5.1 Discussion

Biodiversity

Biodiversity is the totality of genus, species and ecosystem in a region. The wealth of life on the earth today is product of millions of years of evolutionary history. With the passage of time, human cultures have emerged and adapted to the local environment. These socio-econonomic refinements in turn have discovered used and altered local biotic resources. Many areas that appear to be natural in fact bear the marks of million years of human habitation, crop cultivation and resource harvesting. The domestication and breeding of local varieties of crops and livestock have further shaped biodiversity.

The earth's plants, animals and microorganisms interacting with one another and with the physical environment in ecosystems from the foundation of sustainable development. Biotic resources from this wealth of life support human livelihood and make it possible to adapt to the changing needs and environments. The steady erosion of the diversity of genes, species and ecosystems taking place today will undermine progress towards sustainable society. Indeed, the continuing loss of biodiversity is a telling measure of imbalance between human needs and wants and nature's capacity (Peter, 1992). Thus in order to preserve the existing biodiversity a number of conservation measures and strategies are to be adopted. Moreover, there should be an inclination toward discovering unexplored areas in the context of India. Biodiversity survey for terrestrial ecosystem of Gujarat district was done by many researchers. Some major works on floristic and ethno-botanical studies carried out by various researcher showed Kachchh district is a rich area in term of floral diversity.

Notably, 700 species of flowering plants from the Kachchh region (Sabnis and Rao,1983), 574 flowering plants from southeastern Kachchh (Rao, 1981), 518 flowering plants from western Kachchh (Bhatt, 1993) reported 768 species of flowering plants from the district. 251 species of plants from the study area which is less than one percent of the district geographical area (Patel *et al*., 2013).

Vyas, (2001) documented 46 plant species of medicinal values belonging to 26 families in Kachchh district, 30 climbers used as medicinal values from the rural areas of Saraswati river basin of Patan district in north Gujarat (Seliya and Patel, 2009). Similarly biodiversity of aquatic ecosystem of Gujarat was done by many researchers. Some major works remarkably carried out by Dabgar, (2012) reported 73 genera and 82 species belonging to 43 family from wadhwana, 25 species with 22 genera belonging to 18 families from anand city (Patel *et al*., 2014). But biodiversity survey of ponds in Vadodara especially Harani and Gotri remained neglected. Earlier total of 158 angiosperm belonging to 48 families in Harni Pond, vadodara were documented (Phatak and Satakopan, 1957). Similarly Mona D. and Krishnayya, N., (2004) reported 113 species belonging to 46 families were relocated and new documentation of 28 angiosperms belonging to 15 families were made from Harni Pond, Vadodara. Hence, Current study was aimed at identification of aquatic plants growing in these ponds.

DNA Barcoding

Morphological identification is inapplicable when studying population biology. In such cases, barcoding is an efficient and valuable technique. Some ecologists have started using the barcoding approach to identify specific unknown plant samples for practical purposes (Li *et al*., 2009). Ongoing developments of new primers and improvements in sequencing techniques have facilitated the data-emergence process of plant barcoding (Soltis *et al*., 1992; Burgess *et al*., 2011). Recently, plant diversity belowground was determined using rbcL gene sequences as a core plant DNA barcoding marker (Kesanakurti *et al*., 2011). Tsukaya *et al*. (2011) described a new genus based on DNA sequences of the chloroplast *matK* pseudogene and ITS of the nuclear ribosomal DNA.

The generation of *matK* sequences for some plant groups has been reported to be problematic, because this part of the chloroplast genome underwent a large-scale restructuring during evolution (Duffy *et al*., 2009; de Groot *et al*., 2011). None of the currently existing primer sets are likely suitable for all lineages of land plants (Hollingsworth *et al.*, 2009; Li *et al*., 2009; Roy *et al*., 2010) and efforts are now focusing on the development of complex primer assays to achieve reliable amplification and sequencing of land plants.

In conclusion, this study provides preliminary assessment data that will be useful for wider application of DNA barcoding in ecological studies. With the current development of primers, we found that rbcL will be very useful for the barcoding of plant species in Saudi Arabia. However, further protocol development to enhance clean DNA ex-traction, PCR amplification strategies, including the development of new primers, and local authenticated databases could play important roles in efficient utilization of plant barcoding.

