SUMMARY AND CONCLUSIONS

Agriculture has always been the most important livelihood for the masses in India. But the concern among scientists and intellectuals, from different fields has increased with increasing challenges of climate, urbanization and industrialization, pollution etc. that constitute a great threat to agriculture and thus affecting humankind and other biological communities. Environmental degradation due to heavy metal contamination is one of the magnitudes affecting lives on earth directly or indirectly, which is basically due to continuous inputs of industrial effluents that generally carry large amount of toxic metals. Continuous irrigation of agricultural land in the adjoining areas of populated cities with sewage and industrial wastewater leads to heavy metal accumulation in the soil and grown vegetables. As heavy metals are non–biodegradable and non–thermodegradable therefore persist in extreme environments for longer time and thus readily accumulate to toxic levels. Among heavy metals, Cd contamination constitutes a big part. Cadmium is commonly released into the environment from industrial processes and farming practices and has been ranked No.7 among the top 20 toxicants. In agricultural soils the regulatory limit of Cd is continuously increasing due to soil amendment and the intense use of phosphate fertilizers and sewage sludge. Being highly toxic to the plants, Cd is easily taken up by plant roots and can be loaded into the xylem for its transport into above ground parts and enters into the food chain where it may pose serious threats to human health. Apart from affecting the human being, the elevated level of Cd declines growth and development of plants. Although Cd is a non redox metal but it is found to be a strong phytotoxic metal which causes growth inhibition and may leads to plant death. Sensitivity of plants varies with Cd
concentrations and most plants show sensitivity to low Cd which alters the chloroplast ultrastructure, photosynthesis, nitrogen metabolism and antioxidant enzymes. It has also been reported that Cd generates oxidative stress in plants through the overproduction of reactive oxygen species (ROS), which induces changes in the functions of membranes by initiating peroxidation of polyunsaturated fatty acids. Plants possess a wide array of defense strategies to protect themselves from ROS which includes efficient antioxidant enzymes and low molecular weight chemical antioxidants.

Phytohormones also known as plant growth regulators (PGRs) are chemical messengers which regulate the plant growth and development. They are the active members of signal transduction cascade involve in the plant stress responses. However, plants may not synthesize enough endogenous phytohormones for their optimal growth and development under suboptimal climatic, environmental and metal stresses. Noticeably, plants are reported to respond to exogenous application of hormones during certain stage and under specific conditions. Apparently, it is possible that exogenous application of PGRs may enhance plant growth under metal stress. Further, there may be a close link between antioxidant defense system and PGRs action. Therefore, the present work is carried out to investigate the impact of Cd on growth, chlorophyll, carotenoids, protein, nitrate, nitrite and ammonium contents, enzymes of nitrate and ammonium assimilation and antioxidant defense system in leaf tissues of tested seedlings and to find out the possible mechanisms to remove and/or minimize Cd toxicity by using three different growth hormones (plant growth regulators: PGRs) viz. indole acetic acid (IAA), kinetin (KN) and homobrassinoloid (HBL). Further, among all stages of plant growth, seedlings stage is vulnerable to stress, and the growth and development solely depends on this stage. Therefore, for the present study seedlings of two vegetable crops i.e. *Solanum melongena* (eggplant) and *Solanum lycopersicum* (tomato) were selected to carry out the work under the Cd stress where Cd, dose (3 mg Kg⁻¹ sand) is under the safe limit (WHO, 2007) and Cd, dose (9 mg Kg⁻¹ sand) is a toxic dose.
Healthy seeds of *S. melongena* (eggplant) and *S. lycopersicum* (tomato) were surface sterilized with sodium hypochlorite and washed several times with sterilized distilled water. After this, seeds were soaked for 4 h in distilled water and wrapped in sterilized cotton cloth and then kept overnight for germination. Germinated seeds were sown in plastic pots containing acid washed sterilized sand, already mixed with two levels (Cd, and Cd$_2$) of Cd in the form of CdCl$_2$. Seedlings were grown in plant growth chamber (CDR model-GRW-300 DGe, Athens) under photosynthetically active radiation (PAR) of 150 µmol photons m$^{-2}$ s$^{-1}$ with 16:8 h day-night regime and 65-70 % relative humidity at 26±1 ºC. The seedlings were irrigated with half strength Hoagland and Arnon's (1950) nutrient medium and with distilled water on alternate day. During the growth period seedlings were sprayed with PGRs (IAA and KN; 10 µM and HBL; 10 nM). After 30 days of growth, seedlings were harvested and different parameters were analyzed. Growth responses of both test seedlings to Cd and exogenously applied PGRs were studied by analyzing fresh/dry weight, length, pigment contents, Cd accumulation in plants, leaf protein and carbohydrate contents, photochemistry of photosystem II (PS II), nitrogen metabolism and oxidative stress.

