SUMMARY

Both acute and chronic toxicity of copper and nickel on the aquatic macrophyte *Ipomoea aquatica* Forsk. was investigated in the present study. The acute toxicity test was of 15 day duration with relatively high exposure concentration, while lower concentrations were used in the 30 day chronic test. It was observed during the acute toxicity test that Cu concentration of 4.58 mg L\(^{-1}\) was found to be the threshold concentration at and beyond which plant growth was hampered. Plants exposed to 8.14 - 25.45 mg L\(^{-1}\) Cu showed necrosis in stem tissues eventually resulting in plant mortality. However, at relatively low Cu concentrations, the non-necrotic portions of stems could survive and propagate by fragmentation. The appearance of necrosis may be due to suppression of root development by Cu and the resultant translocation of the metal into the stem. In case of Ni exposed plants, significant toxic effects on growth parameters such as shoot height (SH), number of new nodes (NN) and number of new leaf (NL) were observed at and beyond 7.14 mg L\(^{-1}\). The appearance of necrosis in stem tissues was observed in plants exposed to 12.51 and 22.33 mg L\(^{-1}\) Ni due to which reduction in SH and NN occurred. Significant increase in number of dead plants (DP) was observed only in 22.33 mg L\(^{-1}\) Ni. Thus on the basis of the occurrence of necrosis and mortality during these acute toxicity tests, it can be suggested that Cu is more toxic than Ni.

During the chronic toxicity test with Cu, reduction in growth parameters such as SH and NN was observed at and beyond Cu concentrations of 0.25 - 4.58 mg L\(^{-1}\) due to the occurrence of necrosis in stem tissues. On the contrary, 0.13 mg L\(^{-1}\) Cu exposed plants showed significant increment in several growth parameters suggesting that this Cu concentration was the optimum threshold concentration for the growth of *I. aquatica*. The present study on growth parameters at longer duration revealed that while Cu reduced growth at higher concentrations, it promoted
growth at relatively low concentrations. However in case of Ni, control plants showed higher
growth rate than that of Ni exposed plants in terms of various growth parameters, although
appearance of necrosis was observed only at 7.14 mg L⁻¹ during chronic exposure.

Biochemical studies during the chronic toxicity test showed that chlorophyll content was not
affected by Cu concentration of 0.025 - 4.58 mg L⁻¹. Protein content in 0.13 mg L⁻¹ Cu exposed
leaf tissues was found to increase when compared to that in control. Total carbohydrates content
in plants exposed to 2.54 and 4.58 mg L⁻¹ Cu in stem tissues was reduced thereby showing the
toxic effect of Cu. However, no significant difference was observed in ascorbic acid (AsA)
content of Cu exposed plants in both stem and leaf tissues.

Chlorophyll content of I. aquatica was not influenced by different concentrations of Ni
indicating that Ni concentrations used in this experiment were not high enough to cause
deleterious effect on chlorophyll content and at the same time not enough to quantify as
optimum limit that could stimulate the chlorophyll content. Protein content in both stem and
leaf tissues were reduced in Ni concentrations of 2.23 - 7.14 mg L⁻¹ which might be due to
metabolic disorder under Ni toxicity. Total carbohydrates content in leaf tissues of 4.02 mg L⁻¹
Ni exposed plants was reduced. Ascorbic acid content in I. aquatica treated with different
concentrations of Ni was not significantly different from that of control in both stem and leaf
tissues indicating that Ni at these concentrations did not cause severe oxidative stress in I.
aquatica.

Catalase (CAT) activity in stem tissues was inhibited in 0.13 mg L⁻¹ Cu exposed I. aquatica
at the end of 30 day exposure which indicates that Cu at this concentration promoted growth,
and at such benign concentration, reactive oxygen species was produced less. In case of leaf
tissue, CAT activity was significantly increased in 2.54 mg L\(^{-1}\) Cu exposed plants, which might be due to the increased production of reactive oxygen species under Cu toxicity.

