubility (Rhoads *et al.,* 1989; Flemming and Trevors, 1989). In the soil, copper remains immobilized onto the organic materials such as fulvic and humic acids and to clay and mineral surfaces. The bioavailibility in soil is strongly dependent on factors such as pH, cation exchange capacity (CEC), clay content, water hardness and organic matter content (Flemming and Trevors, 1989; Greger, 2004; Rooney *et al.,* 2006). Low pH increases the metal availability since the hydrogen ion has a higher affinity for negative charges on the colloids, thus competing with the metal ions of these sites, therefore releasing metals (Greger, 2004). Rhoads *et al.* (1989) found that growth of tomato plants was reduced at soil pH below 6.5 with soil-copper levels above 150 mg. Thus soil properties have a significant impact in the expression of toxicity of copper in plants.

According to Brun *et al.* (2001) agricultural soil in many parts of the world are contaminated by heavy metals. The use of Bordeaux mixture for almost one century against vine downy mildew has caused severe copper contamination of soil in many wine-producing regions (Van-Zwieten *et al.,* 2004). Copper contamination also caused serious problems in cereals such as rice (Lidon and Henriquesa, 1993), wheat (Lanaras *et al.* 1993) and barley (Vassilev *et al.,* 2003). Graham *et al.* (1986) found that excess fungicidal copper reduced seedling growth in citrus and also inhibited colonization of the roots by mycorrhizal fungus. In citrus orchards, stunted tress were produced with less mycorrhizal colonization under higher Cu concentrations and low pH (<5) conditions of the soil. In India, the major tea cultivation area comprises the eastern sub-Himalayan region where the soil is mainly acidic in nature (pH 4.2-5.8) (Singh and Singh 2006). While this is good for tea cultivation (Sarkar, 1994), but it increases the possibility of Cu^{2+} ions accumulated in the tea garden soils to become more available for absorption by plants which may lead to toxicity.

Copper in tea gardens

An example of an industry in India which depends primarily on copper fungicides is the tea industry. India is second only to China in tea production and the largest consumer of tea in the world. Currently, India produces 23% of total world production. It is the second largest industry in terms of employment and generally drives the economies of the regions where the tea gardens are concentrated, for example Assam and sub-Himalayan West Bengal (Selvakumar and Jeyaselvam, 2012). Tea plants are cultivated extensively as large plantations where it is often allowed to grow under variant soil and climatic condition thereby making them prone to attacks by fungal pathogens. Major diseases include blister blight, brown blight, grey blight and black rot in leaves, and branch canker, thorny blight and pink disease in stems. To control the diseases, copper based fungicides are used excessively in tea gardens of North East India including Assam and sub-Himalayan West Bengal (Barua, 1988). The fungicides that are used most commonly include basic copper sulphate, Bordeaux mixture (a combination of hydrated lime and copper sulphate), Bicoxy (a new formulation of copper oxychloride 50% WP) and various customized formulations of copper sulphate and copper oxychloride (Worthing, 1983; Singh, 2005). A survey covering several tea gardens of the Darjeeling and adjoining Jalpaiguri district of sub-Himalayan West Bengal conducted by the authors has revealed that copper-fungicides are extensively used in the tea gardens of the Dooars and Terai region and also in the hilly regions of West Bengal. Copper based fungicides are used in large scale because they have multisite activity with a low risk of pathogens developing resistance (Van-Zwieten *et al.,* 2004) and are relatively less phytotoxic than Ni based fungicides. In fact, copper based fungicides are highly recommended in literature and are often regarded as the most efficacious and

economic fungicide for controlling the foliar diseases of tea (Singh, 2005).

Mechanisms of Cu^{2+} toxicity

Copper is a redox active metal with an electrochemical potential of -260V. The redox nature of Cu^{2+} ions makes it very useful as a cofactor in electron transfer reactions (Ducic and Polle, 2005). However, the reversible oxidation–reduction property of Cu^{2+} could also result in oxidative stress if Cu^{2+} would be present as a free ion. Heavy metals in general have been recognised as a major toxicant in plant cells due to their capability of generating reactive oxygen species (ROS) such as hydroxyl radical (OH•) superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which can damage the bio-molecules such as membrane lipids, proteins and nucleic acids. During the reduction of oxygen to water, ROS may be produced by a chain of reactions which initially needs energy input but subsequently occur spontaneously. O_2^- is a short-lived and moderately reactive ROS which reduces quinines and transition metal complexes of Fe^{3+} and Cu^{2+} thereby affecting the metal containing transporters and enzymes. O_2^- can additionally combine with protons in aqueous medium and form hydroperoxyl radicals (HO_2•) which can induce lipid auto-oxidation in membranes (Shaw *et al.*, 2004). H_2O_2 is relatively long-lived and moderately reactive which oxidises the thiol groups of some enzymes (e.g. enzymes of the Calvin cycle and Cu-Zn SOD) and inactivates them (Vranova *et al.*, 2002). However, the most reactive of all the ROS is the hydroxyl radical (OH•) which can potentially react with all types of biomolecules and in excess can cause cell death because cells do not have any enzymatic antioxidant system to quench it. The radical is formed from H_2O_2 by the Haber Weiss and Fenton reactions and Cu^{2+} being a redox active metal catalyzes the formation of this most harmful active radical (Arora *et al.*, 2002; Vranova *et al.*, 2002) as summarized below:

$$H_2O_2 + O_2^- \xrightarrow{Cu+,\,Cu2+} OH^- + OH^{\bullet} + O_2$$

Haber Weiss reaction

One of the richest sources of ROS in plants is the chloroplast. These can be formed due to the highly energetic electron transfer reactions triggered by chlorophyll excitation along with an excess supply of oxygen. Singlet oxygen ($^1O_2$) can formed during de-excitation of chlorophyll which causes major oxidative damage to biomolecules. High light intensity can cause over reduction of PS I and generation of excessive NADPH which cannot be utilized by the CO_2 fixation process thereby reducing the $NADP^+$ pools. O_2 which is abundant in the chloroplast can take up electrons from PS I in such a situation, which leads to production of ROS through the Mehler reaction (Sharma *et al.*, 2012). Under conditions of low CO_2 fixation such as cold temperature or low CO_2 availability, excess reduction of an increase in ROS levels can occur even at moderate light intensities. As H_2O_2 or O_2^- are only moderately reactive, therefore, the main responsible factor for the intense biological damage is the metal ion which catalyzes the formation of the highly toxic hydroxyl free radical (OH•) from H_2O_2 (Maksymiec, 1997). Thus ROS may be generated in the plant due to several abiotic as well as biotic causes but true damage is caused by the additional metal toxicity.