Our study took the advantage of using rbcL gene, taking into consideration that the coding rbcL gene is easily amplified and sequenced in most land plants and has an impact in phylogeny investigations by providing a reliable placement of a taxon into a plant family and genus (Kress and Erickson, 2007). rbcL gene is vastly utilized in plant phylogenics and evolution studies. The gene is known for its slow synonymous nucleotide substitution rate and its functional constraint (Wolfe *et al.*, 1987). First suggestions that rbcL gene sequence was appropriate to use in phylogenetics studies were from Ritland and Clegg (1987) and Zurawski and Clegg (1987).

Small scale phylogeny studies based on rbcL sequences were followed (Doebley *et al.*,1990) However, the first collaborative large scale phylogenetic analysis using collected rbcL sequence data for anroad sampling of seed plants was conducted by Chase *et al.*, (1993). The rbcL gene sequence alone and along with other chloroplast and nuclear DNA sequences have been extensively used to resolve plant species phylogenies and evolution.

Phylogenetic methods were applied in a recently conducted study of barcoding species using each barcode locus taken alone and in combinations to evaluate species recovery (Roy *et al.*, 2010). The NJ, MP, and UPGMA methods were used for both single- and multi-locus analyses with 500 bootstrap replicates. When all sequences for a given locus were considered, ITS, *matK*, and *trnH-psbA* were able to form a species-specific clade for only *Berberis pachyacantha*. Not a single species was recovered with *rbcL* using any of the three methods. The clades formed in the trees were mostly mixtures of several species. Therefore, establishing a local barcode database will be valuable for a broad range of potential ecological applications, including the building of community phylogenies (Kress *et al.*, 2009).

Bioaccumulation of Zn, Ni and Cd by *L.polyrhhiza* L. *and L. triscula* L.

Present study shows that Zn, Ni and Cd treatment at different concentration increase linearly in metal accumulation in *Lemna polyrhhiza* L. and *L. triscula* L. Similar result shown by Hasan *et al.* (2007) He reported that metals accumulations in water hyacinth increased linearly with the solution concentration in the order of leaves < stems, roots of water hyacinth.

In the study conducted by Lu *et al*., (2014) who treated 12 plant species (fuzzy water clover, iris – leaved rush, mare's tail, monkeyflower, parrot's feather, sedge, smart weed, smooth cordgrass, striped rush, umbrella plant, water lettuce and water zinnia) with 10 trace elements (As, B, Cd, Cr, Cu, Pb, Mn, Hg, Ni and Se) and reported that with exception of B, all trace elements studied accumulated to substantially higher concentrations (from 5 to 60 folds)in roots than in shoots of all plant species.

The metal accumulation in various parts of aquatic macrophytes is often accompanied by an induction of a variety of cellular changes, some of which directly contribute to metal tolerance capacity of the plants. In their study, Cu, Zn and Ni accumulation in *N. officinale* resulted in considerable physiological changes. Copper uptake occurred rapidly during 24 hour but prolonged incubation depressed the slope of curve. After 48 hours further increase in Zinc level of test plant did not occur. He showed that *N. officinale* were able to accumulate both copper and zinc at upper levels, but was able to accumulate to nickel at low levels (Kara, 2005).

Zaigham Hassan *et al*., (2012) reported that the level of heavy metals increasing in the rivers due to discharge of industrial effluents and civic pollution of various kinds. This is in turn deteriorating the water quality making it unsuitable for both aquatic and human life. In *Amaranthus* plants showed higher Ni accumulation when exposed to 100 and 150 µM Ni compared to lower Ni concentrations (Iori, 2013). Moreover, Mn and Ni accumulation by *L. gibba* after 24, 48 and 72 hours of exposure. The increased concentration of Mn and Ni in the growing media caused am increase in the metal accumulations of metals by plants. Mn accumulation by *L. gibba* was higher than that of the Ni accumulation at each exposure concentration (Doganlar *et al.*, 2012).

Effects of Zn, Ni and Cd ions on Biochemical paramaers of *L.polyrhhiza* L. *and L. triscula* L.

Biochemical parameters such as Chlorophyll, Protein, Carbohydrate, Proline and stress enzyme (Catalase and peroxidase) in plants are commonly used as biomarker for toxicity test (Radic *et al*., 2010). Heavy metals exposition to plants results in significant reduction of growth in plants along with particular alterations in many physiological and metabolic pathways (Burzynski and Klobus, 2004).

Effects of Zn ion toxicity on *L. polyrhhiza* L. and *L. triscula* L.

