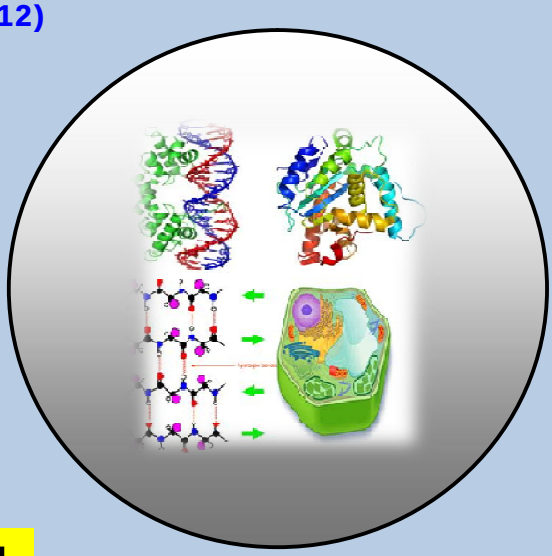
Cadmium induced toxicity on growth, nutrient uptake, enzymes activity and membrane function and its amelioration with phosphorus in young wheat plants (*Triticum aestivum* L.)

By

Ram Kumar, Rohit Kumar, Rajeew Singh and Y.K. Sharma

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)
Scientific Journals Impact Factor: 2.597



J. Biol. Chem. Research
Volume 31 (2) 2014 Pages No. 694-707

Journal of Biological and Chemical Research
(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 2: 694-707 (2014)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Prof. Y.K. Sharma Ram Kumar
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

Received: 01/05/2014 Revised: 31/05/2014 Accepted: 02/06/2014

Cadmium induced toxicity on growth, nutrient uptake, enzymes activity and membrane function and its amelioration with phosphorus in young wheat plants (*Triticum aestivum* L.)

Ram Kumar, Rohit Kumar, Rajeew Singh and Y.K. Sharma
Department of Botany, University of Lucknow, Lucknow- 226007, U.P. India

ABSTRACT

The effects of Cadmium (Cd) and their interaction with P (Phosphorus) on plants growth, antioxidative components and accumulation of Cd, Fe and Zn were studied in winter wheat (Triticum aestivum L.) seedlings. Reduced biomass and chlorophyll contents under the influence of Cd, were observed which was increased on P application. A close relationship existed between lipid peroxidation and tissue metal concentration. The activities of CAT, POX, SOD, APX and GR were increased both in root and shoot at lower dose of Cd but reduced at higher dose while P application enhanced the activity of these enzymes even at higher dose. As compared to control, the content of NP-SH, proline and cysteine were observed to be increased in Cd treated seedlings which was also comparatively higher in Cd+P exposed seedlings. Application of P, reduced the uptake of Cd but enhanced the accumulation of Fe and Zn both in root and shoot tissue. Since Cd is a non-redox element so can't directly produce reactive oxygen species. From this study it was suggested that Cd and Cd+P, promoted enhanced accumulation of Fe (redox metal) which participate in generation of ROS and membrane damage.

Key word: Wheat, Cadmium, Non-protein thiol, Catalase and Superoxide dismutase.

INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metal entered to soil, air and water mainly from industries, mining, burning and leakage of waste and by fertilization with phosphate and sewage sludge. In unpolluted soil Cd is present at concentration of 0.1-0.5 mg kg^{-1}, but in Great Britain, in heavily polluted soils of seawage sludge, concentrations of Cd up to 150 mg kg^{-1} have been identified (Jackson and Alloway, 1991). Long-term consumption of foods containing excess levels of Cd may lead to chronic toxicity and adverse health effects, including kidney tubule dysfunction and reduced bone density, renal and lung cancer (Sorahan and Lancashire, 1997 and Godt *et al*. 2006). Although Cd is a non-essential element for plants, at physiological level, it leads to growth retardation, root damage, chlorosis of leaves and red brown coloration of leaf margins' strongly interferes with plants metabolism. Chlorosis appears may be due to phosphorus deficiency or reduced Mn transport (Godbold and Hutterman, 1985). But mechanism of ROS generation still unknown. Here we have tried to understand how Cd generates reactive oxygen species. Reports also indicated that Cd induces oxidative stress in plant tissues, since increases in lipid peroxidation, protein oxidation and nucleic acid damage are common events occurring in the plants exposed to Cd (Gallego *et al*. 1996 and Romero-Puertas *et al*. 2002). Previous studies have shown that NP-SH and cysteine are involved in the synthesis of a numbers of polypeptide which is involved in the metal detoxification by binding and compartmentalizing them in vacuoles (Cobbett, 2000). The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Hare *et al*. 1999; Kavi Kishor *et al*. 2005; Ashraf and Foolad, 2007; and Sun *et al*. 2007). Unlike Cu and Fe, Cd is a non-redox metal and does not produce reactive oxygen species (ROS) such as the superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). It induces oxidative stress by suppressing the antioxidative defenses (Benavides *et al*. 2005 and Gratao *et al*. 2005). However autoxidation of redox active metal such as Fe^{2+} and Cu^+ results in ROS generation (Halliwell and Gutteridge 1987 and Imlay *et al*. 1988). Many enzymes contain metals in positions important for their activity. The displacement of one metal by another will normally lead to inhibition of enzymes activities. Zn^{2+} and Cd^{2+} were found to displace Mg^{2+} and Ca^{2+} in ribulose1, 5-biphosphate- corboxylase/ oxygenase and calmodulin and inactivating them (Van Assche and Clijsters, 1986). So enhance uptake of Zn^{2+} and Fe^{2+} under Cd and P environment plays a supportive role in toxicity. Several studies shown that either Cd alone or in combination with P increase the uptake of Zn^{2+} and Fe^{2+} which drives the formation of OH^- that lead to oxidative damage. P is a macro-nutrient for plants and indirectly affects Cd accumulation. Application of P may attenuate the phytoavailability of Cd in culture media by P-induced Cd^{2+} adsorption or formation of less soluble metal-phosphate minerals or precipitates. Application of P increases the ionic strength of the culture media lead to decrease in Cd bioavailability for plants. Inhibitory action of P to Cd accumulation benefited by Zn. The Cd and Zn compete for same binding sites in soil, Zn can desorbs Cd from the cation exchange, leading to increased Cd concentrations in soil solutions (Christensen, 1984 and François *et al*. 2009). This work was undertaken to examine Cd tolerance in wheat plants and also determine the ability of P to reduce Cd toxicity in wheat plants.