The hydroxyl radical (OH•) can either add onto the biological molecules or eliminate hydrogen from them by forming water. The hydroxylated biomolecules can in turn hydroxylate other molecules, thereby, initiating a chain of reaction or change to stable oxidised products. The activated hydroxylated molecules can also dismute themselves by forming intermolecular cross links (Shaw *et al.*, 2004). Oxidised Cu^{2+} ions can be actively involved in electron transfer during formation of stable oxidized products. In reactions where the OH• radical eliminates H from biomolecules, it leaves an unpaired electron in the organic molecule thereby forming a reactive organic radical which can then react with oxygen to form peroxy radical (ROO•). The peroxy radical is again a reactive species and can eliminate hydrogen from other biomolecules and change them into organic radical products thereby creating a chain of reactions. The peroxidation reaction is evident in lipid peroxidation reactions that take place in cell membranes to form lipid peroxides (ROOH) (Shaw *et al.*, 2004; Arora

et al., 2002). However, in presence of reduced Cu^{2+} ions which can participate in Fenton reaction (shown below), the highly reactive alkoxy radical (RO^{-}) is formed from the ROOH which is as damaging as the hydroxyl radical thus opening up another cascade of immensely damaging oxidative reactions.

$$ROOH + Cu^{+} \longrightarrow OH^{-} + Cu^{2+} + RO^{-}$$

.A study on the toxicity mechanisms suggest that the generation of reactive oxygen species is a natural phenomenon but is increased to alarming proportions due to presence of stress factors. Presence of Cu^{2+} ions above the threshold limit is immensely stressful to plants due to its redox nature as it can catalyze and enhance the formation of all types of ROS by participating actively in several types of oxidative reactions.

Plant response to Copper toxicity

Plants have developed a wide range of protective mechanisms for mitigating copper toxicity. Primary defence mechanisms prevent metal to enter into the cell via exclusion, or binding of metal to cell wall and other ligands, organic acids, amino acids, glutathione (GSH) or phytochelatins (PCs) to render them harmless (Antosiewicz and Wierzbicka, 1999). Antioxidative mechanisms that control the level of ROS and shield the system before the sensitive parts of the cellular machinery gets damaged are mediated by molecules which have been broadly divided into two types, the high molecular weight enzymatic catalysts and the low molecular weight antioxidants (Pinto *et al.*, 2003). The enzymes involved in scavenging ROS include SOD, catalase (CAT), peroxidases (POD) and glutathione peroxidase and those involved in detoxifying lipid peroxidation products include glutathione-S-transferases (GST), phospholipid-hydroperoxide glutathione peroxidase and ascorbate peroxidase (APX). Table 1 enlists the different enzymes which have been studied in relation to copper toxicity. The low molecular weight compounds that act as cellular antioxidants are ascorbate, glutathione, phenolics, flavonoids, carotenoids and tocopherols. Besides these, a whole array of enzymes is needed for the regeneration of active forms of the antioxidants such as monohydroascorbate reductase and glutathione reductase (Blokhina *et al.*, 2003; Pinto *et al.*, 2003).

Binding of copper and its sequestration

Plant adapt to heavy metal stress by acquiring several strategies, the most prominent being the synthesis of phytochelatins and metallothioneins which contribute to metal detoxification by chelation of the metal ions. Phytochelatins are simple thiol rich metal binding peptides containing glutamate, cystein and glycine in ratios of 2:2:1 to 11:11:1 (Grill *et al.*, 1985; Prasad, 2004). These peptides are synthesized non-translationally from glutathione in the presence of heavy metals by the enzyme phytochelatin synthase (Grill *et al.*, 1989). Apart from being a precursor to phytochelatins, glutathione is also an important antioxidant molecule, which plays a predominant role in protection against free radicals (Alscher, 1989). Copper induced increase in phytochelatin synthesis results in oxidative stress through the depletion of the antioxidant glutathione. De Vos *et al.* (1992) showed that copper tolerance in the plant species *Silene cucubalus* does not depend on the production of phytochelatins but is related to the ability of this plant to prevent glutathione depletion resulting from copper-induced phytochelatin production.

Metallothioneins are low molecular weight proteins with high cystein content, which bind metal ions to form metal thiolates and metal thiolate clusters. Class III metallothioneins are found in plants and is reported to be induced by the presence of a variety of metals including Cd, Cu, Zn, Pb, Hg and Ag (Hamer, 1986; Prasad, 2004). However, phytochelatins rather than metallothioneins are mainly responsible for detoxification of toxic heavy metals (Yadav, 2010). Moreover, metal binding ability is higher in phytochelatins than in metallothioneins on a per-cysteine basis (Mehra and Mulchandani, 1995). In addition, phytochelatins possess the ability to scavenge

Table 1. Enzymes/Metabolites whose levels have been studied after copper exposure

Enzyme/Metabolite	Plant	Location	Reference
Peroxidase	*Zinnia elegans* and *Cosmos sulfureus*	Shoots and roots	Tsay *et al.* 1995
	Zea mays	Leaves and roots	Mocquot 1996
	Helianthus annuus	Leaves and roots	Garcia *et al.* 1999
	Oryza sativa	Leaves	Fang and Kao, 2000
	Capsicum annum	seedlings	Diaz *et al.* 2001
	Phaseolus vulgaris	Leaves and roots	Cuypers *et al.* 2002
	Allium sativum	Leaves and roots	Meng *et al.* 2007
	Erica andevalensis	Leaves, Roots	Oliva *et al.* 2010
	Zea mays	Roots	Zhao et al 2010
	Vigna mungo	seedlings	Solanki *et al.* 2011
	Beta vulgaris	leaves	Morales *et al.* 2012
	Camellia sinensis	Leaves	Saha *et al.* 2012
Catalase	*Avena sativa*	Leaves	Luna *et al.* 1994
	Lycopersicon esculentum	Leaves, stem and roots	Mazhoudi *et al.* 1997
	Oryza sativa	seedlings	Chen *et al.* 2000
	Camellia sinensis	root	Ghanati *et al.* 2005
	Prunus cerasifera	seedlings	Lombardi and Sebastiani, 2005
	Zea mays	roots and shoots	Pourakbar *et al.* 2007
	Vigna mungo	seedlings	Solanki *et al.* 2011
	Atriplex halimus	leaves	Brahim and Muhamed, 2011
	Cucumi sativus	Roots	Iseri *et al.* 2011
Superoxide dismutase	*Nicotiana tabacum*	leaves	Pitcher *et al.* 1991
	Glycine max	root	Chongpraditnun *et al.* 1992
	Nicotiana tabacum and *Pisum sativum*	leaves	Sen Gupta *et al.* 1993 Hartley-Whitaker *et al.* 2001
	Holcus lanatus	Root	Wang *et al.* 2004
	Brassica juncea	Roots	Ghanati *et al.* 2005
	Camellia sinensis	Root	Lombardi and Sebastiani, 2005
	Prunus cerasifera	Root and shoot	Peng *et al.* 2006
	Elsholtzia splendens	Root, stem and leaves	Ke *et al.* 2007
	Daucus carota	Roots and leaves	Meng *et al.* 2007
	Allium sativum	Leaves and roots	Zhang *et al.* 2008
	Elsholtzia haichowensis	Root	Gao *et al.* 2008
	Jatropha curcas	Root, stem and leaves	Tie *et al.* 2012
	Zea mays	Leaves	Azooz *et al.* 2012
	Triticum aestivum cv. Hasaawi	seedlings	
Ascorbate peroxidase	*Avena sativa*	Leaves	Luna *et al.* 1994
	Lycopersicon esculentum	Leaves, stem and roots	Mazhoudi *et al.* 1997
	Phaseolus vulgaris	Leaves and roots	Weckx and Clijsters, 1996
	Oryza sativa	root	Chen *et al.* 2000
	Camellia sinensis	Root	Ghanati *et al.* 2005
	Morus rubra	Leaves	Tewari *et al.* 2006
	Oryza sativa	Root and shoot	Thounaojam *et al.* 2012
	Camellia sinensis	Root and shoot	Hajiboland and Bastani, 2012
	Camellia sinensis	Leaves	Saha *et al.* 2012
γ-glutamylcysteinyl synthetase	*Camellia sinensis*	Leaves	Yadav and Mohanpuria, 2009
	Triticum aestivum	Leaves	Shan *et al.* 2012
Glutathione reductase	*Silene cucubalus*	root	De Vos *et al.* 1992
	Panax ginseng	Roots	Ali et al. 2006

