Phytoremediation Potential of Some Macrophytes for the Removal of Inorganic Pollutants from Municipal Water Sources

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ABDUL BAREY SHAH
M.Sc. Environmental Science, NET (UGC)
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DEPARTMENT OF ENVIRONMENTAL SCIENCE
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
Vidya Vihar, Raebareli Road Lucknow-226025, India
&
CSIR- NATIONAL BOTANICAL RESEARCH INSTITUTE
Rana Pratap Marg, Lucknow U.P. -226001, India

Under the Joint Supervision of

Supervisor
Dr. Rana Pratap Singh
Professor
Department of Environmental Science
BBAU, Lucknow

Co-supervisor
Dr. U N Rai
Senior Principal Scientist
Ecology and Plant Sciences Division
NBRI, Lucknow

2015

Dedicated

To My

Revered Parents

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BABASAHEB BHIMRAO
AMBEDKAR UNIVERSITY
(A Central University)
Vidya Vihar,
Raebareli Road
Lucknow-226025, India

CSIR- National Botanical
Research Institute
Rana Pratap Marg, Lucknow
U.P. -226001, India

Certificate

We have pleasure in forwarding the thesis of **Mr. Abdul Barey Shah**, M.Sc.,(NET-UGC) entitled **“Phytoremediation Potential of Some Macrophytes for the Removal of Inorganic Pollutants from Municipal Water Sources”** for the award of the Degree of Doctor of Philosophy in Department of Environmental Science of the University.

Mr. Abdul Barey Shah has fulfilled the requirements of academic ordinance of Babasaheb Bhimrao Ambedkar (Central) University, Lucknow. The thesis embodies the result of his investigation conducted during the period he worked as Ph.D. research scholar under our joint supervision.

[Rana Pratap Singh]
Supervisor

[U. N. Rai]
Co-Supervisor

Declaration

This is to certify that the material embodied in the present work entitled **"Phytoremediation Potential of Some Macrophytes for the Removal of Inorganic Pollutants from Municipal Water Sources"** is based on candidates original research work. It has not been submitted in part or full for any other diploma or degree of any University. The indebtedness of the candidate to others has been duly acknowledged at relevant places.

Name and Signature
(Candidate)

Countersigned
(Supervisor)

Countersigned
(Co- Supervisor)

Countersigned
(Head of the Department)

PREFACE

Various aquatic ecosystems which serve as an indespensible source of potable water supplies to public are getting contaminated due to discharge of inorganic pollutants particulary toxic heavy metals and nitrogenous species. The minimization of the health and environmental impacts of the presence of inorganic pollutants (heavy metals and nutrients) in aquatic systems requires the application of different treatment processes. This has necessiated the need for economically viable and effective technologies in order to supply safe drinking water and discharge wastewater, that preserves precious natural resources and biological lives. Phytoremediation is one new approach that offers more ecological benefits and a cost-effective alternative. Though, a cheaper method, it requires technical strategy, expert project designers and with field experiences that choose the proper species and cultivators for particular toxic pollutants (metals, nutrients, organics) and regions. A multidisplinary research effort that integrates the work of natural sciences, engineering techniques and policy makers is essential for greater success of green technologies as a potent tool for management of inorganic pollutants in aquatic ecosystems.

Regular monitoring of public water supplies and sources play an important role in minimizing the health hazards due to water contamination. Our study indicates that the application of consortia of macrophytes under mixed cultures was found to extract higher metals and other inorganic pollutants from water. Further, increasing the hydraulic retention time of water by intermittent circulation of water within the designed treatment system was effective for optimum removal of pollutants. In addition, the vermicomposting of the contaminated plant biomass was found to be an integrated approach for stabilizing the contaminated biomass and its safe disposal after water treatment and purification.

Abdul Barey Shah
(M.Sc., UGC-NET)

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Abdul Barey Shah

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List of Abbreviations

ANOVA	Analysis of Variance
cm	Centimeter
ºC	Degree Centigrade
DW	Dry Weight
g	Gram
hr	Hour
mg g^{-1}	Milligram per gram
µg g^{-1}	Microgram per gram
nm	Nanometers
S.D	Standard Deviation
%	Percent
ppm	Parts per million
UV	Ultraviolet
Kg	Kilogram
Fe	Iron
Cd	Cadmium
Cu	Copper
Cr	Chromium
Pb	Lead
DO	Dissolved Oxygen
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
SEM	Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectrophotometer

Chapter 1

Introduction

Introduction

Pure and safe drinking water is an essential and basic need to sustain life and maintenance of human health (Mudiam *et al.*, 2012; Wang and Yu, 2014). Aquatic ecosystems like rivers, lakes, ponds etc., which act as an indispensable source of drinking water for most of the municipal corporations are getting degraded due to discharge of untreated wastewater, overexploitation of water resources, construction of dams and reservoirs, building of flood plains, channels, hydraulic engineering projects, deforestation and introduction of exotic plant and animal species (Wang *et al.*, 2010; Lojko, 2015).

A serious concern in the developing world including India, is the increasing developmental activities in watershed, such as changing land use patterns, increasing discharge of untreated municipal and industrial wastewater, mining activities and runoff from nearby agricultural fields into aquatic ecosystems (Li *et al.*, 2009; Sekomo *et al.*, 2011; Rai *et al.*, 2012), lead to pollution buildup in water resources with hazardous chemicals like heavy metals, nitrates, nitrites and phosphates etc. (Nweke 2009, Rai 2012, Khan *et al.*, 2014). These discharges containing metals and other inorganic pollutants of variable toxicity into the river bodies poses a serious threat not only to the aquatic ecosystem but also to human health (Teixeira *et al.*, 2014). Increased rate of diseases outbreaks both in developed and developing regions have long been recognized due to poor potable water supplies to public (WHO, 2009) and in addition, poor water quality and scarcity can stifle a nation’s economy, negatively impact the environment and fuel conflicts (Rai, 2010; Pham *et al.*, 2011).

The provisions of clean drinking water and its normalized supply play an important role for decreased mortality and improved economic progress in developing countries (Koc, 2010). Monitoring of drinking water at their sources and consumers end is of prime importance for generating databases on the features and chemical characteristics of potable water that can help minimize the health hazards to a large extent (Cieszynska *et al.,* 2012; Faridi *et al.,* 2012). The presence of various inorganic pollutants like toxic heavy metals (Cd, Cu, As, Hg, Cr, Pb, Zn etc.), nitrate, nitrite, ammonium in water need to be removed prior to supply for human and animal consumption. The conventional technologies for water treatment currently at hand are too costly and require energy inputs for their operation (Chalew *et al.,* 2013; Hanks *et al.,* 2015). However, plants and microbes can remove them by extraction, degradation and biomagnification. Use of aquatic plants for water treatment is considered as a sustainable as well as energy efficient and economic process for removal of contaminants from water.

1.1. Inorganic Pollutants in water and their effects on human health

Among the various pollutants, heavy metallic pollution of biospheric media (air, water and soil) is threatening the globe because of their hazardous impacts on life (Mirza *et al.,* 2010; Vesely *et al.,* 2011; Rofkar *et al.,* 2014). “Heavy metal” is a collective term for a group of metals and metalloids with atomic density greater than 4000 kg m^{-3} (Hashim *et al.,* 2011). Although, certain heavy metals are essential for living beings as micro nutrient, however, at higher concentrations these are very toxic and may lead to acute poisoning. Constrains associated with heavy metallic pollution include their non-biodegradability, persistence in the environment, bioaccumulation and biomagnification within the food chains (Singh and Kalamdhad, 2013; Chaudhuri *et al.,* 2014). Heavy metals released by human activities into the environment beyond toxic limits are progressively finding their fate into water bodies thereby making the water unfit for drinking (Zhang *et al.,* 2009; Ishaq and Khan, 2013; Nazeer *et al.,* 2014). River beds act as sinks of heavy metals, as sediments absorb metals in higher quantities than those found in the water column. With the chemical changes in water column and also due to changes in sediment conditions, these metals are released back into the water, thereby acting as secondary source of pollution eventually leading to contamination of entire water column (Huang et al., 2009; Liang et al., 2015). Consumption of water contaminated with heavy metals may lead to their chronic accumulation of in the kidneys, liver, bones of humans resulting in disruption of metabolic activities, leading to cardiovascular, neurological, renal, and bone diseases (US EPA 2001; Jarup, 2003; Johri et al. 2010, Bakirdere *et al.*, 2013; Pourang and Noori, 2014).

Metals such as Hg, Cd, Cr, Cu, Pb, As, Zn and Sn are of great concern due to to their ecotoxicological nature (Ali *et al.*, 2013; Gill *et al.*, 2014). The heavy metals in their ionic forms are most toxic as their stable oxidation states like Cd^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, As^{3+} , Cr^{6+}, etc. react with body's biomolecules and form extremely stable biotoxic compounds which are hazardous to health and are difficult to dissociate (Duruibe *et al.*, 2007). Characteristically, the nature of effect due to heavy metals can be Toxic (acute, chronic, sub-chronic), carcinogenic, mutagenic, neurotoxic or teratogenic (Arora 2008; Gupta et al. 2012), however, their toxicity and targeted organs vary from metal to metal and also depend upon the level of intake, strength of body immune system and duration of exposure (Dieter *et al.*, 2005; Khan *et al.*, 2014). For example, Cd has been classified as human and animal carcinogenic even at very low concentrations and may cause osteomalacia, severely damage the lungs, kidneys and liver (Satarug et al., 2010; Xie et al., 2013); likewise, Cr (VI) inside the living bodies can cause cancer, mutations by destroying DNA protein cross-links, breakage of DNA strands (Mc Carrol et al., 2010; Liu et al., 2014); excessive levels of Cu can result in liver cirrhosis, chronic anemia, gastrointestinal distress (Mandour, 2012; Alam et al., 2012).

The elevated levels of nitrogenous inorganic pollutants (nitrate, nitrite, ammonium) and inorganic phosphates in surface water sources like rivers is due to runoff from agricultural fields, discharge of municipal/industrial sewage etc. lead to many health hazards (Kumari *et al.,* 2013). Inorganic pollutants like nitrate, nitrite, ammonium and phosphate etc. can be assimilated by plants but not by zooplanktons, animals and human beings. Nitrogenous ions especially NO^{-}_{2} can combine with organic pollutants to produce cancer causing nitrosyls and is a big health hazard (Kim Shapiro et al. 2006; Rawat et al. 2012, Duplay et al. 2013). Nitrate-N levels in drinking water of 10mg/l or greater have been linked to methaemoglobinaemia or blue baby syndrome in infants and gastric carcinomas (Tank and Chandel, 2010) and non-Hodgkin's lymphoma in humans (Rao and Puttana, 2000; Rawat and Singh, 2009). Nitrite in human body may lead to the formation of carcinogenic nitrosamine upon reaction with secondary amines, thus posing the risk of stomach, liver and esophageal cancer (Kim Shapiro *et al.,* 2006) and high concentration of ammonium in body can lead to convulsion, coma and even death (Rawat *et al.,* 2012).

1.2. Conventional techniques for water pollution remediation

Being a developing country, India is not having advanced treatment processes and provisions at municipal water treatment plants for the removal of hazardous inorganic pollutants like heavy metals and reactive nitrogen species from potable water prior to public supply. The various conventional techniques e.g. reverse osmosis, ion exchange, coagulation, chemical precipitation, membrane filtration, land application etc. (Mishra and Tripathi, 2008; Pal and Rai, 2010; Dhir and Srivastava, 2011) employed for heavy metal removal are expensive, require high energy and do not completely remove the heavy metals from the water column (Memon and Schroden, 2009) and excessive nutrients (Manuela *et al*., 2010), but merely transfer the pollutants from the wastewater to a sludge residue which is disposed of by land filling (Tolu and Atoke, 2012) whereby the pollutants eventually find their way to fresh water supplies there by contaminating it and thus, do not provide acceptable solution to pollution problem (Li et al., 2010). From an ecological and economic point of view, the need for an alternative ecofriendly and cost-effective technology is recommended as the cleanup of hazardous wastes (Liu et al., 2014). This has necessitated the development and use of alternative, low cost, energy efficient and sustainable techniques for optimal removal of toxic heavy metals from the polluted waters (Vymazal, 2009; Sheng et al., 2013). The application of bio-techniques coupled with engineering methods have recently been gaining attention and are being emphasized for remediation and protection of environment (Uysal and Taner; 2009; Arini et al., 2012; Luca et al., 2013).

1.3. Phytoremediation as an ecofriendly and sustainable water treatment technique

For the efficient management and remediation of water in rivers, the status of water quality and the changes produced therein due to anthropogenic activities is the first step towards establishing effective cleaning system. Aquatic biodiversity is a significant component of aquatic ecosystem and get affected by the quality of water. Various aquatic macrophytes (floating, submerged, rooted and emergent) growing in river courses have shown the potential to accumulate certain toxic pollutants inside their tissues and used to clean the pollution levels (Rai and Tripathi 2009; Souza et al. 2013; Kamel 2013). The plants are differentially tolerant to different inorganic pollutants as they have the capacity to assimilate or scavenge them. These potentials of plants have emerged as a major area of phytotechnological studies and have been evaluated for phytoremediation potential for the removal of toxic pollutants from contaminated soil and water (Rai 2010; Tolu and Atoke 2012; Rawat et al. 2012; Baudhh and Singh 2012).

Phytoremediation that makes use of plant systems to remove and/or detoxify pollutants is gaining global interest by using selective, improved and value added aquatic plants. The macrophytes based treatment systems are low-cost, ecofriendly and aesthetically pleasing technologies and can be used by developing countries for recycling and treatment of wastewater, especially contaminated with heavy/toxic metals, as well as for the treatment of potable water before supply to people (Wang et al. 2002; Jayaweera et al. 2008; Lu et al., 2008; Khan et al. 2009; Rahman and Hasegawa 2011). The selection of appropriate plant species for this process is key component. From phytoremediation perspective, plants should display, (i) high uptake rate; (ii) tolerance to high concentration of metals; (iii) high translocation to shoot system; (iv) efficient system to tolerate high metal levels in plant parts (Singh *et al.* 2006, 2010; Tripathi *et al.* 2007).

Phytoremediation of polluted waters using aquatic macrophytes is recognized as ecofriendly, cost effective and energy efficient technology employing both the biological processes of plants and engineering tools for effective remediation (Bindu *et al.,* 2008; Yadav *et al.,* 2010; Abou-Elela *et al.,* 2013; Upadhyay *et al.,* 2014). The biological mechanisms of phytoextraction and phytofiltration of metals were reviewed by (Raskin *et al.,* 1997; Wu *et al.,* 2010 and Maestri *et al.,* 2010). A review of several field-scale applications shows that phytoremediation is at least 50% less expensive than excavation and it is also cheaper than bioremediation (Vangronsveld et al., 2009). The technology can be used to clean up excessive nutrients, toxic metals, pesticides, solvents, explosives, crude oil, poly aromatic hydrocarbons and landfill leachates (Rawat and Singh, 2010; Bauddh and Singh, 2012a,b, 2015; Sood et al., 2012; Kumar et al., 2013; daSilva et al., 2014). Aquatic macrophytes have been reported to accumulate heavy metals from natural ecosystems (Muzamdar and Das 2014; Shah *et al.,* 2015) as well as from the constructed wetland systems (10000) times greater than in the surrounding water column (Weiss et al., 2006; Yilmaz and Akbulut 2011; Huguenot et al., 2014).

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 (Maltais- landry et al., 2009; Yilmaz and Akbulut, 2011; Farid et al., 2014; Foladori et al., 2014). However, studies on the potential of mono and mixed culture of free floating and submerged plants for removal of multi metals under the effect of sequential processes of circulation is not well documented. Further, developing the knowledge of metal binding sites within the plants, metal localization and class of compounds in which the metal are transported and distributed inside the plant parts needs to be extensively discoursed (Malik, 2007; Ibrahim et al., 2009; Shuvaeva et al., 2013).

One of the main concerns regarding phytoremediation is the biomass disposal after phytoextraction processes (Gomes, 2012). Therefore, efficient methods of disposal are needed. Composting is one of such methods. It enables recycling of organic matter as well as of the nutrients present in wastes. The composting product should meet some requirements in order to be applied for agricultural purposes. It should contain sufficiently high amounts of nutrients (organic matter, N, P, and K) and limited contents of toxic elements. Vermicomposting has been considered as a sustainable, potential and cost effective alternative for the treatment and disposal technique for contaminated plant biomass (Gupta *et al.,* 2007; Gomes 2012; Singh and Kalamdhad, 2013). Through vermicomposting, earthworms ingest, grind and digest organic waste and finally converting it into a much finer, humified, microbially active material by the cooperative action of earthworms and microorganisms (Khwairakpam and Bhargava, 2009).

Heavy metals in composts exist in various chemical forms, which determine their bioavailability, toxicity and transport in the environment. The final content and form of heavy metals is of prime importance. Hence, speciation of heavy metals should be an additional criterion for compost evaluation. It is necessary to carry out speciation analysis. The sequential extraction makes it possible a more precise definition of the forms in which metals exist in compost and a knowledge of the mechanisms involved in the release of the elements to the environment under natural conditions. The mobility and bioavailability of heavy metals and their related eco-toxicity to plants depend strongly on their specific chemical forms or ways of binding rather than total metal concentration (Singh and Kalamdhad, 2013). Sequential chemical extraction can be used to investigate the speciation of heavy metals in various wastes and its compost since it can provide a more accurate measure of risk to the environment than that of total metal concentration obtained after strong acid digestion (Singh and Kalamdhad, 2012a).

Keeping the above perspectives in consideration, following specific objectives were planned for the present study

1. Monitoring and analysis of drinking water of Lucknow city at the municipal water source and users end in order to assess the pollution load with special focus on inorganic (e.g., Nitrate, Nitrite, Ammonia, Phosphate and Heavy Metal) pollutants.
2. Survey of local water bodies for identification and screening of the aquatic plants best adapted for the sequestration, translocation and accumulation of inorganic pollutants like heavy metals e.g. Fe, Cu, Cd, Cr, Pb and inorganic pollutants like Nitrate, Nitrite, Ammonia and Phosphate.
3. To study the phytoremediation potential of selected aquatic macrophytes in mono and mixed cultures for the removal of heavy metals and the other inorganic pollutants from water under simulated conditions.
4. To study the 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.
5. To study the effect of water circulation and hydraulic retention time on removal potential of finally selected plant or plant consortium.
6. Vermicomposting of the contaminated plant biomass and evaluation of its nutritional and toxicological properties to develop low cost and ecofriendly disposal method.

Chapter 2

Review of Literature

Today, water availability both in terms of quality and quantity is a problem all over the world. Mainly water stressed developing countries are experiencing the worse of this problem as the world population and industrialization is increasing and climate change is affecting water resources (EPA, 2000). As far as quantity is concerned, many countries have conflicts and others undergone bilateral/multilateral agreements for water sharing and distribution (Flint, 2004; Rai, 2012). With the enactment of several water legislations and guidelines worldwide coupled with the need for environmental sustainability has necessitated the need for several stringent regulations for drinking water supply and wastewater discharge**.** Wastewater discharges are causing eutrophication and water borne diseases. The situation is getting worse with rapid urbanization where adequate sanitation and wastewater treatment facilities are lacking. Industrialization has contaminated the environments with heavy metals, particularly, in developing countries of the world, where advance treatment technologies are neither available nor affordable (Hooda, 2007)

Since Middle Ages due to human activities, natural aquatic ecosystems, particularly the estuaries and freshwater systems, are not only being polluted to varying degrees, but are also condemned to fairly long-term pollution due to metals deposited in sediments. The heavy metal load from domestic wastewater and sewage alone signifies that this will be a continuing problem for science and humankind. Water in rivers and lakes can become heavily polluted, depending on the volume flow and its proximity to point sources. Toxic metal contamination of aqueous water streams and groundwater poses a major environmental and health problem that is still in need of an effective and affordable technological solution. To achieve unpolluted drinking water and management of water sources, several technologies and processes for the removal of persistent inorganic toxicants including nitrate, ammonia, phosphates and heavy metals are currently in use. These methods include ion exchange, chemical precipitation, disinfection, adsorption by activated carbons, reverse osmosis, nano-filtration and sewage treatment plant (STPs) processes. Most of these methods are expensive, require high energy and are not able to completely remove the heavy metals (Memon and Schroden, 2009) and excessive nutrients (Manuela *et al.*, 2010), but merely transfer the pollutants from the wastewater to a sludge residue which is disposed of by land filling (Tolu and Atoke, 2012) whereby the pollutants eventually find their way to fresh water supplies there by contaminating it and thus, do not provide acceptable solution to pollution problem. From an ecological and economic point of view, the need for an alternative ecofriendly and cost-effective technology is recommended as the cleanup of hazardous wastes by conventional technologies is projected to cost at least $400 billion in the United States alone, (Salt *et al.*, 1995). Methods using living wetland plants to remove metals from water appear to be an alternative. Plants that have a high metal-bioaccumulation capacity and a good tolerance to high metal concentrations over long periods of time are necessary.

Over the past fifteen years phytoremediation technology became an effective method of environment clearing due to the plants ability to accumulate the contaminants at the concentration level thousands times higher than background one. The plants are capable to remove many toxic substances from reservoirs, acquiring the pollutants from waters as food elements, basically through root system using the products of decomposition for their viability (Watai *et al.* 2004). Water plants actively accumulate Pb, Cd, Cu, Fe, Mn, Zn, Cr, As, Ni etc., and other elements and substances. The plants are more tolerant to the inorganic contaminants as they have differential capacity to assimilate or scavenge degradable and non-biodegradable inorganic contaminants. These potentials of plants have emerged as a major area of phytotechnological studies and they have been studied for the phytoremediation potential for removal of toxic contaminants from contaminated soil and water (Rai, 2010; Tolu and Atoke, 2012; Rawat *et al*., 2012; Baudhh and Singh, 2012, Brazini et al., 2012; Vymazal and Svehla, 2013).

2.1. Pollution of Heavy metals in the environment and their toxicity to the living organisms

Heavy metals in surface water systems can come from natural or anthropogenic sources. Currently, anthropogenic sources of metal pollution exceed the natural inputs. Main sources of metal pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, sewage, pesticides, and fertilizers. Energy-production technology and environmental pollution are intimately linked with each other. Energy-intensive processes and chlor-alkali industries for the manufacture of agrochemicals deteriorate the water quality of lakes and reservoirs due to the discharge of various pollutants; especially a range of heavy metals (Rai *et al.*, 2007). Heavy metals constitute a heterogeneous group of elements; having a specific gravity greater than 4.0 and a relatively high density (approximately 5 g/cm3) as their common characteristics (Clijsters et al., 1999). From a general biological as well as plant physiological point of view, essential and non-essential heavy metals can be distinguished. Living organisms require trace amount of some heavy metals, these includes cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), strontium (Sr), vanadium (V) and Zinc (Zn), and they are referred to as essential heavy metals. Non-essential heavy metals of particular concern in the environment include cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), and silver (Ag), (Kennish, 1992). Essential heavy metals play essential roles as components of metalloproteins, as cofactors in enzymatic catalysis, and in a wide array of other cellular processes. At supra-optimal concentration however, they become phytotoxic, induce leaf chlorosis, and reduce growth. At least 20 metals are classified as toxic with half of them emitted into environment in concentrations that pose great risks to human health. The common heavy metals that have been identified in polluted water include arsenic, copper, cadmium, lead, chromium, nickel, mercury and zinc. The danger of heavy metal pollutants in water lies in two aspects of their impact. Firstly, heavy metals have the ability to persist in natural ecosystems for an extended period. Secondly, they have the ability to accumulate in successive levels of the biological chain, thereby causing acute and chronic diseases. For example, cadmium and zinc can lead to acute gastrointestinal and respiratory damages to brain, heart and kidney damages (Nomanbhay and Palanisamy, 2005; Lone et al., 2008). The use of domestic and industrial effluents, which may contain high concentrations of heavy metals on agricultural lands, is a common practice in some parts of the world. These toxic metals, when concentrated on plant tissues can have damaging effects on the plants themselves and may also pose health hazards to man and animals (Athar and Ahmad, 2002). Episodes of the metal pollution such as Minamata Episode due to Methyl mercury, Itai- itai or Ouch Ouch due to Cadmium have taken toll on human populations. Another reason that toxic heavy metals are causing potential concern is that the metals may be transferred and accumulated in the bodies of animals or human beings through the food chain, which potentially causes DNA damage and carcinogenic effects caused by their mutagenic ability (Knasmuller *et al.*, 1998). Examples include Cd, Cr, and Cu, which have been associated with health effects ranging from dermatitis to various types of cancer (Das, Samantaray, and Rout, 1997; McLaughlin, Parker, and Clark, 1999). In addition, some metals occur in the environment as radioactive isotopes (*e.g.*, U238, Cs137, Pt239, and Sr90), which can greatly increase the health risk (Pilon-Smits and Pilon, 2002)

Table 2.1 lists some of the toxic heavy metals with their harmful effects. (Wang et al., 2004; Johnson and Hallberg, 2005; Gardea-Torresdey et al., 2005 Silvia et al., 2006; Baldisserotto et al., 2007 Lon et al.,2008; Singh et al., 2009; Rawat et al., 2009, 2012 ; Apkor and Muchie, 2010, Kumar, 2013).

Table2.1. Effect of more toxic heavy metals reported in aquatic ecosystem on human health and plants

Heavy metal	Effect on Man	Effect on Plants
Cadmium	Damage to brain, Gastrointestinal and respiratory problems, Kidney and Liver damage	Decreases seed germination, lipid content, and plant growth; induces phytochelatins production
Arsenic	Cutaneous and Visceral Malignancies, Black Foot Disease, Severe Vomiting, Diarrhoea	Biochemical dysfunction at cellular level, damage to proteins and lipids.
Lead	Kidney damage, Heart ailments, Reproductive problems, Bone weakness	Reduces chlorophyll production and plant growth; increases superoxide dismutase
Mercury	Foetal Brain damage, Damage to Kidney, Lungs, Heart, Neurological problems	Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulates phenol and proline
Chromium	Haemolysis, renal and liver failure, Allergies, Dermatitis, Foetal deaths, Lung cancers	Decreases enzyme activity and plant growth; produces membrane damage, chlorosis and root Damage
Copper	Gastrointestinal distress, Liver or kidney damage (Long term exposure)	Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area
Iron	Increased pulse rates and respiration, hypertension, drowsiness, congestion of blood vessels	
Zinc	Vomiting, renal damage, cramps	Reduces Ni toxicity and seed germination; increases plant growth and ATP/chlorophyll ratio
Nickel		Reduces seed germination, dry mass accumulation, protein production, chlorophylls and enzymes; increases free amino acids
Manganese	Growth retardation, fever, sexual impotence, muscles fatigue, eye blindness.	Brown spots on mature leaves, intervienal chlorosis and necrosis, deformation of young leaves aand growth retardation

Since heavy metal pollution affects the quality of drinking water supply and wastewater discharge, there by affecting both human and plant lives, great efforts have been made in the last two decades to reduce pollution sources and remedy polluted water resources. Though various technological advancements have been made to remove pollutants from water but certain drawbacks and limitations are associated with them. Table 2, lists the advantages and disadvantages of conventional methods of water treatment (Rai, 2009, Apkor and Muchie, 2010, Barkat, 2011).

Table 2.2 Conventional Methods of Water Treatment

Methods	Advantages	Disadvantages
Chemical precipitation	Convenient, self-operation, low maintenance, low capital cost	Replenishment of chemicals, requirement of extra coagulation and flocculation, toxic sludge generation
Coagulation- flocculation	Settlement of suspended solids in less time, improved sludge settling	Extra operational cost for sludge disposal
Ion exchange	Less time consuming, no sludge generation, high metal removal efficiency, better performance in acidic pH range	Less suitable as few metals are not exchangeable through ion exchange resins, high capital cost
Reverse osmosis	Greater ionic species removal, can also operate at high temperatures, reduces the concentration of dissolved organic compounds	Expensive to procure and operate, elevated pressure makes the technique costly and sensitive to operating conditions
Nanofiltration	Operates at low pressures than reverse osmosis	Costly, membrane fouling

2.2. Phytoremediation of Aquatic ecosystems: A novel, cost effective and ecofriendly technique.

Phytoremediation, the use of plants to remove pollutants from the environment, is a growing field of research in environmental studies because of the advantages of its environmental friendliness, cost effectiveness and the possibility of harvesting the plants for the extraction of absorbed contaminants such as metals that cannot be easily biodegraded for recycling among others(Maine et al., 2004, Skinner et al., 2007, Malik, 2007). Over the last two decades, phytoremediation has become an increasingly recognized pathway for contaminant removal from water and shallow soils and is an aesthetically pleasing, solar-driven, passive technique useful for remediation of shallow plumes with low to moderate levels of contamination (EPA, 2001, Wang et al., 2011). Aquatic macrophytes, which play important roles in aquatic ecosystems, have shown great potential to sequester selected heavy metals and nutrients through their root systems and by uptake through their plant bodies. It has been reported that these plants can accumulate heavy metals 100,000 times greater than in the associated water (Mishra and Tripathi, 2008). Therefore, they have been used for heavy metal and nutrient removal from a variety of sources (Hassan *et al*., 2007; Mishra and Tripathi 2008; Rai, 2010, 2012, Rawat and Singh, 2012). Phytoremediation exploits plant's innate biological mechanisms for the removal of contaminants from the environment for human benefit. The phytoremediation technique relies upon the following processes:

2.3. Phytodegradation

Phytodegradation mainly removes the organic contaminants in the environment by internal and external metabolic processes driven by the plant. It involves the use of plants to uptake, store and degrade contaminants within its tissue. In this process plants metabolize and destroy contaminants within their tissues. Plant enzymes play a significant role in the breakdown of organic pollutants (Newman and Reynolds,2004). During phytodegradation, the plants are able to take-up metal contaminants directly from the soil/water or release exudates that help to degrade pollutants via co-metabolism in the rhizosphere.

2.4. Phytoextraction

This method is used primarily for wastes containing metals whereby plant roots absorb, translocate and store contaminants along with other nutrients and water. Metal compounds that have been successfully phytoextracted include, zinc, copper, nickel, lead, cadmium, arsenic, chromium. The process of phytoextraction is known to be occur either continuously (natural) using hyper-accumulators or induced through the addition of chelates such as EDTA to increase the bioavailability of metals (Utmazian and Wenzel, 2006). Researchers have also realized that phytoextraction can be used for the recovery of precious metals such as gold, silver, platinum, and palladium, which indicates the wide possibilities of the phytoremediation technology with regards to mining (Gardea -Torresdey et al., 2005).

2.5. Rhizofiltration

Usually aquatic plants perform this process. The hyperaccumulating aquatic plants adsorb and absorb pollutants from aquatic environments i.e., water and wastewater (Rahman and Hasegawa, 2011). A suitable plant for rhizofiltration applications can remove toxic metals from solution over an extended period of time with its rapid-growth root system. A variety of plant species have been found to be effective in removing toxic metals such as As, Cu, Cd, Cr , Ni, Pb and Zn from aqueous solutions (EPA, 2001; Rai,2012.,).

2.6. Phytovolatilization

This phytoremediation technique is the plants ability to take up toxic metals from the growth matrix and subsequently transform and volatilize them into the atmosphere through its leaves. There is the transformation of pollutants within the plant body, as the water travels along the plant's vascular system from the roots to the leaves, whereby the contaminants evaporate or volatilize into the air surrounding the plant. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations (Ghosh and Singh, 2005).

2.7. Phytostabilization

Phytostabilization, also referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges (EPA, 2000). The process of phytostabilization depends on the tolerance ability of a plant to a contaminant.It is the use of plant roots to limit metal mobility and bioavailability in the soil. During the process, contaminant are absorbed and accumulated by roots, adsorbed onto the roots, or precipitated in the rhizosphere. When this happens, there is the prevention of mobility of the contaminants, hence reducing their availability in the food chain (Lasat, 2000; Jada and Fulekar, 2009).

2.8. Role of Constructed wetlands in phytoremediation of polluted water

The Ramsar Convention on Wetlands, 1997 defines wetlands as the areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters. Wetlands encompass a broad range of ecosystems, from submerged coastal grass beds to salt marshes, swamp forests, and boggy meadows. In general, the term “wetlands’ refers to transition zones between terrestrial and aquatic systems with soil saturated with water for at least part of the year or covered by shallow water along with characteristic wetland plant species (Kalff, 2002). Wetland ecosystems act as natural filters and have been effectively used for the treating wastewater; and removing toxic chemicals and heavy metals through absorption by plants. Certain wetlands are being engineered and have been designed and constructed to utilize natural processes involving wetland vegetation, soil, and the associated microbial assemblages to assist in treating wastewaters. They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment. They are referred to as constructed wetlands. The use of constructed wetland system is a reasonable option for treating contaminated water by simulating natural wetlands, owing to lower cost, fewer operation and maintenance requirements, and little reliance on energy inputs (Varnell et al., 2009). Constructed wetland (CW) is a biogeochemical and highly efficient system to treat polluted waters generating from different sources such as domestic, highways, mining and industrial sectors and offer an effective alternative for traditional wastewater treatment systems (Khan et al., 2009). Constructed wetlands have been used for a variety of purposes, from rehabilitating areas where wetlands were previously located, to serving very specific functions such as wastewater treatment (Hawkins et al., 1997).This system has been found to be able to remove various pollutants and nutrients from wastewater (J. Vymazal 2007,T Bindu et al., 2008) and has also been successfully used to treat wastewater with high concentrations of nutrients (Gottschall et al., 2007, Haiming et al., 2011). Much interest has been focused on constructed wetlands for removing toxic metals from wastewater and drinking water sources in recent years (Hadad et al., 2006; Maine et al., 2006; Jayaweera et al., 2008).The CW system contains natural processes of aquatic macrophytes that not only accumulate pollutants directly into their tissues but also act as catalysts for purification reactions usually occur in the rhizosphere of the plants (Jenssen et al., 1993). In constructed wetlands, substrate interactions remove most metals from contaminated water (Walker et al., 2002, Liu et al., 2007). The permanent or temporarily anoxic condition in wetland soil helps to create an environment for immobilization of heavy metals in the highly reduced sulfite or metallic form and plants may play an important role in metal removal through filtration, adsorption, cation exchange, and root-induced chemical changes in the rhizosphere (Jainguo et al., 2007, Liu et al., 2007). Numerous factors including pH of water and sediment, mobilization and uptake from the soil, compartmentalization and sequestration within the root, efficiency of xylem loading and transport (transfer factors), distribution between metal sinks in the aerial parts, sequestration and storage in leaf cells, and plant growing and transpiration rates can also effect the remediation processes of the contaminated sites (Hadad et al., 2006; Khan et al., 2006). Most constructed wetlands for wastewater treatment are planted with emergent macrophytes but the design of the systems in terms of media as well as the flow regime varies. The most common systems are designed with a horizontal subsurface flow (HF constructed wetlands), but vertical flow (VF constructed wetlands) systems are becoming more popular (Vymazal, 2005).Among the different types of CWs, Horizontal Sub-surface Flow Constructed Wetlands (HSSFCWs) are most widely used and became low-impact alternatives to more conventional wastewater treatment processes.In a typical HSSFCW, wastewater is maintained at a constant depth and flows horizontally below the surface of the bed has been proven to be efficient in removing pollutants, organic matter and pathogens. Table 3.3 lists some common aquatic macrophytes used for heavy metal removal from aquatic ecosystems. (Liao and Chang, 2004; Prasad *et al*.,2005; Hadad *et al*.,2006; Rai,2007; Rai and Tripathi, 2007a, 2009; Rehman *et al*., 2008; Zhang *et al*., 2009; Dilshad *et al*., 2010; Abida and Hari, 2010; Amin Mojiri, 2011; Usman *et al*., 2011; Hamizah Mokhtar *et al*., 2011; Hegazy *et al*., 2011).

Table 2.3 Some common heavy metal accumulating aquatic macrophytes

Aquatic Macrophytes	Heavy Metal Accumulation
Azolla fililiculoids	Cr, Ni, Zn, Fe, Pb, As, Hg, Cd
Azolla pinnata	Cd, Cu, Zn, Hg
Ceratophyllum demersum	Cu, Cr, Pb, Hg, Fe, Mn, Zn, Ni
Eichhornia crassipes	Cd, Pb, Cu, As, Ni, Cr, Zn, Hg, Co, Al
Hydrilla verticillata	Cu, Hg, Fe, Ni, Pb
Lemna spp.	Pb, Mn, Cu, Cd, Cr, Hg, Ni, Fe
Mentha aquatica	Cd, Zn, Cu, Fe, Hg
Nymphaea alba	Cr, Cd, Pb, Ni, Zn, Mn, Fe,Co
Phragmitis australis	Fe, Mn, Zn, Cu
Potamogeton crispus	Cu, Pb, Mn, Fe, Cd
Salvinia spp	Cu, Fe, Ni, Zn
Spirodela polyrrhiza	As, Hg
Typha domingensis	Fe, Mn, Zn, Al, Ni
Wolfia globosa	As

Chapter 3

Material and Methods

Material and Methods

3.1. Study Area

Lucknow, the capital of Uttar Pradesh (26^o5/N latitude, 80^o56/E longitude, 128 m above the sea level), is spread over an area of 310 km^2 in the central plain of the Indian subcontinent, supporting a population of 36.50 lakh. In India, surface water and groundwater are the main sources of drinking water. Gomti river, a tributary of Ganga River, originates from Fulhar lake near Pilibhit flowing through the Lucknow city meandering for about 12 Km, is a major freshwater ecosystem in India.

The prime source of drinking water in Lucknow city is Gomti river. The water quality of the Gomti river is a major concern for the population of Lucknow. In recent years, Lucknow city has experienced an impressive population growth with expansion in all directions. Drains from all over the city carry and discharge huge loads of industrial effluents, domestic waste, agricultural runoff, sewage, medicinal wastes, washing of clothes and throwing of carcasses of half brunt dead bodies, etc. into river Gomti, resulting a variable increase in the forms and levels of inorganic pollutants such as nitrate, nitrite, phosphate, ammonium, Cd, Pb, Cr, Fe, Cu etc. in the river water and sediments. About 26 drains situated between Gaughat (upstream of Lucknow) and Pipraghat (downstream of Lucknow) discharge about 200-250 MLD of untreated sewage of urban effluent, including municipal and industrial wastewater into Gomti River (Shivani *et al.*, 2011).

Sewage treatment plants (STP), Mohan Meakins (Brewery), Hindustan Aeronautics, Cooperative Milk Dairy, Everyday Flash Light and Telco etc., are certain industrial establishments operating in Lucknow, effluents from these are carried by the drains and these drains dump huge quantities of untreated sewage/effluent into river water body. Sampling station at Gaughat of river Gomti which is the source of raw municipal water supply for Lucknow city along with seven residential sampling sites which are the locations of municipal water supply for various residential areas of Lucknow were chosen for the monitoring of drinking water. Residential areas included Aminabad, Indira Nagar, Aishbagh, Nishatganj, Telibagh, Hazratganj and Charbagh. Further Gomti river at Lucknow city was divided into upstream and downstream sites. Six sites along with the bank of river Gomti were selected for water and plant samples collection from Gaughat (upstream) upto Pipraghat (downstream). Gaughat and Pucca Pull Sites are categorized as upstream sites, while as Hanuman Setu, Nishatganj, Gomti Barrage and Pipraghat sites are categorized as downstream sites.

Experiments under simulated conditions were conducted in NET House of Department.

Plate 3.1. Plant based treatment system designed in NET House of Department

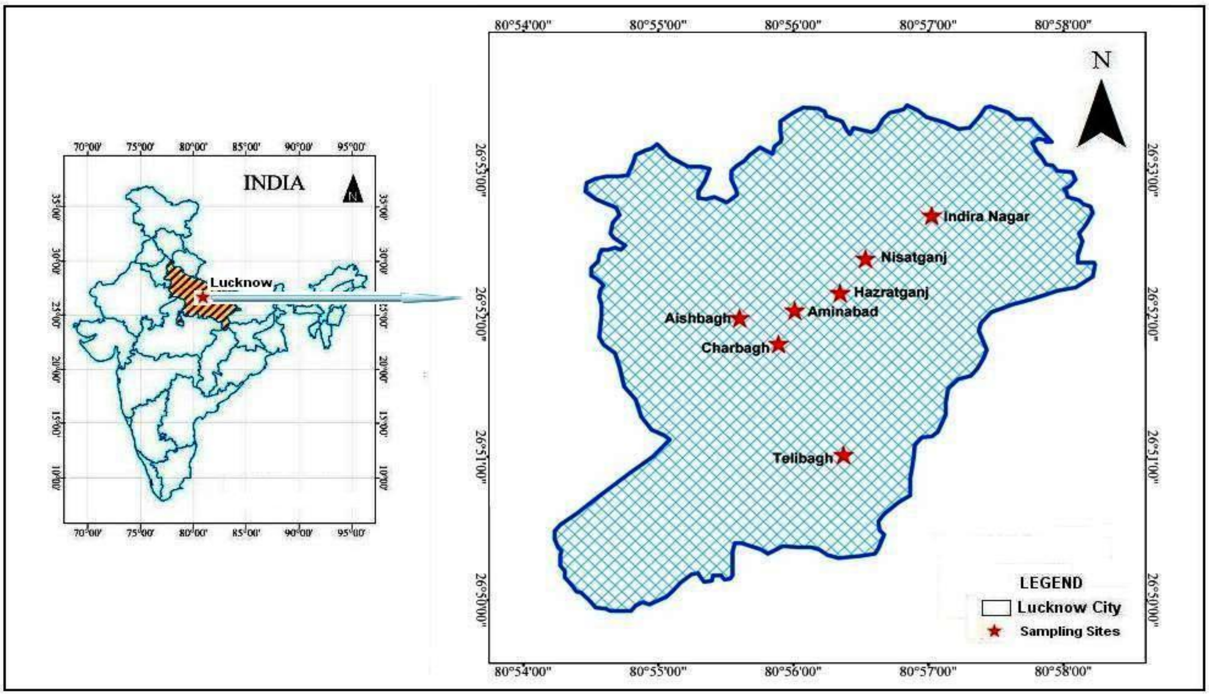


Plate3.2. Map showing seven residential sites of Lucknow city as drinking water sampling sites.

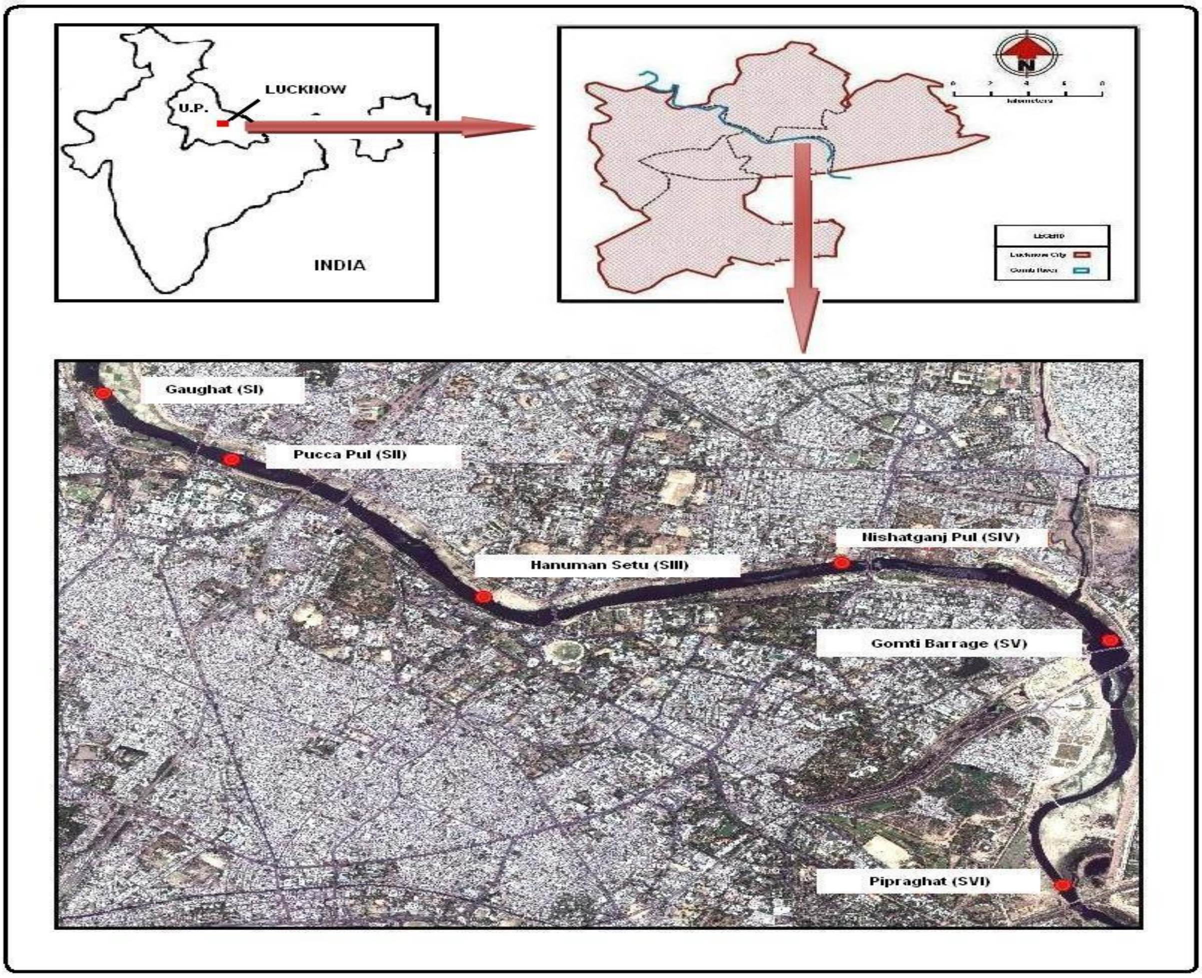


Plate3.3. Map showing river Gomti at Lucknow and location of different sampling sites (I to VI).

3.2 Details of standard protocols used in the experimental analysis

3.2.1. pH: 20 ml of water sample was taken in a beaker. The pH was recorded by dipping the electrode in the sample using potable digital pH meter.

3.2.2. Electrical Conductivity: 20 ml of water sample was taken in a beaker. The EC was recorded by dipping the electrode in the sample using potable digital EC meter.

3.2.3. Nitrate: For nitrate estimation **Catalado method (Catalado et al., 1975)** is used.

Reagents prepared

a) 5% Salicylic Acid- Dissolve 5 gm salicylic acid in 100 ml conc. H_2SO_4.

b) 2 N NaOH- Dissolve 20 gm NaOH in 250 ml distilled water.

Procedure:

- Take 0.5 ml of water sample in a test tube.
- Add 0.5 ml of 5% salicylic acid to it.
- Finally add 9.0 ml of 2N NaOH.
- Orange- yellowish color will appear after 20 min.
- Take O.D. at 410 nm
- **Calculation-**
- Nitrate = K- factor × concentration

K- Factor = Absorbance/ Concentration

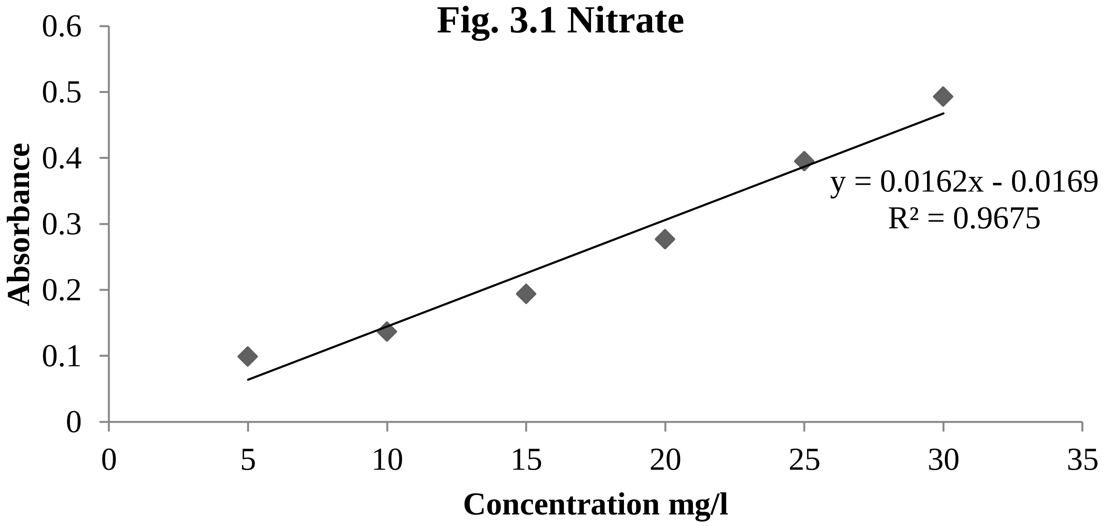


Fig. 3.1 Standard Curve of Nitrate

3.2.4. Nitrite- (Stevens and Oaks, 1973)

Reagents required:

a) NaNO2 stock solution- Dissolve 0.001 gm of NaNO2 in 100ml distilled water.

b) 1% Sulphanilamide (LR) solution: Dissolve 1 gm sulphanilamide in 100 gm of 1 N HCl.

c) 0.01%NED (N-1-napthyl ethyldiamine dihydrochloride): Dissolve 0.01 gm of NED in 100ml distilled water.

Procedure

- Take 1 ml of sample in a test tube.
- Add 1 ml of 1% sulphanilamide to it.
- Finally add 1 ml of 0.01% NED.
- After 10 minutes pink color will appear.
- Take O.D. at 540 nm.

Calculation

Nitrite (µg ml^{-1}) = K- factor × Absorbance

K-Factor = Absorbance/ Concentration

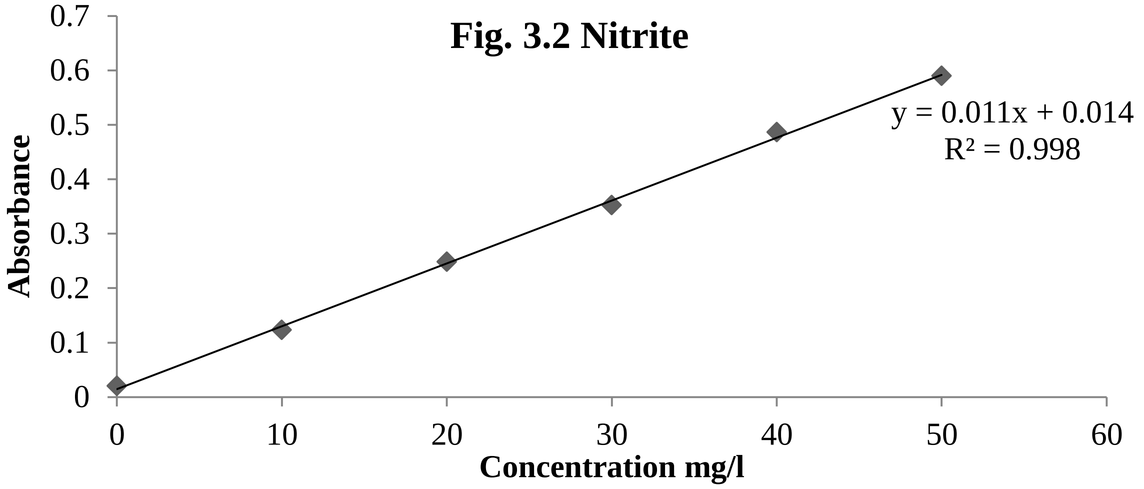


Fig. 3.2. Standard Curve of Nitrite

3.2.5. Ammonium: Ammonium was estimated using **Weather burn method, 1967 by using Nessler's Reagent.**

Reagents prepared

Nessler's Reagent: Dissolve 50 gm KI in 50 ml cold water. Add a saturated solution of mercuric chloride (22gms in 350 ml). Then add 200 ml of 5N NaOH and dilute to 1 L. Keep overnight and then filter.

Procedure-

- Take 2.5 ml of sample.
- Add 1.5 ml of Nessler's reagent to it
- Red to brown color appears, take O.D. at 420 nm.

Ammonium Standard Curve was also prepared separately.

Calculation

Ammonium (µg ml^{-1}) = K- factor × Absorbance

K-Factor = Absorbance/ Concentration

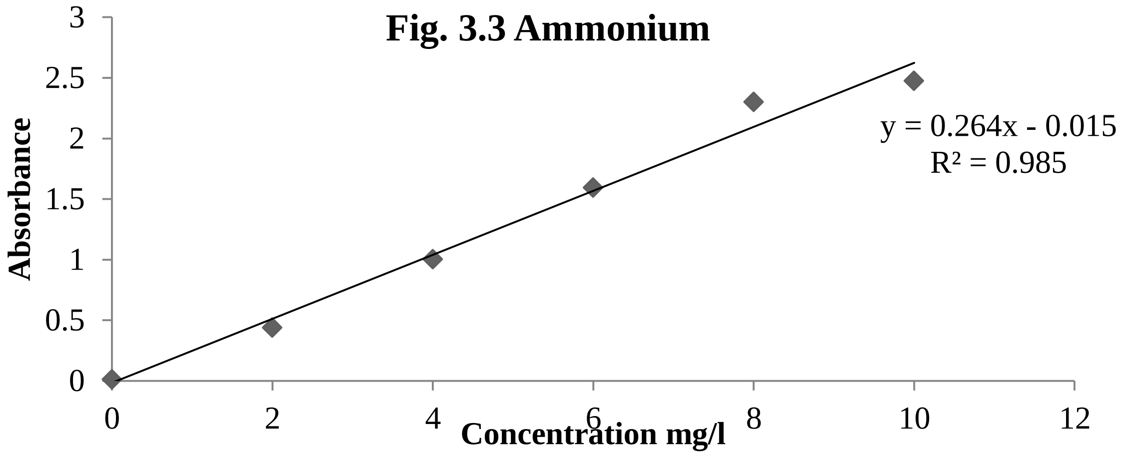


Fig. 3.3 Standard Curve of Ammonium

3.2.6. Phosphate

Phosphate content was estimated using **Ammonium molybdate and stannous chloride method**.

Reagents prepared-

a) Ammonium molybdate- Dissolve 1.25gm of ammonium molybdate in 50 ml conc. H_2SO_4.

b) Stannous chloride- Disslove 0.6 gm stannous chloride in 25 ml glycerol

Procedure-

- Take 10 ml of water sample in a test tube.
- Add 4 ml ammonium molybdate to it.
- Finally add 2-3 drops of stannous chloride to it.
- Take O.D. at 680 nm.

Calculation

Phosphate (µg ml^{-1}) = K- factor × Absorbance

K-Factor = Absorbance/ Concentration

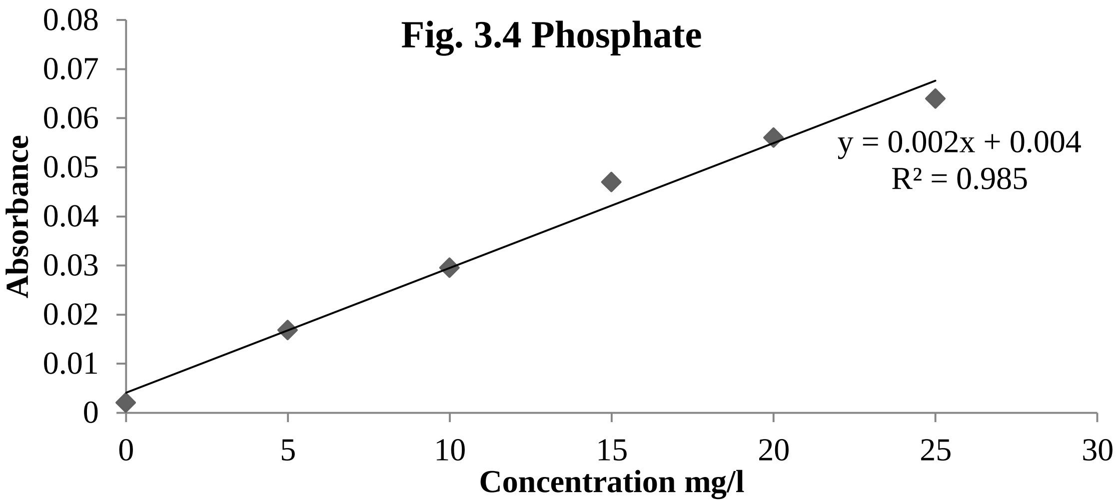


Fig. 3.4 Standard Curve of Phosphate

3.2.7. DO (Dissolved oxygen mg/l)

Reagents prepared

a) Manganous sulphate solution- Dissolve 36.4 gm of manganous sulphate in 100 ml of distilled water.

b) Alkaline Iodide Azide solution- 50 gm of NaOH and 15 gm of KI was dissolved and diluted to 95 ml, 1 gm of NaN3 was dissolved in 4 ml of distilled water. The two solutions were cooled and mixed and the solution was made up to 100ml.

c) Starch indicator- 1 gm of arrow root starch was added with continuous stirring in 200 ml distilled water.

d) Standard sodium thiosulphate solution (0.025 N)- 1.205 gm of sodium thiosulphate was dissolved in 200 ml distilled water which gave 0.025 N. Separately 0.1 gm NaOH was dissolved in 250 ml distilled water. 0.4 ml of this NaOH solution was taken and added in sodium thiosulphate solution.