Stem tissues of plants exposed to 0.025 mg L\(^{-1}\) Cu showed significant increase in guaiacol peroxidase (GPOD) activities as compared to 0.13 - 2.54 mg L\(^{-1}\) Cu, although significant reduction in leaf tissues was observed as compared to control. This irregular pattern of GPOD activity in stem and leaf tissues in plants exposed to 0.025 mg L\(^{-1}\) Cu may be due to restricted mobility of Cu inside the plants.

Enhanced CAT activity was observed in stem tissues of Ni exposed plants (0.11 - 7.14 mg L\(^{-1}\)) and those exposed to 0.02 mg L\(^{-1}\) suggesting antioxidative stress response against Ni toxicity in *I. aquatica*. Guaiacol peroxidase (GPOD) activities in stem tissues were not affected by Ni exposure, although its activity was reduced in leaf tissue in 2.23 and 7.14 mg L\(^{-1}\) Ni.

Metal accumulation studies during the chronic toxicity test showed that the maximum accumulation of both the metals occurred in the roots, suggesting that the metals were confined to the roots, thereby preventing their translocation to stem and leaf.

A 70 day experiment was set up to study the appearance of necrosis in stem tissue of *I. aquatica* under different Cu (0.025 - 4.58 mg L\(^{-1}\)) concentrations. Two stages of necrosis, viz., early necrosis (EN) and advanced necrosis (AN) could be visually recognized in Cu-affected stem. The apical part of stem remained non-necrotic (NN). No necrosis symptoms were observed in plants exposed to 0.025 and 0.08 mg L\(^{-1}\) Cu till the end of the experiment. AN tissues showed less protein and total carbohydrates concentration followed by EN tissues while higher concentrations of these compounds were observed in NN tissue. Conversely, Cu accumulation was the highest in AN, followed by EN, while the least was found in NN portions. The lower protein and carbohydrates content in AN tissues might be due to the disturbance in
their synthesis, metabolism or transportation mechanism. Necrosis could also be identified as a strategy for managing Cu toxicity by *I. aquatica*, where the plant sequestered the major portion of accumulated Cu in the necrotic tissue, while normal metabolic activities were maintained in the non-necrotic portion, which could survive and develop into a new plant by fragmentation. Thus a combination of several properties, viz., retention of most Cu and Ni in the necrotic section of stem; hyperaccumulation of Cu as well as Ni in roots; and propagation by fragmentation through root production from nodes provides an opportunity for possible use of *I. aquatica* in copper and Ni phytoremediation. CAT activity was increased in EN tissues suggesting the antioxidative potential of this enzyme. Significant increase in CAT activity and Cu accumulation was observed in 2.54 and 4.58 mg L\(^{-1}\) Cu exposed plants. Scanning electron microscopic studies of NN, EN and AN tissues revealed the thickening of cortical tissues and vascular bundles elements along with distortion in epidermal cells which was more distinct in AN followed by EN tissues in most of the Cu exposed plants thereby disturbing the normal functioning of the plants.

In case of Ni exposed plants, the appearance of necrosis in 4.02 and 7.14 mg L\(^{-1}\) Ni could be observed, although its occurrence was more uncertain and irregular when compared to that found in Cu contamination. Lowest protein and carbohydrates content were observed in AN followed by EN tissues in 7.14 mg L\(^{-1}\) Ni exposed plants, whereas higher amount of Ni was accumulated in AN, followed by EN, with the lowest in NN tissue. Scanning electron microscopic studies on both 4.02 and 7.14 mg L\(^{-1}\) Ni exposed tissues showed alteration and disruption of cortical tissue and slight impairment of vascular tissue at higher concentrations.

Thus from the present study it can be concluded that on the basis of acute, chronic and necrosis study, Cu was more toxic than Ni on *I. aquatica*. Necrosis of stem tissue, which
comprised an important morphological symptom of Cu and Ni toxicity in *I. aquatica*, may also be viewed as a strategy of the plant to survive against the toxic effects of Cu by its sequestration in thenecrotic part of the stem. And again since this species is semi-aquatic, perennial and easy to grow, so it can be used as a means of evaluating Cu and Ni pollution in water and on soil along with its possible use in Cu and Ni phytoremediation.