PHYTOREMEDIATION OF INORGANIC CONTAMINANTS IN VELLAYANI WETLAND ECOSYSTEM

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"PHYTOREMEDIATION OF INORGANIC CONTAMINANTS IN VELLAYANI WETLAND ECOSYSTEM"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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MEERA. A.V.

In loving memory

of

my Father

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LIST OF ABBREVIATIONS

$^{\circ}C$	Degree Celsius	LC – MS/MS	Liquid chromatography- mass spectrometry
µg	Microgram	kg	Kilogram
Al	Aluminium	K	Potassium
av.	Available	m	Metre
AMF	Arbuscular Mycorrhizal Fungi	mg	Milligram
BCF	Bioconcentration factor	mL	Millilitre
BDL	Below detectable limit	mm	Millimeter
BOD	Biological oxygen demand	MPL	Maximum permissible limit
Cd	Cadmium	MPN	Maximum probable number
CD	Critical difference	N	Nitrogen
CEC	Cation exchange capacity	NH_4	Ammoniacal
CFL	Compact Fluorescent Lamp	No.	Number
cfu	Colony forming units	NO_3	Nitrate
cg	Centigram	NS	Non significant
COD	Chemical oxygen demand	NTU	Nephelometric turbidity unit
CRD	Completely Randomized Design	OC	Organic carbon
DAP	Days after planting	OM	Organic matter
DAT	Days after transplanting	P	Phosphorus
DO	Dissolved oxygen	Pb	Lead
dS	deci Siemens	PGPR	Plant Growth Promoting Rhizobacteria
EC	Electrical conductivity	PO_4	Phosphate
EE	Extraction efficiency	PS I	Photosystem I
EMB	Eosine methylene blue	PSA	Primary Secondary Amine
et al.	And others	QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
Fe	Iron	ROS	Reactive oxygen species
FYM	Farm yard manure	rpm	Revolutions per minute
g	Gram	SP	Sampling point
GC-MS	Gas Chromatography- Mass Spectrometry	t	Tonnes
GIS	Geographic Information System	TDS	Total dissolved solids
ha	Hectare	TF	Translocation Factor
HDPE	High density poly ethylene	USEPA	United States Environmental Protection Agency
Hg	Mercury	viz.	Namely
ISI	Indian Standards Institute	WHO	World Health Organization

Introduction

1. INTRODUCTION

Aquatic resources constitute one of the vital assets of a country. Among all the renewable resources of the planet, water occupies a unique place owing to its essential role in sustaining all life forms. Existing and newly created developments have damaged the quality of nearly most of the streams, rivers and lakes. As Earth's population continues to grow, people are putting ever-increasing pressure on the planet's water resources. In a sense, our water bodies are being "squeezed" by human activities—not so they take up less room, but so their quality is reduced. Poorer water quality means water pollution.

Water pollution is one of the severest issues India is facing right now. The single biggest reason for water pollution is urbanization at an uncontrolled rate. The situation in Kerala is more critical since the state is one among the most thickly populated regions in the world and the population is increasing at a rate of 14% per decade (Harikumar *et al.*, 2017). As a result of the measures to satisfy the needs of the huge population, the aquatic resources of Kerala have been increasingly polluted from the wastes of industrial, domestic and agricultural sectors.

Accelerating industrialization accompanied by uncontrolled disposal of solid wastes and vast consumption of toxic substances which is a growing environmental concern here, posing risks to humans and other organisms. The developments in the last few decades have added huge loads of pollutants especially toxic metals in the water resources and are manifested in the form of fish mortality and depletion of bracts (Bijoy, 2004). Toxic metals unlike organic pollutants are persistent in nature; therefore tend to accumulate in the different components of the environment and makes entry into food chain.

Vellayani wetland ecosystem lies between 8° 24' 09'' to 8° 26' 30'' N latitude and 76° 59' 08'' to 76° 59' 47''E longitude at an elevation of 29 m above mean sea level. This lake is unique in terms of the fresh water it provides and is the sole drinking water source for Kovalam, Vizhinjam, Pachalloor, Vellayani and adjoining areas. Unaware of ecological consequences, this wetland ecosystem is being subjected to indiscriminate anthropogenic interventions like discharge of wastes, intensive agriculture, urbanisation, sand mining, tourism related activities, water sports etc. leading to qualitative and quantitative deterioration of the lake and loss of ecological balance. An earlier study carried out at College of Agriculture, Vellayani on lake water quality indicated wide spread contamination of Al and local contamination of heavy metals like Fe, Pb and Cd (Kamal, 2011). Bio concentration and magnification could lead to high toxicity of these metals in organisms. Apart from their role in ecosystem destabilization, the accumulation of these toxic metals in aquatic food web poses threat to public health together with a long term impact on the integrity of ecosystem.

The heavy metals are released from a variety of sources such as mining, urban sewage, smelters, tanneries, textile industry and chemical industry, automobile servicing centers etc. Their removal usually requires treatment through some technology and the common technologies used for their treatment include reverse-osmosis, ion-exchange, electrodialysis, adsorption etc. Most of them are quite costly, energy intensive and metal specific. Such unprecedented pollution in aquatic ecosystems needs eco-friendly cost-effective remediation technology. Phytoremediation *i.e.* removal of pollutants by the use of plants offers a promising technology for toxic metal removal from contaminated water.

Phytoremediation is a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, with the aim of restoration of contaminated sites. It is largely focused on the use of plants to accelerate degradation of organic contaminants or remove hazardous heavy metals from soils or water. Phytoremediation of contaminated sites is a relatively inexpensive and aesthetically pleasing to the public compared to alternate remediation strategies involving excavation / removal or chemical *in situ* stabilization / conversion.

Vellayani wetland ecosystem is gifted with a diverse group of flora and fauna. Utilizing this flora to decontaminate the fresh water ecosystem and maintain its quality is a matter of great concern under the present circumstances. Phytoremediation capacity of dominant macrophytes of the system viz., *Eicchornea crassipes* for Fe, Al, Cu and Cd and *Nymphoides indica* for Cd and Pb has already been reported (Kamal, 2011). The extent of toxic metal uptake, pattern of distribution and their residence time within plants, and potential release of metals are the major factors deciding the suitability of macrophytes as phytoremediators. Since plant species can differ in rates of metal uptake, allocation and excretion, metal dynamics in wetlands may be influenced by the composition of plant communities. Phytoremediation is considered as an effective, low cost, preferred cleanup option for moderately contaminated areas.

But the major problem encountered in phytoremediation technology is the disposal of the remediated or phytoextracted biomass. Biological methods like composting / vermicomposting and thermal methods like ashing, pyrolysis, incineration, biochar production etc. are being carried out for the disposal of the phytoextracted biomass.

In this context, the present study was undertaken with the following objectives:

a) to track the potential sources of contaminants threatening the Vellayani wetland ecosystem.

b) to suggest viable phytoremediation technologies for decontamination.

Review of literature

2. REVIEW OF LITERATURE

Environmental pollution is one of the greatest problems that the world is facing today, increasing with every passing year and causing grave and irreparable damage to the earth. The resultant ecodegradation happens through five basic types of pollution, namely, air, water, soil, noise and light. Although pollution had been known to exist for a very long time, it had seen the growth of truly global proportions only since the onset of the industrial revolution during the nineteenth century. The industrial revolution brought with it technological progress which had probably become one of the main causes of serious deterioration of natural resources. Factors such as population growth and urbanization invariably place greater demands on the planet earth and stretch the use of natural resources to the maximum. Among the natural resources, the degradation is being manifested in its peak on water resources. Almost all the aquatic resources of the country are experiencing different levels of contamination. The research works carried out in the extent of aquatic degradation and possible ways of phytoremediation are reviewed here.

2.1. ECODEGRADATION OF AQUATIC SYSTEMS

Aquatic resources are the fundamental components for the existence of human beings on earth and fresh water resources contribute only 2.5 percentage of their share. India is blessed with a rich wealth of wetland ecosystems which can support diverse and unique habitats and ecological goods and services (Prasad *et al.*, 2002). But they are under tremendous pressure due to over exploitation resulted from rapid urbanization, industrialization and intensification of agriculture leading to an imbalance in the hydrological, economic and ecological functions they perform (Bassi *et al.*, 2014). Small water bodies like lakes and ponds have a great impact on weaker sections of society by providing income to them and influencing their health (Kumar *et al.*, 2013).

Human interventions in the name of urbanization, industrialization and agricultural activities in an intense scale coupled with disposal of sewage and wastes have caused great impact on these precious resources all over the world. Entry of contaminants into the fresh water bodies through organic and inorganic forms have resulted in pollution of over seventy percentage of Indian surface as well as ground water bodies (Joseph and Claramma, 2010; Chandrasekhar *et al.*, 2014). Wetlands act as sink for contaminants and thereby reduce the impact of point and non-point sources of pollution (Bystorm *et al.*, 2000). But drastic reduction in water inflow has been resulted due to fragmentation of water bodies (Zhao *et al.*, 2006) and large number of water bodies irreversibly converted (Khandekur, 2011).

Aquatic resources of India maintain a rich biodiversity (Molur *et al.*, 2011) and serve as breeding sites for wildlife and provide a refuge for migratory birds (Lalchandani, 2012). Protecting the rich biodiversity of lakes from over exploitation is a must to maintain the ecological sustenance of any nation. They form the habitat for a wide variety of fish and hence all efforts should be taken to preserve them from illegal human interventions, mainly improper waste disposal (Reddy, 2014). Only 26 wetlands of India have been included as Ramsar sites demanding urgent interventions for restoration (Ramsar Convention Secretariat, 2013) though a great portion of our wetlands are under the threat of degradation due to over and misuse of resources by the fast growing population (CPCB, 2008). Another factor that has a great impact on water resources is encroachment in the name of tourism promotion and development (Verma, 2001).

Major reasons for the ecological setback are high population growth and the resultant air and water pollution, unsustainable agricultural activities, non uniform distribution of basic resources and malnutrition and third world debt-crisis. Another important destructional activity is sand mining (Bassi, 2016). Minimizing exposure to environmental risk factors by enhancing air and water quality can contribute significantly to the achievement of the Millennium Development Goals for sustenance of environment, health and development (Tyagi *et al.*, 2014).

2.2. WETLAND RESOURCES OF KERALA

Ground water has much impact on contributing water to lakes, rivers and other wetlands and any adverse effect on surface water quality can affect the quality of ground water as well. In Kerala, ground water resources have very important role as the people depend on these resources for all socio-economic activities (Shaji, 2011). Over use of chemicals in agriculture, improper disposal of solid wastes, retting of coconut husk and sand mining have resulted in large scale deterioration of wetlands of Kerala (Varma *et al.*, 2007; Harikumar, 2012). Sobha *et al.* (2009) reported the heavy metal contamination of wet land resources of Thiruvananthapuram district and Sreebha and Padmalal (2011) about the poor water quality of Chalakudy, Periyar and Muvattupuzha rivers due to sand mining and other illegal activities. Indiscriminate sand mining affected the quality of Neyyar river and affects socio-economic status of people nearby (Shaji and Anilkumar, 2014).