MATERIAL AND METHODS

The seeds of wheat were surface sterilized with 0.01% $HgCl_2$ for 5 min and rinsed extensively in distilled water (d H_2O). Healthy and equal-sized seeds were chosen and soaked in d H_2O for 24hr and germinated in dark at (25^0C). Equal amount of germinated seeds were transferred to separate petridishes (internally lined with filter paper) saturated with Hewitt nutrient solution. After six days five concentration of Cd (0, 0.1, 0.2, 0.4, 1.0 mM) were added to each petridish, while two Cd level i.e. 0.4 and 1.0 mM remediate with 40 ppm phosphorus (P) in separate petri-dishes. Cd was provided as cadmium sulphate ($3CdSO_4.7H_2O$). The test solutions were changed every day. Experiment was conducted in triplicate. After 15 days of incubation plants were harvested and roots of plants were rinsed with distilled H_2O to remove traces of nutrients and Cd from the root surface. Roots and shoots of harvested samples were then used to analyze growth parameter as well as bio-chemical parameter. For the estimation of the metals (Cd, Fe and Zn) plant samples were dried for 48hr at 60^oC in oven. The contents of metals (Cd, Fe and Zn) were determined with double atomic absorption spectrophotometer (AAS Element 4141).

Estimation of metal accumulation by plant

Irrespective of only Cd, the *Triticum aestivum* has been studied for the accumulation of Fe and Zn also. Plant tissues were wet digested with HNO_3:$HClO_4$ (3:1, v/v) by using hot plate. The contents of metals (Cd, Fe and Zn) were determined with double atomic absorption spectrophotometer number AAS Element 4141.

Estimation of photosynthetic pigments

Chlorophyll was estimated by the method of Arnon (1949). Leaves were plucked and washed with distilled water and blotted. 100 mg leaves were taken and ground in 10 ml chilled acetone (85% v/v). Extract was centrifuged at 2000 rpm for 10 minutes. The absorbance of supernatant was read at 663, 645, 510 and 480 nm for chl a, chl b, chl T and carotenoids respectively using the double beam UV-VIS spectrophotometer UV5704SS. The content was expressed in mg g^{-1} fresh weight tissue.

Lipid Peroxidation

The level of lipid peroxidation in plant tissue was measured as thiobarbituric acid reaction by the method of Heath and Packer (1971) in terms of malondialdehyde content. Fresh leaf tissue was homogenized in 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3,500 rpm for 20 min. To a suitable aliquot, 1ml of 20% TCA containing 0.5 %(w/v) TBA as added. The mixture was heated at 95^0C for 30 min and then quickly cooled in ice bath. The contents were centrifuged at 10,000 X g for 15 min and the absorbance was measured at 532 nm using double beam UV-VIS spectrophotometer UV5704SS. Value for non – specific absorbance at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155 Mm^{-1} cm^{-1} and expressed as nmol MDA content g^{-1} FW tissue.

Catalase

Catalase activity was assayed by the modified method of Bisht (1972).The reaction mixture for CAT containing 0.01 mM phosphate buffer (pH 7.0) and 0.5mM H_2O_2 in 10 ml was incubated with suitable aliquot from the extract.

The reaction was run for 5 minutes at room temperature (25^{o}C) and was stopped by the addition of 5ml 2N H_2SO_4, Corresponding zero hour blanks with added H_2SO_4 was also run. The mixture was titrated against 0.1 N $KMnO_4$ and the activity of CAT were expressed as µmol H_2O_2 decomposed / 100 mg fresh weight tissue.

Peroxidase

The peroxidase activity was determined by the method of Luck (1963). The assay system for POX contained 0.5 mM phosphate buffer (pH 6.0), 0.01% (v/v) H_2O_2, 5 mg p-phenylenediamine and extract in 8 ml. The reaction was run at 25^{0}C for 5 minute and stopped with 2ml 5N H_2SO_4. Blanks with added H_2SO_4 were also taken. After centrifugation, OD was measured at 485 nm on double beam UV-VIS spectrophotometer UV5704SS. The enzymes activity was measured as change in optical density per 100 mg fw.

Superoxide Dismutase

The activity of SOD was assayed by the method of Beauchamp and (Fridovich 1971) by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazoliun (NBT). The reduction in NBT was followed by reading the absorbance at 560nm using double beam UV-VIS spectrophotometer UV5704SS. Blanks were run in the same way but without illumination.

Glutathione Reductase

The GR activity was assayed by the method of smith *et al*. (1988). The reaction mixture contained 1. Ml of 0.2 M potassium phosphate buffer (pH 7.5) containing 1mM EDTA, 0.5 ml 3 mM 5, 5'-dithiobis (2-nitrobenzoic acid) in 0.01M phosphate buffer (pH 7.5), 0.25 ml H_2O, 0.1 ml 2 mM NADPH, 0.05 ml enzyme extract and 0.1 ml 20 mM GSSG. The increase in absorbance was monitored for 5 minute at 412 nm. The rate of enzymes activity was calculated using standard curve prepared by known amount of GR (Sigma, USA). Activity of enzyme was expressed as µmoles of GSSG reduced min^{-1} g^{-1} fresh weight.