	Morus rubra	Leaves	Tewari *et al.* 2006
	Zea mays	Roots and leaves	Pourakbar *et al.* 2007
	Oryza sativa	Root and shoot	Thounaojam *et al.* 2012
	Triticum aestivum	Leaves	Shan *et al.* 2012
	Zea mays	Roots	Wang *et al.* 2011
	Zea mays	Leaves	Tie *et al.* 2012
Dehydroascorbate reductase	*Cucumis sativus*	Roots and leaves	Arora *et al.* 2002
	Panax ginseng	roots	Ali *et al.* 2006
	Triticum aestivum	Leaves	Shan *et al.* 2012
Phenylalanine ammonia lyase	*Phyllanthus tenellus*	Leaves	Santiago *et al.* 2000
	Camellia sinensis	leaves	Basak *et al.* 2001
	Camellia sinensis	leaves	Chakraborty *et al.* 2002
	Matricaria recutita	Root and leaves	Kovacik and Backor, 2007
	Glycine max	roots	Chmielowska *et al.* 2008
	Jatropha curcas	Root, stem and leaves	Gao *et al.* 2008
Polyphenol oxidase	*Camellia sinensis*	Leaves	Basak *et al.* 2001
	Jatropha curcas	Root, stem and leaves	Gao *et al.* 2008

ROS and thereby aid in mitigating oxidative stress (Tsuji *et al.*, 2002).

Accumulation of amino acids like proline has been observed in response to several biotic and abiotic stresses in plants. Content of free proline has been found to be related to Cu^{2+} tolerance in plants (Backor *et al.*, 2003; Chen *et al.*, 2004). Excess Cu^{2+} has been found to result in inadequate proline (Thomas *et al.*, 1998) and lead to the malfunctioning of copper exclusion machinery (Chen *et al.*, 2004). Copper complexes with amino acids such as proline, histidine or nicotinamine play important role in xylem sap transport (Liao *et al.*, 2000).

Antioxidant response

Plants possess well developed defence system against ROS which restricts its formation and maneuver its removal. Inside the plant cell, superoxide dismutases (SOD) provide the first line of defence against ROS. The enzyme is located in different cell compartments including mitochondria, chloroplast, glyoxisomes, peroxisomes, microsomes, apoplast and cytosol (Alscher *et al.*, 2002) and catalyzes the disproportionation of O_2^- to H_2O_2 and molecular oxygen (Scandalios, 1993). SOD enzymes are classified based on the metal cofactors: the Cu- Zn SOD, the Mn-SOD and Fe-SOD (Bowler *et al.* 1994). Although each type of SOD predominates in specific cell compartments, their occurrences are not restricted, and all types can be detected in most of the cellular locations (Arora *et al.*, 2002). An increased level of SOD has been correlated to enhanced oxidative stress protection in plants (Sen Gupta *et al.*, 1993). Increase in SOD activity has been reported against copper induced stress in tolerant plants such as *Prunus cerasifera* (Lombardi and Sebastiani, 2005); *Elsholtzia haichowensis* (Zhang *et al.*, 2008); *Elsholtzia splendens* (Peng *et al.*, 2006); *Jatropha curcas* (Gao *et al.*, 2008); *Holcus lanatus* (Hartley-Whitaker *et al.*, 2001); *Daucus carota* (Ke *et al.*, 2007); *Ceratophyllum demersum* (Rama Devi and Prasad, 1998); *Brassica juncea* (Wang *et al.*, 2004); *Hydrilla verticillata* (Srivastava *et al.*, 2006); *Zea mays* (Tie *et al.*, 2012), *Triticum aestivum* cv. Hasaawi (Azooz *et al.*, 2012), *Allium sativum* (Meng *et al.*, 2007) etc. However, Weckx and Clijsters (1996) observed that SOD was not involved in the defence mechanism against copper induced oxidative stress in primary leaves of *Phaseolus vulgaris*. Contradictory results have also been recorded regarding the response of catalase (CAT) against copper stress. Both CAT and peroxidase (POD) are involved in the removal of H_2O_2 that accumulates due to dismutation of O_2^- by SOD. Catalase activity did not increase in Cu^{2+} stressed roots of rice seedlings (Chen *et al.*, 2000) or in black gram (*Vigna mungo*) seedlings (Solanki *et al.*, 2011). On the other hand, CAT activity was reported to increase in

halimus leaves (Brahim and Muhamed, 2011) *Prunus cerasifera* (Lombardi and Sebastiani, 2005), *C. sativus* roots (Iseri *et al.*, 2011) and in maize roots and shoots (Pourakbar *et al.*, 2007) in response to excess Cu^{2+} concentrations. The mobilization of POD in response to Cu^{2+} induced oxidative stress in plants is well accepted (Fang and Kao, 2000; Diaz *et al.*, 2001; Cuypers *et al.*, 2002; Meng *et al.*, 2007; Solanki *et al.*, 2011). Apart from POD and CAT, the enzymes and metabolites of the ascorbate-glutathione cycle are also involved in the removal of H_2O_2. The majority of these enzymes [ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR)] have been found in chloroplasts, cytosol, mitochondria, and peroxisomes (Dat *et al.*, 2000). Glutathione and ascorbate accumulate in these cellular compartments and their redox state is maintained through glutathione reductase (GR), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR). All these enzymes along with ascorbate and glutathione have a pivotal role in defence against ROS induced oxidative damage (Arora *et al.*, 2002; Yruela, 2005; Sharma and Dietz, 2008; Shan *et al.*, 2012). De Vos *et al.*, (1992) observed that glutathione depletion is the major cause of Cu^{2+} induced oxidative damage in Cu^{2+} sensitive *Silene cucubalus* plants. It has been shown that tolerance to a copper-enriched environment, and the accompanying oxidative stress in *Enteromorpha compressa* occurs through the accumulation of copper, activation of ascorbate peroxidase, synthesis of ascorbate (accumulated as dehydroascorbate) and consumption of glutathione and water-soluble phenolic compounds (Ratkevicius *et al.*, 2003).