Procedure-

The sample was collected in BOD bottle. The sample was filled to the neck of the bottle assuring that air bubbles have not been trapped under the stopper and maintained a water seal around the stopper, until ready for the next step and analysis.

- 1ml of manganous sulphate solution was added in the sample followed by 1 ml of alkali iodide azide solution.
- The stopper was placed carefully to avoid any air bubble and mixed by inverting the bottle repeatedly for 15 minutes.

- The precipitate was allowed to settle leaving the clear supernatant.
- The stopper was removed carefully and immediately 1ml of conc. H_2SO_4 was added and bottle was closed and mixed with gentle inversion the precipitate completely dissolves.
- Titrate 100ml content of the bottle with standard sodium thiosulphate solution using starch as indicator.

Calculation-

$$\text{DO (mg/L)} = \frac{\text{ml of titrant} \times \text{normality} \times 8 \times 1000}{V2\,(V1-V)/\,V1}$$

V= Volume of Manganous sulphate and iodide azide solution added.

V1= Volume of BOD bottle (ml)

V2= Volume of the content titrated in ml

3.2.8. BOD (Biochemical Oxygen Demand)

Reagents prepared

a) Manganous sulphate solution- Dissolve 36.4 gm of manganous sulphate in 100 ml of distilled water.

b) Alkaline Iodide Azide solution- 50 gm of NaOH and 15 gm of KI was dissolved and diluted to 95 ml, 1 gm of NaN_3 was dissolved in 4 ml of distilled water. The two solutions were cooled and mixed and the solution was made up to 100ml.

c) Starch indicator- 1 gm of arrow root starch was added with continuous stirring in 200 ml distilled water.

d) Standard sodium thiosulphate solution (0.025 N)- 1.205 gm of sodium thiosulphate was dissolved in 200 ml distilled water which gave 0.025 N. Separately 0.1 gm NaOH was dissolved in 250 ml distilled water. 0.4 ml of this NaOH solution was taken and added in sodium thiosulphate solution.

Procedure: The sample was collected in BOD bottle. The sample was filled to the neck of the bottle assuring that air bubbles have not been trapped under the stopper and maintained a water seal around the stopper, until ready for the next step and analysis.

- 1ml of manganous sulphate solution was added in the sample followed by 1 ml of alkali iodide azide solution
- The stopper was placed carefully to avoid any air bubble and mixed by inverting the bottle repeatedly for 15 minutes.

- The precipitate was allowed to settle leaving the clear supernatant.
- The stopper was removed carefully and immediately 1ml of conc. H_2SO_4 was added and bottle was closed and mixed with gentle inversion the precipitate completely dissolves.
- Titrate 100ml content of the bottle with standard sodium thiosulphate solution using starch as indicator.

Calculation-

$$\text{BOD (mg/L)} = \frac{\text{ml of titrant} \times \text{normality} \times 8 \times 1000}{V2\ (V1-V)/\ V1}$$

V= Volume of Manganous sulphate and iodide azide solution added.

V1= Volume of BOD bottle (ml)

V2= Volume of the content titrated in ml

BOD = initial DO – final DO

3.2.9. Chemical Oxygen Demand (COD)

Reagents prepared-

1) Standard Potassium dichromate solution- Dissolve 12.259gm of potassium dichromate in 1000 ml distilled water.

2) Standard FAS- Dissolve 98gm of ferrous ammonium sulphate in distilled water. Add 30 ml conc. H_2SO_4 to it and maintain it to 1000ml by adding distilled water.

Procedure-

- Take 30 ml of sample and add 10 ml of standard potassium dichromate to it.
- Then add 30 ml of conc. H_2SO_4 and pinch add Ag_2SO_4 to it.
- Keep it for reflux for 2 hours.
- Finally titrate the content with standard FAS solution

Calculation: $$\text{COD (mg/L)} = \frac{(A-B) \times M \times 8000}{\text{ml of sample}}$$

Where, A= ml of FAS used for blank

B= ml of FAS used for sample

M = molarity of FAS

3.2.9. Metal Estimation

Heavy metals viz. Fe, Cu, Cd, Pb and Cr were determined after acid digestion of samples (100ml for water samples and 1gm for soil) with an acid mixture (9 parts nitric acid: 4 parts perchloric acid) at about 100° C. The solution was allowed to evaporate to dryness, and temperature was further raised to 105 ° C to reduce the volume to 0.5-1.0 ml. The solution was filtered through Whatman filter paper No. 40 in a volumetric flask. The residue was re-dissolved and diluted to 15 ml with distilled water. Blanks were also run simultaneously and analyzed to correct for possible external contributions of the metals. Analytical data quality of metals was ensured through repeated analysis (n=3) of EPA quality control in samples. Metal concentration was determined by atomic absorption spectrophotometer (AAS 240 FS, Varian).

Metal Concentration (μg g^{-1}dw) = $\frac{XV}{W}$

Where,

X = Reading in ppm on AAS,

V = Final volume of digested samples (ml)

W = Dry weight of sample/ ml of sample taken

3.2.10. Calculation of Bioconcentration factor (BCF), Translocation factor and Metals removal efficiency

Bioconcentration factor (BCF), expressed as the ratio of metal concentration in plant tissue to that of the water was calculated by:

$$\mathrm{BCF} = \frac{\text{Metal content in plant tissue}}{\text{Initial metal content in water column}}$$

Translocation factor (TF), the ratio of metals in shoot versus root of plants was calculated by the formula of Padmavathiamma and Li (2007).

$$\mathrm{TF} = \frac{\text{Metal content in plant shoot}}{\text{Metal content in plant root}}$$

Metal removal efficiency was calculated on percentage basis according to the equation (Chaudhuri et al., 2011)

$$R\ \% = \frac{Co - Ct}{Co} \times 100$$

Whereas, Co is initial metal concentration in water and Ct is final metal concentration in water at the end of experiment.

3.2.11. Protein estimation: The protein content was then determined by the method described by Lowry et al., (1951). For protein estimation, 500 mg leaves of the samples were homogenized in 3 ml of 10% tricholoroacetic acid (TCA) and centrifuged at 10000rpm for 10 mins. After decanting the supernatants, the pellets were washed and heated for 7 min with 3 ml of 1N NaOH (sodium hydroxide), cooled and centrifuged again at 10,000 rpm for 10 min. The 0.5 ml of extracted sample was taken in 2.5 ml of 0.5% $CuSO_4$ (copper sulphate in 1% potassium sodium tartarate), 48 ml of 5% Na_2CO_3 (sodium carbonate) was added. 0.5 ml (1N) of folin-phenol reagent was added after 10 min. 30-min incubation developed a blue color complex in the mixture. Absorbance was taken at 700nm against a blank without sample. Protein was calculated by a standard curve using bovine serum albumin as the standard.

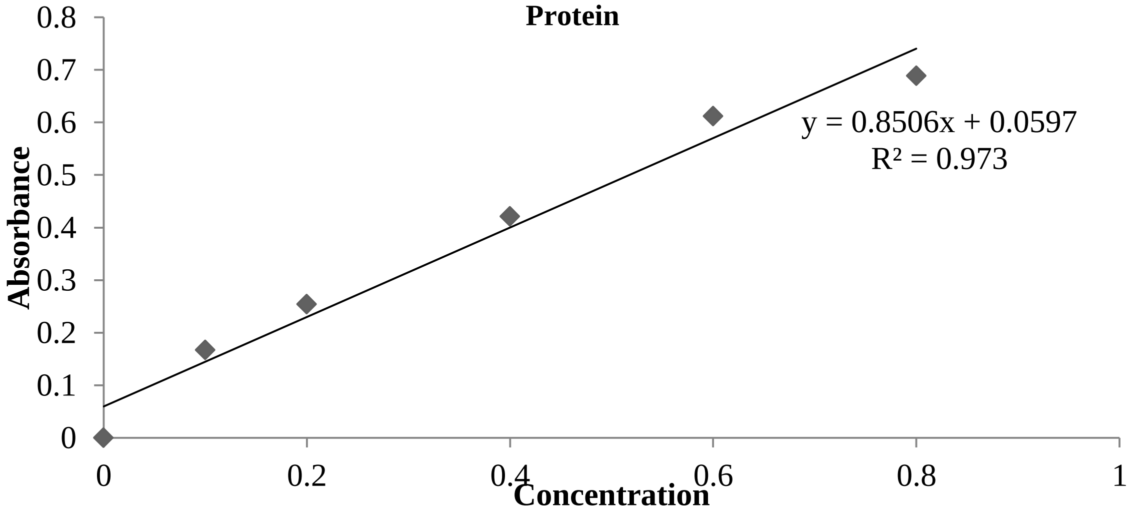


Fig. 3.5. Standard curve for Protein

3.2.12. Proline Estimation: For proline estimation, 500 mg of leaf samples were extracted with 10 ml of 3% sulphosalicylic acid and centrifuged at 5000 rpm to remove cell debris. Supernatant was used to assay the proline content following Bates et al., (1973).

3.2.13. Chlorophyll and carotenoid estimation

Chlorophyll content was estimated as per the protocol of Machalochan & Zalik (1963).

1.0g fresh plant sample was homogenized in 5ml of 80% acetone and centrifuged at 5000rpm for 15 minutes at 4ºC. Optical density of the supernatant was taken on spectrophotometer at 480, 510, 645 and 663nm.

Calculation

Total Chlorophyll (mg/g):20.2(OD645) +4.68(OD663) ×V/1000×W

Total carotenoids (mg/g): [4.695×OD480-0.268] ×chla + chl b

Where

V=volume of 80% acetone; W=weight of sample and OD=optical density.

3.2.14. Scanning Electron Microscopic (SEM) and Fourier Transform Infrared Spectroscopic (FTIR) analysis

Thin cross sections plant samples of 2mm are excised for the sample preparation. The samples were fixed in 2.5% Glutaraldehyde/ Karnovsky's fluid (David et al., 1973), buffered with 0.1M sodium phosphate buffer at pH 7.4. Fixation of the samples was done for 6-8 hours at 4^0C. After this, samples were washed thrice with 0.1M fresh phosphate buffer for 15min each at 4^0C. Post fixation for 2hours in 1% Osmium tetraoxide was performed for the samples. After this, samples were dehydrated using increasing concentration of Acetone 30%, 50%, 70%, 90% and 100%. The duration of treatment was 30 mins at 4^0C for each concentration. Finally, the species were air dried and mounted on stubs, then coated with a thin layer of gold for examining in Scanning Electron Microscope (JEOL, JSM 6490 LV-Japan).

For FTIR analysis of plants samples, dry root and shoot samples were grind properly to form a uniform powder. Then FTIR spectra of the samples prepared as KBr discs were taken by Infrared Spectrophotometer (Nikolet^{TM} 6700) in the wave number of 4000-400 cm^{-1} (Teng et al., 2013).

3.2.15. Organic Carbon and Organic Matter: Organic carbon was determined by method described by Walkley and Black (1934).

Reagents

1N Potassium dichromate$K_2Cr_2O_7$: Dissolve 49.04 gm of $K_2Cr_2O_7$ in distill water and make the volume upto 1 lt

0.5 N Ferrous ammonium sulphates (FAS)-Dissolve 196 gm of FAS in distill water and make volume upto 1 lt.

Concentrated Sulphuric acid (H_2SO_4)

Ortho phosphoric acid (85%)

Diphenyl amine indicator-Dissolve 0.5 gm of Diphenyl amine in a mixture of 40 ml distill water and 20 ml conc.H_2SO_4

Procedure- Take 0.1 gm of soil in a dry conical flask of 500 ml.

- Add 10 ml of 1N $K_2Cr_2O_7$ with the help of Pipette and swirled a little.
- Add 20 ml of conc. H_2SO_4.
- Allow the flask to stand for 30 min and then add 200 ml of distilled water.
- Now add 10 ml of O phosphoric acid and 1 ml of diphenyl amine indicator.
- The colour of the solution changes to Blue.
- Now Titrate with 0.5 N FAS till colour changes Blue to Green.
- Simultaneously run a Blank sample with the same procedure as above.

$$\text{Organic Carbon (OC)} = \frac{N(B-C)\times 0.003\times 100}{\text{Wt of soil (gm)}}$$

N= Normality of FAS; B = Volume of FAS for Blank; C= Volume of FAS required for titration of sample

Actual Organic Carbon = Organic Carbon×1.3

%Organic Matter = Actual Organic Carbon×1.724

3.2.16. Total Phosphorus

Reagents

1. Conc. Sulphuric acid (H_2S0_4)
2. Phenolphthalein indicator
3. Conc. Nitric acid (HNO_3)
4. Sodium hydroxide (NaOH), 1 N: Dissolve 40 g NaOH in 1 L of distilled water.

Procedure

- Take a suitable volume 0.2 gm of sample in a Kjeldahl flask.
- Add 1 ml H_2S0_4 and 5 ml HNO_3
- Digest the sample on a hot plate till the volume becomes nearly 5 ml and Continue the heating further until the solution becomes colorless after complete removal of HNO_3
- Cool and transfer completely to a 100 ml volumetric flask.
- Add 1 drop of phenolphthalein indicator.
- Neutralize the acidity by adding 1 N NaOH. At the end the solution turns pink. Make up the final volume to 100 ml. Read absorbance at 730 nm.

3.2.17. Sequential Extraction of metals: Tessier sequential extraction of metals was employed for metal speciation. The detailed protocol is given in Table 3.1.

Table 3.1 Tessier Sequential Extraction of Metals.

Speciation	Extractant	Environmental Conditions
(1) Exchangeable	8ml of 1.0M $MgCl_2$ (pH=7)	With agitation at 220 rpm for 1 h at 25^0C
(2) Carbonate	8 ml 1.0 M NaOAc (pH=5, adjusted with conc.HOAc),)	With continuous agitation for 5 h at 25^0C
(3) Reducible	20 ml 0.04 M $Nh_2OH.HCl$ in 25% HOAc (v/v)	6h at 96 C in water bath with occasional agitation
(4) Organically bound	3ml 0.02 M HNO_3 and 5 ml 30% H_2O_2 (pH=2, adjusted with conc. HNO_3).	Heated at 85^0C for 2 h
	After 2 h, 3 ml 30% H_2O_2 was added.	Heated at 85^0C for 3 h with occasional agitation.
	After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added.	Agitated for 0.5 at 25^0C
(5) Residual	10 ml of H_2SO_4 and $HCIO_4$ (5:1) mixture.	Heated at 300^0C

Chapter 4

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

Chapter 4. Monitoring of inorganic pollutants and heavy metals in potable water at source of supply and consumers end of Lucknow City

4.1. Introduction

Contamination of drinking water sources and supplies with hazardous chemicals like nitrate, nitrite, ammonium and heavy metals has become an increasing concern in developing countries, including India (Khan *et al.,* 2014). The elevated levels of nitrogenous inorganic pollutants (nitrate, nitrite, ammonium) and inorganic phosphates in river water due to runoff from agricultural fields, discharge of municipal/industrial sewage etc. lead to many health hazards (Kumari *et al.,* 2013). Nitrate-N levels in drinking water of 10mg/l or greater have been linked to methaemoglobinaemia or blue baby syndrome in infants and gastric carcinomas (Tank and Chandel, 2010) and non-Hodgkin's lymphoma in humans (Rao and Puttana, 2000; Rawat and Singh, 2009). Heavy metals released by human activities into the environment beyond toxic limits are progressively finding their fate into water bodies thereby making the water unfit for drinking and also their bioaccumulation in food chains threaten the life of animals and human beings (Zhang *et al.,* 2009; Ishaq and Khan, 2013; Nazeer *et al.,* 2014). Consumption of water contaminated with heavy metals may lead to their chronic accumulation in the kidneys, liver, bones etc. resulting in disruption of various metabolic activities (Bakirdere *et al.,* 2013; Pourang and Noori, 2014). 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 (Cieszynska *et al.,* 2012; Faridi *et al.,* 2012). Earlier reports on drinking water of Lucknow city depict variable concentration of various inorganic contaminants (Mishra and Mishra, 2008; Lohani *et al.,* 2008; Mudiam *et al.,* 2012). However, monitoring of multi metals (Fe, Cu, Cd, Cr and Pb) and reactive nitrogen species (nitrate, nitrite, and ammonium) and phosphate in drinking water before and after municipal treatment has not been investigated in different seasons. The present study is aimed at monitoring the water quality of river Gomti at Gaughat being used as the source of drinking water supply for Lucknow Municipal Corporation and to evaluate the status of post-treatment drinking water quality at the user end during pre-monsoon, monsoon and post monsoon periods.

4.2 Material and Methods

4.2.1. Study Area

Lucknow, the capital of Uttar Pradesh (26^{o}5/N latitude, 80^{o}56/E longitude, 128 m above the sea level), is spread over an area of 310 km^2 in the central plain of the Indian subcontinent, supporting a population of 36.50 lakh (Other details are given in chapter 3). Gomti river, a tributary of Ganga River, originates from Fulhar lake near Pilibhit flowing through the Lucknow city meandering for about 12 Km, is a major freshwater ecosystem in India. Sampling station at Gaughat of river Gomti which is the source of raw municipal water supply for Lucknow city along with seven residential sampling sites which are the locations of municipal water supply for various residential areas of Lucknow were chosen for the monitoring of drinking water. Residential areas included Aminabad, Indira Nagar, Aishbagh, Nishatganj, Telibagh, Hazratganj and Charbagh and these sites in the results and discussion are designated as site1, site2, site3, site4, site5, site6 and site7 respectively.

4.2.2. Water samples collection and analysis

Water samples from Gaughat and other residential areas were collected during August 2012-July 2013. Water samples were collected in triplicates in polypropylene sampling bottles that were acid soaked overnight and rinsed with tap water and then with distilled water. Collected samples were transported and stored in the dark at 4ºC for further analysis (APHA 2005). One bottle from each station was acidified in the laboratory before storage for heavy metal analysis. pH, electrical conductivity (EC), nitrate, nitrite, ammonium and phosphate were monitored monthly, while as for heavy metals seasonal variations during the pre-monsoon, monsoon and post-monsoon was conducted both at the Gaughat and residential areas. Basic parameters such as pH and EC were measured on spot using potable digital pH and EC meters. Nitrate, nitrite, ammonium and phosphate were measured in triplicate by using standard methods for examination of water and wastewater (APHA, 2005). The details of standard protocols are given in chapter 3.

4.2.3. Statistical Analysis

Statistical analysis of data by one way ANOVA followed by Duncan Multiple Range Tests was performed to determine the significance of differences among the mean values using (SPSS Version 20). Results of testing were considered significant if the calculated P-values were ≤ 0.05. Relationships between physicochemical parameters and metal concentration in drinking water at the user end were studied by using Pearson Linear Correlation method.

4.3. Results

4.3.1. Physicochemical characteristics of drinking water at source of supply and consumer end

In the present study, the mean values of pH of water at Gaughat varied between 7.13 in September and 8.63 in February (Table 4.1). These values indicated that pH of drinking water source was slightly alkaline in nature. Significant differences were observed for the pH values of water samples at Gaughat during all months ($p \leq 0.05$). At the consumer end, pH values at different studied sites showed variations from 6.9 at site 1 to 8.66 at Site 6 during May (Fig. 4.1). Statistically significant differences were observed for monthly pH values in drinking water at consumer end sites ($p \leq 0.05$), except in the month of September. The electrical conductivity measurements in water depict the temporal variations in total dissolved solids and major ions present in it.

Fig. 4.1

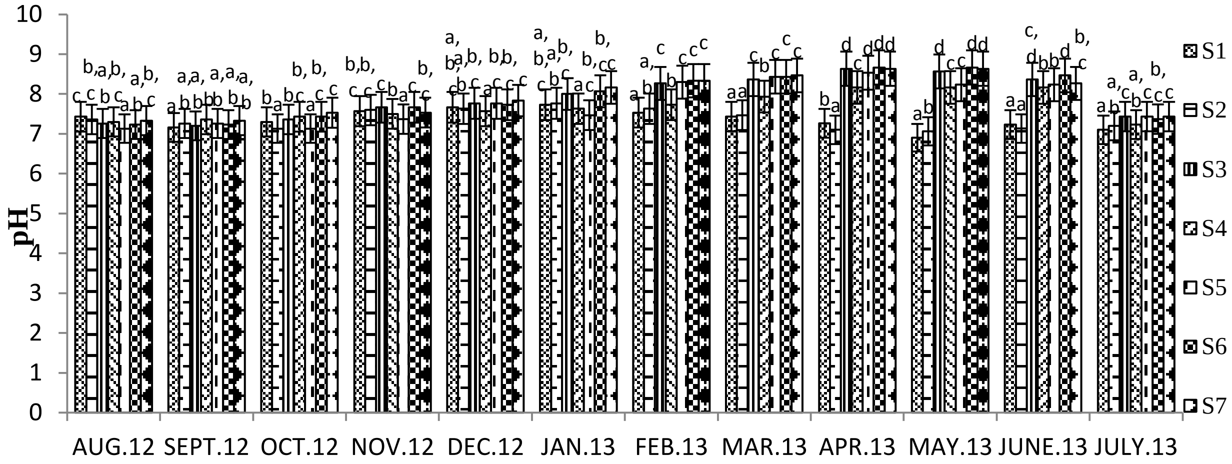


Fig. 4.1 Monthly variations in pH of drinking water at users end of urban Lucknow (Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh.

During the present study, it was found that source of drinking water i.e., Gaughat showed highest EC (571.67µs/cm) in May and lowest value for EC (375.66 µs/cm) was recorded in August (Table 4.1). Monthly variations in EC of water samples at Gaughat were significant ($p \leq 0.05$). At the user end, EC values of drinking water ranged from374.66 µs/cm in July to 475 µs/cm in May at site 7 and 5 respectively (Fig. 4.2).

Fig. 4.2

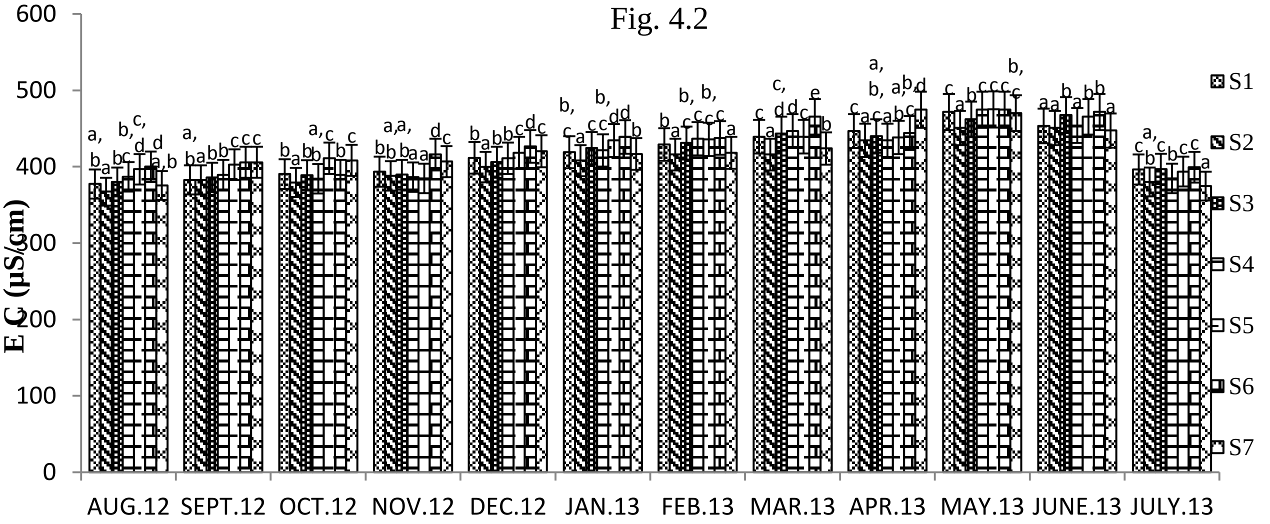
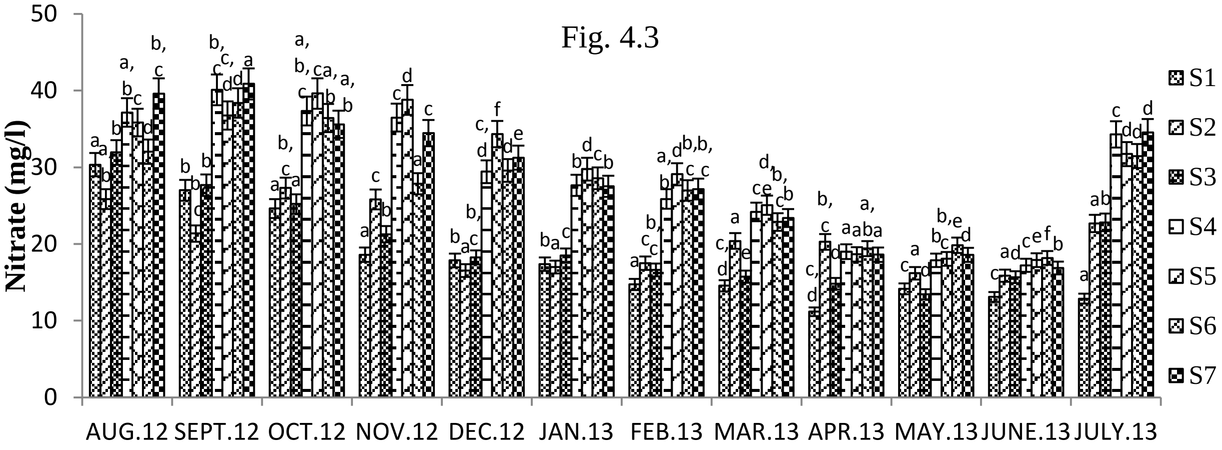


Fig. 4.2 Monthly variations in Electrical conductivity (EC) of drinking water at users end of urban Lucknow (Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh.

Nitrate content in water is typically reported as either nitrate-nitrogen (NO_3-N) or nitrate (NO_3^-). In the present study, the water at Gaughat showed highest NO_3^- (26.25 mg/l) in May and lowest NO_3^- (13.45 mg/l) was reported in August (Table 4.1). While comparing the monthly concentrations of nitrate both at Gaughat water and at user end of all sites, significant differences were observed ($p \leq 0.05$).



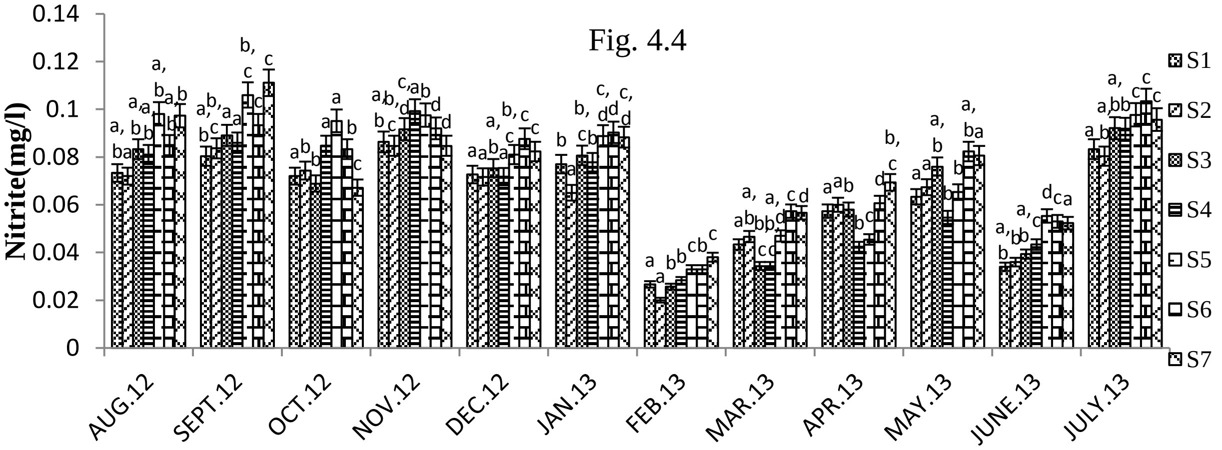


Fig. 4.3 and 4.4. Monthly variations in Nitrate (mg/l) and Nitrite (mg/l) of drinking water at users end of urban Lucknow (Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh.

Nitrate concentration in public water supply at the consumer end showed maximum value (40.86 mg/l) at site 7 in September and minimum value (11.17 mg/l) was analysed at site 1 in April (Fig. 4.3). In the present study, monthly variations in nitrite (NO_2^-) concentration analysed from Gaughat water and consumer end of Lucknow are depicted in Table 4.1 and Fig. 4.4, respectively.

Nitrite levels analysed in water at Gaughat and at various residential sites showed significant differences ($p \le 0.05$). At Gaughat, maximum nitrite (0.082 mg/l) was observed in May and minimum nitrite (0.026 mg/l) in July. Correspondingly, at the user end higher nitrite levels were reported than at the source. Highest nitrite (0.1 mg/l) at site 5 and lowest (0.02 mg/l) at site 2 was analysed in September and February respectively. Ammonium nitrogen (NH_4-N) in water samples at studied sites was found to vary between 2.67 mg/l and 6.9 mg/l at Gaughat (Table 4.1) and at the user end ammonium levels varied from 1.14 mg/l at site 3 to 5.16 mg/l at site 7 during April and August, respectively (Fig. 4.5).

Fig. 4.5

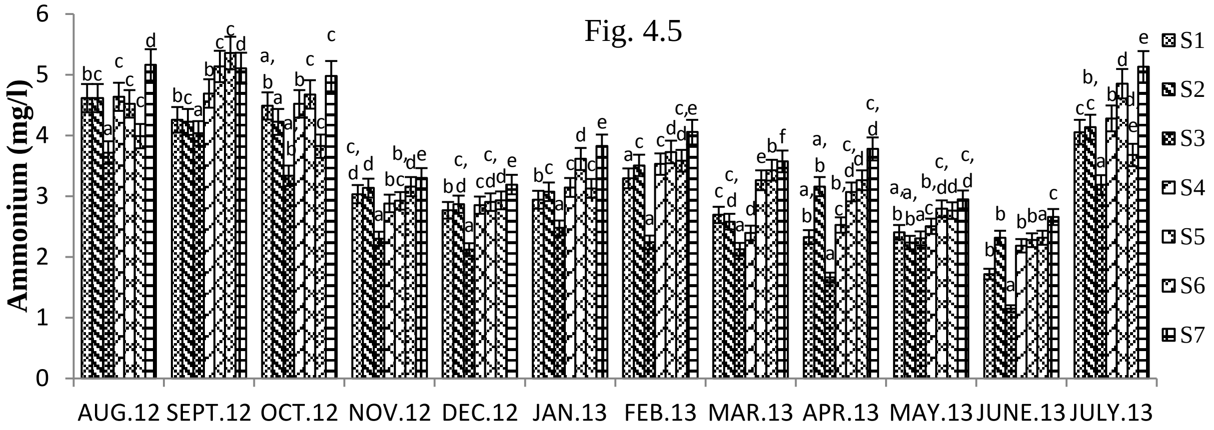


Fig. 4.6

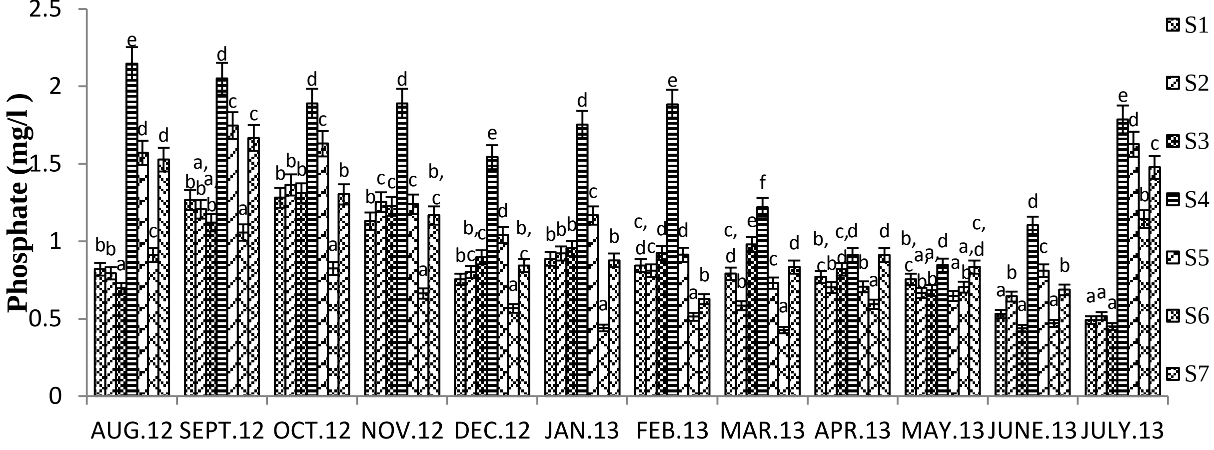


Fig. 4.5 and 4.6 Monthly variations in Ammonium and Phosphate (mg/l) of drinking water at users end of urban Lucknow (Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh.

In the present study variations in phosphate levels of drinking water are depicted in Table 4.1 for Gaughat and Fig. 4.6, for residential sites.

Significant differences were observed for the monthly variations in phosphate contents in drinking water both at source and consumer end ($p \leq 0.05$). For phosphates WHO has not established any specific guideline value for drinking water. At Gaughat maximum phosphate (1.82 mg/l) in October and minimum (0.6 mg/l) in March was observed. From the residential sites, highest phosphate content (2.14 mg/l) at site4 in August and lowest (0.44 mg/l) phosphate at site6 in January was reported. Since phosphates are used at potable water treatment plants, this may be the reason for elevated phosphate contents in drinking water at the residential sites.

Table 4.1 Monthly variation in physicochemical characteristics of Gaughat water of Gomti river used as raw potable water supply for Lucknow city (n=6± S D)

	pH	E. C (µs/cm)	Nitrate (mg/l)	Nitrite (mg/l)	Ammonium (mg/l)	Phosphate (mg/l)
AUG.12	7.36 ± 0.057^{b}	375.66 ± 4.04^{a}	13.45 ± 1.36^{a}	$0.033\pm0.01^{a,b}$	3.89 ± 0.17^{c}	$0.9\pm0.02^{d,e}$
SEPT.12	7.13 ± 0.057^{a}	$379.33\pm4.041^{a,b}$	$14.18\pm1.46^{a,b}$	$0.03\pm0.01^{a,b}$	4.41 ± 0.18^{d}	1.42 ± 0.09^{g}
OCT.12	7.46 ± 0.057^{b}	$383.33\pm2.081^{a,b}$	$17.06\pm0.41^{d,e}$	$0.043\pm0.01^{b,c}$	4.026 ± 0.04^{c}	1.82 ± 0.06^{f}
NOV.12	7.83 ± 0.115^{c}	391.66 ± 2.88^{b}	$18.8\pm0.94^{e,f}$	$0.061\pm0.003^{d,e}$	3.89 ± 0.19^{c}	1.14 ± 0.06^{e}
DEC.12	8.56 ± 0.057^{e}	415 ± 5.0^{c}	20.85 ± 0.95^{g}	$0.06\pm0.003^{d,e}$	3.46 ± 0.12^{b}	$0.89\pm0.04^{d,e}$
JAN.13	8.63 ± 0.115^{e}	425.66 ± 4.04^{c}	$16.37\pm1.62^{c,d}$	$0.054\pm0.005^{b,c}$	2.67 ± 0.18^{a}	0.82 ± 0.01^{d}
FEB.13	8.63 ± 0.057^{e}	435±5.0d	$14.81\pm1.21^{a,b}$	$0.071\pm0.007^{e,f}$	4.78 ± 0.09^{e}	$0.69\pm0.02^{b,c}$
MAR.13	8.1 ± 0.1^{d}	477.66 ± 11.67^{d}	$15.99\pm0.3^{b,c,d}$	$0.067\pm0.004^{d,e}$	5.17 ± 0.17^{f}	0.6 ± 0.02^{a}
APR.13	8.16 ± 0.057^{d}	536.66 ± 15.27^{e}	$19.5\pm0.81^{f,g}$	0.08 ± 0.003^{f}	6.03 ± 0.32^{g}	$0.63\pm0.04^{a,b}$
MAY.13	8.13 ± 0.152^{d}	571.67 ± 10.4^{f}	23.37 ± 1.05^{h}	0.082 ± 0.005^{f}	6.21 ± 0.23^{g}	$0.69\pm0.02^{b,c}$
JUNE13	8.06 ± 0.115^{d}	551.67 ± 10.4^{g}	26.25 ± 0.9^{i}	$0.075\pm0.002^{e,f}$	6.9 ± 0.15^{h}	0.72 ± 0.02^{c}
JULY13	7.76 ± 0.15^{c}	$388.66\pm5.5^{a,b}$	$16.66\pm1.78^{c,d}$	0.026 ± 0.01^{a}	3.95 ± 0.13^{c}	0.81 ± 0.03^{d}
BIS 2003	**6.5 – 8.5**	-	**45**	**0.1**	**0.5**	-

(Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$)

4.3.2. Seasonal variations of metal contents in potable water at Gaughat and consumers end

In the present study, seasonal variations in the average values of heavy metal contents recorded in water at Gaughat during the course of study period are shown in Table 4.2. 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. Except for Pb ($p \geq 0.05$), one way anova showed significant differences between and among the groups of metals (Fe, Cu, Cd and Cr) recorded in water at Gaughat during all the three seasons ($p \leq 0.05$). At Gaughat maximum metal concentration for Fe (1.65 mg/l), Cu (0.108 mg/l) and Cr (0.065 mg/l) were reported in monsoon season, while as maximum Cd (0.052 mg/l) was seen in pre-monsoon and Pb (0.96 mg/l) was observed to be maximum in post-monsoon season. Minimum concentration for Fe (0.33 mg/l) was recorded in premonsoon, Cu (0.077 mg/l) in post-monsoon, Cd (0.03 mg/l) in monsoon, Pb (0.68 mg/l) in monsoon and Cr (0.036 mg/l) in postmonsoon season at Gaughat. Seasonal fluctuations in the levels of heavy metals (Fe, Cu, Cd, Pb and Cr) studied at seven residential sites of urban Lucknow are depicted in Table 4.3. 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.

At the consumer end, the heavy metals studied showed significant differences in all seasons at all sites ($p \leq 0.05$). According to the results, Fe content in the drinking water at consumer end varied from 0.15 mg/l to 5.32 mg/l. Highest Fe content (5.32 mg/l) was recorded at site5 during the monsoon period, followed by Fe (5.24 mg/l) at site7 and Fe (4.75 mg/l) at site6 and lowest Fe concentration (0.15 mg/l) was reported at site 2 during the post monsoon period. Levels of Cu in drinking water at studied sites varied from (0.036 mg/l) to (0.37 mg/l). During the monsoon period highest Cu concentration (0.37 mg/l) was observed at site5, followed by (0.21 mg/l) at site3 during the premonsoon period. Lowest Cu concentration (0.036 mg/l) was observed at site2 during the postmonsoon period. Our result on Cu levels was much lower than U.S. EPA's Drinking Water Equivalent levels (1.3 mg/l) and WHO guideline value (2.0 mg/l). However, Cu levels were above the prescribed permissible levels of BIS (2003). In the present study, Cd was also reported from the drinking water supplies of residential sites of urban Lucknow. Among the reported metals, Cd content in drinking water was found to be least. However, the Cd levels were just above the prescribed limits for drinking water (BIS, 2003). Cd content during the present study showed significant variations at all sites during different seasons ($P \leq 0.05$). Highest Cd content (0.046 mg/l) was observed during the postmonsoon period at site3. The lowest Cd (0.006 mg/l) was recorded at site4 during the premonsoon period.

Chromium levels recorded in the current study were slightly above the prescribed standards of BIS. Maximum Cr (0.051 mg/l) was observed at site6 during the monsoon period, while as minimum Cr concentration (0.013 mg/l) was recorded at site4 during the postmonsoon period. Site6 was followed by site7 for the second highest observed Cr (0.047 mg/l) during the monsoon and (0.046mg/l) in the premonsoon period. In the present study, Pb reported in drinking water from seven residential sites of Lucknow city varied between (0.15 – 0.58 mg/l). Maximum Pb concentration was observed at site 6 (0.58 mg/l) followed by site7 (0.57 mg/l) both during postmonsoon period and at site5 (0.46 mg/l) during the premonsoon period. Minimum Pb concentration (0.15 mg/l) was observed at sites1, 2 and 4 during the post monsoon periods. The Pb levels detected in water supplied to Lucknow city does not fall within the safe limits of Lead in water prescribed by BIS (2003).

Table 4.2 Seasonal variations in metal content (mg/l) in Gaughat water of Gomti river used as raw potable water supply for Lucknow city (n=6± S D)

	Fe	**Cu**	**Cd**	**Pb**	**Cr**
Pre M	0.336 ± 0.016^{c}	0.088 ± 0.206^{a}	0.052 ± 0.004^{a}	0.74 ± 0.18^{a}	0.057 ± 0.003^{b}
M	1.65 ± 0.13^{b}	0.108 ± 0.005^{a}	0.03 ± 0.006^{a}	0.68 ± 0.16^{a}	0.065 ± 0.005^{a}
Po M	1.128 ± 0.007^{a}	0.077 ± 0.022^{b}	0.045 ± 0.005^{b}	0.96 ± 0.061^{a}	0.036 ± 0.004^{c}
BIS 2003	**0.3**	**0.05**	**0.01**	**0.05**	**0.05**

(Different letters signify the statistical differences among metal concentrations ($p \leq 0.05$); whereas Pre M= Premonsoon; M= Monsoon and Po M = Postmonsoon)

4.3.3. Correlations between physicochemical parameters and heavy metals of potable water at the user end of Lucknow municipality

Correlation matrix between physicochemical parameters and heavy metals in the drinking water of residential sites of Lucknow was performed using Pearson correlation analysis (Table 4.4). Nitrate showed significant correlation with pH and conductivity at ($p \leq 0.01$) and with phosphate ($p \leq 0.05$). Nitrite exhibited significant positive correlation with nitrate and ammonium ($p \leq 0.01$). Phosphate also was significantly correlated with EC ($p \leq 0.01$). Among the studied heavy metals, Fe showed positive correlation with nitrite ($p \leq 0.05$). Cadmium was correlated with Cu ($p \leq 0.05$). However, Pb showed significant positive correlation with both ammonium and Cd ($p \leq 0.01$). Highest correlation among metals was observed for Cr with Cu and Pb ($p \leq 0.01$) and with Cd at ($p \leq 0.05$). Significant positive correlation between various parameters reveals the close association between the parameters and also their influence on one another. It is also 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 (Haloi and Sarma, 2012; Mansouri *et al.,* 2012; Agca *et al.,* 2014).

Table 4.3 Seasonal variation in concentration of heavy metal content (mg/l) in potable water at user end in Lucknow City (n=6 ± S D)

	Fe			**Cu**			**Cd**			**Pb**			**Cr**		
	Pre M	M	Po M	Pre M	M	Po M	Pre M	M	Po M	Pre M	M	Po M	Pre M	M	PoM
S1	0.506± 0.02^{a}	3.37± 0.04^{a}	0.16± 0.01^{a}	0.046± 0.01^{a}	0.04± 0.01^{a}	0.044± 0.01^{a}	0.012± 0.001^{a,b}	0.007± 0.004^{a}	0.0098± 0.001^{a}	0.21± 0.02^{a}	0.255± 0.05^{a,b}	0.152± 0.04^{a}	0.0207± 0.0002^{a}	0.0206± 0.0003^{a}	0.0155± 0.004^{a}
S2	0.518± 0.09^{a}	3.38± 0.13^{a}	0.15± 0.01^{a}	0.05± 0.02^{a}	0.06± 0.03^{a}	0.036± 0.01^{a}	0.01± 0.00^{a,b}	0.008± 0.003^{a}	0.0083± 0.001^{a}	0.21± 0.08^{a}	0.29± 0.04^{b}	0.15± 0.04^{a,b}	0.023± 0.005^{a,b}	0.0206± 0.0002^{a}	0.015± 0.001^{a}
S3	1.18± 0.06^{b}	4.16± 0.06^{b}	0.28± 0.05^{b}	0.21± 0.08^{c}	0.2± 0.01^{b}	0.067± 0.04^{a,b}	0.04± 0.009^{d}	0.033± 0.009^{c}	0.046± 0.019^{b}	0.52± 0.03^{c,d}	0.304± 0.014^{b}	0.23± 0.01^{b}	0.026± 0.0005^{b}	0.0308± 0.001^{b}	0.026± 0.0005^{b}
S4	2.09± 0.05^{c}	4.61± 0.08^{c}	0.34± 0.05^{b}	0.11± 0.008^{a,b}	0.29± 0.11^{b,c}	0.102± 0.01^{b}	0.006± 0.002^{a}	0.007± 0.001^{a}	0.0073± 0.001^{a}	0.406± 0.006^{b}	0.18± 0.02^{a}	0.15± 0.03^{a,b}	0.02± 0.0008^{a}	0.018± 0.002^{a}	0.013± 0.005^{a}
S5	2.64± 0.09^{d}	5.32± 0.11^{c}	2.18± 0.05c	0.21± 0.01^{c}	0.37± 0.05^{c}	0.223± 0.02^{c}	0.018± 0.002^{b,c}	0.018± 0.001^{b}	0.012± 0.003^{a}	0.46± 0.02^{b,c}	0.45± 0.03^{c}	0.35± 0.04^{c}	0.046± 0.0008^{c}	0.043± 0.004^{c}	0.033± 0.002^{c}
S6	3.16± 0.05^{e}	4.75± 0.08^{d}	2.36± 0.05^{d}	0.12± 0.02^{b}	0.26± 0.05^{b,c}	0.098± 0.01^{b}	0.022± 0.005^{c}	0.015± 0.002^{a,b}	0.019± 0.009^{a}	0.58± 0.02^{d}	0.57± 0.05^{d}	0.54± 0.05^{d}	0.05± 0.001^{c}	0.051± 0.008^{c}	0.03± 0.00^{b,c}
S7	3.52± 0.08^{f}	5.24± 0.07^{d}	2.43± 0.08^{d}	0.127± 0.02^{b}	0.26± 0.06^{b,c}	0.097± 0.02^{b}	0.014± 0.003^{a,b}	0.018± 0.004^{b}	0.014± 0.002^{a}	0.49± 0.01^{c}	0.56± 0.06^{d}	0.57± 0.03^{d}	0.046± 0.002^{c}	0.047± 0.006^{c}	0.0358± 0.005^{c}
BIS		**0.3**			**0.05**			**0.01**			**0.05**			**0.05**	

(Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh. Pre M= Premonsoon; M=Monsoon and Po M =Postmonsoon.

Table 4.4 Correlation coefficient (r) between physicochemical parameters and heavy metals of potable water at the user end of Lucknow municipality

	pH	E. C	Nitrate	Nitrite	Ammonium	Phosphate	Fe	Cu	Cd	Pb	Cr
pH	1	.621**	-.581**	-0.327	-0.334	-.500*	0.131	-0.122	0.199	0.255	0.178
E.C		1	-.586**	-0.289	-0.12	-.589**	-0.141	-0.283	0.156	0.237	-0.112
Nitrate			1	.805**	.420*	.501*	0.268	-0.158	-0.222	-0.111	-0.043
Nitrite				1	.618**	0.399	.446*	-0.241	-0.151	0.234	0.23
Ammonium					1	.412*	-0.014	0.081	0.139	.589**	0.36
Phosphate						1	0.133	0.282	-0.156	-0.1	-0.024
Fe							1	0.157	-0.235	0.162	0.34
Cu								1	.456*	0.377	.499*
Cd									1	.592**	.452*
Pb										1	.760**
Cr											1

(Values represent Pearson correlation coefficient, significant at **0.01 and *0.05 level).

4.4. Discussion

Safe water supply is recognized as the highest priority task in environmental protection throughout the globe (Adewuyi *et al*., 2014). The declaration of access to pure and safe drinking water as “human birth right” could actually mean “death right” if the water quality of potable sources is not regularly monitored to check their suitability and purity for human consumption (Mebrahtu and Zerabruk, 2011). While comparing the pH of drinking water with drinking water quality standards, it was observed to be slightly above than prescribed by BIS (2003) at sites 3, 6 and 7. It has been reported pH of water systems plays a significant role in metal solubility (Jonnalagadda and Mhere, 2001). Various factors that govern pH fluctuations in water include temperature changes, dissolution of atmospheric CO_2, organic matter decomposition in water, dilution of alkaline substances and metabolic activities of phytoplanktons and other aquatic life (Fawzy *et al.*, 2012). In the current study, the slight alkaline nature of drinking water of Lucknow city is in accordance with the previous reports of Mullai *et al., (*2013) while evaluating pH of Uppanar river of Tamilnadu.

Maximam EC during the summer months reported in the current study may be due to low and lenient flow of water in Gomti river and high temperature during this period leads to the increased salt content in water due to evaporation of water (Bellos and Swadis, 2005). Further increased EC shows the presence of free ions and other chemical substances such as nitrates, chlorides, phosphates in water. For EC of drinking water, WHO guidelines (2011) has prescribed maximum permissible limit of 400 mS/m. At the consumer end, significant differences were observed between all sites for EC in all studied months ($p \leq 0.05$).

Nitrate-N being the most common form of nitrogen present in natural waters is the end product of aerobic decomposition of organic matter (Rai, 2010; Agca *et al.*, 2014). For primary drinking water, USEPA has set a standard of 10 mg/l for nitrogen when reported in nitrate-N form and 45 mg/l when reported in nitrate form. Since nitrate concentration in water samples reported at Gaughat was within the permissible limit 45 mg/l. However, for its use as potable water supply to the Lucknow, it is recommended that nitrate concentration in water at the consumer end should not exceed 10mg/l. Further if water supply has a nitrate level approaching 3-5 mg/l, it is recommended that it should not be used for mixing baby foods or for drinking water for new born infants. Rawat and Singh (2009) have reported as much as 44.83 mg/l of nitrate in the surface waters of Lucknow, which may be due to discharge of municipal and industrial wastes, excessive application of nitrogenous fertilizers, manures and irrigation with untreated water. Dar *et al*., (2010) reported nitrate concentration in summer in 85% of the wells exceeding WHO permissible limits in Kashmir (India). In the extensive agricultural areas of north east Australia, Thorburn *et al*., (2003) concluded that 14-21% of the wells were extensively contaminated with nitrate.

Nitrite levels reported in this study were slightly below the permissible limits prescribed by BIS (2003) at various sites, except at site 5 which showed nitrite concentration in water above permissible levels. Enhanced nitrite production in water may be due to oxidation of ammonium and reduction of nitrate by bacteria (Rawat *et al.,* 2012; Mullai *et al.,* 2013).). Ammonium in water has been seldom reported to be present in water at 1 mg/l unless ammonium fertilizers or other ammonia sources such as wastewater gets added to the water sources or supplies. During the current study, NH_4-N contents were much higher than maximum permissible limit for drinking water of 0.5 mg/l NH_4-N recommended by BIS (2003). The results depict higher ammonium levels in water than previously reported by Rawat and Singh (2009) in surface and groundwater sources of Lucknow city. Excretion of ammonium by the phytoplanktonic organisms may be the reason for higher ammonium levels in water and also their death or decomposition in water storage tanks can lead to increased levels of ammonium and nitrite in the water supply (Damotharan *et al*., 2010). Domestic wastewaters containing detergents, municipal and industrial effluents and agricultural runoff are the main anthropogenic sources for elevated phosphate levels in surface water. In most of the natural surface waters, phosphorus ranges from 0.005 to 0.02 mg/l (WHO, 2011). The high concentration of phosphates are probably due to chemical weathering of rocks, release of soil minerals and also discharge of sewage into aquatic systems (Singh *et al*., 2005).

Heavy metals can enter drinking water by corrosion of distribution pipes as well as from industrial and natural geo-genic sources (Singh *et al.*, 2005; Haloi and Sarma, 2012). The variable metal contents reported in water at Gaughat during the present study depict discharge of wastewater and runoff from the catchment area into river system. Further, seasonal fluctuations in the metal contents may be due to the rain dilution effects. The leaching of metals from the soil minerals and organic matter due to rainfall may influence the concentration of heavy metals in river bodies (Singh *et al.*, 2005; Lohani *et al.*, 2008).

The presence of these metals in the water column used for public supply leads to various types of toxicity, like renal failure is related to the contamination of drinking water with Cd and Pb; liver cirrhosis to the contamination with Fe, Cu and Mo; hair loss to the contamination with Cr and Ni; and chronic anemia to the contamination with Cd and Cu (Jarup, 2003; Johri *et al.,* 2010; Mandour, 2012). Iron content in the drinking water supplies may due to the use iron in coatings of pipes used to transport drinking water, overtime corrosion/deterioration of these iron coatings can lead to excessive iron into water supplies (Mandour, 2012). Iron has been reported to cause liver cirrhosis when present in drinking water. In the present study, iron values exceeded the permissible limits prescribed by BIS (2003) and WHO (2011) for drinking water. Our results on Fe levels in drinking water are much higher than reported by Iqbal *et al.,* (2013) in drinking water of Pakhtunkhwa, Pakistan.

Copper concentration in drinking water varies due to fluctuations in pH and hardness of the water supply (Xu *et al*., 2006). Xu *et al*., 2006 reports excessive levels of Cu concentrations in drinking water of Shanghai China, Chile (1.2 mg/l) and United States (4.8 mg/l). Copper in drinking water supplies largely comes due to leaching from piping and plumbing fixtures. Addition of Cu into waterways is due to natural weathering of rocks and soil/sediments and also anthropogenic sources such as industrial effluents and sewage treatment plant effluents largely contribute to elevated copper levels in water courses (Lokhande *et al*., 2011). Although copper serves as a nutritional requirement for body, exposure to excessive levels of copper can result in number of adverse health effects, most commonly gastrointestinal distress (Alam *et al.,* 2012). Copper can bind with certain enzymes thereby interfering with their protection of cells from free radical damage (Uriu-Adams and Keen, 2005).

Reports of the present study on Cd content in water are much below than recorded by Khan *et al*., (2014), but higher than reported by Mishra and Mishra (2008) in Gomti river. The presence of Cd in drinking water supplies has been correlated with the plumbing of pipelines. Cadmium exposure to humans can lead to both short term (diarrhea, vomiting and destruction of mucous membrane) as well as long term (itai-itai disease, bone and kidney damage) effects (Muhammad *et al.,* 2011; Bakirdere *et al.*, 2013). The present levels of Cr detected in drinking water are less than 0.1 mg/l Cr reported earlier by Lohani *et al.,* (2008) while assessing heavy metal contamination in river Gomti of Lucknow. One of the major sources of Cr in aquatic environments is paint manufacturing industries. Also chromium compounds are used as pigments, mordents and dyes in the textiles and in leather industries as a tanning agent (Lokhande *et al.*, 2011). Cr exposure in drinking water has been reported to cause stomach cancer, alongside anemia and damage to the gastrointestinal tract, lymph nodes, and liver (Beaumont *et al.,* 2008; Adewuyi *et al.*, 2014). Lead is one of the nonessential and toxic metals to human health. Discharge of Pb into surface water is mainly through paints, solders, pipes, building materials etc. (De, 2002). In this study, Pb content in drinking water was higher than reported by Bakirdere *et al.*, (2013) in tap and bottled water samples. Further, Parameswari and Mudgal, (2013) found the highest Pb contents in ground water samples in South India. Since Pb pollution in fresh surface water sources is also due to dry and wet deposition of atmospheric fallouts and highest in those water bodies adjacent to highway roads, this may be the probable reason for greater Pb concentration in water supplies from rivers such as Gomti.

