The current research was conducted to estimate *in vitro* bioaccumulation capacity of heavy metal ions (Zn, Ni, Cd) by two *Lemna* Species i.e. *L. polyrhhiza* L. and *L. triscula* L. The study also focused on biochemical parameters of these two test plants also to observe anatomical alteration through light microscopy and SEM EDX analysis.

A surge in the non destructive technologies such as rhizofiltration and phytoremediation to remove pollution from water and soil as compared to traditional technologies have prompted the researchers interest to study metal remediation through plant species thus developing advanced water purification technology.

Nowadays DNA barcoding provides an efficient molecular tool for taxonomic identifications at the species level and is contributing powerfully to animal and plant biodiversity analysis, and to genetic traceability of livestock and crop species and their food products. At present, molecular techniques based on DNA barcoding seem to be the most reliable and standardizable tool of authentication for identification of plant and animal. Hence we used DNA Barcoding for identification of plants. For selection of plants for phytoremediation study, Total 94 plants were collected from different wetlands of Vadodara out of which for 84 plants DNA isolation and gene sequence analyses were carried out. 57 plants were submitted in BOLD and through this DNA barcode were generated. Based on ecological grouping for example emergent, submerged, rooted with floating leaves and free floating hydrophytes, 12 plants were subjected to phylogenetic tree analysis using rbcL gene sequence. From this analysis it is concluded that *Hydrilla verticilata*, *Vallisneria spiralis, Potamogeton crispum* showed grouping and belongs to Alismatales which are mainly rooted submerged hydrophytes. As per the chart *Potamogeton crispum* showed lowest correlation value with *vallisneria spiralis* and then *Hydrilla verticillata* L.

Similarly *Hydrilla verticillata* L. showed lowest correlation value with *Vallisneria spiralis*. This indicated that these plants are closely related with each other and therefore were therefore grouped together.

As per the analysis, *Equisetum ramosissimum* Desf., *Paspalum vaginatum* Sw., *Ludwigia perrinis* L., *Ludwigia octavalvis* L. belongs to different families but they were grouped in to amphibious plants. In the phylogenic tree *Equisetum ramosissimum* Desf. and *paspalum vaginatum* Sw. showed close relationship. Besides this as per correlation chart *Paspalum distichum* showed close relationship with *Ipomoea aquatica and Hygrophila aristata*. Contradictory results occurred in *Pistia stratiotes* because it was correlating with *Hydrilla verticillata* at the lowest value which depicted that both the plants are correlated and should be grouped together but as per the ecological groups *Hydrilla verticillata* L. belongs to submerged and *Pistia stratiotes* belongs to free floating hydrophytes.

Through biodiversity study we selected *L. polyrhhiza* L. and *L. triscula* L. for *in vitro* study of metal accumulation (Zn, Ni and Cd) as they were growing in majority of wetlands of Vadodara district in abundance all through the year. For metal accumulation study determination of LC 50 value for both the plants at various concentration of metals (For *L. polyrhhia* L. Zn - 1 ppm, 3 ppm, 5 ppm, 7 ppm, 9 ppm; Ni – 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5ppm; Cd – 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm and 0.5 ppm; For *L. triscula L.*- Zn - 1 ppm, 3 ppm, 5 ppm, 7 ppm, 9 ppm; Ni – 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10ppm; Cd – 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm and 0.5 ppm) at different exposure period (For *L. polyrhhia* L. and *L. triscula* L. Zn – 3, 6 and 9 days; Ni – 3,5 and 7 days; Cd- 2, 4 and 6 days), *in vitro* was carried out using Hoagland culture medium. Thus, the study deployed multiple data sources and multiple analysis techniques to test the proposition of using phytoremediation.

SUMMARY

For phytoremediation study the selected plant species, i.e test plants were exposed to different concentration of Zinc, Nickel and Cadmium at different exposure period. Among these metals maximum absorption of Cadmium was recorded both the test plants at a very low concentration at 0.5 mg/ml concentration. The order of accumulation was found to be Cd > Ni > Zn for both the plants. 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.

As metals are found to hinder normal biological processes in plants, the research also includes the effect of these metals on various biochemical parameters like chlorophylls, protein, proline and enzymes such as catalase and peroxidase. It was found that chlorophylls (“a”, “b” and total) were affected by all the metal ions under the study in both the test plants.

Among 3 metal ions under study, Zinc was found to increase chlorophyll content of both the test plants at 1 ppm concentration following 3 days exposure period. This might be due to its role as micronutrient. Though Nickel is also one of the micronutrient it did not enhanced chlorophyll content like Zinc. This suggested that Nickel does not favour the growth of the test plant. It might be non essential element for the plant growth. At higher concentration all other metal ions used in the present study show drastic reduction in chlorophyll content.

Prolonged exposure to metal ions caused inhibitory effect on the synthesis of chlorophylls, protein and carbohydrate contents of the plant. Like chlorophyll, carbohydrate content of the test plants was found to decline in metal treated plants of *L. polyrhhiza* L. and *L. triscula* L. Results of protein from treated plants also revealed reduction in the protein content except in Nickel. This lead us to conclude that Ni ion might have cause production of low molecular weight proteins like glutathione (GSH); which intern may cause oxidative stress in plants. Synthesis of this stress protein participated in cellular detoxification was might have induced under stress conditions. The scientific observations on several of these plants have indicated that glutathione is a major player determining their relative tolerance. Heavy metal stress in general induces ROS and generated oxidative stress. It has been found that in addition to accumulated metal ions, high levels of ROS adversely affected the plants. Glutathione is involved in detoxifying ROS through ascorbate–glutathione cycle. While accumulated metal ions are detoxified by phytochelatins, which are synthesized from glutathione in plants during their exposure to heavy metals. Phytochelatins form complex with metal ions and sequestered them into the vacuole. This mechanism of heavy metal tolerance in plants has strongly suggested that glutathione should not be limiting.