In the present work, when the test plants *(L. polyrhhiza* L. *and L.triscula* L.) were exposed to Zn ion concentration from 3 ppm to 9 ppm significantly reduced total chlorophyll at all the treatment periods. The results were similar to those reported previously Baryla *et al*., (2001) that metal decrease density, size and the synthesis of chlorophyll and inhibition in the activity of some enzymes of Calvin cycle. He showed that leaf chlorosis in *Brassica napas* causes and consequences for photosynthesis and growth. The reason for the loss of chlorophyll following heavy metal exposure was due to the distortion of the chlorophyll ultrastructure, inhibition of the synthesis of photosynthesis pigments and enzymes of the calvin cycle or reduction in the chloroplast density and size which led to the damage of the photosynthetic cycle (Benavides *et al.*, 2005).

The Cd and Zn weakened the capacity of resisting ROS, resulting in lipid peroxidation or deesterifaction, and then led to damages to structure of the chloroplast memberane of *Hydrilla verticillata* L. (Xun, 2004). Jiang *et al*, (2007) had reported that in maize plant combined pollution by Cd and Zn caused the structure of the chloroplast to be changed. However, total chlorophyll content of the test plants was more at 1 ppm Zn ion concentration after 3 days treatment period. This might be due to its role as micronutrient promoting growth at very low concentration. The effects of effect of Zn metal on the activity of many antioxidative enzymes and antioxidant contents in *Hydrilla verticillata* L. The oxidative stress bound up with increased metal accumulation in *Hydrilla verticillata* L. and decrease efficiency of the ascorbate glutathione cycle under the metal stress (Wang, 2009).

The soluble protein contents for all the treatments reached maximum values in all the treatment period studied as compared with the treatment without Zn. The accumulation of Zn in the

soil microorganism led to a breakdown of protein synthesis systems, or the inhibition of protein synthesis or the speeding up of the decomposition of protein. The decline in proteins also leads to decrease in RNA content and increased the activity of hydrolytic enzymes, such as protease and RNAase due to heavy metal stress (Hu *et al*., 2007). Hendawy and Khalid, (2005) reported that *salvia officinalis* under zinc application resulted in a marked decrease of essential oil percentage, total carbohydrates and proline content. The concentration dependent decrease in plant height, fresh weight, chlorophyll, carbohydrate and protein content as well as NR activity under varying concentration of Zn in black gram (Vijaykumar *et al.*, 2007).

Our results are in accordance with these previous reports. It strongly suggests that Zn through micronutrient exhibits its inhibitory effect at higher concentrations. The decline in carbohydrate might be due to alteration in metabolic pathway due to Zn induction in the test plants.

In the current research, it was investigated that the activities of both catalase and peroxidase were significantly higher in treated plants in comparison with the control ones. Greater activities of catalase and Guaicol peroxidase indicated that the tolerant plant were under oxidative stress, a feature often associated with metal tolerance. Heavy metals in soil, water and atmosphere, where plants are living are seen to demonstrate interactions between these heavy metals and the plants. On the other hand, heavy metals show negative effects on plants by inhibiting growth, damaging the structure, affecting the physiological and biochemical activities and decreasing the functions of the plants. The effects and bioavailability of heavy metals depends on many factors including environmental conditions such as pH, species of heavy metals, and organic substances in the media as well as fertilization and the individual plant species.

Plants have their own mechanisms of resistance against the negative effects of heavy metal by combining heavy metals with proteins and developing enzymes and nucleic acids to detoxify heavy metal pollution. Thus, the effects heavy metals on plants are revealed in several aspects and the plants show many kinds of resistance mechanisms. The induction of certain enzymes that detoxify ROS is considered to play an important role in the defense against oxidative stress caused by toxic metal concentrations (Miller *et al*., 2008). Liu *et al.,* (2009) worked on

marigold plant, a number of enzymes regulate H_2O_2 intercellular levels, but CAT, APX and GPX are considered the most important.

Regulation of antioxidant enzymes is performed post translationally under oxidative stress. Therefore it seems that measurement of enzymatic activity is more reliable for the evaluation of *P. indica* and *F. mosseae* effects than expression level. Increase in antioxidant activity was a major target for P. indica in barley leaves. However, it has been showed that *P. indica* is able to up – regulate drought related genes in Arabidopsis leaves colonized by *P. indica* may function more through enhancing enzyme activity than inducing transcription under cadmium toxicity. The alteration of antioxidative defense genes in plants colonized by mycorrhiza – colonized lettuce, Mn – SOD_2 transcripts accumulate under water stress which led to a higher resistance in plants. They also found that expression level of SOD genes were reduced in non stress conditions (Gill and Tuteja, 2010).