4.5. Conclusions

Monitoring of specific inorganic contaminants and heavy metals in this study instead of plethora of parameters can be handy in reflecting the actual water quality supplied by the municipal corporation. Though 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. The present study reveals that the presence of inorganic pollutants and heavy metals in municipal water supplies of urban Lucknow is a major cause of concern for the public health. 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. From the present study, it is concluded that the existing water treatment procedures do not comply with the specific drinking water standards. Therefore, the potable water supplied by the municipal cooperation should be treated prior to supply, for optimal removal of hazardous pollutants, so that public health is protected.

Chapter 5

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

Chapter 5. Water quality evaluation and screening of some naturally occurring macrophytes for remediation of heavy metals in Gomti River at Lucknow

5.1. Introduction

Pollution of the aquatic ecosystems with the inorganic pollutants e.g. heavy metals, nitrates, nitrites and phosphates etc. has become a serious concern in the developing world (Nweke 2009, Rai 2012). In urban areas, deterioration of water quality of rivers is strongly related to the increasing developmental activities in watershed, such as changing land use pattern, increasing discharge of untreated municipal and industrial wastewater and runoff from nearby agricultural fields (Li et al. 2009; Sekomo et al. 2011; Rai et al. 2012). Discharge of untreated or partially treated wastewater containing metals of variable toxicity into the river poses a serious threat not only to the aquatic ecosystem but also human health (Liu et al. 2005).

For the efficient management and remediation of water in rivers, the status of water quality and the changes produced therein due to anthropogenic activities is the first step towards establishing effective cleaning system. Aquatic biodiversity is a significant component of aquatic ecosystem and get affected by the quality of water. Various aquatic macrophytes (floating, submerged, rooted and emergent) growing in river courses have shown the potential to accumulate certain toxic pollutants inside their tissues and used to clean the pollution levels (Rai and Tripathi 2009; Souza et al. 2013; Kamel 2013). The plants are differentially tolerant to different inorganic pollutants as they have the capacity to assimilate or scavenge them. These potentials of plants have emerged as a major area of phytotechnological studies and have been evaluated for phytoremediation potential for the removal of toxic pollutants from contaminated soil and water (Rai 2010; Ajayi & Ogunbayo; Rawat et al. 2012; Baudhh and

Singh 2012). The macrophytes based treatment systems are low-cost, ecofriendly and aesthetically pleasing technologies and can be used by developing countries for recycling and treatment of wastewater, especially contaminated with heavy/toxic metals, as well as for the treatment of potable water before supply to people (Wang et al. 2002; Jayaweera et al. 2008; Lu et al., 2008; Khan et al. 2009; Rahman and Hasegawa 2011).

Previous monitoring studies on Gomti river report variable but alarming contamination of water with certain inorganic and organic pollutants, however, in stream existing macrophytes have not been investigated for their removal efficiency for Fe, Cu, Cd, Cr and Pb, the major heavy metal contaminants of industrial, municipal and agricultural origin from point and non-point sources in different seasons. Therefore, 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 in a semi-arid, urban tropical aquatic ecosystem.

5.2. Material and Methods

5.2.1. Description of the Study Area

Lucknow, the capital of Uttar Pradesh (26^{o}5/N latitude, 80^{o}56/E longitude, 128 m above the sea level), is spread over an area of 310 km^{2} in the central plain of the Indian subcontinent, supporting a population of 36.50 lakh. It has a distinct tropical climate with a marked monsoonal effect. Gomti river, a tributary of Ganga River, originates from Fulhar lake near Pilibhit flowing through the Lucknow city meandering for about 12 Km, is a major freshwater ecosystem in India. River Gomti is the only source for raw water for municipal supply of drinking water for the Lucknow. In the present study, the Gomti river at Lucknow city was divided into upstream and downstream sites. Six sites along with the bank of river

Gomti were selected for water and plant samples collection from Gaughat (upstream) upto Pipraghat (downstream). Gaughat and Pucca Pull Sites are categorized as upstream sites, while as Hanuman Setu, Nishatganj, Gomti Barrage and Pipraghat sites are categorized as downstream sites. Map of river Gomti showing six different sites is presented in Chapter 3.

The description of six sampling sites is as under:

1. Gaughat: This is the upstream site. It is the site where the River Gomti enters the Lucknow City. Here river water at Gaughat is lifted by pumps by Lucknow Municipal Water Corporation and distributed to the city people after filtration
2. Pucca Pull: Here at this site washing of clothes and bathing is performed by the locals.
3. Hanuman Setu: This is in the downstream of river Gomti. Above this site, effluents from industries such as Mohan Meakins, Parag Dairy Works etc. are carried into the river by the drains.
4. Nishatganj: This is also in the downstream of River Gomti. Domestic wastes, agricultural runoff and municipal wastes from the city are discharged at this point.
5. Gomti Barrage: Above this site dead bodies are cremated and burned on the bank of river Gomti. Here unburned human ashes and animal carcasses are added to the river water.
6. Pipraghat: Pipraghat is characterized by the high inputs of domestic and agricultural wastes. Water at this site is highly polluted due to the inflow of pollutants from the above stream. Most of the pollutants and sediments are transported along the course of river and deposited here.

The sites in the results and discussion are designated as Site I, Site II, Site III, Site IV, Site V and Site VI respectively.

5.2.2. Water Sampling and Analysis

Water samples in triplicates were collected in overnight acid soaked polyethylene bottles of 2L capacity from all the study sites during the pre-monsoon (April, May and June) and monsoon periods (July, August and September) of 2013 and brought to the laboratory for further analysis. The samples were preserved with 2ml of conc. HNO_3 per liter and were kept at 4^0C till analyzed. Some parameters such as pH, conductivity were determined onsite using potable digital pH and conductivity meters. For Dissolved Oxygen water samples were fixed onsite in DO bottles and then further analyzed in the laboratory. For the determination of BOD and COD, un-acidified water samples were collected in BOD bottles and brought to the laboratory for analysis. All the physicochemical parameters were determined in triplicate by using standard methods for examination of water and waste water (APHA, 2005). The detailed account of methods is given in Chapter 3.

5. 2.3 Aquatic Plants Collection and Analysis

Samples of aquatic plants naturally occurring at the study sites were randomly collected at the same time and period as with water sampling. The plants of the selected sites were; *Eichhornia crassipes, Pistia stratiotes, Hydrilla verticellata, Jussiaea repens*, *Typha Latifolia, Vallesnaria spiralis* and *Polygonum glabrum.* The plants were kept and sealed in air tight polythene bags and transported to the laboratory at 4^0C. The samples were thoroughly washed with tap water to remove the dirt and foreign objects and then rinsed thrice with the distilled water in the laboratory. Root and shoot of plant samples were separated and oven dried at 90^0C to constant weight. The different metals viz. Fe, Cu, Cd, Cr and Pb in the plant sample parts were determined after acid digestion of dry samples with an acid mixture (9 parts nitric acid: 4 parts perchloric acid) at about 100^0C. All the reagents used were of analytical grade. Sample blanks were also run simultaneously and analyzed to correct for possible external contributions of the metals. Analytical data quality of metals was ensured through repeated analysis (n=3) of EPA quality control in samples. Metal concentration was determined by atomic absorption spectrophotometer (AAS 240 FS, Varian).

5.2.4. Statistical Analysis

Statistical analysis of data by one way ANOVA followed by Duncan Multiple Range tests were performed to determine the significance of differences among the mean values using (SPSS Version 16, Statistical package and MS Excel). Results of testing were considered significant if the calculated P-values were ≤ 0.05. Relationships between physicochemical parameters and metal concentrations in Gomti River water were studied by Pearson Linear Correlation method.

5.2.5 Data Analysis

Metal accumulation and uptake by aquatic plants is also given by Translocation Factor (TF). The Translocation factor (the ratio of metals in shoot versus root) indicates the transfer of accumulated metals from root to shoot of the plants. It was calculated by the formulae (Padmavathiamma and Li 2007).

$$\mathrm{TF} = \frac{\mathrm{Metal\ [Shoot]}}{\mathrm{Metal\ [Root]}}$$

5.3. Results

5.3.1 Physico-chemical characteristics of Gomti River Water

The rivers are dynamic systems and the fluctuations in the physicochemical characteristics of water are governed by complex factors of climate, geomorphology, geochemistry prevailing in the river basin and adjoining drainage systems (Ambedkar &

Muniyan 2012). In the present study, the physico-chemical properties of water at different studied sites during pre-monsoon and monsoon periods are represented in Table 5.1.

Variations in pH of water at six sites during both periods are depicted in Table 5.1. While analyzing pH of river Gomti at Lucknow, irrespective of sites and periods, it is alkaline in nature. Maximam pH (8.66) was recorded downstream at Site V during the pre-monsoon period and minimum pH (7.13) was recorded at Site I in the monsoon period. One Way ANOVA showed significant differences for pH values at different sites during both periods ($p<0.05$). During the present study, EC at different sites showed marked variations. EC value was recorded highest (604.33 µs/cm) during pre-monsoon at Site VI and lowest (352.33 µs/cm) at Site IV in the monsoon period (Table 5.1). Significant differences were observed for EC values at sites and periods ($p<0.05$). Dissolved Oxygen (DO) of the river water during both periods at different sites showed marked statistical differences ($p<0.05$). Lowest value of DO (3.32 mg/l) was observed downstream at Site VI and highest value of DO (7.64 mg/l) was observed at Site I. Both highest and lowest values were recorded during the pre- monsoon period. Maximum biological oxygen demand (BOD) (12.84 mg/l) was recorded at Site VI during the pre-monsoon period and minimum BOD (3.16 mg/l) was recorded at upstream Site I during the same season. Statistically significant differences ($p<0.05$) were observed for BOD values at selected sites and periods. Among different sites chemical oxygen demand (COD) showed maximum value (77.94 mg/l) at downstream end point Site VI during monsoon period. However, minimum COD (14.5 mg/l) was recorded at Site I during the pre- monsoon period. COD values at different sites during pre-monsoon and monsoon periods showed significant differences ($p\leq0.05$).

Table 5.1. Physicochemical characteristics of water samples collected from selected sites of the Gomti River during pre-monsoon and monsoon seasons in India. (n=6, mean± SD)

Seasons	Sites	pH	EC(µs cm^{-1})	DO (mg l^{-1})	BOD (mg l^{-1})	COD (mg l^{-1})	NO$_3$ (mg l^{-1})	NO$_2$ (mg l^{-1})	NH$_4$ (mgl^{-1})	PO$_4$ (mgl^{-1})
Pre-Monsoon	I	8.23^{a} ±0.11	562.6^{b} ±6.42	7.64^{f} ±0.17	3.16^{a} ±0.08	14.5^{a} ±0.52	20.74^{a,b} ±0.61	0.088^{b} ±0.004	5.75^{c} ±0.34	0.70^{a} ±0.07
	II	8.53^{b} ±0.05	541.6^{a} ±12.58	5.89^{e} ±0.18	6.96^{b} ±0.16	29.67^{b} ±1.43	24.36^{c} ±2.08	0.1^{c} ±0.004	4.05^{a} ±0.57	0.90^{b} ±0.07
	III	8.26^{a} ±0.05	556^{a,b} ±5.29	4.62^{b} ±0.27	8.25^{c} ±0.26	40.83^{c} ±0.63	22.41^{b,c} ±0.96	0.082^{b} ±0.003	4.4^{a} ±0.17	0.87^{a,b} ±0.04
	IV	8.13^{a} ±0.05	569^{b} ±2.64	4.94^{c} ±0.04	9.09^{d} ±0.065	43.55^{c} ±0.64	19.50^{a} ±0.76	0.048^{a} ±0.006	4.37^{a} ±0.04	1.02^{b,c} ±0.085
	V	8.66^{c} ±0.05	588^{c} ±6.55	5.27^{d} ±0.08	7.34^{e} ±0.21	47.03^{d} ±0.23	29.08^{d} ±0.67	0.087^{b} ±0.004	5.15^{b} ±0.04	1.12^{c} ±0.01
	VI	8.13^{a} ±0.05	604.3^{c} ±18.14	3.32^{a} ±0.12	12.84^{f} ±0.23	57.97^{e} ±3.36	35.21^{e} ±1.9	0.099^{c} ±0.006	5.73^{c} ±0.14	2.05^{d} ±0.21
Monsoon	I	7.21^{a} ±0.1	362.66^{a} ±6.42	6.14^{d} ±0.14	4.22^{a} ±0.03	25.11^{a} ±0.48	22.76^{b} ±1.57	0.074^{c} ±0.001	6.04^{d} ±0.09	1.42^{a,b} ±0.09
	II	7.46^{c} ±0.05	375^{a} ±57.66	4.99^{c} ±0.27	5.98^{d} ±0.14	40.25^{b} ±0.86	18.1^{a} ±1.47	0.084^{d} ±0.003	4.14^{b} ±0.05	1.23^{a} ±0.06
	III	7.42^{c} ±0.1	455.6^{c} ±17.21	3.68^{a} ±0.58	6.12^{d} ±0.16	52.63^{c} ±2.34	19.94^{a} ±1.2	0.064^{b} ±0.003	5.59^{c} ±0.19	1.84^{b,c} ±0.06
	IV	7.13^{a} ±0.05	352.33^{a} ±4.5	4.27^{b} ±0.06	5.54^{c} ±0.12	64.16^{d} ±2.6	23.4^{b} ±1.9	0.075^{a} ±0.002	3.31^{a} ±0.02	1.58^{a,b} ±0.07
	V	7.6^{c} ±0.17	464.6^{c} ±11.23	5.38^{c} ±0.11	4.63^{b} ±0.1	74.96^{e} ±3.36	24.56^{b} ±1.4	0.045^{d} ±0.004	5.27^{c} ±0.13	2.14^{d} ±0.51
	VI	7.86^{d} ±0.09	495^{c} ±5.35	4.21^{b} ±0.06	7.41^{e} ±0.19	77.94^{e} ±1.53	36.88^{c} ±0.45	0.087^{c} ±0.006	4.44^{b} ±0.35	2.25^{c,d} ±0.22

(Different letters signify the statistical differences among physicochemical parameters at selected sites during pre-monsoon and monsoon seasons ($p < 0.05$). Site I= Gaughat; Site II=Pucca Pull; Site III= Hanuman Setu; Site IV=Nishatganj; Site V=Gomti Barrage and Site VI=Pipraghat. Whereas, EC=Electrical conductivity; DO=Dissolved oxygen; BOD=Biochemical oxygen demand; COD=Chemical oxygen demand; NO$_3$=Nitrate; NO$_2$=Nitrite; NH$_4$= Ammonium; PO$_4$=Phosphate).

Nitrate concentration varied significantly from Site I to Site VI ($p<0.05$) during both periods. Maximum nitrate concentration (36.88 mg/l) was recorded at downstream Site VI during the monsoon period and minimum nitrate concentration (18.1 mg/l) was observed at Site II in the same period. Nitrite concentration in river water showed marked significant variations at different sites ($p<0.05$). Highest nitrite concentration (0.1mg/l) was recorded at Site II during pre-monsoon period and lowest value (0.045mg/l) was observed at Site V in the monsoon period. Ammonium levels in water also showed significant variations at different sites for both periods ($p<0.05$). Maximum value (6.04 mg/l) was recorded at upstream Site I during the monsoon period and minimum value (3.31 mg/l) was also recorded during the monsoon period at Site IV. Phosphate value was recorded highest at downstream Site VI (2.25 mg/l) during the rainy season and lowest value was observed at upstream Site I (0.7 mg/l) during the pre-monsoon period. Concentration of Phosphate in water at different sites showed significant variations during both periods ($p>0.05$).

5.3.2. Metal content in Gomti River at Lucknow

The mean concentration and S.D of metals (Fe, Cd, Cu, Cr, and Pb) recorded at selected sites during both periods are given in Table 5.2. Metal concentration of river water at different sites showed significant differences ($p<0.05$). Metal concentration showed and increasing trend downstream of river Gomti. Metal content in water were in the order of Fe>Cu>Cr>Cd>Pb. Maximum concentration of Fe (5.54 mg/l), Cu (3.74 mg/l), Cr (2.57 mg/l), Cd (1.05 mg/l) and Pb (0.73 mg/l) were recorded during the pre-monsoon period at Site 6. Minimum concentration for metals Fe (1.71 mg/l), Cu (0.094 mg/l), Cr (0.4 mg/l), Cd (0.04 mg/l) at Site I and Pb (0.15 mg/l) at Site IV were recorded during the monsoon season. Significant variations were seen in the metal content of river Gomti in different seasons.

Table 5.2 Metal content (mg/l) of water samples (n=3, mean ±SD) collected from selected sites of the Gomti River during pre-monsoon and monsoon seasons in India.

Sites	Pre Monsoon					Monsoon				
	Fe	Cd	Cu	Cr	Pb	Fe	Cd	Cu	Cr	Pb
I	1.92^{a} ±0.26	0.04^{a} ±0.003	0.14^{a} ±0.02	0.56^{a} ±0.10	0.22^{a} ±0.008	1.71^{a} ±0.61	0.012^{a} ±0.001	0.094^{a} ±0.09	0.4^{a} ±0.05	$0.17^{a,b}$ ±0.02
II	2.67^{b} ±0.29	$0.085^{a,b}$ 0±.005	0.25^{a} ±0.03	0.79^{b} ±0.04	0.32^{b} ±0.02	2.02^{a} ±0.2	0.11^{b} ±0.004	0.118^{a} ±0.02	0.57^{a} ±0.03	$0.24^{c,d}$ ±0.02
III	4.32^{d} ±0.06	$0.14^{b,c}$ ±0.004	1.25^{b} ±0.16	1.08^{c} ±0.009	0.33^{b} ±0.02	3.62^{b} ±0.16	0.14^{b} ±0.04	1.07^{b} ±0.03	0.86^{b} ±0.05	$0.2b^{c}$ ±0.003
IV	3.75^{c} ±0.09	0.16^{c} ±0.01	1.48^{b} ±0.24	$1.25^{c,d}$ ±0.05	0.4^{b} ±0.07	2.99^{c} ±0.26	0.143^{b} ±0.005	1.025^{b} ±0.01	1.02^{b} ±0.05	0.15^{a} ±0.01
V	4.16^{d} ±0.05	0.65^{d} ±0.08	2.88^{c} ±0.25	1.35^{d} ±0.12	0.28^{c} ±0.01	$4.12^{c,d}$ ±0.04	0.16^{b} ±0.03	1.93^{c} ±0.38	0.92^{b} ±0.04	0.25^{d} ±0.02
VI	5.54^{e} ±0.26	1.05^{e} ±0.001	3.74^{d} ±0.16	2.57^{e} ±0.16	0.73^{d}± 0.02	4.44^{d} ±0.08	0.605^{c} ±0.05	2.64^{d} ±0.09	1.39^{c} ±0.21	0.43^{e} ±0.03

(Different letters signify the statistical differences among metals at selected sites during pre-monsoon and monsoon seasons ($p < 0.05$) Site I= Gaughat; Site II=Pucca Pull; Site III= Hanuman Setu; Site IV=Nishatganj; Site V=Gomti Barrage and Site VI=Pipraghat)

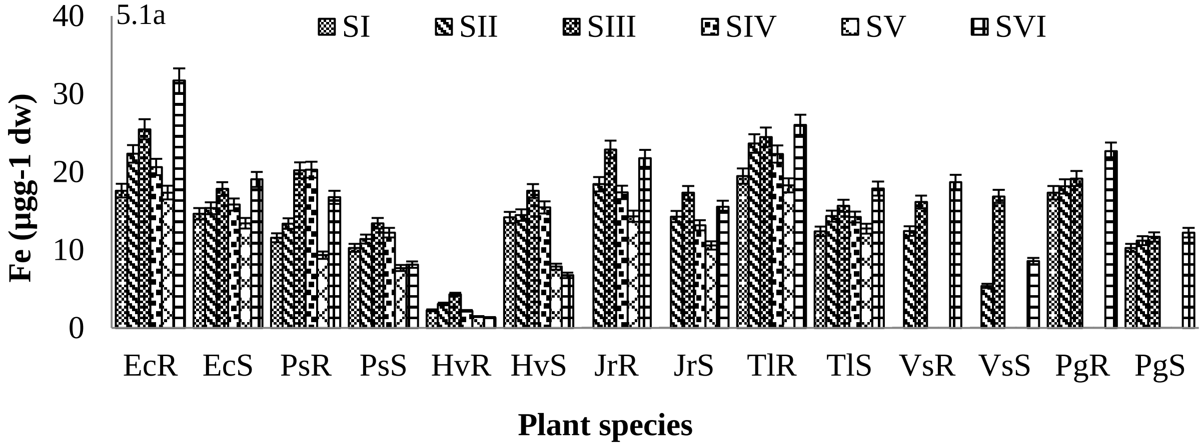
5.3.3. Bioaccumulation of metals by plants

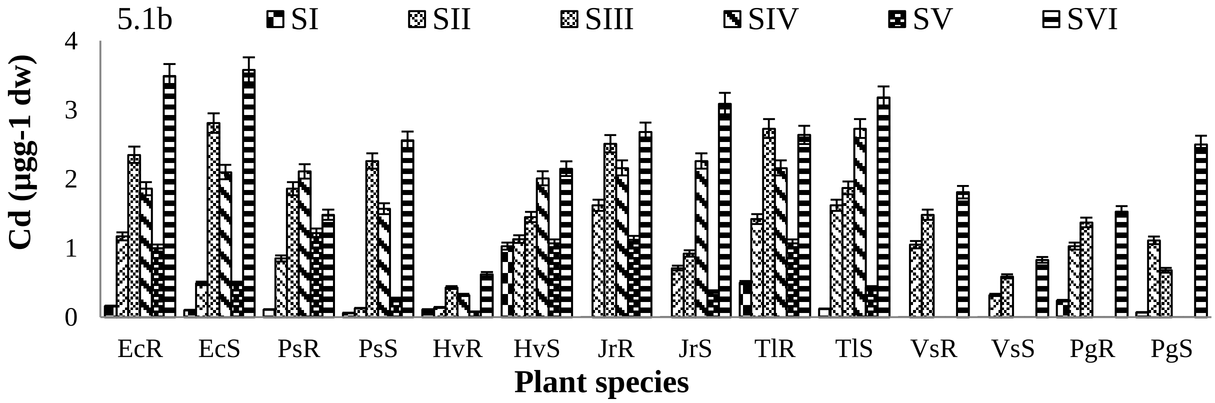
The concentration of metals accumulated by the plant roots and shoot parts collected from six different sites of river Gomti are represented in figures 5.1 during pre-monsoon and 5.2 for monsoon periods. Significant differences were observed for the plants at different sites for metal accumulation ($p<0.05$). Also, metals accumulation in roots of different plants showed significant differences ($p<0.05$). Generally metal concentration in plant roots was higher as compared to plant shoots. However, *Eichhornia crassipes, Pistia stratiotes, Hydrilla verticellata, Typha latifolia and Jussiaea repens* had greater metal accumulation in shoot parts than roots at some sites. Highest concentration of Fe (31.73 µg/g) was observed in the roots of *E. crassipes* at Site VI during the pre-monsoon period (Fig. 5.1a) and lowest root concentration of Fe (0.62 µg/g) was reported in *H. verticellata* at Site V during monsoon period (Fig. 5.2a). For shoot accumulation, highest concentration of Fe (19.06 µg/g) was found for *E. crassipes* at Site VI and lowest shoot concentration (5.403 µg/g) in *V. spiralis* at Site II during the pre-monsoon period (Fig. 5.1a). Cd content in studied plant tissues at different sites showed significant variations in relation to different sites ($p < 0.05$). Cd accumulation showed greater variations in root and shoot of plants. Maximum Cd accumulation (4.19µg/g) was observed in root of *E. crassipes* and minimum Cd accumulation in root was reported for *H. verticellata* at Site V during monsoon period (Fig 5.2b). Highest shoot accumulation for Cd (3.81µg/g) was observed for *P. stratiotes* at Site VI and lowest shoot concentration (0.06µg/g) was also observed in *P. stratiotes* at Site I during the pre-monsoon period (Fig.5.1b).

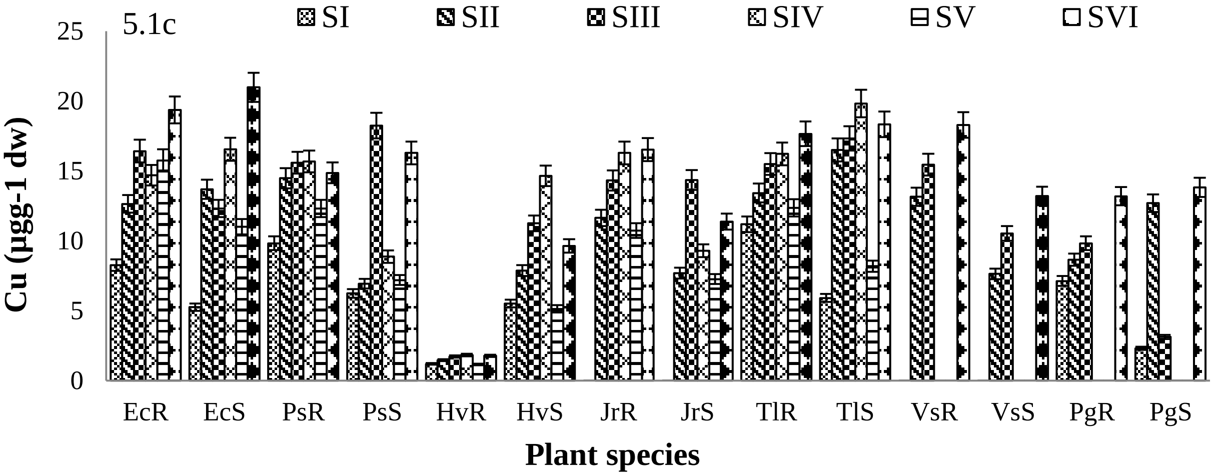
Highest Cu accumulation (21.48µg/g) was observed in shoot of *T. latifolia* during the monsoon period at Site VI (Fig. 5.2c) while as, the lowest shoot concentration of Cu (2.31µg/g) was observed in *P. glabrum* at Site I during pre-monsoon period (Fig. 5.1c). Roots of aquatic plants showing highest copper concentration (20.33 µg/g) was observed for *J. repens* at Site VI and lowest root concentration (0.49 µg/g) was reported for *H. verticellata* at Site I during monsoon period (Fig. 5.2c). All the studied plants showed potential of Cr accumulation. However, *J. repens* and *P. stratiotes* shoot accumulated maximum Cr (8.53 and 7.33 µg/g) at Site III and VI respectively during the monsoon period (Fig. 5.2d). Lowest Cr concentration (0.39µg/g) in shoot was found in *T. latifolia* at Site I. *J. repens* showed highest root concentration of Cr (8.14µg/g) at Site VI during monsoon period and lowest root concentration was reported in *H. verticellata* at Site I (Fig. 5.2d).

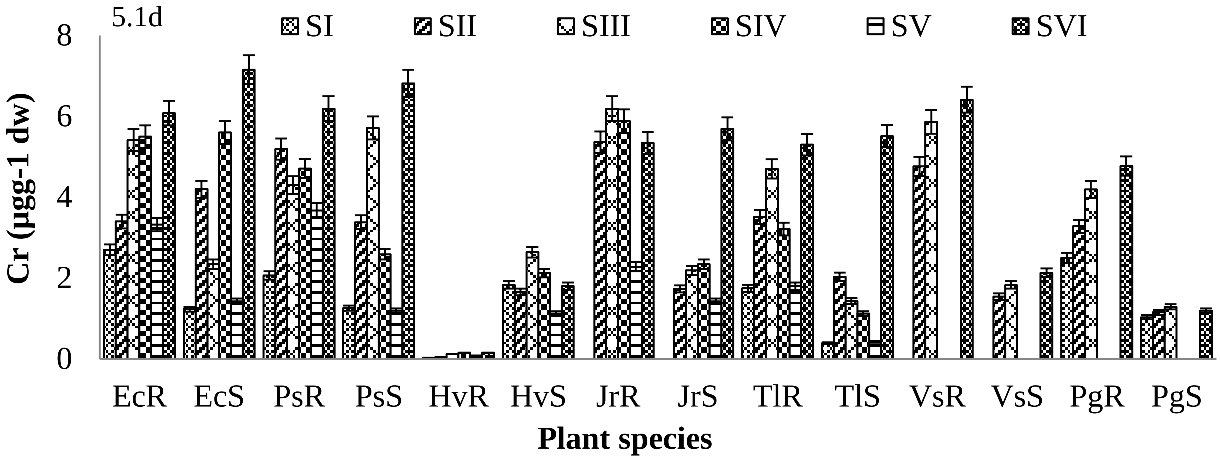
E. crassipes roots accumulated maximum Pb concentration (5.46µg/g) at Site VI during pre-monsoon period (Fig. 5.1e) and minimum root concentration of Pb (0.01 µg/g) was found in *H. verticellata* at Site I during monsoon season (Fig. 5.2e). Highest concentration of

Pb in the shoot of plants was also reported in *E. crassipes* (4.85µg/g) at Site VI and lowest Pb in shoot (0.02µg/g) was found in both *P. stratiotes* and *E. crassipes* at Site I during the monsoon period (Fig. 5.2e).









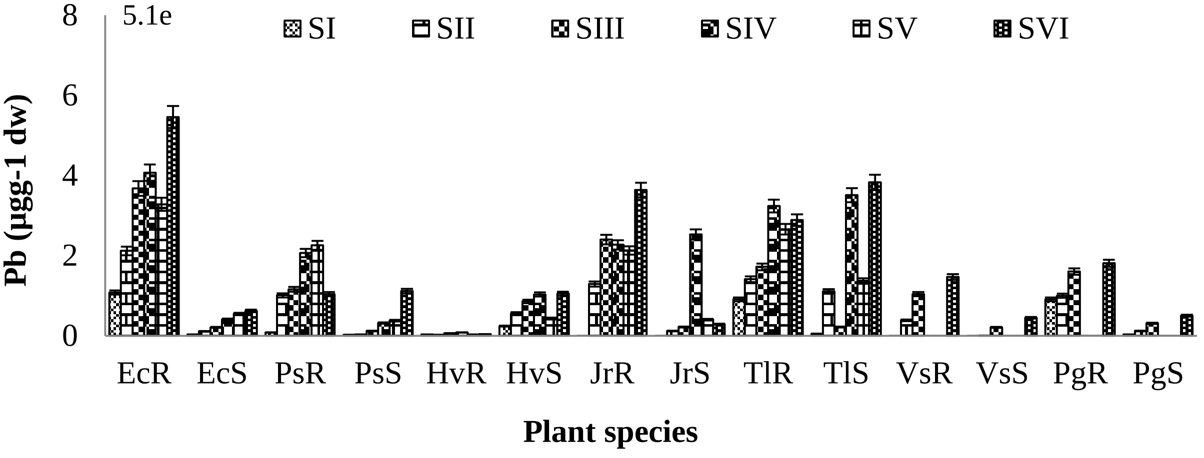
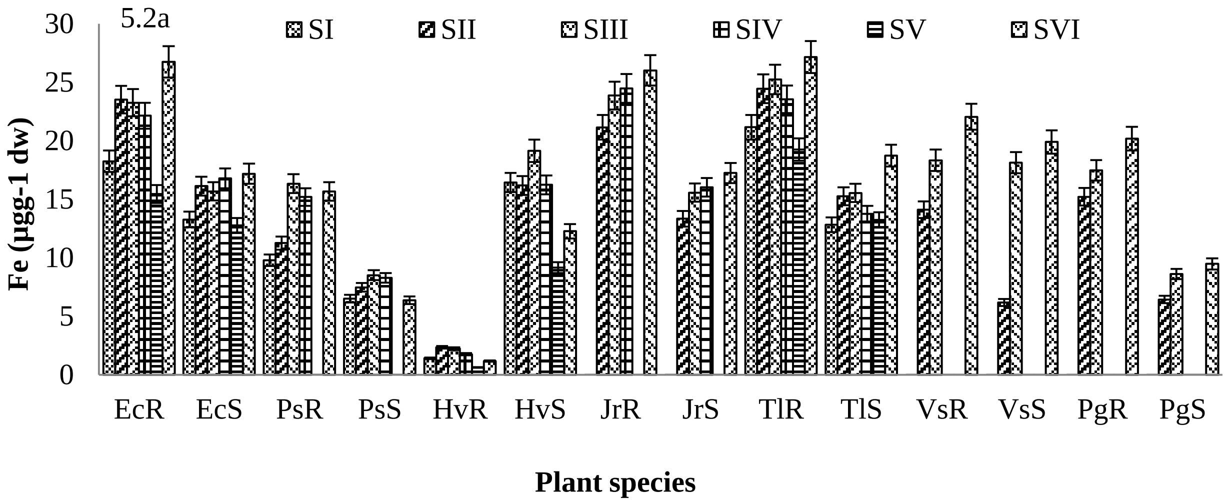
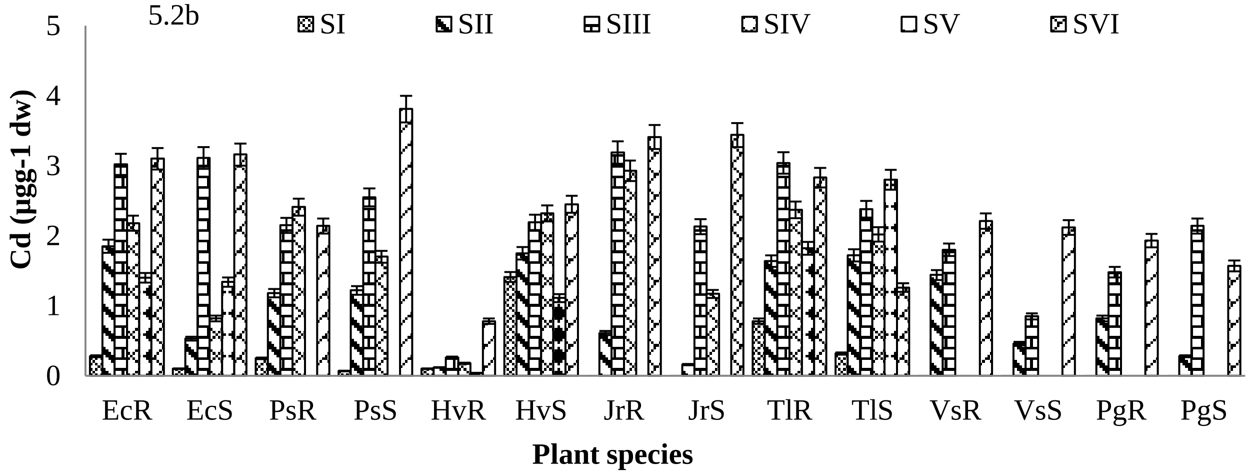


Fig. 5.1. Accumulation of metals (µg g^{-1}dw) in plant species at selected sites of the Gomti River during the Pre-Monsoon period (a-e): a=Fe, b=Cd, c=Cu, d=Cr and e=Pb. EcR=*Eichhornia crassipes* Root, EcS=*Eichhornia crassipes* Shoot, PsR=*Pistia stratiotes* Root, PsS=*Pistia stratiotes* Shoot, HvR=*Hydrilla verticellata* Root, HvS=*Hydrilla verticellata* Shoot, JrR=*Jussiaea repens* Root, JrS=*Jussiaea repens* Shoot, TlR=*Typha latifolia* Root, TlS=*Typha latifolia* Shoot, VsR=*Vallesnaria spiralis* Root, VsS=*Vallesnaria spiralis* Shoot, PgR=*Polygonum glabrum* Root and PgS=*Polygonum glabrum* Shoot; whereas: SI=Gaughat; SII=Pucca Pull; SIII=Hanuman Setu; SIV=Nishatganj; SV=Gomti Barrage and SVI=Pipraghat.


5.2a
SI
SII
SIII
SIV
SV
SVI
Fe (µgg-1 dw)
0
5
10
15
20
25
30
EcR
EcS
PsR
PsS
HvR
HvS
JrR
JrS
TlR
TlS
VsR
VsS
PgR
PgS
Plant species


5.2b
SI
SII
SIII
SIV
SV
SVI
Cd (µgg-1 dw)
0
1
2
3
4
5
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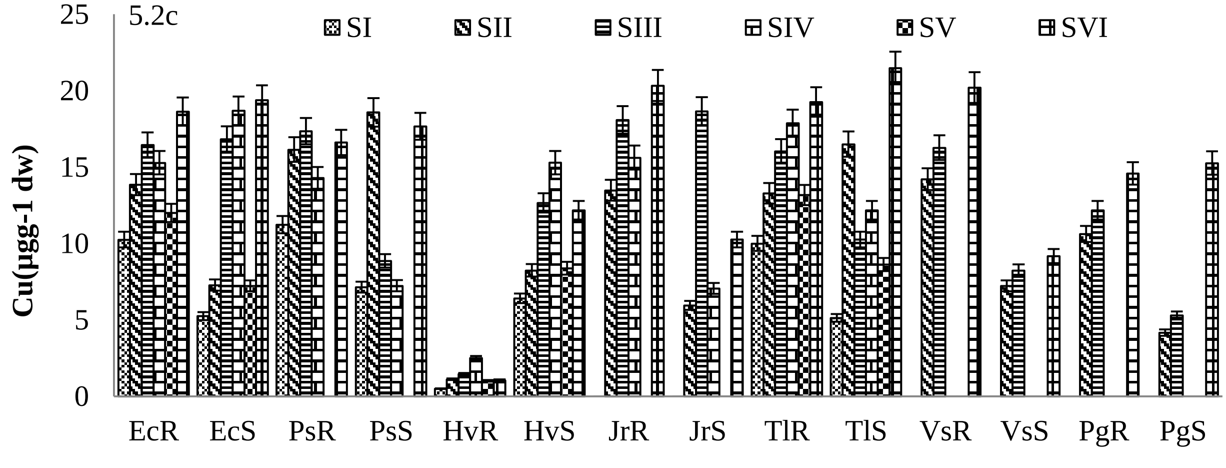
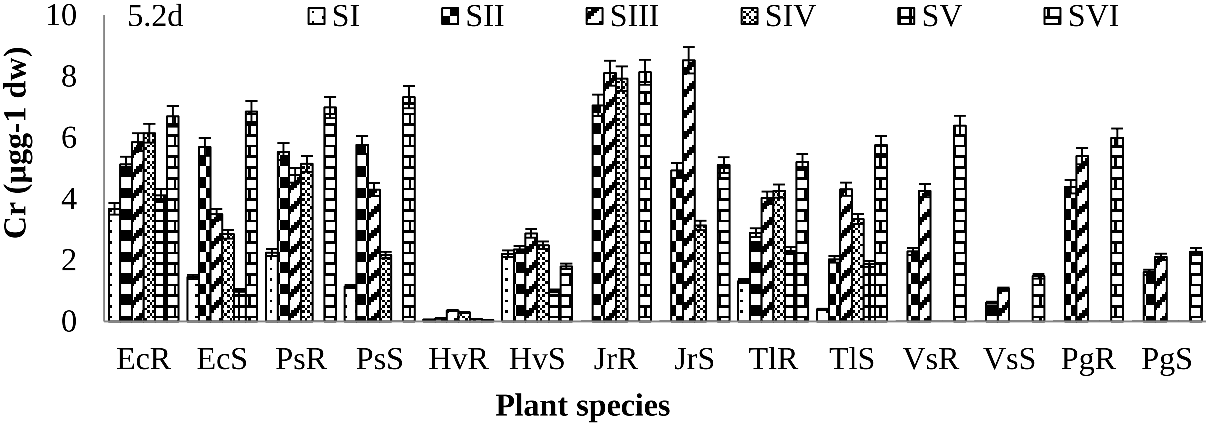
EcS
PsR
PsS
HvR
HvS
JrR
JrS
TlR
TlS
VsR
VsS
PgR
PgS
Plant species


5.2c
SI
SII
SIII
SIV
SV
SVI
Cu(µgg-1 dw)
0
5
10
15
20
25
EcR
EcS
PsR
PsS
HvR
HvS
JrR
JrS
TlR
TlS
VsR
VsS
PgR
PgS
Plant species



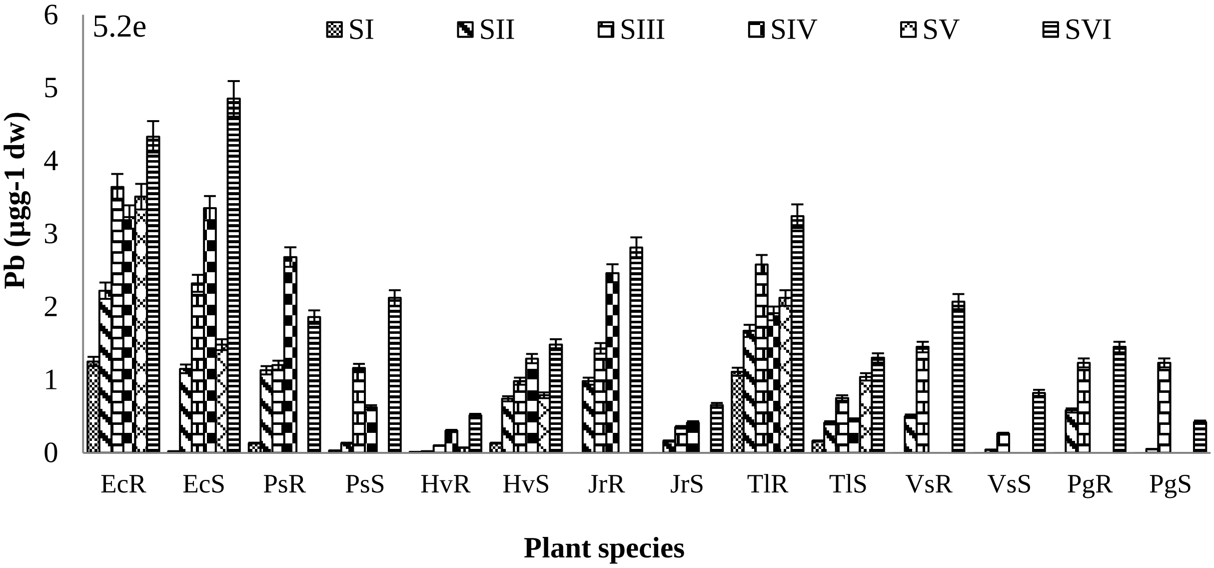


Fig. 5.2. Accumulation of metals (µg g^{-1}dw) in plant species at selected sites of the Gomti River during the Monsoon period (a-e): a=Fe, b=Cd, c=Cu, d=Cr and e=Pb. EcR=*Eichhornia crassipes* Root, EcS=*Eichhornia crassipes* Shoot, PsR=*Pistia stratiotes* Root, PsS=*Pistia stratiotes* Shoot, HvR=*Hydrilla verticellata* Root, HvS=*Hydrilla verticellata* Shoot, JrR=*Jussiaea repens* Root, JrS=*Jussiaea repens* Shoot, TlR=*Typha latifolia* Root, TlS=*Typha latifolia* Shoot, VsR=*Vallesnaria spiralis* Root, VsS=*Vallesnaria spiralis* Shoot, PgR=*Polygonum glabrum* Root and PgS=*Polygonum glabrum* Shoot; whereas: SI=Gaughat; SII=Pucca Pull; SIII=Hanuman Setu; SIV=Nishatganj; SV=Gomti Barrage and SVI=Pipraghat

5.3.4. Correlation between physicochemical parameters and metal content in Gomti river

In the present study, relationship in dynamics of metal concentration with physicochemical characteristics of Gomti river water observed at different sites was evaluated using Pearson Correlation Coefficient (Table 5.3). All heavy metals showed significant positive correlation with COD, phosphate and nitrate and negative correlation with DO. Also, metals showed significant positive correlations with more than one metal. Among physicochemical characteristics, Fe in water showed significant positive correlation with COD (0.898; $p < 0.01$), phosphate (0.743; $p < 0.01$) and nitrate (0.645; $p < 0.05$). Among various metals recorded, Fe showed positive correlation with Cu (0.917), Pb (0.739), Cr (0.808) $p < 0.01$ and with Cd (0.650; $p < 0.05$). With different physicochemical parameters, Cd in water showed significant positive correlation with COD (0.774; $p < 0.01$) and phosphate (0.799; $p < 0.01$). However, Cd didn’t show any significant relation with nitrate. Among various metals recorded, Cd showed positive correlation with Cu (0.842; $p < 0.01$), Pb (0.578; $p < 0.05$) and Cr (0.707; $p < 0.05$). Among physicochemical characteristics, Cu in water showed significant positive correlation with COD (0.901; $p < 0.01$), nitrate (0.761; $p < 0.01$) and phosphate (0.796; $p < 0.01$). Among various metals assessed in water column, Cu showed positive correlation with Pb (0.717) and Cr (0.871) ($p < 0.01$). For Pb significant positive correlation with COD (0.720; $p < 0.01$), nitrate (0.700; $p < 0.01$) and phosphate (0.713; $p < 0.01$) were observed. For metals, Pb also showed significant positive correlation with Cr (0.871; $p <0.01$). Among various physicochemical properties, Cr in water showed significant positive correlation with COD (0.765; $p < 0.01$), nitrate (0.781; $p < 0.01$) and phosphate (0.635; $p < 0.05$)

Table 5.3 Correlation matrix between physicochemical characteristics and metals of the Gomti River water at Lucknow, India.

	pH	E.C	DO	BOD	COD	NO_3	NO_2	NH_4	PO_4	Fe	Cd	Cu	Pb	Cr
pH	1	$.910^{**}$	.215	.404	-.269	.289	.527	.033	-.466	-.048	-.095	.022	-.239	.078
E.C			.025	.563	-.116	.379	.432	.224	-.247	.134	.015	.216	-.165	.172
DO				$-.684^{*}$	$-.704^{*}$	-.396	.244	.224	$-.584^{*}$	$.777^{**}$	-.202	$-.577^{*}$	-.564	-.498
BOD					.294	.541	.148	-.080	.167	.450	.011	.411	.245	.389
COD						.527	-.211	-.254	$.847^{**}$	$.898^{**}$	$.774^{**}$	$.901^{**}$	$.720^{**}$	$.756^{**}$
NO_3							.379	.136	.547	$.645^{*}$	.568	$.761^{**}$	$.700^{*}$	$.781^{**}$
NO_2								.372	-.031	-.109	.188	.000	-.007	.005
NH_4									.172	-.078	.111	.005	-.194	-.176
PO_4										$.743^{**}$	$.799^{**}$	$.796^{**}$	$.713^{**}$	$.635^{*}$
Fe											$.650^{*}$	$.917^{**}$	$.739^{**}$	$.808^{**}$
Cd												$.842^{**}$	$.578^{*}$	$.707^{*}$
Cu													$.717^{**}$	$.871^{**}$
Pb														$.882^{**}$
Cr														1

(Values represent Pearson correlation coefficient and significant at p 0.01=** and p 0.05=*)

5.3.5 Translocation Factor (T.F.)

In the present study, Translocation Factor for metals investigated in selected plants is shown in (Table 5.4). In certain cases, TF of the studied plants for metals was found to be greater than 1, showing their potential of accumulating greater metal contents in different plant parts than roots. Except for Fe, *E. crassipes, P. stratiotes T. latifolia* and *J. repens* showed more than 1 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 also varied during pre-monsoon and monsoon periods. Also significant variations were reported in the TF of plants at different sites.

Table 5.4. Distribution and translocation factor of different metals in plants during the pre-monsoon (PrM) and monsoon (M) seasons in Lucknow, India

Sites	Plant species	Fe		Cd		Cu		Pb		Cr	
		PrM	M	PrM	M	PrM	M	PrM	M	PrM	M
Site I	*E. crassipes*	0.83	0.72	0.62	0.35	0.63	0.51	0.02	0.01	0.45	0.39
	P. stratiotes	0.88	0.66	0.54	0.28	0.67	0.63	0.25	0.23	0.61	0.5
	H. verticellata	6.25	11.65	9.36	14.1	4.63	13.08	8	13	61	36.83
	T. latifolia	0.63	0.60	0.24	0.41	0.52	0.51	0.05	0.14	0.22	0.3
	P. glabrum	0.59	-	0.35	-	0.32	-	0.03	-	0.41	-
Site II	*E. crassipes*	0.68	0.69	0.41	0.28	1.08	0.52	0.05	0.51	1.2	1.11
	P. stratiotes	0.85	0.66	0.15	1.03	0.47	1.15	0.02	0.11	0.65	1.04
	H. verticellata	4.6	6.88	8.07	14.58	5.39	7.42	28	37	41.5	23.5
	J. repens	0.77	0.63	0.43	0.26	0.66	0.44	0.09	0.16	0.32	0.69
	T. latifolia	0.6	0.62	1.14	1.03	1.22	1.24	0.78	0.24	0.57	0.7
	V. spiralis	0.43	0.43	0.3	0.31	0.58	0.5	0.02	0.08	0.32	0.27
	P. glabrum	0.61	0.42	1.07	0.34	1.46	0.39	0.12	0.08	0.35	0.36
Site III	*E. crassipes*	0.69	0.67	1.19	1.02	0.75	1.02	0.005	0.63	0.43	0.59
	P. stratiotes	0.66	0.52	1.21	1.18	1.17	0.51	0.1	0.96	1.32	0.9
	H. verticellata	4.07	8.58	3.37	8.42	6.54	8.72	14.33	9.8	22	8
	J. repens	0.75	0.65	0.36	0.66	1.0	1.03	0.09	0.24	0.35	0.62
	T. latifolia	0.64	0.61	0.68	0.78	1.17	0.64	0.12	0.29	0.3	0.98
	V. spiralis	1.01	0.98	0.39	0.47	0.68	0.5	0.2	0.17	0.31	0.24
	P. glabrum	0.6	0.49	0.49	1.44	0.31	0.43	0.19	1.0	0.3	0.39
Site IV	*E. crassipes*	0.76	0.75	1.12	0.37	1.12	1.22	0.1	1.03	1.01	0.46
	P. stratiotes	0.6	0.54	0.74	0.7	0.56	0.5	0.15	0.23	0.54	0.42
	H. verticellata	7.0	9.22	6.28	12.88	8.0	6.09	12.87	4.3	14.13	8.58
	J. repens	0.75	0.65	1.04	0.39	0.57	0.45	1.11	0.16	0.39	0.39
	T latifolia	0.63	0.58	1.26	0.85	1.22	0.68	1.08	0.23	0.34	0.78
Site V	*E. crassipes*	0.77	0.82	0.49	0.95	0.69	0.6	0.16	0.42	0.42	0.24
	P. stratiotes	0.82	-	0.22	-	0.58	-	0.16	-	0.32	-
	H. verticellata	5.35	14.79	13.37	27.75	4.46	8.29	14.33	11.2	14	12.5
	Jr repens	0.73	-	0.33	-	0.67	-	0.18	-	0.62	-
	T. latifolia	0.69	0.68	0.4	1.53	0.66	0.65	0.51	0.49	0.23	0.81
Site VI	*E. crassipes*	0.6	0.64	1.02	0.89	1.08	1.04	0.11	1.12	1.17	1.02
	P. stratiotes	0.48	0.4	1.72	1.78	1.09	1.06	1.07	1.13	1.1	1.04
	H. verticellata	5.05	10.39	3.46	3.14	8.18	11.71	26.25	2.9	12	36
	J. repens	0.71	0.66	1.15	1.0	0.68	0.84	0.07	0.23	1.06	0.62
	T. latifolia	0.68	0.68	1.2	0.36	1.03	1.11	1.32	0.4	1.03	1.1
	V. spiralis	0.45	0.9	0.45	0.95	0.72	0.45	0.3	0.39	0.36	0.23
	P. glabrum	0.53	0.46	1.63	0.81	1.04	1.04	0..27	0.28	0.24	0.38

(Whereas; Site I= Gaughat; Site II=Pucca Pull; Site III= Hanuman Setu; Site IV=Nishatganj; Site V=Gomti Barrage and Site VI=Pipraghat; *E. crassipes=Eichhornia crassipes, P. stratiotes=Pistia stratiotes, H. verticellata=Hydrilla verticellata, J. repens=Jussiaea repens, T. latifolia=Typha latifolia, V. spiralis=Vallesnaria spiralis* and *P. glabrum=Polygonum glabrum*; (-) indicates the plant species were not present during that season at the site.

5.4. Discussion

5.4.1. Physicochemical Characteristics of water

Gomti river though affected by the hydrological factors, anthropogenic activities such as recreation, settlements growth, industrial establishments, agricultural development in the watershed impact the water quality of river (Agarwal et al. 2007), leading to changes in the physicochemical characteristics of river water.

pH of aquatic ecosystems and the changes therein are mainly due to metabolic activities such as photosynthetic processes of macrophytes and phytoplankton's, respiration of aquatic animals and plants as well as due to variations in temperature (Vymazal 2007). In the present study, it was observed that pH of Gomti river at Lucknow is alkaline in nature. Similar results pertaining to the study were reported by Anukool and Shivani (2011), while analyzing pH of river Gomti at different stations. Our findings are in conformity with Kress and Herut (1998). Fawzy et al. (2012) while assessing pH of river Nile ascribed alkaline nature of water due to high plant and plankton density.

Results showed that conductivity of Gomti River at Lucknow increased downstream, reaching a maximum at Site VI, which could be attributed to the dumping of huge load of untreated municipal and industrial wastewater containing chlorides, phosphates, nitrates, sulphates etc. by the drains (Rai 2010a). Dissolved Oxygen content of river reduced drastically from upstream Site I to downstream Site VI during warm periods as high temperatures and nutrient inputs lead to an increase in algal blooms and other aquatic diversity, resulting in significant decrease in DO levels of water (Copper et al. 2002, Bellos et al. 2004). Present results on maximum BOD in the downstream of river emphasized on increasing organic pollution due to development of cow sheds on the river banks and washing of faecal matter of animals and dead plants into river. Very high BOD values at Pipraghat of River Gomti were also reported by Anukool and Shivani (2011). COD of river Gomti at Lucknow was as high as 77.94 mg/l, depicting high chemical inputs into river system. High COD values of river Gomti during monsoon period indicate discharges of agricultural runoff, industrial sewage carried by the rains into the river during the period.

The level of inorganic pollutants like nitrate, ammonium and phosphate were found to be maximum during the rainy season. Similar results were obtained by Rosli and Yahya (2012) while assessing nutrient loading in a tropical river system. Adeyomo et al. (2008) reported higher levels of nitrate and phosphate in water of Ibadan river system during the rainy due to discharge of sewage into rivers and runoff from agricultural fields and storm water. The natural sources of phosphate (PO_4) are weathering of phosphorus bearing rocks and decomposition of organic matter. Domestic wastewater containing detergents, leaching of chemical fertilizers from the terrestrial systems after heavy rainfall is the major source of inorganic nutrient loading into rivers (Bellos and Sawidis 2005).

Compared to nitrate concentration, nitrite and ammonium levels were very less and fluctuated significantly. Nitrite concentration in freshwaters is usually very low, 0.001 mg/l NO_2-N and rarely higher than 1.0 mg/l NO_2-N (WHO 2002; Fawzy et al. 2012). The changing values of ammonium and nitrite in river signify the occurrence of micro-organisms that are responsible for redox reactions of these pollutants (Chandra et al. 2012). Since Gomti river water is used for drinking purpose by the Lucknow, levels of nitrate, nitrite and phosphate concentration in river water above the prescribed limits of BIS (2003) and EPA (2009) may have harmful consequences on the health of people dwelling along the river as well as on river Gomti itself due to eutrophication.

5.4.2. Metal content in Gomti river water

Gomti river water at Lucknow showed varying concentration of five heavy metals investigated. This behavior is influenced due to urbanization of the Lucknow City (Singh et al. 1997; Lohani et al. 2008). In the present study the pollution problem in river Gomti is evident as the concentration of all five heavy metals (Fe, Cu, Cd, Cr and Pb) were definitely higher than the critical ranges stated for drinking water standards (BIS 2003, WHO 2008 and EPA 2009). Compared to monsoon period, metal concentration was higher in the pre-monsoon period. The lesser values during the monsoon period could be due to the rain dilution effect as the water level of river during the monsoon period goes very high. Generally metal concentration increased downstream from Gaughat (Site I) towards Pipraghat (Site VI) depicting influence of downstream effluents into river.