Stress in tea

A literature survey revealed that several studies have been conducted on different types of abiotic stresses in tea. Plants of different cultivars of tea have been grouped into the tolerance classes: susceptible and resistant, in response to drought stress (Chakraborty *et al.*, 2002; Damayanti *et al.*, 2010), cold stress (Upadhyay, 2012) and heavy metal stress (Yadav and Mohanpuria, 2009). Several parameters have been identified such as rates of photosynthesis and transpiration, relative water content, stomatal conductance and leaf total soluble sugar content (Damayanti *et al.*, 2010), root and shoot extension (Burgess and Carr, 1997), levels of proline and antioxidative enzymes (Chakraborty *et al.*, 2002; Upadhyay and Panda, 2004; Upadhyay *et al.*, 2008), morphological characters (Waheed *et al.*, 2012) etc. in order to screen tea cultivars for drought tolerance. Additionally, studies on alterations in bioconstituents that determined quality of tea in the tea clones under soil moisture revealed a decrease in PAL activity in both tolerant and susceptible clones which correlated with a lower flavonol content and quality deterioration (Jeyaramaja *et al.*, 2003).

Tea plants exposed to excess heavy metals have shown several alterations in physiological and biochemical parameters. Increased level of lipid peroxidation and a reduction in photosynthetic rate, transpiration rate, chlorophyll and protein content and biomass production were found in plants exposed to excess Cd (Mohanpuria *et al.*, 2007; Shi *et al.*, 2008). Oxidative stress was evident as the transcript levels of glutathione biosynthetic genes showed up-regulation while glutathione-S-transferase (GST), the enzyme which help in sequestration of high levels of metal ions to vacuole, did not show any change on Cd exposure (Mohanpuria *et al.*, 2007). Hajiboland and Bastani (2012) observed that CO_2 assimilation and dry matter production decreased while antioxidant enzyme activity and proline content increased significantly in tea plants under Boron deficiency and water stress. Mukhopadhyay *et al.* (2013) observed that both deficiency and excess in zinc caused a considerable decrease in shoot and root fresh and dry masses. Zinc stress decreased net photosynthetic rate, transpiration rate, stomatal conductance, and content of chlorophylls *a* and *b and increased the content of* superoxide anion, malondialdehyde, hydrogen peroxide, and phenols. Although the activities of ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase as well as expression of respective genes were up-regulated, the authors concluded that the overall antioxidant

system did not afford sufficient protection against oxidative damage (Mukhopadhyay *et al.*, 2013). Treatment of tea plants with excess heavy metals such as mercury (II) and nickel (II) decreased the chlorophyll content of the leaves, along with a significant reduction in Hill activity (Basak *et al.*, 2001). The activities of antioxidative enzymes viz. Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was increased by Aluminium in the roots of cultured tea cells and also in intact plants (Ghanati *et al.*, 2005). Aluminum (Al) inhibited tea pollen tube growth but the effect was found to be alleviated by fluorine (Konishi and Miyamoto, 1983) which is accumulated by tea plants normally in high excess (Ruan *et al.*, 2004). Tea plants tolerated fluorine at concentrations < 0.32 mM (Li *et al.*, 2011). Fresh and dry mass, chlorophyll content and net photosynthetic rate decreased while proline, malondialdehyde and hydrogen peroxide contents increased with increasing fluorine concentrations. Activity of antioxidant enzymes also showed significant alterations thereby suggesting that antioxidant defence system of leaves did not sufficiently scavenge excessive reactive oxygen species generated due to excess fluorine (Li *et al.*, 2011).

Cu^{2+} stress in tea

Although copper based fungicides are being used in tea gardens for several decades (Sarmah, 1960), we know little about the role of excess Cu^{2+} on tea plants and at what concentrations it may be considered as a pervasive threat (Saha *et al.*, 2012). Only a few studies have focused on Cu^{2+} toxicity in tea (Basak *et al.*, 2001; Yadav and Mohanpuria, 2009; Saha *et al.*, 2012) and these have revealed that number physiochemical parameters are altered on exposure to excess copper. For example, the chlorophyll and protein contents were found to decrease in Cu^{2+} treated plants (Basak *et al.*, 2001; Yadav and Mohanpuria, 2009; Saha *et al.*, 2012). Germination of tea seeds were also affected in presence of excess Cu^{2+}. Substantial reduction in the length and biomass of root and shoot was observed (Mandal et al., 2013). Excess Cu^{2+} caused an increase in lipid peroxidation, phenolics and antioxidative enzyme levels such as POD, SOD and APX in multiple cultivars of tea (Saha *et al.*, 2012). A significant difference among cultivars was noted where the more sensitive cultivar seemed to lose its antioxidative capacity at Cu^{2+} concentrations higher than 400 µM while the more tolerant cultivar was able to withstand a maximum of 600 µM of Cu^{2+} ions. Two new isozymes were also found to be induced in the leaves of tea exposed to high concentration of Cu^{2+} (Saha *et al.*, 2012). Yadav and Mohanpuria (2009) observed that expression of the enzymes γ-glutamylcysteinyl synthetase, glutathione synthetase and phytochelatin synthase was elevated more in the tolerant tea cultivar than the susceptible one when exposed to excess Copper and Aluminium.

Conclusion

Heavy metal stress is one of the major problems that limit agricultural productivity of plants. Plants show relative differences in their heavy metal tolerance capacity among the species and also among cultivars of the same species. Copper stress in general induces ROS and generates oxidative stress. It has been found that in addition to accumulated metal ions, high levels of ROS adversely affected the plants. Such ROS related damages have been observed in tea cultivars also. Although of the negative impact of excess Cu^{2+} in tea plants have been documented, the level of Cu^{2+} accumulation caused due to long term application of Cu^{2+}-based fungicides in tea gardens and its bioavailability under tea garden conditions are yet to be studied. Additionally, more detailed studies on mechanisms of Cu^{2+} toxicity in the tea plant, especially at the gene level are necessary. Identification of genetic determiners of tolerance may make the resistant cultivars a potential source for genetic manipulation of other important elite cultivars.

