Chapter 9. Summary and Conclusions

The contamination of freshwater ecosystems due to inorganic pollutants like heavy metals and nitrogenous species like nitrate, nitrite ammonium is threatening the life on globe because of their hazardous impacts. Constrains associated with heavy metallic pollution include their non-biodegradability, persistence in the environment, bioaccumulation and biomagnification within the food chains. The elevated levels of nitrogenous inorganic pollutants (nitrate, nitrite, and ammonium) and inorganic phosphates in surface water sources due to runoff from catchment, discharge of municipal/industrial sewage, may lead to different health ailments among the consumers.

Since inorganic pollution affects the quality of municipal water supply, importantly drinking water sources, there by affecting human and plant lives, great efforts have been made in the last two decades to reduce pollution sources and remediate polluted water resources. Many conventional/traditional methods of water treatment like Chemical precipitation, Coagulation- flocculation, Ion exchange, Reverse osmosis, Nanofiltration etc., are employed for water treatment, however certain limitations like expensive to procure and operate, sensitivity to operating conditions, toxic sludge generation, extra operational cost for sludge disposal etc., make these techniques unaffordable and unsustainable. Cost-effective, more cleaner and ecofriendly alternatives are required to sustainably remediate polluted water in order to minimize the health hazards. The efforts made in this field include the use of green technologies like bioremediation and phytoremediation.

Many studies have reported the use of aquatic macrophytes for the remediation of domestic and industrial wastewater. Most of the research work carried in this field has been conducted using hydroponic cultures of single plant species i.e., monocultures, most of which are invasive in character like *Eichhornia crassipes.* Further disposal of metal contaminated biomass needs to be taken into consideration. For phytoremediation technique

to be made successful it is recommended that plants should be perennial in nature, have high biomass production, must be able to tolerate and absorb multimetals without showing symptoms of toxicity and survive under different biotic and abiotic stresses.

The present work was undertaken to evaluate the drinking water quality of municipal water at source and consumers end of Lucknow city and further, studies were conducted to demonstrate the potential of different aquatic macrophytes for the remediation of inorganic pollutants from freshwater ecosystems like Gomti River, under natural and simulated pilot scale microcosm conditions. Simultaneously, mono and mixed cultures of three different aquatic plants i.e., free floating *Pistia stratiotes*; submerged *Hydrilla verticillata* and emergent *Typha latifolia* were employed to remediate the water contaminated with heavy metals (Cu, Cd, Cr and Fe) and other inorganic pollutants like nitrate, nitrite ammonium and phosphate. A comparative analysis of selected plants under the effect of intermittent water circulation in constructed microcosm system was studied for the remediation potential of multimetals and other inorganics. Also, possible changes in plant biomass, productivity, molecular FTIR (Fourier Transform Infrared) Spectroscopy and Scanning Electron Microscopic analysis of the plants before and after pollutants removal was carried out. The generated plant biomass was subjected to vermicomposting and evaluation of nutritional and toxicological properties of the developed vermicompost was carried out to develop low cost and ecofriendly disposal method of contaminated plant biomass.

Monitoring of inorganic pollutants and heavy metals in potable water at source of supply and consumers end of Lucknow City

Regular monitoring of drinking water at the source of supply and at consumer end is of prime importance for generating the database on overall feature and chemical characteristics of water that can help minimize the health hazards to a large extent. The objective of this

study was the monitoring of the water quality of river Gomti at Gaughat being used as the source of drinking water supply for Lucknow Municipal Corporation by analyzing multi metals (Fe, Cu, Cd, Cr and Pb) and reactive nitrogen species (nitrate, nitrite, and ammonium) and phosphate and to evaluate the status of post-treatment drinking water quality at the user end during pre-monsoon, monsoon and post monsoon periods.