Intense agricultural activities (Agrawal *et al.*, 2010) and dumping of hotel / slaughter house / domestic wastes have resulted in much deterioration of Sasthamcotta lake (George and Koshy, 2008). Ashtamudi and Vembanad lakes are also in a deteriorated condition (Sitaram, 2014) as evidenced by the low level of dissolved oxygen (DO) which may affect the biodiversity of the lake (Sujatha *et al.*, 2009; Suma *et al.*, 2011). Indiscriminate sand mining over the years had adversely affected the ground water recharge capacity of the lake (Vishnu *et al.*, 2015). Kavvayi wetlands, the biggest wetland ecosystem of northern Kerala is under the threat of pollution hazards due to indiscriminate human activities as revealed by the presence of heavy metals like Fe, Mn, Cu, Pb and Cd in both water and sediments and organic carbon (OC) pesticides like aldrin. Coliform bacteria were also detected from certain sites (Harikumar, 2016).

Heavy metal contamination (Fe, Pb and Cd) was noticed in Meenachil river (Nair *et al.*, 2011). Presence of heavy metals in excess of the desirable units was detected in Vembanad lake, Kerala (Sobha *et al.,* 2011b; Thampatti and Beena, 2014).

2.3. VELLAYANI WETLAND ECOSYSTEM

Vellayani wetland ecosystem comprising a fresh water lake and adjoining padasekharams extends through three panchayaths namely, Kalliyoor, Pallichal, Venganoor and two zones of Thiruvananthapuram Corporation namely, Nemom and Thiruvallam. The area has been drastically reduced to less than 240 ha (Job *et al.*, 2014) from 750 ha in 1926. It is the only fresh water source to the people of Venganoor, Vizhinjam, Pachalloor, Kovalam, Vellayani and nearby areas (KAU, 2009; Sobha *et al.*, 2011a).

Lake water was found to be contaminated with organic residues as evidenced by high BOD, NH_4-N, Al and heavy metals. *E. coli* was also found to be beyond the maximum permissible limit (MPL). Proper treatment measures must be carried out to ensure the drinking status of the water (Kamal, 2011). Land degradation of the ecosystem was also mentioned by Meera and Thampatti (2016). This precious water resource is currently under the threat of pollution owing to unplanned developmental activities and constructions in the name of tourism. It is severely affected by encroachment and illegal sand mining (Gopinath and Kumar, 2014). Dharmapalan (2014) has reported on the poor quality of lake water during pre monsoon season owing to increased pH, Fe, total dissolved solids (TDS), DO, BOD, PO_4 (phosphate) and NO_3 (nitrate) content. Presence of allochthonous materials lowered the values during monsoon season (Radhika *et al.*, 2004; Radhika and Devi, 2007).

Comparative analysis of two fresh water lakes of Kerala viz., Sasthamcotta and Vellayani lake revealed more accumulation of NO_3, PO_4 and Fe contents in Vellayani lake compared to Sasthamcotta lake due to intensive agricultural activities

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(John *et al.*, 2014). According to Reghunathan *et al.* (2016), the carrying capacity of Vellayani lake has been reduced drastically and there are several factors operating in and around the lake system that adversely affect the quality of lake water. Gopinath and Kumar (2014) and Lekshmi *et al.* (2014) also reported the deteriorated condition of Vellayani lake. Comparative analysis of Vellayani and Veli lakes of Trivandrum district revealed that condition of fresh water lake, Vellayani is far better than the other which is situated in the middle of the city (Abhijna, 2016).

Freshwater ecosystems are the most important functional units that sustain human life on earth. Main reason for deterioration of these fresh water resources is the lack of awareness among the people about the value of the ecosystem services they provide. This fresh water lake supports livelihood for people living in and around the lake system (Vijayan *et al.*, 2015).

2.4. SOURCES OF CONTAMINATION

Rapid enhancement in the population rate and improper effluent discharge from industries and sewage drains without adopting any pre treatment are the major factors contributing to the pollution of water bodies (Kahlown *et al.* 2006). The chemical nature of pollutants and sediments that enter the aquatic system from various sources is also a major problem of many water bodies in India (Sharma *et al.*, 2010). Aquatic bodies of India are under varied stages of degradation due to indiscriminate human activities which include rapid urbanization, encroachment, dumping of domestic wastes, sewage drains, industrial effluents and over use of chemicals in agriculture (Ramya *et al.*, 2012). Lake water pollution resulting from intense agricultural activities has become a common phenomenon (Sharma *et al.*, 2014) and heavy load of N and P were detected in Lake Uluabat basin, a fresh water lake situated in Marmara region of Turkey (Katip and Karear, 2013).

Encroachment of reservoir area for urban development and excessive diversion of water for agriculture are major reasons that contribute to deterioration of wetland resources (Verma, 2001). Release of pesticides and fertilizers from agricultural fields and discharge of waste water from domestic area has resulted in widespread eutrophication of most of the water bodies of Asia (Prasad *et al.*, 2002; Liu and Diamond, 2005). Some common examples for deterioration of lakes due to encroachment include Charkop lake (Maharashtra), Ousteri lake (Puducherry), Pallikaranai marshland (Bangalore) (Prasad *et al.*, 2009).

Release of dishwasher detergents and laundry wastes can cause eutrophication problem due to enrichment of nutrients mainly phosphates and can adversely affect aquatic fauna due to depletion of oxygen. This necessitates the importance of treating sewage for removal of PO_4 and NO_3 (Pattusamy *et al.*, 2013). Water bodies near brick kiln industries in the state of Assam are in a highly degraded condition and had adversely affected the biodiversity (Dey and Dey, 2015). Large scale reclamation of ecosystem for agricultural purposes and diversion of domestic wastes and sewage effluents directly to water have enhanced the pollution of Kolleru lake, Andhra Pradesh (Reddy, 2014). Mahajan and Billore (2014) reported heavy eutrophication in Nagchoon pond of Khandwa district, Madhya Pradesh.

2.5. HEAVY METAL POLLUTION

Heavy metal pollution in water bodies is a serious environmental problem, threatening not only the aquatic ecosystems, but also human health. As heavy metals are non-biodegradable, removal of these metals from the aquatic system is the only remedy available for water decontamination (Cheng *et al.*, 2002). Activities in connection with religious performances have drastically affected the water quality of Waral Devi lake, Thane, Mumbai showing higher levels of heavy metals viz., Cu and Zn immediately after festival occasion (Momin *et al.*, 2014).

The biomagnification of heavy metals within aquatic organisms and its adverse effects on the nervous, reproductive and cardio vascular systems has been reported (Mishra *et al.*, 2006). Disposal of plastic wastes, batteries, fertilizer materials, untreated industrial effluents etc. releases heavy metals including Cd into the aquatic environment which causes several causalities like osteoporosis, kidney failure, infertility and improper brain development (WHO, 2011).

Wide spread pollution due to the presence of heavy metals like Fe, Zn, Pb, Cd, Ni, Cu and Cr in water bodies of India is very common (Lokeshwari and Chandrappa, 2006). Higher concentration of Fe, Pb, Cu, Ni, Al, and Na above MPL in Bholakpur area of Hyderabad (Rasheed *et al.*, 2012); excessive levels of Fe and Cr in Cauvery river (Rajendran *et al.*, 2015); Pb contamination in river bodies receiving untreated effluents from sugar factories (Qureshi *et al.*, 2015); Cd, Cu, Zn and Al contamination in ground water near hospital areas of Kanyakumari district (Shylasree and Indirani, 2014) have already been reported.

2.6. BIOLOGICAL CONTAMINATION

Drinking water sources should be devoid of any pathogenic microorganisms and presence of coliform bacteria in water is an indication of faecal contamination (WHO, 1993). Water bodies throughout the country seem to be contaminated with coliforms which indicate the direct entry of sewage drains into the lake. Heavy contamination with coliform bacteria in river Ganges and river Yamuna (Singh *et al.*, 2013) has already been reported. *E. coli* population in river Pamba was particularly very high during the pilgrimage season though the river was infested with coliforms all the year round (Jalal and Kumar, 2013). According to Kumar *et al.* (2015), immediate interventions are needed to prevent further degradation of Achencovil river, Kerala as it is polluted with coliforms. Water from Vellayani lake, Trivandrum also needs proper treatment prior to consumption due to coliform contamination (COA, 2009; Kamal, 2011).

2.7. ABATEMENT OF POLLUTION THROUGH PHYTOREMEDIATION

Several health hazards are associated with the contamination of water bodies with heavy metals. But the conventional methods employed to remove the metals from a polluted system like coagulation, flocculation, osmosis, stabilization etc. are highly expensive. In addition they further aggravate deterioration with the release of chemicals being used and hence these methods are not at all environmentally safe (Rai, 2009).

A fast emerging 'green technology' which employs the use of plants to decontaminate or remove toxic metals from a system is referred as phytoremediation. Phytoremediation is an ideal technology for wetland restoration. The plants growing in the contaminated areas will absorb the elements from the sediment / soil / water by roots. The absorbed elements travel from root through cell sap and finally get precipitated in vacuole or cell membrane, thereby reduces the level of contamination in sediment / soil / water (Cunningham and Ow, 1996). It is relatively cheap and is very successful over other methods (Qian *et al.*, 1999; Lasat, 2002; Peer *et al.*, 2005). The concept of extraction of metals by macrophytes was actually given by Chaney (1983). Efficiency of macrophytes to extract metal from contaminated site depends on the metal hyperaccumulation capacity and biomass production (McGrath and Zhao, 2003).

The ability of a species to carry out effective remediation of heavy metals depends on biomass production (Liu *et al.*, 2014; Zhang *et al.*, 2014). According to Evlard *et al.* (2014) biomass production alone cannot substantiate the remediation potential of plants. Free floating macrophytes have more phytoremediation potential compared to emergent types as revealed by the high bioconcentration factor (BCF) and translocation factor (TF) obtained for them (Ndeda and Manohar, 2014). The potential to produce huge biomass and easy uprooting makes water hyacinth (Das *et al.*, 2016) to play an efficient role in phytoremediation.

Thampatti and Beena (2014) reported the poor quality of water in Kuttanad, Kerala due to contamination with NO_3-N, S, Fe, Mn and Al. They evaluated different aquatic macrophytes for their phytoremediation ability and found *Hydrilla verticillata* as the best phytoextractor for Fe, Zn, Cu and Al followed by *Eichhornea crassipes*; *Cyperus sp* as good phytoextractor for Mn and *E. crassipes* for Cd and Pb.