Acknowledgement

S. Mandal wishes to thank the University Grants Commission, India, for Rajiv Gandhi National Fellowship [No. F.14-2(SC)/2008(SA-III)].

References

Abdel-Ghany, S.E., Muller-Moule, P., Niyogi, K.K., Pilon, M., and Shikanai, T. (2005). Two P-Type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. *Plant Cell,* 17(4): 1233–1251.

Ali, M.B., Hahn, E.J., and Paek, K.Y. (2006). Copper induced changes in the growth, oxidative-metabolism and saponins production in suspension culture roots of *Panax ginseng* in bioreactors. *Plant Cell Reports,* 25(10): 1122-1132.

Alscher, R.G. (1989). Biosynthesis and antioxidant function of glutathione in plants. *Physiologia Plantarum,* 77: 457–464.

Alscher, R.G., Erturk, N., and Heath, L.S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany, 53,* 1331-1341.

Andres-Colas, N., Sancenon, V., Rodriguez-Navarro, S., Mayo, S., Thiele, D.J., Ecker, J.R., *et al.* (2006). The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. *Plant Journal,* 45: 225–236.

Antosiewicz, D., and Wierzbicka, M. (1999). Location of lead in *Allium cepa* L. cell by electron microscope. *Journal of Microscopy,* 195: 139–146.

Arduini, I., Godbold, D.L., and Onnis, A. (1996). Cadmium and copper uptake and distribution in Mediterranean tree seedlings. *Physiologia Plantarum,* 97: 111-117.

Arora, A., Sairam, R.K., and Srivastava, G.C. (2002). Oxidative stress and antioxidative system in plants. *Current science,* 82: 1227-1238.

Azooz, M.M., Abou-Elhamd, M.F., and Al-Fredan, M.A. (2012). Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. *Australian Journal of crop science,* 6: 688-694.

Backor, M., Fahselt, D., Davidson, R.D., and Wu, C.T. (2003). Effects of copper on wild and tolerant strains of the lichen photobiont Trebouxia ericl (Chlorophyta) and possible tolerance mechanisms. *Archives of Environmental Contamination and Toxicology,* 45: 159-67.

Barua, K.C. (1988). Some aspects of disease control in tea. Field management in tea. Tea Research Association. Tocklai experimental station, pp. 119-124.

Basak, M., Sharma M., and Chakraborty, U. (2001). Biochemical responses of *Camellia sinensis* (L.) O. Kuntze to heavy metal stress. *Journal of Environmental Biology,* 22(1): 37-41.

Blokhina, O., Virolainen, E., and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of botany,* 91: 179-194.

Bowler, C., Van Camp, W., and Montagu, V., and Inze, D. (1994). Superoxide dismutase in plants. *Critical Reviews in Plant Science,* 13: 199-218.

Brahim, L., and Mohamed, M. (2011). Effects of copper stress on antioxidative enzymes, chlorophyll and protein content in *Atriplex halimus. African Journal of Biotechnology,* 10: 10143-10148.

Brun, L.A., Maillet, J., Hinsinger, P., and Pepin, M. (2001). Evaluation of copper availability to plants in copper-contaminated vineyard soils. *Environmental Pollution,* 111: 293-302.

Burgess, P.J. and Carr, M.K.V. (1997). Responses of young tea (*camellia sinensis*) clones to drought and temperature. 3. shoot extension and development. *Experimental Agriculture,* 33: 367-383.

Chakraborty, U., Dutta, S., and Chakraborty, B.N. (2002). Response of Tea Plants to Water Stress. *Biologia Plantarum,* 45: 557-562.

Chen, C.T., Chen, T.H., Lo, K.F., and Chiu, C.Y. (2004). Effects of proline on copper transport in rice seedlings under excess copper stress. *Plant Science,* 166: 103-111.

Chen, L.M., Lin, C.C., and Kao, C.H. (2000). Copper toxicity in rice seedlings: Changes in antioxidative enzyme activies, H_2O_2 level, and cell wall peroxidase activity in

roots. *Botanical Bulletin of Academia Sinica,* 41: 99-103.

Chmielowska, J., Deckert, J., and Diaz, J. (2008). Activity of peroxidases and phenylalanine ammonia-lyase in lupine and soybean seedlings treated with copper and an ethylene inhibitor. *Biological Letters,* 45: 59-67.

Chongpraditnum, P., Mori, S., and Chino, M. (1992). Excess copper induces a cytosolin Cu, Zn-superoxide dismutase in soybean root. *Plant Cell Physiology,* 33: 239-244.

Chu, C.C., Lee, W.C., Guo, W.Y., Pan, S.M., Chen, L.J., Li H.M., and Jinn, T.L. (2005). A copper chaperone for superoxide dismutase that confers three types of copper/zinc superoxide dismutase activity in Arabidopsis. *Plant Physiology,* 139: 425-436.

Clemens, S., Palmgren, M.G., and Kramer, U. (2002). A long way ahead: Understanding and engineering plant metal accumulation. *Trends in Plant Sciences,* 7: 309-315.

Cohu, C. M., and Pilon, M., (2007) Regulation of superoxide dismutase expression by copper availability. *Physiologia Plantarum,* 129: 747-755.

Company, P., and Gonzalez-Bosch, C. (2003). Identification of a copper chaperone from tomato fruits infected with *Botrytis cinerea* by differential display. *Biochemical and Biophysical Research Communications,* 304(4): 825-830.

Cuypers, A., Vangronsveld, J., and Clijsters, H. (2002). Peroxidases in roots and primary leaves of Phaseolus vulgaris Copper and Zinc Phytotoxicity: a comparison. *Journal of Plant Physiology,* 159: 869-876.

Damayanthi, M.M.N., Mohotti, A.J., and Nissanka, S.P. (2010). Comparison of Tolerant Ability of Mature Field Grown Tea (*Camellia sinensis* L.) Cultivars Exposed to a Drought Stress in Passara Area. *Tropical Agricultural Research,* 22(1): 66-75.

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D., and Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences,* 57: 779-795.

De Vos, C.H.R., Vonk, M.J., Vooijs, R., and Schat, H. (1992). Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *silene cucubalus. Plant Physiology,* 98, 853-858.

Diaz, J., Bernal, A., Pomar, F., and Merino, F. (2001) Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annum* L.) seedlings in response to copper stress and its relation to lignification. *Plant Science,* 161: 179-188.

Ducic, T., and Polle, A. (2005). Transport and detoxification of manganese and copper in plants. *Brazilian Journal Plant Physiology,* 17: 103-112.