The pH values indicated that drinking water at source was slightly alkaline in nature. The electrical conductivity in water which depicts the temporal variations in total dissolved solids and major ions present in it was found higher at the source of drinking water i.e., Gaughat than at the user end. The nitrate content concentration in public water supply at the consumer end showed maximum value. Correspondingly, at the user end higher nitrite levels were reported than at the source. Ammonium nitrogen (NH_4-N) in water samples at studied sites was found to be variable both at the source and residential sites. However, phosphate levels in drinking water of Lucknow city were higher at the user end than at the source of supply. The use of phosphates at potable water treatment plants may have been responsible for elevated phosphate levels at the residential sites.

It was observed that in municipal water supply most of the metals recorded were above the permissible limits prescribed by BIS (2003) for Drinking Water Specifications. Metal contents recorded at Gaughat were in the order of Fe>Pb>Cu>Cr>Cd. At the user end metal levels reported in the water supply exhibited same trend as detected at Gaughat i.e. Fe>Pb>Cu>Cr>Cd. However, the concentrations of these metals at user end were correspondingly higher than the background levels. Correlation matrix between physicochemical parameters and heavy metals in the drinking water of residential sites of Lucknow hypothesized that metals showing significant positive correlation among each other signify that they may be leached out from the same sources and their mobility in the environment may be the same.

Although water is treated at municipal treatment plants, post-treatment concentrations can be compared to the standard prescribed limits, which indicate whether the treated water can be reused for public supply. It was observed that among the studied sites Hazratganj and Charbagh were most polluted as water samples from these sites contained maximum metals contents. Further, the alkaline nature of this potable water can increase the metal toxicity at the consumer end. It is recommended that potable water supplied by the municipal cooperation should be treated prior to supply, using green technologies, for optimal removal of hazardous pollutants, so that public health is protected.

Water quality evaluation and screening of some naturally occurring macrophytes for remediation of heavy metals in Gomti River at Lucknow

The study is aimed at monitoring the water quality of river Gomti from upstream (Gaughat) to downstream (Pipraghat) of Lucknow and to evaluate the potentials of endogenous mix macro-flora as pollution bio-monitors and for phytoremediation of multiple pollutants under natural aquatic ecosystem. While analyzing pH of river Gomti at Lucknow, irrespective of sites and periods, it is alkaline in nature. EC value was recorded highest during pre-monsoon. Dissolved Oxygen (DO) of the Gomti river water at selected sites showed marked differences with both highest and lowest values during the pre- monsoon period. Maximum biological oxygen demand (BOD) was recorded during the pre-monsoon period. Among different sites chemical oxygen demand (COD) showed maximum value during monsoon period. Nitrate concentration varied significantly at selected sites during both periods. Highest nitrite concentration was recorded at Site II during pre-monsoon period whereas, maximum ammonium levels in water was recorded at upstream Site I during the monsoon period. Phosphate value was recorded highest at downstream Site VI during the rainy season.

Metal concentration showed and increasing trend downstream of river Gomti. Metal content in water were in the order of Fe>Cu>Cr>Cd>Pb. Metal concentration of river water at different sites showed significant differences ($p<0.05$). Metal accumulation by selected plants varied from plant to plant and also from one season to other. Generally plants accumulated greater metal content in roots than shoot parts. However, *Eichhornia crassipes, Pistia stratiotes, Hydrilla verticellata, Typha latifolia and Jussiaea repens* had greater metal accumulation in shoot parts than roots at some sites. The metals accumulation by selected aquatic plants was in the order of Fe > Cu > Cr > Pb > Cd. *E. crassipes* accumulated higher concentration of Fe; *P. stratiotes* accumulated highest Cd in shoot; highest Cu accumulation was in shoot of *T. latifolia* while as *J. repens* and *P. stratiotes* shoots accumulated maximum Cr and maximum Pb accumulation was found in *E. crassipes* roots followed by *T. latifolia.* The differential metal uptake by the roots and shoots resulting in partitioning and translocation of metals in the vascular system of plants may be due to anatomy and morphology of different plant taxa coupled with their sorptive potentialities, plant growth rate and physiological conditions of each plant species. Except for Fe, *E. crassipes, P. stratiotes T. latifolia* and *J. repens* showed more than 1 Translocation Factor (TF) for Cd, Cr, Cu and Pb at different sites during both periods. *Hydrilla verticellata* recorded highest TF for all five metals at all sites and periods. Translocation factor by the plants was in the order of *H. verticellata> E. crassipes> P. stratiotes > T. Latifolia > J. repens >P. glabrum> V. spiralis.* Pearson Correlation Coefficient was used to evaluate the relationship in dynamics of metals concentration with physicochemical characteristics of river water. All the heavy metals showed significant positive correlation with COD, phosphate (PO_4) and nitrate. Individually, each metal showed positive correlations with other metals. In aquatic environment, accumulation of heavy metals and subsequent transformations due to physico-