All the metals reached their maximum value at downstream Site VI during the pre-monsoon period. Lohani et al. (2008) while assessing seasonal variations of heavy metals in river Gomti at Lucknow also reported that metal concentration was higher during the pre-monsoon period. Pollutants such as herbicides, pesticides, fertilizers and chemicals can make their way in to the river through drains of the whole city (Dutta et al. 2010). Rainfall and the subsequent leaching of organic matter and minerals influence the concentration of heavy metal in river water. In aquatic ecosystems, due to interaction of metals with suspended particulate matter of water column, the metals tend to settle and accumulate in the bottom sediments of rivers (Rai, 2008). The accumulation and remobilization of metals in river systems are the two important mechanisms that regulate their concentration in an aquatic environment (Linnik and Zubenko 2000; Vardanyan and Ingole 2006 and Ishaq and Khan 2103).

5.4.3. Bioaccumulation of metals by plants in Gomti river at Lucknow

Bioavailability of metals in water for accumulation is influenced by various factors such as pH, temperature, redox potential, chemical speciation, seasonal changes, sediment type, salinity and organic matter (Du Laing et al. 2007 and Shuping et al. 2011). The present study focused on the quantification of levels of Fe, Cu, Cd, Cr and Pb in the plant tissues. Heavy metal uptake, translocation and accumulation potentialities by native aquatic macrophytes growing naturally at different six sites in River Gomti at Lucknow were evaluated. Both root and shoot tissues were assessed for metal accumulation and translocation. Generally metal levels were much higher in the roots as compared to shoot of the plants, which are in consistent with the results of others (Vesk et al. 1999; Chandra and Kulshreshtra 2004; Rieumont et al. 2007; Rai et al. 2012). The reason for this may be ascribed to low mobility of metals from root to shoot due to formation of complex compounds with -COOH group, which inhibits metal translocation to shoot parts of plants (Cradwell et al. 2002). However at certain sites, it was observed that plants accumulated metals in higher proportions in shoots than root. Kim et al. (2003) stated that differential metal accumulation by different plant species which may be due to compartmentalization and translocation in the vascular system of plants.

Results of this study showed that heavy metal accumulation in seven aquatic plants were in the order of Fe > Cu > Cr > Pb > Cd. With the exception of *H. verticellata* a submerged plant, free floating aquatic plants generally accumulated greater amount of metals than rooted and emergent plants. The results are in line with the observations of Ebrahimpour and Mushrifah (2008). *H. verticellata* showed maximum accumulation of all five metals in the shoot parts. The reason for this may be that submerged aquatic plants readily absorb available elements directly from the water through foliar surfaces (Baldantoni et al. 2004).

Iron (Fe) though an essential element for living organisms may damage the tissues at excessive levels due to formation of free radicals (Engin et al. 2013). Maximum Fe accumulation was found in the roots of the plants. The reason for greater root concentration in plants may be due to the formation of iron hydroxide plaques that are mobilized and precipitated onto root surfaces (Weis and Weis 2004). The formation of iron plaques on root surfaces may form a barrier to the uptake of cationic nutrients and metals and may immobilize metals (Taggart et al. 2009). This would have resulted in lesser translocation of Fe to the shoot of plants. Generally, Fe accumulation in all seven plants was notably higher along downstream sites. However, plants collected from sites III and VI had accumulated highest Fe content in their roots. This may be due to probable draining of effluents containing Fe spare parts from automobile works situated on the banks of river into river water and subsequent accumulation of Fe by plant roots. Fe accumulation by the studied plants at different sites could be arranged as: *E. crassipes* > *T. latifolia* > *J. repens* >*P. stratiotes* > *P. glabrum* >*H. verticellata* >*V. spiralis.* Fe accumulation by aquatic plants has also been reported by Rai (2012).

Cadmium (Cd) a non- essential metal for living organisms, is a metal of concern for its greater mobility in the environment and is one of most toxic heavy metal for living world (Wang and Zhou 2005). Cd accumulation was found to be greater in shoots than the roots of certain plants. The distribution of Cd within the plants is quite variable and thus explains the rapid translocation from root to aerial parts of plants (Pendais and Pendais 2001; Duman 2007). Similar findings pertaining to Cd accumulation were observed for the shoots of *P. stratiotes, J. repens* and *H. verticellata.* Greater shoot accumulation by *P. stratiotes* indicates this species may be useful vehicle for absorbing and accumulating Cd from polluted water (Shuvaeva et al. 2013). The *H. verticellata* showing higher Cd content in the shoot might adsorb Cd from water via the leaves. Cd accumulation at different sites by the studied plants could be arranged in the order of: *P. stratiotes* > *J. repens* > *E. crassipes* > *T. latifolia* >*H. verticellata* > *V. spiralis* > *P. glabrum.*

Copper (Cu) is a micronutrient required for plants at very low concentrations. However, it becomes highly toxic at elevated concentrations. Our findings on Cu accumulation by plants showed that generally roots of all plants contained higher concentration of Cu than shoot. But *T. latifolia* an emergent plant showed higher Cu accumulation capabilities in the shoot parts. This was followed by *E. crassipes* shoots. These findings are contradictory to the observations of Fawzy and Badr (2006), who reported greater Cu accumulation in roots of *T. latifolia* and *E. crassipes*. Since, most of the studied plants accumulated higher concentrations of Cu in the roots at sites with higher pH (> 8.0). These results are in line with the findings of Weis and Weis (2004), who reported that at higher pH conditions (> 8.0) the presence of plaque enhanced Cu uptake into roots. Similar results were also observed for Cu accumulation by *C. papyrus* plant growing in an urban natural wetland of Rwanda (Sekomo et al. 2011). Cu accumulation at different sites by the studied plants could be arranged in the order of: *T. latifolia* > *E. crassipes* >*P. stratiotes* > *H. verticellata* > *J. repens* > *V. spiralis* > *P. glabrum.*

Chromium (Cr) a non-essential element for plants is very toxic at excessive concentrations (Rai et al. 1995; Mishra and Tripathi, 2008). Cr accumulating potential was shown by all plants at selected sites. However, *J. repens* and *P. stratiotes* are the plants that showed elevated levels of Cr in the shoot parts than roots at site III and IV, respectively. This may be due to their potential of concentrating Cr in various plant organs without showing symptoms of toxicity. Chromium levels above 0.5 mg/kg dw is considered as toxic to plants (Allen, 1989). However, plants accumulated more Cr than toxic levels. E. *crassipes* accumulated higher Cr content in shoot than roots. Similar findings were also reported by Tiwari et al. (2007) for highest bioaccumulation of Cr (10.1 mg/kg) by E. *crassipes.* Cr bioaccumulation by plants at six different sites was found in the order of: *J. repens* > *P. stratiotes* > *E. crassipes* > *V. spiralis* > *T. latifolia* >*H. verticellata* > *P. glabrum.*

Lead (Pb) is a highly toxic metal even at low doses. Lead content in plants leads to reduced photosynthetic pigments, structural changes in chloroplasts and a decrease in enzyme activity for CO_2 assimilation (Wang et al. 2011; Dirilgen et al. 2011). All plants showed maximum Pb accumulation in plant roots than shoot parts, with exception of *E. crassipes* at Site VI. The greater Pb concentration in roots of plants signifies lesser translocation of Pb to aerial parts of aquatic plants. Vesely et al. (2011) also reported 10 times higher Pb in plant roots than in leaves of *P. stratiotes.* Pb is reported as the least bioavailable metal and highest accumulation occurs in the root of plant species (Pendais and Pendais 2001). The reason for greater accumulation of Pb in roots than shoots is that, Pb can move inside the root tissues through the apoplast and gets accumulated near the endoderm. Endoderm acts as a partial barrier to the translocation of Pb through root to the shoot (Verma et al. 2005). Pb bio-accumulation by plants at six different sites was found in the order of: *E. crassipes* > *T. latifolia* > *J. repens* > *P. stratiotes* >*V. spiralis* > *H. verticellata* > *P. glabrum.*

5.4.4. Translocation factor of metals for plants. Translocation factor serves the purpose of indicating metal mobility inside the plant species from root to shoot parts.

It shows metal accumulating capabilities of plants in different plant parts other than roots. The present study showed that, translocation factor for metal remediation by the plants was in the order of *H. verticellata*> *E. crassipes*> *P. stratiotes* > *T. Latifolia* > *J. repens* >*P. glabrum*> *V. spiralis.* The exceptional highest translocation factor of *H. verticellata* for all metals is due to that euphydrophtes (plants that have completely submerged leaves) uptake of metals through leaves becomes important when the metal concentrations in the surroundings are high (Guilizoni 1991; Denny 1995). The differential metal uptake by the roots and shoots 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 (Ahmad et al. 2013). A higher value of translocation factor (>1), depicts that the plant efficiently translocate metals from root to the shoot parts (Braker and Brooks 1989), however (<1) value of TF signifies that the specific plant genera could serve as a potential plant for phytostabilisation (Archer and Caiwell 2004; Rai et al. 2012).

5.5. Conclusions

The study demonstrates that river Gomti is subjected to alarming inputs of heavy metal pollution and other inorganic pollutants due to urbanization, industrialization and agricultural activities in the watershed. 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 increased metals accumulation by plants was directly proportional to greater metal content in water, which reflects that these species could be used in ecological surveys as in situ bio-monitors of water quality due to their ability to concentrate metal pollutants in tissue parts and to predict the level and kind of environmental pollution. Further studies should be conducted in the river Gomti during all seasons round the year to evaluate the dynamics of metal accumulation and release back into river by these macrophytes for efficient water quality management.

Chapter 6

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

Chapter 6. Phytoremediation efficacy of *Pistia stratiotes* and *Hydrilla Verticellata* under mono and mixed cultures for heavy metals removal: Mechanism of metal sorption by SEM and FTIR studies

6.1. Introduction

Metals such as Hg, Cd, Cr, Cu, Pb, As, Zn and Sn are of great concern with regards to their ecotoxicology (Ali et al., 2013; Gill et al., 2014). Cd has been classified as human and animal carcinogenic even at very low concentrations and may cause osteomalacia, severely damage the lungs, kidneys and liver (Satarug et al., 2010; Xie et al., 2013); likewise, Cr (VI) inside the living bodies can cause cancer, mutations by destroying DNA protein cross-links, breakage of DNA strands (Mc Carrol et al., 2010; Liu et al., 2014); excessive levels of Cu can result in liver cirrhosis, chronic anemia, gastrointestinal distress (Mandour, 2012; Alam et al., 2012).

The various conventional techniques e.g. reverse osmosis, ion exchange, coagulation, chemical precipitation, membrane filtration, land application etc. (Mishra and Tripathi, 2008; Pal and Rai, 2010; Dhir and Srivastava, 2011) employed for heavy metal removal are expensive, require high energy and do not completely remove the heavy metals from the water column, besides generating toxic sludge which when disposed-off release the pollutants that eventually find their way into water resources (Aravindhan et al., 2004; Tolu and Atoke, 2012; Liu et al., 2014). This has necessitated the development and use of alternative, low cost, energy efficient and sustainable techniques for optimal removal of toxic heavy metals from the polluted waters (Vymazal, 2009; Sheng et al., 2013). The application of bio-techniques coupled with engineering methods have recently been gaining attention and are being emphasized for remediation and protection of environment (Uysal and Taner; 2009; Arini et al., 2012; Luca et al., 2013).

Aquatic macrophytes have been reported to accumulate heavy metals from natural ecosystems (Muzamdar and Das 2014; Shah et al., 2015) as well as from the constructed wetland systems (10000) times greater than in the surrounding water column (Weiss et al., 2006; Yilmaz and Akbulut 2011; Huguenot et al., 2014). 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 (Maltais- landry et al., 2009; Yilmaz and Akbulut, 2011; Farid et al., 2014; Foladori et al., 2014). However, studies on the potential of mono and mixed culture of free floating and submerged plants for removal of multi metals under the effect of sequential processes of circulation is not well documented. Further, developing the knowledge of metal binding sites within the plants, metal localization and class of compounds in which the metal are transported and distributed inside the plant parts needs to be extensively discoursed (Malik, 2007; Ibrahim et al., 2009; Shuvaeva et al., 2013).

The present study is undertaken to evaluate the effect of intermittent water recirculation on the phytoremediation potential of mono and mixed cultures of *Pistia stratiotes* and *Hydrilla verticellata* under multi metal stress in a pilot scale microcosm treatment system. Also attempts on the 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 were conducted and presented.

6.2. Materials and methods

6.2.1. Plants collection and their acclimatization

The experimental plants *Pistia stratiotes* and *Hydrilla Verticellata* were collected from the Gomti River and local ponds of Lucknow city. The plants were washed with running tap water to remove any attached particles. They were acclimatized for 15 days in Hoagland solution (Hoagland and Arnon, 1950) in constructed cemented ponds. The selection of these plants was employed due to their high biomass production and also potential of accumulating multiple metals under natural aquatic ecosystems in our previous reports (Shah et al., 2015). After 15 days of acclimatization, fully grown and healthy plants were transferred to the treatment tubs. 200 grams of each plant was put into tubs for their monocultures, covering 80% of surface area of water in the tub. For mixed culture, 100 grams of each plant was put together in the tubs.

6.2.2. Experimental treatment set up and mode of operation

A bench scale microcosm water treatment system, with intermittent flow and circulation, comprising of different parallel treatments of individual and mixed cultures of *Pistia stratiotes* and *Hydrilla Verticellata* were set up in the Net House located at field station of Department of Environmental Science, BBAU Lucknow. Each set up as a series, consisted of a 50 liters storage tank, three treatment polypropylene tubs of 20 liters capacity each and a collection tub. The components of the each independent treatment system were fixed in such a way that water flow was from the storage tank through the treatment tubs to the collection tub. Water from the storage tank was manually drained towards the tubs through a fitted tap value at the bottom of each tank and tubs. Plant biomass was immediately inserted into the tubs after each tub was filled with 15 liters of water.

Simulated metals contaminated water was intermittently circulated between the treatment tubs. Initially water in the tubs was given a hydraulic retention time of three days and on the fourth day fitted tap values were opened and water flowed through the tubs. From the storage tank water flow through the treatment tubs to collection tub and back to recirculation was done for 10 hours/circulation day. The flow rate of water in the setup was maintained at 1.5 liters/hour. Finally water was received in the collection tub located at the end of the each treatment series and water was put back manually into the storage tank for recirculation. The intermittent circulation of water was done on 4th, 8th, 12th, 20th, 24th and 28th days of treatment.

The experiment was performed in the month of November 2013 and repeated again in December.

6.2.3. Plant cultures and treatment conditions

Mono and mixed cultures of free floating *Pistia stratiotes* and submerged *Hydrilla verticellata* were tested for their metal accumulation and removal capacities form multi metal solution of Cu (5ppm); Fe (5ppm); Cd (2.5 ppm) and Cr (2.5 ppm). Different treatments for the experiment were: T1= Monoculture of *Pistia stratiotes* in metals contaminated water under the effect of water circulation; T2= Monoculture of *Hydrilla verticellata* in metals contaminated water under the effect of water circulation; T3= Monoculture of *Pistia stratiotes* in metals contaminated water without circulation

T4= Monoculture of *Hydrilla verticellata* in metals contaminated water without circulation

T5= Mixed culture of *Pistia stratiotes* and *Hydrilla verticellata* in metals contaminated water under the effect of water circulation

T6= Mixed culture of *Pistia stratiotes* and *Hydrilla verticellata* in metals contaminated water without water circulation

T7= Control for metals contaminated water without plants under the effect of water circulation was used to determine the possible adsorption of metals onto the tubs

T8 = Control for plants (Mixed culture) without metals under water circulation.

6.2.4. Plant sampling and analysis

Plant samples from each treatment were collected on 5th, 10th, 15th, 20th, 25th and 30th day from the start of the experiment. On each sampling day, randomly plants were selected from each treatment and rinsed with deionized water and divided into root and shoot components for further analysis.

Metals analysis: Root and shoot plant samples were oven dried at 90 °C to a constant weight and metals (Cu, Fe, Cd and Cr) in the plant parts were determined in 1 gram (dry weight) of each root and shoot sample after acid digestion of dry samples with an acid mixture (9:4 nitric acid: perchloric acid) at about 100 °C. Metal concentrations were determined by using atomic absorption spectrophotometer (AAS 240 FS, Varian) following standard protocols. Analytical data quality of metals was ensured through repeated analysis (n=3) of EPA quality control in samples.

Chlorophyll and Carotenoid analysis: On each sampling day, leaves of plants from each treatment and control were used for the determination of chlorophyll and carotenoid content. Chlorophyll and carotenoid content in leaves was calculated by the method Machalachlan and Zalik (1963). The details are given in Chapter 3.

Protein and proline analysis: On each sampling day, leaves of plants from each treatment and control were used for the determination of protein and proline content. For protein estimation, 500 mg leaves of the samples were homogenized in 3 ml of 10% tricholoroacetic acid (TCA) and centrifuged at 10000rpm for 10 mins. The protein content was then determined by the method described by Lowry et al., (1951) using bovine serum albumin as the standard. For proline estimation, 500 mg of leaf samples were extracted with 10 ml of 3% sulphosalicylic acid and centrifuged at 5000 rpm to remove cell debris. Supernatant was used to assay the proline content following Bates et al., (1973). The detailed methods have been described in chapter 3.

6.2.5. Metals removal efficiency, Bioconcentration factor and Translocation factor

100ml water samples in triplicates were collected from each treatment setup at the end of the experiment to determine the removal efficiencies of metals by each plant cultures. Metal contents viz. Fe, Cu, Cd and Cr were determined after acid digestion of samples with an acid mixture (9 parts nitric acid: 4 parts perchloric acid) at about 100º C. Metal concentration was determined by atomic absorption spectrophotometer (AAS 240 FS, Varian). Blanks were also run simultaneously and analyzed to correct for possible external contributions of the metals. Metal removal efficiency was calculated on percentage basis according to the equation (Chaudhuri et al., 2011)

$$\mathrm{R}\ \% = \frac{\mathrm{Co} - \mathrm{Ct}}{\mathrm{Co}} \times 100$$

Whereas, Co is initial metal concentration in water and Ct is final metal concentration in water after 30 days.

Bioconcentration factor (BCF), expressed as the ratio of metal concentration in plant tissue to that of the water was calculated by:

$$\mathrm{BCF} = \frac{\text{Metal content in plant tissue}}{\text{Initial metal content in water column}}$$

Translocation factor (TF), the ratio of metals in shoot versus root of plants was calculated by the formula of Padmavathiamma and Li (2007).

$$\mathrm{TF} = \frac{\text{Metal content in plant shoot}}{\text{Metal content in plant root}}$$

6.2.6. SEM and FTIR analysis

The sorption characteristics of metals on to plant parts and inside the tissues of untreated and metals treated samples were observed with the help of Scanning Electron Microscope. Thin cross sections of the untreated and metals treated plant root, shoot and leaves of 2mm were excised and collected for the sample preparation. The samples were fixed in 2.5% Glutaraldehyde/ Karnovsky’s fluid (David et al., 1973), buffered with 0.1M sodium phosphate buffer at pH 7.4. Fixation of the samples was done for 6-8 hours at 4^0C. After this, samples were washed thrice with 0.1M fresh phosphate buffer for 15min each at 4^0C. Post fixation for 2hours in 1% Osmium tetraoxide was performed for the samples. After this, samples were dehydrated using increasing concentration of Acetone 30%, 50%, 70%, 90% and 100%. The duration of treatment was 30 mins at 4^0C for each concentration. Finally, the species were air dried and mounted on stubs, then coated with a thin layer of gold for examining in Scanning Electron Microscope (JEOL, JSM 6490 LV-Japan).

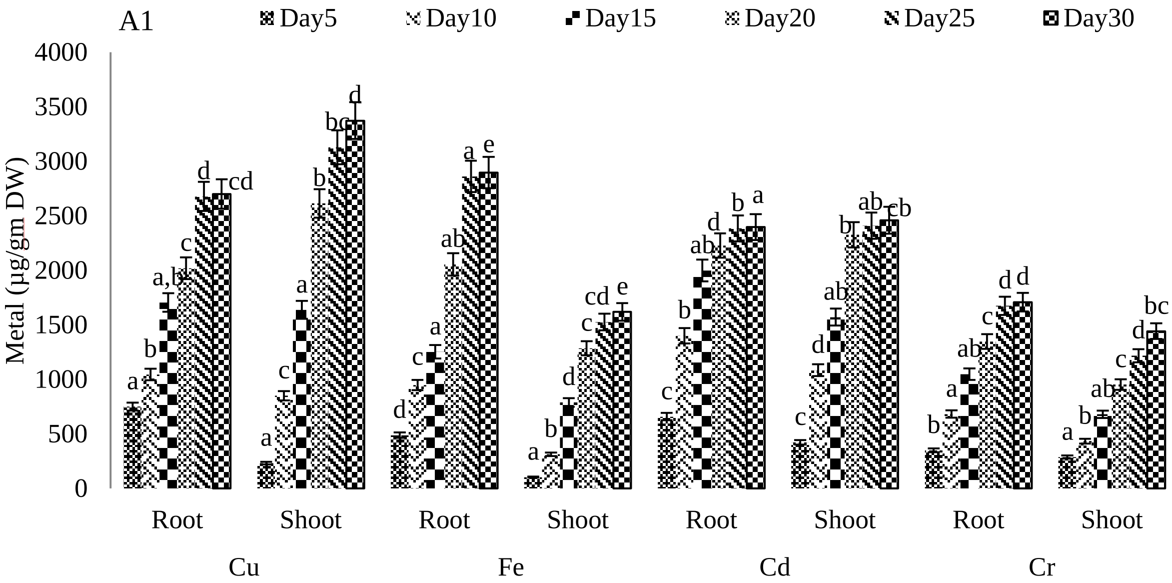
For FTIR analysis of plants, both untreated and metals treated dry root and shoot samples were grind properly to form a uniform powder. Then FTIR spectra of the samples prepared as KBr discs were taken by Infrared Spectrophotometer (Nikolet^{TM} 6700) in the wave number of 4000-400 cm^{-1} (Teng et al., 2013).

6.3. Results

6.3.1. Metals accumulation by selected plants under mono and mixed cultures

In the present study the concentration of Cu, Fe, Cd and Cr accumulated by *Pistia stratiotes* and *Hydrilla verticellata* in root and shoot under mono and mixed culture with and without effect of circulation are presented in figure 6.1 and 6.2, respectively. Significant differences were observed for the metals accumulation by both plants under mono and mixed cultures ($p \leq 0.05$). In the monocultures, water circulation has a positive effect on enhanced metal accumulation and also on the survival of plants. In *Pistia stratiotes* roots concentrations of Cu, Fe, Cd and Cr reached a maximum of 2697.74, 2895.1, 2395 and 1708.3 µg/gm, respectively due to water recirculation on 30th day, while as for shoot Cu (3371.54 µg/gm) and Cd (2459 µg/gm) was observed to be highest on 30th day of treatment Fig.6.1 (A1). Metals accumulation was increased by 30% for Cu, 25% for Cd, 45% for Fe and 28% for Cr in *Pistia stratiotes* monoculture under the circulation effect than the non-circulating conditions. *Hydrilla verticellata* also accumulated considerable amount of all the four metals; however, shoot accumulation was greater than roots Fig. 6.1 (B1). Metals accumulation in the shoot of *Hydrilla verticellata* under monoculture coupled with the effect of water circulation was in the order of Cd>Fe>Cu>Cr. While comparing with the metals accumulation by *Hydrilla verticellata* under monocultures, metals accumulation increased for Cd (36.5%), Fe (23.25%), Cu (21.67%) and Cr (21.53%) in-case of water circulating effect than non-circulating conditions.

While comparing metals accumulation by monoculture under circulating and non-circulating treatments, it was observed that in case of non-circulating conditions, metal accumulation was only upto 25 days, after which *Pistia stratiotes* and *Hydrilla verticillata* were not able to survive and decomposed within the tubs Fig. 6.1 (C1 and D1), respectively . Generally, it was seen that both plants showed metals accumulating characteristics, however, *Pistia stratiotes* accumulated more metals than *Hydrilla verticillata* under no circulation of water. For *Pistia stratiotes* roots accumulation was greater than shoot with Cu (1891.93 µg/gm) > Cd (1790.66 µg/gm) > Fe (1587.33 µg/gm) > Cr (1240.1 µg/gm). For *Hydrilla verticillata* metals accumulation in shoot was seen to be greater than root with Fe (1329.16 µg/gm) > Cu (1248.06 µg/gm) > Cr (1086.93 µg/gm) > Cd (1059.56 µg/gm).



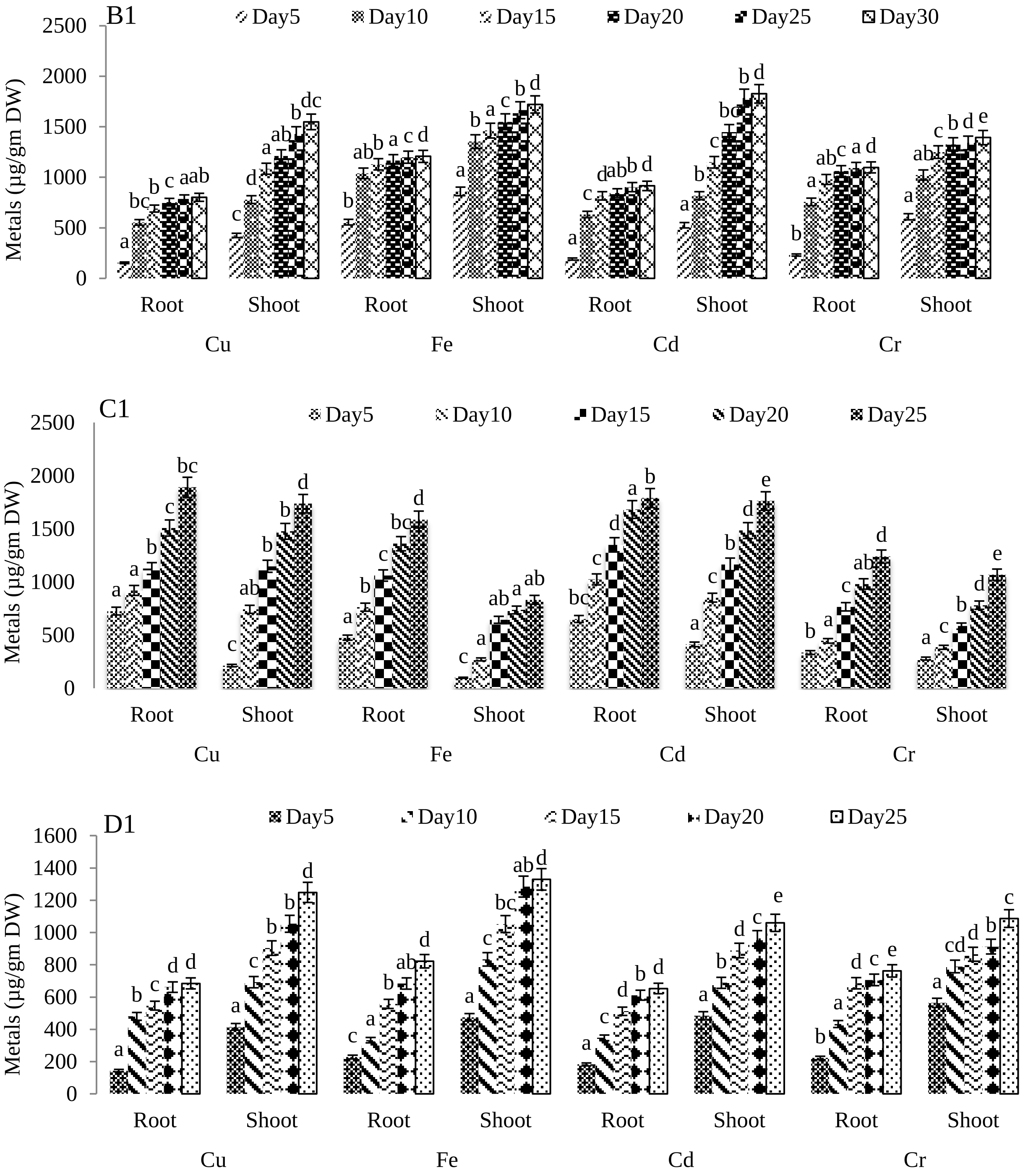
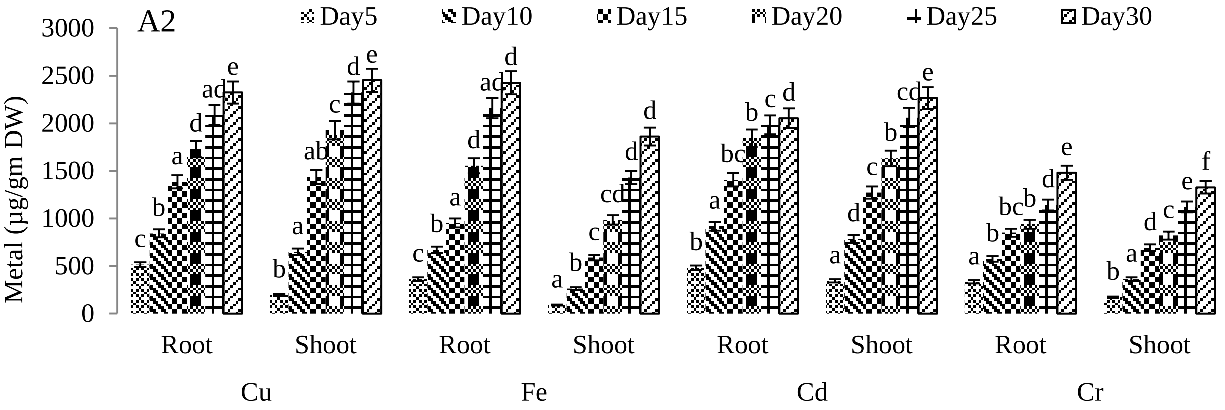
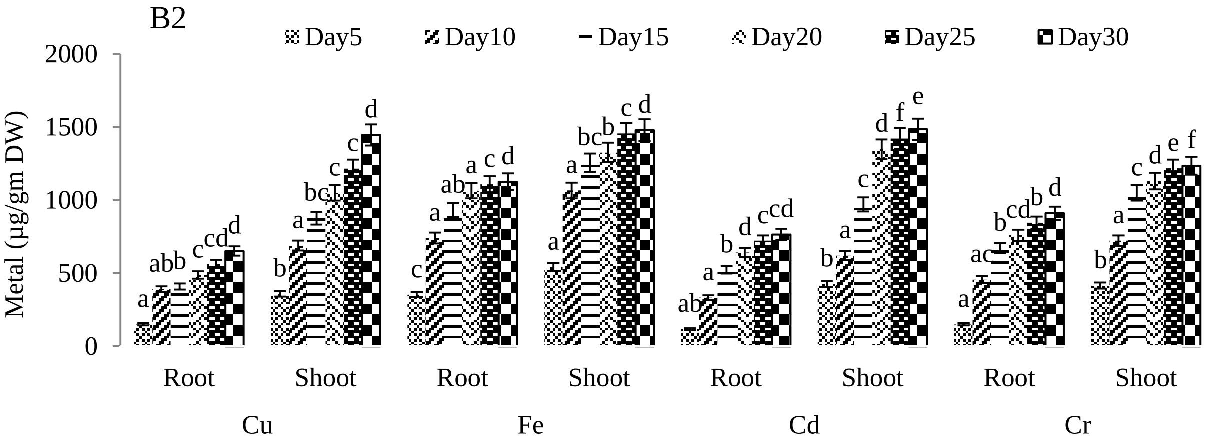


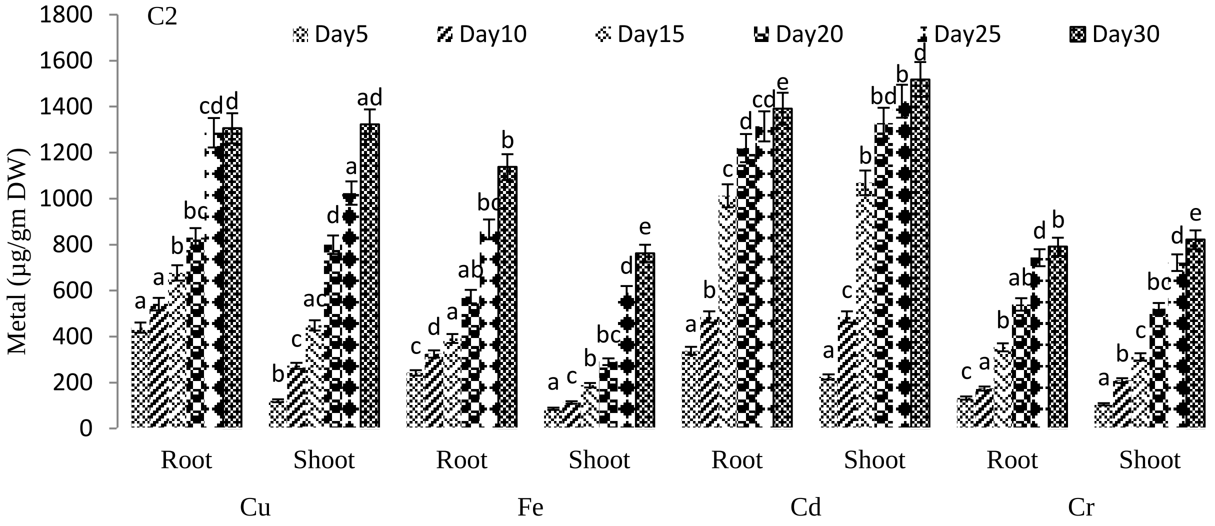
Figure 6.1. [A1, B1, C1 and D1] Accumulation of Cu, Fe, Cd and Cr from multimetal solution by *Pistia stratiotes* under monoculture with circulating (A1) and non-circulating conditions (C1); Fig. B1 and D1 represent metals accumulation by *Hydrilla verticillata* under monoculture with circulating and non-circulating conditions, respectively. Different letter represent the significant differences between the mean values of metals accumulation during the study period ($p < 0.05$).

In the present study, circulation of multimetal contaminated water on different days of treatment between the tubs of *Pistia stratiotes* and *Hydrilla verticillata* grown under mixed culture had a significant effect on the enhanced metals removal and concurrent accumulation by the both plants (Figure 6.2). Significant differences were observed for metals accumulation by *Pistia stratiotes* and *Hydrilla verticillata* under mixed culture during the 30 days treatment period treatment period ($p<0.05$). Under mixed culture, it was observed that *Pistia stratiotes* had accumulated greater metal concentrations than *Hydrilla verticellata*. Metals accumulation by *Pistia stratiotes* was in the order of Cu>Cd>Fe>Cr (Fig. 6.2 A2), while as for *Hydrilla verticillata* metals accumulation was observed as Fe>Cd>Cr>Cu (Fig. 6.2 B2) under mixed culture. Comparing root and shoot accumulation of metals, it was observed that *Pistia stratiotes* accumulated higher metals in both parts that *Hydrilla verticillata* under mixed culture with water circulation. *Pistia stratiotes* accumulated Cu (2452.54 µg/gm), Cd (2265.23 µg/gm), Fe (1861.61 µg/gm) and Cr (1327.8 µg/gm) in shoot, for Hydrilla verticillata Cu (1446.4 µg/gm), Cd (1484 µg/gm), Fe (1478.3 µg/gm) and Cr (1235.8 µg/gm) was accumulated in the shoot. Metal accumulation increased by 50% for Cu, 41.5% for Fe, 52.4% for Cd and 54.5% for Cr in mixed culture for *Pistia stratiotes* with circulation than in non-circulating water and for *Hydrilla verticillata* metal accumulation increased by 40% for Cu, 32% for Fe, 25% for Cd and 34% for Cr under mixed culture in water circulating conditions than the non-circulation of water.

In the present study, metals accumulation by the selected plants under mixed culture tubs with no water circulation are presented in Fig. 6.2 (C2 and D2). It was observed that phytoextraction of metals and their subsequent bioaccumulation within the both plants was until 30 days. Metals accumulation varied considerably in root and shoot of both plants, however, maximum Cu (1322.54 µg/gm) and Cd (1517.9 µg/gm) was accumulated in the shoot of *Pistia* *stratiotes* and Fe (1079.3 µg/gm) and Cr (900.96 µg/gm) was accumulated maximum in the shoot of *Hydrilla verticillata*.







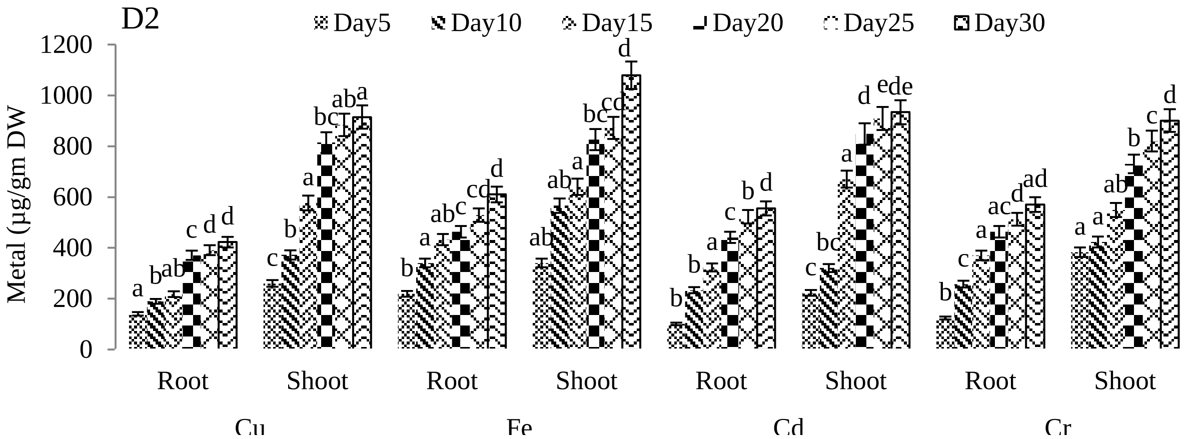


Figure 6.2. [A2, B2, C2 and D2] Accumulation of Cu, Fe, Cd and Cr from multimetal solution by *Pistia stratiotes* under mixed culture with circulating (A2) and non-circulating conditions (C2); Fig. B2 and D2 represents metals accumulation by *Hydrilla verticillata* under mixed culture with circulating and non-circulating conditions, respectively. Different letter specify the significant differences between the mean values of metals accumulation by *Pistia stratiotes* and *Hydrilla verticillata* during the study period ($p < 0.05$).

6.3.2. Metal removal efficiencies, bioconcentration factor and translocation factor of the plants under different cultures

In the present study, the results obtained for the removal efficiencies of selected plant cultures treating metal contaminated water under circulating and no circulating conditions are presented in table 6.1. Among the studied treatments, 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, under mixed culture due to water circulation, 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%).

The phytoextraction potential of plants under different culture was evaluated by both bioconcentration factor (BCF) and translocation factor (TF) (Table 6.1). It was observed that BCF of the plants enhanced under the effect of circulating multimetal contaminated water between cultures. It was found that *Pistia stratiotes* showed highest BCF for Cd (1941.5), Cu (1302.2) and Cr (1259.2) under monoculture. Also BCF of *Pistia stratiotes* for Cd (1566) and Cr (1123.2) was highest under mixed culture compared to *Hydrilla verticillata* which was (899) for Cd and (859) for Cr. Under monoculture, *Hydrilla verticillata* showed highest BCF for Cd (1096) followed by Cr (996), Fe (585.4) and Cu (496.8). 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. Also *Pistia stratiotes* under monoculture showed TF >1 for Cu (1.2) and Cd (1.02). Further in the mixed culture TF of *Pistia stratiotes* was greater than 1 for Cu (1.05), Cd (1.1) and Cr (1.03).

Table 6.1. Effect of intermittent circulation on the metal removal efficiencies (%), Bioconcentration factor (BCF) and Translocation factor (TF) by plant cultures (mean ± S.D.)

Treatments	%Removal				Treatments	BCF				TF			
	Cu	Fe	Cd	Cr		Cu	Fe	Cd	Cr	Cu	Fe	Cd	Cr
T1	78.3 ±2.19	68.5 ±1.76	73.5 ±2.12	65.4 ±1.67	T1	1302 ±3.78	902.6 ±4.1	1941 ±6.75	1259 ±4.22	1.2 ±0.06	0.55 ±0.01	1.02 ±0.02	0.86 ±0.02
T2	58.4 ±1.56	60.75 ±1.45	50.45 ±2.01	55.38 ±1.72	T2	469.8 ±2.43	585.4 ±3.13	1096 ±4.43	996 ±3.45	1.92 ±0.1	1.42 ±0.02	1.99 ±0.06	1.26 ±0.04
T3	62.45 ±1.97	48.5 ±1.21	60.8 ±1.78	47.88 ±1.32	T3	627.8 ±3.12	483.8 ±2.43	1420 ±4.85	922.3 ±3.17	0.91 ±0.03	0.51 ±0.01	0.98 ±0.08	0.8 ±0.02
T4	48.5 ±1.21	45.9 ±1.23	45.33 ±1.13	46.5 ±1.21	T4	386.4 ±2.87	430.2 ±2.32	684.4 ±2.34	783.6 ±2.65	1.82 ±0.04	1.31 ±0.03	1.62 ±0.06	1.13 ±0.03
T5	90.4 ±1.98	85.76 ±1.94	92.95 ±2.1	82.33 ±1.79	T5 *Pistia stratiotes*	955 ±3.98	857 ±3.68	1566 ±5.47	1123 ±4.78	1.05 ±0.02	0.76 ±0.03	1.1 ±0.02	1.03 ±0.03
					Hydrilla verticellata	419 ±2.43	520 ±3.11	899 ±4.32	859 ±3.11	2.2 ±0.05	1.3 ±0.04	1.93 ±0.05	1.39 ±0.04
T6	78.5 ±1.34	75.75 ±1.65	78.5 ±2.1	74.15 ±1.54	T6 *Pistia stratiotes*	525 ±2.76	379.6 ±2.1	1162 ±3.98	644.4 ±2.61	1.01 ±0.02	0.66 ±0.02	0.96 ±0.01	0.87 ±0.02
					Hydrilla verticellata	267.2 ±1.91	341.4 ±1.98	596 ±2.21	588 ±3.14	2.1 ±0.05	1.7 ±0.06	1.6 ±0.05	1.5 ±0.05
T7	2.82 ±0.21	3.71 ±0.18	1.5 ±0.13	2.15 ±0.16									

6.3.3. Effect on the protein and proline content in leaves of *Pistia stratiotes* and *Hydrilla verticillata* under different cultures

The changes in the protein content in leaves of *Pistia stratiotes* and *Hydrilla verticillata* of metals treated and control under different water circulating conditions have been represented in figures 6.3 (3A-3E). Mono and mixed culture with circulation and no circulation of metals contaminated water had a profound effect on the protein content of both plants. Protein content significantly increased for monocultures of *Pistia stratiotes* (R^2=0.935) and *Hydrilla verticillata* (R^2=0.952). Also, under mixed cultures coupled with circulating effect of water both plants showed a significant increase in the levels of protein as for *Pistia stratiotes* and *Hydrilla verticillata* value of R^2= 0.957 and 0.904, respectively. Further it was observed that plants under mono and mixed cultures treated in no water circulation, there was a significant decrease in the protein content, however, both plants under mixed culture showed an increase in the protein content until 20 days and thereafter, it decreased R^2=0.659 and 0.431 for *Pistia stratiotes* and *Hydrilla verticillata*, respectively. In case of control, no significant increase in the protein content for both plants was observed R^2= 0.3107 for *Pistia stratiotes* and 0.3717 for *Hydrilla verticillata.*

The accumulation of proline in the leaves of *Pistia stratiotes* and *Hydrilla verticillata* under mono and mixed cultures with multimetals stress and control treatment are depicted in figure 6.4 (4A-4E). 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. Proline significantly increased in *Pistia stratiotes* under both cultures with the circulation of metals contaminated water R^2= 0.958 and 0.949 under mono and mixed culture, respectively. In case of *Hydrilla verticillata* proline accumulation increased considerably with time, however, proline accumulation under mixed culture (R^2=0.855) was greater than mono culture (R^2=0.63).

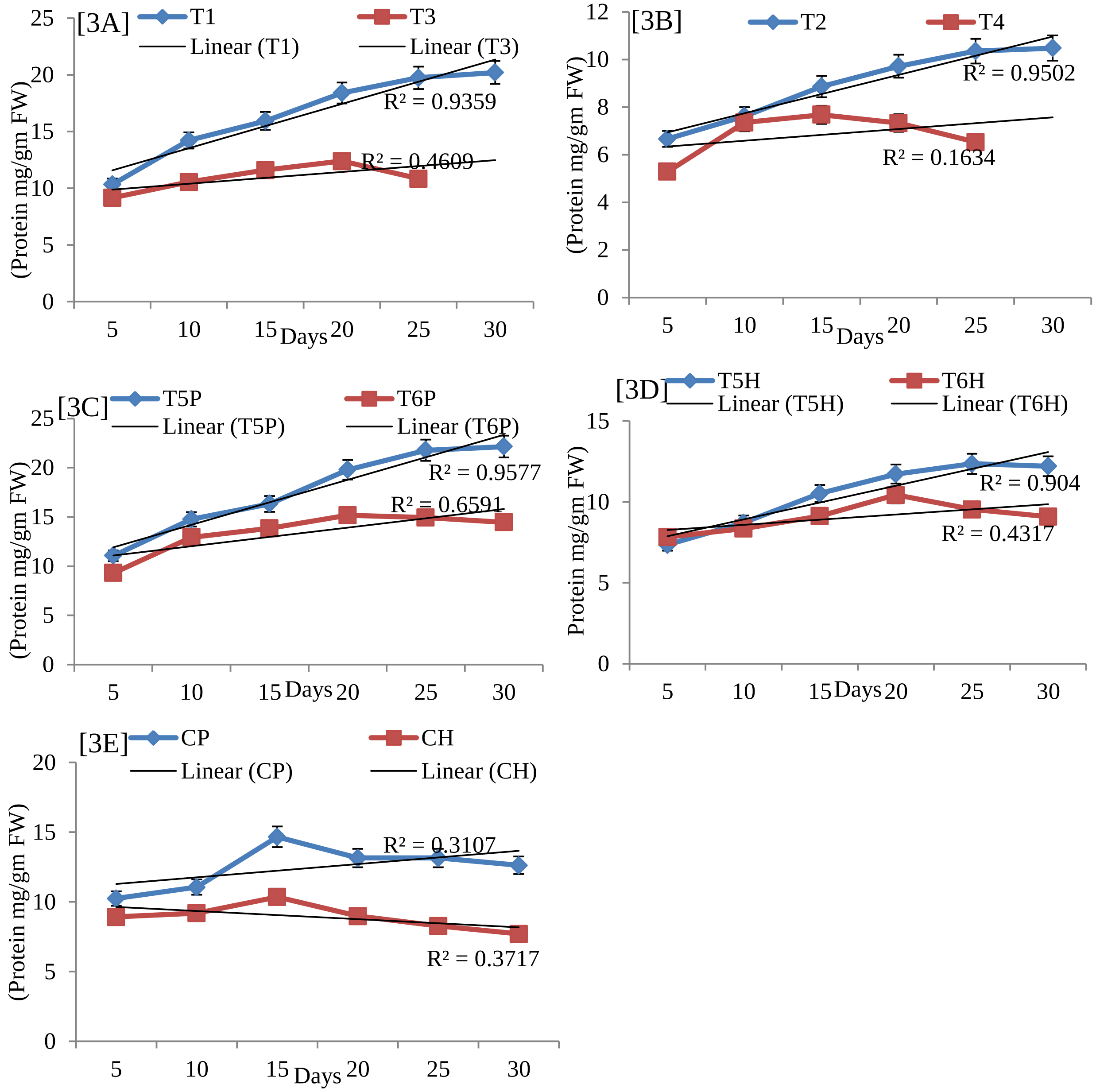


Figure 6.3. (3A-3E) Effect of multi-metals contaminated water and its intermittent circulation on protein content of *Pistia stratiotes* and *Hydrilla verticellata* under different treatment cultures.

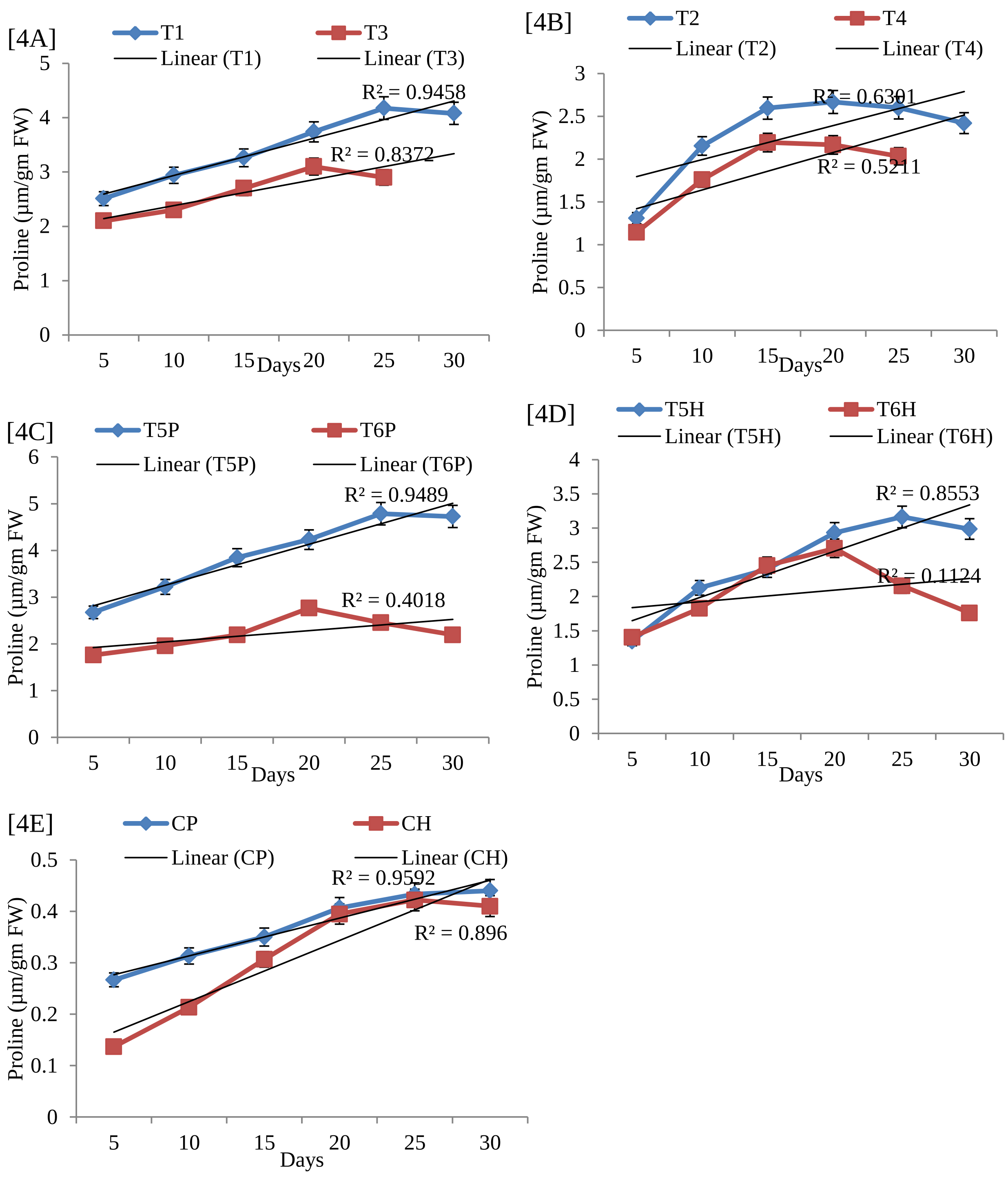


Figure 6.4. (4A-4E) Effect of multi-metals contaminated water and its intermittent circulation on proline content of *Pistia stratiotes* and *Hydrilla verticellata* under different treatment cultures.

6.3.4. Effect on the total chlorophyll and carotenoid content in leaves of *Pistia stratiotes* and *Hydrilla verticillata* under different cultures

The changes in the chlorophyll and carotenoid content in leaves of *Pistia stratiotes* and *Hydrilla verticillata* of metals treated and control under different water circulating conditions have been represented in figure 6.5 (5A-5E) and figure 6.6. (6A-6E), respectively. The water circulation coupled with mixed culture of plants had a synergistic effect on the chlorophyll and carotenoid content of both plants.

Metal treated monoculture of plants with under non-circulating conditions showed decline in the total chlorophyll and carotenoid content for both plants. The contents increased significantly by day 20, however, a significant decrease in the total chlorophyll content of plants in monoculture under non-circulating conditions was reported. Instead, the total chlorophyll for *Pistia stratiotes* monoculture increased (R^2= 0.816) and also for *Hydrilla verticellata* (R^2= 0.727). It also increased under mixed culture with circulation (R^2= 0.976; *Pistia stratiotes*) and (R^2=0.9593; *Hydrilla verticellata*) instead of R^2= 0.414 and 0.443, respectively under non circulating conditions.

The carotenoid content of *Pistia stratiotes* and *Hydrilla verticellata* under different treatments varied considerably. The carotenoid contents increased significantly by day 15, however, a significant decrease in monoculture of both plants under non-circulating conditions was reported. Plants in mixed culture showed a significant increase in carotenoid content (R^2 = 0.5241) for *Pistia stratiotes* and (R^2 = 0.545) for *Hydrilla verticellata,* instead of (R^2 = 0.4488) for *Pistia stratiotes* and (R^2 = 0.3248) for *Hydrilla verticellata* in mixed cultures under non circulating conditions.

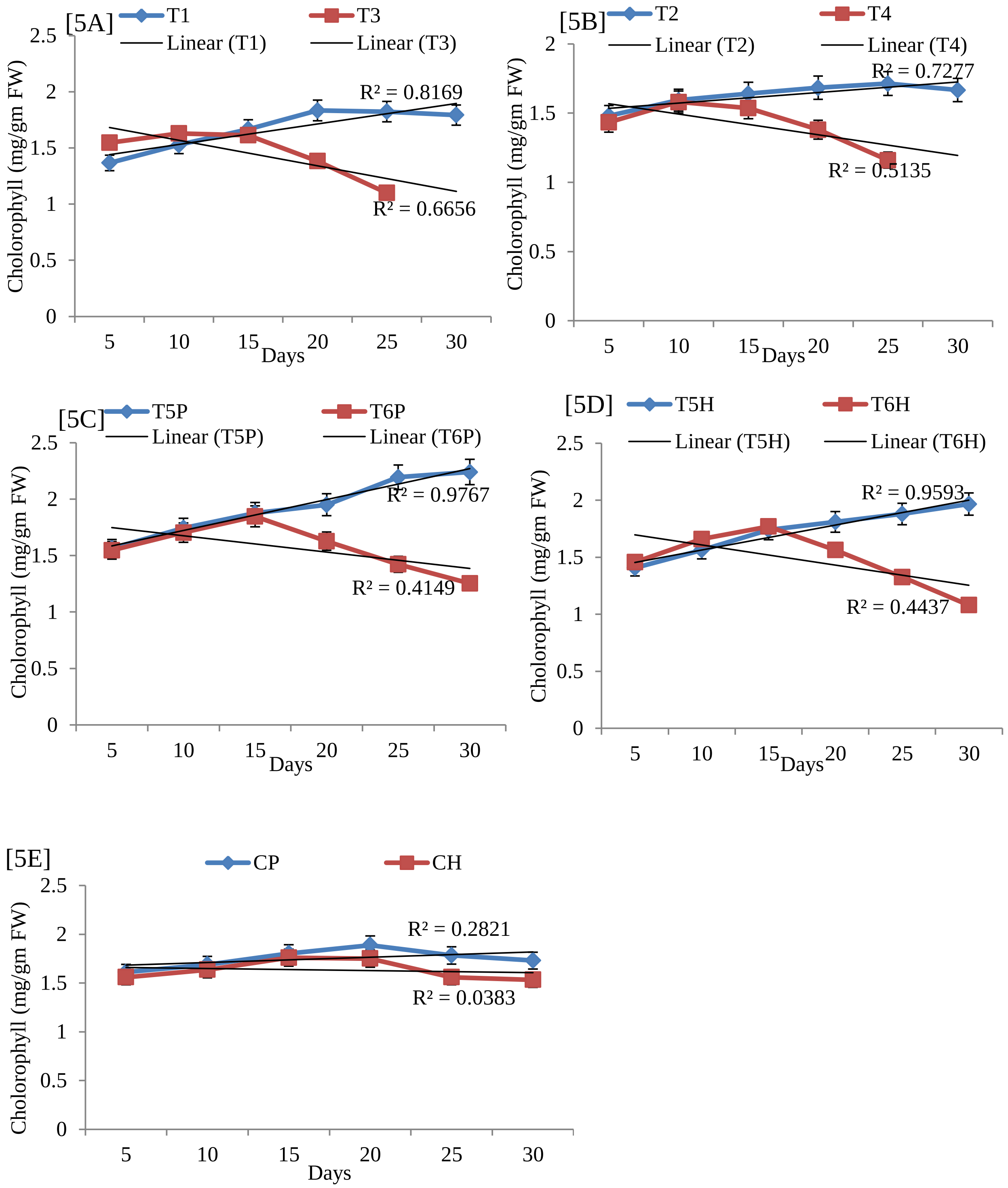


Figure 6.5. (5A-5E) Effect of multi-metals contaminated water and its intermittent circulation on chlorophyll content of *Pistia stratiotes* and *Hydrilla verticellata* under different treatment cultures.

