6.0 Conclusion

In the present work, attempts were made to examine biodiversity of ponds of Vadodara city for selection of plant species for in vitro phytoremediation study, metal ions (Zn, Ni and Cd) accumulation by the selected plants, effects of these ions on plants by examining various biochemical parameters (Chlorophylls, Carbohydrates, Protein, Proline, Catalase peroxidase) and observations on anatomical alterations in metal treated plants through light microscopy and SEM EDX analysis.

In biodiversity study, we reported total of 94 aquatic plant species belonging to emergent, submerged, rooted with floating leaves and free floating hydrophytes. When the data was compared with earlier reports for the same wetlands, it showed that aquatic plants were reducing in population in Vadodara region to the point of extinction. These aquatic plants should be conserved to sustain the rich biodiversity for vadodara region for the benefit of humanity.

As *Lemna polyrhhiza L.* and *L. triscula* L. were found growing in abundance in majority of surveyed wetlands these two plant species were selected for phytoremediation study. For phytoremediation study, the selected plant species (Test plants) were exposed to different concentration of Zinc, Nickel and Cadmium ions at different exposure periods. Among these metal ions, cadmium was absorbed in maximum amount by both the test plants at very low concentration of 0.5 mg/ml concentration. The order of accumulation was found to be Cd > Ni > Zn for both the plant species. Hence, these plants can be used to remediate aquatic environment contaminated with the cadmium metal ion. The study also proves that the aquatic plants can sequester metal ions in their cells but they showed differential uptake capacities towards absorption of different metals.

Zn, Ni and Cd treated plants showed decrease in the level of Chlorophyll, Carbohydrates, proline, Catalase, Peroxidase. This proves toxicity of these metal ions when concentration and exposure period increased. Experimentally, among all the parameter analysed, Nickel treated test plants (*L. polyrhhiza* L. *and L. triscula* L.) revealed increased protein content even if concentration of the metal ion and exposure period had increased. This lead us to conclude that Ni ion might have caused depletion of low molecular weight proteins like glutathione (GSH); which in turn may caused oxidative stress in plants. Synthesis of this stress protein participated in cellular detoxification might have induced under stress conditions.

The current research reported increase in the level of antioxidative enzymes *i.e*. catalase and peroxidase with increasing concentration of tested metal ions and treatment periods. Greater activities of proline, catalase and guaicol peroxidase indicated that the test plant were under oxidative stress, a feature often associated with metal tolerance. The enhanced levels of these enzymes are known to enhance plant respiration and it may cause further consumption of net photosynthesis and may enhance plant catabolism.

As Cadmium was found to be the most toxic metal ion even at lower concentration, in the present research to view anatomical abnormalities, light microscopy and SEM EDX analysis were carried out only for cadmium treated plants (0.5 mg/ml) for both the test plants.

The control samples showed mesophyll cells full of chloroplasts and uniformly distributed and intact thick outer epidermis. The cells have retained their original shape and size as well as intercellular spaces. Whereas 0.5 ppm Cd treated plants showed gradual loss in chlorophyll pigment, disturbance in the arrangement of mesophyll cell and disorganization in epidermal layer. *L.triscula* L. Cd treated cells in addition to all these abnormalities also showed significant change in structure of vascular system which revealed expansion in the xylem and phloem tissues. This might be due to the fact that increased volume of vessel provides easy movement of water under stress condition.

Based on light microscopy observations it is clear that both the test plants more or less showed similar abnormalities at cellular level. Therefore SEM - EDX study was carried out only for *L. polyrhhiza* L. for control and treated plant.

Surface morphology of control plant showed non shiny, porous, non crystalline surface with fixed shape and wasfound to maintanin evenness through out the observed surface where as SEM images of Cadmium treated reveled shiny white surface which is an evidence that Cadmium metal ion have got absorbed on the surface. Moreover, the surface of treated plant was found to be more porous than the control surface. It was heterogenous and rough which are the indications of plant to be a good bioindicator of cadmium contaminated environment. The observed crystalline of the treated plant might be due to sequestration of molecules like metallothianine on the surface.

L. polyrhhiza L. and *L.triscula* L. are valuable species for the study of metal hyperaccumulation mechanisms, which is of particular importance in the context of the potential use of this plant in phytoremediation. The phytoremidiation technique is more cost effective than other conventional techniques in treating large volumes of wastewater with low concentration of pollutants. However, more work is needed to optimize the design and management of an aquatic plant based system so as to get maximum efficiency in metal removal. The system should have a confined environment such as constructed wetland. In addition, knowledge of water chemistry, presence of humic acid in the system, harvesting techniques, metal recovery technology and safe disposal of used plants will have to be worked out before large scale application is adopted.