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Copper-induced changes in growth and antioxidative mechanisms of tea plant (*Camellia sinensis* (L.) O. Kuntze)

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Tea is the oldest, most popular, non-alcoholic caffeine containing beverage in the world. Tea plants are prone to the attack of many diseases which can be controlled by the treatment of pesticides. These pesticides contain heavy metal which prolonged accumulation can lead to the damage of crop yield both in quantity and quality. In the present study, we observed the effects of high concentration of Cu stress on physiological and biochemical parameters. The tea cultivars (S_3A_3 and TS-491) were collected from the Rosekandi Tea Estate, Silchar, Assam. The accumulation of Cu in the different parts of the tea plants had a positive correlation with the Cu stress. The accumulation of Cu was higher in roots than in leaves and also the new stems. The results show gradual decrease in the photosynthetic activity with the increase in the concentration of the Cu stress in both the cultivars. The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased with the increase in the Cu concentration but remarkably in different manner in both the cultivars. The activities of antioxidants ascorbate peroxidase (APX), CAT, SOD and POD in cultivar S_3A_3 increased up to 500 µM and at 600 µM showed a low rate of increase whereas TS-491was tolerant up to 400 µM. The responses in the oxidative stress were characterized by an accumulation of malondialdehyde (MDA). Phenol content has also positive correlation with increase in the concentration in both the cultivars up to 500 µM. Finally it was concluded that Cu is tolerant to the cultivars of tea plant to some extent (specifically at lower concentration), but at higher concentration (beyond 400 µM) of Cu with exposure time, tea plant had a strong inhibition of growth by damaging the normal metabolism.

Key words: Tea plants, copper stress, physiological characters, lipid peroxidation, reactive oxygen species.

INTRODUTION

Camellia sinensis (L.) O. Kuntze is an economically important crop for production of tea leaves. Tea is the oldest, most popular, non-alcoholic caffeine containing these are toxic to plants when they are present in soil or the growth media is above the permissible level (Xu and Shi, 2000). Sometimes, heavy metals like Cu, Cd, As, Zn, beverage in the world. Copper (Cu), cadmium (Cd), nickel (Ni), manganese (Mn) and zinc (Zn) are the essential micronutrients required for the plant life but concentra- Mn, Ni, etc. are hazardous pollutant even at very low con- tion (Ross 1994, Prasad and Strzalka 2002). Due to rapid industrialization and urbanization, there is an elevated

emission of toxic heavy metals which enters the biosphere and affects the growth and development of plants (Nriagu and Pacyna, 1988; Kabata- Pendias, 2001). Pesticides and fertilizers are the other major sources of heavy metal which are directly taken up by plants. As Cu is an essential element, it participates in number of physiological processes, and is an essential cofactor for many metalloproteins, but due to excess amount of copper present in cells, causes problems by inhibiting the plant growth which impairs important cellular processes like photosynthetic electron transport (Demirevska-kepova et al., 2004). Since copper is both an essential cofactor and a toxic element, involving a complex network of metal trafficking pathways that must prevent accumulation of the metal in the freely reactive form (metal detoxification pathways) and ensure proper delivery of this element to target metalloproteins is necessary. Cu as fungicides and pesticides are very effective in the control of the diseases in tea plants and are widely used (Gallagher et al., 2001; Singh, 2005), but higher concentration can cause adverse effect in plants by lowering the chlorophyll content, delay in flowering, reduction in the number and quality of shoots which leads to fall down in the quality of tea (Setia, Kaur, and Setia, 1989). The accumulation of Cu at the higher concentration through direct contact or from the food chain is harmful to human beings who consume tea. Heavy metals such as iron, copper, zinc, nickel, manganese, lead and cadmium can cause oxidative stress, producing enzymatic and non-enzymatic antioxidative reaction responses and lipid peroxidation in plants. The exposure of Cu can cause toxicity to the plants which is due to oxidative damage to the biological macromolecule by redox cycling, depletion of glutathione and alteration of homeostatis (Stohs and Bagchi, 1995). Due to the accumulation of Cu in excessive manner leads to the production of reactive oxygen species (ROS) which can damage lipids, nucleic acids, proteins, amino acids, carbohydrates and other complex molecules produced from all these in cells (Pietrini et al., 2003). To overcome such stress, plants evolved much effective mechanism to detoxify the ROS (Dat et al., 2000). A group of effective antioxidants are present in plants to maintain the antioxidant potential in the cells, which detoxify the production of reactive oxygen species. Many researches showed that amounts of antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD) increase in plants when highly exposed to heavy metals (Acar et al., 2001). Peroxidase plays a crucial role in physiological events which are related to diminishing growth of plants by lignification, cross-connection of cell wall polysaccharides, oxidation of indole-3-acetic acid (IAA), cell elongation and phenol oxidation (Mocquot et al., 1996). Rate of ROS formation and efficiency, and capacity of detoxification mechanisms in plants determine the level of damage of cells under stress conditions. Hence in this study, we observed the effects of Cu stress at different concentrations on some physiological indicators such as growth (shoot and root length), rate of photo-synthesis, enzymatic activity of scavengers of ROS such as SOD, peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), malondialdehyde (MDA) on two cultivars of tea plants *viz.* clonal tea plants, S_3A_3 and the tea plants produced from seeds, TS-491.

MATERIALS AND METHODS

Experimental condition

Three months old plants of the two cultivars viz. S_3A_3 (clonal propagated) and TS-491(developed from seeds) were collected from the Rosekandy Tea Estate, Assam, India. The plants were then transferred to Hoagland solution as nutrient media and allowed to get stable for seven days. The plants were treated with $CuSO_4$ at different concentrations of 50, 200, 300, 400, 500 and 600 µM in the nutrient solution (Hoagland and Amon, 1938). The control plants were left as untreated, and allowed to grow only in Hoagland solution. The top four leaves were collected for measuring the

various enzymatic activities after 2^{nd}, 4^{th} and 7^{th} days of the treatment.

Morphological and growth analysis

The roots, stem and leaves of the two cultivars S_3A_3 and TS-491 were collected and analyzed for the morphological characters like growth of the different parts of the plants, appearance of new leaves, new stems etc.

