SUMMARY

The present thesis has embodied some important findings regarding the cellular and physiological responses of *Salvinia* plants under various concentration of aluminium (Al) in *in-vitro* condition. Therefore, wide ranges of variation in cellular responses of the plants were observed under Al toxicity and also with the interaction of exogenously applied putrescine (Put). Finally, on the basis of hyperaccumulating potential, *Salvinia* plants were also preliminary trialed to check the ability of Al absorption from different sources of contaminated water bodies. Overall, this present investigation has suggested the possibilities of *Salvinia* plants for bio-indication of Al toxicity as well as its scope for phytoremediation purposes with the view of plants hyperaccumulating efficiency.

*Salvinia* (*Salvinia natans* L.) were collected from unpolluted marshy land and further cultured under laboratory controlled condition with various concentrations (0, 240, 360, 480µM) of Al salt. In addition plants were also fed with 1 mM put as supplemented with the highest concentration of Al (480µM). The plants were allowed to grow under such salt concentration for 3, 5 and 7 days and on completion of treatments; plants were harvested.

Initially *Salvinia* plants (*Salvinia natans* L.) were evaluated on their growth status and accumulation of Al at the tissue level. A significant deterioration of the growth and development was the feature under Al exposure. The physiological parameters attributing the growth were determined from dry weight adopting specific derivations. The relative growth rate (RGR) was decreased with increasing of Al concentration and duration. In case of net assimilation rate (NAR), a significant deterioration was the featured which also recorded a maximum fall under highest dose of Al salt concentration (480µM) and also with maximum duration of incubation (7 days). The fall of RGR and NAR was retrieved significantly with the application of Put. In coordination with subdued growth parameters a sufficient amount of Al accumulation was the evidence for metal induced toxicity in plants. The significant accumulation of Al in tissue was measured by atomic
absorption spectrophotometer (AAS) and concomitantly deposition was detected by
scanning electron microscope (SEM) micrograph coupled with EDXS system, which
showed the level of Al deposition in dose dependent manner. Interestingly, we noticed
that Al absorption by *Salvinia* plant was reduced significantly under the application of
Put and also it might be restricted the localization of Al mostly in cortical zone of root.
Though there are no true roots in *Salvinia*, however, rhizoids like structure developed
from third leaf of each whorl, which acts as a root. These modified roots are highly
affected due to generation of ROS and thereby cell death in root tip was detected through
histochemical staining by Evan’s blue. More so, the Evan’s blue uptake was measured
spectrophotometrically and it showed in proportionate manner with Al concentration. We
have mentioned earlier that Al could be inducing oxidative damages to the tissue by
generation of different ROS, hence, as expected total chlorophyll content was reduced
significantly in our present experiment. However, Put mitigated the ROS effect therefore
total chlorophyll content was also increased. The extra cellular generation of super oxide
\( \text{O}_2^- \) and hydrogen peroxide \( \text{H}_2\text{O}_2 \) were more confirmed in their abundance in affected
tissues when we checked it through their biosynthetic as well as activity inhibitors viz.,
imidazole (IMZ), sodium azide (NaN\(_3\)). This was more studied when the generation of
ROS at the sub-cellular level as was detected with membrane bound NADH/NAD(P)H-
oxidase activity in the apoplastic spaces. Moreover, Put found to be corroborated with its
specific sites of action where the biosynthesis of ROS was blocked by those inhibitors. A
significant accumulation of lipid peroxides and carbonyl derivates in the affected tissues
characterized the *Salvinia* plants prone to oxidative stress. Interestingly Put has
diminished the damages significantly for both the cases.

As already mentioned that antioxidation is the predominant measure for alleviation of
metal induced oxidative stress so the plants are well tuned with some existing
metabolites. Classically those are called antioxidant moieties which are of low molecular
weight. In the present experiment few such compounds have been monitored with the
*Salvinia* plants with special reference to Put application. Therefore, in defence of
oxidative damages, plant has displayed both enzymatic and non-enzymatic strategies.
Thus, we find the total phenolics were increased linearly with increasing concentration of
Al and maximum value was recorded at 7 days of Al exposure. The phenolics comprises of the flavonoids which has similar trend and in both the cases, PA could be acting as substitute since a significant down regulation was noticed. Amongst those glutathione with its two alternative forms GSSG and GSH is interesting to note to maintain a stable ratio under stressful condition. The maximization of this ratio was recorded in *Salvinia natans* L. under highest concentration of Al (480µM). Put here played to be a good stabilizer of redox since it declined the GSH:GSSG content. Ascorbate played an important role for stress tolerance in plants. Along with glutathione, another predominant antioxidant in plant system comes in functioning for maintaining the redox pool, is the ascorbate. Ascorbate accumulation increased significantly under Al stress and this was well reduced with the application of Put. Reducing potential is the total non-thiol containing compounds that essentially make an index for reducing status of the tissues. Thus, we measured the reducing potential of the tissues and found a fair increase against control for each duration (3, 5, 7 days) and also with each treatment of Al exposure, however, maximum at 7 days which was down regulated with Put.