Shah *et al*., (2001) reported that in rice plant changes in antioxidative enzyme activities in response to heavy metal stress are known to be dependent on heavy metal concentration. Activities of these enzymes might increase in order to cope with the oxidative stress imposed by heavy metals on plants, as was repeatedly found in our experiments. Alternatively, they might be diminished if the toxic effects of higher concentration of heavy metals were greater than can be tolerated and combated by the antioxidant enzymes, as is the case in the present experiment, particularly catalase activity.

On the other hand, decreased activity of peroxidase in 6 and 9 days Zn treated plant indicated that the plants were under tremendous heavy metal stress. This might have resulted in the accumulation of ROS (reactive oxygen species).

The ability of plants to produce ROS was due to increased activity enzymes required to regenerate ascorbate and glutathione. He worked on legume nodules and explained that ROS products are reported to cause damage to the biomolecules by peroxidation, electrophilic substitution reaction, reduction of memberane lipids, proteins, chloroplast pigments, enzymes, nucleic acids etc. (Becana *et al*., 2000).

It is evident that each of Zn can separately cause significant reduction in most of the recorded structural parameters with detrimental physiological consequences. It might be concluded that exposure of *L.poyrhhiza* L. to toxic levels of Zn triggers a number of closely inter related s structural and functional events in the stressed plants.

Effects of Ni ion toxicity on *L. polyrhhiza* L. and *L. tricula* L.

Nickel is able to inhibit a large number of plant enzymes such as those of calvin cycle and chlorophyll biosysnthesis. Singh, *et al*., (2010) worked on *Vigna mungo* L. and he found that based on the results it can be concluded that chlorophyll, carotenoid, and proline contents were seriously affected carotenoid, and proline contents were seriously affected more as compared to chlorophyll b whereas carotenoids were less affected then Chlorophyll a and b. The amount of proline increased in plants under stress caused by these heavy metals. Nickel proved to be more toxic than lead at all the three concentrations (10, 50 and 100 µM). The deleterious effects of heavy metals may be alleviated in plants if provided with appropriate concentration and form of nitrogen in nutrient medium. Further more research is needed in order to evaluate the effect of different heavy metals on various crops.

Stimulation in the low levels of Ni on carbohydrate synthesis, in case of corn plant, may be attributed to an activation effect on the enzymatic system connected with the carbohydrates anabolism cycle. On the other hand, the observed decline in carbohydrate with respect to high level of Ni was observed which might be due to its role on enzymatic reactions related to the cycles of carbohydrate catabolism (Rabie *et al.*, 1992). A similar result were reported by John *et al*., (2008) with *Spirodela polyrrhiza* L. stressed by Cd and Pb. In that study, it seems that, there might have been greater ROS generation due to high Ni load in plants and as a result, more oxidative stress which resulted in the decline of protein concentration caused by oxidative damage and decrease in the total carbohydrate content corresponded with the photosynthetic inhibition or stimulation of the respiration rate.

Protein accumulation in leaves was also seen in other plants treated with heavy metals (Heiss *et al.*, 2003). Induction of total soluble proteins under heavy metals stress indicates that this organism is adapted to produce specific proteins during metal stress conditions. Plant cells have developed defense mechanisms against heavy metals, which are highly toxic compounds and these proteins can confer heavy metal tolerance on these organisms. It was suggested that these induced proteins might be important to repair proteins damaged by heavy metals. (Hall, 2002).

Gajewska and Sklodowska, (2005) were reported that the nickel-induced increase of soluble protein concentration and strong stimulation of proline accumulation in roots and leaves of the bean plants. The exposure of the cyanobacterium to 25 and 50 µM nickel treatment increased the proline activity from 6.5 [µg (g FW) -1] to 7.67 and 8.45. At 75 and 100 µM concentration the proline accumulation was 9.75 and 11.18 [ìg (g FW) -1], respectively. Enhanced production of proline in cells of cyanobacterium could be linked with detoxification against Ni induced oxidative stress. It suggested that it may be involved in the mechanisms of osmoregulation (Cris, 2012). Accumulation of proline in plants subject to Ni stress has been well documented (Prasad *et al*., 2002).