Determination of chlorophyll content

The chlorophyll content of the matured leaves of both treated and control plants of both cultivars was determined following the protocol of Hegedus et al. (2001). 0.1 g leaf extract was dissolved in 80% acetone and optical density was determined using UV -VIS spectrophotometer (Thermo Fisher) at 645 and 663 nm, respectively. The values of optical density was assayed and

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Abbreviations: ROS, Oxygen species; **POD,** peroxidase; **APX,** ascorbate peroxidase; **CAT**, catalase; **SOD,** superoxide dismutase; **PVP,** polyvinylpyrrolidone; **NBT,** nitrobluetetrazolium; **MDA,** malondialdehyde

expressed as µg g^{-1} fresh weight.

Assay of enzyme activity

For determining the enzyme activities, 0.5 g leaves were dippedi

liquid nitrogen and then homogenized in a chilled mortar and pestle in 5 ml 50 mM cold phosphate buffer of pH 7.8 containing 2% polyvinylpyrrolidone (PVP). The filtrate homogenate was centrifuged at 13000 *g* for 20 min at 4°C and the supernatant were used for determining the enzyme activities. The antioxidant ability of leaves was determined by measuring the POD activity at absorbance of 470 nm due to the oxidation of guaiacol (Wu and von Tiedemann, 2002). SOD activity was measured by the inhibition of nitrobluetetrazolium (NBT) reduction (Krivosheeva et al., 1996). CAT activity was measured with the help of the absorbance at 240 nm (Pinhero et al., 1997).

Determination of lipid peroxidation and phenolic content:

The 0.5 g fresh weight of leaves was homogenized in a pre-chilled mortar pestle in 5 ml of 50 mM cold Na-phosphate buffer (pH 7.8), with 0.1 mM EDTA and 1% (w/v) PVP. After centrifugation at 13,000 *g* for 30 min at 4°C, supernatant was used for further analysis. The level of peroxidation was determined in terms of 2-thiobarbituric acid (TBA) (Liu et al., 1996). To determine the total phenolic components, the 0.5 g of leaves were extracted in ethanol and then the content was estimated with the changes in absorbance at 520 nm (Mahadevan and Sridhar, 1996).

Statistical analysis

All the experiments were done in triplicate and the mean were taken. Statistical analysis of mean values and standard deviations (SD) were performed for all the data. The significant difference was set between treatments at $p < 0.01$ or $p < 0.05$.

RESULTS

Effect of high concentration of copper on tea morphological characters

Tea plants showed different degrees of symptoms in different concentrations of copper stress. With the increase in the concentration of copper, the roots suffered mostly which is followed by leaves and then new stems (data was not given). The leaves first developed some brown spots, yellow patches, become dry and then fell off. At the highest concentration of 500 and 600 µM, less number of new leaves appeared, and most of the leaves became withered in both the cultivars. The number of new stems was very less and gradually the growth rate decreased. The fresh and dry weights of roots and leaves were analyzed and the results reflected that growth rate of the copper treated plants with respect to the control was less. The dry weight of the organs of the treated plants decreased with the increase in the concentration of the copper in both cultivars with respect to exposure days. In the highest concentration (600 µM), the inhibition was the strongest with the smallest growth rate of roots, leaves and stem compared to tea plants treated with lower concentrations.

Effect of Cu on chlorophyll content

Chlorophyll is an important pigment which plays a vital role in the process of photosynthesis. Table 1 shows that there was a significant negative correlation between the concentration of Cu and the chlorophyll contents in the tea plants. With the exposure of high concentration of copper, the total chlorophyll content of both the cultivars decreased. The level of decrease of chlorophyll content is high in TS-491 than the cultivar S_3A_3. From Table 1A, we can conclude that at higher concentration of 400, 500 and 600 µM, there was a gradual decrease in the chlorophyll content in cultivar S_3A_3 whereas from Table 1B, we observed the decrease was more than two fold in cultivar TS-491 compared to S_3A_3. In S_3A_3, at control, the total chlorophyll content was 90.12 µg g^{-1} fresh weight on 2nd day and chlorophyll content decreased gradually as the Cu concentration increased; whereas, maximum decrease in chlorophyll content was found to be started from 400 µm onwards where the content has degraded and ended up at 42.13 µg g^{-1} fresh weight on 7th day at 600 µm Cu concentration. Whereas in TS-491, the chlorophyll content was 94.12 µg g^{-1} fresh weight on 2nd day at control, and from 300 µM concentration onwards, the content degraded more than two folds and ended up at 18.09 µg g^{-1} fresh weight on 7th day at 600 µm Cu concentration. The observation indicates that compared to cultivar S_3A_3, cultivar, TS-491 was more sensitive to copper stress.

Effect of high concentration of Cu on antioxidative enzymes

Plants are exposed to abrupt stress daily and seasonal changes in the environment, and they have a wide spectrum of developmental responses and biochemical adaptations to stress condition. During normal metabolic activities and due to the consequences of environmental condition, O_2 is capable of giving rise to reactive oxygen species (Mittler et al., 2004). ROS include the superoxide radical, hydroxyl radical and hydrogen peroxide which are toxic to plants (Dismukes et al., 2001, Vellosillo et al., 2010). The activities of APX, CAT, SOD and POD gradually decreased with increase in the concentration of Cu.

It was observed that with respect to control, there was a significant increase in the APX activity in S_3A_3 cultivar up to the 4th day of 600 µM concentration, but there was a slight decrease in the APX activity on the 7th day of600 µM (Figure 1). On the other hand, there was only a

Table 1. Effects of different concentration of Cu on total chlorophyll contents of cultivar (A) S3A3 (B) TS-491.

Treatment (µM)	2nd day	4th day	7th day
	µg g^{-1} fresh weight		
(A) S_3A_3			
control	90.12±0.52	88.92±0.78	86.58±0.93
50	86.25±0.38	85.65±0.67	82.29±1.4
200	83.31±0.26	82.62±0.81	80.19±1.36
300	81.02±0.29	79.93±0.51	75.14±0.86
400	71.92±0.42	68.23±0.86	63.89±0.99
500	65.23±0.36	62.18±0.91	56.12±0.69
600	54.23±0.39	50.19±0.57	42.13±0.86
(B) TS491			
control	94.14±1.08	92.01±1.46	89.03±1.49
50	90.13±1.09	87.32±1.26	84.28±0.99
200	83.93±0.92	80.02±1.21	78.21±1.22
300	73.32±1.03	70.89±1.22	67.12±1.36
400	61.07±0.99	52.87±0.87	49.89±1.32
500	49.27±1.29	36.87±1.27	32.13±1.36
600	36.23±1.28	23.9±0.99	18.09±1.21