2.8. METHODS OF PHYTOREMEDIATION

Depending upon the process by which plants / microbes are removing or reducing the toxic effect of contaminants from the soil and water, phytoremediation technology can be broadly classified as follows (Prasad, 2011).

a) Phytoextraction or phytoaccumulation - refers to the uptake and translocation of metal contaminants in the soil by plant roots with subsequent transport to the aerial plant organs. Certain plants called hyperaccumulators absorb unusually large amounts of metals in comparison to other plants and concentrate them in the aerial portions (Chaney, 1983; Baker *et al.*, 1994; Brooks *et al.*, 1998; Salt *et al.*, 1998).

b) Phytosequestration -It is the ability of plants to sequester certain contaminants in the rhizosphere through exudation of phytochemicals and on the root through transport proteins and cellular processes. It reduces the mobility of the contaminant and prevents migration to soil, water, and air through either phytochemical complexation in the root zone or transport protein inhibition on the root membrane and / vacuolar storage in the root cells (Cunningham *et al.*, 1997).

c) Rhizofiltration - It is the adsorption or precipitation of contaminants onto plant roots or absorption into the roots that are in solution surrounding the root zone. The acclimatized plants against contamination are planted in the contaminated area and the roots extract the contaminants along with water. As the roots become saturated with contaminants, they are harvested and incinerated (Dushenkov, *et al.*, 1995; Zhu *et al.*, 1999; Raskin and Ensley, 2000; Gardea-Torresdey *et al.*, 2004).

d) Phytodegradation or phytotransformation: It is the breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of compounds (such as enzymes) produced by the plants. Pollutants are degraded, incorporated into the plant tissues,

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and used as nutrients. In phytodegredation, organic pollutants are converted by internal or secreted enzymes into compounds with reduced toxicity (Black, 1995; Salt *et a*l., 1998; Suresh and Ravishankar, 2004).

e) Rhizodegradation - It is the breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the rhizosphere and is a much slower process than phytodegradation. Microorganisms (yeast, fungi, or bacteria) consume and digest organic substances for nutrition and energy (Kuiper *et al.*, 2004; Yadav *et al.*, 2010).

f) Phytostabilization: It is the use of certain plant species to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone. This process reduces the mobility of the contaminant and prevents migration to the ground water or air, and it reduces bioavailability for entry into the food chain (Berti and Cunningham, 2000; Stoltz and Gregor, 2002).

g) Phytovolatalization- It is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant. In this process, the soluble contaminants are taken up with water by the roots, transported to the leaves, and volatized into the atmosphere through the stomata (Newman *et al.*, 1997; Davis *et al.*, 1998). The best example of this is the volatilization of mercury (Hg) by conversion to the elemental form in transgenic *Arabidopsis* and yellow poplars containing modified bacterial mercuric reductase (*merA*) (Heaton *et al.*, 1998; Rugh *et al.*, 1998; Dushenkov, 2003).

The dominating families that include hyperaccumulators are Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae. Brassicaceae had the largest number of taxa viz. 11 genera and 87 species. *Thlaspi* species are known to hyperaccumulate more than one metal *i.e. T. caerulescence* - Cd, Ni, Pb, and Zn; *T.*

goesingense - Ni and Zn and *T. ochroleucum* - Ni and Zn and *T. rotundifolium* - Ni, Pb and Zn (Prasad and Freitas, 2003). Aquatic plants in freshwater, marine and estuarine systems act as receptacle for several metals. Several aquatic macrophytes viz., *Eichhornea crassipes, Hydrilla verticillata, Typha angustata, Ipomoea aquatica* etc. can remove Zn, Cu, Pb, Ni and Cd from lakes and maintain water quality (Weis *et al.,* 2004; KAU, 2008; Kamal, 2011, Anil *et al*., 2013, Thampatti *et al*., 2016).

A study conducted at College of Agriculture, Vellayani (Thampatti *et al*., 2007; KAU, 2009) also revealed the phytoremediation potential of *Bacopa monnieri* and *Hydrocotyl asiatica* for Fe, Al, Zn and Cd. Fe and Zn were accumulated mainly in root and Cd and Zn in shoot portion. The metal extraction by the plants enhanced with increasing levels of the above metals in soil. The phytoremediation potential of *B. monnieri* and *H. asiatica* has to be looked seriously since these plants possess high medicinal value and is an important ingredient of many ayurvedic preparations. Thampatti *et al.* (2016) reported the good phytomining ability of *Eichhornea crassipes* for Fe from acid sulphate soils of Kuttanad.

Among the different methods of phytoremediation, phytoextraction by hyperaccumulators is the most efficient one as it helps in removal of the phytoextracted biomass from the contaminated sites (Sun *et al*., 2011). But phytoremediation cannot be used as a primary treatment method for highly contaminated areas with heavy metals like Cd, Zn, Cr and Pb, because of the prolonged time period required for the complete clean up. Crop plants even possess hyperacumulation capacity and it was found that corn was better than sunflower for Zn, Cd and Pb accumulation (Mathew, 2001).

2.9. PHYTOREMEDIATION ABILITY OF EMERGENT MACROPHYTES

Al accumulation potential of *Typha domingensis* was reported by Hegazy *et al.* (2011) and it was retained mainly in the root indicating the rhizofiltration potential. According to Othman *et al.* (2014), *Riccia fluitans* is a very effective phytoremediator capable of removing pollutants viz., Pb, Mn and Zn from aquatic environment. *Typha latifolia* possess the ability to remove Al from contaminated environment (Kumari *et al.*, 2016).

According to Rachmadiarti *et al.* (2012), *Limnocharis flava* possess the ability to remove higher quantity of Pb as evidenced by BCF>1 and TF<1. *L. flava* has an effective potential in removing heavy metals Pb and Cd from waste water and these metals were accumulated in greater quantities within the roots (Bindu *et al.*, 2010; Anning *et al.*, 2013). The phytoremediation potential of *L .flava* for Fe was reported by Kamrudzaman *et al.* (2012). According to Marbaniang and Chaturvedi (2014), the emergent aquatic macrophyte *Scirpus mucronatus* possess phytoremediation capacity for the heavy metal, Pb. The role of *S. grossus* as a hyperaccumulator of Pb was given by Tangahu *et al.* (2013). Efficiency of *Colocasia esculenta* in removing Pb and Cd from waste water was reported by Madera-Parra *et al.* (2015). Indian lotus (*Nelumbo nucifera*) has phytoextraction potential to remove Cd from contaminated water (Mishra *et al.*, 2009; Kamal, 2011; Meera and Thampatti, 2016).

2.10. PHYTOREMEDIATION ABILITY OF FLOATING MACROPHYTES

Role of *Eichhornea crassipes* and *Pistia stratiotes* as phytoremediators of Al was suggested by Klumpp *et al.* (2002). These macrophytes possess the ability to concentrate metals both in the root and aerial parts. According to Sukumaran (2013), *E. crassipes* is an ideal macrophyte to remove Pb from industrial effluents associated with separating rare earth metals. Several workers have reported the potential of *E. crassipes* to remove Cd from contaminated water (Narain *et al.*, 2011; Khankhane *et al.*, 2014). Among the macrophytes tested for phytoremediation potential, *E.crassipes* followed by *P. stratiotes* and *Salvinia polyrrhiza* showed the efficiency in Fe removal without causing any toxic symptoms on plant growth (Mishra and Tripathi, 2008; Preetha and Kaladevi, 2014). Thampatti *et al.* (2016) reported the effective phytoextraction potential of *E. crassipes* for Fe.

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According to Schor- Fumbarov *et al.* (2003), *Nymphaea aurora*, is very efficient in removing Cd from waste water. Rai (2008) has reported on the Cd accumulation potential of *Azolla pinnata*. Hyperaccumulation capacity of water lilly, *Nympheae* sp. for Pb based on the BCF and TF was reported by Shuaibu and Nasiru (2011). The macrophyte showed BCF and TF values >1, indicating its Pb accumulation potential.

Rhizofiltration removes contaminants from water and aqueous waste streams, such as agricultural runoff, industrial discharges, and nuclear material processing wastes (Salt *et al.* 1998; Suresh and Ravishankar, 2004). Absorption and adsorption by plant roots play a key role in this technique, and consequently large root surface areas are usually required. *E. crassipes* an invasive weed of Vembanad wetlands, Kerala was very successful in removing Zn, Fe, Cd and Pb from the contaminated backwater system by concentrating the metals in its roots as well as in shoots. *E. crassipes* and *P. stratiotes* removed Fe, Al, Cd, Pb and S from contaminated water through rhizofiltration (KAU, 2009).

Aquatic macrophytes viz., *Typha* sp., *Eichhornea* sp., *Salvinia* sp., *Pistia* sp., *Azolla* sp. and *Lemna* sp. were successful in degrading organic contaminants of dairy effluents and *Typha* sp. was able to reduce the BOD value up to 65.4% (Dipu *et al.*, 2010). Efficiency of aquatic weeds like *E. crassipes* and *P. stratiotes* for phytoremediation of latex factory effluent was done in constructed wetlands and *E. crassipes* showed more effectiveness in reducing COD, BOD, pH and total solids of the effluent (Dipu *et al.*, 2011; Dipu *et al.*, 2013).

The biological filtration processes using different fodder grasses in the sewage farm reduced BOD, COD and TDS of sewage water and removed Cd and Cu in the sewage. Hybrid Napier accumulates inorganic contaminants more compared to that of the para grass cultivated in the sewage farm (Chitra and Jaya, 2005).

2.11. PHYTOREMEDIATION ABILITY OF SUBMERGED MACROPHYTES

Submerged aquatic macrophte, *Ceratophyllum demersum* can be successfully utilized in removal of Pb (Keskinkan *et al.*, 2004). Accumulation of heavy metals in the stems and leaves of submerged aquatic macrophytes was lower than that of roots which is an indication of their rhizofiltration potential (Baldantoni *et al.*, 2005). As reported by Norouznia and Hamidian (2014), *Potamogeton crispus* is an efficient phytoremediator in aquatic system capable of removing heavy metals like Pb and Cd.

Kumar *et al.* (1989) reported on the metal accumulation potential of three macrophytes viz., *Vallisneria spiralis*, *Hydrilla verticillata* and *Azolla pinnata* for the metals Al, Fe , Si and Mn while Kumar *et al.* (2008) reported on the Pb retention potential of *V. spiralis*. Two submerged macrophytes, *Rotala rotundifolia* and *Myriophyllum intermedium* were found to hyperaccumulate Pb and can be used in the phytoremediation programme (Marbaniang and Chaturvedi, 2014).

Phytormediation potential of aquatic macrophytes (emergent, floating, and submerged) both under natural and constructed wetlands is well known (Dhir *et al.*, 2009). On comparison, it could be seen that submerged and floating macrophytes have the potential to accumulate more metals than emergent types (Kamel, 2013).

2.12. PHYTOREMEDIATION ABILITY - TERRESTRIAL MACROPHYTES

Cynadon aethiopicus showed very high BCF and TF for Al revealing its phytoextraction potential. There was no excessive production of ROS or DNA damage indicating the ability of the plant to withstand Al stress (Yetneberk *et al.*, 2013). *Cynadon dactylon* has got the potential to remove Pb from a contaminated system and highest accumulation was noticed in roots (Soleimani *et al.* 2009; Mahmoud and Ghoneim, 2016) while its Cd extraction potential was reported by Kumar *et al.* (2015).

Commolina bengalensis and *Cynadon dactylon* were found to extract large quantities of Fe and Al from acid sulphate soils. Under graded doses of Fe and Al,

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the same plants survived well without showing enhanced extraction with graded doses without any toxicity symptoms. They excluded the absorption of Fe and Al under high concentration, confirming their phytostabilzation potential (KAU, 2008).

