

Effect of Cadmium on Growth, Photosynthesis and Nitrogen Metabolism of Crop Plants

Wasifa Noor^{1*}, Shahid Umar², Mohammad Yaseen Mir¹, Durdana Shah¹, Gousia Majeed³, Sabira Hafeez³, Sabba Yaqoob³, Aadil Gulzar³ and Azra N. Kamili¹

¹Centre of Research for Development, University of Kashmir, Srinagar-190006, J & K, India

²Department of Botany, Jamia Hamdard, New Delhi, India

³Department of Environmental Sciences, University of Kashmir, Srinagar-190006, J & K, India

*Corresponding author: wasifanoor3@gmail.com

Abstract

Cadmium (Cd) toxicity is one of the major abiotic stresses that adversely affect the growth, development, productivity of crop plants. It is highly soluble in water and therefore is an extremely powerful pollutant. Cadmium disturbs the function of chloroplasts by accumulating to higher levels in aerial parts. It inhibits the enzymes needed for chlorophyll biosynthesis as well as enzymes for carbon dioxide fixation i.e Ribulose-1, 5-biphosphate carboxylase (RUBPCase) and phosphoenol pyruvate carboxylase (PEPCase). Proline acts as an osmolyte for osmotic adjustment. It reduces the reactive oxygen species levels generated by Cd stress and protects the plasma membrane integrity of crop plants. Moreover nitrate assimilation is inhibited by Cadmium which is a potential inhibitor of photosynthetic process. By replacing molybdenum, cadmium inhibits nitrate reductase activity and thereby decreases the nitrate assimilation by plants.

Keywords: Cadmium, plant growth, photosynthesis and nitrogen metabolism

Introduction

Growth, development, productivity of crop plants is adversely affected by Cd toxicity which is one of the major abiotic stresses. Cd is highly toxic and has greater solubility in soil and water and thus acts as powerful pollutant affecting plant growth adversely (Nazar *et al.*, 2012). Photosynthesis electron chain PS II is inhibited, enzyme structure altered by Cd toxicity by interacting with sulfhydryl groups, lipid peroxidation inhibition of ATPase activity, disruption of channels and transporters (Van Assche and Clijsters, 1990). Cd can affect the plants from very beginning of their life cycle i.e., seed germination to production of grains. The activity of enzymes needed for the chlorophyll biosynthesis and CO₂ fixation is inhibited by the Cd by disturbing the function of chloroplast (Boddi *et al.*, 1995; Krupa and Baszynski 1997) as the higher levels of Cd accumulates in the aerial organs (Pence *et al.*, 2000). Superoxide anion (O₂⁻), hydroxyl (OH) free radicles and H₂O₂ are the ROS formed due to high concentration of Cd (Dixit *et al.*, 2001). Plants metabolic reactions is damaged due to the production of reactive oxygen species (ROS), such as superoxide anion (O₂⁻) hydroxyl (OH) radicles and hydrogen peroxide (H₂O₂) as a result of excess concentration of Cd (Takeda *et al.*, 1995; Nazar *et al.*, 2012). Oxidative DNA damage, DNA strand breaks, DNA-protein cross links, chromosomal aberrations, disregulation of gene expression resulting in the increase in proliferation, depressed apoptosis, and changed DNA repair are caused due to Cd toxicity (Nazar *et al.*, 2012). Photosynthesis is inhibited due to increased levels of Cd as it causes the inhibition of seed germination, cell growth, plant growth as well as nutrient uptake which ultimately leads to retarded growth and productivity. The Calvin cycle enzymes, photosynthesis and carbohydrate metabolism are disturbed due to accumulation of Cd and also causes changes in the antioxidant metabolism, affects the stomatal opening by interacting with the water balance and lowers the crop productivity. Chlorophyll biosynthesis, photosynthesis, stomatal behavior, enzymes of Calvin cycle and

electron transport are very sensitive to Cd. and thiobarbituric acid reactive substances(TBARS) and electrolyte leakage causing oxidative stress (Mobin and Khan *et al.*, 2007) that causes decrease in growth and productivity by imposing oxidative damage to the membrane, proteins, photosynthetic pigments, biomolecules such as, nucleic acids and lipids and eventually causing death of plants.

Since the toxic effects of Cd on plant development, particularly on photosynthetic functions are known therefore efforts have been made to counteract the toxicity of Cd. Among various strategies mineral nutritional input may impart dual effects on plant functions as they benefit the plant or may reduce toxicity generated by Cd.