Deng *et al*., (2004) worked on 12 wetland plant species and mentioned that metals accumulated by wetland plants were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. Hussain *et al*., (2011) worked on *bacopa monnieri* and founded that ion the roots of *S. mucronatus* the accumulation of Cr, Cd, As, Ni and Pb is directly proportional with the increase of metals concentration in the medium.

The effect of nickel on the activity of some antioxidant enzymes (Catalase and Peroxidase) showed significant increase at higher concentration of nickel ion concentration. Ni in very low concentration (0.05 mM) increased the activities of peroxidase (POD), SOD, and guaiacol peroxidase (GOPX) (Gajewska *et al*., 2006) but the high concentration of Ni reduced the activities of many cellular antioxidant enzymes both in vitro and vivo plants so for the capability of plants to remove the ROS and finally lead to oxidative stress The 2 weeks old plants of pea under the Ni stress (10, 100, and 200 µM for 1, 3, 6 and 9 days) significantly reduced the activities of SOD in both roots and leaves and activity of APX in roots however, the activities of catalase (CAT) remain unaltered (Gajewska *et al.*, 2006).

The activities of POD, SOD and glutathione reductase (GR) were increased, while the activity of CAT reduced in the seedling (6 days) of pigeonpea (*C. cajan* L. Millspaugh) under the Ni stress (0.5 mM) (Rao and Sresty, 2000). It was demonstrated that the activities of POD and CAT significantly decreased under the Ni stress (0.5 mM for 8 days) (Pandey and Sharma, 2002). The same trend was observed for other enzymes like POD, SOD and CAT in leaves of *Hydrocharis dubia* in response to Ni stress (0.5, 1, 2, 3, and 4 mM Ni treatments of 3 days (Papadopoulos *et al.*, 2007). Lipid peroxidation product malondialdehyde (MDA) content in roots and shoots was increased in the plant of pigeonpea under the Ni stress (0.5 to 1.5 mM) (Rao and Sresty, 2000).

Similar results were observed in wheat, Alyssum species and corn (Boominathan *et al.*, 2002). Ni caused the depletion of low molecular weight proteins like glutathione (GSH); this may caused oxidative stress in plants (Rao and Sresty, 2000). Peroxidase and catalase are two major systems for enzymatic removal of H_2O_2 and peridative damage of cell walls is controlled by the potency of the antioxidatuve peroxidase enzyme system (Velikova *et al.*,2000). Previously Mocquot was found a positive relationship between increased peroxidase and catalase enzyme activity and the amount of heavy metals such as Cu, Pb and Zn in plant tissue showed that the increase in the activity of catalase and peroxidase with high levels of nickel is known to enhance plant respiration and this may cause further consumtion of plant net photosynthesis and enhanced plant catabolism (Mocquot *et al.*, 1996).

In oxidative stress, total soluble proteins is usually studied as an index of metabolic changes, because, under stress conditions, ROS cause serious damage by interaction with cellular components such as proteins, nucleic acids and lipids (Sabatini *et al*., 2009). Increase in total soluble proteins under Ni stresses can be considered as a plant tolerance mechanism, or in other words, the synthesis of stress proteins that participate in cellular detoxification was induced under stress conditions (Sabatini *et al.*, 2009).

Effects of Cd ion toxicity on *L. polyrhhiza* L. and *L. tricula* L.

Various abiotic stresses decrease the chlorophyll content in plants (Ahmad *et al*. 2003). Several reports show chlorophyll biosynthesis inhibition by metals in higher plants. Saquib *et al*.,(2010) worked on *Croton bonplandianum*. He reported and concluded that significant decrease in photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, photosynthetic pigments and photosynthetic area. These pigments including chlorophyll "a", chlorophyll "b", total chlorophyll and carotenoids were severely affected in the stressed polluted environment and showed a significant reduction in the polluted sample with percent variation having 89, 74, 83 and 20%, respectively.

The heavy metals in higher concentrations concerned the physiological behavior of plants and degrade the activities of photosynthetic enzymes and block the electron transport chain which reduced the chlorophyll contents (Thapar et al., 2008). Lead is a toxic element discharged in the environment due to automobile activities. Lead at 50 mg^{-L} concentration inhibited photosynthetic and transpiration rates and stomatal conductance of two mung beans (*Vigna radiata*) cultivars ($Mung^{-1}$ and $Mung^{-6}$) and concluded that the photosynthetic inhibition is due to stomatal limitations with a considerable reduction in chlorophyll "b" content of mung bean (Ahmad *et al*., 2008).