Alternanthera philoxeroides found in both aquatic and terrestrial environments is a potential scavenger of heavy metals (Bingzhong *et al.,* 2007). Pb remediation potential of *A. philoxeroides* was reported by Nan *et al.* (2013) based on BCF and TF and of *Wedelia trilobata* by Patel *et al.* (2014).

2.13. HYPERACCUMULTORS

The hyperaccumulation potential of macrophytes was determined based on BCF and TF. BCF is defined as the ability of a plant to accumulate a particular metal in its plant part with respect to its concentration in the soil substrate (Baker *et al.*, 1994; Raskin *et al.*, 1994; Zayed *et al.*, 1998) while TF is the ratio of metal concentration in shoot to that in the root (Luo *et al.*, 2005; Yoon *et al.*, 2006).

When BCF is more than one, it indicates that the plant is an accumulator while less than one, means the plant is an excluder (Baker, 1981). According to Baker and Brooks (1989), hyperaccumulators are plants that contain more than 1000 mg kg^{-1} of Cu, Cd, Cr, Pb, Ni, Co or more than 10,000 mg kg^{-1} of Zn and Mn in the dry matter. For Cd and other rare metals, it is 100 mg kg^{-1} by dry weight. A high value for TF indicate the efficiency of the plant to translocate metal from the root to shoot and such plants (> 1) are referred as hyperaccumulators (Ma *et al.*, 2001). They possess the phytoextraction ability to remove contaminants from the growth medium to the above ground portions and the biomass can be uprooted and removed.

E. crassipes obtained very high BCF in the root when grown in Cd contaminated water (Lu *et al.*, 2004). According to Jayaweera *et al.* (2008) rhizofiltration is the main mechanism involved in its phytoremediation process. It had the potential to concentrate metals more in the roots which is a specific feature of aquatic macrophytes, especially floating macrophytes (Ndimele *et al.*, 2014). Das *et*

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al. (2016) confirmed the hyperaccumulation potential of water hyacinth for Cd based on the values of BCF and TF.

The phytostabilisation effect of *L. flava* for Cd based on BCF and TF was reported by Abhilash *et al.* (2009). Accumulation capacity was highest in roots while extraction was highest in the aerial parts. *P. stratiotes* also indicated Cd hyperaccumulation capacity (Das *et al.*, 2014).

Sekabira *et al.* (2011) reported that *C. dactylon* has more of phytostabilisation nature as revealed by the higher BCF and lower TF. *A. philorexoides* can be considered as a hyperaccumulator for Cd based on the very high BCF (36.78) and TF (7.38) obtained for the plant (Nan *et al.*, 2013) and its hyperaccumulation potential for Fe was reported by Khankhane *et al.* (2014).

2.14. MECHANISM OF HEAVY METAL TOLERANCE

Accumulation of heavy metals inside the plant body results in certain physiological changes and synthesis of certain enzymes to tolerate the metal stress. Major changes that occur inside the plant cell to activate metal absorption include enhancement in the bioavailability of metal in the rhizosphere region leading to an increased uptake of metal towards the plasma membrane. Inside the cell wall, chelation of metal may occur by binding with various proteins like phytochelatin or, metallothionein or form a bond with the cell wall or get sequestered into the cell vacuole (Hall, 2002; Yadav, 2010). The various mechanisms are the following:

2.14.1. Solubilization of the metal from the soil matrix

Most of the heavy metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: (1) acidification of the rhizosphere through the action of plasma membrane proton pumps and (2) secretion of ligands capable of chelating the metal. But no plant species has been identified to handle high concentrations of toxic metals if they are present in solution. Hence phytoremediator plants should be modified to handle the extreme situations (Prasad, 2011).

2.14.2. Uptake into the root

Soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells. For movement through xylem, which is more efficient, the metals must cross a membrane, probably through the action of a membrane pump or channel. Most toxic metals are thought to cross these membranes through pumps and channels intended to transport essential elements. Excluder plants survive by enhancing specificity for the essential element or pumping the toxic metal back out of the plant (Hall, 2002).

2.14.3. Transport to the leaves

Once loaded into the xylem, the flow of the xylem sap will transport the metal to the leaves, where it must be loaded into the cells of the leaf, again crossing a membrane. The cell types where the metals are deposited vary between hyperaccumulator species. For example, *T. caerulescens* was found to have more Zn in its epidermis than in its mesophyll (Kupper *et al.,* 1999), while *Arabidopses halleri* preferentially accumulates its Zn in its mesophyll cells instead of its epidermal cells.

2.14.4. Detoxification / Chelation

At any point along the pathway, the metal could be converted to a less toxic form through chemical conversion or by complexation. Various oxidation states of toxic elements have very different uptake, transport, and sequestration or toxicity characteristics in plants. Chelation of toxins by endogenous plant compounds can have similar effects on all of these properties as well. Two major classes of heavy metal chelating peptides are known to exist in plants – metallothioneins and phytochelatins. As many chelators use thiol groups as ligands, the sulfur biosynthetic pathways have been shown to be critical for hyperaccumulator function (Pickering *et al.,* 2003) and for possible phytoremediation strategies. Oxidative stress is one of the most common effects of heavy metal accumulation in plants, and the increased antioxidant capabilities of hyperaccumulators allow tolerance of higher concentrations of metals (Freeman *et al.*, 2004).

2.14.5. Sequestration / Volatilization

The final step for the accumulation of most metals is the sequestration of the metal away from any cellular processes it might disrupt. Sequestration usually occurs in the plant vacuole, where the metal / metal-ligand must be transported across the vacuolar membrane. Metals may also remain in the cell wall instead of crossing the plasma membrane into the cell, as the negative charge sites on the cell walls may interact with polyvalent cations (Wang and Evangelou, 1994). Selenium may also be volatized through the stomata.

2.14.6. Microbial assistance

The plant roots deposit high amounts of photosynthetically derived hydrocarbons viz., rhizodeposits such as exudates, secretions, plant mucilages, mucigel, and root lysates to soil. These organic substances (e.g. organic acids of low and high molecular weight, sugars, and amino acids) play an important role in interactions of plants with their environment and consequently in the stimulation of microbial degradation of soil contaminants by plants. They stimulate the growth of microorganisms in the root zone of plants leading to an enhanced abundance of bacteria and fungi. This so-called rhizosphere effect is supported by physical impacts of the plant roots on the soil. No single plant or microbe excels in a) immobilization, b) removal, and c) destruction properties, nor does any single species show maximum uptake of all toxic metals or faster degradation of all organic contaminants. Therefore, successful treatment of soils with mixed waste requires a combination of plant species with appropriate remediation properties, and also the inclusion of plant species hosting rhizosphere communities (bacteria and fungi) active against specific contaminants that are present (Rajkumar *et al.*, 2010).

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Rhizosphere microorganisms, which are closely associated with roots, have been termed Plant Growth Promoting Rhizobacteria (PGPR). Further, rhizosphere microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests (Rajkumar *et al.*, 2009; Mackova *et al.,* 2006). Among the rhizosphere microorganisms involved in plant interactions with the soil milieu, the PGPR and Arbuscular Mycorrhizal Fungi (AMF) have gained prominence all over the world to treat soil (Ma *et al.,* 2011).

Like phytodegradation, rhizosphere degradation involves the enzymatic breakdown of organic pollutants. These breakdown products are either volatilized or incorporated into the microorganisms and soil matrix of the rhizosphere. The types of plants growing in the contaminated area influence the amount, diversity, and activity of microbial populations (Jones *et al.*, 2004; Kirk *et al.*, 2005). Grasses with high root density and legumes that fix nitrogen have high evapotranspiration rates and are associated with different microbial populations. These plants create a more aerobic environment in the soil that stimulates microbial activity that enhances oxidation of organic chemical residues (Narayanan *et al.,* 1998; Jones *et al.,* 2004; Kirk *et al.,* 2005). Secondary metabolites and other components of root exudates also stimulate microbial activity, a byproduct of which may stimulate degradation of organic pollutants (Pieper *et al.*, 2004).

Phytoremediation potential of paddy varieties to remediate an oily sludge was evaluated and found a maximum of 51.4% of total petroleum hydrocarbons of oily sludge was removed within 90 days. There was a significant drop in percent germination and seed sterility. Among the paddy varieties tested, Pokkali variety was superior to others in effecting phytoremediation (Joseph, 2007). *Pseudomonas fluorescens* was found to be effective in reducing Al uptake by paddy variety Uma and its accumulation in rice grains grown in acid sulphate soil (KAU, 2009).

2.15. HEAVY METAL ACCUMULATION BY MACROPHYTES

Free floating aquatic plants are capable of absorbing heavy metals through biosorption or adsorption to the cell walls followed by their uptake in a slow manner leading to bioaccumulation. Carboxyl groups present on cell wall enhances this absorption mechanism by these macrophytes (S´anchez-Galv´an *et al.*, 2008).

According to Ezaki *et al.* (2013), Al translocated to the shoot portion get accumulated in the trichomes and spikes of the leaves while a small part is secreted as sap from the tip area. In response to Al accumulation in roots, certain polyphenolic compounds like anthocyanin are produced but no damage due to reactive oxygen species (ROS) has been detected. Chelation of Al with organic acids can result in low accumulation of Al in the root tips. Anthocyanin can chelate with Al, Fe^{3+} and Cd (Kondo *et al.*, 1987; Dai *et al.*, 2012). These results suggest that anthocyanin can diminish Al toxicity through the formation of stable chelation complexes.

Accumulation of Cd in phytoremediators results in the production of low molecular weight poly-peptides that help in further phytoextraction (Prasad, 2001). Liu *et al.* (2012) had isolated an enzyme, viz., phytochelatins synthease from Indian lotus whose expression increased with increased accumulation of Cd in the leaves. Major portion of Cd extracted by *Sedum plumbizincicola* get sequestered in cell wall by creating modification in pectin esterase activity and pectin catabolic process (Peng *et al.*, 2017).

Beneficial microbial consortium like PGPR can enhance uptake of metals from soil by producing siderophores which can enhance metal uptake or by the production of Acc- deaminase enzyme which can reduce ethylene stress and thus the effect of heavy metals (Glick, 2003).

Alternanthera sessilis is capable of accumulating Pb and Cd in plant parts. The stumilation to accumulate these metals is effected through the production of enzymes like superoxide dismutase, catalase, polyphenol oxidase and peroxidase

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(Chinmayee *et al.*, 2014). On accumulation of heavy metals like Pb, activity of PS I increases resulting in maintenance of photophosphorylation and RUBISCO enzyme activity decline (Dhir *et al.*, 2011). According to Mandal *et al.* (2013), *Salvinia natans* possess the phytoextraction ability for Al. Accumulation of Al is associated in some management in the activities H^+/ATPase and preoxidase enzymes affecting the species to tolerate the accumulation.

Secretion of various organic acids in the rhizosphere results in chelation of metals which is further enhanced by the production of some chelating agents. Changes in pH and resultant redox reactions can solubilize and accumulate trace element at low levels, even from nearly insoluble precipitates (Tangahu *et al.*, 2011).