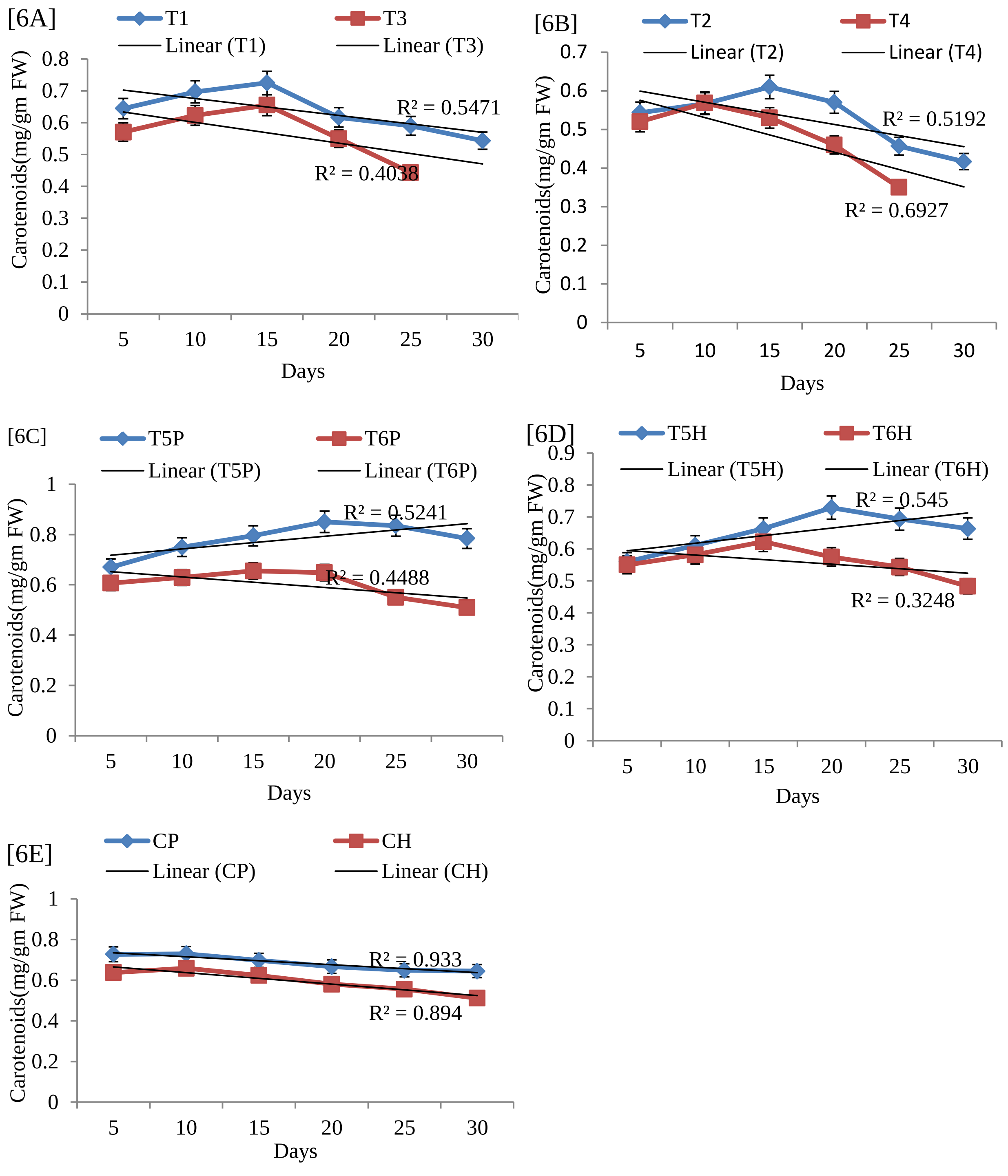


Figure 6.6. (6A-6E) Effect of multi-metals contaminated water and its intermittent circulation on carotenoid content of *Pistia stratiotes* and *Hydrilla verticellata* under different treatment cultures.

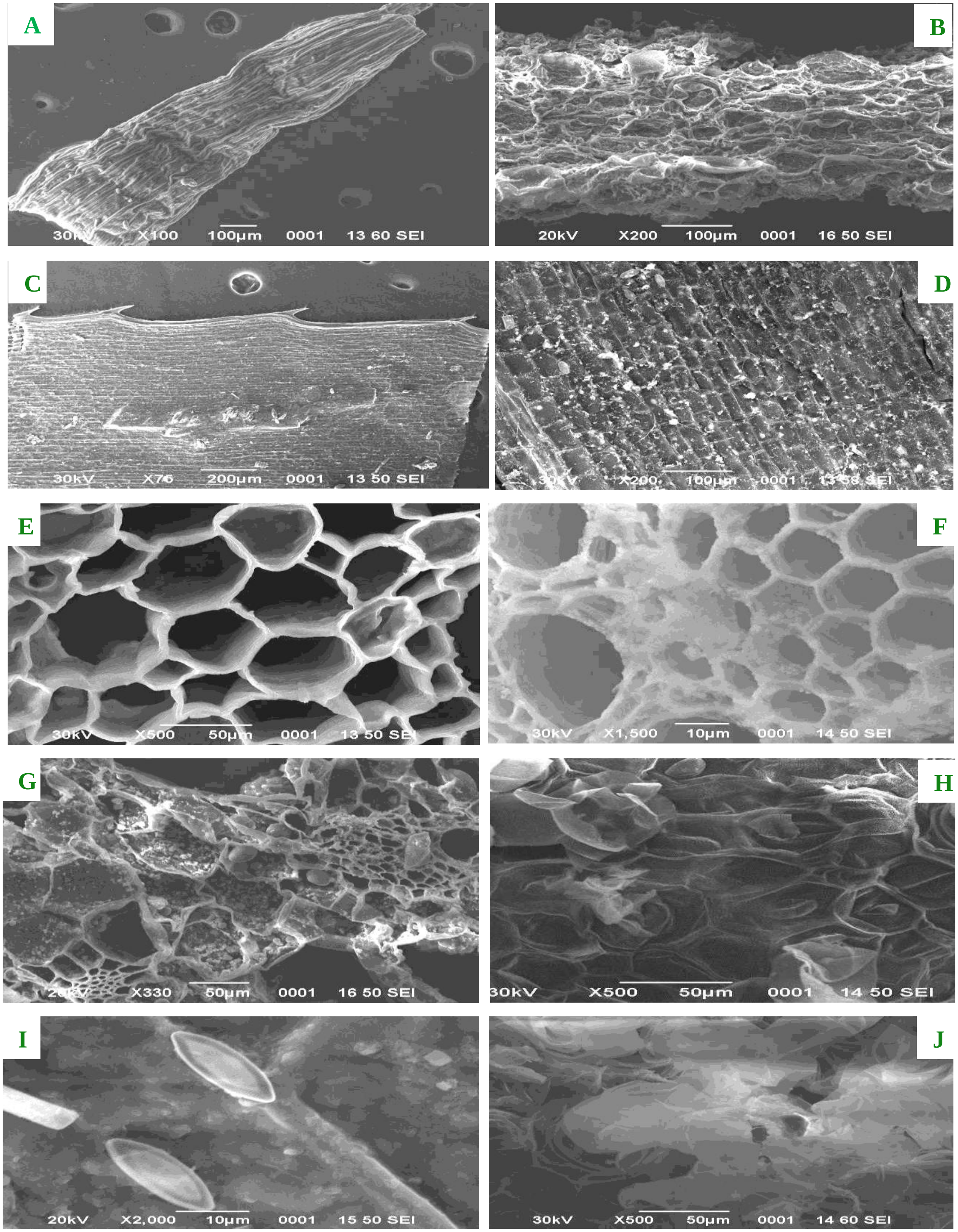


Plate 6.1. (A-J). Scanning electron microscopic (SEM) studies of *Pistia stratiotes* and *Hydrilla verticellata* parts before and after metals treatment

6.3.5. Scanning Electron Microscopic (SEM) and Fourier Transform Infrared (FTIR) studies of *Pistia stratiotes* and *Hydrilla verticillata*

In the current study, metal localization; partitioning within the plant parts using Scanning Electron Microscopic (SEM) and infrared spectroscopic technique was used to study the metal binding sites within the plant parts and the role of different functional groups of biomolecules as metal chelators. The SEM images of different plant parts are presented in plate 6.1, fig. (A-J). Fig. A represents the untreated root (control) and fig. B represents the metals treated root showing root deformations due to metals deposition onto them. Fig. C depicts the leaf surface of control plant while as fig. D shows the adsorption of metals onto the leaf surface. Xylem and phloem vessels show clumps of metals deposition in treated parts than the untreated parts (Fig. E and F). SEM micrographs of untreated plants show visible stomata (Fig. Hand I), while as Fig. J, represents the metals in clumps adhered to the stomata that may result in considerable damages in stomata. 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.

The FTIR spectra of *Pistia stratiotes* and *Hydrilla verticillata* of untreated and metals treated root and shoot biomass and their corresponding specific IR bands and major functional groups have been presented in plates (6.2-6.9) and table (6.2) and table (6.3) for *Pistia stratiotes* and *Hydrilla verticillata*, respectively. The FTIR spectroscopic studies depicted a wide range of absorption bands at different frequencies. The presence of 3407.4 cm^{-1} band peak in untreated *Pistia stratiotes* root to its sharp decrease in the band intensity at 3404.4 cm^{-1} in metal treated root can be assigned to NH stretching of amide group. Further an intense decrease in the band intensity from 3412.0 cm^{-1} in untreated *Pistia stratiotes* shoot to 3365.3 cm^{-1} in metal laden shoot may be assigned to hydroxyl (OH) group. The absorption bands at 1631.5 cm^{-1} and 1627.6 cm^{-1} in metal treated root and shoot of *Pistia stratiotes* can be assigned to amide bond of proteins and peptides. A sharp band at 1153.2 cm^{-1}and it's shifting to 1154.0 cm^{-1} in metal treated shoot may be assigned to Thiol and sulfhydryl groups of biomolecules (Yeliz, 2012). The presence of 1029 and 1028 cm^{-1} frequency band peaks represents the C-O, C-C bonding of polysaccharides and celluloses in the plants.

The metal free spectrum of root and shoot biomass of *Hydrilla verticillata* shows major characteristic frequency bands at 3419.2 and 3449.0 cm^{-1} which corresponds to NH and OH groups. The presence of spectral band at 2976.2 cm^{-1} and its sharp shift at 2937.0 cm^{-1} depicts the stretching of -CH_2 group in protein and phospholipids (Akhter at al., 2010). Band at 2882.7 cm^{-1} and 2882.3 cm^{-1} in the untreated root and shoot biomass of *Hydrilla verticillata* represents the C=H stretching of –CH_3 group. The peaks at 1624; 1643.6 cm^{-1} and 1319.0; 1320.5 cm^{-1} corresponds to amide-I group of proteins, C=O stretching and amide-III band component of α –helix protein. The peaks at 1239, 1241.1 cm^{-1} represent the P=O stretching of phosphodiesters and shifting peaks from 1108.2 to 1117.0 cm^{-1} may be attributed to the presence of secondary alcoholic (OH) groups. Also sharp peak bands at 1071.0; 1072.7 and 1068.1 cm^{-1} corresponds to functional groups of carboxylic and phosphate groups in the binding of metals and formation of complexes (Tang et al., 2013). The occurrence of absorption bands at 1108.2; 1239; 1321.3; 1320 and 1420 cm^{-1} in the metals treated biomass are in consistent with the reports of Malik, (2007) and Shuveau et al., (2013).

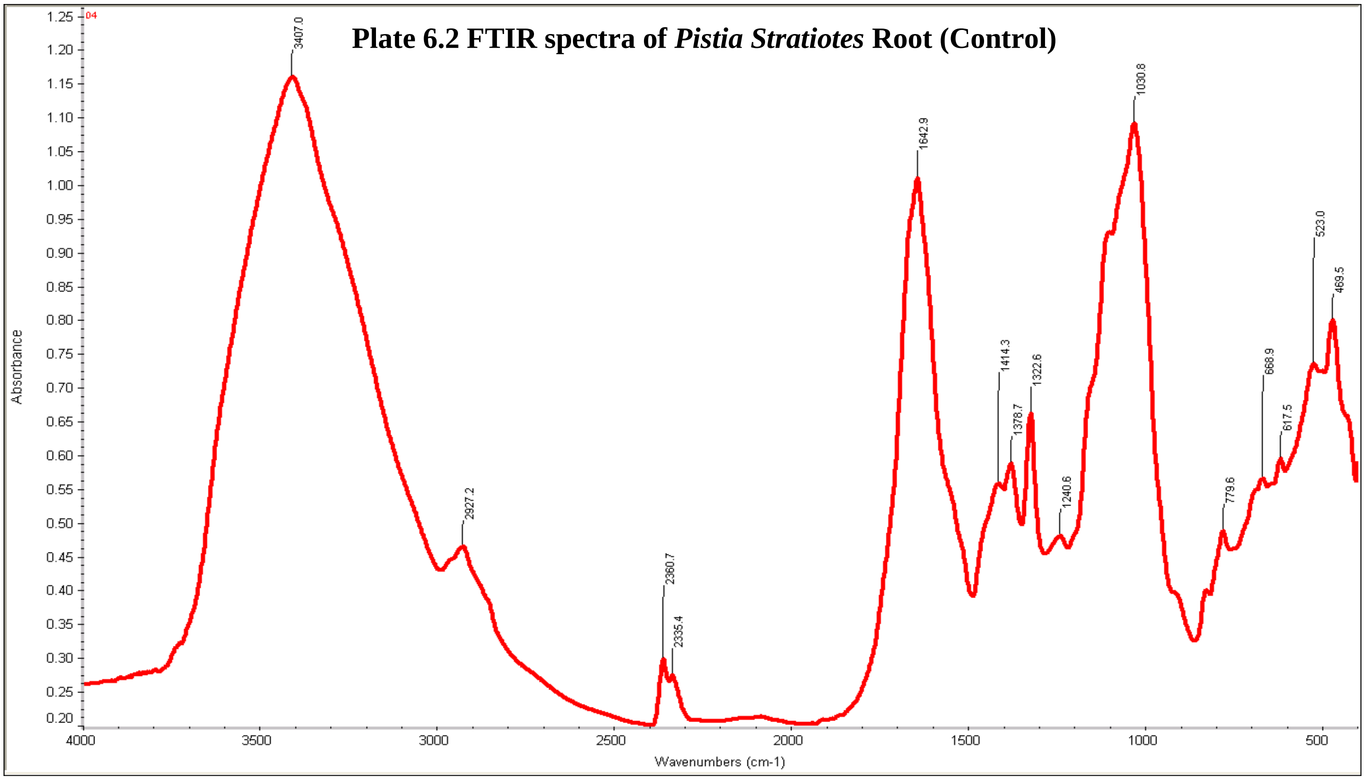


Plate 6.2 FTIR spectra of *Pistia Stratiotes* Root (Control)

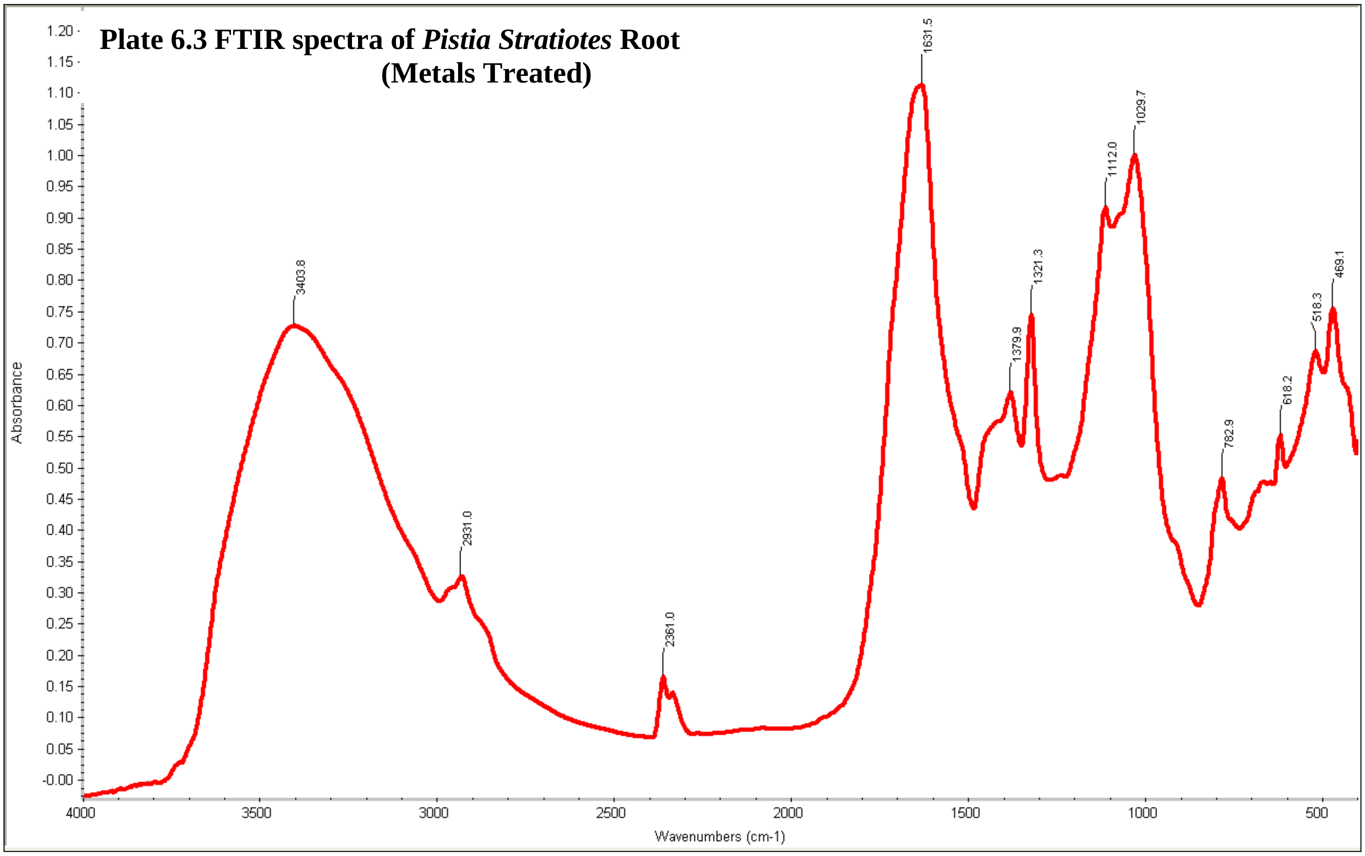


Plate 6.3 FTIR spectra of *Pistia Stratiotes* Root (Metals Treated)

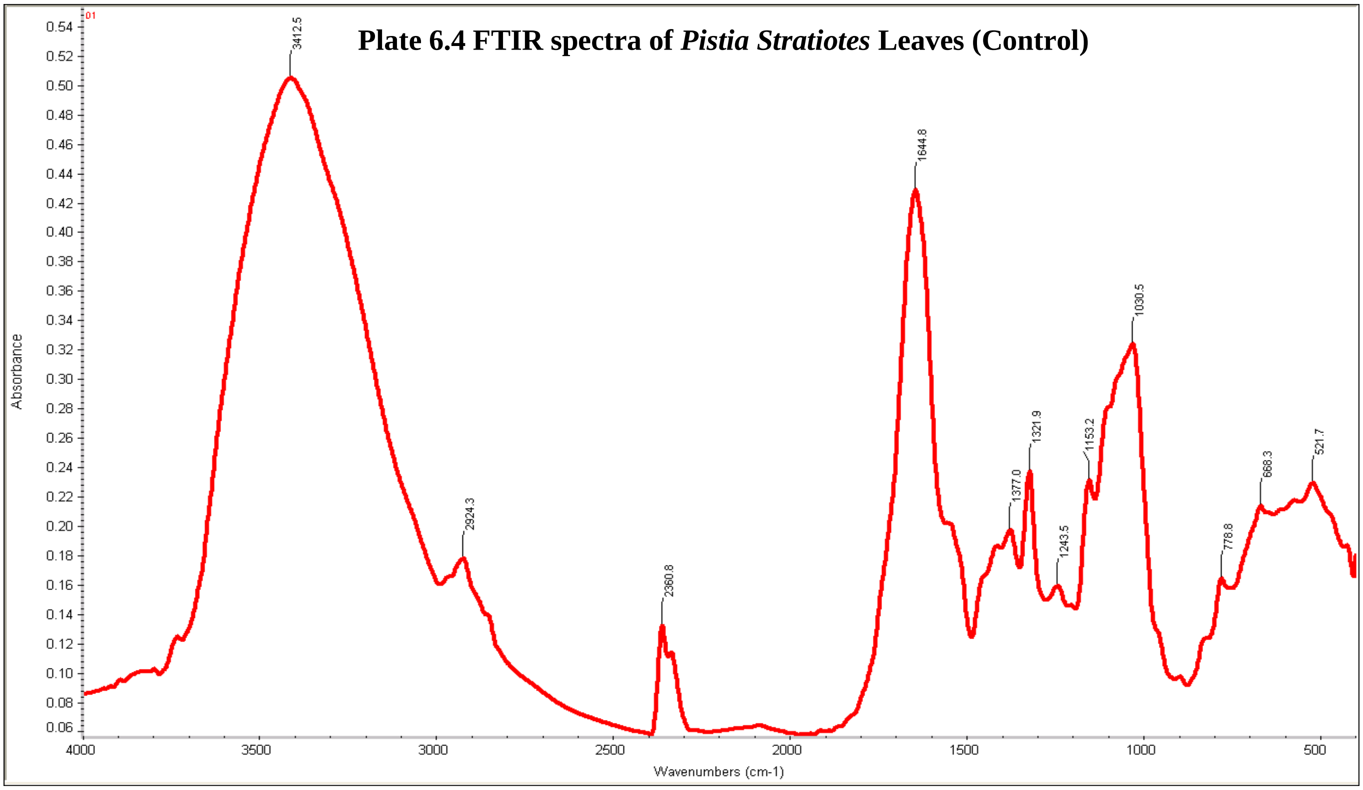


Plate 6.4 FTIR spectra of *Pistia Stratiotes* Leaves (Control)

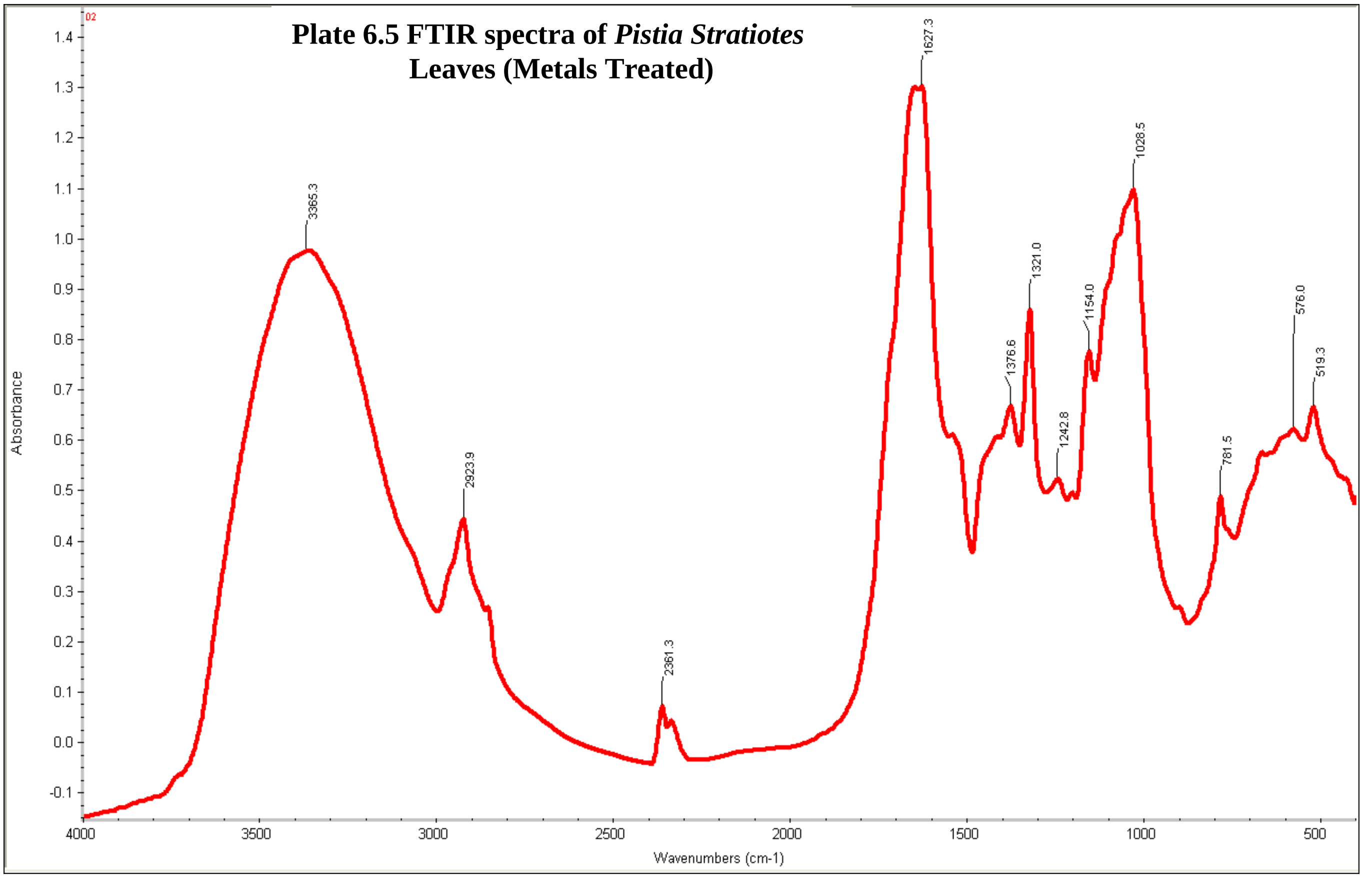


Plate 6.5 FTIR spectra of *Pistia Stratiotes* Leaves (Metals Treated)

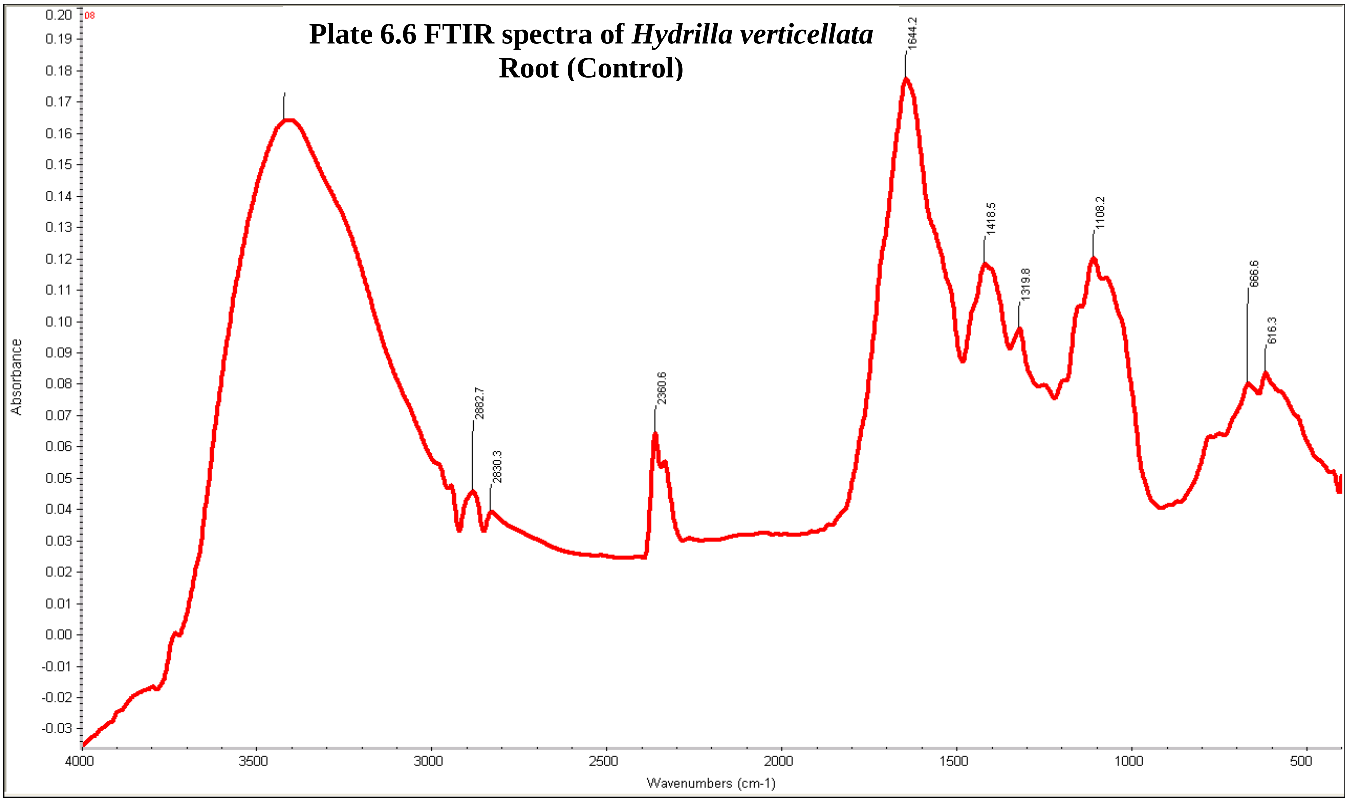


Plate 6.6 FTIR spectra of *Hydrilla verticellata* Root (Control)

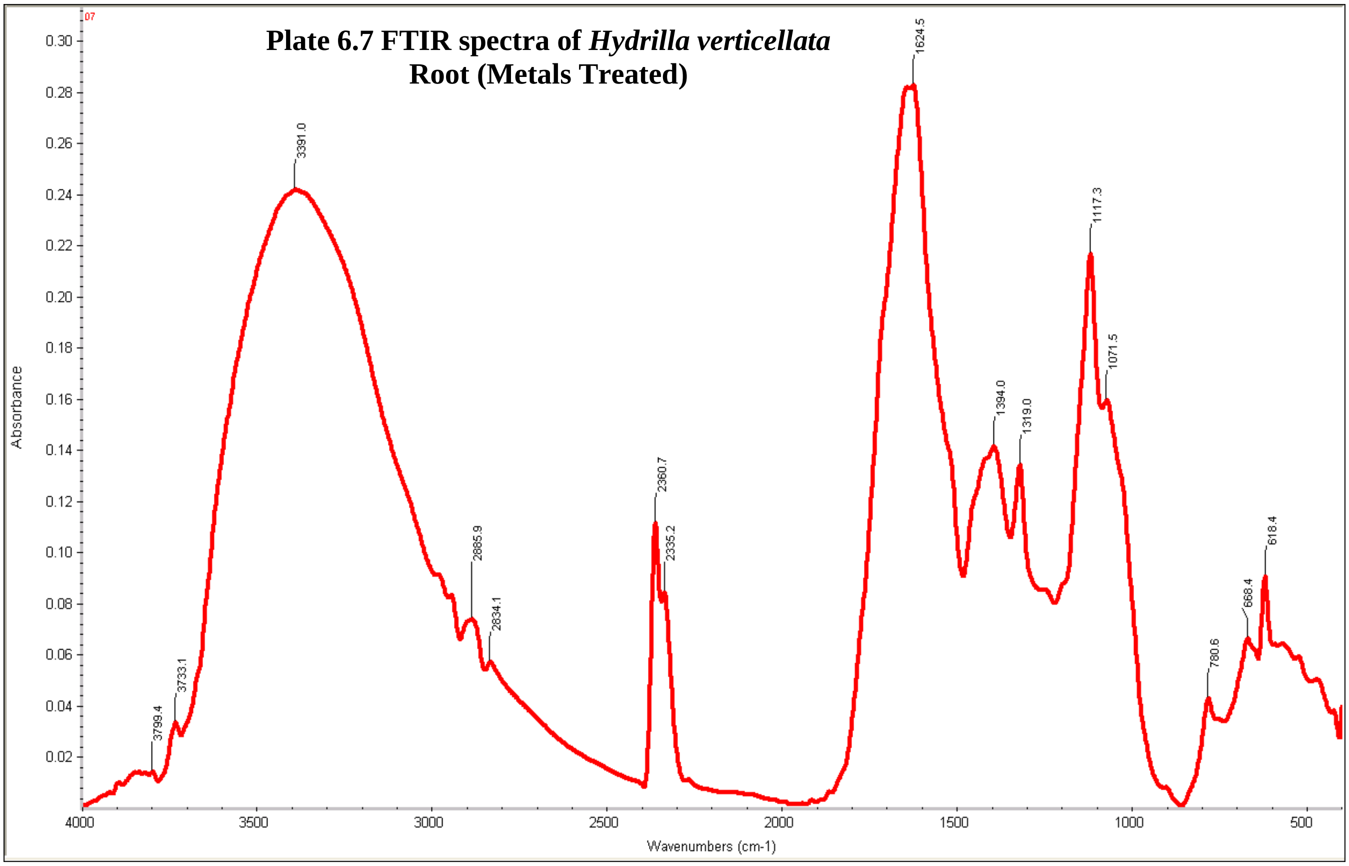


Plate 6.7 FTIR spectra of *Hydrilla verticellata* Root (Metals Treated)

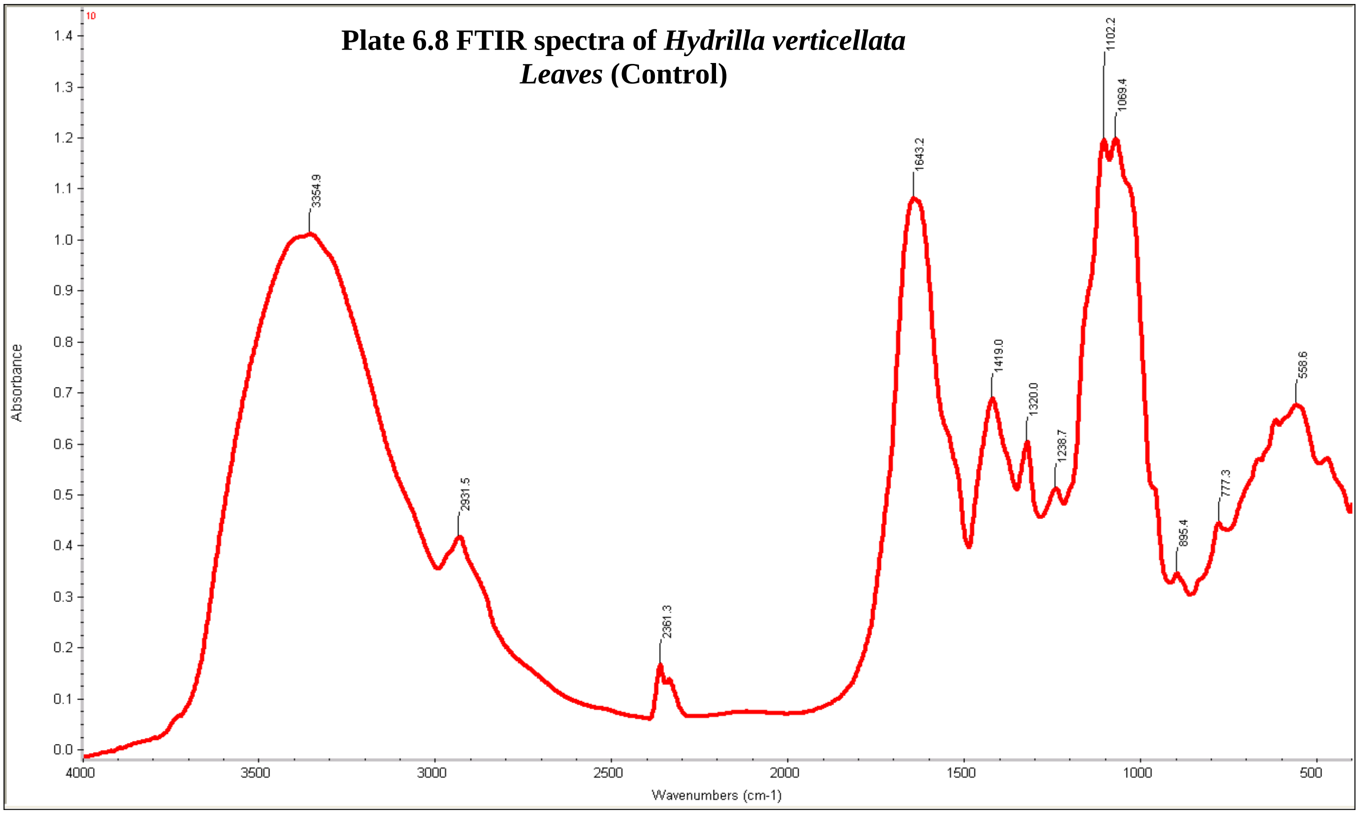


Plate 6.8 FTIR spectra of *Hydrilla verticellata Leaves* (Control)

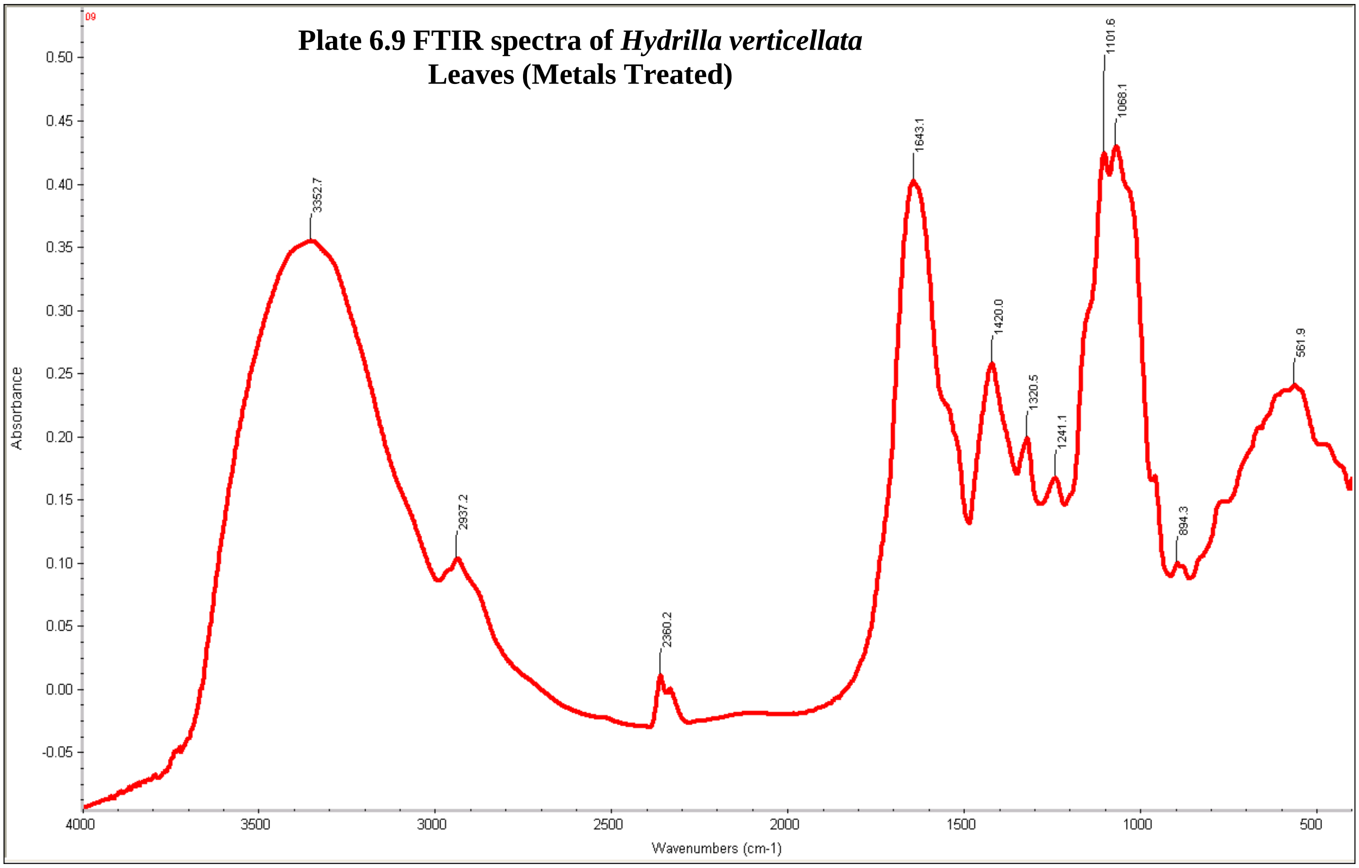


Plate 6.9 FTIR spectra of *Hydrilla verticellata* Leaves (Metals Treated)

Table6.2. Representing the description of FTIR spectra of metals treated and untreated plant roots and leaves of *Pistia stratiotes*

Observed wavenumber cm^{-1} and band assignment of *Pistia stratiotes* (metals untreated and treated)					**Band Assignment**
Representing range of frequency (cm^{-1})	1. *Pistia Stratiotes* (Root Control)	2. *Pistia Stratiotes* (Root Treated)	3. *Pistia Stratiotes* (Leaves Control)	4. *Pistia Stratiotes* (Leaves Treated)	
3500-3300	**3407.4**	**3403.4**	**3412.0**	**3365.3**	**N-H stretching of Amide-A, O-H stretching of Hydroxyl groups**
3000 - 2800	2927.8	2931.5	2924.2	2923.9	Asymmetric vibration C-H of CH_2 , protein and phospholipids CH_2 and CH_3 groups (Lata et al., 2011)
2400 - 2300	2360.0	2361.0	2360.8	2361.5	Asymmetric stretching band of CO_2 hydrates; metal complexation with –SH groups (Naumann, 2000)
1680-1640	**1642.9**	**1631.5**	**1644.8**	**1627.6**	**Amide- I(Proteins), C=O stretching (Jilie et al., 2007)**
	1378.6	1379.6	1377.6	1376.6	Stretching C-O, deformation C-H, deformation N-H
1350 - 1300	**1322.6**	**1321.8**	**1321.9**	**1321.0**	**Amide III band components of α – helix proteins**
1250-1220	1240.8	1239	1243.5	1242.8	P=O stretching (asymmetry) of PO^{2-}phosphodiesters (Naumann, 2000)
1200-1000		1112.0	1153.2	1154.0	SO_3 asymmetric, ester groups, Thiol and Sulfhydryl groups
1100- 1000		**1029.0**		**1028**	**C-O bonding due to polysaccharide (Das, 2007)**
	1030.8		1030.5		Carboxylic groups; O-CH_3 stretching of methoxy groups (Movasaghi et al., 2008)
770 -620	668.9; 779	782	778.8; 668.3	781	Amide IV band (OCN bending) (Mohan, 2005)
630 -530	617	518.3	521.5	576	Amide VI band (OCN) deformation (Mohan, 2005)
	523.0	469.1		519	C-Br Stretching

Table6.3. Representing the description of FTIR spectra of metals treated and untreated plant roots and leaves of *Hydrilla verticillata*

Observed wavenumber cm^{-1} and band assignment of *Hydrilla verticillata* (metals untreated and treated)					**Band Assignment**
Representing range of frequency (cm^{-1})	5. *Hydrilla verticillata* (Root Control)	6. *Hydrilla verticillata* (Root Treated)	7. *Hydrilla verticillata* (Leaves Control)	8.*Hydrilla verticillata* (Leaves Treated)	
3900 - 3500		**3799; 3733**			**O-H &N-H stretching vibrations (**Naumann 2000)
3500-3300	3419.2	3391	3354.9	3352	N-H and O-H groups stretching and overlapping
3000 - 2800			**2931.2**	**2937**	**Presence of CH_2 group in protein and phospholipids**
2900 - 2800	2882.7	2885			C=H stretching of -CH3 group (Naumann 2000)
		2834.5			CH stretching of CH2 or CH3 groups in fatty acids or their deformations (Akhter etal., 2010)
2400 - 2300		2360.2			Asymmetric stretching band of CO_2 hydrates (Naumann, 2000)
	2360.6		2361.3	2360.2	CN-SH groups, Asymmetric stretching band of CO_2 hydrates (Naumann, 2000)
2300 - 2250		2335			NH_2^+ asymmetry Stretching (Mohan, 2005)
1680-1640	**1644.2**	**1624**	**1643.2**	**1643.6**	**Amide- I (protein), C=O stretching COH vibrations (Jilie et al., 2007; Ibrahim et al., 2011)**
1450-1400	1418.5		1419.6	1420.0	C=O symmetric stretching of aliphatic ketones (Tang et al., 2013)
1350 - 1300	**1319.8**	**1319.0**	**1320.0**	**1320.5**	**Amino substituted alkyl group (Tang et al., 2013)Amide III band components of α – helix proteins (Adriana, 2011)**
1250-1220			1238.7	1241.1	Ethers and Anhydride; P=O stretching (asymmetry) of PO^{2-} phosphodiesters (Naumann, 2000)
1200-1000	1108.2	1117.0			Secondary Alcohols, C-C bonds; SO_3 asymmetric (Yeliz, 2012)
			1102.2	1101.6	C-O, C-C ring, polysaccharides, cellulose (Shetty et al., 2006)
1100- 1000		1071.0	1069.4	1068.1	C-O bonding due to polysaccharide and C-O stretching vibration of cellulose (Das, 2007, Ibrahim et al., 2009)
			895.7	894.3	C-H out-of-plane bending vibrations (Jilie et al., 2007)
630 -530		618.4	558.6	561.0	Amide VI band (OCN) deformation ; C-Br stretching (Mohan, 2005)

6.4.Discussion:

In the present study the concentration of Cu, Fe, Cd and Cr accumulated by *Pistia stratiotes* and *Hydrilla verticellata* in root and shoot under mono and mixed culture with and without effect of intermittent circulation of metal contaminated water was evaluated. The efficiency of phytoremediation varies significantly between species as different mechanisms of ion uptake are operative in each species, based on their genetic, morphological, physiological and anatomical characteristics (Rehman and Hasegawa, 2011).

In the monocultures, water circulation has a positive effect on enhanced metal accumulation and also on the survival of plants. Generally, it was seen that both plants showed metals accumulating characteristics, however, *Pistia stratiotes* accumulated more metals than *Hydrilla verticillata* under non-circulation of water. Circulation of multimetal contaminated water on different days of treatment between the tubs of *Pistia stratiotes* and *Hydrilla verticillata* grown under mixed culture had a significant effect on the enhanced metals removal and concurrent accumulation by the both plants. Comparing root and shoot accumulation of metals, it was observed that *Pistia stratiotes* accumulated higher metals in both parts that *Hydrilla verticillata* under mixed culture with water circulation.

Due to the intermittent recirculation, the number of passages of water through the bed increases in comparison with the other conventional techniques, 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, as it is well known that the efficiency of pollution removal in CWs depends greatly upon HRT (Maltais-Landry et al., 2009; Foladori et al., 2014). Further, intermittent recirculation with water storage in the bed has the following benefits: (1) a longer contact between pollutants and biomass in the bed; (2) an enhancement of the natural re-aeration during each recirculation step. Differences in the bioaccumulation rates and TFs among the two plant species reflect the abundance of the metals studied and the intrinsic abilities of the plants to sequester the metals (Anning et al., 2013).

One of the prominent physiological changes due to metal toxicity in plants is chlorosis, resulting a subsequent change in the chlorophyll and carotenoid contents (Chaudhuri et al., 2013). The water circulation coupled with mixed culture of plants had a synergistic effect on the chlorophyll and carotenoid content of both plants. Similar observation was also reported by Mohan and Hosetti (1997), where cadmium and lead also reduced chlorophyll content of *Lemna minor*. According to them cadmium suppressed chlorophyll content by inhibiting its biosynthesis through interaction with different essential enzymes like Amino levulinic acid, required in chlorophyll synthesis pathway.

Under mixed cultures coupled with circulating effect of water both plants showed a significant increase in the levels of protein for *Pistia stratiotes* and *Hydrilla verticillata*. However, the contents decreased towards the end of experiment. The effect of varying metal content on leaf soluble protein has been reported to be decreasing in many plants (Best et al., 2011; Muneer et al., 2011; Bauddh and Singh, 2012). It has been described that leaf protein levels decreases more effectively when multiple abiotic stresses occur simultaneously. Abiotic stresses such as heavy metals are known to cause oxidative damage to plants through the formation of reactive oxygen species, which cause damage to membrane lipids and proteins etc., However, to resist the damage plants have an antioxidant defense system by producing antioxidant compounds as stress metabolites (Chugh et al., 2011; Bauddh and Singh, 2012). Proline is one such metabolite known to accumulate under metal stresses. It plays an important role in osmoregulation and osmo-tolerance. Proline content in *Pistia stratiotes* and *Hydrilla* *verticillata* was observed to be increasing in both cultures under metal contents and circulating water conditions.

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 sorption of metal ions within the plant cells is of great significance in understanding the mechanism of heavy metal tolerance and partitioning within the vascular plants (Vollenweider et al., 2006). Metals absorption and storage has been reported in leaf epidermis and support tissues of leaf veins as in case of Cd (Shinmachi et al., 2003). Subcellular compartments have been found to be rich in metal binding sites. The deposition of metal clumps in xylem and phloem channels confirms the metal translocation to upper plant parts.

It has been observed that cell wall plays an important role in metal accumulation. The importance of cell wall has been recognized for its role in metal accumulation. Accumulation of metals and concentrated in the cell walls of plants has been reported by Xu et al., (2004). The presence of functional groups such as carboxylic (glutronic acids in pectin) and hydroxyl (in cellulose) can act as strong binding sites for metal cations in aqueous solution by means of chelation, complexation, ion exchange etc. (Sachez-Galvan et al., 2008; Srivastava et al., 2011).

Metal ions have been studied to be highly reactive with Thiol, amino or hydroxyl groups and the biomolecules carrying such functional groups may have a greater tendency to act as metal chelators within the plant cells. The presence of bands at 3391 and 3352 cm^{-1} in metal treated Hydrilla verticillata roots and shoot have been characteristically assigned to N-H (amide) and OH (hydroxyl) groups stretching (Lata et al., 2011). The biomolecules containing the chelators for metal ions have been demonstrated as small proteins (metallothioniens), peptides, gluta thione and phytochelatins.

The presence of frequency bands in 2927 cm^{-1} in *Hydrilla verticillata* leaves have been assigned to asymmetric vibrations of CH_2 groups of proteins and phospholipids (Lata et al., 2011). Also presence of carboxylic and methoxy group bands at 1030.8 and 1030.5 cm^{-1} in untreated root and shoot biomass of plants depict their role in metal binding. The band at 1322.6 cm^{-1} in untreated root of *Pistia stratiotes* and its shifting to 1321.8 cm^{-1} in metal treated biomass depicts the amide band components of α – helix proteins (Adriana, 2011). Carboxylation reaction has been found to be the major mechanism involved in the binding of metals within the plants. The degree of carboxylation depends upon the ratio of intensity between the C=O and C-O stretching vibrations in the spectra (Ibrahim et al., 2009). The presence of both groups C=O and C-O and their shifting patterns depicts that carboxylation reaction may have led to changes in the intensity at these frequency groups and may have played a role in metal binding within the plants (Javed et al., 2011).

6.5.Conclusions

Plants adaptability to heavy metal stress is multigenic as well as they help in detoxification. In the study, aquatic plant *Pistia stratiotes* and *Hydrilla verticellata* considerably reduced the concentration of metals from water and it may be one of the cost effective treatment system. The mixed culture of plants was more effective in metals removal from water.

Further recirculation increased the Hydraulic Retention Time of water in the treatment system, thereby enhancing metal removal. The major frequency band changes and shifts at (3403.4, 3365.3, 1631.5, 1321.8, 1030cm^{-1}) were observed in both metal treated plants root and shoot biomass, which correspond to specific band frequencies of OH, NH, COOH and these functional groups act as metal binding sites within the plant cells, depicting their role in metals accumulation within the plant parts. The study was intended to corroborate the hypothesis that intermittent water circulation and mixed culture of plants can improve the removal rates of metals and eventually offer a greater potential for the reduction of its area requirement. The treatment system is easy and cheap to construct and operate and can be a suitable alternative for water treatment and purification.

Chapter 7

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

Chapter 7. Comparative study on remediation of nitrate, nitrite, ammonium and phosphate from water using *Pistia stratiotes* and *Hydrilla verticillata*

7.1. Introduction

Agriculture, fishing, industries and municipal drainage have become an increasing concern for point and non-point pollution sources of nitrogen and phosphorous in many countries, especially developing countries (Wu, 2011; Souza *et al.,* 2013). Excessive release of nitrogen and phosphorus may enter freshwater ecosystems such as lakes, rivers, leads to undesirable eutrophication of surface waters and different diseases among animals and humans due to consumption of water contaminated with nitrate, nitrite, ammonium, which is the most widespread problem to water environment quality around the world (Rawat *et al*., 2012; Rezania *et al*., 2015). Nitrite has been shown to cause methaemoglobinaemia in animals. Humans appear to be more sensitive to nitrite-induced methaemoglobin formation. Nitrogenous ions especially NO^{-}_{2} can combine with organic pollutants to produce cancer causing nitrosyls which is a big health hazard (Kim Shapiro et al. 2006; Duplay et al. 2013).

The removal of reactive nitrogenous species (Nitrate, Nitrite and Ammonium) from drinking water is a challenging problem which demands high cost technologies (Abou-Elela, 2013). On the other hand phosphorus also needs to be removed from waste water entering the fresh water sources. Plants are considered to play an important role in nutrient removal from constructed wetlands (Vymazal and Kropfelova, 2011). Macrophytes are considered to be the main biological component of constructed wetlands that directly take up nitrogen and phosphorous, which are key nutrients in the life cycles of wetland plants for the growth and reproduction (Xu et al., 2009). Research indicates that different macrophytes have different nutrient preferences. Although most wetland plants prefer absorbing NH4–N in anoxic wetland substrates some wetland plants prefer absorbing NO3–N (Zhang et al., 2009). Mostly the macrophytes have been employed to remediate the wastewater. The study was undertaken to remediate nitrate, nitrite, ammonium and phosphate from drinking water under simulated conditions.

7.2. Material and Methods

7.2.1. Collection of plants and experimental design

The experimental plants *Pistia stratiotes* and *Hydrilla Verticellata* were collected from the Gomti River and local ponds of Lucknow city. The plants were washed with running tap water to remove any attached particles. They were acclimatized for 15 days in Hoagland solution (Hoagland and Arnon, 1950) in constructed cemented ponds. After 15 days of acclimatization, fully grown and healthy plants were transferred to the treatment tubs. Cemented tubs filled with 20 liters of simulated water were employed to evaluate the phytoremediation potential of *Pistia stratiotes* and *Hydrilla verticillata* for the removal of inorganic pollutants. 100 grams of each plant was put into 4 tubs for their monocultures, covering 80% of surface area of water in the tub. A control treatment without any plant was also setup. Simulated water was having the following concentration of Nitrate: 40.00 mg/l; Nitrite: 10.00 mg/l; Ammonium: 10.00 mg/l and Phosphate: 5 mg/l.