Fang, W.C., and Kao, C.H. (2000). Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Science,* 158: 71-76.

Flemming, C.A., and Trevors, J.T. (1989). Copper toxicity and chemistry in the environment: a review, *Water, air, and soil pollution,* 44(12): 143-158.

Gao, S., Yan, R., Cao M., Yang W., Wang, S., and Chen, F. (2008). Effects of copper on growth, antioxidant enzymes and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedling, *Plant Soil Environment,* 54(3): 117-122.

Garcia, A., Baquedano, F.J., Navarro, P., and Castillo, F.J. (1999). Oxidative stress induced by copper in sunflower plants. *Free Radical Research,* 31: 45-50.

Ghanati, F., Morita, A., and Yokota, H. (2005). Effects of aluminum on the growth of tea plant and activation of antioxidant system. *Plant Soil,* 276: 133–141.

Graham, J.H., Timmer, L.W., and Fardelmann, D. (1986). Toxicity of fungicidal copper in soil to *citrus* seedling and vesicular-arbuscular mycorrhizal fungi. *Phytopathology,* 76: 66-70.

Greger, M. (2004). Metal availability, uptake, transport and accumulation in plants. *In:* M.N.V. Prasad (ed.), *Heavy Metal stress in plants.* Narosa Publishing House, New Delhi, pp. 1-28.

Grill, E., Loffler, S., Winnacker, E.L. and Zenk, M.H. (1989). Phytochelatins, the heavy-

metal-binding peptides of plants, are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). *Biochemistry,* 86: 6838-6842.

Grill, E., Winnacker, E.L., and Zenk, M.H. (1985). Phytochelatins: the principal heavy-metal complexing peptides of higher plants. *Science,* 230: 674-676.

Hagemeyer (2004) Ecophysiology of plant growth under heavy metal stress. *In:* M.N.V. Prasad (ed.), Heavy Metal stress in plants. Narosa Publishing House, New Delhi, pp. 201-222.

Hajiboland, R. and Bastani, S. (2012). Tolerance to water stress in boron-deficient tea (*Camellia sinensis*) plants. *Folia Horticulturae,* 24: 41-51.

Hamer, D.H. (1986). Metallothionein[1, 2]. *Annual Review of Biochemistry,* 55: 913-951.

Hartley-Whitaker, J., Ainsworth, G., and Meharg, A.A. (2001). Copper- and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. *Plant Cell Environment,* 24: 713-722.

Himelblau, E., and Amasino, R.M. (2000). Delivering copper within plant cells. *Current Opinion in Plant Biology, Physiology and metabolism,* 3: 205-210.

Iseri, O.D., Korpe, D.A., Yurtcu, E., Sahin, F.I., and Haberal, M. (2011). Copper-induced oxidative damage, antioxidant response and genotoxicity in *Lycopersicum esculentum* Mill. and *Cucumis sativus* L. *Plant Cell Reports,* 30: 1713-1720.

Jeyaramraja, P.R., Pius, P.K., Raj Kumar, R., and Jayakumar, D. (2003). Soil moisture stress-induced alterations in bioconstituents determining tea quality. *Journal of the Science of Food and Agriculture,* 83: 1187-1191.

Kampfenkel, K., Kushnir, S., Babiychuk, E., Inze, D., and Van Montagu, M. (1995). Molecular characterization of a putative *Arabidopsis thaliana* copper transporter and its yeast homologue. *The Journal of Biological Chemistry,* 270(47): 28479-28486.

Karmakar, K.G., and Banerjee, G.D. (2005). *The Tea Industry in India*: A Survey, National Bank for Agriculture and Rural Development, Tea trade, Vol. 39, p. 177.

Ke, W., Xiong, Z., Xie, M., and Luo, Q. (2007). Accumulation, subcellular localization and ecophysiological responses to copper stress in two *Daucus carota* L. populations. *Plant Soil,* 292: 291-304.

Kieselbach, T., Hagman, A., Anderson, B., and Schroder, W.P. (1998). The thylakoid lumen of chloroplasts: Isolation and characterization. *The Journal of Biological Chemistry,* 273: 6710-6716.

Kliebenstein, D.J., Monde, R.A., and Last, R.L. (1998). Superoxide Dismutase in Arabidopsis: An Eclectic Enzyme Family with Disparate Regulation and Protein Localization. *Plant Physiology,* 118: 637-650.

Konishi, S., and Miyamoto, S. (1983). Alleviation of Aluminum Stress and Stimulation of Tea Pollen Tube Growth by Fluorine. *Plant and Cell Physiology,* 24(5): 857-862.

Kovacik, J. and Backor, M. (2007). Phenylalanine ammonia-lyase and phenolic compounds in chamomile tolerance to cadmium and copper excess. *Water, Air, and Soil Pollution,* 185: 185-193.

Lanaras, T., Moustakas, M., Symeonidis, L., Diamantoglou, S., and Karataglis, S. (1993). Plant metal content, growth responses and some photosynthetic measurement on field-cultivated wheat growing on ore bodies enriched in Cu. *Physiologia Plantarum,* 88: 307-314.

Lequeux, H., Hermans, C., Lutts, S., and Verbruggen, N. (2010). Response to copper excess in Arabidopsis thaliana: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. *Plant Physiology Biochemistry,* 48(8): 673-682.

Li C., Zheng Y., Zhou J., Xu J., and Ni D. (2011). Changes of leaf antioxidant system, photosynthesis and ultrastructure in tea plant under the stress of fluorine. *Biologia Plantarum,* 53(3): 563-566.

Liao, M.T., Hedley, M.J., Woolley, D.J., Brooks, R.R., and Nichols, M.A. (2000). Copper uptake and translocation in chicory (*Cichorium intybus* L. cv Grasslands Puna) and tomato (*Lycopersicon esculentum* Mill. Cv Rondy) plants grown in NFT system. II. The role of nicotianamine and histidine in xylem sap copper transport. *Plant Soil,* 223: 243-252.

Lidon, F.C. and Henriquesa, F.S. (1993). Effects of copper toxicity on growth and the uptake and translocation of metals in rice plants. *Journal of Plant Nutrition,* 16: 1449-1464.

Lidon, F., and Henriques, F.S., (1991). Limiting step on photosynthesis of rice plants treated with varying copper levels. *Journal of Plant Physiology,* 138: 115-118.

Lombardi, L., and Sebastiani, L. (2005). Copper toxicity in *Prunus cerasifera*: growth and antioxidant enzymes responses of in vitro grown plants. *Plant Science,* 168: 797-802.

Luna, C.M., Gonzalez, C.A., and Trippi, V.S. (1994). Oxidative damage caused by an excess of copper in oat leaves. *Plant Cell Physiology,* 35: 11-15.