2.16. DISPOSAL OF PHYTOREMEDIATED BIOMASS

Disposal of the huge biomass that results from phytoextraction of heavy metals / pollutants is a matter of great concern (Dipu *et al.*, 2011). Direct disposal of powdered *Salvinia natans* grown in waste water from electorplating industries did not affect the germination of *Triticum aestivum* seeds; and plant height and chlorophyll content revealing the possibility of using such metal loaded biomass directly to soil (Dhir and Srivastava, 2012). Slow release of metals from the biomass may be the reason for not inhibiting plant growth.

Phytoremediated biomass could successfully be utilised for biogas production resulting in two way benefit—*ie.* for treating the industrial effluent and for energy production (Verma *et al.*, 2007). Phytoremediated *Typha, Eichhornea, Salvinia, Lemna, Azolla* and *Pistia,* were used for biogas production. Gas production was slow during the initial period while it increased with time and surpassed the biogas production from the traditional cow dung slurry (Dipu *et al.*, 2011).

Phytoremediated biomass of *E. crassipes* produced more biogas compared to *V. spiralis* (Singhal and Rai, 2003). Biogas production was quicker in water hyacinth water chestnut (Verma *et al.*, 2007). The high organic matter in phytoextracted biomass suggests the good potential for conversion to biogas (Thilakar *et al.*, 2012).

Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health (Wong and Selvam, 2006). Composting and vermicomposting are the best-known processes for biological stabilization of green waste by transforming them into safer and more stabilized composts that can be used as a soil conditioner in agricultural applications (Gabhane *et al.*, 2012). Application of *Eichhornea* compost resulted in higher yield for amaranthus and there was no heavy metal accumulation, which implies the use of processed biomass as safe organic source for raising crops (Sasidharan *et al.*, 2013).

Composting results in efficient reduction of biomass (Cao *et al.*, 2010). Composting of contaminated biomass (water hyacinth) showed that heavy metals are largely confined to unavailable residual position and addition of FYM can further reduce the mobility of metals (Singh and Kalamdhad, 2012). Reduced metal availability with composting was also reported by Reyes and Cuevas (2015). Application of water hyacinth compost on tomato positively affected plant growth but not on tomato fruit production. Heavy metal concentration in tomato was below the MPL for Pb, Cu and Zn and an application rate of 74 t ha^{-1} were found to be most promising (Mashavira *et al.*, 2015).

Contaminated soil mixed with compost will result in less mobility of metals and become relatively unavailable for plant uptake. OM component of the compost - humic and fulvic acids present complexation surfaces or ligands for effective binding of Cu (Fontanilla and Cuevas, 2010). It resulted in minimal translocation of Cu to the shoot from roots (Reyes and Cuevas, 2015).

The phytoextracted biomass of both *Pistia stratiotes* and *Salvinia natans* were effectively used for the production of biofuels, viz., bio-ethanol and bio-methanol,

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using genetically engineered microbes. In this manner pollution can be mitigated and aquatic ecosystem can be protected (Thilakar *et al.,* 2012).

Combustion of biomass under reduced oxygen conditions produces black coloured carbon rich residue "biochar". Because of the large surface area and CEC, both organic and inorganic contaminants get adsorbed on its surface resulting in reduced mobility in soil. Combined use of compost, manure and biochar can be the best mechanism for reducing pollutions hazards in soil (Beesley *et al*., 2011; Houben *et al*., 2013).

Biochar incorporation has reduced the availability of Cd and Pb in soil while increased the plant available P and K. This has resulted with the efficient partitioning of these metals in soil with biochar application and also resulted in more biomass production (Park *et al*., 2011). Comparative evaluation of biochar and ash of metal contaminated waste showed phytotoxicity with respect to availability of Cu and increased soil pH. But biochar was able to retain more Pb in soil compared to ash (Luccini *et al*., 2014). Phytoextraction of heavy metals from soil through hyperaccumulators and converting it to biochar offers double extraction of heavy metals from soil and limits the leaching losses from soil (Paz-Ferreiro *et al*., 2014; Brendova *et al*., 2015).

According to Keller *et al.* (2005), incineration is a viable option to treat the phytoextracted biomass and it is possible to recover the metal from the residues.

The major problems with phytoremediation technology are complete removal of contaminants are not possible, not practical in highly contaminated areas, slower and faces extreme difficulty in the disposal of phytoremediated biomass. At the same time it had the advantages of low cost, higher aesthetic value and safe for the removal of toxic organics and heavy metals and eliminates secondary wastes (Thampatti and Sudharmaidevi, 2014).

Materials and methods

3. MATERIALS AND METHODS

The present investigation entitled "Phytoremediation of inorganic contaminants in Vellayani wetland ecosystem" was carried out at the College of Agriculture, Vellayani during the period 2013 to 2016. The main objectives were to track the potential sources of contaminants threatening the wetland ecosystem and suggest suitable phytoremediation technologies for decontamination.

3.1. General description of the study area

3.1.1. Geography

Vellayani lake and its catchment represents the study area. It includes the Vellayani lake and its surroundings extended over 8°24'09" to 8°26'30" latitude and 76°59'08" to 76°59'47" longitude. The ecosystem covers three panchayaths viz., Kalliyoor, Pallichal and Venganoor and two corporation zones viz., Nemom and Thiruvallam. It lies on the south - east of Thiruvananthapuram district at an altitude of 29 m above mean sea level covering an area of 245 ha. The lake area was about 750 ha in 1926 which showed a drastic reduction due to indiscriminate anthropogenic activities.

3.1.2. Hydrology

The original length of the lake is 3.15 km with a depth of 2-3 m which may reaches up to 5-6 m during rainy season. Pallichal and Njendinjil are the main streams providing water to this lake. This largest freshwater lake of Thiruvananthapuram district serves as the sole drinking water source for Kovalam, Vizhinjam, Pachalloor, Vellayani and nearby areas. The proposed international transshipment terminal at Vizhinjam will have to depend on this rainfed water resource for its daily requirements. Much impact on this ecosystem for food and water has resulted in increased siltation of the lake and lowering of water level. Major part of the lake is static and water that flows along Kannukalichal gets emptied into Karamana river through Madhupalam spillway.

3.1.3. Geology

During the past, the tidal water had reached much inward along the lower basin of Karamana river. The regression of the sea followed and the deeper portions of the tributary became the Vellayani lake.

3.1.4. Biodiveristy

The freshwater ecosystem is gifted with a rich diversity of flora and fauna. This wetland is particularly famous for the lotus cultivation being done here. About 41 species of freshwater fishes have been documented from the lake. It is a suitable habitat for a large number of aquatic and migratory birds. This biodiversity richness supports the livelihood of a large group of common man residing there who derive their income through activities like fishing, bird rearing, floriculture and agricultural production.

3.1.5. Climate

Data on temperature, relative humidity, rainfall, number of rainy days and evaporation during the study period were collected from the Meteorological Observatory, College of Agriculture, Vellayani (Table 1). The region experiences a humid tropical climate. It mainly depends on south-west and north-east monsoons for fresh water supply.

3.1.6. Land use

Vellayani wetland ecosystem is an agriculture predominant area. The major crops raised in the area include coconut, banana, rice, vegetables (cucumber, snake gourd, bitter gourd, ivy gourd, amaranthus, cowpea, okra and chilly), cassava and sweet potato. Fishing, duck rearing, lotus cultivation etc. are other major sources of income generation activities being carried out here.

Table 1. Weather data during the period of study (January 2014 to May 2016)

Period	Temperature (°C)		Reltive Humidity (%)		Rainfall (mm)	Number of rainy days	Evaporation (mm day^{-1})
	max.	min.	max.	min.			
2014							
January	30.61	21.53	93.41	73.64	28.5	2	3.2
February	31.33	22.32	92.35	69.46	21.0	3	4.0
March	32.43	22.88	91.90	66.54	31.5	2	4.3
April	32.40	24.47	91.20	73.60	115	9	3.0
May	31.88	24.73	90.03	78.45	280.4	11	3.4
June	30.76	25.21	92.46	79.16	88.0	10	3.3
July	29.99	24.30	91.90	77.50	104.2	9	3.6
August	29.50	23.74	90.70	81.20	551.5	16	5.1
September	30.20	24.19	90.80	78.96	219.4	10	3.8
October	30.52	23.82	86.93	82.40	230.2	12	3.7
November	30.18	23.38	93.46	77.23	137.3	11	1.7
December	31.20	23.25	94.30	78.30	133.5	5	2.5
2015							
January	30.61	21.56	93.87	65.00	8.0	2	3.0
February	31.53	22.34	91.78	64.71	0	0	4.1
March	32.42	23.65	89.93	67.12	56.1	4	4.2
April	32.74	24.48	92.00	75.00	182.6	9	4.0
May	32.14	25.31	90.71	83.29	406.0	8	3.4
June	31.43	24.47	90.97	82.97	346.9	19	3.9
July	31.31	24.60	89.10	79.87	53.5	5	4.0
August	31.68	24.57	89.52	76.42	80.2	4	4.2
September	31.41	24.40	91.40	83.77	289.8	15	4.5
October	31.29	24.03	92.45	80.54	399.1	19	3.8
November	31.54	23.79	92.70	79.57	254.1	18	3.6
December	31.48	23.38	93.94	83.16	259.3	11	3.4
2016							
January	32.52	22.64	91.40	72.50	0.4	0	4.2
February	32.91	23.17	92.50	73.30	43.6	1	4.5
March	34.40	24.64	90.20	68.50	1.9	1	3.8
April	35.29	26.62	90.83	77.03	22.6	2	3.6
May	33.81	25.42	89.00	77.00	463.3	13	3.4

max.- maximum min.- minimum

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3.2. Components of investigation

The investigation consisted of four parts:

I Peripatetic survey of the catchment area of Vellayani lake

II Evaluation of native macrophytes for possible hyperaccumulation capacity for Fe, Al, Pb and Cd

III Performance of selected hyperaccumulators under graded doses of metals to identify selective retention sites

IV Bioavailability of phytoextracted metals and comparison of common disposal methods

PART I

3.2.1. PERIPATETIC SURVEY OF THE CATCHMENT AREA OF VELLAYANI LAKE

Based on a previous study (Kamal, 2011), seven sites of the Vellayani wetland ecosystem were reported to be contaminated with heavy / toxic metals viz., Fe, Al, Pb and Cd. Hence a peripatetic survey was carried out in the catchment area of Vellayani lake along the rivulets that contribute water to these selected sites during 2014. Three rivulets were selected from each site, and water and sediment samples were taken from five sampling points under each rivulet during pre monsoon and post monsoon seasons. These samples were subjected to different physical, chemical and biological analyses and interpreted with standard permissible limits set by ISI/ WHO/ USEPA and the extent of contamination was determined.

3.2.1.1. Selection of sites

The sites selected for the present study were Palapoor, Pallichalthodu, Reservoir bund, Arattukadavu RB, Valiyavilagam, Mannamvarambu and Manamukku. Three rivulets that contribute water to each site were identified (Table 2) and samples collected from five sampling points per rivulet.