Growth is the resultant of metabolic processes of plants and used as an indicator for testing sensitivity of plants under stress. The growth responses of *S. melongena* and *S. lycopersicum* seedlings were measured as fresh and dry weight, shoot and root length and leaf area per plant. Results revealed that both the doses of Cd, decreased growth as compared to control and the reduction in growth was more prominent under Cd, treatment and the effect was greater in *S. melongena* seedlings. On the other hand, foliar application of PGRs (IAA, KN and HBL) alleviated Cd-induced toxicity on growth parameters in both the test seedlings. Among the PGRs, HBL appeared to be more effective than KN followed by IAA and the alleviating effect was more prominent under Cd, treatment in *S. lycopersicum* seedlings.

Cadmium accumulation in roots and shoots was studied in both the test seedlings exposed to Cd, and Cd$_2$ doses alone and together with the foliar application of PGRs to find
out its impact on growth. Further, data of Cd accumulation may also be correlated with biomass accumulation pattern in both seedlings exposed to PGRs application under Cd toxicity. Cadmium accumulation in the roots and shoots of both the test seedlings increased with rising concentration of Cd in the sand, and Cd content in roots of both the seedlings was found to be several fold greater than that of shoot. Upon PGRs application, Cd content declined in roots and shoots as compared to Cd treatments alone and among the tested PGRs, HBL was found to be more effective than KN followed by IAA. Furthermore, Cd content in shoots of both the test seedlings upon PGRs application declined more rapidly than that of roots and this effect was greater in *S. lycopersicum* seedlings.

Photosynthetic pigments i.e. chlorophyll (*Chl a and Chl b*) and carotenoids (*Car*) were measured in both the test seedlings exposed to PGRs under Cd toxicity because they are important constituents of light harvesting complexes and play vital role in photosynthesis. Results revealed that Cd significantly decreased contents of *Chl a, Chl b* and *Car* in both the test seedlings and the decrease was greater in *S. melongena* than *S. lycopersicum*. Exogenous PGRs (IAA, KN and HBL) alleviated Cd-induced toxicity on pigments contents in both the seedlings. Among the PGRs, HBL appeared to be more effective than KN followed by IAA, and the alleviating effect was more prominent under Cd, stress in *S. lycopersicum* seedlings.

Photosynthesis is one of the biochemical processes that provides organic compounds for the growth and development of the plant. Therefore, to understand the mechanism of growth reduction in both the test seedlings, the impact of Cd on photosynthesis was studied. Cadmium significantly inhibited photosynthetic (oxygen yield) and carbonic anhydrase (CA) activity in both the test seedlings in Cd concentration dependent manner; however, inhibition was more in *S. melongena* than *S. lycopersicum seedlings*. Under this condition exogenous PGRs improved the photosynthetic and CA activity in both the seedlings showing better result in *S. lycopersicum* and this response to PGRs showed the order: HBL>KN> IAA.
To ascribe the impact of test stressor and PGRs on photochemistry of PS II in both the test seedlings, chlorophyll a fluorescence kinetics-JIP test was analyzed. Both the test seedlings showed characteristic OJIP transient rise in control and there was a decrease in O-J, J-I and I-P rise under Cd treatment in concentration depended manner. The reduction in Fv/Fm signifies towards the inability of PS II in reducing the primary acceptor (QA) under Cd stress, while decline in size and number of active photosynthetic centres (Fv/F0) is indicating either a decreased rate of photochemistry as the primary electron acceptor pool became increasingly oxidized, or a reduction of the pool size of QA associated with PS II activity. Increased F0/Fv value under Cd stress points toward strong damaging effect on oxygen evolving complex which could occur as a result of replacement of manganese (Mn) as well as substitution of calcium (Ca) by Cd. Furthermore, to analyze the efficiency of electron transport of PS II as evident by decrease in value of quantum efficiency of electron transfer (Ψe) as well as trapped exciton (ϕE0) which can move an electron through electron transport chain beyond QA, under Cd exposure also indicated an inhibition of the downstream of QA electron transfer. The significantly lowered performance index (PI_{ABS}) value of plants exposed to Cd was the result of lowered values of Fv/Fm (ϕP0), Ψe and ϕE0. Furthermore, the damaging effect on PS II was also demonstrated by substantial increase in specific energy flux parameters (ABS/RC, TR/RC, ET/RC, DI/RC) that could be due to the decrease in active RC following Cd stress. Exogenous PGRs improved the efficiency of PS II in Cd treated seedlings by restoring the functional and structural attributes such as quantum yield, size and number of photosynthetic centre and water splitting complex as evidence by increased Fv/Fm and Fv/F0 values and decreased value of F0/Fv. In addition to this, significant reduction in energy fluxes (ABS/RC, TR/RC, ET/RC, DI/RC) per reaction centre following exogenous PGRs in Cd treated seedlings further indicated towards the improvement in photosynthesis hence, considerable alleviation in photosynthetic oxygen yield was noticed in both the test seedlings. The result depicts that exogenous PGRs showed more pronounced effect under Cd, treatment in both the test seedlings and HBL was more effective than KN followed by IAA. The damaging effect
induced by Cd in both the test seedlings and consequent improvement in the PS II photochemistry by PGRs was greater in *S. lycopersicum*.