Ascorbate Peroxidase

The activity of APX was measured according to the Nakano and Asada (1981) by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM^{-1} cm^{-1}). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm and enzymes activity was expressed as µmol of ascorbate oxidized min^{-1} g^{-1}.

Non-Protein Thiol

Non protein thiol group was estimated by the method of Ellman (1959). Leaves were plucked and washed with distilled water. 700mg plant tissue grinds in 6.67% sulphosalicylic acid. Extract was centrifuged at 13,000 rpm for 10 minute at 4^{o}C. Reaction mixture contains 5mM of EDTA, 0.6 mM of DTNB and 120mM of phosphate buffer. The absorbance of supernatant was read at 412nm using double beam UV-VIS spectrophotometer UV5704SS and NP-SH contents expressed as mmol g^{-1} FW tissue.

Proline

The extraction and determination of proline was performed according to the method of (Bates et al. 1973).

Plant tissue was homogenized in 3% sulphosalicylic acid. Extracts(2ml) were held for 1h in boiling water by adding 2 ml ninhydrin regent and 2 ml glacial acetic acid, after which cold toluene (4ml) was added. For the absorbance of supernatant was read at 520nm using double beam UV-VIS spectrophotometer UV5704SS and value calculated as μmol g^{-1} FW against standard proline.

Cysteine

Cysteine content in control and metal exposed plants was estimated following the method of (Gaitonde, 1967). Reaction mixture (3ml) contained one ml each of supernatant, glacial acetic acid and acid nin-hydrin reagent. Mixture was heated for 15min at 95° C, and then cooled rapidly to room temperature and absorbance was recorded at 560 nm. Cysteine content was calculated from the standard curve prepared using known concentration of cysteine (L-cysteine hydrochloride, sigma) and is expressed as nmol g^{-1} fw.

Statistical Analysis

The experimental data were tested for significance by using least significant difference (LSD) to compare means of different treatments that have an equal number of replications. All statistical test were performed with analysis tools from Microsoft office excel 2007.

RESULTS AND DISCUSSION

In this experiment, I had studied the effect of Cd and its amelioration with P on imbalance absorption of some micronutrients (Fe and Zn) that led to disturb in growth of wheat seedlings. Results showed that Cd exposure reduced FW, DW and length of shoot and root in dose dependent manner. The inhibition of growth is commonly observed event (Stolt *et al.* 2003; Sharma *et al*. 2004 and Ranieri *et al*. 2005). It has been suggested that this growth reduction is due to the competition for the same uptake system between Cd and other ions required for plants development (Alcantara *et al*. 1994; Lozano- Rodriguez *et al*. 1997 and Rivetta *et al*. 1997). Shoot length and root length decreased by 42.8 and 80.9% when exposed to 1.0mM Cd. However, FW and DW of shoot decreased up to 36 and 61% and that of root 74 and 64.5% respectively. This could be due to direct contact of cadmium to root with toxic metals. The differential effect of Cd on root and shoot growth could be accounted by the fact that Cd is accumulated mainly in roots and to a minor extent in shoot (Fernandes and Henriques, 1991). Similar results were observed in respect to root and shoot length and their FW and DW on other plants species and were supported by (Parviz Malekzadeh *et al*. 2007; Surjendu, *et al*. 2007). The content of Chl T, and carotenoids in the Cd treated plants were decreased significantly in leaves of *T. aestivum.* The Chl T and Chl a, significantly ($P<0.01$) decreased by 38.9, 65.9% and 37.0, 66% at 0.4 and 1.0 mM Cd respectively. Padmaja *et al*. (1990) had speculated that addition of Cd reduced aminolevulinic acid dehydratase (ALAD) activity whereas Kupper *et al*. (1998) reported that Mg^{2+} which is the central ion in the chlorophyll is replaced by the Cd. It had also reported that the loss of chlorophyll was due to distortion of the chlorophyll ultrastructure, inhibition of the synthesis of photosynthetic pigments and enzymes of the Calvin cycle, or reduction in the chloroplast density and size which led to the damage of the photosynthetic cycle (Baryla *et al*. 2001 and Benavides *et al*. 2005).

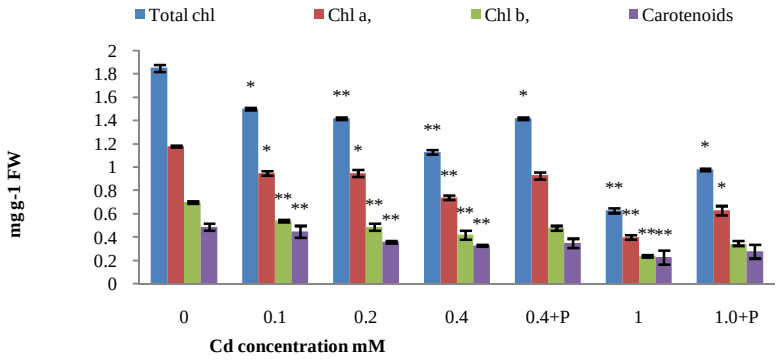


Figure 1. The effect of Cd and its amelioration with P (40ppm) on pigment contents of wheat (*Triticum aestivum* L.) seedlings observed at 15 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 level.