Worldwide problem and great threat to environment is metal contamination, as these metals accumulate in soils in excess, and enters the food chain. Through anthropogenic activities Cd is a phytotoxic metal often added to agricultural soils. Cd is highly toxic metal released into the environment by mining industrial activities, use of fertilizers, sewage sludge, and atmospheric deposition affects plants due to its increased concentration in the agricultural soil (di Toppi and Gabbrielli 1999). Cd has long biological half-life thus for the agricultural system it is considered as the major environment problem. It is highly toxic even at low concentration (Wagner 1993). At the molecular level, Cd has been attributed to (1) displacement of essential metal ions from biomolecules (Rivetta *et al.*, 1997), (2) production of ROS by autoxidation and Fenton reaction (Van Assche and Clijsters 1990; Gallego *et al.*, 1996; Chaoui A *et al.*, 1997) and (3) blocking of essential functional groups in biomolecules (Schutendubel and Polle 2002).

Effect of cadmium toxicity on growth

Inhibition and abnormalities of growth in plants is caused due to Cd toxicity. The effect of various stressors is monitored by measuring the growth of whole plant or plant parts. Plant growth is inhibited by high concentrations of Cd (Anita *et al.*, 1990) and alters plant metabolism even at low concentration (Van Assche and Clijsters 1990). It has also been reported in many cereals and leguminous crops growth and processes of growth are inhibited due to Cd (Dubey and Dwivedi, 1987; Singh *et al.*, 1988). Cd inhibited root and shoot growth considerably but has negligible effect on seed germination (Mishra *et al.*, 1994). Due to Cd toxicity the main root becomes rigid, twisted and brown while inhibition in the lateral root formation occurs (Krantev *et al.* 2008; Yadav 2010; Rascio and Navari-Izzo 2011). Epidermal and cortical cell layers present in the apical region dividing in disordered manner and their abnormal enlargement are the main reasons indicated. Because of altered physiological phenomena reduction in the growth and biomass yield occurs which is mainly due to the increased levels of Cd in the growth media (Demirevska-kepava *et al.*, 2006). In many other cases also drop in root/shoot elongation has also been reported (Gruenhage, 1985; Pahlsson, 1989). Decreased root hydraulic water conductance, root growth retardation, suberization and damage to external and internal structures of root are the symptoms due to increased levels of Cd, translocation of nutrients and normal absorption are also affected (Moral *et al.*, 1994). Strong effect on photosynthetic enzymatic activities occurs due to the high concentration of metal (Baszynski *et al.*, 1980; Van Assche and Clijsters, 1990).

In Ultisol at 100 and 200 µg Cd/g soil the yields of maize shoots grown showed reduction. The level of 100 Cd/g soil and 200 Cd/g soil turned out to be highly toxic in the Ultisol and at these concentration visual toxicities started to appear. Scorching of leaf tips, necrosis, interveinal whitening of leaves followed by withering and stunting of plants were the toxicity symptoms showed by plants (Ramachandran and D'Souza, 1998).

Effect of cadmium toxicity on photosynthesis

Photosynthesis, respiration, water and nutrient uptake are affected by exposure of Cd which results in plant growth inhibition and even plant death (Baszynski *et al.* 1980; di Toppi and Gabbrielli 1999). Plant species like *Brassica juncea* (Mobin and Khan 2007), *Brassica napus* (Baryla *et al.* 2001) and *Helianthus annuus* (Di Cagno *et al.*, 2001) signified that long term exposure of Cd inhibits the photosynthesis. Photosynthetic pigments, mainly the biosynthesis of chlorophyll (Baszynski *et al.* 1980) and are the primary site of action of Cd. The activities of enzymes of chlorophyll biosynthesis and CO₂ fixation is inhibited thus disturbing the function of chloroplast (Boddi *et al.* 1995; Krupa and Baszynski 1995) as the Cd is accumulated in the aerial organs at high levels (Pence *et al.* 2000), especially in the chloroplasts and also interferes in the pigment protein complexes aggregation of the photosystems (Horvath *et al.*, 1996). Internal chloroplast membranes has been observed to breakdown in Cd treated rape seedlings (Filek *et al.*, 2010). The Calvin cycle enzymes are restricted due to the presence of H₂O₂ in the chloroplast thus decreasing the carbon assimilation which results in the Cd-induced-ROS damaged chloroplast (Takeda *et al.*, 1995). Ribulose-1, 5-biphosphate carboxylase (RUBPCase) and phosphoenol pyruvate carboxylase (PEPCase) which are the key enzymes of CO₂ fixation are the main targets of Cd toxicity. PSII electron transport and especially oxygen evolving complex are also altered due to the influence of Cd (Clijters and Assche 1985). Decrease in maximum photochemical efficiency of PSII and weakening of net CO₂ assimilation rate was observed by increasing Cd concentrations from metal to 10 and 50 µM, which was supposedly due to Rubisco activity decrease (Dias *et al.*, 2013). It has been observed that there is reduction in the amount of chlorophyll in leaf of *Zea mays* (Root *et al.*, 1975). Decrease in the Chlorophyll a and b was also observed (Kalita *et al.*, 1993). (Naguib *et al.*, 1986) reported in wheat and rice seedlings presence of Cd resulted in the symptoms of iron deficiency. Thus decrease in iron uptake caused the inhibition of protein synthesis in turn resulting in chloroplast inhibition which might have resulted in the reduction in chlorophyll content. With increasing concentrations of salt solution applied Chlorophyll a and b contents decreased progressively. At higher Cd levels the pigment suffered extreme reduction but the reduction in the chlorophyll content was observed at the lowest concentration. Heavy metals may interfere with the structural components of chloroplasts, the synthesis of proteins thus resulting in the impairment of chlorophyll development (Keshan and Mukherji, 1992).