chemical and biological processes are the important mechanisms for their changing levels in water.

From the study it is concluded variations in metal accumulation by plants from site to site could be attributed to the dwelling of plants at distinct microhabitats, their growth patterns, metal availability for absorption and metal levels in water column. It is worthy to note that plants accumulated greater metals than the corresponding levels in in water, which reflects that these species could be used in ecological surveys as in situ bio-monitors of water quality and potential candidates for phytoremediation due to their ability to concentrate metal pollutants in their tissue parts.

Phytoremediation efficacy of *Pistia stratiotes* and *Hydrilla Verticellata* under mono and mixed cultures for heavy metal removal: Mechanism of metal sorption by SEM and FTIR studies

Phytoremediation of polluted waters using aquatic macrophytes is recognized as ecofriendly and energy efficient technology, employing the biological processes of plants and engineering tools for effective remediation. Effect of circulation on the remediation of polluted waters has been shown to enhance the treatment capacity, increase the hydraulic retention time, reduce the area required for treatment and facilitate operation at higher loads. The potential of mono and mixed culture of native plants (*Pistia stratiotes* and *Hydrilla verticellata*) for removal of multi metals (Cu, Cd, Fe and Cr) under intermittent sequential processes of water circulation in a pilot scale microcosm treatment has been evaluated. Further, metal localization; partitioning and the role of different functional groups as binding sites for metal sequestration within the plants by Scanning Electron Microscopic (SEM) and Fourier Transform Infrared (FTIR) Spectroscopic studies was conducted.

Most constructed wetlands in the world are low in plant diversity or even monocultures, and one attempt to improve the role of plants in constructed wetlands is to increase the plant diversity and to construct mixed wetlands. Maximum removal of metals was observed in the water subjected to intermittent circulation of multimetal contaminated water under mixed culture of *Pistia stratiotes* and *Hydrilla verticillata.* Although due to water circulation under mixed culture, maximum removal for Cd (92.95%), Cu (90.4%), Fe (85.76%) and Cr (82.33%) was obtained, in case of monocultures highest removal was observed for Cu (78.3%), Cd (73.5%), Fe (68.5%) and Cr (65.4%) independently by *Pistia stratiotes*. Under monoculture with multimetal water circulation, *Hydrilla verticillata* was able to remove Fe (60.75%), Cu (58.4%), Cr (55.38%) and Cd (50.45%). Due to the intermittent recirculation, the number of passages of metal contaminated water through the treatment tubs increases in comparison with the non-circulating treatments where the passage occurs only once. Thus, the hydraulic retention time (HRT) in the systems becomes longer and the removal of pollutants is expected to be greater. It was observed that BCF of the plants enhanced under the effect of circulating multimetal contaminated water between cultures. While evaluating the translocation ability of *Pistia stratiotes* and *Hydrilla verticillata* plants for multimetals under different treatments, it was observed that *Hydrilla verticillata* exhibited TF >1 for all metals under selected cultures.

The water circulation coupled with mixed culture of plants had a synergistic effect on the chlorophyll and carotenoid contents of both plants. The chlorophyll and carotenoid contents increased under the mixed culture with water circulation. Plants under mono and mixed cultures treated in non-circulating conditions, the protein content significantly decreased, however, both plants under mixed culture showed an increase in the protein content until 20 days and thereafter, it decreased. Proline content in *Pistia stratiotes* and *Hydrilla verticillata* was observed to be increasing in both cultures until 25 days and

thereafter, a decrease in the proline content was observed. Proline accumulation was greater in *Pistia stratiotes* than *Hydrilla verticillata* in all treatments with a marked effect of water circulation in the accumulation of proline content.