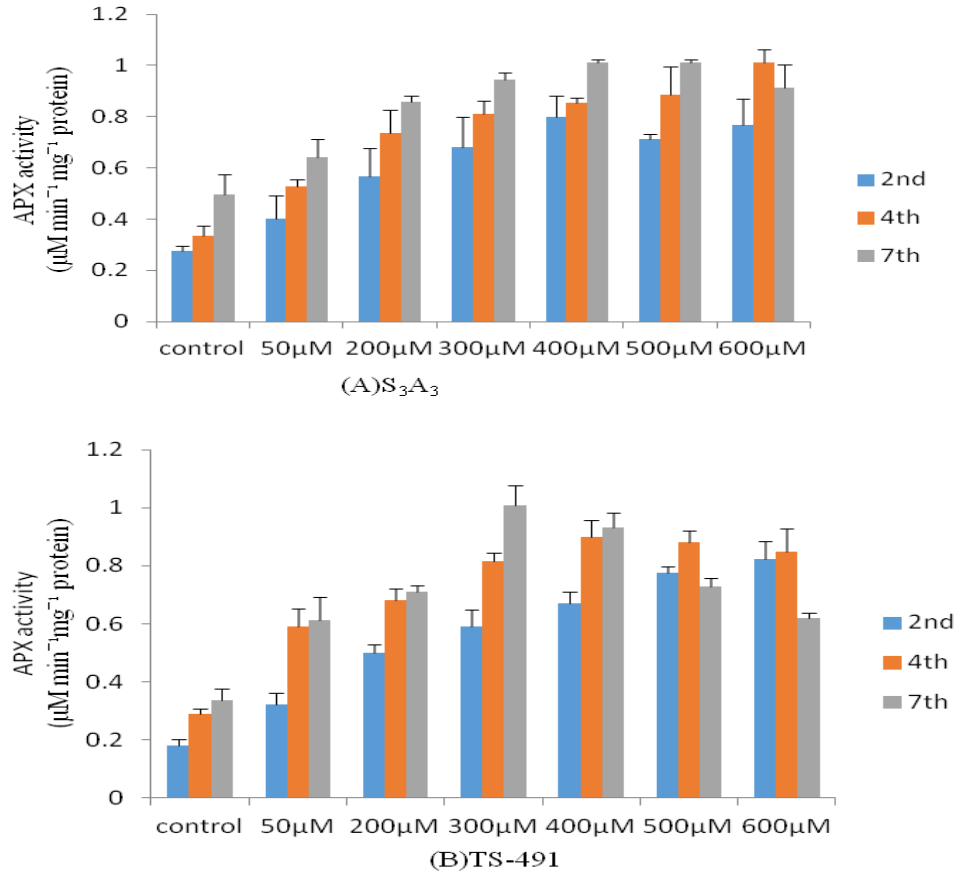

Figure 1. Effect of increasing concentrations of Cu on APX activity in leaves of two cultivars of tea S_3A_3 and TS-491.

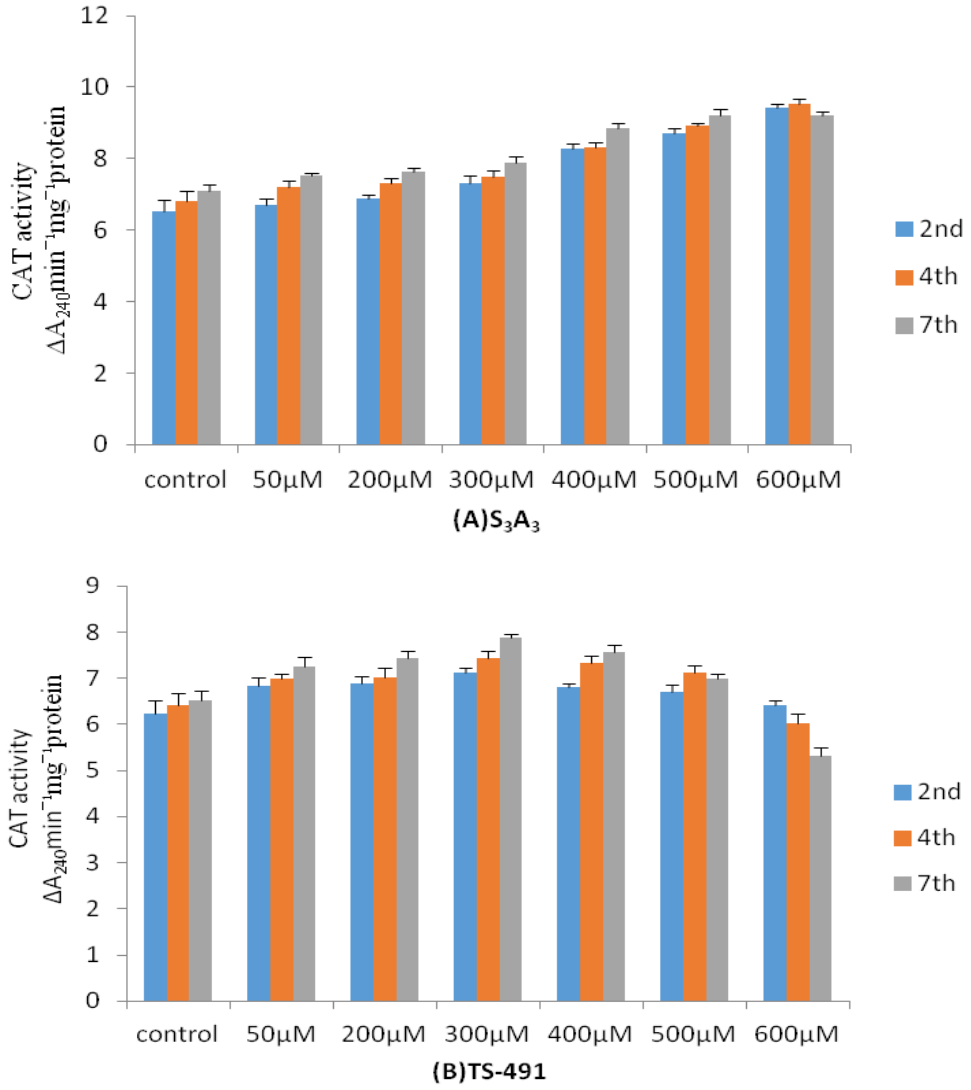

Figure 2. Effect of increasing concentrations of Cu on CAT activity in leaves of two cultivars of tea S_3A_3 and TS-491.