In connection to other pathways for antioxidation, enzymatic cascades are most important, which comprises of a set of reactions with consecutive reduction of molecular oxygen into water. Thus, super oxide dismutase (SOD), peroxidases [viz. guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione peroxidase (GuPX), wall bound peroxidase (WPOD)] and glutathione reductase (GR) were assayed both in-vitro as well as in in-vivo conditions. These were over expressed in *Salvinia* plants under Al toxicity, on the other hand catalase (CAT) showed opposite trend. Another enzyme, glutathione-S-transferase (GST) is the key effective antioxidant in plant system, which happens to be a common marker against the antioxidative system for xenobiotics by making provision of glutathione. In support to normal antioxidizing enzymes the conjugant enzyme systems so studied were PAL, DHAR and GST. The GST activity was increased by several folds over the control under Al exposure in *Salvinia* plants. Put, on the other hand down regulated the enzyme activity significantly. Dehydro ascorate reductase (DHAR) plays a vital role to monitor the ascorbate accumulation in plants. DHAR activity also measured in *Salvinia* plant through both in in-vitro assay as well as
through in gel staining. It showed up regulation with the courses of Al concentration. In contrast, put mitigated the DHAR activity significantly. From the biosynthetic pathway of phenolics few enzymes were observed as rate regulatory. Phenylalanine ammonia lyase (PAL) belonging to one such category. In the present experiment the activity of PAL was highly up regulated under the exposure of Al, whereas, Put moderated the PAL activity.

In an osmotic relationship of Salvinia plants, Al had some impact on it and that was evident from water deficit or dehydration in plant tissues, though it was measured indirectly. Thus, the development of proline, compatible solutes for encountering osmotic stress was down regulated, this is in accordance with earlier reports where aquatic species Lemna, Hydrilla and even Salvinia exhibited similar trends. In a similar way the rate limiting enzymes for proline biosynthesis: $\gamma$-glutamyl kinase ($\gamma$-GK) and $\gamma$-glutamyl phosphate reductase ($\gamma$-GPR) had also concomitantly down regulated through in-vitro assay. However, Put modulated the activities of both the enzymes. Glycine betaine is a quaternary amine in nature; it has a vital role in protection of enzyme like RuBisCo for maintenance of osmotic potentiality. These metabolites when assayed, it shows a stepper up regulation as expected under Al concentration for the maintenance of membrane integrity. Put as evident is effective to down regulate the ROS, therefore, glycine betaine concentration reduced.

In compliance with the strategies of osmotic stress under metal (Al in the present case) toxicity, the membrane bound proton ATPase (H\textsuperscript{+}/ATPase) activity was determined from the Salvinia plants. The functional aspects of H\textsuperscript{+}/ATPase clearly showed an up regulation throughout the duration of stress period. It also showed the increasing trend with increasing concentration of Al, whereas, in-vitro assay of H\textsuperscript{+}/ATPase activity was modulated under application of Put. The activity of H\textsuperscript{+}/ATPase had also been tested with the help of its functional promoter (KCl) and inhibitor (V\textsubscript{2}O\textsubscript{5}). In addition, we have also run the membrane bound protein in SDS-PAGE to analyze the membrane bound protein profile.
*Salvinia* being a typically C₃ plant essentially accomplishes the photo-respiratory loss of acquired carbon as default physiological trait. Therefore, as NADP-ME is one of the most important photosynthetic enzymes, in our present experiment we have studied this. Though it functions commonly in C₄ plants for CO₂ enrichment as well as other aspects of plant growth and development more specifically for stress tolerance, still, it is also essential for C₃ plants to replenish the carbon deficit in anaploretic pathways. In the present experiment, we have measured the NADP-ME activity in *Salvinia* plant under light and dark condition. Preliminarily, plant responded well in activities under the light as compared to dark. More interestingly, when we assayed this enzyme activity with different Al concentration under light and dark separately, then we recorded a linear induction of NADP-ME activity in dose dependent manner. However, a significant reduction of NADP-ME activity was showed with the application of Put. Photosynthesis related another important enzyme is PEPC. It is ubiquitous in nature and it catalyzes the PEP thereby oxaloacetate and Pi are formed. The PEPC activity was up regulated under Al stress, thus, its adaptive responses to stress in the plant has been featured. However, Put somehow modulates the PEPC activity.