In this study results shown that the application of P enhanced pigment synthesis, lead to photosynthetic growth and yield. We speculated that the application of P may promote plant growth and increase the yields so that the concentration of Cd in the plants was perhaps diluted with respect to the control plants, and the toxicity was reduced. The external P may form phosphate deposits with Cd and decrease bioavailability of Cd for plants. The accumulation of Cd in shoot increased with increase in Cd concentrations ($P < 0.01$), reached a maximum of 94.6 and 97.4 µg g^{-1} DW at 0.4 and 1.0mM Cd treatment respectively while in root Cd value reached to 121.4 and 126.2 µg g^{-1} DW at 0.4 and 1.0mM Cd treatment respectively. Concentration of Cd in shoots (35.73 µg g^{-1}) was lower than in roots (113.5 µg g–1), indicating that wheat seedlings had relatively low transportation rate of Cd from root to shoot (Figure 4). On interaction with P at 0.4 and 1.0mM Cd treatment, accumulation of Cd in shoot significantly ($P<0.05$) decreased by 29.6 and 26.3% respectively while decrease in root Cd concentration was 2.3 and 1.8% respectively. Application of P at 0.4 and 1.0 mM Cd level reduced Cd absorption by 14.4 and 13.0%. This reduced absorption of total Cd could be due to less availability of free Cd in culture medium because some of the Cd gets. In this experiment it was noticed that Cd absorbed and accumulated more efficiently in roots and their translocation inhibited toward shoot in presence of P. At lower level of Cd (0.1 and 0.2mM) iron accumulation enhanced in shoot by 63.0 and 10.0% respectively however there was no significant difference in total iron at that level. Lower level of Cd enhanced translocation of iron from root to shoot. Also translocation of Zn from root to shoot increased at lower Cd levels while total Zn accumulation in root and shoot was not significantly affected at 0.1, 0.2 and 0.4mM Cd treatment. However P supplementation significantly ($P<0.05$) enhanced the Zn accumulation in root by 50.0% at 0.4mM Cd treatment. In the present study, we also investigated the response of root and shoot of wheat seedlings to Cd-induced oxidative damage. Plant cell membranes are generally considered to be primary sites of metal injury (Smeets *et al*. 2005). In this study, results showed that fatty acid of shoot plasma membrane were more prone to oxidation than root plasma membrane and application of P have no mitigatory effect on membrane damage while it declined the uptake of Cd up to 29.3%.

Table 1. The effect of different concentration of Cd and its amelioration with P (40ppm) on length, FW, DW and moisture % of shoot and root of wheat (*Triticum aestivum* L.) seedlings observed at 15 days.

Treatment Cd (mM) Parameter	0.0	0.1	0.2	0.4	0.4+P	1.0	1.0+P
Shoot length (cm)	14.0±0.29	11.0±0.29**	10.8±0.44**	9.0±0.29**	13.8±0.22**	8.0±0.17**	10.0±0.29**
Shoot FW (mg plant^{-1})	94±2.3	84±2.3	65±2.9**	61±0.9**	87±1.7**	60±2.9**	65±1.4
Shoot DW(mg plant^{-1})	14.0±0.6	7.8±0.1**	6.3±0.2**	5.6±0.1**	10±1.1**	5.4±0.2**	8.4±0.02*
Shoot moisture %	85±1.18	91±1.15**	90±0.58**	91±1.01**	89±1.18*	91±0.88**	87±1.15*
Root length (cm)	11.5±0.29	5.0±0.3**	4.6±0.2**	3.0±0.1**	5.7±0.2*	2.2±0.1**	4.2±0.1*
Root FW (mg plant^{-1})	67±01.5	20±1.2**	20±0.9**	18±0.7**	27±1.7*	17±0.6**	18±0.6
Root DW (mg plant^{-1})	4.5±0.29	2.0±0.3**	1.7±0.1**	1.7±0.1**	2.3±0.1	1.6±0.1**	1.7±0.2
Root moisture %	93.3±0.57	90.0±1.2**	91.0±1.2**	91.5±0.9**	92.0±1.2	91.2±0.7**	90.6±0.9

± represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels. Amelioration with 40ppm P was given at 0.4 and 1.0mM Cd levels.

This suggests that membrane damage is due to enhanced accumulation of iron. The aplication of P enhanced the Fe accumulation both in root and shoot tissue which is a redox active metal ion (Weckx and Clijsters, 1996), initiated the synthesis of iron-containing enzyme lipoxygenase (Thompson *et al*., 1987) which led to membrane destabilization. However the P application reduced the Cd uptake. This result shows that Cd is not directly participating in the ROS production. The enhanced MDA accumulation could be due to higher accumulation of Fe that initiated the production of reactive oxygen species. Results showed that proline accumulation gradually increased both in root and shoot tissue at all tested Cd levels. Proline accumulation significantly (P<0.01) increased up to 249% in shoot and 220% in root at 1.0mM Cd treatment. P application reduced the proline content both in root and shoot. This suggests proline accumulation directly related to entrance of Cd in the plants. This result suggested that proline synthesis directly correlated with metal accumulation and plant tolerance. The enhancement of proline level can be used as an indicator of the species tolerance to cadmium toxicity (Wu *et al.* 1995). Proline has been reported to play important roles in osmoregulation, protecting enzymes denaturization, acting as a source of carbon and nitrogen, stabilizing the machinery of protein synthesis, regulating the cytosolic acidity and/ or scavenging hydroxyl radical (Saradhi and Saradhi, 1991).