Effect of cadmium toxicity on proline

Free proline in plants forms a non-toxic Cd-protein complex (Sharma *et al.*, 1998). Proline acts as an osmolyte for osmotic adjustment and plays principal role in stabilizing subcellular structures. It reduces cytoplasmic acidosis and maintains appropriate NADP⁺/NADPH ratios compatible with metabolism (Hare *et al.*, 1999) and may also act as protein compatible hydrotrope (Srinivas and Balasubramanian 1999). Proline accumulation in plants in response to heavy metals is to maintain the osmotic balance in the cells. Cell membrane is protected by the accumulation of proline and thus protects against the oxidative damage (Singh *et al.*, 2003).

Effect of cadmium toxicity on nitrogen metabolism

80% of the nutrients absorbed by plants is contributed by N which is an essential macronutrient and acts as important component in structural, metabolic and genetic components. One of key enzyme of nitrogen assimilation is nitrate reductase (NR) whose activity is regulated by nutritional and environmental factors (Srivastava, 1995). Nitrate assimilation is inhibited by Cd which is a potential inhibitor of photosynthetic process. Inhibition of nitrate

uptake and the activity of enzymes which are required in nitrate assimilation pathway by Cd toxicity have been studied in many plants (Hernandez *et al.*, 1997; Boussama *et al.*, 2006). Cd inhibits the activities of NR and thus decreasing the nitrate assimilation by plants (Chaffei *et al.*, 2004; Wang *et al.*, 2008). Gouia *et al.* (2000) treated *Phaseolus vulgaris* (bean plant) with 100 μM concentration of Cd and reported decrease in NR activity by 80% after 7 days of exposure. *Brassica juncea* was treated with 100 mg kg^{-1} Cd and exposed for 30 days which resulted the decrease in NR activity by 53% (Irfan *et al.*, 2014). Furthermore, decrease in NR activity under different concentrations of Cd has been reported in many crops which are mentioned in **table 1**. By inhibiting NR activity in shoots Cd reduces the uptake of nitrate from roots to shoots (Hernandez *et al.*, 1997; Chaffei *et al.*, 2003). Reducing the activity of existing enzyme molecules or by repressing the synthesis of enzyme, Cd reduces the NR activity. (Campbell, 1988). Thus by replacing molybdenum Cd inhibits NR activity and thereby affecting nitrate binding. The conversion of nitrate to nitrite is done by NR which is the key enzyme. Decline in enzymatic activity because of Cd has been reported in some plant systems (Chug *et al.*, 1992). As the Cd concentration enhances, total protein in the leaves is also enhanced (Mishra *et al.*, 1994).