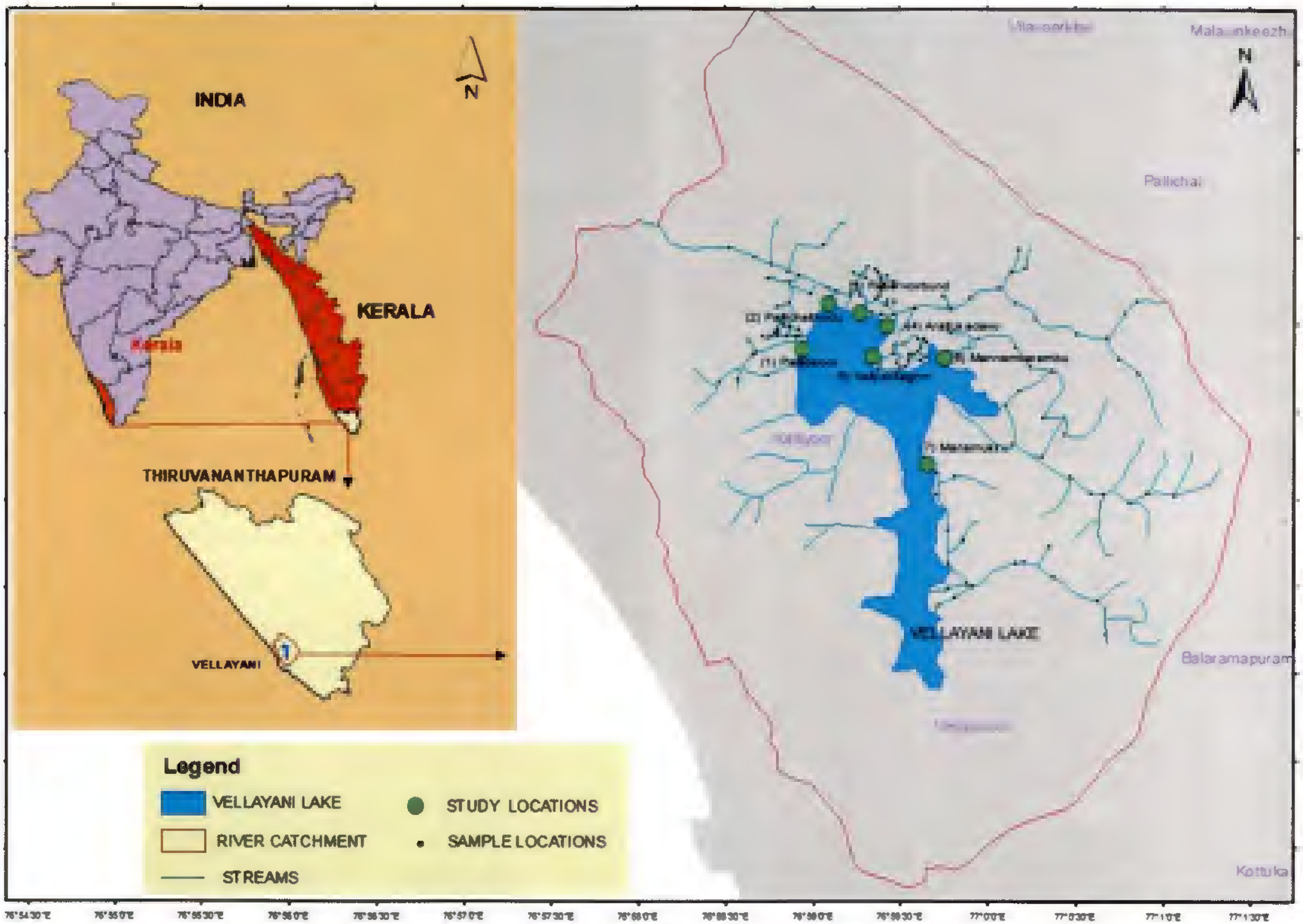

Plate 1. Location Map of Study Area

Table 2. Sites selected from Vellayani wetland ecosystem for the study

Code No.	Major sites	Sub sites		
		1	2	3
I	Palappoor	Palappoor	Punchakkari	Pappanchani
II	Pallichalthodu	Pallichalthodu	Pallinada	Sivodayam
III	Reservoir bund	Reservoir bund	Vayalvaram	Thennor
IV	Arattukadavu RB	Arattukadavu	Pallichalkalungu	Cherupalamandham
V	Valiyavilagam	Valiyavilagam	Mukkalloormoola	Kanthari
VI	Mannamvarambu	Mannamvarambu	Keepadvevila	Papparatheri
VII	Manamukku	Manamukku	Njedinjil	Narakamthattu

Water and sediment samples were collected from five sampling points in each rivulet at a distance of 250 m apart, using GIS tools during the pre monsoon (April – May, 2014) and post monsoon (January – February, 2015) seasons. Details of geo coded sampling points are presented in Table 3. A brief description of the selected sites is provided in Table 4 and the location map in Plate 1.

3.2.1.2. Collection of water and sediment samples

Surface water and sediment samples were collected from the geo coded sampling points during both the pre and post monsoon seasons, taking the sampling point at the lake as the first sampling point (Plate 2). Water samples were collected in high density poly ethylene (HDPE) containers and sediments in polythene bags, clearly labelled and transported to the laboratory for analysis. The samples were kept in deep freezer at -15°C till analyses were completed. Fresh water samples were used for microbiological analysis. The standard procedure followed for the analysis of water and sediment analyses are given in Tables 5 and 6, respectively.

PART II

3.2.2. EVALUATION OF NATIVE MACROPHYTES FOR POSSIBLE HYPERACCUMULATION CAPACITY FOR Fe, Al, Pb AND Cd

The dominant aquatic and terrestrial macrophytes flourishing along the sampling points of Vellayani wetland ecosystem during the pre monsoon period (April – May, 2014) were identified as per the descriptions of Gamble (1928 - 1932).

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Plate 2. Sites selected for the study in Vellayani wetland ecosystem

Site 1. Palappoor

Site 2. Pallichalthodu

Site 3. Reservoir bund

Site 4. Arattukadavu RB

Plate 2. Sites selected for the study in Vellayani wetland ecosystem (continued)

Site 5. Valiyavilagam

Site 6. Mannamvarambu

Site 7. Manamukku

Lake (eastern side)

Table 3. Geo reference coordinates of the identified sampling points

Selected sites	Sub sites - Rivulets					
	Rivulet 1		Rivulet 2		Rivulet 3	
	N	E	N	E	N	E
	8° 26' 26.511"	76° 58' 55.750"	8° 26' 26.431"	76° 58' 45.727"	8° 26' 10.936"	76° 58' 28.317"
	8° 26' 26.443"	76° 58' 54.174"	8° 26' 27.665"	76° 58' 47.385"	8° 26' 11.450"	76° 58' 34.656"
Palappoor	8° 26' 26.638"	76° 58' 51.559"	8° 26' 29.099"	76° 58' 48.053"	8° 26' 24.935"	76° 58' 38.258"
	8° 26' 28.784"	76° 58' 55.402"	8° 26' 26.431"	76° 58' 43.129"	8° 26' 13.163"	76° 58' 44.678"
	8° 26' 29.703"	76° 58' 52.196"	8° 26' 30.248"	76° 58' 50.404"	8° 26' 20.234"	76° 58' 40.095"
	8° 26' 48.867"	76° 58' 51.806"	8° 26' 40.669"	76° 59' 13.447"	8° 26' 45.221"	76° 58' 52.492"
	8° 26' 51.122"	76° 58' 46.196"	8° 26' 44.753"	76° 59' 14.666"	8° 26' 46.558"	76° 58' 45.284"
Pallichalthodu	8° 26' 56.760"	76° 58' 27.245"	8° 26' 46.615"	76° 59' 18.535"	8° 26' 41.217"	76° 58' 59.429"
	8° 27' 5.317"	76° 58' 2.227"	8° 26' 48.225"	76° 59' 14.440"	8° 26' 37.820"	76° 58' 52.389"
	8° 26' 58.174"	76° 59' 4.193"	8° 26' 50.214"	76° 59' 19.056"	8° 26' 36.466"	76° 58' 48.443"
	8° 26' 35.861"	76° 59' 17.270"	8° 26' 36.832"	76° 59' 25.721"	8° 26' 46.075"	76° 59' 23.497"
	8° 26' 40.676"	76° 59' 16.806"	8° 26' 38.437"	76° 59' 28.650"	8° 26' 47.141"	76° 59' 25.884"
Reservoir bund	8° 26' 34.503"	76° 59' 20.628"	8° 26' 38.730"	76° 59' 25.900"	8° 26' 48.195"	76° 59' 22.983"
	8° 26' 33.977"	76° 59' 24.088"	8° 26' 40.726"	76° 59' 23.300"	8° 26' 49.458"	76° 59' 21.998"
	8° 26' 34.252"	76° 59' 28.284"	8° 26' 43.114"	76° 59' 24.069"	8° 26' 45.157"	76° 59' 28.565"
	8° 26' 36.131"	76° 59' 35.459"	8° 26' 48.567"	77° 0' 8.533"	8° 26' 33.323"	77° 0' 46.661"
	8° 26' 35.843"	76° 59' 47.663"	8° 26' 49.794"	77° 0' 13.236"	8° 26' 37.621"	77° 0' 58.728"
Arattukadavu RB	8° 26' 39.965"	76° 59' 45.253"	8° 26' 56.123"	77° 0' 10.736"	8° 26' 13.732"	77° 0' 52.107"
	8° 26' 39.646"	76° 59' 54.448"	8° 26' 35.532"	77° 0' 14.350"	8° 26' 29.376"	77° 1' 1.940"
	8° 26' 29.759"	76° 59' 32.608"	8° 26' 29.450"	77° 0' 23.943"	8° 26' 19.632"	77° 1' 4.296"
	8° 26' 15.451"	76° 59' 23.074"	8° 26' 22.784"	76° 59' 37.231"	8° 26' 15.692"	76° 59' 32.137"
	8° 26' 17.632"	76° 59' 29.239"	8° 26' 24.157"	76° 59' 39.572"	8° 26' 18.018"	76° 59' 33.167"
Valiyavilagam	8° 26' 20.877"	76° 59' 28.184"	8° 26' 25.697"	76° 59' 38.537"	8° 26' 19.989"	76° 59' 33.201"
	8° 26' 23.187"	76° 59' 28.625"	8° 26' 19.669"	76° 59' 38.905"	8° 26' 18.244"	76° 59' 37.548"
	8° 26' 24.051"	76° 59' 31.882"	8° 26' 25.413"	76° 59' 36.508"	8° 26' 20.412"	76° 59' 35.017"
	8° 26' 25.609"	76° 59' 56.998"	8° 26' 7.547"	77° 0' 11.296"	8° 25' 36.072"	77° 0' 30.308"
	8° 26' 27.598"	76° 59' 47.608"	8° 25' 49.214"	77° 0' 9.195"	8° 25' 31.898"	77° 0' 40.118"
Mannamvarambu	8° 26' 22.582"	77° 0' 10.058"	8° 25' 44.873"	77° 0' 18.928"	8° 25' 29.030"	77° 0' 48.886"
	8° 26' 15.651"	77° 0' 14.987"	8° 25' 58.305"	77° 0' 19.484"	8° 25' 33.115"	77° 1' 10.127"
	8° 26' 14.455"	77° 0' 22.644"	8° 25' 57.334"	77° 0' 38.923"	8° 25' 42.802"	77° 1' 1.420"
	8° 25' 39.177"	76° 59' 42.835"	8° 25' 1.377"	76° 59' 52.970"	8° 25' 8.442"	77° 0' 12.846"
	8° 25' 37.997"	76° 59' 49.312"	8° 25' 7.976"	76° 59' 47.126"	8° 25' 13.582"	77° 0' 21.069"
Manamukku	8° 25' 31.987"	76° 59' 42.862"	8° 24' 59.150"	76° 59' 46.634"	8° 25' 2.997"	77° 0' 22.752"
	8° 25' 19.394"	76° 59' 46.803"	8° 24' 56.342"	76° 59' 49.075"	8° 24' 54.908"	77° 0' 37.859"
	8° 25' 16.307"	76° 59' 51.819"	8° 24' 53.666"	76° 59' 43.507"	8° 24' 43.811"	77° 0' 40.600"

Plant density for individual species was calculated to assess their predominance in the selected site. Processed samples were analysed to estimate the metal contents viz., Fe, Al, Pb and Cd. Sediment / soil samples from the rhizosphere region of the selected macrophytes were also collected to assess the metal and microbial load. Bio concentration factors for the above elements were calculated.