Proline accumulation could probably also be related to a decrease in electron transport in plants under stress conditions (Venekamp, 1989). As a result there would be an accumulation of NADH and H^+. An increase in NADH might even affect substrate level phosphorylation besides inhibiting important metabolic reactions that need NAD^+. In the present study, proline content increase in root and leaves could be due to its role against metal-induced oxidative stress. This could be supported by the results obtained by P application. Application of P reduced metals concentration, as the metal accumulation reduced the proline synthesis also declined in same proportion. The present observation revealed that the increased level of cysteine mitigates cadmium toxicity. Cysteine involved in the synthesis of heavy metal-binding peptides which involved in buffering cytosolic metal concentration. The accumulation of cystiene in the shoots and roots significantly ($p<0.01$) increased with the increase in Cd concentrations. In the present study, NP-SH content showed an increasing trend in root and leaves of wheat seedlings following Cd exposure. The results showed conformity to the finding of Mishra *et al*. (2006) in Bacopa monnieri. The antioxidant property of NP-SH depends on the oxidation of -SH group of the tripeptide to disulfide form (Noctor and Foyer, 1998; Sanita di Toppi and Gabbrielli, 1999). The plant exhibited high amounts of NP-SH synthesis during Cd exposure, indicates its ability to tolerate cellular metal load. In this study it was observed that NP-SH synthesis was directly proportional to metal accumulation. As the Cd concentration in root tissues was higher than shoot, similarly it was observed that NP-SH synthesis was higher in root than shoot. Inspite, application of P inhibit Cd uptake which results decrease in Cd accumulation in shoot and root tissue, NP-SH synthesis also declined. It is not well known how P influenced NP-SH synthesis. It is believed that NP-SH synthesis is directly proportional to metal concentration and when the P application reduces Cd concentration, NP-SH synthesis also declines. In this study, a significant increase in SOD activity was observed both in root and shoot up to 0.2 mM Cd, then decreased at higher dose of Cd.

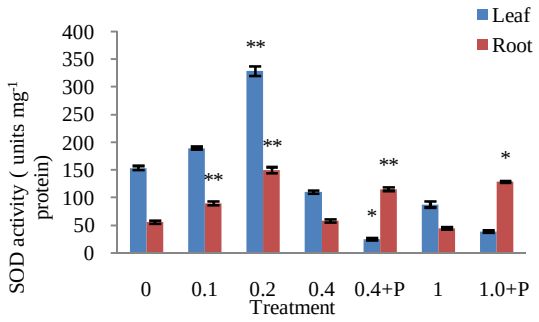


Figure 2. The effect of Cd and its amelioration with P (40ppm) on activity of SOD in the leaf and root of wheat (*Triticum aestivum* L.) seedlings observed at 15 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

SOD activity in shoot significantly ($P<0.01$) increased up to 114% at 0.2mM Cd level while inroot significant ($P<0.01$) enhancement in SOD activity was observed at lower level of 0.1Cd. However, activity of SOD increased at 0.4mM Cd+ P in root and shoot up to 99 and 12.5% respectively compared to plant exposed to 0.4mM Cd alone. This enhancement in SOD activity due to P application could be due to enhanced accumulation of iron that participates in Fe-SOD isoform.

This trend of SOD activity was reported in *Sesbania drumondii* (Israr *et al*. 2006), and two wheat cultivar (Ezatollah *et al*. 2007) under Cd stress. Increase in SOD activity at moderate Cd level may be linked to an increase in superoxide radical (O^{-}_{2}) formation as well as de novo synthesis of enzyme protein which in turn may be associated with an induction of genes of SOD by O^{-}_{2} mediated signal transduction(Fatima and Ahmed 2005). CAT activity significantly (P<0.01) increased both in shoot and root tissue at low doses of Cd then decreased on further increment in the Cd concentration. CAT activity significantly (P<0.01) increased by 400 and 125% in shoot and root respectively at 0.2 mM Cd treatment. Reduction in CAT activity was observed only in root at higher Cd supply. Application of P significantly increased the activity of CAT both in root and shoot.

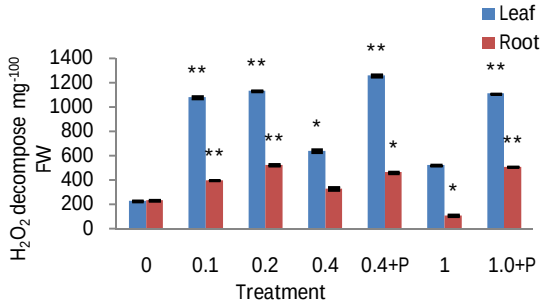


Figure 3. The effect of Cd and its amelioration with P (40ppm) on activity of CAT in the leaf and root of wheat (*Triticum aestivum* L.) seedlings observed at 15 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Hydrogen peroxides and lipid peroxides are the substrate of POX enzymes. In metal stress condition H_2O_2 production increases and membrane faces peroxidation from heavy metal.

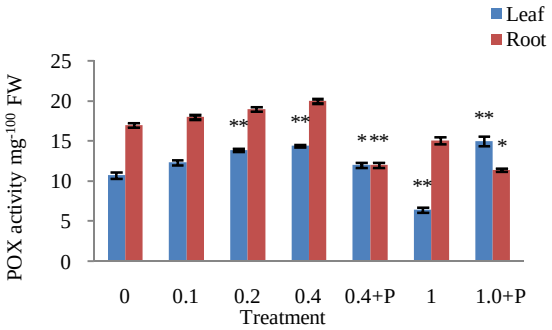


Figure 4. The effect of Cd and its amelioration with P (40ppm) on activity of POX in the leaf and root of wheat (*Triticum aestivum* L.) seedlings observed at 15 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Enhanced production of H_2O_2 and lipid peroxides is injurious for plants growth. To overcome this problem, plants possess a chain of defense mechanism including morphological adaptation to over expression of antioxidative enzymes. One of such enzyme is peroxidase whose synthesis rises up when plants are exposed to stress. Results show that, in shoot, activity of POX were increased by 29.6 and 34.4% at 0.2 and 0.4mM Cd treatment and decreased by 10.7% at 1.0mM Cd compared to control. While in root POX activity was not significant at any levels of Cd supply, but P application at 0.4mM cd, decreased in POX activity up to 16.8 and 40% in shoot and root respectively.