marginal increase in the APX activity with the exposure of Cu up to 200 µM in TS491 cultivar; but further increase in the concentration of Cu resulted in a significant increase (more than two fold) in the activity specially from the 2nd day of 300 µM and continued up to 4th day of 400 µM compared to the lower concentration, finally the APX activity started to decline from 7th day of 500 µM in TS-491. Catalase has double function, as a primary enzymatic mechanism it generally decomposes the toxic H_2O_2 generated during oxygen metabolism by the aerobic organisms and it also catalyses the oxidation of H donors with the consumption of one mole of peroxide (Havir and Mchale, 1987). From Figure 2A and B, it was observed that there was a significant increase in the CAT activity occurs in both the cultivars. S_3A_3 is found to be more tolerant compare to the cultivar TS-491. The increase in the CAT activity of S_3A_3 cultivar continued even at higher concentration of Cu up to 4th day of 600 µM; but, there was slight decrease in the activity on the 7th day of 600 µM. whereas in TS-491 the decrease in the CAT activity was observed from 400 µM itself. The same trend of result has been found in the activity of SOD in both the cultivars. From Figure 3A, it can be observed that in the cultivar S_3A_3 the activity showed a positive correlation with the increasing concentration. There was increase in the SOD activity by one fold with the subsequent increase in the concentration with respect to the exposure days. Whereas in cultivar TS-491, the increase in the activity was up to 400 µM concentration and with further increase in the concentration the activity was

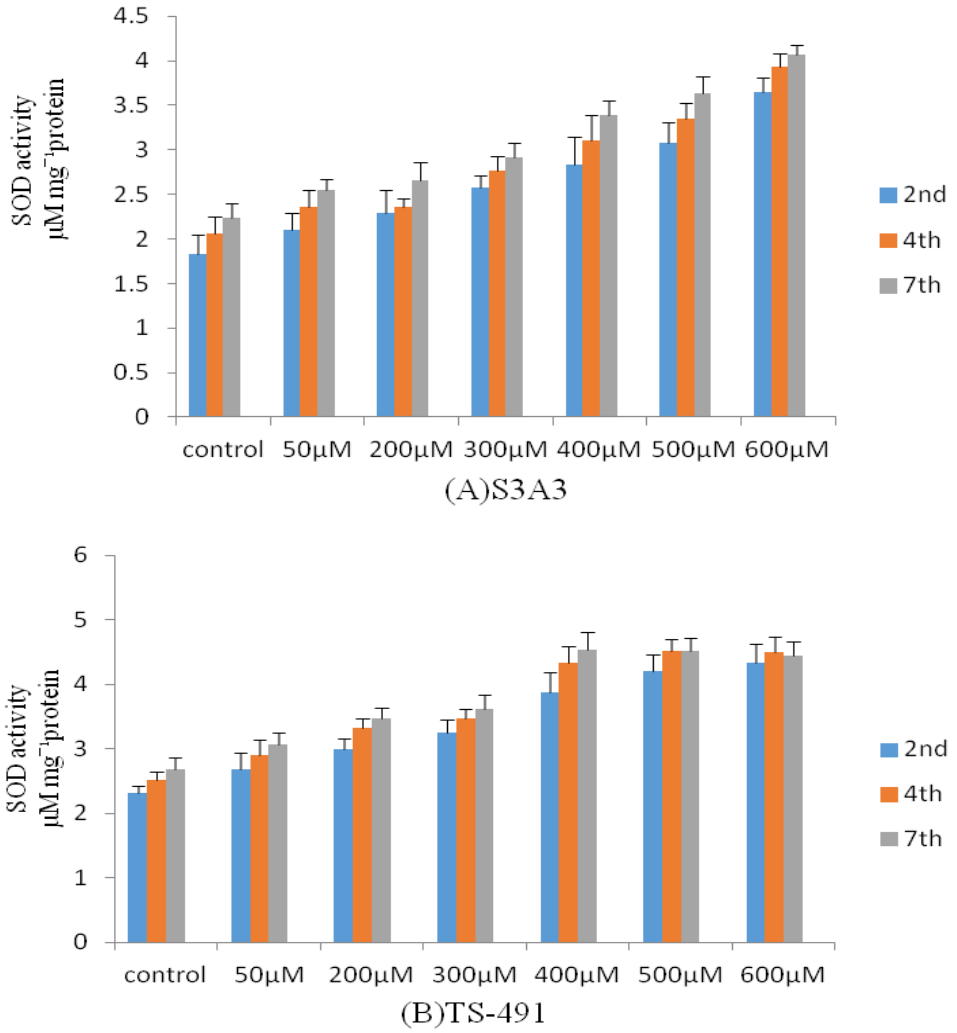

Figure 3. Effect of increasing concentrations of Cu on SOD activity in leaves of two cultivars of tea S_3A_3 and TS-491.

declined (Figure 3B). In cultivar S_3A_3, the POD activity has increased with the exposure of higher concentration of Cu (600 µM) (Figure 4A). In cultivar TS-491, the increase in the activity was more than two fold up to 400 µM concentration of Cu, but the level of increase in the activity lowered at 500µM whereas at 600µM there was total decline in the activity of POD from 7th day onwards. POD plays an important role in plant respiratory metabolism and physiological resistance. From the result, it can be observed that S_3A_3 cultivar is more tolerant to higher concentration of Cu compared to TS-491. The adverse action of biotic and abiotic stress affects the active oxygen metabolism of plants.