Maksymiec, W. (1997) Effect of copper on cellular processes in higher plants. *Photosynthetica,* 34: 321-342.

Mandal, S., Saha, A. and Saha, D. (2013). Effect of copper on seed germination, root elongation and shoot elongation of seedlings of commercially cultivated tea varieties. *NBU Journal of Plant Sciences,* 7: 43-49.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants,* 2nd ed. Academic Press, London.

Mazhoudi, S., Chaoui, A., Ghorbal, M.H., and El Ferjani, E. (1997). Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum,* Mill.) *Plant Science,* 127: 129-137.

Mehra, R.K., and Mulchandani, P. (1995). Glutathione-mediated transfer of Cu (I) into phytochelatins. *Biochemical Journal,* 307: 697-705.

Meng, Q., Zou, J., Zou, J., Jiang, W., and Liu, D. (2007). Effect of Cu^{2+} concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (*Allium Sativum* L.). *Acta Biologica Cracoviensia Series Botanica,* 49: 95-101.

Mocquot, B., Vangronsveld, J., Clijsters, H., and Mench, M. (1996). Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents, and enzyme activities. *Plant and Soil,* 182(2): 287-300.

Mohanpuria, P., Rana, N.K., and Yadav, S.K. (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze. *Environmental Toxicology,* 67: 368-374.

Morales, J. M. L., Roddrigues-Monroy, M., and Sepulveda-Jimenez, G. (2012). Betacyanin accumulation and guaiacol peroxidase activity in *Beta vulgaris* L. leaves following copper stress. *Acta Societatis Botanicorum Poloniae,* 81(3): 193-201.

Mukhopadhyay M., Das A., Subba P., Bantawa P., Sarkar B., Ghosh P., and Mondal T.K. (2013) Structural, physiological, and biochemical profiling of tea plantlets under zinc stress. *Biologia Plantarum,* 57(3): 474-480.

Tie, S.G., Tang, Z.J., Zhao Y.M., and Li, W., (2012). Oxidative damage and antioxidant response caused by excess copper in leaves of maize. *African Journal of Biotechnology,* 11: 4378-4384.

Oliva, S.R., Mingorance M.D., Valdes B., and Leidi, E.O. (2010). Uptake, localisation and physiological changes in response to copper excess in Erica andevalensis. *Plant Soil,* 328: 411-420.

Patsikka, E., Kairavuo, M., Sersen, F., Aro, E.M., and Tyystjarvi, E. (2002). Excess copper predisposes photosystem II to photoinhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll. *Plant Physiology,* 129(3): 1359-1367.

Peng, H.Y., Yang, X.E., Yang, M.J., and Tian, S.K. (2006). Responses of antioxidant enzyme system to copper toxicity and copper detoxification in the leaves of *Elsholtzia splenden. Journal of Plant Nutrition,* 29(9): 1619-1635.

Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse D., and Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *Journal of Phycology*, 39: 1008–1018.

Pitcher L.H., Brennan E., Hurley A., Dunsmuir P., Tepperman J.M., and Zilinskas B.A. (1991) Overproduction of Petunia Chloroplastic Copper/Zinc Superoxide Dismutase Does Not Confer Ozone Tolerance in Transgenic Tobacco. *Plant Physiology*, 97: 452-455.

Pourakbar, L., Khayami, M., Khara, J., and Farbodnia, T. (2007). Copper–induce change in antioxidative system in maize (*Zea mays* L.). *Pakistan Journal of Biological Science*, 10(20): 3662-3667.

Prasad, M.N.V. (2004). *Heavy Metal Stress in Plants*. Springer, Narosa Publishing House, New Delhi.

Rama Devi, S., and Prasad, M.N.V. (1998). Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: response of antioxidant enzymes and antioxidants. *Plant Science*, 138: 157-165.

Ratkevicius, N., Correa, J.A., and Moenne, A. (2003). Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in northern Chile. *Plant, Cell and Environment*, 26: 1599-1608.

Rhoads, F.M., Olsou, S.M., Manning, A. (1989). Copper toxicity in tomato plants. *Journal of environmental Quality*, 18: 195-197.

Rooney, P.C., Zhao, F.J., and McGrath, S.P. (2006). Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils. *Environmental Toxicology and Chemistry*, 25(3): 726-732.

Ruan, J., Ma, L., Shi, Y., and Han, W. (2004). The Impact of pH and calcium on the uptake of fluoride by tea plants (*Camellia sinensis* L.). *Annals of Botany*, 93: 97-105.

Saha, D., Mandal, S., and Saha, A. (2012). Copper induced oxidative stress in tea (*Camellia sinensis*) leaves. *Journal of Environmental Biology*, 33: 861-866.

Sancenon, V., Puig, S., Mira, H., Thiele, D.J., and Penarrubia, L. (2003). Identification of a copper transporter family in *Arabidopsis thaliana*. *Plant Molecular Biology*, 51: 577-587.

Santiago, L.J.M., Louro, R.P., and Oliveira, D.E.D. (2000). Compartmentation of phenolic compounds and phenylalanine ammonia-lyase in leaves of *Phyllanthus tenellus* roxb. and their induction by copper sulphate. *Annals of Botany*, 86: 1023-1032.

Sarkar, A.N. (1994). Integrated horticultural development in Eastern Himalayas. MD Publications Pvt. Ltd., New Delhi.

Sarmah, K.C. (1960). *Disease of tea and associated crops in north east India. In:* Indian Tea Association, Memo. No. 26, Tocklai Experimental Station, Assam, India, pp. 6-42.

Scandalios, J.G. (1993). Oxygen Stress and Superoxide Dismutases. *Plant Physiology*, 101: 7-12.

Schubert, M., Petersson, U.A., Haas, B.J., Funk, C., Schroder, W.P., and Kieselbach, T. (2002). Proteome map of the chloroplast lumen of *Arabidopsis thaliana*. *Journal of Biological Chemistry*, 278: 8354-8365.

Selvakumar, M., and Jeyaselvam, M. (2012). Tea industry: A Tonic for the Indian Economy. Facts for you, pp. 11-17. http://www.flymag.com/admin/issue/pdf/Tea_April_12pdf

Sen Gupta, A., Heinen, J.L., Holaday, A.S., Burket, J.J., and Allen, R.D. (1993). Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. *Plant Biology*, 90: 1629-1633.

Shan, C., Dai, H., and Sun, Y. (2012). Hydrogen sulfide protects wheat seedlings against copper stress by regulating the ascorbate and glutathione metabolism in leaves. *Auastralian Journal of crop science*, 6(2): 248-254.

Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M., (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in

Plants under Stressful Conditions. *Journal of Botany,* doi:10.1155/2012/217037

Sharma, S.S., and Dietz, K. (2008). The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science,* 14: 43-50.