The analysis of scanning electron microscopy of untreated and metals treated plant root and shoot parts revealed that metals was mainly localized in the root epidermis and exodermis tissues. The control plants showed a well-developed xylem. Metals were found to be concentrated in the vascular bundles and translocated to upper plant parts through xylem. Metal localization revealed that metal contents increased in the inner epidermis, cortex, and bundle cell walls of roots. Metals treated plant parts showed varied levels of ultra-morphological changes such as compactness of fronds, closed stomata, decrease in the number of stomata with considerable damages in the stomata. The presence of functional groups such as carboxylic (glutronic acids in pectin) amide (-NH)and hydroxyl (in cellulose) can act as strong binding sites for metal cations in aqueous solution by means of chelation, complexation, ion exchange etc. The major frequency band changes and shifts at (3403.4, 3365.3, 1631.5, 1321.8, 1030cm^{-1}) were observed in metals treated plant root and shoot biomass, which correspond to specific band frequencies of OH, NH, COOH. Therefore, these functional groups act as metal binding sites within the plant cells, depicting their role in metal accumulation within the plant parts.

Phytoremediation potential of *Pistia stratiotes* and *Hydrilla Verticellata* for removal of inorganic pollutants (nitrate, nitrite, ammonium and phosphate) from water

The removal of reactive nitrogenous species (Nitrate, Nitrite and Ammonium) from drinking water is a challenging problem which demands high cost technologies. Research indicates that different macrophytes have different nutrient preferences. The study was undertaken to evaluate the potential of two different aquatic macrophytes i.e., free floating *Pistia stratiotes*

and submerged *Hydrilla Verticellata* for the removal of inorganic pollutants (nitrate, nitrite, ammonium and phosphate) from water under simulated conditions.

The changes in the temperature, pH, conductivity and the removal of Nitrate, nitrite, ammonium and phosphate were observed during the study period. Fluctuations in temperature, pH and electrical conductivity were observed in simulated nutrient contaminated water treated with *P. stratiotes* and *H. verticillata* with no significant linear trend. *P. stratiotes* was observed to be better remediator than *H. verticillata* as *P. stratiotes* was able to remove 90.83% nitrate, 70.06% nitrite, 80.88% ammonium and 93.05% phosphate over the 25 days of study period. The biomass of *P. stratiotes* and *H. verticillata* increased significantly during the study period. A linear increase in the biomass of both plants was reported with decreasing concentration of nitrate, nitrite, ammonium and phosphate in the water column. Therefore, nutrient removal was positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*.

Study on consortia of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia* for removal of heavy metals from water under water circulation effects and vermicomposting of the generated plant biomass

Different aquatic macrophytes have different growth speed, growth rhythm, root morphology and distribution; hence, it could be deduced that mixed cultures of plants may have better removal rates for pollutants because of the temporal and spatial compensation in plant growth, root distribution and pollutant/nutrient preference. The effect of water circulation and consequent increased hydraulic retention time (HRT) was evaluated on selected mono and mixed culture of three different aquatic macrophytes i.e., free floating *Pistia stratiotes*, submerged *Hydrilla Verticellata* and emergent *Typha latifolia*. The generated plant biomass was disposed-off by subjecting it to vermicomposting for possible

production of a vermicompost. The nutritional and toxicological properties of the developed vermicompost were evaluated.

T. latifolia monoculture was found to be better remediator under water circulating conditions. The removal rates of metals increased significantly under mixed cultures of *P. stratiotes* and *T. latifolia* with intermittent water circulation. The consortium of all the three plants was found to be efficient mixed culture in the removal of multimetals (Cu, Fe, Cd and Cr) from water and also enhancing the better survival chances of each plant species. Highest bioconcentration factor (BCF) was observed for *Typha latifolia* (>1000), under water circulation at intervals than non-circulating conditions. The translocation factor (TF) of plants also increased significantly under intermittent circulating conditions.