Effect of phenolic contents and lipid peroxidation

Phenols are aromatic compounds with hydroxyl groups, which offer resistance to diseases and pests in plants. From Figure 5A and B, it can be observed that the cultivar S_3A_3 has a significant increase in the total phenolic compound up to 500 µM and at 600µM; the level of increasing in the activity has slowed down slightly. On the other hand in TS491, there was increase in phenolic content up to 4th day of 500 µM and then there was a decline in the activity from 7th day of 500 µM. MDA is the final product of lipid peroxidation and its content reflect stress tolerance of plants (Liu et al., 2001; Sugiyama, 1994). It can be observed that with the increase in the concentration of Cu, the MDA content of tea cultivars increased and showed a significant positive correlation with Cu concentration. The level of increase in the peroxidation activity was less in S_3A_3 compared to TS-491 (Figure 6A and B). This shows that high concentration of Cu can lead to lipid peroxidation which

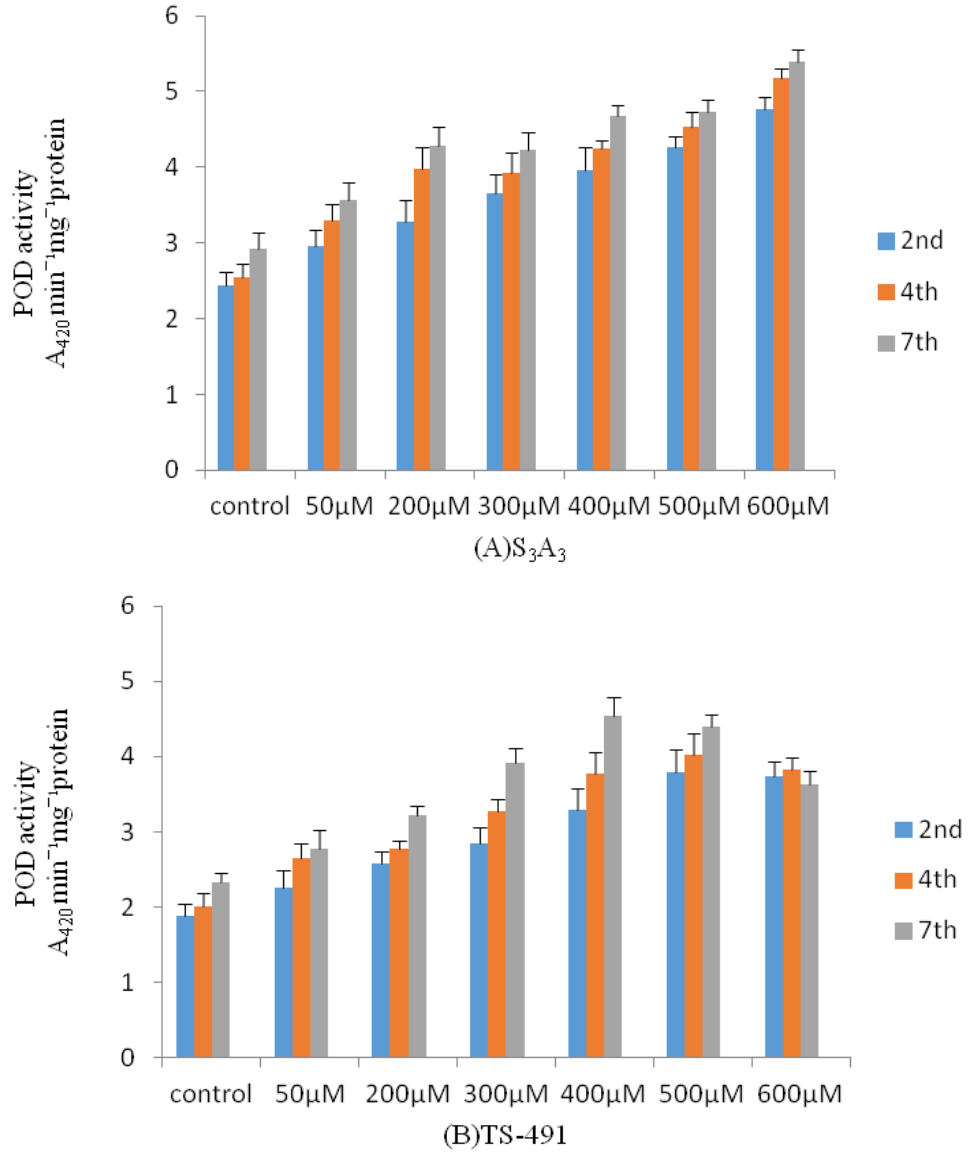

Figure 4. Effect of increasing concentrations of Cu on POD activity in leaves of two cultivars of tea S_3A_3 and TS-491.

causes damage to the balance of ROS scavenging activities of tea plants.

DISCUSSION

Heavy metals play a vital role in the physiological process of plants. In trace amounts, several ions are required for metabolism, growth and development which are present in soil or as in growth media. Number of proteins and enzymes are found to contain heavy metals which make them essential for the growth and development of plants and also helps in maintaining the optimum metabolism; but when the amount exceeds, it creates problem in the plants by leading to cellular damage (Avery, 2001; Schutzendubel and Polle, 2002; Gaetke and Chow, 2003). Though Cu is an essential component for both the photosynthetic and respiratory electron chains, but excess of Cu can cause changes in permeability in membrane, chromatin structure, synthesis of protein, enzyme activity, photosynthesis and respiratory processes through its phytotoxic effect and also can causes lipid peroxidation and lead to activation of senescence (Baryla et al., 2000). Cu in Free State binds irreversibly to SH group which are involved in the catalytic action of enzymes (Van Assche and Clisjters,