The decline in chlorophyll content in plants exposed to Cd^{2+} and Pb^{2+} stress is believed to be due to: (a) inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALAdehydratase) and protochlorophyllide reductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; (b) impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; (c) Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters 1990); (d) the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule.

Our results of decrease in chlorophyll content corroborated with the findings of Siedlecka and Krupa, (1996) who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery.

The decrease in carbohydrate content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Higher starch accumulation in damaged leaves of *Tilia argentea* and *Quercus cerris* may result both in the higher resistance of their photosynthetic apparatus (Prokopiev, 1978) and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate carboxylase (Stiborova *et. al*. 1987).

Our studies of soluble protein content coincides with the findings of Singh and Sinha (2005) who found decrease in soluble protein content in *B. juncea* when grown on various amendments of tannery waste containing heavy metals. Decrease in the protein content has also been found in aquatic plants when treated with metalliferous wastewater. However, increased concentration of salt stress increases protein content in *Pisum sativum* (Ahmad and Jhon, 2005). The increase in protein content with lower concentrations of salt (Na_2CO_3) and decrease with higher concentration of salt (Na_2CO_3) in mulberry (Ahmad *et al*., 2006). Palma et al reported that decrease in protein content in *B. juncea* may be because of enhanced protein degradation process as a result of increased protease activity which is found to increase under stress conditions (Palma *et al*., 2002). It is also likely that these heavy metals may have induced lipid peroxidation and fragmentation of proteins due to toxic effects of reactive oxygen species which led to reduced protein content.

Proline, amino acid is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress. Plants have been shown proline accumulation under environmental stress (Ahmad *et al*., 2008). Proline accumulation in shoots of *B. juncea*, *Triticum aestivum* and *Vignaradiata* in response to Cd^{+} toxicity has been demonstrated by (Dhir *et al*.,2004). Similar results of increasing proline content by Cd^{+} was also reported by in sunflower (Zengin and Munzuroglu, 2006).

Oliveira *et al*., (2001) reported that the two species studied here were aquatic plants, and therefore, during the experiment, the leaves maintained direct contact with the nutrient solution. The leaves likely contributed to an increase in Cd absorption, especially in *Salvinia.*

Corroborating this hypothesis, in an experiment where leaf contact with the nutrient solution was not allowed, a significant decrease in leaf Cd concentration was observed in *Salvinia* but not in water hyacinth. For this reason, *Salvinia* leaves probably showed higher Cd concentrations. Additionally, the leaves of this species showed higher free Cd concentrations. Thus, although the water hyacinth absorbed more Cd, most of it was retained in the roots and/or was kept in a bound and less toxic form than in *Salvinia*.

Several studies have demonstrated that the excessive absorption of heavy metals by plants induces the production of reactive oxygen species (ROS) in plant tissues (Singh *et al*., 2006). In both aquatic species, there were strong increases in H_2O_2 and $O_2^{\cdot -}$, increasing lipid peroxidation, especially in the leaves of *Salvinia*. These ROS cause imbalances in the antioxidative defenses of plants and induce oxidative stress (Edreva, 2005). Plant antioxidative defenses against ROS may involve antioxidative enzymes and nonenzymatic antioxidants, including ascorbate and glutathione (GSH) in addition to tocopherol, flavonoids, alkaloids and carotenoids (Apel and Hirt, 2004). Glutathione peroxidase (GPX), like APX, detoxifies H_2O_2 to H_2O, but uses GSH directly as the reducing agent. The regeneration of GSH is made possible by the reduction of GSSG by glutathione reductase (GR), closing the GPX cycle (Apel and Hirt, 2004). In general, GR activity increases in plants under oxidative stress. This has been observed in *Raphanus sativus* (Vitoria *et al*., 2001), *Crotalaria juncea* (Pereira et al., 2002), *Beta vulgaris* and*Beta maritima* (Bor *et al*., 2003), especially in the leaves. The observed inhibition of GR in Cd-treated plants was paralleled with a decrease in GSH concentration (data not published), lowering the amount of substrate available for GPX. The expected reduction in GPX activity, however, was observed only in water hyacinth. In Salvinia, on the contrary, despite a reduction in GSH concentration, GPX activity increases of over 60% were observed in Cd-treated plants. GPX appears to be capable of using reduced substrates other than GSH, including lipid hydroperoxides. Nevertheless, GPX activity was always higher in water hyacinth, indicating a higher capacity of this species to scavenge the ROS induced by Cd.