Polyamine (PA), Put in the present case applied with highest concentration of Al (480 µM), acted as a modulator in different cellular activities. It is quite expected to have a change of plant’s own PA profile and its concentration, therefore it generate the resistance against metal induced oxidative damages. Interestingly the PA profiles in the *Salvinia* plant was reflected in two forms: free and conjugated PA. With the ongoing concentration of Al, *Salvinia* plants show maximum conjugated or bound PA than soluble or free form. This suggests the accumulation of PA under Al concentration in *Salvinia* plant favoured more to use in conjugation of bio molecules. However, this study was performed through spectro-fuorometric assay, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques. In accordance to PA accumulation, S-adenosyl methionine decarboxylase (SAMDC), the enzyme involved in PA biosynthesis was also evaluated in our experiment and found a typical up regulation with ongoing concentration of Al. Responses of plants to Al stress are reflected in many ways at the cellular level. Amongst those, expression of heat shock protein (Hsp)
established a very common cellular trait with regards to Al toxicity. In the present experiment, the expression of Hsp-70 in protein level was studied under Al concentration as well as with the interaction of Put. However, we found that the expression was induced with the courses of Al and moderated by the use of Put. Another cellular trait was found variable according to Al concentration and that was detected in *Salvinia* plants, is glutathione peroxidase (GuPX). This gene was expressed in an ascending order with regards to Al concentration when its activity was studied; whereas it was down regulated under Put supplementation, however, not significantly.

Nitrogen metabolism: toxicity due to metal accumulation has seriously curtailed the mineral nutrition of plants where nitrogen metabolism could be indexed for plants growth and development. With this concept, *Salvinia* plants in the present experiment have been evaluated for nitrogen assimilation with reference to nitrogen metabolising enzyme. A significant deterioration of assimilated inorganic nitrogen was observed. More so, in our present experiment the changes in nitrogen metabolising enzymes viz., glutamine synthetase (GS), glutamine-2-oxo-glutarate aminotransferase (GOGAT) and glutamate dehydrogenase (GDH) were clearly observed under Al exposure. However, PA in all the cases acted as a modulator to stabilize the nitrogen metabolism.

*Salvinia* plants were subdued in fixation and assimilation of carbohydrate under Al toxicity. The accumulation of carbohydrate by plants was significantly depreciated, which was evaluated through the study of different carbohydrate profiles. The availability of total carbohydrate and its depletion was correlated with the photosynthetic process. Therefore, in a consequence, it has been proven that sugar accumulation in tissues is fully dependent on concurrent photosynthesis. However, in the present experiment total carbohydrate and reducing sugar were down regulated, whereas, acid invertase, sucrose phosphate synthase (SPS) and malate dehydrogenase (MDH) were up regulated under Al exposure. Put had modulating role to maintain a steady pool of organic acid and as a whole carbohydrate metabolism in plants.
Finally at the extreme condition, the genotoxicity of Al is manifested into nuclear level which showed the DNA fragmentation through the lysis of nuclear membrane as studied by comet assay techniques. From the increase in comet tail, a direct indicative of DNA lysis was noticed in *Salvinia* plants. From the reduction of comet tail length, it is also proven that Put acts as an effective elicitor in protection of nuclear membrane and genetic material from reactive oxygen species (ROS).

**Parameters studied under exogenously applied hydrogen peroxide**

In association with Al induced ROS, the *Salvinia* plant had also responded well with direct exposure of H$_2$O$_2$. The cellular responses of plants under exogenous application of H$_2$O$_2$ and its impacts on oxidative damages have been studied by taking various physiological and biochemical parameters. Similar trend, as shown earlier under the Al doses, was recorded in both the cases: enzymatic and non enzymatic study. Clear degenerative effects on *Salvinia* upon H$_2$O$_2$ treatments and its modulation with Put were observed. This may undoubtedly suggests that *Salvinia* plants are prone to Al toxicity, which is mediated by reactive oxygen species like H$_2$O$_2$. 