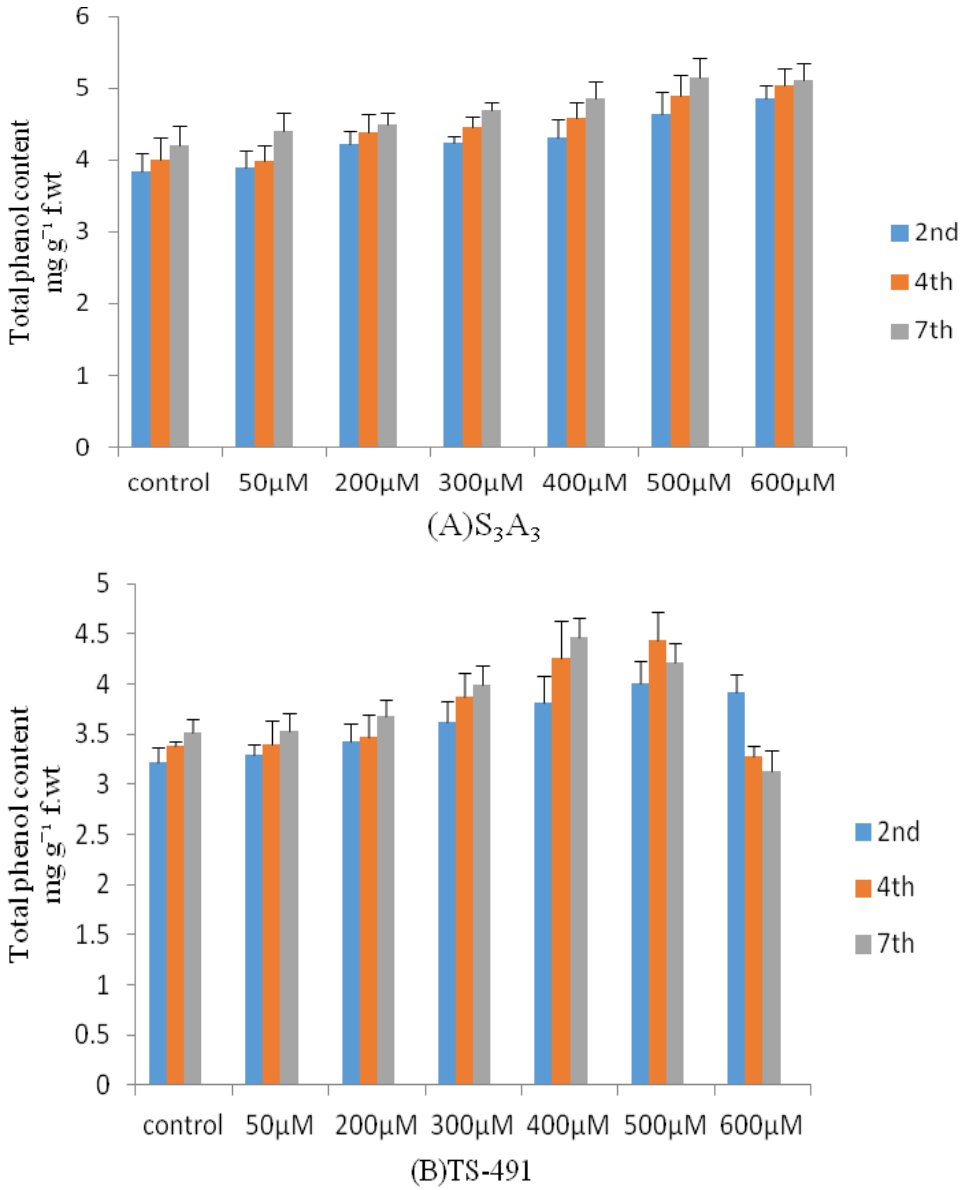

Figure 5. Effect of increasing concentrations of Cu on total phenol content in leaves of two cultivars of tea S_3A_3 and TS-491.

1990). In the present investigation, we observed that the chlorophyll content decrease with increase in the concentration of Cu. Other investigators also reported that with increase in the concentration of heavy metals, the activities of photosynthetic enzymes degrade which results in the reduction of chlorophyll content (Thapar et al., 2008). The content of chlorophyll has a close relation with photosynthesis (Liu et al., 2001), Cu ion is inserted directly to the reaction center especially PSII which due to the high irradiance causes direct damage to the reaction center (Kupper et al., 1996, 1998, 2002). Cu can change the pigment and protein composition of photo-synthetic membranes and interferes with the biosynthesis of photosynthetic activity. Declination in the chlorophyll content is the primary bio indicator of toxicity of Cu (De Vos et al., 1992). The same result of decrease in chlorophyll content was observed in tea plants when exposed to Cr stress (Tang et al., 2011). Many other authors also observed the same trend of decreasing of chlorophyll content with increase in Cu concentration (Rama Devi and Prasad, 1998; Mohanpuria et al., 2007; Saha et al., 2011). The present study indicates that the excess of Cu concentration decrease the chlorophyll content which in turn can cause inhibition to photosystem