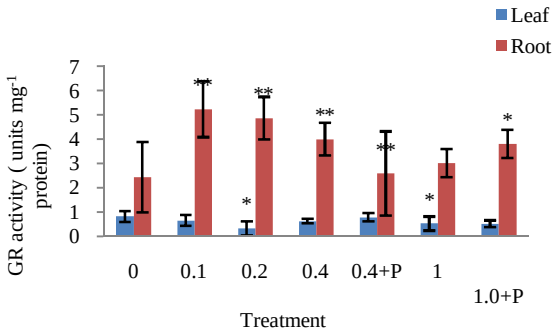


Figure 5. The effect of Cd and its amelioration with P (40ppm) on activity of GR in the leaf and root of wheat (*Triticum aestivum* L.) seedlings observed at 15 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Table 3. The effect of different concentration of Cd and its amelioration with P (40ppm) on accumulation of Cd, Fe and Zn and the concentration of MDA, Proline, Cysteine and NP-SH in the roots of wheat (*Triticum aestivum* L.) seedlings observed at 15 days.

Treatment Cd (mM) Parameter	0.0	0.1	0.2	0.4	0.4+P	1.0	1.0+P
Cd (µg g^{-1}DW)	11.67±0.88	113.5±1.25**	117.6±0.37**	121.4±0.67**	118.5±0.52	126.2±0.52**	120.7±0.30
Fe (µg g^{-1}DW)	20.76±01.4	11.75±1.15	16.37±0.88	53.82±0.67**	74.0±1.73*	32.47±0.58	41.34±0.58
Zn (µg g^{-1}DW)	15.51±0.57	14.57±0.58	11.45±0.29	10.7±0.59*	16.0±0.28*	10.3±0.18*	12.5±0.28
MDA (nmol g^{-1}FW)	6.5±0.04	9.8±0.04	16.0±0.20**	16.5±0.24**	16.5±0.27	16.8±0.48**	18.0±0.36
Proline(µmol g^{-1}FW)	0.50±0.03	0.85±0.06	1.16±0.09**	1.25±0.09**	0.85±0.08	1.60±0.06**	1.06±0.08*
Cysteine(mM g^{-1}FW)	2.65±00.17	4.65±0.12**	5.12±0.26**	5.65±0.12**	4.65±0.17	6.65±0.12**	5.35±0.20*
NP-SH(mM g^{-1}FW)	0.63±0.01	0.96±0.07	2.57±0.11**	3.40±0.06**	3.11±0.07	3.73±0.06**	3.71±0.09

± represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Amelioration with 40ppm P was given at 0.4 and 1.0mM Cd levels. P application reduces POX activity; may due to enhanced accumulation of Fe and lipid peroxides. This result suggested that, CAT is more efficient in decomposing H_2O_2 than POX. In this study reduced activity of SOD, CAT and POX at higher dose of Cd may be due to enhanced production of O^-_2, H_2O_2 and peroxides. SOD, CAT and POX are unable to catalyze such an intense production of ROS in to their final product that causes severe damage. Enhanced formation of O^-_2 at higher Cd concentration can be supported by increased production of MDA content and enhanced accumulation of Fe in shoot that help in the increased production of O^-_2 by participating in the Fenton and Heber Wises reaction. GR catalyzes the reduction of GSH, a molecule involved in many metabolic regulatory and antioxidative processes.

With increasing concentration of Cd , there was a sharp decline in GR activity in shoot tissues, decreasing 57.8% significantly (P<0.01) at 0.2mM Cd concentration, whereas in root tissues its activity were increased at 0.1 mM Cd by 116%. Application of P enhanced the GR activity up to 25% in shoot at 0.4mM Cd where as no such response was found in root. GR play a crucial role in determining the tolerance of a plant under various stresses (Chalpathi *et al*. 2008). Studies showed that GR activity increased in the presence of Cd in Capsicum annuum (Leon et al. 2002), Triticum aestivum (Khan, 2007) and Brassica juncea (Mobin, 2007). In this study, P application enhances the GR activity in root tissues which subsequently promote the tolerance against the Cd toxicity. Result shows that the activity of APX increased at lower doses of Cd, while decreased at higher dose. In shoot APX activity significantly (P<0.01) increased up to 45% at 0.4mM Cd then decreased to higher level of Cd. While in roots APX activity slightly increased by 2.4% at 0.1mM Cd then decreased significantly (P<0.01) 40.8% at 1.0mM Cd treatment.

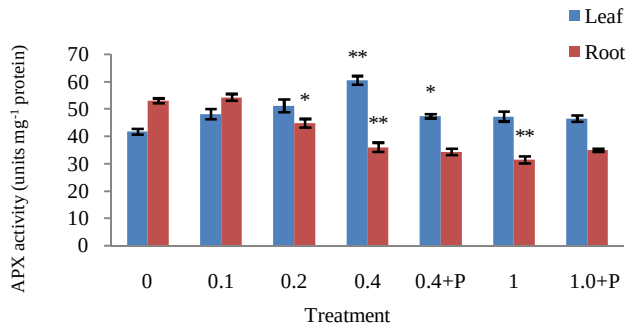


Figure 6. The effect of Cd and its amelioration with P (40ppm) on activity of APX in the leaf and root of wheat (*Triticum aestivum* L.) seedlings observed at 15 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

In our findings, Cd enhanced the accumulation of Fe in root and shoot, being a metal to performed Fenton and habber-weiss reaction. Results shows that Fe, uptake in the Cd treated plants was greater than that in control plants, which is in agreement with the findings by Harnandez *et al.* (1998), who found that Fe in pea plants was higher than that recorded in control plants. Our results indicated that Cd treatment had negative influence on uptake of Zn while Fe uptake influenced by Cd concentration. Verna and Minhas (1987) found that P fertilization increased the translocation of Zn into above ground part of wheat and maize (Zea mays L.). Thus phosphorus application reduced Cd accumulation in plant tissue but enhanced the accumulation of Fe and Zn.

CONCLUSION

Cadmium inhibited plants growth through inhibition of antioxidant enzymes activity, micronutrient uptake and excess accumulation of redox metal like Fe which caused severe membrane damage and eventually cell death. Otherwise, plants responded to Cd toxicity by elevating synthesis of osmoregulator proline, metal binder NP-SH and cysteine, antioxidant enzymes like CAT, POX, SOD, APX and GR. An important point to note that enhanced accumulation of Fe in presence of Cd and P increased the frequency of membrane damage.