Table 1. Nitrate Reductase activity decreased in various crops exposed to variable concentrations of cadmium

Cd concentration	Exposure time	Plant species	Decrease in NR activity %age	References
100 μM	7 days	<i>Phaseolus vulgaris</i> (bean plant)	80	Gouia <i>et al.</i> , 2000
0, 50, 25 and 100 mg kg^{-1}	30 and 60 days	<i>Cicer arietinum</i> L.	–	Faizan <i>et al.</i> , 2011
100 mg kg^{-1}	30 days	<i>Brassica juncea</i>	53	Irfan <i>et al.</i> , 2014
100 μM	4, 8 or 12 h	<i>Lycopersicon esculentum</i> Mill.	27.7	Hayat <i>et al.</i> , 2011
100 μM	10- 25 day	<i>Arachis hypogaea</i> L.	20.87 – 79.41	Dinakar <i>et al.</i> , 2009
50 mg kg^{-1}	30 day	<i>Vigna mungo</i>	98.3	Mohammad Mobin (2013)
50 μM	24 h	<i>Cucurbita pepo</i> L.	60	El-Shora and Ali (2011)

Effect of cadmium toxicity on sugar

In growing seedlings of rice (*Oryza sativa* L.) cultivars Ratna and Jaya the changes in activities of sugar metabolism and the content of sugar were studied in response to Cd. An increase in the content of total soluble sugars and reducing sugars, and decrease in the content of non-reducing sugars was observed when treated with 100 μM or 500 μM Cd (NO_3)₂ in the growth medium for 5 to 20 days exposure. In shoots sugar content was greater than in roots when treated with Cd. In maize total sugar content also decreased with increasing levels of Cd. At high Cd levels there is decrease in net photosynthetic rate which might be the reason of decreased sugar content in maize as also reported by (Wichman *et al.*, 1983) and (Keul *et al.*, 1979).with increased Cd levels protein content in maize increased significantly which might be due to concentration effect because addition of Cd reduced the dry matter yield.

Discussion

It has long been recognized that the environmental pollutants have direct impact on the quality and productivity of various plants. Cd is one of the environmental pollutant which directly or indirectly affects the mineral uptake because of which almost every morpho-physiological and chemical processes in the plant were affected and thus leads to an influence on growth, development and yield of the plants however the degree of impact is greatly governed by the nutritional status of the plant and thus if the plants are provided with the appropriate amount of nutrient elements, the damage caused by the Cd may be reduced.

References

- Anita, R., Chugh, L.K. Sawhney, V. and Sawhney, S.K. 1990. *Proceedings of environmental pollution* (Eds.: S.K. Arora, M. Singh and R.P. Aggarwal). Haryana Agricultural University Press, Hisar, India. pp. 66-77.
- Baryla A, Carrier P, Franck F, Coulomb C, Sahut C & Havaux M 2001. Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on Cd-polluted soil: causes and consequences for photosynthesis and growth. *Planta* **212**: 696–709
- Baszynski, T., Krol, M., Wolinska, D. Drupa Z. and Tukendorf. 1980. Photosynthetic activities of Cd treated tomato plants. *Physiol. Plant.* **48**:365-370
- Böddi B., Oravec A.R. and Lehoczki E. 1995. Effect of Cd on organization and photoreduction of protochlorophyllide in dark-grown leaves and etioplast inner membrane preparations of wheat. *Photosynthetica* (Prague) **31(3)**: 411-420
- Boussama, N., Ouariti, O., Suzuki, A. and Ghorbal, M. H. 2006. Cd stress on nitrogen assimilation. *J. Plant Physiol.* **155**, 310–317
- Campbell WH (1988). Higher plant NR and its role in regulation of nitrate assimilation. *Physiol Plant.* **74**: 214-219
- Chaffei C, Pageau K, Suzuki A, Gouia H and Ghorbel MH, Masclaux-Daubresse C. 2004. Cd toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. *Plant Cell Physiol* **45**: 1681–93
- Chaffei, C., Gouia, H. and Ghorbel, M.H. 2003. Nitrogen metabolism of tomato under Cd stress conditions. *J. Plant Nutr.* **26**: 1617–1634
- Chaoui A, Mazhoudi S., Ghorbal M. H., El Ferjani E. 1997. Cd and Zinc induction of lipid peroxidation and effect on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant sci.* **127**: 139-147
- Chug, L.K., Gupta, V.K. and Sawhney, S.K.. 1992. Effect of Cd on enzymes of nitrogen metabolism in pea seedlings. *Phytochemistry.* **31**:395-400.
- Clijsters H, and Van Assche F. 1985. Inhibition of photosynthesis by heavy metals. *Photosynthesis Research* **7**: 31-40
- Demirevska, K., Simova-Stoilova, L., Stoyanova, Z. and Feller, U. 2006. Cd stress in barley: Growth, leaf pigment and protein composition and detoxification of reactive oxygen species. *Journal of Plant Nutrition*, **29**: 451-468
- Di Cagno R, Guidi L, De Gara L and Soldatini GF 2001. Combined Cd and ozone treatments affect photosynthesis and ascorbate-dependent defences in sunflower. *New Phytologist*, **151**: 627–636
- Di Toppi, L.S. and R. Gabbrielli. 1999. Response to Cd in higher plants, *Environ. Exp. Bot.* **41**: 105-130
- Dias MC, Pinto G, Correia C, Moutinho-Pereira J, Silva S, Santos C 2013 Photosynthetic parameters of *Ulmus minor* plantlets affected by irradiance during acclimatization. *Biol Plant* **57**: 33–40
- Dinakar N., Nagajyothi, P.C. Suresh, S. Damodharam, T. and C. Suresh. 2009. Cd induced changes on proline, antioxidant enzymes, nitrate and nitrite reductases in *Arachis hypogaea* L. *J. Environ. Biol.* **30(2)**: 289-294
- Dixit P., Mukherjee P. K., Ramachandran V. and Eapen S. 2001. Glutathione transferase from *Trichoderma virens* enhances Cd tolerance without enhancing its accumulation in transgenic *Nicotiana tabacum*, *PLoS One* **6(1)**.