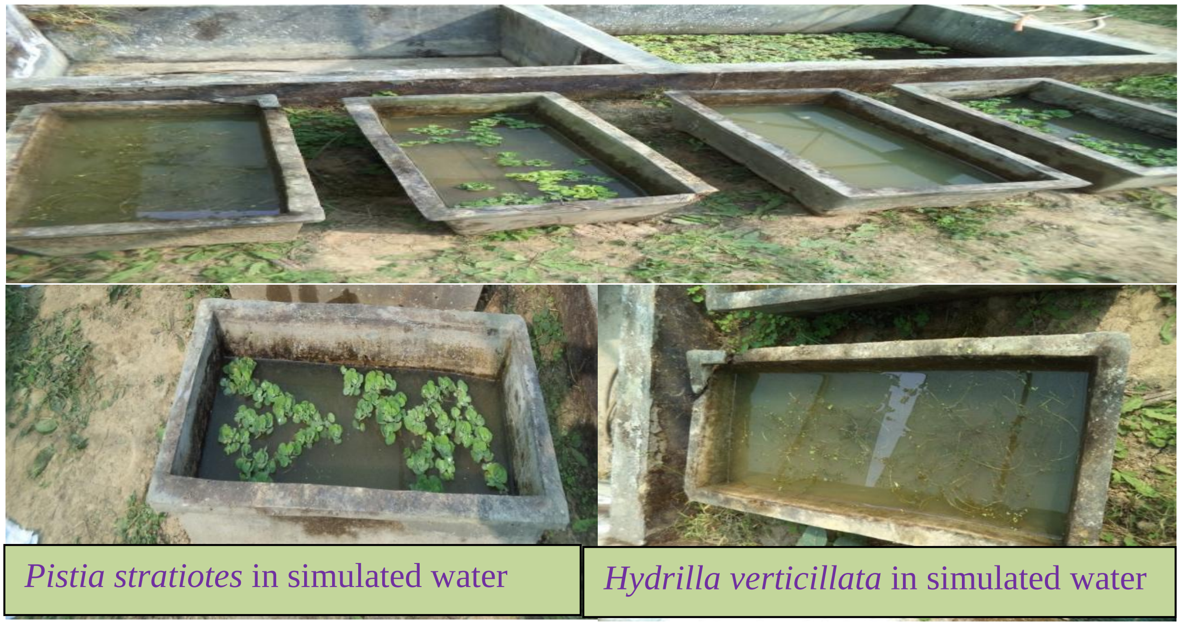


Plate7.1. Showing the experiment conducted in the net house of DES, to evaluate the efficiency of selected plants for removal of nitrate, nitrite, ammonium and phosphate.

7.2.2. Water sampling and analysis

Water samples from each treatment were collected on 5th, 10th, 15th, 20th and 25th day from the start of the experiment from each treatment including the control. On each sampling day, randomly plants were selected from each treatment and rinsed with deionized water and divided into root and shoot components for further analysis. The changes in the temperature, pH, conductivity of water and the removal of nitrate, nitrite, ammonium and phosphate from the simulated water were observed during the study period. The details of the protocols are given in chapter 3.

7.2.3. Plant sampling and biomass analysis

Plant samples from each treatment were collected on 5th, 10th, 15th, 20th and 25th day from the start of the experiment. On each sampling day, randomly plants were selected from each treatment and rinsed with deionized water and divided into root and shoot components for biomass analysis. The biomass of plants was expressed on dry weight (DW) basis. The dry weight of the samples was recorded after drying it in hot air oven at 60° C for 48 hr. until the concordant values were obtained.

7.3. Results

7.3.1. Changes in the physicochemical characteristics of nutrient simulated water due to treatment with *Pistia stratiotes* and *Hydrilla verticillata*

In the present study the changes in the temperature, pH and electrical conductivity (EC), nitrate, nitrite ammonium and phosphate of nutrient simulated water were observed due to treatment with *Pistia stratiotes* and *Hydrilla verticillata* are presented in Tables 6.1 and 6.2. The marked variations were observed in temperature, pH and electrical conductivity of water treated with *Pistia stratiotes* and *Hydrilla verticillata.* The temperature in water treated with *P. stratiotes* decreased with time reaching minimum of 23.33 ° C from a maximum of 24.66° C at the beginning of experiment. In case of water treated with *H. verticillata,* temperature increased from 24.6 ° C to 25.43 ° C at the end of the experiment. The pH of water treated in both case decreased with time and reached 7.21 and 7.19 for water treated with *P. stratiotes* and *H. verticillata,* respectively. A sharp decrease in EC (µs/cm) for both treatments was observed. EC decreased from 2.06 (µs/cm) to 0.17 (µs/cm) in water treated with *P. stratiotes* and decreased from 2.02 (µs/cm) to 0.32 (µs/cm) in water treated with *H. verticillata.*

Table 7.1. Changes in Temperature, pH and Electrical Conductivity (EC) of water due to *Pistia stratiotes* and *Hydrilla verticillata* (n=6±SD)

Parameter	Days	*Pistia stratiotes*	*Hydrilla verticillata*	Control
Temperature (°C)	Day5	24.66 ± 0.05	24.6±0.05	24.64± 0.01
	Day10	23.66±0.01	25.06±0.15	24.43± 0.01
	Day15	22.33±0.05	25.16±0.05	24.33± 0.02
	Day20	23.1±0.03	25.23±0.52	23.21± 0.03
	Day25	23.33±0.01	25.43±0.02	23.1± 0.01
pH	Day 5	7.56±0.05	7.57±0.01	7.55± 0.02
	Day10	6.8±0.01	7.63±0.03	7.49± 0.03
	Day15	6.67±0.02	7.31±0.09	7.32± 0.02
	Day20	7.13±0.02	7.46±0.01	7.22± 0.01
	Day25	7.21±0.01	7.19±0.02	7.17± 0.05
EC(µs/cm)	Day5	2.06±0.01	2.02±0.01	2.02± 0.05
	Day10	0.66±0.015	1.06±0.03	2.00± 0.05
	Day15	0.32±0.03	0.71±0.026	1.96± 0.05
	Day20	0.26±0.015	0.41±0.05	1.87± 0.05
	Day25	0.17±0.03	0.32±0.01	1.65± 0.05

An overall decrease in the four inorganic pollutants i.e., nitrate, nitrite ammonium and phosphate was observed with water treated with *P. stratiotes* and *H. verticillata.* Nitrate content in water decreased from (40 mg/l) to (3.7 mg/l) and (7.83 mg/l) treated with *P. stratiotes* and *H. verticillata*, respectively at the 25th day of the experiment. Nitrite content in water treated with *P. stratiotes* decreased from (10mg/l) to (3.28 mg/l) and (2.23 mg/l) in case of treatment with *H. verticillata.* For ammonium the final content in water at the end of the experiment was (1.98 mg/l) in case of *P. stratiotes* and (1.23 mg/l) for *H. verticillata.* The phosphate content in water reached a final concentration of (0.69 mg/l) from (5 mg/l) treated with *P. stratiotes* and (0.44 mg/l) in *H. verticillata* treated water. From the control treatment the changes in concentration of selected pollutants was not significant.

Table 7.2. Changes in nitrate, nitrite , ammonium and phosphate during water treatment with *Pistia stratiotes* and *Hydrilla verticillata* (n=6±SD)

Parameter	Days	*Pistia stratiotes*	*Hydrilla verticillata*	Control
Nitrate (mg/l)	Day 5	31.01±0.14	35.79±0.30	39.45±0.02
	Day10	23.96±0.16	28.66±0.40	39.1±0.01
	Day15	13.41±0.19	17.52±0.4	38.65±0.02
	Day20	7.08±0.15	14.88±0.12	37.93±0.03
	Day 25	3.7±0.18	7.83±0.38	37.65±0.02
Nitrite (mg/l)	Day 5	8.52±0.05	10.7±0.3	9.87±0.01
	Day10	7.29±0.14	8.6±0.4	9.88±0.03
	Day15	6.33±0.38	7.52±0.4	9.72±0.02
	Day20	4.59±0.43	4.8±0.1	9.7±0.01
	Day 25	3.28±0.34	2.23±0.3	9.67±0.03
Ammonium(mg/l)	Day 5	8.35±0.8	8.62±0.5	9.87±0.02
	Day10	7.14±0.3	7.54±0.4	9.66±0.03
	Day15	6.72±0.05	4.35±0.5	9.56±0.03
	Day20	2.45±0.07	3.35±0.2	9.45±0.02
	Day 25	1.98±0.2	1.23±0.3	9.23±0.05
Phosphate(mg/l)	Day 5	4.41±0.8	4.19±0.2	4.91±0.01
	Day10	3.63±1.8	2.82±0.6	4.9±0.02
	Day15	2.18±0.2	1.45±1.1	4.88±0.03
	Day20	1.27±0.2	1.04±0.6	4.86±0.03
	Day 25	0.69±0.5	0.44±0.4	4.87±0.01

7.3.2. Percentage removal of nitrate, nitrite, ammonium and phosphate from water treated with *Pistia stratiotes* and *Hydrilla verticillata*

The percent removal of studied inorganic pollutants by *Pistia stratiotes* and *Hydrilla verticillata* has been presented in table 6.3. *P. stratiotes* was more efficient for the removal of nitrate and phosphate. 90.83% of nitrate and 91.07% of phosphate was removed from water treated with *P. stratiotes*. However, nitrite and ammonium was maximum removed by submerged macrophyte *H. verticillata.* The removal percentage for nitrite and ammonium by *Hydrilla verticillata* was 77.48% and 81.81%, respectively. The removal of pollutants from the control treatment was not significant.

Table 7.3. Percent removal of nitrate, nitrite, ammonium and phosphate in water during application of *Pistia stratiotes* and *Hydrilla verticillata*

	Parameter	Day1 (mg/l)	Day 25 (mg/l)	% Remediation
P. stratiotes	NO_3^-	40.01±0.14	3.790±0.18	90.83
	NO_2^-	10.02 ± 0.05	3.28 ± 0.34	70.06
	NH_4^+	10.05± 0.8	1.98±0.2	80.88
	PO_4^-	5.01±0.8	0.69±0.5	93.05
H. verticillata	NO_3^-	40.01 ±0.14	7.82±0.053	81.08
	NO_2^-	10.02 ±0.03	2.23± 0.3	77.48
	NH_4^+	10.02 ±0.5	1.23 ±0.3	81.81
	PO_4^-	5.01 ±0.2	0.46± 0.4	91.07
Control	NO_3^-	40.01 ±0.14	37.65±0.02	5.54
	NO_2^-	10.02 ±0.03	9.67±0.03	3.21
	NH_4^+	10.02 ±0.5	9.23±0.02	7.68
	PO_4^-	5.01 ±0.2	4.87±0.01	2.04

7.3.3. Changes and correlation of biomass accumulation with nutrient removal by *Pistia stratiotes* and *Hydrilla verticillata*.

In the present study, the changes in the biomass accumulation expressed as dry weight (DW/plant) of *Pistia stratiotes* and *Hydrilla verticillata* are given in table 6.4. The correlation of biomass accumulation with nutrient removal by the plants has been presented in figures 6.1 (A-H). The biomass of both plants increased with the removal and concurrent accumulation of inorganic nutrients from the water. Increase in root and shoot biomass of *Pistia stratiotes* and *Hydrilla verticillata* was observed during the treatment phase. *P. stratiotes* accumulated greater biomass both in root and shoot parts than *H. verticillata* at the end of the experiment.

Table 7.4. Changes in biomass of *Pistia stratiotes* and *Hydrilla verticillata* during the treatment phase (n=6±SD)

Parameter	Days	*P. stratiotes*		*H. verticillata*	
		Shoot	Root	Shoot	Root
Biomass (mg/plant DW)	Day5	0.47±0.04	0.223±0.04	0.75±0.03	0.19±0.04
	Day10	0.9±0.06	0.342±0.04	1.17±0.01	0.27±0.06
	Day15	2.1±0.06	0.481±0.05	2.71±0.04	0.57±0.04
	Day20	6.85±0.04	0.831±0.01	3.15±0.02	1.45±0.02
	Day25	9.55±0.04	3.24±0.06	5.57±0.03	2.27±0.02

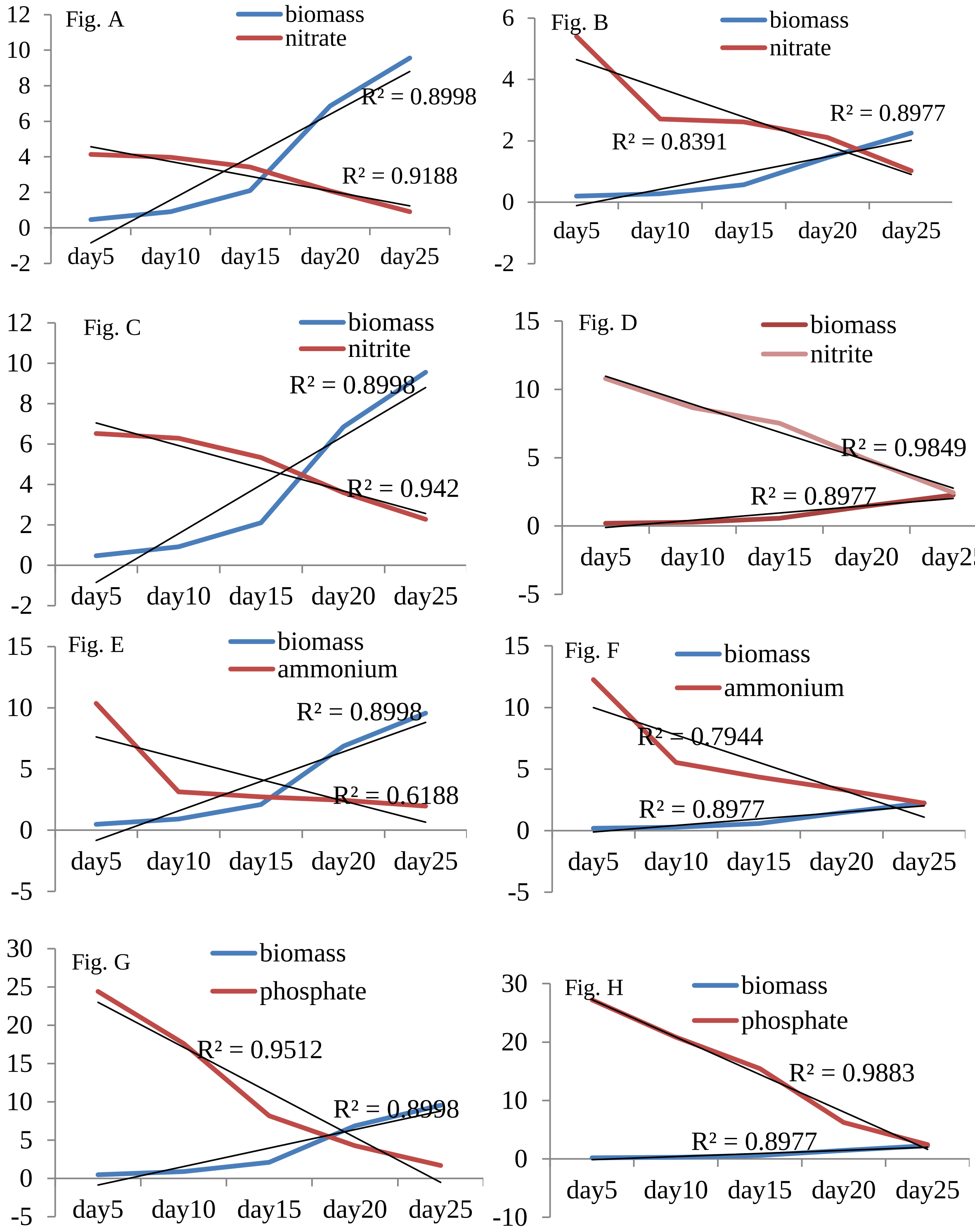


Figure7.1. (A-H). Fig. A and B: Correlations of biomass accumulation with nitrate removal by *P. stratiotes* and *H. verticillata*, respectively. **Fig. C and D:** Correlation of biomass accumulation with nitrite removal by *P. stratiotes* and *H. verticillata*, respectively. **Fig. E and F:** Correlation of biomass accumulation with ammonium removal by *P. stratiotes* and *H. verticillata*, respectively. **Fig. G and H:** Correlation of biomass accumulation with Phosphate removal by *P. stratiotes* and *H. verticillata*, respectively.

Nitrate removal was observed to be positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*. The value of R^2 was found to be (0.9188) for *P. stratiotes*, while as for *H. verticillata* it was (0.8391).Also nitrite removal was positively correlated with plant biomass accumulation. The value of R^2 was found to be (0.942) for *P. stratiotes*, while as for *H. verticillata* it was (0.9849).Ammonium removal was observed to be positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*. The value of R^2 for *P. stratiotes* was found to be (0.6188) for *P. stratiotes,* while as for *H. verticillata* it was (0.7944). Phosphate removal was found to be positively correlated with plant biomass accumulation for *P. stratiotes* and *H. verticillata*. The value of R^2 for *P. stratiotes* was found to be (0.9512) for *P. stratiotes,* while as for *H. verticillata* it was (0.9883). Therefore, the removal of pollutants and their accumulation within the plants was significantly correlated with the increase in the biomass of both *P. stratiotes* and *H. verticillata.*

7.4. Discussion

The current study was undertaken to evaluate the comparative potential of two different aquatic macrophytes *Pistia stratiotes* and *Hydrilla verticillata* for the removal of inorganic pollutants from the simulated water under NET house conditions of Department. The changes in temperature, pH, EC, nitrate, nitrite, ammonium and phosphate were observed during the 25days study period. The removal efficiency for pollutants and biomass accumulation by the plants were evaluated. The fluctuations in temperature were evident during the study period in both treatments. This may be due to minimal temperature stratification within the system (Tripathi *et al*; 2013).

The pH in water determines the extent of acidity or basicity in water. Our study pertaining to the changes in pH reveals that pH of simulated water after treatment with selected plants was reduced. Reduction in pH may be due to the plant uptake of significant amount of soluble nutrients by rhizosphere or by acidifying the rhizosphere, via excreting H^+ in exchange for cation and ending organic acids and carbon dioxide (Gikas and Tsihrintzis, 2012). The changes in EC reveal that EC of water was significantly reduced during the treatment. This is expected due the removal of dissolved nitrogen and phosphorus salts (Souza *et al*, 2013).

The inorganic nutrients in water were nitrate, nitrite, ammonium and phosphate degrades the quality of water and depletes the dissolved oxygen present in water. Nitrite is a natural component of the nitrogen cycle in ecosystems, and its presence in the environment is a potential problem due to its well documented toxicity to animals (Sinha and Nag, 2011). It has been reported in various water sources (Bingbing *et al*., 2009). It is therefore, necessary to remove it from water in order to reduce its harm to the animal and human consumers of the water which cannot assimilate nitrite like bacteria and plants (Alonso and Camargo, 2009). Our study pertaining to the changes in the inorganic nutrients reveals that there is a significant decrease in the concentration of inorganic pollutants from the treatment system. Similar results pertaining to the current study were reported by Rawat *et al*., (2012). The potential rate of nutrient uptake by plant is limited by its net productivity (growth rate) and the concentration of nutrients in the plant tissues (Vymazal, 2007). Decrease in the concentration of nitrate could be due to the increased plant uptake rather than microbial denitrification. Kadlec *et al.,* (2009) and Bindu *et al.,* (2008) reported the removal of nitrate might be due to uptake by the plant roots. Denitrification is believed to be the major pathway for ammonia removal in the constructed wetland (Rai et al., 2013).

The mechanism of the phosphorus removal could be reported to occur by sorption, complexation, precipitation and assimilation into microbial and plant biomass. The better removal of phosphorus in the planted system in the presented study could be attributed to plant uptake and microbial assimilation. The main role of wetland plants with respect to removal of phosphate are direct uptake and provision of suitable conditions for microorganisms that use phosphorus as a nutrient (Mbuligwe; 2004). Nutrient removals by plants accounted for 15–80% N and 24–80%P (Greenway and Woolley, 2001). Different species, nutrient loading rates and climates accounted for different nutrient uptake by plants in various studies (Vymazal, 2007).

Growth characteristics like biomass were also observed during the study period. The changes in growth parameters reveal that the biomass of both the plants increased continuously throughout the experiment. Increase in biomass could be due the uptake of pollutant and all other nutrient in water by the plant. Nutrients removal coupled with biomass accumulation and plant productivity varied widely with the species. This variation resulted partly from relative differences in climate, intrinsic species and possible ecotype as well as growth characteristics (Brisson and Chazarenc, 2009).

7.5. Conclusions

Removal of inorganic pollutants from water can help reduce eutrophication of water bodies and also improve the quality of water thereby preventing human and animal life from different health aliments. The studied plants were able to remove nitrate, nitrite, ammonium and inorganic phosphate from simulated water. Both plants showed growth and development by increasing their biomass from day 5 until day 25. Moreover, plants were able to survive in high concentration of nutrients and effectively removed all the four nutrients/pollutants from water. Analysis of data revealed that *Pistia stratiotes* can be utilized as a hyper-accumulator of nitrate and phosphate, while as *Hydrilla verticellata* could serve the purpose of stabilizing nitrite and ammonium in contaminated waters.

Chapter 8

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

Chapter 8. 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

8.1. Introduction

Different wetland plants have different growth speed, growth rhythm, root morphology and distribution; hence, it could be deduced that mixed wetlands may have better removal rates because of the temporal and spatial compensation in plant growth, root distribution, and nutrient preference (Kongroy et al., 2012; Turker et al., 2014). Aquatic plant based treatment systems are ecofriendly and cost-effective technologies which can be adopted by developing counties like India for recycling/treatment of waste water, especially contaminated by heavy/toxic metals (Rai et al., 2012). Potential productivity of plants in nutrient enriched wastewaters has lead to its selection for phytoremediation of various industrial effluents and the produced biomass as a feed stock for vermicomposting production to achieve safe disposal of contaminated biomass.

Composting followed by land application represents one of the most economical ways for the treatment and final disposal of plant biomass after phytoremediation of pollutants because it combines material recycling and biomass disposal at the same time (Villasenor et al., 2011). 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 (Deka et al., 2011 and Gabhane et al., 2012).

The present study is undertaken to remediate metals contaminated water using consortia of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia* under mixed cultures and vermicomposting of the generated plant biomass.

8.2. Material and Methods

8.2.1. Plants collection and their acclimatization

The experimental plants *Typha latifolia*, *Pistia stratiotes* and *Hydrilla Verticellata* were collected from the Gomti River and local ponds of Lucknow city. The plants were washed with running tap water to remove any attached particles. They were acclimatized for 15 days in Hoagland solution (Hoagland and Arnon, 1950) in constructed cemented ponds. After 15 days of acclimatization, fully grown and healthy plants were transferred to the treatment tubs. 200 grams of each plant was put into tubs for their monocultures, covering 80% of surface area of water in the tub. For mixed culture, 100 grams of each plant was put together in the tubs and for the consortium of all three plants 70 grams of each plant was put together in the tub.

8.2.2. Experimental treatment set up and mode of operation

A bench scale microcosm water treatment system, with intermittent flow and circulation, comprising of different parallel treatments of individual and mixed cultures of *Typha latifolia*, *Pistia stratiotes* and *Hydrilla Verticellata* were set up in the Net House located at field station of Department of Environmental Science, BBAU Lucknow. The experiments were performed in the month of May 2014. Other details of experimental setup are given in Chapter 6 section 6.2.2

8.2.3. Plant cultures and treatment conditions

Mono and mixed cultures of free floating *Typha latifolia, Pistia stratiotes* and submerged *Hydrilla verticellata* were tested for their metal accumulation and removal capacities form multi metal solution of Cu (5ppm); Fe (5ppm); Cd (2.5 ppm) and Cr (2.5 ppm). Different treatments for the experiment were: T1= *Typha latifolia* monoculture with water recirculation at intervals; T2= *Typha latifolia* monoculture without circulation; T3= *Pistia stratiotes* and *Typha latifolia* mixed culture with water recirculation at intervals;

T4 = *Pistia stratiotes* and *Typha latifolia* mixed culture without water recirculation; T5= *Typha latifolia and Hydrilla verticellata* mixed culture with water recirculation; T6= *Typha latifolia and Hydrilla verticellata* mixed culture without water circulation; T7= *Typha latifolia, Pistia stratiotes and Hydrilla verticellata* mixed culture with water recirculation; T8 = *Typha latifolia, Pistia stratiotes and Hydrilla verticellata* mixed culture without water recirculation; T9= Control for water without plants with recirculation at intervals

8.2.4. Metals accumulation, Bioconcentration factor and Translocation factor

Plant samples from each treatment were collected on 5th, 10th, 15th, 20th, 25th and 30th day from the start of the experiment and divided into root and shoot components for further analysis.

Metals analysis: Root and shoot plant samples were oven dried at 90 °C to a constant weight and metals (Cu, Fe, Cd and Cr) in the plant parts were determined in 1 gram (dry weight) of each root and shoot sample after acid digestion of dry samples with an acid mixture (9:4 nitric acid: perchloric acid) at about 100 °C. Metal concentrations were determined by using atomic absorption spectrophotometer (AAS 240 FS, Varian) following standard protocols. Analytical data quality of metals was ensured through repeated analysis (n=3) of EPA quality control in samples.

Bioconcentration factor (BCF), expressed as the ratio of metal concentration in plant tissue to that of the water was calculated by:

$$\mathrm{BCF} = \frac{\text{Metal content in plant tissue}}{\text{Initial metal content in water column}}$$

Translocation factor (TF), the ratio of metals in shoot versus root of plants was calculated by the formula of Padmavathiamma and Li (2007).

$$\mathrm{TF} = \frac{\text{Metal content in plant shoot}}{\text{Metal content in plant root}}$$

8.2.5. Vermicomposting of the contaminated plant biomass

For developing the vermicompost, the experiments were conducted in plastic bin of sizes 450 × 300 × 450 mm in the laboratory net house. The reactor was designed for a total weight of 0.5 kg for 45 days of vermicomposting period. Acclimatized 250 earthworms (adult and juvenile) species *Eisena fetida* collected from Biotech Park, Kursi Road Lucknow were randomly picked from the culture and added to each treatment.

The composition for the development of vermicompost in different trials was as follow:

- Trial 1: *Pistia stratiotes* biomass + earthworms
- Trial 2: *Typha latifolia* biomass + earthworms
- Trial 3: *Hydrilla verticellata* biomass + earthworms
- Trial 4: *Pistia stratiotes* biomass+ *Hydrilla verticellata* biomass + earthworms
- Trial 5: *Pistia stratiotes* biomass+ *Typha Latifolia* biomass+ earthworms
- Trail 6: *Pistia stratiotes* biomass+ *Typha Latifolia* + *Hydrilla verticellata* biomass + earthworms
- Trail 7: CONTROL (*Pistia stratiotes* biomass+ *Typha Latifolia* + *Hydrilla verticellata* biomass without earthworms)

These mixtures were manually turned at every 10th day, in order to provide suitable aeration to the earthworms. All the containers were kept in the dark under identical ambient conditions of NET House DES.

8.2.6. Determination of pH, Nitrate, Nitrite, Phosphate, Total phosphorus, Total organic carbon and % Total organic matter in vermicompost. The details of protocols are given in Chapter 3.

8.2.7. Sequential Extraction of metals: For sequential extraction of metals form the developed vermicompost, the conventional method designed and developed by Tessier et. al., (1979) for heavy metal speciation into five fractions (exchangeable, carbonate, reducible, organically complexed and residual) was employed. The details of the technique are given in Chapter 3.

8.3. Results

8.3.1. Accumulation of Fe, Cu, Cd and Cr from water by selected plant cultures of *Pistia stratiotes, Hydrilla Verticellata* and *Typha latifolia* under circulating and no circulating conditions under intermittent circulation

In the present study the concentration of Cu, Fe, Cd and Cr accumulated by *Typha latifolia Pistia stratiotes* and *Hydrilla verticellata* in roots and shoot under mono and mixed culture with and without effect of circulation are presented in table 8.1 for Cu and Fe and table 8.2 for Cd and Cr.

Table 8.1. Accumulation of Cu and Fe (µg/gm dw) by *Typha latifolia Pistia stratiotes* and *Hydrilla verticellata* under different culture treatments (n=6±S.D.)

	Cu (µg/gm dw)		Fe (µg/gm dw)	
Treatments	Root	Shoot	Root	Shoot
T1	1708.66±13.05	1736±10.14	1392.65±6.45	1086.17±4.3
T2	1015.33±5.5	1236±10.14	933.25±6.11	842.84±6.11
T3P	2086.36±8.87	2323.8±8.22	1160.13±6.77	1030.26±7.79
T3T	1486.36±8.87	1523.83±8.22	1460.13±6.77	1030.26±7.79
T4P	1026.9±3.55	1047.76±9.54	961.8±9.95	644.23±6.82
T4T	815.33±5.5	936±10.14	633.25±6.11	742.84±6.11
T5H	563.38±7.37	1216.86±6.7	1108.18±9.4	1456.2±7.15
T5T	1385.22±8.76	1436.3±10.37	952.33±6.19	588.2±4.5
T6H	356.71±4.59	922.9±4.4	708.18±2.64	1012.87±4.69
T6T	790.56±5.09	942.96±3.87	644.23±6.82	372.04±4.7
T7P	842.5±10.69	950.06±8.35	671.34±10.92	263.2±10.31
T7H	409.35±9.72	877.3±4.68	932.13±8.76	1256.68±8.86
T7T	1126.9±3.55	1154.43±4.36	1061.8±9.95	644.23±6.82
T8P	517.56±3.8	649.73±7.25	412.34±3.7	162.86±10.4
T8H	222.68±5.87	678.96±5.93	626.8±5.16	856.6±8.86
T8T	627.5±3.58	854.3±4.58	761.8±9.95	544.2±6.8

Considerable differences were observed for metals accumulation by plants under different cultures. Metals accumulation varied from plant to plant and the effect of different consortium of plants on metals accumulation was profound. Cu was maximum accumulated by *Pistia* *stratiotes* roots (2086.36 µg/gm DW) and shoot (2323.8 µg/gm DW) in mixed culture with *Typha latifolia* under the water circulation effects. However, maximum Fe accumulation was observed in *Typha latifolia* roots (1460 µg/gm DW) under intermittent water circulation. Shoot accumulation for Fe was reported to be maximum in *Hydrilla verticellata* (1456.2 µg/gm DW). Overall, it was observed that plants under the mixed culture with intermittent circulation of water accumulated greater metal ions than those under non-circulating effects.

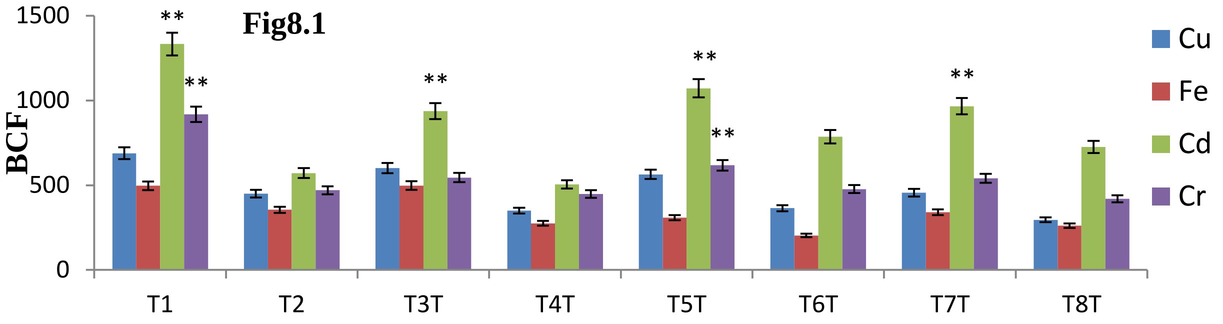
Table 8.2. Accumulation of Cd and Cr (µg/gm dw) by *Typha latifolia Pistia stratiotes* and *Hydrilla verticellata* under different culture treatments (n=6±S.D.)

	Cd (µg/gm dw)		Cr (µg/gm dw)	
Treatments	Root	Shoot	Root	Shoot
T1	1696.6±6.65	1636±10.14	951.98±1.22	1345.44±1.4
T2	693.33±5.85	736±10.14	442.11±7.05	754.32±3.54
T3P	1984.9±9.81	2060.2±4.06	1142.1±9.32	1121.66±5.11
T3T	984.95±9.81	1360.2±4.06	642.1±9.32	721.66±5.11
T4P	1250.3±6.25	1065.8±10.04	766.7±8.1	585.43±7.65
T4T	626.6±55.98	636±10.14	342.11±7.05	554.32±3.54
T5H	722.53±6.27	1422.6±7.32	845.94±3.44	1217.22±4.54
T5T	1407.07±6.04	1272.09±4.86	851.2±6.63	692.03±6.15
T6H	623.2±6.2	1025.96±4.55	615.94±3.44	823.5±5.12
T6T	1105.85±2.67	861.8±9.95	577.8±3.9	615.03±3.84
T7P	918.82±8.4	984.94±8.26	573.76±8.84	561.76±6.64
T7H	523.06±5.46	970.3±7.64	671.72±7.4	1049.3±7.53
T7T	1250.3±6.25	1165.86±10.04	766.7±8.16	585.43±7.65
T8P	628.48±3.55	714.943±3.05	457.1±8.18	555.1±3.89
T8H	419.73±7.67	721.63±5.56	571.72±7.46	877.3±5.59
T8T	850.33±6.25	965.86±10.04	566.7±8.16	485.43±7.65

Cd was observed to be highest accumulated by *Pistia stratiotes* roots (1984.9 µg/gm DW) and shoot (2060.2 µg/gm DW) under water circulation effects. It was followed by to be greater accumulated by *Typha latifolia* in both root and shoot parts (1696.6 µg/gm DW) and (1636.0 µg/gm DW), respectively. In case of Cr, maximum accumulation was observed in shoot of *Typha latifolia* (1345.44 µg/gm DW) followed by shoot *Hydrilla verticillata* (1217.22 µg/gm DW) in mixed cultures under water circulation.

8.3.2. Bioconcentration factor (BCF) and Translocation factor (TF) of *Typha latifolia* under different cultures and water circulation effects

The BCF and TF of *Typha latifolia* under different treatment conditions have been presented in figures 8.1 and 8.2, respectively. Significant differences were observed for BCF of *Typha latifolia* under different plant combinations. BCF around 1400 for Cd was observed to be highest for *T. latifolia* in monoculture under intermittent water circulation effect. It was followed by 900 for Cr. Lowest BCF was observed for Fe and Cr for *Typha latifolia* under different cultures.



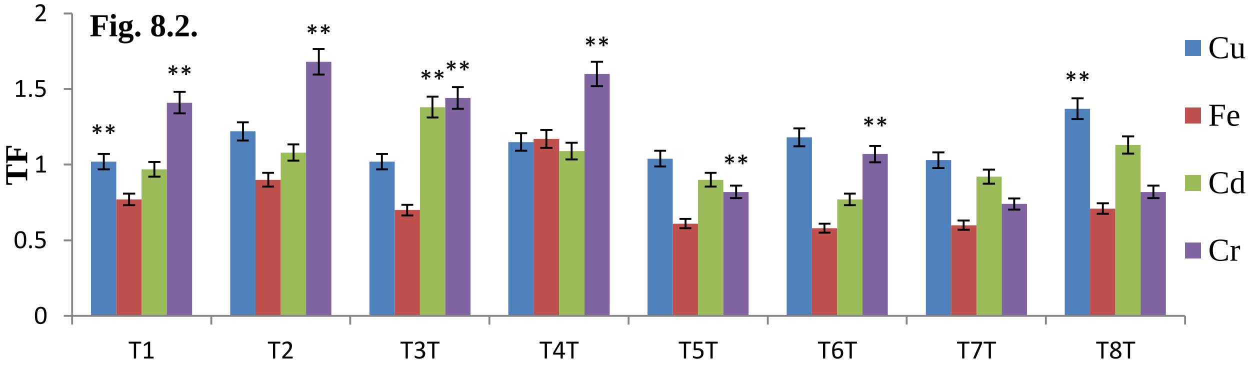


Fig. 8.1 and 8.2. BCF and TF of *Typha latifolia* for Cu, Fe, Cd and Cr in different treatment. **represents significant results.

The translocation factor of *Typha latifolia* for metals is presented in figure 8.2. Highest TF was observed for Cr followed by Cd and Cu under various plant cultures in different treatments. Fe and observed to be least translocated in all the treatments.

8.3.3. Changes in the levels of pH, nitrate, nitrite phosphate and total phosphorus of the developed vermicompost on different days

The vermicomposting of the contaminated plant biomass under different trails was very effective in enhancing the nutritional status of developed vermicompost towards the end of the experiment. Except for control treatment significant changes in the observed chemical characteristics were seen at the maturity of compost. The changes in the pH of the plant biomass subjected to vermicomposting are presented in Fig.8.3. Significant differences were observed for pH changes among trails on different days ($p<0.05$). Except for the control treatment, pH of all trails increased during the vermicomposting period. Highest pH (>8) was observed for trails 1 and 4. The nitrate content also showed significant differences among all trails, except control. Highest nitrate content was observed in trail 6 and 4 towards the end.

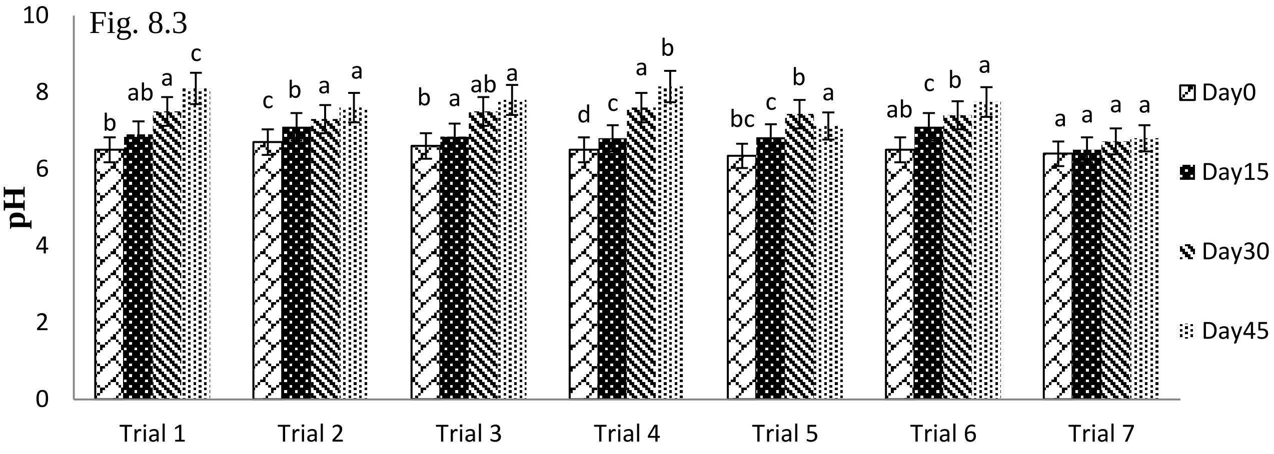


Fig. 8.3. Changes in pH of different plant biomass trails during vermicomposting

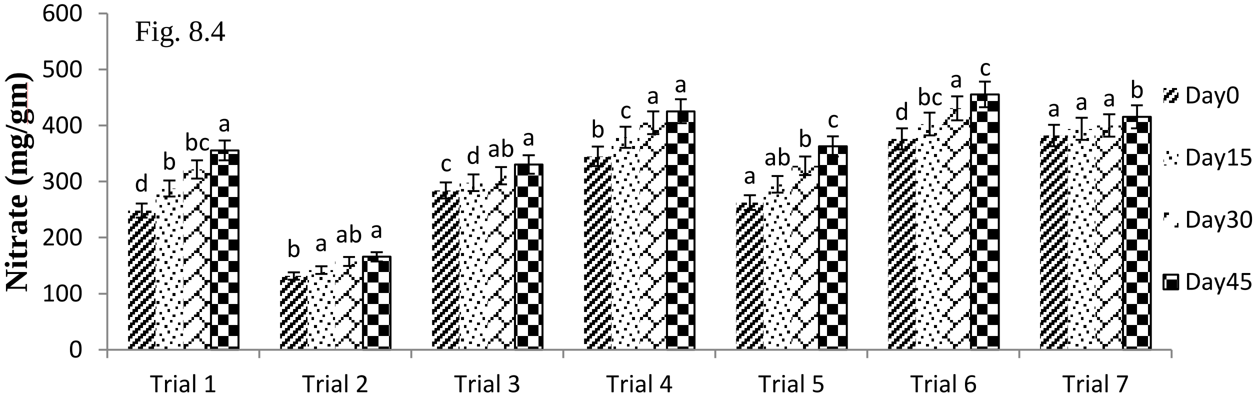


Fig. 8.4. Changes in nitrate content of different plant biomass trails during vermicomposting

The changes in the levels of nitrite, phosphate and total phosphorus of different trails are depicted in figures 8.5, 8.6 and 8.7, respectively. Nitrite content increased with time of composting. Highest increase was observed in trail 6 and 4. Similarly, phosphate content also increased in different trails. Significant differences were observed for total phosphorus concentrations among different days of vermicomposting. Total phosphorus was also reported highest in trail 6.

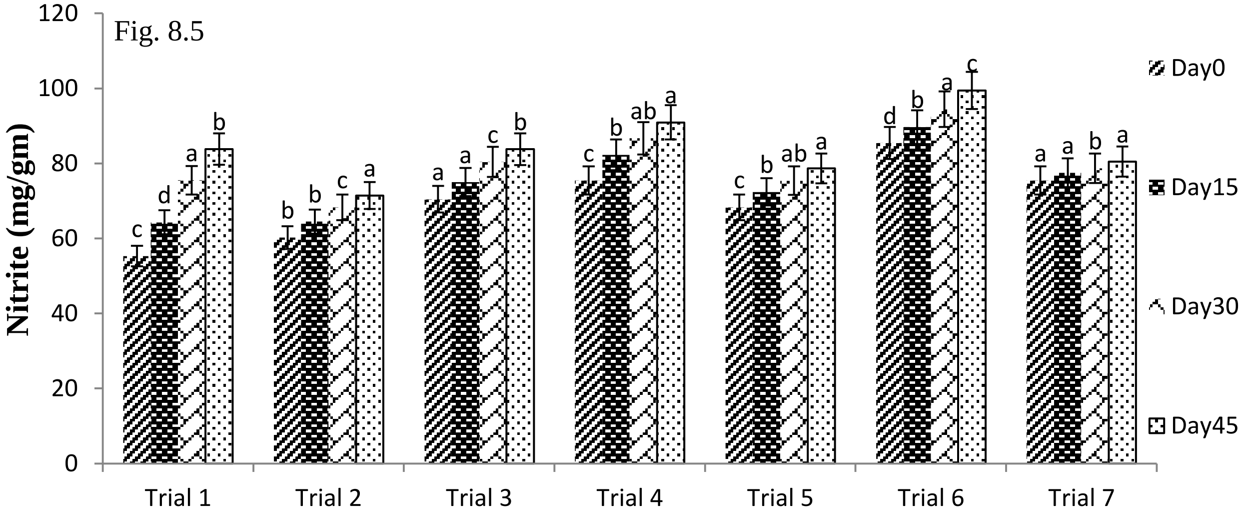


Fig. 8.5. Changes in nitrite content of different plant biomass trails during vermicomposting

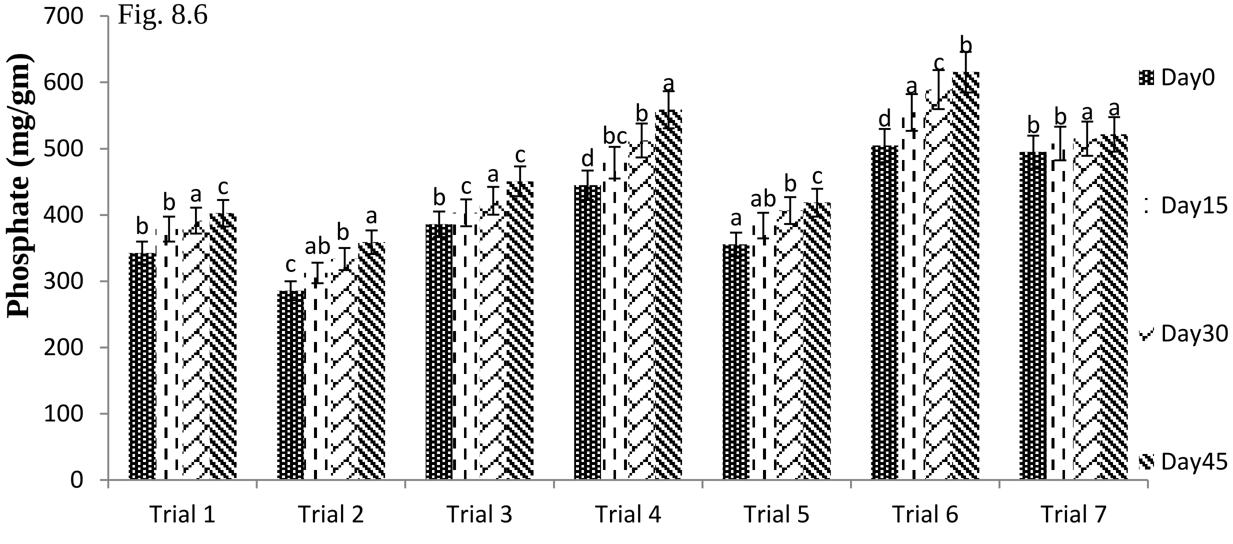


Fig. 8.6. Changes in phosphate content of different plant biomass trails during vermicomposting

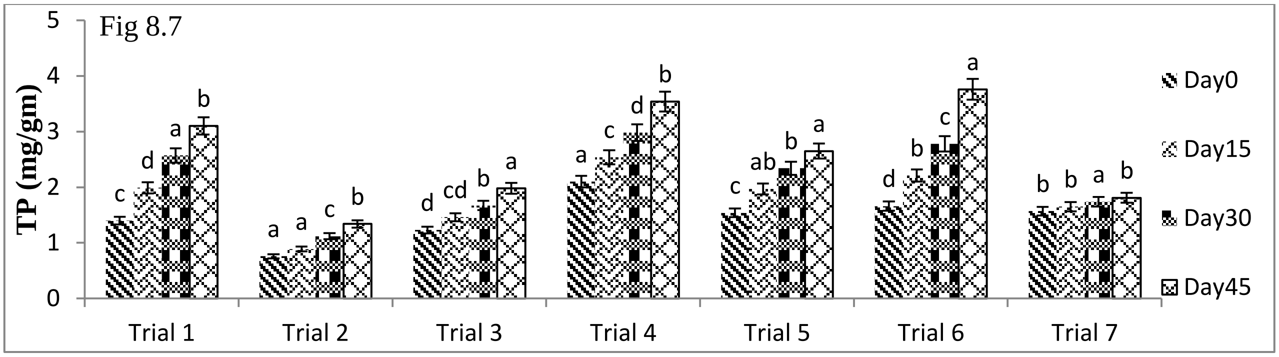


Fig. 8.7. Changes in total phosphorus of different plant biomass trails during vermicomposting

The changes in the % organic carbon and organic matter of different vermicomposting trails of plant biomass are represented in figures 8.8 and 8.9, respectively. The organic carbon and organic matter content of all trails showed significant differences between different days of composting ($p<0.05$). A decrease in both organic carbon and organic matter content was observed for all trails.

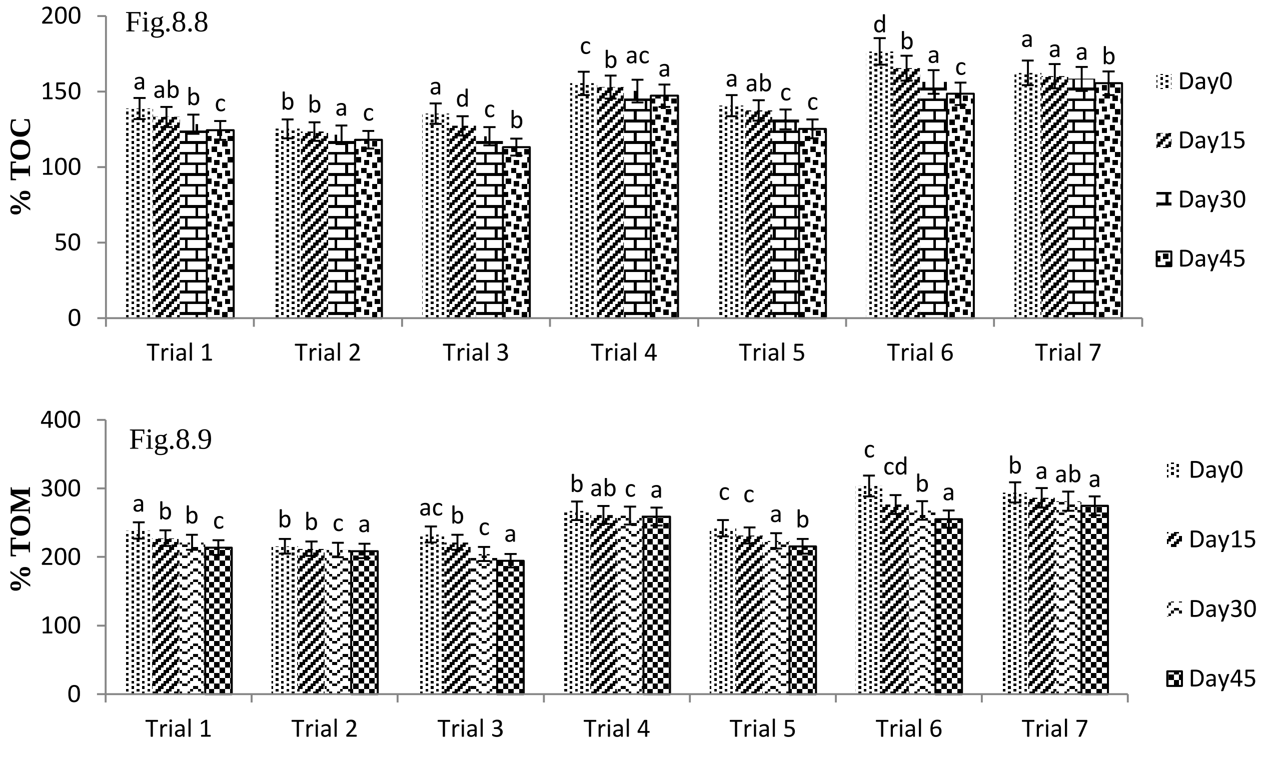


Fig. 8.8 and 8.9. Changes in % organic carbon and organic matter of different plant biomass trails during vermicomposting

8.3.4. Heavy metal speciation during vermicomposting of plant biomass.

Table 8.3, 8.4, 8.5 and 8.6 illustrates the concentration of Cu, Fe, Cd and Cr as different chemical species in selected trails during the composting process. Except for control trail, significant differences were observed for among the Exchangeable, Carbonate, Reducible, Organically Bound and Residual forms of metals among different trails ($p<0.05$). Cu was converted into organically bound and residual fractions in different trails. Among trails 1, 4 and 6 greater residual fractions of Cu were observed in the final metal content after vermicomposting. The initial exchangeable and carbonate fractions got significantly reduced and the final content of metals was found to be containing residual and organically bound fractions. Similarly, Fe was also converted into less mobile fractions like reducible and residual in the final developed compost. Trail 4 and 6 were observed for greater Fe contents in case of reducible and residual fractions.

Table 8.3. Speciation of Cu (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initial Conc.	0.72 ± 0.02^{a}	0.97 ± 0.03^{ab}	2.1 ± 0.051^{c}	2.54 ± 0.03^{b}	5.56 ± 0.05^{c}
	Final Conc.	0.31 ± 0.01^{d}	1.32 ± 0.05^{c}	1.93 ± 0.13^{ab}	4.43 ± 0.05^{b}	7.33 ± 0.05^{a}
Trail 2	Initial Conc.	0.72 ± 0.02^{c}	0.94 ± 0.02^{c}	1.12 ± 0.01^{b}	2.2 ± 0.02^{ab}	5.48 ± 0.04^{a}
	Final Conc.	0.68 ± 0.02^{d}	1.12 ± 0.05^{a}	1.21 ± 0.01^{a}	1.67 ± 0.03^{b}	6.1 ± 0.1^{c}
Trail 3	Initial Conc.	1.76 ± 0.27^{c}	0.68 ± 0.01^{c}	1.21 ± 0.03^{d}	1.98 ± 0.05^{b}	4.87 ± 0.03^{a}
	Final Conc.	0.32 ± 0.01^{a}	1.12 ± 0.06^{a}	2.34 ± 0.1^{b}	2.98 ± 0.07^{b}	7.98 ± 0.1^{c}
Trail 4	Initial Conc.	1.45 ± 0.11^{c}	1.87 ± 0.04^{b}	2.45 ± 0.07^{bc}	4.3 ± 0.04^{a}	6.88 ± 0.06^{a}
	Final Conc.	1.12 ± 0.06^{e}	2.98 ± 0.11^{d}	5.78 ± 0.12^{a}	6.11 ± 0.05^{c}	9.12 ± 0.11^{b}
Trail 5	Initial Conc.	1.89 ± 0.08d	1.56 ± 0.1b^{c}	2.11 ± 0.03^{c}	2.76 ± 0.01^{b}	4.45 ± 0.04^{a}
	Final Conc.	1.12 ± 0.05^{a}	2.34 ± 0.13^{a}	3.31 ± 0.05^{a}	$5.72\pm0.02_{b}$	6.74 ± 0.05^{b}
Trail 6	Initial Conc.	2.11 ± 0.12^{a}	2.87 ± 0.11^{b}	3.65 ± 0.06^{b}	5.78 ± 0.03^{c}	8.21 ± 0.04^{a}
	Final Conc.	0.87 ± 0.02^{c}	3.89 ± 0.21^{d}	5.87 ± 0.11^{b}	7.98 ± 0.07^{a}	12.56 ± 0.12^{c}
Trail 7	Initial Conc.	1.32 ± 0.02^{b}	1.62 ± 0.15^{c}	2.05 ± 0.03^{a}	3.83 ± 0.04^{a}	7.62 ± 0.04^{ac}
	Final Conc.	0.92 ± 0.01^{a}	1.68 ± 0.12^{a}	3.18 ± 0.04^{a}	3.11 ± 0.05^{a}	6.12 ± 0.03^{b}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Table 8.4. Speciation of Fe (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initial Conc.	0.51±0.01^{b}	0.82±0.02^{c}	1.87±0.1^{d}	2.54±0.2^{a}	4.53±0.15^{a}
	Final Conc.	0.23±0.02^{c}	1.17±0.04^{d}	2.29±0.12^{ab}	3.56±0.23^{b}	6.44±0.17^{a}
Trail 2	Initial Conc.	0.81±0.02^{e}	1.15±0.03^{b}	1.55±0.1^{c}	1.84±0.12^{d}	6.29±0.16^{a}
	Final Conc.	1.14±0.024^{a}	1.54±0.02^{ab}	1.33±0.04^{b}	2.11±0.1^{c}	6.85±0.13^{d}
Trail 3	Initial Conc.	1.81±0.021^{b}	0.73±0.01^{c}	1.43±0.04^{a}	2.15±0.1^{ab}	5.28±0.1^{a}
	Final Conc.	0.21±0.01^{d}	1.33±0.04^{e}	2.35±0.13^{bc}	3.11±0.21^{b}	8.24±0.21^{a}
Trail 4	Initial Conc.	2.19±0.1^{a}	2.33±0.12^{ab}	4.8±0.12^{c}	5.8±0.23^{b}	10.5±0.24^{c}
	Final Conc.	1.31±0.34^{bc}	3.45±0.11^{a}	6.72±0.15^{a}	5.92±0.25^{a}	11.51±0.3^{b}
Trail 5	Initial Conc.	1.72±0.12^{e}	1.34±0.12^{c}	1.43±0.11^{d}	1.94±0.1^{ab}	3.32±0.12^{a}
	Final Conc.	1.42±0.05^{cd}	1.94±0.21^{d}	2.31±0.12^{b}	3.42±0.14^{c}	4.17±0.15^{a}
Trail 6	Initial Conc.	1.63±0.04^{c}	1.82±0.14^{a}	3.21±0.11^{a}	4.55±0.16^{b}	7.33±0.14^{a}
	Final Conc.	0.84±0.01^{d}	1.08±0.05^{cd}	3.05±0.12^{c}	6.48±0.11^{b}	12.43±0.11^{a}
Trail 7	Initial Conc.	1.98±0.1^{a}	2.26±0.13^{a}	4.75±0.13^{a}	5.88±0.12^{b}	8.95±0.11^{a}
	Final Conc.	2.75±0.14^{a}	2.36±0.12^{a}	3.45±0.12^{a}	5.01±0.11^{a}	7.88±0.13^{b}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Table 8.5. Speciation of Cd (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initial Conc.	0.21±0.01^{d}	0.35±0.01^{c}	1.12±0.04^{bc}	2.01±0.04^{c}	3.23±0.06^{a}
	Final Conc.	0.08±0.01^{b}	0.54±0.02^{b}	1.04±0.03^{c}	3.51±0.07^{c}	5.14±0.1^{a}
Trail 2	Initial Conc.	0.42±0.02^{a}	0.76±0.022^{a}	1.21±0.04^{c}	1.02±0.02^{b}	5.92±0.1^{d}
	Final Conc.	0.69±0.02^{c}	2.48±0.04^{cd}	2.67±0.1^{b}	3.61±0.05^{b}	5.35±0.07^{a}
Trail 3	Initial Conc.	0.32±0.02^{a}	0.43±0.03^{b}	1.37±0.05^{d}	2.85±0.01^{c}	4.78±0.04^{e}
	Final Conc.	0.16±0.01^{d}	1.33±0.042^{c}	3.55±0.11^{b}	5.74±0.1^{ab}	6.37±0.05^{a}
Trail 4	Initial Conc.	1.09±0.03^{c}	1.33±0.032^{c}	3.86±0.1^{b}	4.58±0.06^{b}	6.5±0.07^{a}
	Final Conc.	0.65±0.01^{d}	2.25±0.023^{b}	4.12±0.07^{c}	6.88±0.12^{a}	8.76±0.06^{ac}
Trail 5	Initial Conc.	0.53±0.01^{c}	0.76±0.02^{c}	1.06±0.03^{c}	2.14±0.03^{b}	2.2±0.01^{a}
	Final Conc.	0.2±0.03^{c}	1.32±0.05^{b}	1.98±0.05^{b}	2.64±0.04^{ab}	3.43±0.02^{a}
Trail 6	Initial Conc.	1.13±0.05^{b}	1.43±0.03^{b}	2.27±0.08^{c}	3.21±0.05^{d}	5.23±0.03^{a}
	Final Conc.	0.41±0.03^{e}	0.78±0.01^{d}	2.5±0.05^{b}	8.98±0.13^{c}	9.13±0.05^{a}
Trail 7	Initial Conc.	0.87±0.02^{a}	1.06±0.03^{a}	2.15±0.03^{b}	4.91±0.03^{b}	5.95±0.06^{a}
	Final Conc.	1.05±0.04^{a}	0.89±0.02^{a}	3.49±0.1^{b}	3.01±0.06^{c}	5.58±0.05^{a}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Chemical speciation of Cd (mg/kg) during vermicomposting of plant biomass is presented in table 8.5. Cd fractions were also found to be organically bound and reducible in nature in the final compost. Highest reducible fractions of Cd were reported in trails 6, 4 and 3. Organically bound fractions of Cd were also observed highest in the same trails.