Composting and vermicomposting are the best-known processes for biological stabilization of green waste by transforming them into a safer and more stabilized material (compost) that can be used as a soil conditioner in agricultural applications. The pH of different vermicompost trails of plant biomass increased significantly ($p<0.05$) over the 45 days of composting period. The nitrate, nitrite and ammonium content in different trails increased towards the end of the composting period. The highest nutrient concentration of nitrogenous species and phosphate was observed in the vermicomposting of plant biomass of *Pistia stratiotes* and *Hydrilla Verticellata* mixed culture followed by consortia of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia*. Total phosphorus was observed to be increased in final compost of monoculture of *P. stratiotes; P. stratiotes* and *H. verticillata* mixed culture and consortia of all three plants. During the process of composting, the emittance of carbon dioxide from the composted biomass lead to decreased organic carbon and organic matter at the end of the compost. In case of control trail, no significant differences were observed for the said chemical changes ($p>0.05$).

The availability of the metals to the living organisms particularly plants and microbes depend on physicochemical and biological properties of the vermicompost rather than total metal contents. Increase in the pH during the vermicomposting was most important factor for reducing bioavailability of heavy metals. The exchangeable fraction of metals was dominant in the initial plant biomass but in the final vermicompost it was converted into less mobile fractions such as reducible, oxidizable and residual. The exchangeable fraction of all metals (Cd, Cu, Cr and Fe) was reduced in all trials, except the control treatment. Therefore, it is concluded that vermicomposting of metal treated plant biomass mixed with cattle manure by using *Eisenia fetida* was very effective for enhancing the nutritional status of the developed vermicompost and reducing most bioavailable fractions (exchangeable and carbonate) and enhancing inert fraction (residual) of heavy metals.

The study presented in this thesis provides some new insights in understanding the mechanism involved in phytoremediation of contaminated water using consortia of aquatic macrophytes. Following are the specific conclusions from the present study:

- Gomti river water, an indispensable source of drinking water supply to Lucknow Municipal Corporation, was found to contain nitrate, nitrite and heavy metals (Fe>Pb>Cu>Cr>Cd), which are beyond the prescribed standards of BIS (Drinking Water Specifications) and WHO standards for drinking water.
- *Pistia stratiotes* was found to be an efficient phytoremediator for Cd from water, submerged *Hydrilla verticillata* was observed to be a good remediator of Fe and Cr under different cultures. Typha latifolia efficiently removed and accumulated Cd, Cu and Cr in mono and mixed cultures with intermittent water circulation.
- Phytoremediation with a mixed culture of *Pistia stratiotes* and *Hydrilla verticillata* coupled with intermittent water circulation was more effective in pollutants removal, than relying on a single plant species. Due to the intermittent recirculation

of water, the number of passages of contaminated water through the plant beds increases. Thus, the hydraulic retention time becomes longer and the removal of pollutants is greater.

- The growth parameters of both plants like total chlorophyll, carotenoid, protein content are the central part of energy manifestation of plants, therefore any significant alteration in their levels is likely to cause a marked effect on entire metabolism of plants. Their concentration decreased in selected mono cultures of plants, however, in the mixed culture, the concentration increased with circulation effect.
- Scanning electron microscopy of *Pistia stratiotes* and *Hydrilla verticillata* root and shoot parts revealed that metals were mainly localized in the root epidermis and exodermis tissues. Metals were found to be absorbed within the plants and translocated to upper plant parts through xylem. The presence of functional groups such as Carboxylic (-COOH), Amide (-NH) and Hydroxyl (-OH) in the FTIR spectra of *Pistia stratiotes* and *Hydrilla verticillata* depict their role as strong binding sites for metal cations and help in the sorption of metals from water column.
- Toxicity of heavy metals in the developed vermicompost depends on the different forms of metals rather than its total concentration. Due to vermicomposting of contaminated plant biomass using *Eisenia fetida*, the exchangeable forms of the metals got reduced and were converted into less mobile fractions such as reducible, organically bound and residual.