3.2.2.1. Collection of macrophytes

Using Quadrat method (Bartlett, 1948), population of dominant macrophytes from one square metre area was enumerated to assess the plant density. These selected macrophytes from unit area were uprooted and washed thoroughly with water to remove all the sediment / soil particles. After measuring the wet weight, they were kept in polythene bags, labelled properly and brought to the laboratory. Shoot and root portions were separated, cleaned and air dried in shade and later in hot air oven at 60 ± 5°C. The dried biomass was powdered and stored in butter paper covers for further analysis.

3.2.2.2. Collection of rhizosphere sediment / soil

From the rhizosphere region of the identified aquatic / terrestrial macrophytes, sediment / soil samples were collected during the pre monsoon period, stored in polythene bags and carried to the laboratory for analysis. These samples were kept in deep freezer at -15°C and subjected to physicochemical analysis. Fresh samples were used for the estimation of microbial load viz., bacteria, fungi and actinomycete count.

PART III

3.2.3. PERFORMANCE OF SELECTED HYPERACCUMULATORS UNDER GRADED DOSES OF METALS TO IDENTIFY RETENTION SITES

Dominant macrophytes of the ecosystem showing higher BCF values either in shoot or root were subjected to an initial screening trial under varied doses of Fe

(500, 750 and 1000 mg kg^{-1}), Al (250, 500 and 750 mg kg^{-1}), Pb (10, 20 and 30 mg kg^{-1}) and Cd (10, 20 and 30 mg kg^{-1}).

Table 4. Brief description of the sites selected for the investigation

Sl. No.	Name of the site	Description
1	Palappoor	Intensively cultivated with vegetables and banana, many padasekharams are left fallow where the aquatic weed, *Scirpus grossus* dominates, habitat for migratory birds, pump house to supply water to Vandithadam plant, dumping of domestic and hotel waste
2	Pallichalthodu	Moderately thick vegetation dominated by coconut, one canal joins the lake, water diverted through Kannukalichal to Madhupalam spillway, *Nympheaea nouchali* and *Eichhornea crassipes* seen in abundance
3	Reservoir bund	Less number of human dwellings present, moderately thick riparian vegetation dominated by *Pandanus kaida* and *Eichhornea crassipes*
4	Arattukadavu RB	Thick riparian vegetation dominated by *Colacasia esculenta, Pandanus kaida* and *Salvinia molesta,* area associated with some rituals of Vellayani Devi temple, semiurban settlement, automobile workshop unit present, hotel and domestic wastes dumped in large quantities
5	Valiyavilagam	Shore partially protected by granite rubbling, *Monochoria vaginalis* and *Eichhornea crassipes* are the dominant vegetation, non functional old pump house is located here
6	Mannamvarambu	Area well protected by granite rubbling, dense growth of *Colacasia esculenta*, sports hostel under Kerala State Sports Council present, less human settlement
7	Manamukku	Mixed vegetation dominated by coconut and vegetables especially amaranthus, Njedinjil stream joins the lake, poultry unit present

The selected macrophytes were raised in the sediment collected from the native ecosystem using water from the lake and grown for a period of 15 days. Then they were uprooted, washed properly, dried in shade and later in hot air oven at 60 ± 5°C. The dried powdered biomass was kept in butter paper covers for metal analysis.

Based on growth performance and metal content, nine macrophytes each were selected for the above elements. These plants were further grown in pots under three graded doses of each of the above metals to determine the hyperaccumulation capacity. Each experiment was replicated five times. The lowest dose was fixed based on the highest level of metal identified in the sediment / soil. The macrophytes were grown for 45 days and later analysed to determine the metal content and best phytoextractor for each metal were identified.

3.2.3.1. Phytoextractor for Iron

Macrophytes selected for pot culture experiment under graded doses of Fe were *Alternanthera tenella, Buchloe dactyoides, Colacasia esculenta, Cynadon dactylon, Eichhornea crassipes, Eleocharis dulcis, Limnocharis flava, Monochoria vaginalis* and *Nymphoides indica*. They were raised in plastic troughs filled with 10 kg sediment collected from the Vellayani wetland ecosystem. Water used to nurture the plants was also collected from the same site. Treatments @ 1000, 2000 and 3000 mg Fe kg^{-1} were applied as ferrous ammonium sulphate, thoroughly mixed and macrophytes weighing 250 g were planted. They were grown for a period of 45 days, uprooted, properly cleaned, shoot and root portions were separated and fresh weight was noted. Plant samples were air dried in shade for a few days and then in hot air oven at 60 ± 5°C. Dried samples were powdered and stored in butter paper covers for further analysis.

Water and sediment samples from each pot were collected at 45 DAP, stored in HDPE containers and stored at -15°C in deep freezer for further analysis. Fresh rhizosphere sediment samples were used for the estimation of microbial load. Metal content of water, sediment and macrophytes were calculated. Based on the total quantity of metal accumulated by the plant, best phytoextractor was selected. Based on TF, major site of retention was identified.

3.2.3.2. Phytoextractor for Aluminium

Graded doses of Al @ 750, 1000 and 1250 mg Al kg^{-1} were applied to the plastic troughs filled with 10 kg of sediment collected from the native ecosystem, using aluminium nitrate. Macrophytes selected were *Nymphoides indica, Salvinia molesta, Colacasia esculenta, Eleocharis dulcis, Limnocharis flava, Monochoria vaginalis, Buchloe dactyoides, Panicum repens* and *Cynadon dactylon.* After thoroughly mixing Al salt with sediment, macrophytes weighing 250 g were grown for 45 days. Water collected from the same site was used to nurture the macrophytes.

Plants were uprooted 45 DAP, thoroughly cleaned, shoot and root portions were separated and fresh weight was determined. After air drying in shade for a few days, kept in hot air oven at 60 ± 5°C. Dried samples were powdered and stored in butter paper covers for further chemical analysis. Water and sediment were collected from the pots 45 DAP, stored in HDPE containers and kept in deep freezer at -15°C for further analysis. Fresh sediment samples were analysed to estimate the microbial load. Metal content of sediment and macrophytes were determined and based on the total quantity of Al extracted, the best phytoextractor was selected and based on TF, major site of retention was identified.

3.2.3.3. Phytoextractor for Lead

Nine macrophytes viz., *Nelumbo nucifera, Nymphoides indica, Pistia stratiotes, Eichhornea crassipes, Limnocharis flava, Scirpus grossus, Colacasia esculenta, , Buchloe dactyloides* and *Alternanthera tenella* were grown in plastic troughs treated with Pb @ 50, 75 and 100 mg Pb kg^{-1}. Each trough was filled with 10 kg sediment collected from the native ecosystem. Lead nitrate was used to supply the element. The macrophytes were planted after thoroughly mixing the Pb source with

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sediment and grown for 45 days. Plants were carefully uprooted 45 DAP, cleaned properly, root and shoot portions separated and fresh weight was taken. Samples were air dried for a few days and kept in hot air oven at 60 ± 5°C. Dried samples were powdered and stored in butter paper covers for further chemical analysis.

From each pot, water and sediment samples were collected in HDPE containers and stored in deep freezer for further analysis. Metal content of sediment and plant samples were determined and based on the metal content of the macrophyte, best phytoextractor for Pb was identified and based on TF major site of retention was identified.

3.2.3.4. Phytoextractor for Cadmium

Plastic troughs were filled with 10 kg sediment and graded doses of Cd @ 50, 75 and 100 mg kg^{-1} were applied using cadmium nitrate as the source material. *Nelumbo nucifera*, *Nymphoides indica*, *Pistia stratiotes*, *Eichhornea crassipes*, *Limnocharis flava*, *Eleocharis dulcis*, *Colacasia esculenta*, *Alternanthera tenella* and *Sphagneticola trilobata* were grown for 45 days. Macrophytes of 250g weight were planted in the troughs. At 45 DAP, they were uprooted, thoroughly washed, separated the root and shoot portions and fresh weights were noted. These samples were kept in shade for air drying and later dried in hot air oven at 60 ± 5°C. Dried biomass was powdered and stored in butter paper covers for further analysis.

Water and sediment samples collected from each pot 45 DAP were stored in HDPE containers and kept in deep freezer at -15 °C for further analysis. Based on Cd content in different macrophytes, the best phytoextractor for Cd was determined and based on TF, major site of retention was identified.

PART IV

3.2.4. BIOAVAILABILITY OF PHYTOEXTRACTED METALS AND COMPARISON OF COMMON DISPOSAL METHODS

Phytoextractors identified based on the performance under graded doses of metal (3.2.3) were *E. crassipes* for Fe, *M. vaginals for Al and L. flava* for both Pb and Cd. The selected phytoextractors were grown for 60 days in plastic troughs containing seven kg sediment spiked with the corresponding metals (2000 mg kg^{-1} for Fe, 1000 mg kg^{-1} for Al and 75 mg kg^{-1} for Pb and Cd). The water from Vellayani lake was used to maintain the plants. Plants were uprooted at 60 DAP, cleaned properly; fresh weight was noted and kept in shade for air drying. Biomass resulted from each metal was separated into four equal parts and was processed into ordinary compost, vermicompost, biochar and ash respectively.

3.2.4.1. Ordinary compost preparation

Known quantity of phytoextracted biomass for each metal was separately placed in plastic troughs mixed with farm yard manure (FYM) in 6:1 proportion. Periodical turnings were given and the compost was ready by 75^{th} day. The compost prepared for each metal was taken out, weighed to assess the recovery percentage, converted to fine powder and kept in HDPE containers for further use in pot culture experiment.

3.2.4.2. Vermicompost preparation

Known quantity of phytoextracted biomass obtained on spiking the selected phytoextractor with the particular metal was placed in four separate troughs, mixed with FYM in 6:1 proportion and earthworms (*Eudrillus eugeneae)* were introduced into the biomass mixture. Periodical turnings were given for aeration and adequate moisture was maintained. Vermicompost was ready by 45^{th} day. Vermicompost for each metal was taken out; quantity was determined, sieved and stored in HDPE containers for further use in the pot culture study.