Shaw, B.P., Sahu, S.K., and Mishra, R.K. (2004). *Heavy metal induced oxidative damage in terrestrial plants. In:* M.N.V. Prasad (ed.), *Heavy Metal stress in plants.* Narosa Publishing House, New Delhi. pp. 84-119.

Sheldon, A.R., and Menzies N.W. (2005). The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant and Soil,* 278: 341-349.

Shi, Y., Ruan, J., Ma, L., Han, W., and Wang, F. (2008). Accumulation and distribution of arsenic and cadmium by tea plants. *Journal of Zhejang University Science B,* 9(3): 265-270.

Shikanai, T., Muller-Moule, P., Munekage, Y., Niyogi, K.K., and Pilon, M. (2003). PAA1, a P-Type ATPase of Arabidopsis, Functions in Copper Transport in Chloroplasts. *The Plant Cell,* 15: 1333–1346.

Singh, A.K., and Singh, V.B. (2006). Significance of limiting in horticultural crop production. *In:* V.B. Singh (ed.), *Horticulture for sustainable income and environmental protection.* Concept Publishing Company, New Delhi, pp. 88-93.

Singh, I.D. (2005). *The planters guide to tea culture and manufacture,* N.B. Modern Agencies, Siliguri, India, pp. 132-138.

Solanki, R., Poonam, A., and Dhankhar, R. (2011). Zinc and copper induced changes in physiological characteristics of vigna mungo (L.) *Journal of Environmental Biology,* 32: 747-751.

Srivastava, S., Mishra, S., Tripathi, D.R., Dwivedi, S., and Gupta, K.D. (2006). Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. *Aquatic Toxicology,* 80: 405-415.

Tewari, R.K., Kumar, P., and Sharma, P.N. (2006). Antioxidant responses to enhanced generation of superoxide anion radical and hydrogen peroxide in the copper-stressed mulberry plants. *Planta,* 223(6): 1145-1153.

Thomas, J.C., Malick, F.K., Endreszl, C., Davies, E. C., and Murry, K.S. (1998). Distinct response to copper stress in the halophyte Mesembryanthemum crystallinum. *Physiologia Plantarum,* 102: 360-368.

Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L., and Panda, S.K. (2012). Excess copper induced oxidative stress and response of antioxidants in rice. *Plant physiology* and *biochemistry,* 53: 33-39.

Tsay, C.C., Wang, L.W., and Chen, Y.R. (1995). Plant in response to copper toxicity. *Taiwania,* 40: 173-181.

Tsuji, T., Ishizaki, T., Okamoto, M., Higashida, C., Kimura, K., Furuyashiki, T., Arakawa, Y., Birge, R.B., Nakamoto, T., Hirai, H., and Narumiya, S. (2002). ROCK and mDia1 antagonize in Rho-dependent Rac activation in Swiss 3T3 fibroblasts. *The Journal of Cell Biology,* 157: 819-830.

Upadhyaya, H. (2012). Changes in Antioxidative Responses to Low Temperature in Tea [*Camellia sinensis* (L) O. Kuntze] Cultivars. *International Journal of Modern Botany,* 2(4): 83-87.

Upadhyaya, H., Panda, S.K., and Dutta, B.K. (2008). Variation of physiological and antioxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery, *Acta Physiologiae Plantarum,* 30: 457-468.

Upadhyaya, H., and Panda S.K. (2004). Responses of *Camellia sinensis* to drought and rehydration. *Biologia Plantarum,* 48: 597-600.

Van-Zwieten, L., Merrington, G., and Van-Zwieten, M. (2004). Review of impacts on soil biota caused by copper residues from fungicide application. SuperSoil. 3rd Australian New Zealand Soils Conference, 5–9 December, University of Sydney, Australia. Published on CDROM. Website www.regional.org.au/au/asssi/

Vassilev, A., Lidon, F., Ramalho, J.C., Doceumatos, M., and Dagraca, M. (2003). Effects of excess cu on growth and photosynthesis of barley plants. Implication with a screening test for Cu tolerance. *Journal of Central European Agriculture,* 4: 225-235.

Vaughn, K.C., Lax A.R., and Duke, S.O. (1988). Polyphenol oxidase: The chloroplast oxidase with no established function. *Physiologia Plantarum,* 72: 659-665.

Vranova, E., Inze, D., and Van Breusegem, F. (2002). Signal transduction during oxidativestress. *Journal of Experimental Botany,* 53: 1227-1236.

Waheed, A., Hamid, F.S., Shah, A.H., Habib, H., Khalid, A., Abbasi, F.M., Ahmad, N., Aslam, S., and Sarwar, S. (2012). Response of different tea (*Camellia sinensis* L.) clones against drought tress. *Journal of Materials and Environmental Science,* 3(2): 395-410.

Wang, S.H., Yang, Z.M., Yang, H., Lu, B., Li, S.Q., and Lu, Y.P. (2004). Copper-induced stress and antioxidative responses in roots of *Brassica juncea* L. *Botanical Bulletin of Academia Sinica,* 45, 203-212.

Wang, Y.Z., Nie, L.H.,Tie, S., Xie, D., Zhu,W., Qi, J., and Yue, R. (2011). Effects of excess copper on the oxidative stress in roots of maize seedlings. *African Journal of Agricultural Research,* 6(21): 4998-5004.

Weckx, J.E.J., and Clijsters, H.M.M. (1996). Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a root assimilation of toxic amounts of copper. *Physiologia Plantarum,* 96: 506–512.

Weigel, M., Varotto, C., Pesaresi, P., Finazzi, G., Rappaport, F., Salamini, F., and Leister, D. (2003). Plastocyanin is indispensable for photosynthetic electron flow in *Arabidopsis thaliana. Journal of Biological Chemistry,* 278: 31286-31289.

Worthing, C.R. (1983). The pesticides manual: A world compendium. The British Crop Protection Council, Croydon, England. http://isbndb.com/d/publisher/british_crop_protection _counci.html

Yadav, S.K. (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany,* 76: 167-179.

Yadav, S.K., and Mohanpuria, P. (2009). Responses of *Camellia sinensis* cultivars to Cu and Al stress. *Biologia Plantarum,* 53(4): 737-740.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology,* 17: 145-156.

Zhang, H., Xia, Y., Wang, G., and Shen, Z. (2008). Excess copper induces accumulation of hydrogen peroxide and increases lipid peroxidation and total activity of copper–zinc superoxide dismutase in roots of *Elsholtzia haichowensis. Planta,* 227: 465-475.

Zhao, S., Liu, Q., Qi, Y., and Duo, L. (2010). Responses of root growth and protective enzymes to copper stress in turfgrass. *Acta Biologica Cracoviensia Series Botanica,* 52(2): 7-11.