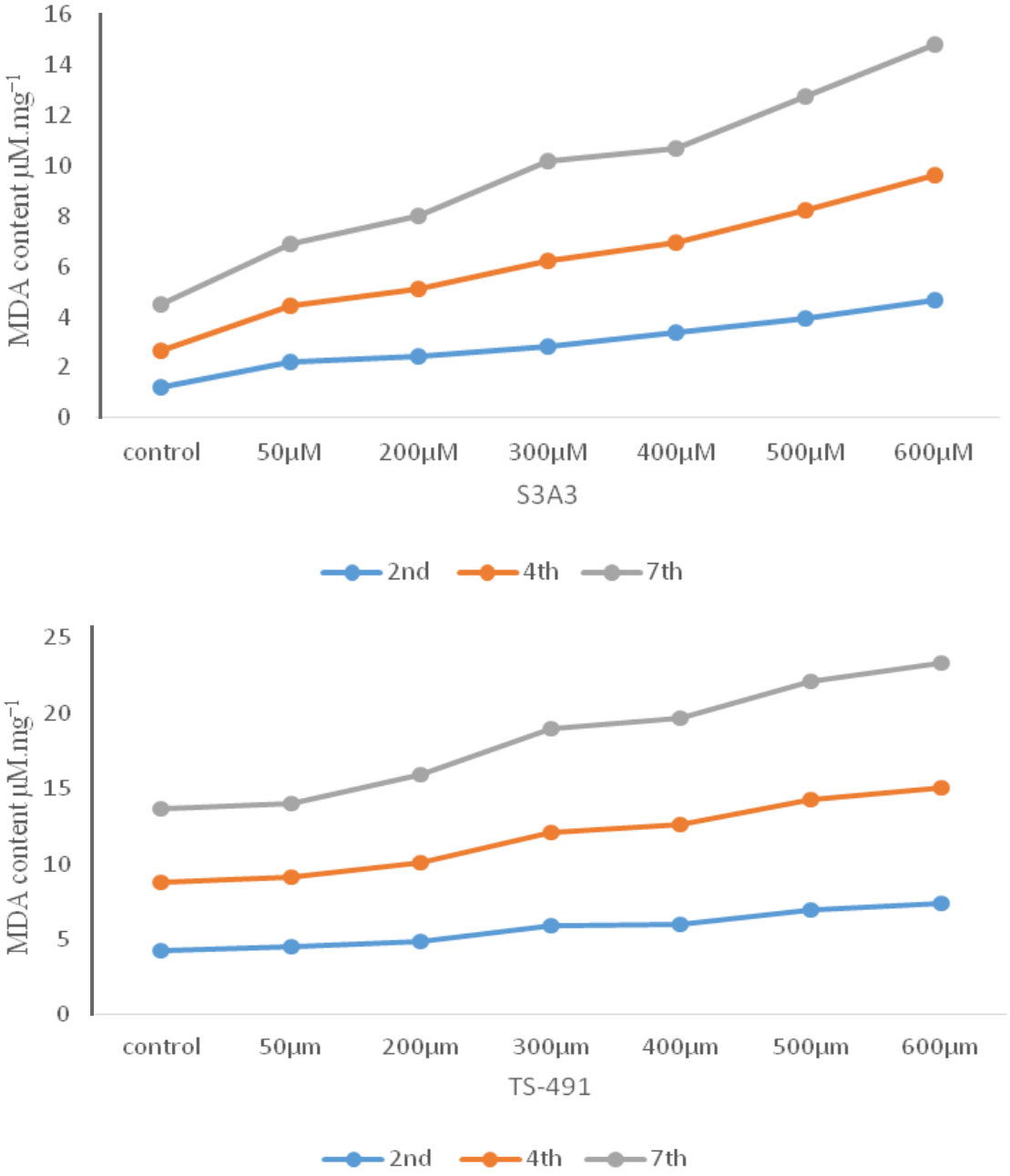

Figure 6. Effect of increasing concentrations of Cu on MDA content in leaves of two cultivars of tea S_3A_3 and TS-491

(Saha et al., 2012).

The different parts of the plants come in contact through the absorption of roots in the solution. In this study, we found that with the increase in the concentration of Cu, the level of it also increased in the different parts of the plants. The roots found to accumulate highest level of Cu as they are in direct contact with the Cu stress present in the nutrient media. The deposition of Cu in organ are in the following order roots> leaves> stems. The accumulating ability of root was stronger than other parts of the tea plants (Tang et al. 2011). In our study both the cultivars showed different symptoms due to different concentration of Cu and in the later phases of the experiment, the growth of the tea plants were inhibited. Tang et al., 2008 reported the same results when they grow tea plant in nutrient solution incorporated with Cu stress. Cu toxicity was found to affect the growth of *Alyssum montanum* (Ouzounidou,

1994), Singh and Tewari, 2003 also observed similar results in *Brassica juncea* when treated with Cu.

Many oxidative stresses also get generated due to the accumulation of Cu in higher concentration and this as a whole cause the inhibition in the photosynthetic reactions which has been observed by many authors (Rocchetta and Kupper, 2009). The decreased concentration of the chloroplastic pigments may be the result of reduced synthesis and/or enhanced oxidative degradation of these pigments by the enhancement of oxidative stress (Romero-Puertas et al., 2002). Due to the emergence of reactive oxygen species, there is occurrence of oxidative stress. The ROS are superoxide radicals, hydroxyl radicals, singlet oxygen and H_2O_2, these damage the cells by degrading the nucleic acids (Pietrini et al., 2003). Increase in the APX activities in tea suggests that the anti-oxidative mechanism induced by Cu was invaded in detoxification of H_2O_2. Many other authors also observed the increase in APX activities due to accumulation of stress. Accumulation of Cu showed increase in APX activity in *Pheseolus vulgaris* (Gupta et al., 1999) and *Camellia sinensis* (Saha et al., 2011).

Copper was inhibitory to CAT but relatively less effective in producing oxidative damage probably due to the fairly enhanced levels of carotenoids, Cu/Zn SOD, POD and glutathione reductase (GR). CAT is also an important enzyme which catalyses H_2O_2 by breaking down it directly to form H_2O and O_2. Initially there was increase in CAT activity in cultivar TS-491 but from 4^{th} day, there was decline in the activity. The increase in SOD and decrease in CAT activity in response to excess supply of heavy metals has been widely reported (Chaoui et al., 1997; Pandey and Sharma, 2002; Cho and Seo, 2005; Lombardi and Sebastiani, 2005). The decrease in CAT activity results in the inactivation of its reaction with superoxide ions which leads to weaken the effective detoxification of H_2O_2 (Kono and Fridovich, 1982). Cho and Seo (2005) reported that oxidative stress in response to Cd toxicity is due to H_2O_2 accumulation. Cu toxicity causes oxidative stress by generating oxygen species ROS which as a result cause lipid peroxidation. The oxidative damage in the heavy metal-stressed tea plants could be due to accumulation of H_2O_2 as a consequence of enhanced activity of total SOD and inhibition of CAT. The significant increase in CAT activity at lower concentration with the Cu exposure has also been reported in tea plant (Saha et al., 2011). However, the decrease or unaffected CAT activity due to Cu was reported in oat leaf (Luna et al., 1994), tomato seedlings (Mazhoudi et al., 1997); whereas, significant increase in CAT activity was also reported in *Prunus cerasifera* plantlets due to Cu stress (Lombardi and Sebastiani, 2005). Tang et al., 2011, also observed reduced CAT activity in tea plants due to increase in the Cr concentration.

In the present investigation, we observed increase in SOD activity in both the cultivars with the increase in the concentration of Cu. SOD is one of the stress resistant enzymes which can catalyze O_2^- radicals to H_2O_2 and O_2. The decrease or increase in the level of these metals causes adverse effect in the activity. The same result has been observed by other authors (Saha et al., 2011; Wang et al., 2004; Hartley-Whitaker et al., 2001). SOD is an important antioxidant enzyme that catalyzes a disproportionate amount of superoxide anion (O^{2-}) to hydrogen peroxide (H_2O_2) (Bowler et al., 1992). Excess supply of heavy metals led to increased accumulation of ascorbate, which is associated with increased activities of APX and GR. The heavy metal-induced oxidative stress also triggers the ascorbate-glutathione cycle for detoxification of hydrogen peroxide which could be a common strategy for counteracting the over-production of the ROS (Foyer and NoCtor, 2005).

Peroxidase (POD) includes a group of specific enzymes such as NAD-peroxidase, NADP-peroxidase, fatty acid peroxidase etc. POD catalyses the dehydrogenation of a large number of organic compounds such as phenols, aromatic amines, hydro-quinone etc. POD catalyses H_2O_2 dependent oxidation of substrates which takes part in improving the mechanical protection in plants (Dong et al., 2006). The present study observes the increase in the POD activity in both the cultivars. In TS-491, increase in the activity was only up to 500 µM concentration whereas in S_3A_3 the increase was up to 600 µM. The elevation in the activity of POD shows that it catalyses the H_2O_2 into H_2O and prevent the accumulation of H_2O_2 and O_2; which may be due to ionic micro environment or tissue specific gene expression in plants. The increase in POD helps in reduction of harmful affect caused by the free redicals to structure and function of the membrane (Sun et al., 2006; Pauls and Thompson 1984; Vetanovetz and Peterson, 1990). Saha et al., 2011 also reported the increase in the activity of POD with the increase in the Cu concentration up to 400 µM.