Although ROS are continuously formed in normal metabolism, cells must be able to quickly and efficiently scavenge these reactive species to reach a homeostasis. When ROS generation is not adequately regulated, its accumulation may cause oxidative damage to cells. Glutathione S-transferase (GST) is an enzyme with a determinant function in the detoxification processes. It catalyzes the conjugation of several xenobiotics to reduced glutathione (GSH) (Davis and Swanson, 2001).

This enzyme may also protect plants from oxidative injury, functioning as the glutathione peroxidase, by using glutathione to reduce organic hydroperoxides produced by the oxidative degradation of membrane lipids and/or nucleic acids (Dixon *et al*., 2002). It may also induce the accumulation of hydroperoxides or other by-products of ROS action, leading to GST inhibition (Nagalakshmi and Prasad, 2001), as was observed here in the roots of both species.

Observed enzyme activity reductions are indicative of a limited protection against oxidative stress (Schutzendubel and Polle, 2002). The intensity and direction of the antioxidative response to Cd appeared to be dependent on the plant species, tissue analyzed, metal identity (Rout and Shaw, 2001), duration of metal exposure (Hegedus *et al*., 2001) and stage of plant development (Rout and Shaw, 2001). The explanation for such antioxidative enzyme activity reductions is not fully known yet. Apparently, excessive metal accumulation can inhibit enzymes by binding to catalytic active groups or causing protein denaturation (Das *et al*., 1997). Furthermore, toxic metals can induce ROS production and accumulation, which can cause protein oxidation and enzyme inhibition (Mittler, 2002).

Wojcik *et al*., (2005) found higher metal accumulation in roots than in shoots of hydroponically grown *Thlaspi caerulescens*. Some literature data show a higher Cd accumulation in shoots than in roots (Roosens *et al*., 2003) as well, although other authors reported a higher Cd content in roots than in shoot.

Effect of the metal ions on plant Anatomical structure

Light Microscopy

Anatomy of Control and Cd treated plants of *L. polyrhiza* L.

Excess cadmium triggers a wide range of biochemical effects and structural disturbances in plants often accompanied by visual toxicity symptoms. These were studied in detail by Djebali *et al*., 2002, 2005, 2008 and Zoghlami *et al*., 2006. Such effects were very well noticed in the test plants after treatment with Cd ion.

Cd induced significant reduction in the total chlorophyll content of *Lemna polyrhiza* L. In our study chloroplast degradation was noticed when the Cd treated cells were observed under microscope. The observations were similar to those reported previously in *Brassica napus* by Baryla *et al*., (2001). The decrease in chlorophyll content was also reported in *Helianthus annuus* L. (sunflower) by Zengin and Munzuroglu, (2006) and in *Prunus dulcis* (almond) by Elloumi *et al*., (2007). The reason for the loss of chlorophyll following heavy metal exposure was due to the distortion of the chlorophyll ultrastructure, inhibition of the synthesis of photosynthetic pigments and enzymes of the Calvin cycle or reduction in the chloroplast density and size which led to the damage of the photosynthetic cycle.

The study also reported loss in arrangement of mesophyll cells of Cd treated test plants, leading to disorganization in the plant structure. Such observations were earlier reported by Djball *et al.*, 2005 and Garato *et al.*, 2009. This proves that Cd can induce alteration in size and shape of the plant cells.

The test plants, *Lemna Polyrrhiza* L. when exposed to different Cd ion concentration caused disruption and ornamentation of epidermal cells. Chmielewska and Chwil, 2001, Sridhar, 2007 and Andre *et al*., 2006 also noticed such changes in epidermal cells in different plants exposed to different metals. Thus, the study confirms leaf morphogenesis disorders characterized and reflected by plant leaf epidermis, mesophyll cells and chloroplast deterioration.

Exposure to heavy metals leads to a reduction in the size of mesophyll cells (Sridhar *et al.*, 2005) and the collapse of palisade and spongy parenchyma cells (Sirdhar *et al*., 2005), which could justify the thinned leaf blade observed in the treatments exposed to contamination. Conditional on anatomical plasticity, some species develop modified leaf tissues that allow better adaptability to different stress conditions (Melo et al., 2007). Reduction in size and number of conducting elements of the xylem in response to heavy metals has been reported by Sandalio *et al*. (2001).