Particularly, role of glutathione, phytochelatin, cysteine synthesis and glyoxalase pathway genes have been reported in imparting heavy metal stress tolerance. Additionally, several natural plant species have been identified showing such plants documented the involvement of glutathione in the mechanism of heavy metal stress tolerance. However, this need further detailed account of experimental validation. These natural heavy metal accumulators could be a potential source for genetic manipulation of other important agricultural crop plants.

As a response of heavy metal stress, proline accumulation was found in both the experimental test plants. Heavy metal stress is one of the major problem affecting agricultural productivity of plants. Natural flora show relative differences in their heavy metal tolerance capacity. Some plants grow well in a soil enriched with toxic levels of heavy metals while others could not grow. Therefore, attempts were made by various researchers to generate transgenic plants using several different genes regulating glutathione levels in plants.

The increase in the level of antioxidative enzymes i.e. catalase and peroxidase with increasing concentration of tested metal ions and treatment periods were estimated and recorded in the present study. In both the test plants we found enhanced level of proline, catalase and Guaicol peroxidase. The results are indicative of generation of oxidative stress in metal treated plants. This is feature usually in response to metal tolerance under stress condition.

Uptake and excess of metals by plants initiate a variety of metabolic reactions, finally leading to global phytotoxic responses e.g. dwarf growth and chlorosis. The reduction in the photosynthetic pigments due to disorganization of chloroplast was reported by many researchers: which suggested that chlorophylls ("a", "b" and total chlorophyll) get inhibited due to metal induction. The chloroplast is the organelle most affected by metal contamination. Heavy metals bind to the thiol (–SH) and nitrogen containing groups in the enzymes in the plants thus blocking the catalytically active sites.

This way they are reported to be "**enzyme blockers**". Some essential metal ions form metallo-enzymes after their incorporation into the cells and cause inactivation of enzymes. Many of them can form the lipid soluble organ metallic compounds that accumulate within the cells and organelles impairing their function.

It is known that when metal ion gets accumulated in the plant cell it causes several abnormalities even at gene level. The plant under metal stress morphologically show symptoms like chlorosis, necrosis, stunted growth etc. whereas at cellular level they show cell plasmolysis, degradation of cell organelles, thinning or thickening of cell walls, increase the number of cell vacuoles, thickening of xylem vessels etc. Therefore, the study was conducted on control and treated plant to observed anatomical abnormalities through light microscopy. In general, all the metal ions under study caused distruction in epidermal cell, disintegration of cytoplasmic contents and chloroplasts and pith disintigration. *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 increased volume of vessel provides easy movement of water under stress condition.

Through electron microscopic observations on surface morphology of control and treated plants SEM analysis was performed for cadmium treated (0.5 ppm) *L. polyrhhiza L.* Surface morphology of control plant showed non shiny, porous, non crystalline surface with fixed shape and were found to maintanin evenness throughout 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 was found to be more porous than the control surface. The surface observed was heterogenous and rough it is in indications of plant as a good bioindicator of cadmium contamination. In addition to this, in the SEM study, the surface was found to be crystalline which might be due to sequestration of molecules like metallothianine. SEM analysis has proved that treated plants have promising metal adsorbing characteristics. It is clear that Sublethal concentrations of Cd ion caused alterations in surface structure. Such ultrastructural aberrations occurring in *L.polyrhhiza L.* exposed to Cd, at the cell level might be due to alterations caused by Cd metal ion. The observed crystalline depositions are of metals sequestered by specific molecules like metallothioneins.

In the current research, EDX analysis was performed to determine the elemental composition of the biosorbents before and after the Cd ion adsorption. The quantitative analysis using EDX of control plant showed that carbon and oxygen are the major constituents that is 56.72 % and 31.49 % respectively. The spectrum also showed the presence of magnesium 0.52 %, aluminium 1.55%, silicon 0.39%, phosphorous 0.65%, sulphur 0.34, chlorine 1.43% , potassium 2.64%, calcium 2.29%, copper 1.99%. In control plant, EDX analysis revealed complete absence of Cd ion.

The graph of EDX analysis clearly showed absence of Cd metal ion peak in control plants. The quantitative analysis using EDX of treated plant showed that carbon and oxygen are the major constituents that is 50.50 % and 39.87 % respectively in the biosorbents. The spectrum also showed the presence of magnesium 0.45 %, aluminium1.55%, silicon 0.31%, phosphorous 0.45%, sulphur 0.50, chlorine 3.24% , potassium 2.43%, calcium 1.24%, copper 1.99%. Cadmium ion with concentration of 0.30 %. This is a strong evidence suggesting that the plant after treatment of Cadmium ion have absorbed the metal ion. This is further confirmed by the occurance of peak in Cadmium treated plant. Elemental analysis of the same shows percentage increas in constituents like carbon, phosporous, sulphur and chlorine but at the same time showed decrease in oxygen, silicon and calcium. This results suggest that absorption of cadmium ion in treated plant affects absorption of other metal ions also.