Application of P partially alleviated Cd toxicity, through inhibition of Cd uptake and increase in the synthesis of important biomolecules. However it promoted enhanced accumulation of Fe which led to membrane damage. An important field for future study would be to develop technique for phyto-remediation of Cd through selecting Cd hyperaccumulator plants and screen out micronutrient influencing uptake of Cd by selected plants.

ACKNOWLEDGEMENTS

The author would like to acknowledge Council of Scientific and Industrial research, New Delhi, for financial support and Dr. Rajeev Gopal for the critical reading of the manuscript.

REFERENCES

Alcantara, E., F. J. Romera., M. Canete and M. D. De La Guardia. 1994. Effects of heavy metals on both induction and function of root Fe (III) reductase in Fe-deficient cucumber (*Cucumis sativus* L.) plants. *J. Exp. Bot.* 45: 1893-1898.

Arnon, D. T. 1949. Copper enzymes in isolated Chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1-15.

Ashraf, M. and M. R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*. 59: 206-216.

Baryla, A., P. Carrier, F. Franck, C. Coulomb, C. Sahut and M. Havaux. 2001. Leaf chlorosis in oilseed rape plants (Brassica napus) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. *Planta*. 212: 696–709.

Bates, R. P., Waldren and I. D. Teare. 1973. Rapid determination of free proline for water stress studies, *Plant and Soil*. 39:205–207.

Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutases: Improved assays and assay applicable to acrylamide gels. *Analyt. Biochem*., 44:276-287.

Benavides, M. P., S. M. Gallego, and M. L. Tomaro. 2005.Cadmium toxicity in plants. *Braz. J. Plant Physiol.,* 17: 21-34.

Chalapathi Rao, A. S. V. and A. R. Reddy. 2008. Glutathione reductase: a putative redox regulatory system in plant cells. in: N.A. Khan, S. Singh, S. Umar (Eds.), Sulfur Assimilation and Abiotic Stresses in Plants. *Springer*. The Netherlands pp. 111-147.

Cobbett, C. S. 2000. Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.*123:825–832.

Ellman, G. 1959. *Arch. Biochem. Biophy.,* 8:82-70.

Euler, H. and K. Von and Josephson. 1927. *Liebige Ann.* 452:158.

Ezatollah, K. and M. Afsaneh. 2007. Sustainable agricultural attitudes and behaviors: a gender analysis of Iranian farmers; *Environment, Development and Sustainability*; 7:1573-2975.

Fernandes, J. C. and F. S. Henriques. 1991. Biochemical, physiological, and structural effects of excess copper in plants. *Bot Rev*., 57:246–273.

Gaitonde, M. K. 1967. A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids', *Biochem. J.* 104:627–633.

Gallego, S. M., M. P. Benavides and M. L. Tomaro. 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science* 121: 151-159.

Godbold, D. L. and A. Huttermann. 1985. Effect of zinc, cadmium and mercury on root elongation of Picea abies (Karst.) seedlings, and the significance of these metals to forest die-back. *Environmental Pollution*. 38: 375–381.

Godt, J., F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Branderburg, A. Reich. 2006. The toxicity of cadmium and resulting hazards for human health, J Occup Med Toxicol, 1, 22.

Gratao, P. L., A. Polle, P. J. Lea, and R. A. Azevedo. 2005. Making the life of heavy metal-stress plants a little easier. *Functional Plant Biology.* 32: 481–494.

Hare, P. D., W. A. Cress, and J. van Staden. 1999. Proline biosynthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot*., 50:413-434.

Harnandez, A., R. P. Mellado and J. L. Martinez. 1998. Metal Accumulation and Vanadium-induced multidrug resistenceby environmental isolates of Escherichia hermanii and enterobacter cloacae. *Applied and Environmental Microbiology*. 64:4317-4320.

Heath, R. L. and L. Packer. 1968. Photo oxidation in isolated chloroplast .I. Kinetics and Stoichiometry of fatty acid peroxidation. *Arch. Biochem. Bio. Physic*., 125:189-198.

Hewitt, E. J. 1966. Sand and Waterculture Methods Used in the Study of Plant Nutrition. Commonwealth Agricultural Bureau, England.33.

Imley, J. A., and S. M. Chin, S. Linn. 1988. Toxic DNA damage by hydrogen peroxide through the fenton reaction in vivo and in vitro, Science 240, 640-642.

Israr, M., S. Sahi, R. Datta and D. Sarkar. 2006. Bioaccumulation and physiological effects of mercury in *Sesbania drummonii*. *Chemosphere*. 65:591–598.

Jackson A. P., B. J. Alloway. 1991. Bioavailability of cadmium to lettuce and cabbage in soils previously treated with sewage sludges. Plant Soil, 132:179–186.

Kavi Kishor, P. B., S. Sangam, R. N. Amrutha, P. S. Laxmi, K. R. Naidu, K. R. S. S. Rao, S. Rao, K. J. Reddy, P. Theriappan, and N. Sreenivasulu. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci*. 88:424-438.

Khan, N.A., S. Samiullah, Singh, R. Nazar, (2007). Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress*. J. Agro.Crop Sci.,* 193: 435-444.

Küpper, H., F. Küpper, M. Spiller. 1998. Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. J. Exp. Bot. 47, 259-266.

Leon, A. M., J. M. Palma, F. J. Corpas, M. Gomez, M. C. Romero-Puertas, D. Chatterjee, R. M. Mateos, L. A. del Rio, and L. M. Sandalio. 2002. Antioxidant enzymes in cultivars of pepper plants with different sensitivity to candium. *Plant Physiol. Biochem.,* 40:813-820.