In contrast to photosynthetic activity, respiratory oxygen uptake was increased by Cd in concentration dependent manner in both the test seedlings. Exogenous PGRs: IAA, KN and HBL under Cd stress showed declining trend in respiration oxygen uptake in both the seedlings and this effect was greater in case of *S. lycopersicum*.

Nitrogen is an important bio-element which is incorporated into the biosphere through assimilatory processes carried out by microorganisms and plants. Nitrate assimilation is carried out by successive action of nitrate reductase (NR) and nitrite reductase (NiR) and results into the formation of ammonium. The ammonium formed is incorporated into carbon skeletons (glutamate) mainly through the glutamine synthetase-glutamate synthase cycle (GS-GOGAT cycle). However, recent studies showed that ammonium may also incorporate into glutamate by the action of aminating glutamate dehydrogenase. Alteration in activities of nitrogen assimilating enzymes may also adversely affect the growth and development of plants. To elucidate the nitrogen metabolism under Cd stress and exogenous PGRs treatments, nitrogen (NO$_3^-$, NO$_2^-$, NH$_4^+$) contents in leaf tissue, NR, NiR, GS, GOGAT and GDH activities were determined in both the seedlings. Cd declined NO$_3^-$ and NO$_2^-$ contents and activity of NR, NiR, GS and GOGAT while enhanced the content of NH$_4^+$ and activity of GDH enzyme in its concentration dependent manner in test seedlings. Exogenous PGRs application improved the nitrogen metabolism in both the seedlings, hence exhibited reverse trend in these parameters under Cd stress in both the seedlings. Furthermore, the PGRs induced improvement was greater in *S. lycopersicum* and their order is: HBL>KN> IAA.

In order to understand the impact of Cd and PGRs on growth of both the seedlings the status of oxidative stress was analyzed. ROS: SOR and H$_2$O$_2$, and MDA (lipid peroxidation) contents and electrolyte leakage (%) were increased significantly in test seedlings by Cd in concentration dependent manner, which was higher in *S. melongena*. 

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**Summary and Conclusions**

(108)
Upon foliar application of PGRs in Cd treated seedlings both the ROS and MDA contents and also electrolyte leakage exhibited declining trend and decrease was greater in *S. lycopersicum*. Effect of HBL was found to greater than KN followed by IAA.

To understand the oxidative stress in detail the activity of enzymatic antioxidants: SOD, POD, CAT, GST, APX, GR and DHAR and contents of nonenzymatic: AsA+DHA, GSH+GSSG, proline, cysteine and NP-SH were analyzed. Under Cd stress the activity of SOD, POD, CAT and GST and also of ascorbate-glutathione cycle enzymes i.e. APX, GR and DHAR and the contents of nonenzymatic antioxidants: proline, cysteine and NP-SH were found to increase in both the test seedlings in Cd concentration dependent manner. Notwithstanding to this, values of AsA, AsA+DHA, DHA, AsA/DHA, GSH, GSH+GSSG, GSSG and GSH/GSSG were declined in both the seedlings under Cd stress. Upon PGRs treatments the activity of tested antioxidant enzymes as well as contents of nonenzymatic antioxidants: proline, cysteine and NP-SH were further enhanced. Further, exogenous PGRs caused significant improvement in the contents of AsA, AsA+DHA, DHA, AsA/DHA, GSH, GSH+GSSG, GSSG and GSH/GSSG. Further, application of HBL increased the activity of antioxidants enzymes as well as contents of non-enzymatic antioxidants more efficiently in *S. lycopersicum* followed by KN than IAA. Similar response was also observed in isoenzymes patterns of SOD and POD under Cd stress alone and upon the application of PGRs: HBL, KN and IAA.

The conclusion drawn from the present study is that Cd at both the doses (Cd, and Cd,) suppresses the growth of *S. melongena* and *S. lycopersicum* seedlings by inhibiting photosynthesis and the activity of enzymes involved in nitrogen metabolism, and inducing oxidative stress despite of enhanced antioxidant system particularly enzymatic antioxidants and the damaging effect was greater in *S. melongena* seedlings. Exogenous IAA, KN and HBL caused significant reduction in Cd uptake and translocation from root to shoot, enhanced activity/ production of antioxidants and improved the photosynthetic efficiency and activity of enzymes of nitrogen metabolism hence, alleviated the toxic effect.
of Cd on growth. The alleviation was more prominent under Cd stress in _S. lycopersicum_ seedlings. Overall, results demonstrate that IAA, KN, HBL application may improve the vegetables in general and _S. melongena_ and _S. lycopersicum_ in particular growing in Cd contaminated soil, a condition prevailing in nearby areas of the cities.