- Dubey, R. S and Dwivedi, R. S. 1987. Effect of heavy metal on seed germination and seedling growth of soybean. *Nat. Acad. Sci. Letters*. **10**:121-124
- El-Shora H.E. and Ali A.S. 2011. Changes in activities of nitrogen metabolism enzymes in Cd induced marrow seedlings. *Asain Journal of Plant Sciences*. **10(2)**: 117-124
- Faizan S, Kausar, S., Perveen, R. 2011. Varietal differences for Cd-induced seedling mortality, foliar toxicity symptoms, plant growth, proline and NRactivity in chickpea (*Cicer arietinum* L). *Biology and Medicine*. **3 (2)**: 196-206
- Filek M, Gzyl-Malcher, B., Zembala, M., Bednarska, E., Laggner, P. and Kriechbaum M. 2010. Effect of selenium on characteristics of rape chloroplasts modified by Cd. *J Plant Physiol*. **167(1)**: 28-33
- Gallego S.M., Benavides M. P. and Tomaro M. L. 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science* **121**: 151–159
- Gouia H., Ghorbala, M. H. and Christian Meye. 2000. Effects of Cd on activity of NRand on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiol. Biochem*. **38**: 629–638
- Gruenhage, L. 1985. Effect of heavy metal on growth and heavy metal content of *Allium porrum* and *Pisum sativum*. *Angewandte Botanik.*, **59(1-2)**: 11-28. Gruenhage
- Hare, P.D., Hare, W.A. Cress, J. and Van Staden. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot*. **50**: 413–434
- Hayat S., S. A. Hasan, Aqil A. 2011. Growth, nitrate reductase activity and antioxidant system in Cd stressed tomato (*Lycopersicon esculentum* Mill.) cultivars. *Biotechnol. Agron. Soc. Environ*. **15(3)**: 401-414
- Hernandez JA, Olmos E, Corpas F, Sevilla F. and Del Rio L. A. 1997. Salt induced oxidative stress in chloroplasts of pea plants. *Plant Sci*. **105**: 151–167
- Horvath G., Droppa, M., Oravec, A. Raskin V. I. and Marder J. B. 1996. Formation of the photosynthetic apparatus during greening of Cd poisoned barley leaves. *Planta*. **199**: 238-243
- Irfan M., Ahmad A. and Hayat S. 2014. Effect of Cd on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. *Saudi Journal of Biological Sciences*. **21**: 125–131
- Kalita, M.C., P.Devi and I.Bhattacharya. 1993. Effect of Cd on seed germination, early seedling growth and chlorophyll content of *Triticum aestivum*. *Indian J. Plant Physio*. **36 (3)**: 189-190
- Keshan, T. J. and Mukherji, S. 1992. Effect of Cd toxicity on chlorophyllase content, hill activity and chlorophyllase activity in *Vigna radiate* L. leaves. *Indian J. Plant Physio*. **35 (.3)**: 225-230
- Keul, M., Andrei, R., Lazar-keul, G. and Vintila, R. 1979. Accumulation and effects of Lead and 444 5 Cd on wheat and maize. *Studii si cercetari be Biologie, Biologie Vegetala*. **31**:49-54
- Krantev A., Yordanova R., Janda, T., Szalai G. and Popova L. 2008. Treatment with salicylic acid decreases the effect of Cd on photosynthesis in maize plants. *J. Plant Physiol*. **165**: 920-931
- Krupa Z., Baszynski T. 1995. Some aspects of heavy metals toxicity towards photosynthetic apparatus – direct and indirect effects on light and dark reactions, a review. *Acta Physiol. Plantarum*, **17**: 177-190.
- Mishra S. N., Seema Bhutani and Singh, D. B. 1994. Influence of nitrate supply on Cd toxicity in *Brassica juncea* during early seedling growth. *Indian J. Plant Physiol*. **37 (1)**: 12-16
- Mobin, M., and Khan, N. A. 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to Cd stress, *J Plant Physiol* **164**: 601-61
- Mohammad, Mobin. 2013. Effects of Cd-induced oxidative stress on growth and nitrogen assimilation in blackgram (*Vigna mungo* (L.) Hepper). *Journal of Agricultural Sciences*. **58**: 31-39
- Moral R., I. G-omez, J. N. Pedreno, and J. Matax. (1994). Effects of Cd on nutrient distribution, yield and growth of tomato grown in soilless culture. *J.Plant Nutr.*, **17**: 953-962.
- Naguib M. I., Hamed A. A. and Wakeel, S.A. A. I. 1986. Effect of Cd on growth criteria of some crop plants. *Egyptian J. Bot*. **25**, 1-12.
- Nazar R., Noushina I., Asim M., Iqbal, M. Khan, R., Shabina S., Nafees A. K. 2012. Cadmium toxicity in plants and role of mineral nutrients in its alleviation. *American Journal of Plant Sciences* **3**:1476-1489
- Pahlsson AMB 1989 Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water Air Soil Pollut*. **47**:287-319.

- Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L. D., Lasat, M. M., Garvin, D. F., Eide, D., and Kochian, V. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc Natl Acad Sci USA* **97**: 4956–4960
- Ramachandran V. and Souza, T. J. D 1998. Behaviour of Cd in contrasting Indian soils. *Journal of Environmental Science and Health*. **33 (8)**: 1583-1605
- Rascio, N. and Navari-Izzo F. 2011. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science* **180**: 169–181.
- Rivetta A., Negrini N. and Cocucci M. 1997. Involvement of Cd²⁺ calmodulin in Cd²⁺ toxicity during the early phases of radish (*Raphanus sativus* L.) seed germination. *Plant Cell Environ.* **20**: 600-608
- Root, B. A., Miller, R. J. and Koeppel, D. M. 1975. Uptake of Cd-its toxicity and effect on the iron ratio in hydroponically grown corn. *J. Environ. Qual.* **4**:473-476
- Schutzendubel A., Polle A. 2002. Cd and H₂O₂ induced oxidative stress in *Populus x canescens* roots. *Plant Physiol. Biochem.* **40**: 577-584
- Sharma S. S., Schat H. and Vooijs R. 1998. In vitro alleviation of heavy metal-induced enzyme inhibition by proline. *Phytochemistry* **49**: 1531–1535.
- Singh D. N., H. S. Srivastava and R. P. Singh. 1988. Nitrate assimilation in pea leaves in the presence of Cd. *Water, Air, Soil Pollut.* **42**:1-5
- Singh P. K. and Tewari R. K. 2003. Cd toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. *J. Environ Biol* **24(1)**: 107- 112
- Srinivas, V., and Balasubramanian, D. 1995 Proline is a protein-compatible hydrotrope. *Langmuir* **11**: 2830-2833
- Srivastava, H. S. 1995. Nitrate reductase, In: N nutrition in higher plants. *Assoc. Publishing Company, New Delhi*, p. 145-164
- Takeda T, Yokota A, and Shigeoka S. 1995. Resistance of photosynthesis to Hydrogen peroxide in algae. *Plant physiol* **36**:1089-1095
- Van, Assche F., and H. Clijsters. 1990. Effects of metal on enzyme activity in plants. *Plant Cell Environment* **13**:195-206
- Wagner G. J. 1993. Accumulation of Cd in crop plants and its consequences to human health. *Adv Agron* **51**: 173-213.
- Wang, R., Chen, S., Zhou, X., Shen, X., Deng, L., Zhu, H., Shao, J., Shi, Y., Dai, S. and Fritz, E. 2008 Ionic homeostasis and reactive oxygen species control in leaves and xylem sap of two poplars subjected to NaCl stress. *Tree Physiol* **28**: 947–957
- Wichman, G., Lieman, F. and Knosel, D. 1983. Studies on the influence of low Cd concentration on metabolism in vegetables. *Angewandte Botanik.* **57**:331-334
- 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