Table 8.6. Speciation of Cr (mg/kg) during vermicomposting of plant biomass

		Exchangeable	Carbonate	Reducible	Organically Bound	Residual
Trail 1	Initail Conc.	0.17 ± 0.01^{b}	0.22 ± 0.01^{b}	0.92 ± 0.03^{b}	1.61 ± 0.03^{c}	2.39 ± 0.02^{ac}
	Final Conc.	0.1 ± 0.001^{c}	0.38 ± 0.01^{c}	0.84 ± 0.02^{b}	2.61 ± 0.05^{a}	4.4 ± 0.05^{a}
Trail 2	Initail Conc.	0.23 ± 0.01^{b}	0.62 ± 0.02^{cd}	1.01 ± 0.03^{d}	1.32 ± 0.03^{c}	3.52 ± 0.04^{a}
	Final Conc.	0.45 ± 0.02^{e}	1.83 ± 0.04^{de}	2.03 ± 0.05^{c}	2.71 ± 0.06^{b}	4.21 ± 0.08^{ab}
Trail 3	Initail Conc.	0.21 ± 0.02^{c}	0.31 ± 0.01^{c}	1.07 ± 0.03^{b}	1.85 ± 0.04^{ab}	3.28 ± 0.02^{a}
	Final Conc.	0.06 ± 0.01^{d}	1.56 ± 0.03^{c}	2.95 ± 0.05^{ab}	4.24 ± 0.05^{b}	5.98 ± 0.04^{a}
Trail 4	Initail Conc.	0.93 ± 0.03^{c}	1.57 ± 0.03^{d}	4.39 ± 0.06^{ab}	4.89 ± 0.06^{b}	5.35 ± 0.05^{a}
	Final Conc.	0.15 ± 0.01^{d}	3.03 ± 0.06^{cd}	3.87 ± 0.04^{b}	5.28 ± 0.07^{c}	9.26 ± 0.1^{a}
Trail 5	Initail Conc.	0.41 ± 0.02^{ab}	0.53 ± 0.03^{a}	0.94 ± 0.02^{c}	1.83 ± 0.02^{b}	2.42 ± 0.03^{c}
	Final Conc.	0.23 ± 0.02^{a}	1.12 ± 0.06^{c}	1.45 ± 0.03^{b}	2.04 ± 0.03^{d}	3.73 ± 0.03^{bc}
Trail 6	Initail Conc.	1.38 ± 0.05^{b}	1.72 ± 0.04^{c}	2.79 ± 0.05^{ac}	3.79 ± 0.05^{a}	6.44 ± 0.05^{a}
	Final Conc.	0.33 ± 0.01^{d}	0.18 ± 0.01^{b}	1.05 ± 0.02^{c}	7.65 ± 0.06^{a}	12.87 ± 0.14^{a}
Trail 7	Initail Conc.	0.77 ± 0.02^{c}	1.42 ± 0.02^{bc}	3.95 ± 0.05^{b}	4.18 ± 0.05^{a}	5.32 ± 0.04^{ab}
	Final Conc.	0.65 ± 0.03^{a}	1.79 ± 0.03^{b}	2.73 ± 0.01^{b}	3.81 ± 0.03^{c}	4.88 ± 0.05^{a}

Different letters represent the significant statistical differences among different metal forms ($p<0.05$)

Table 8.6 represents the chemical speciation of Cr during the initial and final stages of vermicomposting of contaminated biomass of selected plants. Significant differences were reported for all fractions of Cr in selected trails during the final compost analysis ($p<0.05$). The highest reducible fractions in case of Cr were observed for trails 2 and 6. Reducible fractions in the final compost were also dominant in Cr speciation after organically bound fractions. Trails 2 and 3 had increased reducible fractions of Cr in the final compost. Final concentration of Exchangeable fractions of Cr was found to be lesser than the initial contents in all the trails of composting.

8.4. Discussion

Data obtained from the present research showed that *T. latifolia* performed better in accumulation of metals from monoculture than other cultures, under the same operational conditions and over the same length of time. This indicates that removal efficiencies differ according to the plant species in the treatment system; therefore, the selection of plant species is a key component to the success of CWs designed for wastewater treatment (Turker, 2014). This study revealed a similar allocation patterns of metals in the emergent macrophyte species as the most wetland plants having higher concentrations of metals in their belowground biomass than in their shoot tissues (Mays and Edwards 2007; Vymazal et al. 2007). Mixed consortia of plants resulted in significant variations in metals accumulation.

pH is a parameter which greatly affects the composting process. The pH values during the vermicomposting were within the optimal range for the development of bacteria 6.0–7.5 and fungi 5.5–8.0 (Amir et al., 2005). The pH value of a solution strongly influences the speciation and bio-sorption availability of the heavy metals (Wang et al., 2009). During the composting process, carbon dioxide is emitted from the composting mass as a metabolic end product. Thus the total organic carbon content of the composting mass decreases as composting proceeds (Singh et al., 2009). Similar results pertaining to the current study were observed for different trails of composting. Humic substances have a capacity to interact with metal ions and the ability to buffer pH and to act as a potential source of nutrients for plants (Amir et al., 2005; Singh et al., 2009). The increase in the nitrate, nitrite, phosphate and total phosphorus content of the final developed compost adds to the nutritional value of compost.

Chemical speciation of metals is the process of identifying different species or forms in a material (Cai et al., 2007). The determination of total heavy metal content does not provide useful information about the threat of bioavailability, which depends on their chemical forms. Chemical speciation of heavy metals during composting is a useful technique for determining the chemical forms in which these metals are present (Hsu and Lo, 2001). The reduction in the exchangeable and carbonate fractions of selected metals by vermicomposting was effective in reducing the toxicity of developed compost towards the end of experiment. Earthworms are capable of reducing possible toxic effects of heavy metals by utilizing them for physiological metabolism (Jain et al., 2004).

8.5. Conclusions

From the current study it is concluded that using multiple plants in consortia for water treatment is effective in removing the metals. The metals containing biomass of plants used for production of vermicompost showed enhanced nutritional qualities at the termination of composting process. Metal availability was also reduced to a large extent. Increase in the pH during the vermicomposting was most important factor for reducing bioavailability of heavy metals. 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.

Chapter 9

Summary and Conclusion

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 physicochemical 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.

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Publications

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- **Abdul Barey Shah**, U N Rai, Rana Pratap Singh **(2015)**. Correlations between some hazardous inorganic pollutants in the Gomti River and their accumulation in selected macrophytes under aquatic ecosystem. ***Bulletin of Environmental Contamination and Toxicology***. DOI 10.1007/s00128-015-1546-0. 94:783–790 [**Impact Factor ISI= 1.216**]

- **Abdul Barey Shah,** Rana Pratap Singh **(2015)**. Monitoring of hazardous inorganic pollutants and heavy metals in potable water at the source of supply and consumers end of a tropical urban municipality. ***International Journal of Environmental Research*** **(Accepted)** [**Impact Factor ISI= 1.818**]

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- **Abdul Barey Shah** and Shazia Majeed Sofi **(2014)**. A Study of Seasonal Variation in Biomass of three Medicinal Plants (*Malva Neglecta*, *Rumex Patientia* and *Taraxacum Officinale*) of Kashmir Valley, India. ***International Research Journal of Environment Sciences*** 3(1), 38-42.

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- **Abdul Barey Shah**, U N Rai, Rana Pratap Singh **(2015)**. Intermittent circulation of multi-metal contaminated water for enhancing the phytoremediation efficacy of *Pistia stratiotes* and *Hydrilla Verticellata* under mono and mixed culture: Mechanism of metal sorption by SEM and FTIR studies**. Ecological Engineering (Communicated)** [**Impact Factor ISI= 3.405**]

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Correlations Between Some Hazardous Inorganic Pollutants in the Gomti River and Their Accumulation in Selected Macrophytes Under Aquatic Ecosystem

Abdul Barey Shah[1] · U. N. Rai[2] · Rana Pratap Singh[1]

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Abstract Water quality of the Gomti River and phytoremediation potential of native macrophytes dwelling therein at six different sites were evaluated. River water showed high biochemical oxygen demand, chemical oxygen demand, nitrate, ammonium and phosphate (12.84, 77.94, 36.88, 6.04 and 2.25 mg L^{-1}, respectively). Gomti water was found to be contaminated with different metals like Fe, Cd, Cu, Cr and Pb (5.54, 1.05, 3.74, 2.57 and 0.73 mg L^{-1}, respectively). Macrophytes growing in the river accumulated considerable amounts of Fe, Cd, Cu, Cr and Pb in different parts. Among the studied plants, *Eichhornia crassipes* showed maximum remediation potential for Fe, Cd and Pb; *Jussiaea repens* for Cr; and *Pistia stratiotes* for Cd. However, in *Typha latifolia*, Cu accumulation was maximum. Except for Fe, translocation factor of *E. crassipes*, *P. stratiotes*, *Hydrilla verticellata* and *T. latifolia* was >1 for the studied metals, showing their potential to accumulate multiple metals in different plant parts.

Keywords Bioaccumulation · Metals · Phytoremediation · Translocation factor

In urban areas, deterioration of water quality of rivers like the Gomti is strongly related to the increasing developmental activities in the watershed, such as changing land use pattern, increased discharge of untreated municipal and industrial wastewater, and runoff from nearby agricultural fields (Rai et al. 2012). Discharge of untreated wastewater containing metals of variable toxicity into rivers poses a serious threat not only to the aquatic ecosystem, but also to human health (Rai 2010; Sun et al. 2014). Consumption of water contaminated with metals may lead to their chronic accumulation in the kidneys, liver and bones of humans, resulting in disruption of metabolic activities, which can also lead to cardiovascular, neurological and renal diseases (Jarup 2003; Johri et al. 2010). Other inorganic pollutants like nitrogenous ions (especially NO_2^-) present in water can combine with organic pollutants to produce cancer causing nitrosyls in human beings. Various aquatic macrophytes (floating, submerged, rooted, and emergent) growing in river courses have shown the potential to accumulate certain toxic pollutants inside their tissues and are used to monitor pollution levels (Souza et al. 2013). These plant potentials have emerged as a major area of phytotechnological studies and have been evaluated for phytoremediation potential for the removal of toxic pollutants from contaminated water and soil (Bauddh and Singh 2012; Chiranjeevi et al. 2013). Macrophyte based treatment systems can be used by developing countries for recycling of wastewater and treatment of potable water, especially those contaminated with metals (Khan et al. 2009; Rahman and Hasegawa 2011).

Some monitoring studies on the Gomti River report variable, but alarming, contamination of water with certain inorganic and organic pollutants (Agarwal et al. 2007; Lohani et al. 2008). However, in-stream macrophytes have not been investigated for their removal efficiency for Fe, Cu, Cd, Cr and Pb, the major metal contaminants of industrial, municipal and agricultural origin during different seasons. The present study is aimed at monitoring water

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✉ Rana Pratap Singh
ranapratap@bbau.ac.in

[1] Department of Environmental Science, Babasaheb Bhimrao Ambedkar (Central) University, Lucknow 226025, India

[2] Plant Ecology and Environmental Science Division, CSIR-NBRI, Rana Pratap Marg, Lucknow 226001, India

quality of the Gomti River from upstream (Gaughat) to downstream (Pipraghat) of Lucknow and to evaluate the potential of endogenous mixed macroflora as pollution biomonitors for phytoremediation of multiple pollutants in a semi-arid, urban tropical aquatic ecosystem.

Materials and Methods

The Gomti River, a freshwater ecosystem and a major tributary of the Ganges in India, flowing through the Lucknow city, is the only source of municipal water supply for the city. Various drains situated between Gaughat (upstream of Lucknow) and Pipraghat (downstream of Lucknow) discharge untreated sewage, including municipal and industrial wastewater into the Gomti River. Six sites along the bank of the Gomti were selected for water and plant sample collection from Gaughat (upstream) up to Pipraghat (downstream). Gaughat and Pucca Pull were categorized as upstream sites, while Hanuman Setu, Nishatganj, Gomti Barrage and Pipraghat sites were downstream. Triplicate water samples were collected in acid soaked 2 L, polyethylene bottles from the study sites during the pre-monsoon (April, May, June) and monsoon (July, August and September) period of 2013 and brought to the laboratory for further analysis. pH and electrical conductivity were determined onsite using portable digital pH and conductivity meters. Physicochemical parameters were determined using standard methods for the examination of water and waste water (APHA 2005). Aquatic plant samples naturally occurring at the study sites were randomly collected at the same time as water sampling. Whole plants including roots and shoots were carefully harvested at sampling sites. Plants at the selected sites were *Eichhornia crassipes*, *Pistia stratiotes*, *Hydrilla verticellata*, *Jussiaea repens*, *Typha latifolia*, *Vallesnaria spiralis* and *Polygonum glabrum*. The plants were put and sealed in air tight polyethylene bags and transported to the laboratory and kept there at 4°C. Thoroughly washed root and shoot plant samples were separated and oven dried at 90°C to a constant weight and metals (Fe, Cu, Cd, Cr and Pb) in the plant parts were determined after acid digestion of dry samples with an acid mixture (9:4 nitric acid:perchloric acid) at about 100°C. Metal concentration was determined by atomic absorption spectrophotometer (AAS 240 FS, Varian). Analytical data quality of metals was ensured through repeated analysis ($n = 3$) of EPA quality control in samples. The translocation factor (the ratio of metals in shoot vs. root of plants) was calculated by the formula of Padmavathiamma and Li (2007).

Statistical analysis of data by one way analysis of variance (ANOVA) and Duncan multiple range tests were performed to determine the significance of differences among the mean values using SPSS (Version 16). Relationships between physicochemical parameters and metal concentrations in Gomti River water were studied by Pearson linear correlation method.

Results and Discussion

The physicochemical properties of water at sample sites during pre-monsoon and monsoon periods are represented in Table 1. Statistically significant differences ($p < 0.05$) were observed for physicochemical characteristics of Gomti River water at selected sites and periods. Irrespective of sites and periods, pH of Gomti water at Lucknow is alkaline. Maximum pH (8.66) was observed during the pre-monsoon period downstream at Gomti Barrage, while electrical conductivity (EC) was highest (604.33 μs cm^{-1}) during pre-monsoon at Pipraghat. Dissolved oxygen (DO) was lowest at Pipraghat (3.32 mg L^{-1}) during the pre-monsoon period, while the maximum biochemical oxygen demand (BOD) was recorded at Pipraghat (12.84 mg L^{-1}), depicting high organic pollution at the site. Chemical oxygen demand (COD) showed a maximum value (77.94 mg L^{-1}) at Pipraghat during the monsoon period. Among the inorganic nitrogenous compounds, nitrate (NO_3) concentration reached a maximum value (36.88 mg L^{-1}) downstream at Pipraghat during the monsoon period, while as highest nitrite (NO_2) concentration (0.1 mg L^{-1}) was recorded upstream at Pucca Pull during the pre-monsoon period.

Ammonium levels in river water were highest (6.04 mg L^{-1}) upstream at Gaughat during the monsoon period. Phosphate was highest downstream at Pipraghat (2.25 mg L^{-1}) during the rainy season. Domestic wastewater containing detergents and leaching of chemical fertilizers from terrestrial systems after heavy rainfall leads to inorganic nutrient loading of nutrients into rivers (Bellos and Sawidis 2005; Rai and Tripathi 2009). Gomti River water at Lucknow showed varying concentration of five metals investigated. Mean concentrations of different metals (Fe, Cd, Cu, Cr, and Pb) in Gomti water are presented in Table 2. Metal content in river water was in the order of Fe > Cu > Cr > Cd > Pb. Maximum concentration of Fe (5.54 mg L^{-1}), Cu (3.74 mg L^{-1}), Cr (2.57 mg L^{-1}), Cd (1.05 mg L^{-1}) and Pb (0.73 mg L^{-1}) were recorded during the pre-monsoon period downstream at Pipraghat. The concentration of all five metals in the Gomti River was higher than the critical ranges stated for drinking water standards (EPA 2009). Compared to the monsoon period, metals concentration was higher during the pre-monsoon period. The lesser values during the monsoon period could be due to a dilution effect. Since metals tend to settle and accumulate in the sediments of

Table 1 Physicochemical characteristics of water samples (n = 3, mean ± SD) collected from selected sites of the Gomti River during pre-monsoon and monsoon seasons in India

Seasons	Sites	pH	EC (µs cm^{-1})	DO (mg L^{-1})	BOD (mg L^{-1})	COD (mg L^{-1})	NO_3 (mg L^{-1})	NO_2 (mg L^{-1})	NH_4 (mg L^{-1})	PO_4 (mg L^{-1})
Pre-monsoon	I	$8.23^{a} \pm 0.11$	$562.6^{b} \pm 6.42$	$7.64^{f} \pm 0.17$	$3.16^{a} \pm 0.08$	$14.5^{a} \pm 0.52$	$20.74^{a,b} \pm 0.61$	$0.088^{b} \pm 0.004$	$5.75^{c} \pm 0.34$	$0.70^{a} \pm 0.07$
	II	$8.53^{b} \pm 0.05$	$541.6^{a} \pm 12.58$	$5.89^{e} \pm 0.18$	$6.96^{b} \pm 0.16$	$29.67^{b} \pm 1.43$	$24.36^{c} \pm 2.08$	$0.1^{c} \pm 0.004$	$4.05^{a} \pm 0.57$	$0.90^{b} \pm 0.07$
	III	$8.26^{a} \pm 0.05$	$556^{a,b} \pm 5.29$	$4.62^{b} \pm 0.27$	$8.25^{c} \pm 0.26$	$40.83^{c} \pm 0.63$	$22.41^{b,c} \pm 0.96$	$0.082^{b} \pm 0.003$	$4.4^{a} \pm 0.17$	$0.87^{a,b} \pm 0.04$
	IV	$8.13^{a} \pm 0.05$	$569^{b} \pm 2.64$	$4.94^{c} \pm 0.04$	$9.09^{d} \pm 0.065$	$43.55^{c} \pm 0.64$	$19.50^{a} \pm 0.76$	$0.048^{a} \pm 0.006$	$4.37^{a} \pm 0.04$	$1.02^{b,c} \pm 0.085$
	V	$8.66^{c} \pm 0.05$	$588^{c} \pm 6.55$	$5.27^{d} \pm 0.08$	$7.34^{e} \pm 0.21$	$47.03^{d} \pm 0.23$	$29.08^{d} \pm 0.67$	$0.087^{b} \pm 0.004$	$5.15^{b} \pm 0.04$	$1.12^{c} \pm 0.01$
	VI	$8.13^{a} \pm 0.05$	$604.3^{c} \pm 18.14$	$3.32^{a} \pm 0.12$	$12.84^{f} \pm 0.23$	$57.97^{e} \pm 3.36$	$35.21^{e} \pm 1.9$	$0.099^{c} \pm 0.006$	$5.73^{c} \pm 0.14$	$2.05^{d} \pm 0.21$
Monsoon	I	$7.21^{a} \pm 0.1$	$362.66^{a} \pm 6.42$	$6.14^{d} \pm 0.14$	$4.22^{a} \pm 0.03$	$25.11^{a} \pm 0.48$	$22.76^{b} \pm 1.57$	$0.074^{c} \pm 0.001$	$6.04^{d} \pm 0.09$	$1.42^{a,b} \pm 0.09$
	II	$7.46^{c} \pm 0.05$	$375^{a} \pm 57.66$	$4.99^{c} \pm 0.27$	$5.98^{d} \pm 0.14$	$40.25^{b} \pm 0.86$	$18.1^{a} \pm 1.47$	$0.084^{d} \pm 0.003$	$4.14^{b} \pm 0.05$	$1.23^{a} \pm 0.06$
	III	$7.42^{c} \pm 0.1$	$455.6^{c} \pm 17.21$	$3.68^{a} \pm 0.58$	$6.12^{d} \pm 0.16$	$52.63^{c} \pm 2.34$	$19.94^{a} \pm 1.2$	$0.064^{b} \pm 0.003$	$5.59^{c} \pm 0.19$	$1.84^{b,c} \pm 0.06$
	IV	$7.13^{a} \pm 0.05$	$352.33^{a} \pm 4.5$	$4.27^{b} \pm 0.06$	$5.54^{c} \pm 0.12$	$64.16^{d} \pm 2.6$	$23.4^{b} \pm 1.9$	$0.075^{a} \pm 0.002$	$3.31^{a} \pm 0.02$	$1.58^{a,b} \pm 0.07$
	V	$7.6^{c} \pm 0.17$	$464.6^{c} \pm 11.23$	$5.38^{c} \pm 0.11$	$4.63^{b} \pm 0.1$	$74.96^{e} \pm 3.36$	$24.56^{b} \pm 1.4$	$0.045^{d} \pm 0.004$	$5.27^{c} \pm 0.13$	$2.14^{d} \pm 0.51$
	VI	$7.86^{d} \pm 0.09$	$495^{c} \pm 5.35$	$4.21^{b} \pm 0.06$	$7.41^{e} \pm 0.19$	$77.94^{e} \pm 1.53$	$36.88^{c} \pm 0.45$	$0.087^{c} \pm 0.006$	$4.44^{b} \pm 0.35$	$2.25^{c,d} \pm 0.22$

Different letters signify the statistical differences among physicochemical parameters at selected sites during pre-monsoon and monsoon seasons ($p < 0.05$). Site I = Gaughat; Site II = Pucca Pull; Site III = Hanuman Setu; Site IV = Nishatganj; Site V = Gomti Barrage and Site VI = Pipraghat

EC electrical conductivity, *DO* dissolved oxygen, *BOD* biochemical oxygen demand, *COD* chemical oxygen demand, NO_3 nitrate, NO_2 nitrite, NH_4 ammonium, PO_4 phosphate

Table 2 Metal content (mg L^{-1}) of water samples (n = 3, mean ± SD) collected from selected sites of the Gomti River during pre-monsoon and monsoon seasons in India

Sites	Pre-monsoon					Monsoon				
	Fe	Cd	Cu	Cr	Pb	Fe	Cd	Cu	Cr	Pb
I	$1.92^{a} \pm 0.26$	$0.04^{a} \pm 0.003$	$0.14^{a} \pm 0.02$	$0.56^{a} \pm 0.10$	$0.22^{a} \pm 0.008$	$1.71^{a} \pm 0.61$	$0.012^{a} \pm 0.001$	$0.094^{a} \pm 0.09$	$0.4^{a} \pm 0.05$	$0.17^{a,b} \pm 0.02$
II	$2.67^{b} \pm 0.29$	$0.085^{a,b} \pm 0.005$	$0.25^{a} \pm 0.03$	$0.79^{b} \pm 0.04$	$0.32^{b} \pm 0.02$	$2.02^{a} \pm 0.2$	$0.11^{b} \pm 0.004$	$0.118^{a} \pm 0.02$	$0.57^{a} \pm 0.03$	$0.24^{c,d} \pm 0.02$
III	$4.32^{d} \pm 0.06$	$0.14^{b,c} \pm 0.004$	$1.25^{b} \pm 0.16$	$1.08^{c} \pm 0.009$	$0.33^{b} \pm 0.02$	$3.62^{b} \pm 0.16$	$0.14^{b} \pm 0.04$	$1.07^{b} \pm 0.03$	$0.86^{b} \pm 0.05$	$0.2b^{c} \pm 0.003$
IV	$3.75^{c} \pm 0.09$	$0.16^{c} \pm 0.01$	$1.48^{b} \pm 0.24$	$1.25^{c,d} \pm 0.05$	$0.4^{b} \pm 0.07$	$2.99^{c} \pm 0.26$	$0.143^{b} \pm 0.005$	$1.025^{b} \pm 0.01$	$1.02^{b} \pm 0.05$	$0.15^{a} \pm 0.01$
V	$4.16^{d} \pm 0.05$	$0.65^{d} \pm 0.08$	$2.88^{c} \pm 0.25$	$1.35^{d} \pm 0.12$	$0.28^{c} \pm 0.01$	$4.12^{c,d} \pm 0.04$	$0.16^{b} \pm 0.03$	$1.93^{c} \pm 0.38$	$0.92^{b} \pm 0.04$	$0.25^{d} \pm 0.02$
VI	$5.54^{e} \pm 0.26$	$1.05^{e} \pm 0.001$	$3.74^{d} \pm 0.16$	$2.57^{e} \pm 0.16$	$0.73^{d} \pm 0.02$	$4.44^{d} \pm 0.08$	$0.605^{c} \pm 0.05$	$2.64^{d} \pm 0.09$	$1.39^{c} \pm 0.21$	$0.43^{e} \pm 0.03$

Different letters signify the statistical differences among metals at selected sites during pre-monsoon and monsoon seasons ($p < 0.05$). Site I = Gaughat; Site II = Pucca Pull; Site III = Hanuman Setu; Site IV = Nishatganj; Site V = Gomti Barrage and Site VI = Pipraghat rivers, the accumulation and remobilization of metals in river systems are two important mechanisms that regulate their concentration in an aquatic environment (Vardanyan and Ingole 2006; Ishaq and Khan 2013).

In the present study, quantification of Fe, Cu, Cd, Cr and Pb in plant tissues was also examined.

The concentration of metals accumulated by the plant root and shoot parts collected from six different sites of the Gomti River are presented in Figs. 1 and 2. Metal accumulation by selected plants varied by plant and season. Generally plants accumulated greater metal content in roots than shoots. Due to formation of complex compounds with carboxylic acid (–COOH) group, low mobility of metals from root to shoot may occur (Cardwell et al. 2002). Metals accumulation in selected aquatic plants was in the order of Fe > Cu > Cr > Pb > Cd. Significant differences were observed for metal accumulation by the plants at different sites ($p < 0.05$). Roots of *E. crassipes* accumulated higher concentrations of Fe (31.73 µg g^{-1}) at Pipraghat during the pre-monsoon period. The reason for greater Fe concentration in roots of plants may be due to the formation of iron hydroxide plaques that are mobilized and precipitated onto root surfaces (Weis and Weis 2004). Plants collected from Hanuman Setu and Pipraghat had accumulated the highest Fe content in their roots. This may be due to draining of effluents containing Fe from automobile works situated on the banks of river banks and subsequent accumulation by plant roots. Maximum Cd accumulation was observed in root of *E. crassipes* (4.19 µg g^{-1}) at Gomti Barrage during the monsoon period. *P. stratiotes* accumulated the highest Cd in shoot (3.81 µg g^{-1}) at Pipraghat during the pre-monsoon period. Greater shoot accumulation by *P. stratiotes* indicates this species may be useful for absorbing and accumulating Cd from polluted water (Shuvaeva et al. 2013). Similar findings pertaining to Cd accumulation were observed for the shoots of *J. repens* and *H. verticellata.* The distribution of Cd within the plants is quite variable and thus explains the rapid translocation from root to aerial parts of plants (Fawzy et al. 2012). Highest Cu accumulation (21.48 µg g^{-1}) was in shoot of *T. latifolia* during the monsoon period at Pipraghat. Roots of aquatic plants showing the highest Cu concentrations (20.33 µg g^{-1}) were observed for *J. repens* at Pipraghat. Bioaccumulation of Cu by plant roots was higher at sites with water pH > 8, as it was reported that at higher pH (>8.0) the presence of plaque enhanced Cu uptake into roots (Weis and Weis 2004). Similar results were also shown for Cu accumulation by *Cyperus papyrus* plant growing in an urban natural wetland of Rwanda (Sekomo et al. 2011). *J. repens* and *P. stratiotes* shoots accumulated the most Cr (8.53 and 7.33 µg g^{-1}) at Hanuman Setu and Pipraghat, respectively during the monsoon period. This may be due to their

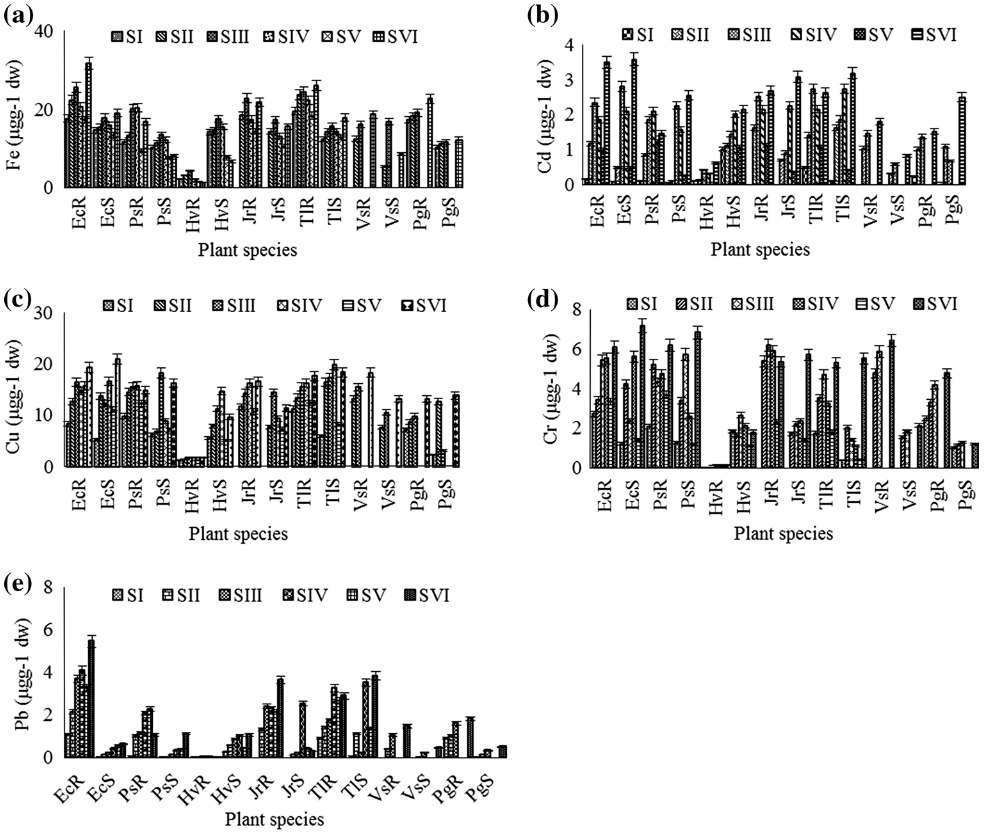

Fig. 1 Accumulation of metals (µg g^{-1} dw) in plant species at selected sites of the Gomti River during the pre-monsoon period: **a** = Fe, **b** = Cd, **c** = Cu, **d** = Cr and **e** = Pb (EcR = *Eichhornia crassipes* Root, EcS = *Eichhornia crassipes* Shoot, PsR = *Pistia stratiotes* Root, PsS = *Pistia stratiotes* Shoot, HvR = *Hydrilla verticellata* Root, HvS = *Hydrilla verticellata* Shoot, JrR = *Jussiaea repens* Root, JrS = *Jussiaea repens* Shoot, TlR = *Typha latifolia* Root, TlS = *Typha latifolia* Shoot, VsR = *Vallesnaria spiralis* Root, VsS = *Vallesnaria spiralis* Shoot, PgR = *Polygonum glabrum* Root and PgS = *Polygonum glabrum* Shoot; whereas: SI = Gaughat; SII = Pucca Pull; SIII = Hanuman Setu; SIV = Nishatganj; SV = Gomti Barrage and SVI = Pipraghat)

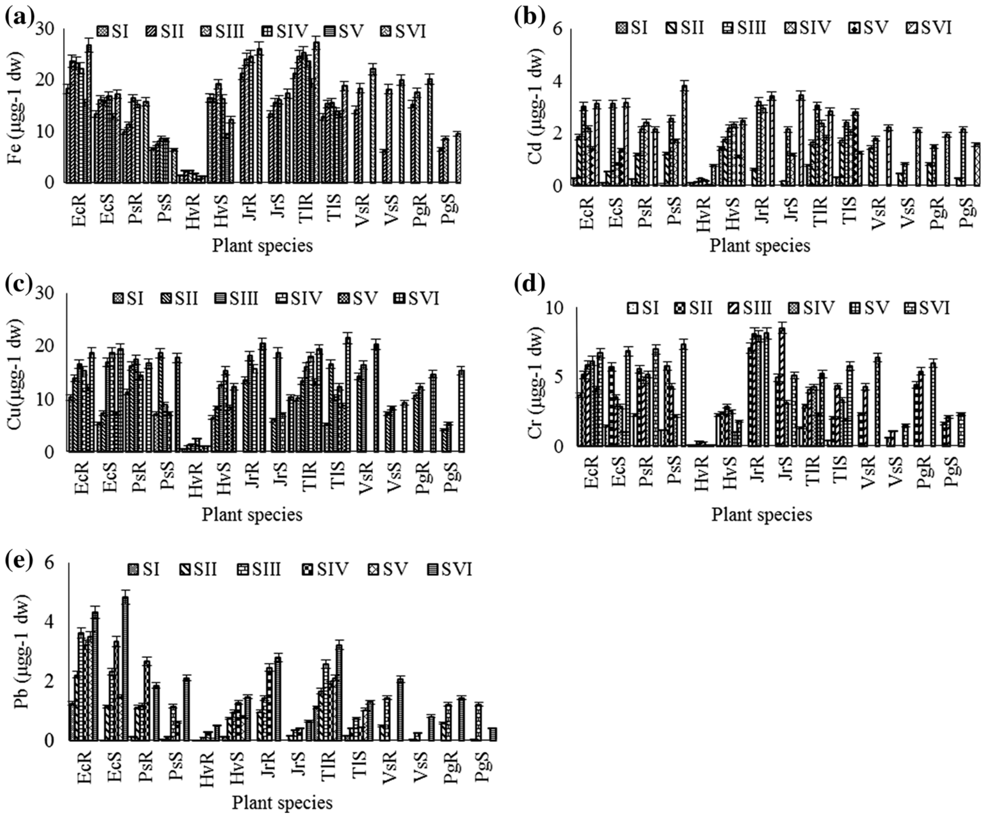

Fig. 2 Accumulation of metals (µg g^{-1} dw) in plant species at selected sites of the Gomti River during the monsoon period: **a** = Fe, **b** = Cd, **c** = Cu, **d** = Cr and **e** = Pb (EcR = *Eichhornia crassipes* Root, EcS = *Eichhornia crassipes* Shoot, PsR = *Pistia stratiotes* Root, PsS = *Pistia stratiotes* Shoot, HvR = *Hydrilla verticellata* Root, HvS = *Hydrilla verticellata* Shoot, JrR = *Jussiaea repens* Root, JrS = *Jussiaea repens* Shoot, TlR = *Typha latifolia* Root, TlS = *Typha latifolia* Shoot, VsR = *Vallesnaria spiralis* Root, VsS = *Vallesnaria spiralis* Shoot, PgR = *Polygonum glabrum* Root and PgS = *Polygonum glabrum* Shoot; whereas: SI = Gaughat; SII = Pucca Pull; SIII = Hanuman Setu; SIV = Nishatganj; SV = Gomti Barrage and SVI = Pipraghat)

potential of concentrating Cr in various plant organs without showing symptoms of toxicity.

Chromium levels above 0.5 mg kg^{-1} dw is considered toxic to plants (Allen 1989); however, plants analysed for Cr showed greater concentrations both in the root and shoot parts than the toxic levels. Maximum Pb accumulation by *E. crassipes* roots (5.46 µg g^{-1}) was at Pipraghat during the pre-monsoon period. The greater Pb concentration in roots of plants signifies lesser translocation of Pb to aerial parts of aquatic plants. Vesely et al. (2011) also reported ten times higher Pb in plant roots than in leaves of *P. stratiotes*. 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 (Ahmad et al. 2014).

Translocation factor (TF) for the metals investigated in selected plants is shown in Table 3. TF indicates metal mobility from root to shoot parts, demonstrating metal accumulation capability of plants in different plant parts other than roots. Except for Fe, *E. crassipes*, *P. stratiotes T. latifolia* and *J. repens* had a TF > 1 for Cd, Cr, Cu and Pb at different sites during both periods. *H. verticellata* recorded highest TF for all five metals at all sites and periods. The exceptionally TF of *H. verticellata* for all metals may be due to the plants whorled leaves resulting in a large surface area and thus conferring metal uptake through them when a metal concentrations in the surrounding water were high (Srivastava et al. 2011). When TF > 1, the plant efficiently mobilizes and translocates metals from root to the shoot and may be useful for the phytoextraction of metals from water ecosystems (Baker and Brooks 1989); however TF < 1 signifies that the specific plant genera could serve as a potential plant for phytostabilisation (Rai et al. 2012). TF < 1 depicts greater metal accumulation in root than shoot of plants and this may enhance plants own ability to tolerate metal concentrations that are usually toxic (Weis and Weis 2004).

The relationship in dynamics of metals concentration with physicochemical characteristics of river water at different sites was evaluated using Pearson correlation

Table 3 Distribution and translocation factor of different metals in plants during the pre-monsoon (PrM) and monsoon (M) seasons in Lucknow, India

Sites	Plant species	Fe		Cd		Cu		Pb		Cr	
		PrM	M	PrM	M	PrM	M	PrM	M	PrM	M
Site I	*E. crassipes*	0.83	0.72	0.62	0.35	0.63	0.51	0.02	0.01	0.45	0.39
	P. stratiotes	0.88	0.66	0.54	0.28	0.67	0.63	0.25	0.23	0.61	0.5
	H. verticellata	6.25	11.65	9.36	14.1	4.63	13.08	8	13	61	36.83
	T. latifolia	0.63	0.60	0.24	0.41	0.52	0.51	0.05	0.14	0.22	0.3
	P. glabrum	0.59	–	0.35	–	0.32	–	0.03	–	0.41	–
Site II	*E. crassipes*	0.68	0.69	0.41	0.28	1.08	0.52	0.05	0.51	1.2	1.11
	P. stratiotes	0.85	0.66	0.15	1.03	0.47	1.15	0.02	0.11	0.65	1.04
	H. verticellata	4.6	6.88	8.07	14.58	5.39	7.42	28	37	41.5	23.5
	J. repens	0.77	0.63	0.43	0.26	0.66	0.44	0.09	0.16	0.32	0.69
	T. latifolia	0.6	0.62	1.14	1.03	1.22	1.24	0.78	0.24	0.57	0.7
	V. spiralis	0.43	0.43	0.3	0.31	0.58	0.5	0.02	0.08	0.32	0.27
	P. glabrum	0.61	0.42	1.07	0.34	1.46	0.39	0.12	0.08	0.35	0.36
Site III	*E. crassipes*	0.69	0.67	1.19	1.02	0.75	1.02	0.005	0.63	0.43	0.59
	P. stratiotes	0.66	0.52	1.21	1.18	1.17	0.51	0.1	0.96	1.32	0.9
	H. verticellata	4.07	8.58	3.37	8.42	6.54	8.72	14.33	9.8	22	8
	J. repens	0.75	0.65	0.36	0.66	1.0	1.03	0.09	0.24	0.35	0.62
	T. latifolia	0.64	0.61	0.68	0.78	1.17	0.64	0.12	0.29	0.3	0.98
	V. spiralis	1.01	0.98	0.39	0.47	0.68	0.5	0.2	0.17	0.31	0.24
	P. glabrum	0.6	0.49	0.49	1.44	0.31	0.43	0.19	1.0	0.3	0.39
Site IV	*E. crassipes*	0.76	0.75	1.12	0.37	1.12	1.22	0.1	1.03	1.01	0.46
	P. stratiotes	0.6	0.54	0.74	0.7	0.56	0.5	0.15	0.23	0.54	0.42
	H. verticellata	7.0	9.22	6.28	12.88	8.0	6.09	12.87	4.3	14.13	8.58
	J. repens	0.75	0.65	1.04	0.39	0.57	0.45	1.11	0.16	0.39	0.39
	T latifolia	0.63	0.58	1.26	0.85	1.22	0.68	1.08	0.23	0.34	0.78
Site V	*E. crassipes*	0.77	0.82	0.49	0.95	0.69	0.6	0.16	0.42	0.42	0.24
	P. stratiotes	0.82	–	0.22	–	0.58	–	0.16	–	0.32	–
	H. verticellata	5.35	14.79	13.37	27.75	4.46	8.29	14.33	11.2	14	12.5
	J. repens	0.73	–	0.33	–	0.67	–	0.18	–	0.62	–
	T. latifolia	0.69	0.68	0.4	1.53	0.66	0.65	0.51	0.49	0.23	0.81
Site VI	*E. crassipes*	0.6	0.64	1.02	0.89	1.08	1.04	0.11	1.12	1.17	1.02
	P. stratiotes	0.48	0.4	1.72	1.78	1.09	1.06	1.07	1.13	1.1	1.04
	H. verticellata	5.05	10.39	3.46	3.14	8.18	11.71	26.25	2.9	12	36
	J. repens	0.71	0.66	1.15	1.0	0.68	0.84	0.07	0.23	1.06	0.62
	T. latifolia	0.68	0.68	1.2	0.36	1.03	1.11	1.32	0.4	1.03	1.1
	V. spiralis	0.45	0.9	0.45	0.95	0.72	0.45	0.3	0.39	0.36	0.23
	P. glabrum	0.53	0.46	1.63	0.81	1.04	1.04	0..27	0.28	0.24	0.38

E. crassipes = *Eichhornia crassipes*, *P. stratiotes* = *Pistia stratiotes*, *H. verticellata* = *Hydrilla verticellata*, *J. repens* = *Jussiaea repens*, *T. latifolia* = *Typha latifolia*, *V. spiralis* = *Vallesnaria spiralis* and *P. glabrum* = *Polygonum glabrum* whereas; Site I = Gaughat; Site II = Pucca Pull; Site III = Hanuman Setu; Site IV = Nishatganj; Site V = Gomti Barrage and Site VI = Pipraghat; (–) indicates the plant species were not present during that season at the site

coefficient (Table 4). All metals showed significant positive correlation with COD, phosphate (PO_4), nitrate and negative correlation with DO. Individually, each metal showed positive correlations with other metals. Among physicochemical characteristics, Fe in water showed a significant positive correlation with COD (0.898; $p < 0.01$), phosphate (0.743; $p < 0.01$) and nitrate (0.645; $p < 0.05$).

Among various metals recorded, Fe showed a positive correlation with Cu (0.917), Pb (0.739), Cr (0.808; $p < 0.01$) and with Cd (0.650; $p < 0.05$). Cd in water showed a positive correlation with COD (0.774; $p < 0.01$)

Table 4 Correlation matrix between physicochemical characteristics and metals of the Gomti River water at Lucknow, India

	pH	E.C	DO	BOD	COD	NO_3	NO_2	NH_4	PO_4	Fe	Cd	Cu	Pb	Cr
pH	1	0.910**	0.215	0.404	−0.269	0.289	0.527	0.033	−0.466	−0.048	−0.095	0.022	−0.239	0.078
E.C			0.025	0.563	−0.116	0.379	0.432	0.224	−0.247	0.134	0.015	0.216	−0.165	0.172
DO				−0.684*	−0.704*	−0.396	0.244	0.224	−0.584*	0.777**	−0.202	−0.577*	−0.564	−0.498
BOD					0.294	0.541	0.148	−0.080	0.167	0.450	0.011	0.411	0.245	0.389
COD						0.527	−0.211	−0.254	0.847**	0.898**	0.774**	0.901**	0.720**	0.756**
NO_3							0.379	0.136	0.547	0.645*	0.568	0.761**	0.700*	0.781**
NO_2								0.372	−0.031	−0.109	0.188	0.000	−0.007	0.005
NH_4									0.172	−0.078	0.111	0.005	−0.194	−0.176
PO_4										0.743**	0.799**	0.796**	0.713**	0.635*
Fe											0.650*	0.917**	0.739**	0.808**
Cd												0.842**	0.578*	0.707*
Cu													0.717**	0.871**
Pb														0.882**
Cr														1

Values represent Pearson correlation coefficient and significant at ** $p = 0.01$ and * $p = 0.05$

and phosphate (0.799; $p < 0.01$). However, Cd didn't show any significant relation with nitrate. Among various metals, Cd showed a positive correlation with Cu (0.842; $p < 0.01$), Pb (0.578; $p < 0.05$) and Cr (0.707; $p < 0.05$). Among physicochemical characteristics, Cu in water showed a significant positive correlation with COD (0.901; $p < 0.01$), nitrate (0.761; $p < 0.01$) and phosphate (0.796; $p < 0.01$). Cu showed a positive correlation with Pb (0.717) and Cr (0.871; $p < 0.01$). For Pb, a significant positive correlation with COD (0.720; $p < 0.01$), nitrate (0.700; $p < 0.01$) and phosphate (0.713; $p < 0.01$) were observed. Pb also showed a positive correlation with Cr (0.871; $p < 0.01$). Among various physicochemical properties, Cr showed a significant positive correlation with COD (0.765; $p < 0.01$), nitrate (0.781; $p < 0.01$) and phosphate (0.635; $p < 0.05$). In the aquatic environment, accumulation of metals and subsequent transformations due to physicochemical and biological processes are important mechanisms for their changing levels in water (Rai 2010).

The present study reveals that the Gomti River is subjected to alarming inputs of metals and other inorganic pollutants. 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 the water column. It is worth noting that plants accumulated more metals than the corresponding levels in water, indicating these species could be used in ecological surveys as in situ biomonitors of water quality. Further studies should be conducted in this river during all seasons throughout the year to evaluate the dynamics of metal accumulation and release back into river by these macrophytes for efficient water quality management.

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Abdul Barey <shahbarey8@gmail.com>

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Short running Title: Potable water quality monitoring

Monitoring of hazardous inorganic pollutants and heavy metals in potable water at the source of supply and consumers end of a tropical urban municipality

Abdul Barey Shah and Rana Pratap Singh*
Department of Environmental Science, Babasaheb Bhimrao Ambedkar (Central) University, Lucknow-226025, India
**Corresponding Author Email: cceseditor@gmail.com*

Abstract

River water is not only an indispensable source for irrigation but also plays a vital role for drinking water supply for most of the urban municipalities. Water from rivers is pumped at specific sites and after treatment at municipal water treatment plants supplied as domestic potable water supply. The present study was undertaken to assess the suitability of Gomti river water at Gaughat being used as the source of water supply for Lucknow city and to evaluate post-treatment potable water quality at the consumer end by monitoring the levels of inorganic pollutants (nitrate, nitrite, ammonium and phosphate) and heavy metals. Municipal water supply at Gaughat showed marked variations in the levels of pH (7.13-8.63) and electrical conductivity (375.66-571.67µS/cm). The amount of nitrate, nitrite, ammonium and phosphate was observed 26.25, 0.082, 6.9 and 1.82 mg/l respectively at Gaughat. Also, the levels of heavy metals in the municipal water source at Gaughat varied significantly for Fe (0.33-1.65 mg/l), Cu (0.077-0.108 mg/l), Cd (0.03-0.052 mg/l), Pb (0.68-0.96 mg/l) and Cr (0.036-0.065 mg/l). Water at the user end was also contaminated as the concentration of analysed inorganic pollutants and heavy metals were correspondingly higher than observed at the source. While comparing potable water at the user end of Lucknow municipality with the BIS (Drinking Water Specifications) and WHO standards for drinking water, the concentration of all studied heavy metals and other inorganic contaminants were much above the permissible levels, thus posing a serious threat to the public health.

Key Words: Drinking water, Gomti river, heavy metals, nitrate, nitrite, public health

Introduction

Pure and safe drinking water is an essential and basic need to sustain life and maintenance of human health (Urashima, 2008; Mudiam *et al.,* 2012; Wang and Yu, 2014). River water is often used for domestic needs and also supplied as potable water by municipal corporations. Contamination of drinking water sources and supplies with hazardous chemicals like nitrate, nitrite, ammonium and heavy metals has become an increasing concern in developing countries, including India (Khan *et al.,* 2014). Increased rate of diseases outbreaks both in developed and developing regions have long been recognized due to poor potable water supplies to public (WHO, 2009) and in addition, poor water quality and scarcity can stifle a nation's economy, negatively impact the environment and fuel conflicts (Rai, 2010; Pham *et al*., 2011).

The elevated levels of nitrogenous inorganic pollutants (nitrate, nitrite, ammonium) and inorganic phosphates in river water due to runoff from agricultural fields, discharge of municipal/industrial sewage etc. lead to many health hazards (Kumari *et al.*, 2013). Nitrate-N levels in drinking water of 10mg/l or greater have been linked to methaemoglobinaemia or blue baby syndrome in infants and gastric carcinomas (Tank and Chandel, 2010) and non-Hodgkin's lymphoma in humans (Rao and Puttana, 2000; Rawat and Singh, 2009). Nitrite in human body may lead to the formation of carcinogenic nitrosamine upon reaction with secondary amines, thus posing the risk of stomach, liver and esophageal cancer (Kim Shapiro *et al.*, 2006) and high concentration of ammonium in body can lead to convulsion, coma and even death (Rawat *et al.*, 2012).

Heavy metals released by human activities into the environment beyond toxic limits are progressively finding their fate into water bodies thereby making the water unfit for drinking and also their bioaccumulation in food chains threaten the life of animals and human beings (Zhang *et al.*, 2009; Ishaq and Khan, 2013; Nazeer *et al.*, 2014). Characteristically, heavy metals are carcinogenic and neurotoxic in nature, however, their toxicity and targeted organs vary from metal to metal and also depend upon the level of intake, strength of body immune system and duration of exposure (Dieter *et al.*, 2005; Khan *et al.*, 2014). Consumption of water contaminated with heavy metals may lead to their chronic accumulation in the kidneys, liver, bones etc. resulting in disruption of various metabolic activities (Bakirdere *et al.*, 2013; Pourang and Noori, 2014).

The provisions of clean drinking water and its normalized supply play an important role for decreased mortality and improved economic progress in developing countries (Koc, 2010). 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 (Cieszynska *et al.*, 2012; Faridi *et al.*, 2012). Being a developing country, India is not having advanced treatment processes and provisions at municipal water treatment plants for the removal of hazardous inorganic pollutants like heavy metals and reactive nitrogen species from potable water prior to public supply.

Although some of the monitoring studies on drinking water of Lucknow city have evaluated variable concentration of various inorganic contaminants (Mishra and Mishra, 2008; Lohani *et al.*, 2008; Mudiam *et al.*, 2012). However, monitoring of multi metals (Fe, Cu, Cd, Cr and Pb) and reactive nitrogen species (nitrate, nitrite, and ammonium) and phosphate in drinking water before and after municipal treatment has not been investigated in different seasons. Therefore, the present study is aimed at monitoring the water quality of river Gomti at Gaughat being used as the source of drinking water supply for Lucknow Municipal Corporation and to evaluate the status of post-treatment drinking water quality at the user end during pre-monsoon, monsoon and post monsoon periods in a semi-arid, tropical city of India.

Materials and Methods

Lucknow, the capital of Uttar Pradesh (26^{o}5/N latitude, 80^{o}56/E longitude, 128 m above the sea level), is spread over an area of 310 km^2 in the central plain of the Indian subcontinent, supporting a population of 36.50 lakh. It has a distinct tropical climate with a marked monsoonal effect. The year is divided into three distinct seasons *i.e*., summer (March to June), rainy (July to October) and winter (November to February). The temperature ranges from a minimum of 5^0 C in winter to a maximum of 47^0 C in summer. The mean average relative humidity is 60% with a rainfall of 1006.8 mm. Gomti river, a tributary of Ganga River, originates from Fulhar lake near Pilibhit flowing through the Lucknow city meandering for about 12 Km, is a major freshwater ecosystem in India. Lucknow Jal Sansthan, an Organisation of Govt. of UP, has been entrusted to provide safe and potable drinking water to the city dwellers. Sampling station at Gaughat of river Gomti which is the source of raw municipal water supply for Lucknow city along with seven residential sampling sites which are the locations of municipal water supply for various residential areas of Lucknow were chosen for the monitoring of drinking. Residential areas included Aminabad, Indira Nagar, Aishbagh, Nishatganj, Telibagh, Hazratganj and Charbagh and these sites in the results and discussion are designated as site1, site2, site3, site4, site5, site6 and site7 respectively. Water samples from Gaughat and other residential areas were collected during August 2012-July 2013. Water samples were collected in triplicates in polypropylene sampling bottles that were acid soaked overnight and rinsed with tap water and then with distilled water. Collected samples were transported and stored in the dark at 4ºC for further analysis (APHA 2005). One bottle from each station was acidified in the laboratory before storage for heavy metal analysis. pH, electrical conductivity (EC), nitrate, nitrite, ammonium and phosphate were monitored monthly, while as for heavy metals seasonal variations during the pre-monsoon, monsoon and post-monsoon was conducted both at the Gaughat and residential areas. Basic parameters such as pH and EC were measured on spot using potable digital pH and EC meters. Nitrate, nitrite, ammonium and phosphate were measured in triplicate by using standard methods for examination of water and wastewater (APHA, 2005). Heavy metals viz. Fe, Cu, Cd, Pb and Cr were determined after acid digestion of samples with an acid mixture (9 parts nitric acid: 4 parts perchloric acid) at about 100º C. Blanks were also run simultaneously and analyzed to correct for possible external contributions of the metals. Analytical data quality of metals was ensured through repeated analysis (n=3) of EPA quality control in samples. Metal concentration was determined by atomic absorption spectrophotometer (AAS 240 FS, Varian). Statistical analysis of data by one way ANOVA followed by Duncan Multiple Range Tests was performed to determine the significance of differences among the mean values using (SPSS Version 20). Results of testing were considered significant if the calculated P-values were ≤ 0.05. Relationships between physicochemical parameters and metal concentration in drinking water at the user end were studied by using Pearson Linear Correlation method.

