Conclusions

From the present study investigating Ni$^{2+}$-induced phytotoxicity in relation to alterations in oxidative metabolism, and carbohydrate and nitrogen metabolism, following conclusions can be drawn:

- In a laboratory bioassay, (Ni$^{2+}$) inhibited seedling growth (length and dry biomass) and altered associated biochemical processes in *Triticum aestivum* (wheat) and *Zea mays* (maize) in a dose-dependent manner.

- The reduction in seedling growth was corroborated by higher accumulation of Ni$^{2+}$ content in the test plants. Effect of Ni$^{2+}$ was found to be more pronounced on roots than shoots of test plants. *Triticum aestivum* was found more sensitive to Ni$^{2+}$ than *Zea mays*.

- Ni$^{2+}$ severely affected the protein content and activities of various hydrolyzing enzymes (proteases) and oxido-reductase (polyphenol oxidases and peroxidases) enzymes.

- Ni$^{2+}$ affected root growth by inducing oxidative stress as indicated by enhanced accumulation of malondialdehyde (MDA, an indicator of lipid peroxidation), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($^\cdot OH$).

- Ni$^{2+}$-treatment induced ROS generation in a time- and dose-dependent manner. Enhanced generation of reactive oxygen species (ROS) upon Ni$^{2+}$ treatment was also confirmed by *in situ* histochemical localization of ROS.

- Ni$^{2+}$ exposure increased electrolyte leakage from the tissue, thereby suggesting damage to cell membranes and it was confirmed by *in situ* histochemical detection studies.
To overcome the Ni\textsuperscript{2+}-induced stress, there was an induction of antioxidant enzymes—superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR)—and non-enzymatic antioxidants like ascorbates (total and reduced) and non-protein thiols, thereby suggesting their role in providing protection to tissue by quenching ROS.

Ni\textsuperscript{2+} affected carbohydrate metabolism by altering the activities of various enzymes involved in sugar metabolism. Accumulation of carbohydrate content was accompanied by the alterations in starch hydrolyzing enzymes (\(\alpha\)-amylase, \(\beta\)-amylase and starch phosphorylase), sucrose hydrolyzing enzymes (acid and alkaline invertases) and phosphorolytic enzymes (acid and alkaline phosphatases). The reduction in reducing sugar content in \textit{Z. mays} roots suggested a disturbance in phloem translocation under Ni-stress.

Excess of Ni\textsuperscript{2+} altered the nitrogen metabolism as indicated by a decline in the activities of nitrate (nitrate reductase, NR and, nitrite reductase, NiR) and ammonium (glutamine synthetase, GS, and glutamate synthase, GOGAT) assimilating enzymes. It suggested a reduction of \(\text{NO}_3^-\) uptake by the plants under metal stress and inability of GS/GOGAT activity to cope up with increasing ammonium ion levels under Ni\textsuperscript{2+}-stress. The activities of various enzymes of nitrogen metabolism altered in \textit{Z. mays} seedlings in response to Ni\textsuperscript{2+} exposure.

Ni\textsuperscript{2+} exposure caused various morphological, anatomical and ultra-structural changes in 4-day old seedlings of \textit{Z. mays}, as revealed by light microscopy and transmission electron microscopy. Under Ni\textsuperscript{2+}-stress there was a thinning of cell wall, formation of folds and amoeboid protrusions. Further, there was appearance of vesicles and intercellular spaces in the cytoplasm due to cell wall disintegration. Ni\textsuperscript{2+}-treatment resulted in an increase in the number of mitochondria and disintegration of nucleolus, which finally disappeared.