The present study shows the resemblance with the result of Cu stress on tea plant where they showed significant increase in the phenol content below 400 µM concentration (Saha et al., 2011). Phenolic compounds act as reducing agents, hydrogen donor and singlet oxygen quenchers which as a whole make it as an important antioxidant (Rice-Evans et al., 1997). The significant increase in the activity shows that the compound helps in the binding of the heavy metal Cu. The hydroxyls groups of phenol help in chelation by binding heavy metal (Jung et al., 2003). It was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen et al., 1993; Gülçin, 2005).

When the plants are grown under some stress involved in the environment, then excess of free radicals accumulate in the cells which results in the lipid peroxidation

consequently resulting to increase in the malon-dialdehyde (MDA) production as last product of this activity (Chaoui et al., 1997). Increased in MDA content is an indicator of physiological stress and aging (Quariti et al., 1997). Many authors also reported that exposure of heavy metals causes increase in MDA levels (Asada 1992; Gille and Singler 1995; Ozounidou 1994). In our study, we observed the increase in MDA content in both the cultivars with the increase in Cu concentration which shows similarity with the findings of Cu stress with other authors (Saha et al., 2011, Rama Devi and Prasad, 1998; Mohanpuria et al., 2007). The difference among the cultivars in response to Cu has also been reported by many authors (Ciscato et al., 1997)

Conclusion

In conclusion, our data demonstrates that among the two cultivars, TS-491 was more sensitive to the higher concentration of Cu compared to S_3A_3. The higher concentration beyond 400 µM of Cu became toxic to the tea plant. It leads to the decline in the growth by retarding many biological functions. The accumulation of Cu was higher in roots leading to the production of ROS, which are followed by lipid peroxidation and many more antioxidant enzymes. The cooperation and the interaction between the antioxidant enzymes detoxify the ROS.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Asada K (1992). Ascorbate peroxidase - a hydrogen peroxide scavenging enzyme in plants. Physiol. Plant. 85:235-241.

Avery SV (2001). Metal toxicity in yeasts and role of oxidative stress, Adv. Appl. Microbiol. 49:111-142.

Bowler C, Van Montagu M, Inze D (1992). Superoxide dismutase and stress tolerance, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:83-116.

Chaoui A, Mazhoudi S, Ghorbal M.H, Ferjani E (1997). Cadmium and zinc induction of lipid peroxidation and effect on antioxidant enzyme activities in bean (*Phaseolus vulgaris L.*). Plant Sci. 127:139-147.

Cho UH, Seo NH (2005). Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation, Plant Sci. 168:113-120.

Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Vaan Breusegem F (2000). Dual action of the active oxygen species during plant stress responses. Cell. Mol. Life Sci. 57:779-795.

Dong, Wu V, Zhang GP (2006). Influence of Cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*), Chemosphere. 64:1659-1666.

Foyer CH, Noctor G (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses, Plant Cell. 17:1866-1875.

Gaetke LM, Chow CK (2003). Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicol. 189:147-163.

Gallagher DL, Johnston KM, Dietrich AM (2001). Fate and transport of copper-based crop protectants in plasticulture runoff and the impact of sedimentation as a best management practice. Water Res. 35:2984-2994.

Gille G, singler K (1995). Oxidative stress in living cells. Folia Microbiologica 40:131-152.

Havir EA, Mchale NA (1987). Biochemical and development characterization of multiple forms of catalase in tobacco leaves, Plant Physiol. 84:450-455.

Hegedus A, Erdei S, Horvath G (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress, Plant Sci. 160:1085-1093. Hoagland DR, Arnon DI (1938). The water culture method for growing plants without soil, Circ. Calif. Agr. Exp. Sta. 52:347-461.

Krivosheeva A, Tao DL, Ottander C, Wingsle G, Dube SL, Oquist G (1996). Cold acclimation and photoinhibition of photosynthesis in Scots pine, Planta. 200:296-305.

Kupper H, Ku¨pper F, Spiller M (1998). In situ detection of heavy metal substituted chlorophylls in water plants, Photosynth. Res. 58:125-133.

Liu DY, Xie JC, Yang SY, Shen H (2001). Effects of Copper mine tallings on growth and development and physiological function of wheat, Chinese J. Appl. Ecol. 12:126-128.

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

Luna CM, Gonzalezca, Trippivs (1994). Oxidative damage caused by an excess of copper in oat leaves, Plant Cell Physiol. 35:11-15.

Mazhoudi, Chaouia, Ghorbalmh, Elferjanie (1997). Response of antioxidant enzymes to excess copper intomato (Lycopersicon esculentumMill). Plant Sci. 127:129-137.

Mocquot B, Vangronsvel DJ, Clijsters H (1996). Mench M Copper toxicity in young maize (Zea maysL.) plants: Effects on growth, mineral and chlorophyll contents, and enzyme activities, Plant and Soil. 82:287-300.

Mohanpuria P, Rana NK, Yadav SK (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of Camellia sinensis (L.) 0. Kuntze, Environ. Toxicol. 22:368-374.

Ozounidou G (1994). Root growth and pigment composition in relationship to element uptake in Silene compacta plants treated with copper, J. Plant Nutr. 17:933-943.

Rama Devi S, Prasad MNV (1998). Copper toxicity in Ceratophyllum demersum L. (coontail), a free floating macrophyte: Response of antioxidant enzymes and antioxidants, Plant Sci. 138:157-165.

Rice-Evans C, Miller N, Paganga G (1997). Antioxidant properties of phenolic compounds, Trends Plant Sci. 2:152-159.

Rocchetta I, Kupper H (2009) Chromium and copper induced inhibition of photosynthesis in Euglena Gracilis analysed on single cell level by fluorescence kinetic microscopy, New Phytol. 182:405-420.

Thapa R, Srivastava AK, Bhargava P, Mishra Y, Rai LC (2008). Impact of different abiotic stress on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated Anabaena doliolum, J. Plant Physiol. 165: 306-316.

Van Assche F, Clijsters H (1990). Effect of metals on enzyme activity in plants. Plant Cell and Environment 13:195-206.

Wang RZ (2004). Plant functional types and their ecological responses to salinization in saline grasslands, Northeastern china. Photosynthetica. 42:511-519.