Duckweed (*L. minor*) was found to be an efficient hyperaccumulator of heavy metals and to exhibit mortality at higher concentrations of metals. Wojcik *et al*., (2005) found higher metal accumulation in roots than in shoots of hydroponically grown *Thlaspi caerulescens*. Some literature data show a higher Cd accumulation in shoots than in roots (Roosens *et al*., 2003) as well, although other authors reported a higher Cd content in roots than in shoots.

Our studies corroborate with Brennan and Shelley (1999) who found higher accumulation of Pb in the roots than shoots of maize. The bioaccumulation of single metal is known to be influenced by the presence of other metals, resulting in inhibited or enhanced bioaccumulation of one metal in the mixture (An *et al*., 2004). Several studies reported that the presence of one metal studies reported that the presence of one metal Videa *et al*., (2002). Our studies show a higher accumulation of Cd than Pb, which confirms the results of An *et al*., (2004) who observed lesser uptake of Cd in the shoots of *Cucumis sativus* in presence of Pb.

Anatomy of Control and Cd treated plants of *L. triscula* L.

The addition of Zn at low concentration had a favorable effect on the growth of plants, which may be attributed to the fact that the plants utilize Zn as a micronutrient for their growth (Lu *et al*., 2004). But its enhanced concentration caused toxic effect on the plants. It was earlier reported that in long term experiment (24 days), *Eichhornia crassipes* exposed to 9 mg/L of Zn resulted in 30% reduction in weight (Delgado *et al*..1993).

Stress caused by the presence of high zinc ion levels in the medium contributed to the disruption of the growth and development of the *Lemna triscula* L. Our study shows that Zn treatment at different concentration increase linearly in metal accumulation in *Lemna triscula* L. Such results were also reported by Stratford *et al*., (1984) and Lu *et al.*, (2004) in water hyacinth.

In the previous study significant changes in root, stem and leaves of water hyacinth were observed (Warrier *et al*., 2008). The changes in anatomical features at 9 mg/ml (9 days) Zn treated *L. triscula* L. were also observed in the current research. These alterations include disorganization in epidermis, breakage in aerenchyma of the cortex and expansion of xylem. Chmielewska, (2001) and Sridhar, (2007) have earlier reported disorganized epidermis in Glycine max exposed to lead and *Hordeum vulgare* exposed to Zinc and Cadmium metal.

The relative volume of the cortex became reduced in treated plant as compare to control due to disorganization of the parenchyma tissues (Panou-Filotheou *et al*, (2006) and Shridhar et al., 2007). One of the significant observations was that that zinc stress in *Lemna triscula* L. resulted in an increase of volume of xylem. The higher number of vessel may facilitate the movement of water (Paunou-Filotheou and Bosabalidis, 2004) may be due to some physiological activities caused by the metal stress (Martens, *et al.*, 2002 and Tahseen *et al*., 2004).

The information available in this work is an important step towards obtaining a better understanding of the structural changes caused by Zn and its effects on metabolic processes. The study envisaged the toxic effect of excessive Zn leading to development of stress symptoms. These symptoms comprise characteristic structural alterations apparently reflecting divergence from at least some normal metabolic patterns.

SEM EDX Analysis

SEM can be used to visualize the surface morphology of the plant before and after metal binding, allowing for direct observation of any change. (Rzize *et al*., 2004) analyzed the effect of metal binding to *Sargassum vulgaris* using combined SEM with EDX. SEM analysis revealed that there were significant morphological changes, including shrinking and layer sticking in the sea week after metal binding.

Our results were similar to the Baruah et al. who reported that SEM EDX micrograph of control plant did not show the presence of Pb. SEM-EDX analysis was performed to localize Pb at tissue level in plants shown in hydroponics culture plus $Pb(NO_3)_{2.}$ 30mg/ml (Baruah *et al*. 2012).

However, Sandalio Rao *et al*. (2001) showed that in treated plant at an intensity of 20 kV, the characteristic peak of lead was seen inside the leaf and root indicating that this demonstrated that Pb accumulated in higher proportion on the root surface, decreasing in concentration towards centre. He worked on *Tectona grandis* L. leaf powder and reported that SEM studies indicated that the cadmium loaded leaf powder has a tendency to form agglomerates.

Our results were similar to Zulfi Abdullah *et al*., (2013) he worked on effect of Cu (II) on *Annona muricata L* leaves who showed that SEM images of *Annona muricata* L. leaves have porous surface which indicate that it have good potential as biosorbent.