Results and Discussion

Physicochemical characteristics of drinking water at source of supply and consumer end

Safe water supply is recognized as the highest priority task in environmental protection throughout the globe (Adewuyi *et al.*, 2014). The declaration of access to pure and safe drinking water as “human birth right” could actually mean “death right” if the water quality of potable sources is not regularly monitored to check their suitability and purity for human consumption (Mebrahtu and Zerabruk, 2011). In the present study, the mean values of pH of water at Gaughat varied between 7.13 in September and 8.63 in February (Table 1). These values indicated that pH of drinking water source was slightly alkaline in nature. Significant differences were observed for the pH values of water samples at Gaughat during all months ($p \leq 0.05$). At the consumer end, pH values at different studied sites showed variations from 6.9 at site 1 to 8.66 at Site 6 during May (Fig.1). Statistically significant differences were observed for monthly pH values in drinking water at consumer end sites ($p \leq 0.05$), except in the month of September. While comparing the pH of drinking water with drinking water quality standards, it was observed to be slightly above than prescribed by BIS (2003) at sites 3, 6 and 7. It has been reported pH of water systems plays a significant role in metal solubility (Jonnalagadda and Mhere, 2001). Various factors that govern pH fluctuations in water include temperature changes, dissolution of atmospheric CO_2, organic matter decomposition in water, dilution of alkaline substances and metabolic activities of phytoplanktons and other aquatic life (Fawzy *et al.*, 2012). In the current study, the slight alkaline nature of drinking water of Lucknow city is in accordance with the previous reports of Mullai *et al., (*2013) while evaluating pH of Uppanar river of Tamilnadu.

The electrical conductivity measurements in water depict the temporal variations in total dissolved solids and major ions present in it. During the present study, it was found that source of drinking water i.e., Gaughat showed highest EC (571.67µs/cm) in May and lowest value for EC (375.66 µs/cm) was recorded in August (Table1). Monthly variations in EC of water samples at Gaughat were significant ($p \leq 0.05$). At the user end, EC values of drinking water ranged from374.66 µs/cm in July to 475 µs/cm in May at site 7 and 5 respectively (Fig.2). Maximam EC during the summer months reported in the current study may be due to low and lenient flow of water in Gomti river and high temperature during this period leads to the increased salt content in water due to evaporation of water (Bellos and Swadis, 2005). Further increased EC shows the presence of free ions and other chemical substances such as nitrates, chlorides, phosphates in water. For EC of drinking water, WHO guidelines (2011) has prescribed maximum permissible limit of 400 mS/m. At the consumer end, significant differences were observed between all sites for EC in all studied months ($p \leq 0.05$).

Nitrate content in water is typically reported as either nitrate-nitrogen (NO_3-N) or nitrate (NO_3^-). Nitrate-N being the most common form of nitrogen present in natural waters is the end product of aerobic decomposition of organic matter (Rai, 2010; Agca *et al.*, 2014). For primary drinking water, USEPA has set a standard of 10 mg/l for nitrogen when reported in nitrate-N form and 45 mg/l when reported in nitrate form. In the present study, the water at Gaughat showed highest NO_3^- (26.25 mg/l) in May and lowest NO_3^- (13.45 mg/l) was reported in August (Table 1). While comparing the monthly concentrations of nitrate both at Gaughat water and at user end of all sites, significant differences were observed ($p \leq 0.05$). Nitrate concentration in public water supply at the consumer end showed maximum value (40.86 mg/l) at site 7 in September and minimum value (11.17 mg/l) was analysed at site 1 in April (Fig.3). Since nitrate concentration in water samples reported at Gaughat was within the permissible limit 45 mg/l. However, for its use as potable water supply to the Lucknow, it is recommended that nitrate concentration in water at the consumer end should not exceed 10mg/l. Further if water supply has a nitrate level approaching 3-5 mg/l, it is recommended that it should not be used for mixing baby foods or for drinking water for new born infants. Rawat and Singh (2009) have reported as much as 44.83 mg/l of nitrate in the surface waters of Lucknow, which may be due to discharge of municipal and industrial wastes, excessive application of nitrogenous fertilizers, manures and irrigation with untreated water. Dar *et al.*, (2010) reported nitrate concentration in summer in 85% of the wells exceeding WHO permissible limits in Kashmir (India). In the extensive agricultural areas of north east Australia, Thorburn *et al.*, (2003) concluded that 14-21% of the wells were extensively contaminated with nitrate.

Table 1. Monthly variation in physicochemical characteristics of Gaughat water of Gomti river used as raw potable water supply for Lucknow city (n=3± S D)

	pH	E. Conductivity (µs/cm)	Nitrate (mg/l)	Nitrite (mg/l)	Ammonium (mg/l)	Phosphate (mg/l)
AUG.12	7.36±0.057^{b}	375.66±4.04^{a}	13.45±1.36^{a}	0.033±0.01^{a,b}	3.89±0.17^{c}	0.9±0.02^{d,e}
SEPT.12	7.13±0.057^{a}	379.33±4.041^{a,b}	14.18±1.46^{a,b}	0.03±0.01^{a,b}	4.41±0.18^{d}	1.42±0.09^{g}
OCT.12	7.46±0.057^{b}	383.33±2.081^{a,b}	17.06±0.41^{d,e}	0.043±0.01^{b,c}	4.026±0.04^{c}	**1.82±0.06**^{f}
NOV.12	7.83±0.115^{c}	391.66±2.88^{b}	18.8±0.94^{e,f}	0.061±0.003^{d,e}	3.89±0.19^{c}	1.14±0.06^{e}
DEC.12	8.56±0.057^{e}	415±5.0^{c}	20.85±0.95^{g}	0.06±0.003^{d,e}	3.46±0.12^{b}	0.89±0.04^{d,e}
JAN.13	8.63±0.115^{e}	425.66±4.04^{c}	16.37±1.62^{c,d}	0.054±0.005^{b,c}	2.67±0.18^{a}	0.82±0.01^{d}
FEB.13	**8.63±0.057**^{e}	435±5.0d	14.81±1.21^{a,b}	0.071±0.007^{e,f}	4.78±0.09^{e}	0.69±0.02^{b,c}
MAR.13	8.1±0.1^{d}	477.66±11.67^{d}	15.99±0.3^{b,c,d}	0.067±0.004^{d,e}	5.17±0.17^{f}	0.6±0.02^{a}
APR.13	8.16±0.057^{d}	536.66±15.27^{e}	19.5±0.81^{f,g}	0.08±0.003^{f}	6.03±0.32^{g}	0.63±0.04^{a,b}
MAY.13	8.13±0.152^{d}	**571.67±10.4**^{f}	23.37±1.05^{h}	**0.082±0.005**^{f}	6.21±0.23^{g}	0.69±0.02^{b,c}
JUNE13	8.06±0.115^{d}	551.67±10.4^{g}	**26.25±0.9**^{i}	0.075±0.002^{e,f}	**6.9±0.15**^{h}	0.72±0.02^{c}
JULY13	7.76±0.15^{c}	388.66±5.5^{a,b}	16.66±1.78^{c,d}	0.026±0.01^{a}	3.95±0.13^{c}	0.81±0.03^{d}
BIS 2003	**6.5 – 8.5**	**-**	**45**	**0.1**	**0.5**	**-**

(Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$)

In the present study, monthly variations in nitrite (NO_2^-) concentration analysed from Gaughat water and consumer end of Lucknow are depicted in Table 1 and Fig.4 respectively. Nitrite levels analysed in water at Gaughat and at various residential sites showed significant differences ($p \leq 0.05$). At Gaughat, maximum nitrite (0.082 mg/l) was observed in May and minimum nitrite (0.026 mg/l) in July. Correspondingly, at the user end higher nitrite levels were reported than at the source. Highest nitrite (0.1 mg/l) at site 5 and lowest (0.02 mg/l) at site 2 was analysed in September and February respectively. Nitrite levels reported in this study were slightly below the permissible limits prescribed by BIS (2003) at various sites, except at site 5 which showed nitrite concentration in water above permissible levels. Enhanced nitrite production in water may be due to oxidation of ammonium and reduction of nitrate by bacteria (Rawat *et al.,* 2012; Mullai *et al.,* 2013). Ammonium nitrogen (NH_4-N) in water samples at studied sites was found to vary between 2.67 mg/l and 6.9 mg/l at Gaughat (Table 1) and at the user end ammonium levels varied from 1.14 mg/l at site 3 to 5.16 mg/l at site 7 during April and August, respectively (Fig. 5). Ammonium in water has been seldom reported to be present in water at 1 mg/l unless ammonium fertilizers or other ammonia sources such as wastewater gets added to the water sources or supplies. During the current study, NH_4-N contents were much higher than maximum permissible limit for drinking water of 0.5 mg/l NH_4-N recommended by BIS (2003). The results depict higher ammonium levels in water than previously reported by Rawat and Singh (2009) in surface and groundwater sources of Lucknow city. Excretion of ammonium by the phytoplanktonic organisms may be the reason for higher ammonium levels in water and also their death or decomposition in water storage tanks can lead to increased levels of ammonium and nitrite in the water supply (Damotharan *et al*., 2010).

Domestic wastewaters containing detergents, municipal and industrial effluents and agricultural runoff are the main anthropogenic sources for elevated phosphate levels in surface water. In most of the natural surface waters, phosphorus ranges from 0.005 to 0.02 mg/l (WHO, 2011). For phosphates WHO has not established any specific guideline value for drinking water. In the present study variations in phosphate levels of drinking water are depicted in Table 1 for Gaughat and Fig.6 for residential sites. Significant differences were observed for the monthly variations in phosphate contents in drinking water both at source and consumer end ($p \leq 0.05$). At Gaughat maximum phosphate (1.82 mg/l) in October and minimum (0.6 mg/l) in March was observed. From the residential sites, highest phosphate content (2.14 mg/l) at site4 in August and lowest (0.44 mg/l) phosphate at site6 in January was reported. The high concentration of phosphates are probably due to chemical weathering of rocks, release of soil minerals and also discharge of sewage into aquatic systems (Singh *et al*., 2005). Since phosphates are used at potable water treatment plants, this may be the reason for elevated phosphate contents in drinking water at the residential sites.

Seasonal variations of metal contents in potable water at Gaughat and consumer end

Heavy metals can enter drinking water by corrosion of distribution pipes as well as from industrial and natural geo-genic sources (Singh *et al.,* 2005; Haloi and Sarma, 2012). In the present study, seasonal variations in the average values of heavy metal contents recorded in water at Gaughat during the course of study period are shown in Table 2. 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. Except for Pb ($p \geq 0.05$), one way anova showed significant differences between and among the groups of metals (Fe, Cu, Cd and Cr) recorded in water at Gaughat during all the three seasons ($p \leq 0.05$). At Gaughat maximum metal concentration for Fe (1.65 mg/l), Cu (0.108 mg/l) and Cr (0.065 mg/l) were reported in monsoon season, while as maximum Cd (0.052 mg/l) was seen in pre-monsoon and Pb (0.96 mg/l) was observed to be maximum in post-monsoon season. Minimum concentration for Fe (0.33 mg/l) was recorded in premonsoon, Cu (0.077 mg/l) in post-monsoon, Cd (0.03 mg/l) in monsoon, Pb (0.68 mg/l) in monsoon and Cr (0.036 mg/l) in postmonsoon season at Gaughat. The variable metal contents reported in water at Gaughat during the present study depict discharge of wastewater and runoff from the catchment area into river system. Further, seasonal fluctuations in the metal contents may be due to the rain dilution effects. The leaching of metals from the soil minerals and organic matter due to rainfall may influence the concentration of heavy metals in river bodies (Singh *et al.*, 2005; Lohani *et al.*, 2008). Nevertheless, the presence of these metals in the water column used for public supply leads to various types of toxicity, like renal failure is related to the contamination of drinking water with Cd and Pb; liver cirrhosis to the contamination with Fe, Cu and Mo; hair loss to the contamination with Cr and Ni; and chronic anemia to the contamination with Cd and Cu (Jarup, 2003; Johri *et al.*, 2010; Mandour, 2012).

Table 2. Seasonal variations in metal content (mg/l) in Gaughat water of Gomti river used as raw potable water supply for Lucknow city (n=3± S D)

	Fe	**Cu**	**Cd**	**Pb**	**Cr**
Pre M	0.336 ± 0.016^{c}	0.088 ± 0.206^{a}	**0.052 ± 0.004^{a}**	0.74 ± 0.18^{a}	0.057 ± 0.003^{b}
M	**1.65 ± 0.13**^{b}	**0.108 ± 0.005^{a}**	0.03 ± 0.006^{a}	0.68 ± 0.16^{a}	**0.065 ± 0.005^{a}**
Po M	1.128 ± 0.007^{a}	0.077 ± 0.022^{b}	0.045 ± 0.005^{b}	**0.96 ± 0.061^{a}**	0.036 ± 0.004^{c}
BIS 2003	**0.3**	**0.05**	**0.01**	**0.05**	**0.05**

(Different letters signify the statistical differences among physico-chemical parameters $p \leq 0.05$; whereas Pre M= Premonsoon; M= Monsoon and Po M = Postmonsoon)

Seasonal fluctuations in the levels of heavy metals (Fe, Cu, Cd, Pb and Cr) studied at seven residential sites of urban Lucknow are depicted in Table 3. 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.

At the consumer end, the heavy metals studied showed significant differences in all seasons at all sites ($p \leq 0.05$). According to the results, Fe content in the drinking water at consumer end varied from 0.15 mg/l to 5.32 mg/l. Highest Fe content (5.32 mg/l) was recorded at site5 during the monsoon period, followed by Fe (5.24 mg/l) at site7 and Fe (4.75 mg/l) at site6 and lowest Fe concentration (0.15 mg/l) was reported at site 2 during the post monsoon period which may due to the use iron in coatings of pipes used to transport drinking water, overtime corrosion/deterioration of these iron coatings can lead to excessive iron into water supplies (Mandour, 2012). Iron has been reported to cause liver cirrhosis when present in drinking water. In the present study, iron values exceeded the permissible limits prescribed by BIS (2003) and WHO (2011) for drinking water. Our results on Fe levels in drinking water are much higher than reported by Iqbal *et al.*, (2013) in drinking water of Pakhtunkhwa, Pakistan.

Copper concentration in drinking water varies due to fluctuations in pH and hardness of the water supply (Xu *et al*., 2006). Levels of Cu in drinking water at studied sites varied from (0.036 mg/l) to (0.37 mg/l). During the monsoon period highest Cu concentration (0.37 mg/l) was observed at site5, followed by (0.21 mg/l) at site3 during the premonsoon period. Lowest Cu concentration (0.036 mg/l) was observed at site2 during the postmonsoon period. Our result on Cu levels was much lower than U.S. EPA’s Drinking Water Equivalent levels (1.3 mg/l) and WHO guideline value (2.0 mg/l). However, Cu levels were above the prescribed permissible levels of BIS (2003). Xu *et al*., 2006 reports excessive levels of Cu concentrations in drinking water of Shanghai China, Chile (1.2 mg/l) and United States (4.8 mg/l). Copper in drinking water supplies largely comes due to leaching from piping and plumbing fixtures. Addition of Cu into waterways is due to natural weathering of rocks and soil/sediments and also anthropogenic sources such as industrial effluents and sewage treatment plant effluents largely contribute to elevated copper levels in water courses (Lokhande *et al*., 2011). Although copper serves as a nutritional requirement for body, exposure to excessive levels of copper can result in number of adverse health effects, most commonly gastrointestinal distress (Alam *et al.*, 2012). Copper can bind with certain enzymes thereby interfering with their protection of cells from free radical damage (Uriu-Adams and Keen, 2005).

In the present study, Cd was also reported from the drinking water supplies of residential sites of urban Lucknow. Among the reported metals, Cd content in drinking water was found to be least. However, the Cd levels were just above the prescribed limits for drinking water (BIS, 2003). Cd content during the present study showed significant variations at all sites during different seasons ($P \leq 0.05$). Highest Cd content (0.046 mg/l) was observed during the postmonsoon period at site3. The lowest Cd (0.006 mg/l) was recorded at site4 during the premonsoon period. Reports of the present study on Cd content in water are much below than recorded by Khan *et al*., (2014), but higher than reported by Mishra and Mishra (2008) in Gomti river. The presence of Cd in drinking water supplies has been correlated with the plumbing of pipelines. Cadmium exposure to humans can lead to both short term (diarrhea, vomiting and destruction of mucous membrane) as well as long term (itai-itai disease, bone and kidney damage) effects (Muhammad *et al.*, 2011; Bakirdere *et al.*, 2013). .

Table 3 Seasonal variation in concentration of heavy metal content (mg/l) in potable water at user end in Lucknow City (n=3± S D)

	Fe			**Cu**			**Cd**			**Pb**			**Cr**		
	Pre M	M	Po M	Pre M	M	Po M	Pre M	M	Po M	Pre M	M	Po M	Pre M	M	PoM
S1	0.506±0.02^{a}	3.37±0.04^{a}	0.16±0.01^{a}	0.046±0.01^{a}	0.04±0.01^{a}	0.044±0.01^{a}	0.012±0.001^{a,b}	0.007±0.004^{a}	0.0098±0.001^{a}	0.21±0.02^{a}	0.255±0.05^{a,b}	0.152±0.04^{a}	0.0207±0.0002^{a}	0.0206±0.0003^{a}	0.0155±0.004^{a}
S2	0.518±0.09^{a}	3.38±0.13^{a}	0.15±0.01^{a}	0.05±0.02^{a}	0.06±0.03^{a}	0.036±0.01^{a}	0.01±0.00^{a,b}	0.008±0.003^{a}	0.0083±0.001^{a}	0.21±0.08^{a}	0.29±0.04^{b}	0.15±0.04^{a,b}	0.023±0.005^{a,b}	0.0206±0.0002^{a}	0.015±0.001^{a}
S3	1.18±0.06^{b}	4.16±0.06^{b}	0.28±0.05^{b}	0.21±0.08^{c}	0.2±0.01^{b}	0.067±0.04^{a,b}	0.04±0.009^{d}	0.033±0.009^{c}	**0.046±0.019^{b}**	0.52±0.03^{c,d}	0.304±0.014^{b}	0.23±0.01^{b}	0.026±0.0005^{b}	0.0308±0.001^{b}	0.026±0.0005^{b}
S4	2.09±0.05^{c}	4.61±0.08^{c}	0.34±0.05^{b}	0.11±0.008^{a,b}	0.29±0.11^{b,c}	0.102±0.01^{b}	0.006±0.002^{a}	0.007±0.001^{a}	0.0073±0.001^{a}	0.406±0.006^{b}	0.18±0.02^{a}	0.15±0.03^{a,b}	0.02±0.0008^{a}	0.018±0.002^{a}	0.013±0.005^{a}
S5	2.64±0.09^{d}	**5.32±0.11^{c}**	2.18±0.05c	0.21±0.01^{c}	**0.37±0.05^{c}**	0.223±0.02^{c}	0.018±0.002^{b,c}	0.018±0.001^{b}	0.012±0.003^{a}	0.46±0.02^{b,c}	0.45±0.03^{c}	0.35±0.04^{c}	0.046±0.0008^{c}	0.043±0.004^{c}	0.033±0.002^{c}
S6	3.16±0.05^{e}	4.75±0.08^{d}	2.36±0.05^{d}	0.12±0.02^{b}	0.26±0.05^{b,c}	0.098±0.01^{b}	0.022±0.005^{c}	0.015±0.002^{a,b}	0.019±0.009^{a}	**0.58±0.02^{d}**	0.57±0.05^{d}	0.54±0.05^{d}	0.05±0.001^{c}	**0.051±0.008^{c}**	0.03±0.00^{b,c}
S7	3.52±0.08^{f}	5.24±0.07^{d}	2.43±0.08^{d}	0.127±0.02^{b}	0.26±0.06^{b,c}	0.097±0.02^{b}	0.014±0.003^{a,b}	0.018±0.004^{b}	0.014±0.002^{a}	0.49±0.01^{c}	0.56±0.06^{d}	0.57±0.03^{d}	0.046±0.002^{c}	0.047±0.006^{c}	0.0358±0.005^{c}
BIS	**0.3**			**0.05**			**0.01**			**0.05**			**0.05**		

(Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh. Pre M= Premonsoon; M=Monsoon and Po M =Postmonsoon

Table 4 Correlation coefficient (r) between physicochemical parameters and heavy metals of potable water at the user end of Lucknow municipality

	pH	E. C	Nitrate	Nitrite	Ammonium	Phosphate	Fe	Cu	Cd	Pb	Cr
pH	1	.621**	-.581**	-0.327	-0.334	-.500*	0.131	-0.122	0.199	0.255	0.178
E.C		1	-.586**	-0.289	-0.12	-.589**	-0.141	-0.283	0.156	0.237	-0.112
Nitrate			1	.805**	.420*	.501*	0.268	-0.158	-0.222	-0.111	-0.043
Nitrite				1	.618**	0.399	.446*	-0.241	-0.151	0.234	0.23
Ammonium					1	.412*	-0.014	0.081	0.139	.589**	0.36
Phosphate						1	0.133	0.282	-0.156	-0.1	-0.024
Fe							1	0.157	-0.235	0.162	0.34
Cu								1	.456*	0.377	.499*
Cd									1	.592**	.452*
Pb										1	.760**
Cr											1

(Values represent Pearson correlation coefficient, significant at **0.01 and *0.05 level).

Chromium levels recorded in the current study were slightly above the prescribed standards of BIS. Maximum Cr (0.051 mg/l) was observed at site6 during the monsoon period, while as minimum Cr concentration (0.013 mg/l) was recorded at site4 during the postmonsoon period. Site6 was followed by site7 for the second highest observed Cr (0.047 mg/l) during the monsoon and (0.046mg/l) in the premonsoon period. The present levels of Cr detected in drinking water are less than 0.1 mg/l Cr reported earlier by Lohani *et al.*, (2008) while assessing heavy metal contamination in river Gomti of Lucknow. One of the major sources of Cr in aquatic environments is paint manufacturing industries. Also chromium compounds are used as pigments, mordents and dyes in the textiles and in leather industries as a tanning agent (Lokhande *et al.,* 2011). Cr exposure in drinking water has been reported to cause stomach cancer, alongside anemia and damage to the gastrointestinal tract, lymph nodes, and liver (Beaumont *et al.,* 2008; Adewuyi *et al.,* 2014)

Lead is one of the nonessential and toxic metals to human health. Discharge of Pb into surface water is mainly through paints, solders, pipes, building materials etc. (De, 2002). In the present study, Pb reported in drinking water from seven residential sites of Lucknow city varied between (0.15 – 0.58 mg/l). Maximum Pb concentration was observed at site 6 (0.58 mg/l) followed by site7 (0.57 mg/l) both during postmonsoon period and at site5 (0.46 mg/l) during the premonsoon period. Minimum Pb concentration (0.15 mg/l) was observed at sites1, 2 and 4 during the post monsoon periods. The Pb levels detected in water supplied to Lucknow city does not fall within the safe limits of Lead in water prescribed by BIS (2003). In this study, Pb content in drinking water was higher than reported by Bakirdere *et al.*, (2013) in tap and bottled water samples. Further, Parameswari and Mudgal, (2013) found the highest Pb contents in ground water samples in South India. Since Pb pollution in fresh surface water sources is also due to dry and wet deposition of atmospheric fallouts and highest in those water bodies adjacent to highway roads, this may be the probable reason for greater Pb concentration in water supplies from rivers such as Gomti.

Correlations between physicochemical parameters and heavy metals of potable water at the user end of Lucknow municipality

Correlation matrix between physicochemical parameters and heavy metals in the drinking water of residential sites of Lucknow was performed using Pearson correlation analysis (Table 4). Nitrate showed significant correlation with pH and conductivity at ($p \leq 0.01$) and with phosphate ($p \leq 0.05$). Nitrite exhibited significant positive correlation with nitrate and ammonium ($p \leq 0.01$). Phosphate also was significantly correlated with EC ($p \leq 0.01$). Among the studied heavy metals, Fe showed positive correlation with nitrite ($p \leq 0.05$). Cadmium was correlated with Cu ($p \leq 0.05$). However, Pb showed significant positive correlation with both ammonium and Cd ($p \leq 0.01$). Highest correlation among metals wasobserved for Cr with Cu and Pb ($p \leq 0.01$) and with Cd at ($p \leq 0.05$). Significant positive correlation between various parameters reveals the close association between the parameters and also their influence on one another. It is also 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 (Haloi and Sarma, 2012; Mansouri *et al.,* 2012; Agca *et al.,* 2014).

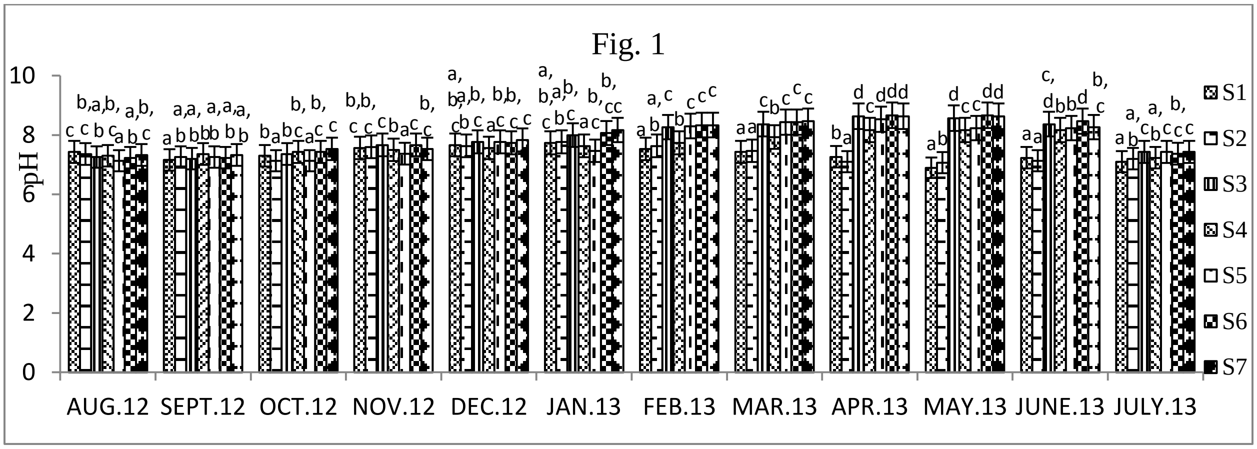


Fig. 1

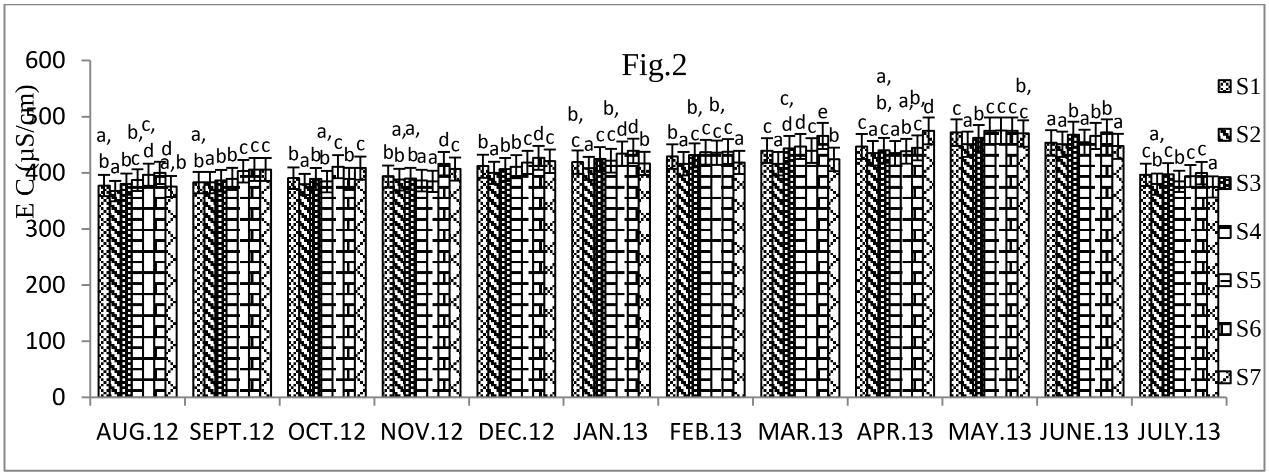


Fig.2

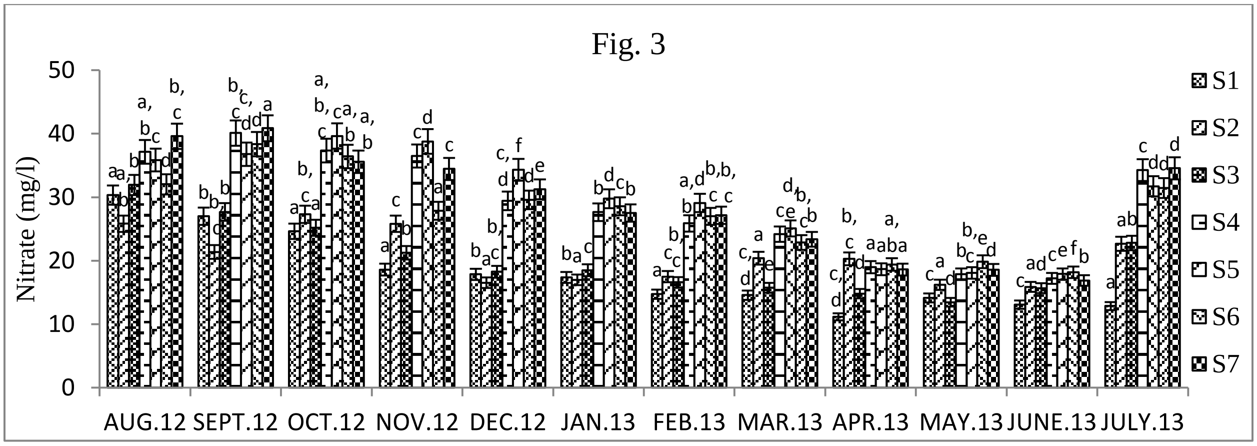
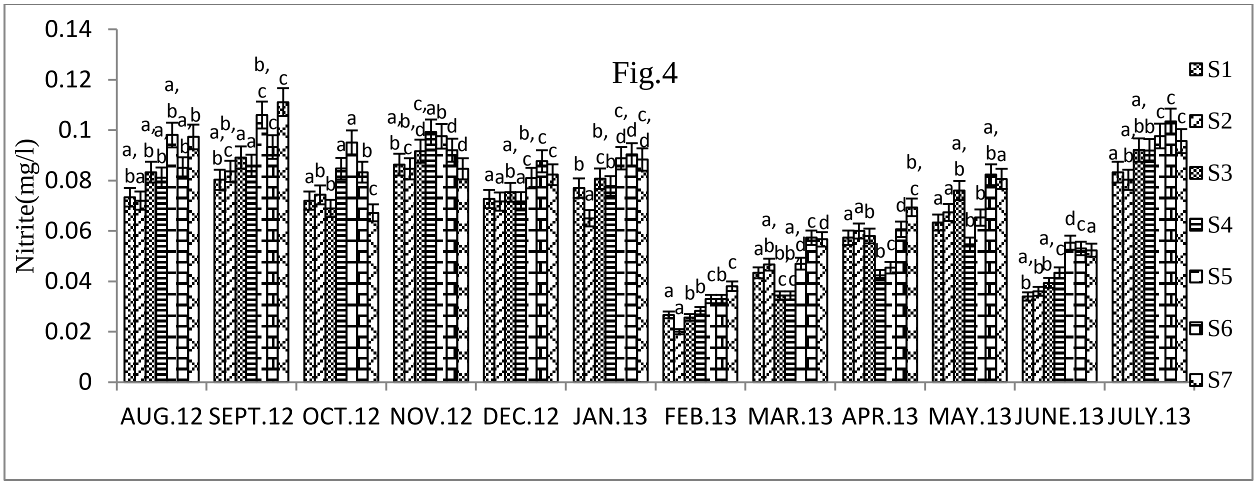
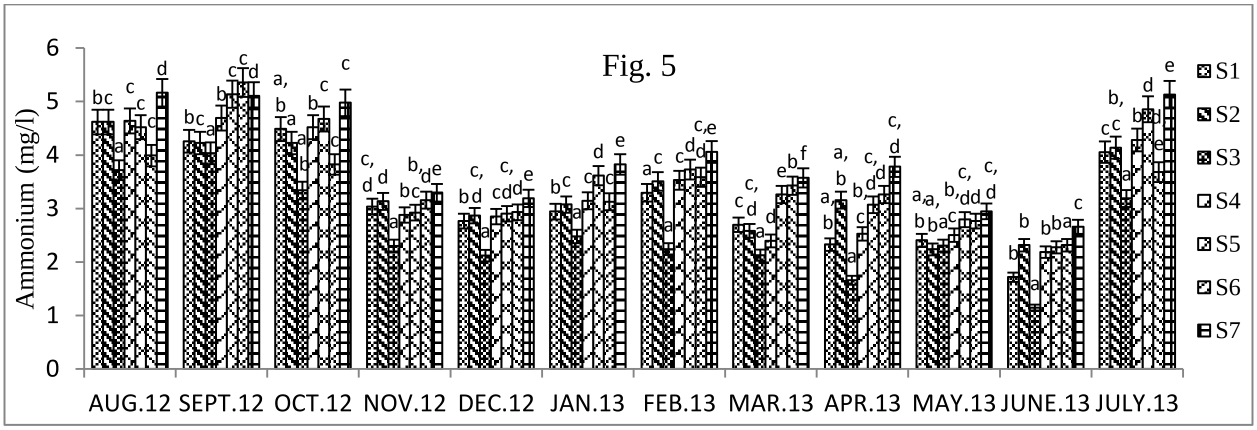
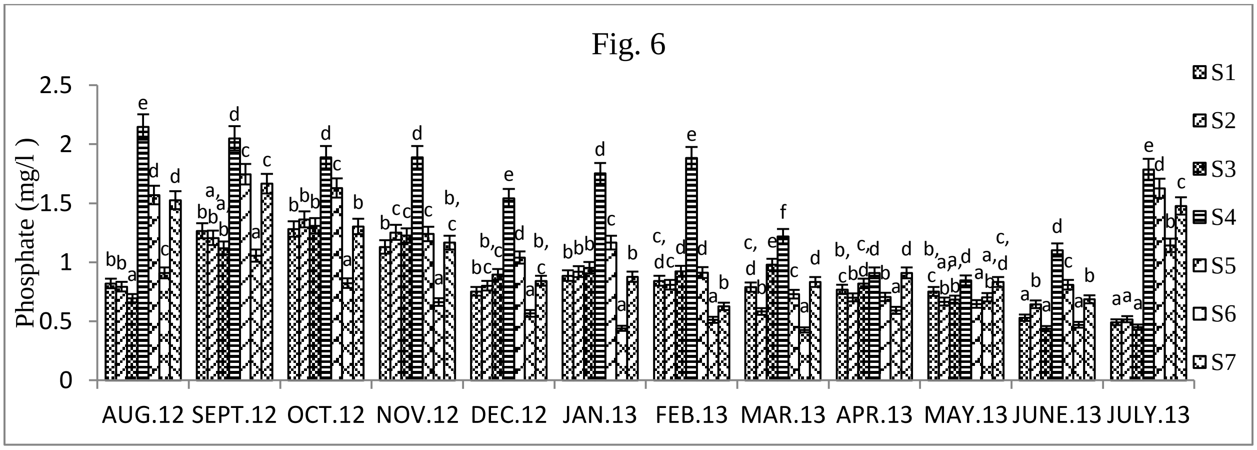


Fig. 3







(Fig. 1 to 6) Monthly variations in pH (Fig.1), EC (Fig.2), Nitrate (Fig.3), Nitrite (Fig.4), Ammonium (Fig.5) and Phosphate (Fig.6) of drinking water at user end of urban Lucknow (Different letters signify the statistical differences among physico-chemical parameters ($p < 0.05$). S1= Aminabad, S2=Indira Nagar, S3=Aishbagh, S4=Nishatganj, S5=Telibagh, S6=Hazratganj and S7=Charbagh.

Conclusions

Monitoring of specific inorganic contaminants and heavy metals in this study instead of plethora of parameters can be handy in reflecting the actual water quality supplied by the municipal corporation. Though 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. The present study reveals that the presence of inorganic pollutants and heavy metals in municipal water supplies of urban Lucknow is a major cause of concern for the public health. 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. From the present study, it is concluded that the existing water treatment procedures do not comply with the specific drinking water standards. Therefore, the potable water supplied by the municipal cooperation should be treated prior to supply, for optimal removal of hazardous pollutants, so that public health is protected.

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Phyto-remediation of Inorganic Pollutants from Aquatic Ecosystems

Abdul Barey Shah* and Rana Pratap Singh*

Abstract

Pollution of the bio-spheric media (air, water, soil), with toxic metallic and non-metallic inorganic pollutants, is a serious concern throughout the globe. The removal of heavy metals and excessive nutrients from water bodies is of great importance in the protection of aquatic biodiversity and human health. Various technologies are being employed to ameliorate the pollutants from water. Phytoremediation is an economical, ecofriendly and aesthetically pleasing technology that makes the use of plant systems to remove and/or detoxify pollutants from the environment. Phytoremediation of aquatic systems is gaining global interest for removing heavy metals and excessive nutrients from aquatic ecosystems by selective, improved and value added aquatic plants. This review is a critical analysis of the use of potential aquatic plants in phytoremediation.

Keywords: Phytoremediation, constructed wetlands, heavy metals, conventional treatments

Introduction

Water being the most precious gift of nature, is the life blood of the planet and the state of this resource affects all natural, social and economic systems (UNWWAP, 2009). As far as quantity is concerned, many countries have conflicts and others undergone bilateral/multilateral agreements for water sharing and distribution (Flint, 2004; Rai, 2012). It is said that if there would be any world war on the mother earth in future, it would be for possession of water and not for anything else. There is no doubt that water and sustainable development are inextricably linked. Once viewed as an infinite and bountiful resource, water today is considered as a resource in crisis. Without adequate supply and management of water resources, especially freshwater, not only socio-economic development will cease, but survival of life on earth will suffer greatly.

Water resources are sources of water that are useful or potentially useful. The total volume of water on earth is about 1400 million km^3, of which only 2.5% or about 35 million km^3 is fresh water (USNAS, 2008). Most of freshwater occurs in the form of permanent ice or snow or in deep underground aquifers. The principal sources of freshwater which can be available for human use are, Surface waters which include lakes, rivers, reservoirs and streams and Groundwater that is water underground in aquifers (highly permeable rocks, soil, and sand).

The usable portion of these sources is only about 200000 km^3 of water- less than 1% of all fresh water and only 0.01% of all water on earth (Martin *et al.*, 2006; Rai, 2009).Thus, fresh water is a critical, finite, vulnerable, renewable resource on the earth. It is reported that 2.6 billion people do not have access to safe drinking water and sanitation (Nelson and Murray, 2008; Rai, 2008) and further coupled with the depletion of freshwater resources could very well create the resource crisis of the 21st century bringing up agricultural, industrial, domestic as well as international conflicts (Shelef *et al.*, 2011). Lack of access to improved drinking water is still a serious problem in large portions of Asia where an estimated 675 million people are deprived of safe drinking water (UNICEF, 2004). In India, about 85 % of rural population is solely dependent on ground water, which is depleting at a fast rate and is also contaminated with many inorganic pollutants (Rawat *et al.*, 2012). In the urban areas though about 60% of the population is depended on surface water sources, the availability and quality of drinking water is not adequate.

Pollution of the aquatic ecosystems with inorganic pollutants like heavy metals and excessive nutrients has become a serious concern in the developing world (Basile *et al.*, 2012).The release of these toxic contaminants in the environment has dramatically increased as a result of anthropogenic activities mainly linked to burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and urban sewage (Singh *et al.*, 2011; Mojiri, 2012). Global water quality is rapidly being altered trough the disposal of inorganic contaminants,

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*Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Raebareli Road, Lucknow-226025

particularly deleterious heavy metals into lakes, ponds and reservoirs (Rai, 2008, 2010). The production processes carried out at high temperature introduce in the atmosphere metals both as vapours and dust particles that finally lay down on soil and into the water (Nguyen *et al.*, 2010). Amongst the pollutants many inorganic contaminants e.g., nitrate, nitrite, ammonia, phosphate, cadmium, mercury, arsenic, lead, chromium, nickel, zinc, copper etc, are more notorious being persistent and non biodegradable. They are not oxidised during the course of time in natural aquatic ecosystems but contaminate the physical, chemical and biological environment of aquatic ecosystem. Aquatic biodiversity is a significant component of aquatic ecosystem and get affected by the quality of water. The plants are more tolerant to the inorganic contaminants as they have differential capacity to assimilate or scavenge degradable and non-biodegradable inorganic contaminants. These potentials of plants have emerged as a major area of phytotechnological studies and they have been studied for the phytoremediation potential for removal of toxic contaminants from contaminated soil and water (Rai, 2010; Tolu and Atoke, 2012; Rawat *et al.*, 2012; Baudhh and Singh, 2012). This article contains a critical attempt to analyse the potentials of aquatic macrophytes for cleanup of surface water resources.

Sources and Human Health Impacts of Inorganic Water Pollutants: As a result of rapid industrialisation, increasing human population and urbanization has caused the occurrence and accumulation of pollutants in the environment by manifolds (Torresdey *et al.*, 2005; Martin Gonzalez *et al.*, 2006). Further, because of poor management plans more pollutants being introduced into water courses and an increasing number of surface water bodies and groundwater have come under serious threat of degradation. Human activities accelerate the rate at which excessive inorganic nutrients and heavy metals exceed their desired level in the aquatic ecosystems. According to EPA, 1998 types of pollutants entering the aquatic systems come from two source i.e., point sources which includes industries, sewage treatment plants, combined sewer overflows, resource extraction and land disposal and nonpoint sources which includes runoff from agricultural land, urban runoff, atmospheric deposition, and other human-related activities increase the flux of both inorganic nutrients including heavy metals and organic substances into aquatic ecosystems. According to Rajaram and Das (2008) and Annual Report of Central Pollution Control Board (2009) water polluting sources in India and their effect on ecosystems are summarised as under (Table I):

Table I. Different Sources of Water Pollution

Polluting Sources	Effect on Ecosystem
Domestic sewage from towns and cities	Organic pollution of rivers, eutrophication of lakes, water borne diseases.
Industrial effluents (point sources)	
Industrial and mines runoff	Organic and inorganic pollutants, toxic pollutants in food chain
Agricultural runoff	Fertilizers leading to eutrophication, pesticides in food chain

Among the inorganic pollutants, heavy metal and excessive nutrient contamination in water environment is a serious problem that threatens not only the aquatic ecosystem but also human health (Liu *et al.*, 2007). The various health hazards in human beings is due to the heavy metals owing to their property of persistence and biomagnifications (Rai2008c, Rai,2010, Apkor,2010).The heavy metals linked most often to human poisoning are lead, mercury, arsenic and cadmium (Singh *et al.*, 2011). Other heavy metals, including iron, copper, zinc, chromium etc., are actually required by the body in small amounts, but can also be toxic at larger doses. Table II, list some of the toxic heavy metals and nutrients with their harmful effects. (After Wang *et al.*, 2004; Johnson and Hallberg, 2005; Silvia *et al.*, 2006; Lon *et al.*,2008; Singh *et al.*, 2009; Rawat *et al.*, 2009, 2012 ; Apkor and Muchie, 2010).

Remediation Measures

To achieve unpolluted drinking water and management of water sources, several technologies and processes for the removal of persistent inorganic toxicants including nitrate, ammonia, phosphates and heavy metals are currently in use. These methods include ion exchange, chemical precipitation, disinfection, adsorption by activated carbons, reverse osmosis, nano-filtration and sewage treatment plant (STPs) processes. Most of these methods are expensive, require high energy and are not able to completely remove the heavy metals (Memon and Schroden, 2009) and excessive nutrients (Manuela *et al.*, 2010), but merely transfer the pollutants from the wastewater to a sludge residue which is disposed of by

Table II. Effect of heavy metals and certain plant nutrients on human health

Heavy metal	Effects on man
Cadmium	Damage to brain, Gastrointestinal and respiratory problems, Kidney and Liver damage
Arsenic	Cutaneous and Visceral Malignancies, Black Foot Disease, Severe Vomiting, Diarrhoea
Lead	Kidney damage, Heart ailments, Reproductive problems, Bone weakness
Mercury	Foetal Brain damage, Damage to Kidney, Lungs, Heart, Neurological problems
Chromium	Haemolysis, renal and liver failure, Allergies, Dermatitis, Foetal deaths, Lung cancers
Copper	Gastrointestinal distress, Liver or kidney damage (Long term exposure)
Iron	Increased pulse rates and respiration, hypertension, drowsiness, congestion of blood vessels
Zinc	Vomiting, renal damage, cramps
Manganese	Growth retardation, fever, sexual impotence, muscles fatigue, eye blindness.
Fluoride	Sponging/fluorosis of bones and teeth, metabolic alterations in soft tissues
Nutrients and their effects	
Nitrates	Methaemoglobinemia (Blue Baby Syndrome), Cancer, Alzheimer's disease, Neural tube defects
Nitrite	Carcinogenic, Reproductive and Developmental defects, heart and lung ailments
Phosphates	Kidney damage, osteoporosis
Ammonia	Taste and odour problems, decreased disinfection efficiency

land filling (Tolu and Atoke, 2012) whereby the pollutants eventually find their way to fresh water supplies there by contaminating it and thus, do not provide acceptable solution to pollution problem. Table III, lists the advantages and disadvantages of conventional methods of water treatment (Rai, 2009 and Apkor and Muchie, 2010).

Thus, conventional techniques do not harmonize with basic principles of water conservation, do not enable reclamation and reuse of water and nutrients, generate toxic sludge as by product and use chemicals, harmful to environment and people, in the treatment process (EPA, 2004). Developing cost effective and environmentally friendly technologies for the remediation of waters polluted with toxic substances is a topic of global interest. The use of plant species for cleaning polluted soils and waste waters named as phytoremediation has gained increasing attention from many researchers since last decade, as an emerging cheaper and ecologically friendly technology for metal removal and other inorganic contaminants such as nitrate, nitrite, ammonia etc. from bio-spheric media. Numerous plant species have been identified and tested for their natural traits in the uptake and accumulation of different heavy metals and nutrients. (Lone *et al.*, 2008). Aquatic macrophytes, which play important roles in aquatic ecosystems, have shown great potential to sequester selected heavy metals and nutrients through their root systems and by uptake through their plant bodies. It has been reported that these plants can

Table III. Conventional Methods of Water Treatment

Methods	Advantages	Disadvantages
Chemical precipitation	Convenient, self operation, low maintenance, low capital cost	Replenishment of chemicals, requirement of extra coagulation and flocculation, toxic sludge generation
Coagulation-flocculation	Settlement of suspended solids in less time, improved sludge settling	Extra operational cost for sludge disposal
Ion exchange	Less time consuming, no sludge generation, high metal removal efficiency, better performance in acidic pH range	Less suitable as few metals are not exchangeable through ion exchange resins, high capital cost
Reverse osmosis	Greater ionic species removal, can also operate at high temperatures, reduces the concentration of dissolved organic compounds	Expensive to procure and operate, elevated pressure makes the technique costly and sensitive to operating conditions
Nanofiltration	Operates at low pressures than reverse osmosis	Costly, membrane fouling

accumulate heavy metals 100,000 times greater than in the associated water (Mishra and Tripathi, 2008). Therefore, they have been used for heavy metal and nutrient removal from a variety of sources (Hassan *et al.*, 2007; Mishra and Tripathi 2008; Rai, 2010, 2012, Rawat and Singh, 2012).

Phytoremediation as a novel technique

Water treatment technology needs to be appropriate and sustainable. It also needs to be less costly, easy to operate and maintain and efficient in removing both inorganic and organic toxic pollutants. The use of special plants for environmental clean-up is an emerging technology known as phytoremediation (Dar *et al.*, 2011). Phytoremediation is based on the fact that a living plant can be considered as a solar driven pump, which can extract, concentrate, degrade, volatilize or vaporize soluble toxic substances from the soil, water or air through their natural water and mineral uptake, transport, partitioning, assimilation and transpiration systems (Singh *et al.,* 2007). It is also referred to as green remediation, botanoremediation, agro-remediation or vegetative remediation (Pivetz, 2001). Phytoremediation is an emerging technology that is rapidly gaining interest and promises effective and inexpensive cleanup of hazardous waste sites of water (Rawat *et al.*, 2009,2012) and soil(Baudhh and Singh, 2012) contaminated with heavy metals, excessive nutrients, hydrocarbons, pesticides, and chlorinated solvents. The removal of inorganic toxicants including nutrients and heavy metals from water through phytoremediation occurs by any of three mechanisms: rhizofiltration, phytoextraction and phytostabilisation (UNEP, 2010).

Role of Aquatic macrophytes

Using aquatic plants for water treatment is in line with the golden rules of sustainable development (Atoke and Tolu, 2012). Green plants are not only the lungs of nature with an ability of purifying impure air by photosynthesis but some species have also the unique ability to uptake tolerate and even hyperaccumulate heavy metals and other toxic substances from soil and water through their roots and concentrate them in roots, stems and leaves. Many researchers report that heavy metals are taken up by the roots of the plants and translocated to the shoots and other plant tissues where they are concentrated (Kelly and Guerin, 2008; Jadia and Fulekar, 2009). Aquatic plants have variety of capacity in removing and accumulating heavy metals and some plant species can take much more heavy metals than others such as Duckweed (Lemna minor, Yagmur and Taner, 2009; Divya *et al.*,2012), Cattail(Typha latifolia, Ye *et al.*, 2001), Common reed(Phragmites australis, Aslam *et al.*, 2007), Water hyacinth(Eichhornia crassipes, Tolu and Atoke, 2012). There are also literatures citing that some plant species can accumulate specific heavy metals and nutrients such as Spirodella polyrhiza and Polygonum hydropiper for zinc(Liu et al., 2007), Pistia strioates for nitrate and nitrite(Rawat *et al.*, 2010, 2012) and for cadmium and lead (Tomas *et al.*, 2011), Azolla pinnata for chromium(Rai, 2009) and mercury (Rai and Tripathi, 2009), Leptodictyum riparium for copper (Basile, 2012), Lemna minor for lead (Divya *et al.*, 2012), Typha domingensis for aluminium, iron, zinc and lead (Hegazy *et al.*, 2011)etc. To date more than 400 plant species from at least 45 families have been identified as metal hyperaccumulators. Hyperaccumulator are species capable of accumulating metals at levels 100 fold greater than those typically measured in shoots of the common nonaccumulator plants(Lone *et al.*, 2008). Aquatic plants have shown to improve the waste effluennts from oxidation ponds and integrated treatment systems(Liao and chang, 2004)and also removal of appreciable amounts of inorganic forms of nitrogen and phosphorus found in domestic sewage, industrial and municipal waste waters, municipal public water supplies(Jayaweera *et al.*, 2008; UNEP, 2010; Nesir, 2010; Rawat and Singh, 2009, 2012). Removal of biological oxygen demand (BOD) and suspended solids from water streams by aquatic plants have also been carried out successfully(Nesir, 2010).

Constructed wetlands are one of the many types of natural systems that makes use of aquatic macrophytes and can be used for treatment and control of water pollution. Since 1950's constructed wetlands have been used effectively to treat different waste waters with different confrigutions, scales and designs throughout the world. Constructed wetlands offers an alternative to conventional waste water treatment system. Constructed wetlands can be used for removal of various types of contaminats from municipal and industrial waste water(Krongroy *et al.*, 2012). In addition to treating water constructed wetlands can provide several advantages such as water reuse, landscape improvement, recreartion, biodiversity conservation and wildlife habitat maintenance(Jing *et al.*, 2008). Dilshad and Ahmad 2010, revealed that more replicated constructed wetland cells have the highest heavy metal removal efficiency.Constructed wetlands with emerging macrophytes are being managed as water quality improving systems. Liu *et al.*, 2007 designed a small scale plot constructed wetland and studied the uptake and distribution of Cd, Pb and Zn by 19 wetland plants and revealed that the removal efficiency of metals from water was more than 90%.

Table IV, lists some common aquatic macrophytes used for heavy metal removal from aquatic ecosystems. (Data collected from Liao and Chang, 2004; Prasad *et al.*,2005; Hadad *et al.*,2006; Rai,2007; Rai and Tripathi, 2007a, 2009; Rehman *et al.*, 2008; Zhang *et al.*, 2009; Dilshad *et al.*, 2010; Abida and Hari, 2010; Amin Mojiri, 2011; Usman *et al.*, 2011; Hamizah Mokhtar *et al.*, 2011; Hegazy *et al.*, 2011).

Table IV. Some heavy metal accumulating Aquatic Macrophytes

Aquatic Macrophytes	**Heavy Metal Accumulation**
Azolla fililiculoids	Cr, Ni, Zn, Fe, Pb
Azolla pinnata	Cd, Cr, Zn, Hg
Ceratophyllum demersum	Cu, Cr, Pb, Hg, Fe, Mn, Zn, Ni
Eichhornia crassipes	Cd, Pb, Cu, As, Ni, Cr, Zn, Hg, Co, Al
Hydrilla vertticillata	Cu, Hg, Fe, Ni, Pb
Lemna spp.	Pb, Mn, Cu, Cd, Cr, Hg, Ni, Fe
Mentha aquatica	Cd, Zn, Cu, Fe, Hg
Nymphaea alba	Cr, Cd, Pb, Ni, Zn, Mn, Fe,Co
Phragmitis australis	Fe, Mn, Zn, Cu
Potamogeton crispus	Cu, Pb, Mn, Fe, Cd
Salvinia spp	Cu, Fe, Ni, Zn
Spirodela polyrrhiza	As, Hg
Typha domingensis	Fe, Mn, Zn, Al, Ni
Wolfia globosa	As

Table V, mentions the removal of inorganic nutrients by some aquatic plants from water. (Kutty *et al.*, 2009; Esi Awuah *et al.*, 2010; Abida *et al.*, 2010; Rawat *et al.*, 2009; Singh *et al*; 2012)

Table V. Nutrient removal by some common macrophytes

Aquatic Macrophytes	Inorganic Nutrient removal
Azolla pinnata	Nitrate, Phosphate
Eichhornia crassipes	Ammonia, Phosphates and Nitrate
Lemna spp	Ammonia, Nitrate
Pistia stratiotes	Nitrate, Nitrite, Ammonia, Phosphates
Slavinia spp	Nitrate, Phosphates
Trapa natans	Nitrite, Nitrate
Typha latifolia, *Ceratophyllum demersum*	Phosphates

Factors affecting Phytoremediation process

The efficacy of the detoxification or remediation function of aquatic macrophytes depends on a) sediment geochemistry, b) water physico-chemistry (Adriano *et al.*, 2004), c) plant physiology (Prasad, 2004) d) plant genotype and e) nature of contaminant or pollutant (Pilon-Smiths, 2005). Trace element and nutrient removal by wetland vegetation can be greatly enhanced by selection of appropriate wetland plant species. The selection is based on the types of elements to be remediated, the geographic location, microclimate, hydrologic conditions, soil properties, and known accumulation capacities of the species. Knowledge of the capabilities of different wetland plant species to absorb and transport trace elements under different conditions is important to know.

Conclusions and future prospects

The minimization of the health and environmental impacts of the presence of inorganic pollutants (heavy metals and nutrients) in aquatic systems requires the application of different treatment processes. This has necessiated the need for economically viable and effective technologies in order to supply safe drinking water and discharge wastewater , that preserves precious natural resources and biological lives. Phytoremediation is one new approach that offers more ecological benefits and a cost-effective alternative. Though, a cheaper method, it requires technical strategy, expert project designers and with field experiences that choose the proper species and cultivators for particular toxic pollutants(metals, nutrients organics) and regions. Phytoremdiation technoogy needs more attention in various areas such as gene manipulation, harvesting and recycling tools. A multidisplinary research effort that integrates the work of natural sciences, environmental engineers and policy makers is essential for greater success of green technologies as a potent tool for inorganic pollutants management in aquatic ecosystems.

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