Lozano-Rodriguez, E., L. E. Hernandez, P. Bonay, and R. O. Carpenta-RuiZ. 1997. Distribution of cadmium in shoot and root tissues of maize and pea plants: physiological disturbance. *J Exp. Bot.,* 48: 123-128.

Luck, H. 1963. In Methods of Enzymatic Analysis, 2nd edn. (Bergmeyer, H. U., ed.), pp. 885-888, Verlag Chemie, Weinheim, and Academic Press, NewYork.

Mishra, S., S. Srivastava, R. D. Tripathi, R. Govindarajan, S. V. Kuriakose, M. N. V. and Prasad. 2006. Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L*. Plant Physiol Biochem* 44:25–37.

Mobin, M. and N. A. Khan. 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J. Plant Physiol.* 164: 601-610.

Nakano, Y., and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxides in spinach chloroplasts. *Plant Cell Physiol*. 22: 867–880.

Noctor, G. and C. Foyer. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol Biol.,* 49: 249–279.

Padmaja, K., D. D. K. Prasad, A. R. K. Prasad. 1990. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* Seedlings by cadmium acetate. Photosynthetica 24, 399-405.

Parviz malekzadeh, jalil khara and shadi Farshian (2007). Copper toxicity influence on antioxidant enzymes activity in tomato plants and role of arbuscular mycorrhzal fungus Glomus etunicatum in the tolerance of toxicity. *Pak. Jor. of bio. Sci.* 10(12): 2008-2013.

Ranieri, A., A. Castagna, F. Scebba, M. Careri, I. Zagnoni, G. Predieri, M. Pagliari, L. and Sanita di Toppi. 2005. Oxidative stress and phytochelatin characterisation in bread wheat exposed to cadmium excess. *Plant Physiol. Biochem.*, 43: 45–54.

Rivetta, A., N. Negrini, M. and Cocucci. 1997. Involvement of Ca^{2+}-calmodulin in Cd^{2+} toxicity during the early phases of radish (*Raphanus sativus* L.) seed germination. *Plant Cell Environ*., 20: 600–608

Romero-Puertas, M. C., J. M. Palma M. Gomez, L. A. Del Rio and L. M. Sandalio. 2002. Cadmium causes the oxidative modification of proteins in pea plants. *Plant, Cell and Environment*. 25: 677-686.

Sanita di Toppi, L., and R. Gabbrielli. 1999. Response to cadmium in higher plants. *Environ Exp Bot*., 41: 105–130.

Saradhi, A., P. P. and Saradhi. 1991. Proline accumulation under heavy metal stress. - *J. Plant Physiol.* 138:554-558.

Sharma, R. K., M. Agrawal, and F. M. Marshall. 2004. Effect of wastewater irrigation on heavy metal accumulation in soil and plants. Paper presented at a national seminar, Bangalore University, Bangalore. Abstract No. 7, p. 8.

Smeets, K., A. Cuypers, A. Lambrechts, B. Semane, P. Hoet, and A. Laere. 2005. Induction of oxidative stress and antioxidative mechanisms in Phaseolus vulgaris after Cd application. *Plant Physiol Biochem.,* 43: 437–444.

Smith, I. K., T. L. Vierheller, and C.A. Thorne. 1988. Assay of glutathione reductase in crude tissue homogenates using 5,50-dithiobis (2-nitobenzoic acid), Anal. Biochem. 175 : 408–413.

Sorahan, T. and R. J. Lancashire. 1997. Lung cancer mortality in a cohort of workers employed at a cadmium recovery plant in the United States: an analysis with detailed job histories", *Occupational and Environmental Medicine,* 54: 194-201.

Stolt, J. P., F. E. C. Sneller, T. Bryngelsson, T. Lundborg, and H. Schat. 2003. Phytochelatin and cadmium accumulation in wheat. *Environ. Exp. Bot*. 49: 21-28.

Sun, L.Y., S. Aryee and K. S. Law. 2007. High-performance human resources practices, citizenship behaviour, and organisational performance: A relational perspective. Academy of Management Journal 50, pp. 558-577.

Surjendu Kumar Dey, Priyanka Priyadarshani Jena and Satyajit Kundu (2009). Antioxidative efficiency of *Triticum aestivum* L. exposed to chromium stress. *Journal of Environmental Biology* 30(4): 539-544.

Thompson, J. E., G. Paliyath, J. H. Brown and C. L. Duxbury. 1987. The involvement of active oxygen in membrane deterioration during senescence. In: Thomson WW, Nothnogel EA, Huffaker RC (Eds.), Plant Senescence: Its Biochemistry and Physiology. *Am. Soc. Plant Physiologists.* Rockville, pp. 146-155.

Van Assche F., H. Clijster. 1986. Inhibition of photosynthesis in phaesiolus vulgaris by treatment with toxic concentration of Zinc; effect on ribulose 1,5- bisphosphate carboxilase/ oxugenase. Journal of plant physiology. 125: 355-360.

Venekamp, J. H. (1989). Regulation of cytosol acidity in plants under conditions of drought. *Physiol. Plant.* 76: 112–117.

Verna T. S., R. S. Minhas. 1987. Zinc and Phosphorus interaction in a wheat-maize cropping system. Fert. Res. 13: 77-86.

Weckx, J. and H. Clijsters. 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseofus aufgaris* as a result of root assimilation of toxic amounts of copper. *Physiol. Plant,* 96: 506-512.

Wu, J. T., S. C. Chang and K. S. Chen. 1995. Enhancement of intracellular proline level in cellof anacystis nidulans (Yanobacteria) exposed to deleterious concentrations of copper. *J. physiol.* 31: 376-379.

Corresponding author: Dr. Ram Kumar, Department of Botany, University of Lucknow, Lucknow- 226007, U.P. India
Email: ramkumar320031@gmail.com