3.2.4.3. Biochar production

Known quantity of phytoextracted biomass generated on spiking the selected phytoextractor with the particular metal was placed in four separate stainless steel containers and fed to the heating unit of biochar production chamber. Resultant biochar for each metal was weighed, converted into fine powder and stored in HDPE containers for further use in pot culture studies.

3.2.4.4. Ashing of phytoextracted biomass

Known quantity of phytoextracted biomass obtained on spiking the selected phytoextractor with the particular metal was ashed separately under high temperature in controlled conditions. Resultant ash for each metal was weighed and kept in HDPE containers for use in the pot culture experiment.

3.2.4.5. Metal availability and phytoextraction by amaranthus

Four separate pot culture experiments using the processed materials for each of the heavy / toxic elements viz., Fe, Al, Pb and Cd were carried out to assess the availability of each metal in the treated biomass and the quantity extracted by the test plant amaranthus.

Amaranthus, variey Arun was grown for 30 days. Experiment was laid out in completely randomized design (CRD) with three replications and six treatments.

T_1: FYM @ 25 g kg^{-1} of soil

T_2: Compost prepared from 100 g phytoextracted biomass per kg of soil

T_3: Vermicompost prepared from 100 g phytoextracted biomass per kg of soil

T_4: Ash prepared from 100 g phytoextracted biomass per kg of soil

T_5: Biochar prepared from 100 g phytoextracted biomass per kg of soil

T_6: Absolute control

Each pot was filled with 500 g garden soil collected from non- contaminated area and mixed with 50 g coir pith compost. Treatments were applied to the pots and amaranthus seed was sown. Plants were retained for 30 days, and then uprooted, cleaned and fresh weight was noted. The samples were air dried in shade followed by

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oven drying at 60 ± 5°C. Dried samples were powdered and stored in butter paper covers for analysis. Growth medium and plant samples were analysed for the metal concentration. Based on the metal concentration in plant and soil samples, safe method for disposal of phytoextracted biomass was assessed.

3.6. Analysis of water, sediment / soil and plant samples

Standard procedures used for the physical, chemical and biological analyses of water, sediment / soil and plant samples are listed below.

3.6.1. Water

Standard procedure employed for the physical, chemical and biological analyses of water are listed in Table 5.

Table 5. Standard methods followed for analysis of water

Parameters	Method	Reference
A. Physical		
Colour (hazen units)	Florel-Ule colour scale method for qualitative recognition	Gupta (1999)
Temperature	Mercury thermometer for surface temperature	Gupta (1999)
Turbidity	Nephelometry based on scattering of light (2020we Portable Turbidity Meter)	Gupta (1999)
Suspended solids	Filtration followed by gravimetric method	Gupta (1999)
B. Chemical		
pH	Potentiometry (Cyber Scan pH510, EuTech Instruments)	Gupta (1999)
EC	Conductivity meter EC-TDS Analyzer (CM 183, Elico, India)	Gupta (1999)
NH_4-N and NO_3-N	Macro-Kjeldahl distillation and titrimetry	Bremner and Keeney (1965)
P	Colorimetric (ammonium molybdate – ascorbic acid) method (Double Beam UV-VIS Spectrophotometer 2201, Systronics)	Watanabe and Olsen (1965)
BOD	Modified Winkler's method (Incubation followed by titration) (BOD incubator)	APHA (2012)
COD	Refluxing and titration (COD reflux unit)	APHA (2012)
Fe, Al, Pb and Cd	Emission spectroscopy(ICP OES Optima 8000, PerkinElmer Inc., USA)	Wei and Yang (2010)
Pesticide residues	GC- MS (modified AOAC method)	Sharma, 2013
C. Biological		
Coliforms	Multiple tube fermentation technique- EMB agar - gram staining	Collee *et al.*, 1996

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3.6.2. Sediment / Soil

Table 6 represents the standard procedure followed for the physical, chemical and biological analyses of sediment / soil.

Table 6. Standard methods followed for analysis of sediment / soil

Parameters	Method	Reference
A. Physical		
Texture	Hydrometer method	Piper (1942)
B. Chemical		
pH (1:2.5)	pH meter (Cyber Scan PC510, EuTech Instruments, Singapore)	Jackson (1973)
EC (1:2.5)	Conductivity meter EC-TDS Analyzer (CM 183, Elico, India)	Jackson (1973)
Organic carbon	Wet digestion method	Walkley and Black (1934)
NH_4-N and NO_3-N	2M KCl extraction and macro-Kjeldahl distillation	Bremner and Keeney (1965)
P	Bray No. 1 extraction and spectrophotometry	Jackson (1973)
Fe, Al, Pb and Cd	i) Available metal estimation: 0.1N HCl extraction (Emission spectroscopy)	Jackson (1973)
	ii) Total metal estimation : Nitric-Perchloric acid (9:4) digestion (ICP OES Optima 8000, PerkinElmer Inc., USA	Wei and Yang (2010)
Pesticide residues	GC- MS and LC- MS/ MS (modified QuEChERS method)	Asensio-Ramos *et al.* (2010)
C. Biological		
Bacteria	Serial dilution and plate count method - Nutrient Agar medium	Johnson and Kurl (1972)
Fungi	Serial dilution and plate count method - Rose Bengal Agar medium	Johnson and Kurl (1972)
Actinomycetes	Serial dilution and plate count method - Kenknight's medium	Johnson and Kurl (1972)

3.6.3. Macrophytes and processed biomass

Standard procedure followed for the elemental analysis of plant samples and processed materials (viz.,compost, vermicompost, ash and biochar) prepared from phytoextracted biomass is given in Table 7.

Table 7. Standard methods followed for the chemical analysis of plant material

Parameter	Method	Reference
Fe, Al, Pb and Cd	Nitric-Perchloric acid (9:4) digestion- Emission spectroscopy (ICP OES Optima 8000, PerkinElmer Inc., USA	Hesse (1971)

3.6.4. Analysis of pesticide residues

3.6.4.1. Water (Modified AOAC method)

Non-ionic residues in water were extracted by liquid-liquid partitioning method using dichloromethane. Residues of organochlorine, organophosphorus and pyrethroids can be extracted by this method. 750 mL of water sample was transferred to a 1 litre separating funnel and added 150 g sodium chloride. Dichloromethane was added at sequential intervals to partition the organic and aqueous layers. Initially 75 mL was added and shaken for 5 min. in a separating funnel at 250 rpm. Separated lower organic layer was collected in a round bottom flask and further partitioning carried out two more times using 40 mL dichloromethane. Decanted organic layer collected in the flask was concentrated on a rotary evaporator at 40 ˚C with 20 mL hexane and the process was repeated twice. The residue was evaporated to dryness in Turbo vap evaporator and made the final volume with 1 mL n-hexane and detected using GC-MS.

3.6.4.2. Sediment (Modified QuEChERS method)

Pesticide residue present in sediment samples were analysed following QuEChERS method with slight modifications. Soil sample was extracted with acetonitrile along with magnesium sulphate and sodium citrate- sodium hydrogen citrate sesquihydrate. 10 g sediment sample was taken in a 50 ml centrifuge tube and added 20 mL acetonitrile. Shaken vigorously for one minute followed by the addition of magnesium sulphate (4 g) and sodium chloride (1 g) and centrifuged at 3300 rpm for 5 minutes. 10 mL of supernatant was transferred to a 15 mL centrifuge tube containing magnesium sulphate (1.5 g) and PSA - Primary Secondary Amine (250 mg) and again centrifuged for 10 min. at 4400 rpm. 4 mL each of supernatant was collected in two vials and evaporated to dryness in rotavapour at 40°C and made to 1 mL using cyclohexane for GC- MS and in methanol for LC-MS/MS analysis.

3.6.5. Computed indices (Chaney *et al.*, 1995)

$$\text{Bio concentration factor (BCF)} = \frac{\text{Concentration in plant part}}{\text{Concentration in growing medium}}$$

$$\text{Translocation factor (TF)} = \frac{\text{Concentration in shoot}}{\text{Concentration in root}}$$

$$\text{Extraction efficiency (EE)} = \frac{\text{Metal extraction by treatment - control}}{\text{Quantity of added metal}} \times 100$$

3.7. Statistical analysis

Data generated from the investigation were statistically analysed (Cochran and Cox, 1965) by applying Analysis of variance (ANOVA) in Factorial CRD (Completely Randomised Design) for Part I (3.2.1.) and Part III (3.2.3) and CRD for part 4 (3.2.4). For Part I, sites at seven levels and sampling points at five levels were taken as the two factors with three replications. For Part III, macrophytes at nine levels and graded doses of metals at three levels were considered as the two factors and replicated five times.

Pearsons correlation coefficients were calculated to assess the relation between rhizosphere metal content and plant metal accumulation / extraction and rhizosphere microbial load.

Results

4. RESULTS

The study entitled "Phytoremediation of inorganic contaminants in Vellayani wetland ecosystem" was carried out at the College of Agriculture, Vellayani during 2013–16 with the objectives to track the potential sources of contaminants in Vellayani wetland ecosystem and suggest viable phytoremediation technologies for decontamination. The study comprised of four parts which include : (i) peripatetic survey in the catchment area of Vellayani wetland ecosystem covering the seven contaminated sites viz., Palappoor, Pallichalthodu, Reservoir bund, Arattukadavu RB, Valiyavilagam, Mannamvarambu and Manamukku; (ii) evaluation of native macrophytes for their hyperaccumulation capacity for Fe, Al, Pb and Cd; (iii) performance of selected hyperaccumulators under graded doses of toxic metals and (iv) comparison of common disposal methods of selected phytoextractors based on the bioavailability of phytoextracted metals to the plants. The data generated from the study were statistically analysed and the results are presented below.

Part I

4.1. Peripatetic inventory of the catchment area of Vellayani lake

A peripatetic survey was carried out along the catchment area of the selected sites (Table 2 and 3) of the Vellayani wetland ecosystem to identify the major sources of contamination and dominant macrophytes along the contaminated sites. Water and sediment samples were collected for analysis.

4.1.1. Sources of contamination

Based on the peripatetic survey, it was observed that the potential sources of contaminants (Table 8) that threatens the Vellayani wetland ecosystem were domestic wastes from hotels / houses, sewage, cleaning of vehicles and animals, washing of clothes, drainage from agricultural fields and discharge from automobile workshops and servicing centers (Plate 3).

Plate 3. Sources of contamination in Vellayani wetland ecosystem

Waste disposal

Lake encroachment

Detergent addition

Agricultural drain

Automobile service station

Sewage drain

Table 8. Sources of contamination in selected sites of Vellayani wetland ecosystem

Site No.	Name of the site	Sources of contamination
I	Palappoor	Non point pollution from agricultural fields Dumping of domestic and hotel waste to the lake Washing of clothes Cleaning of vehicles
II	Pallichalthodu	Non point pollution from agricultural fields Cleaning of vehicles and cattle wading Disposal of waste to the lake
III	Reservoir bund	Non point pollution from agricultural fields Dumping of waste
IV	Arattukadavu RB	Semiurban settlement area and consequent pollution Automobile workshops and servicing centre Hotel and domestic wastes Washing of clothes Cleaning of vehicles
V	Valiyavilagam	Non point pollution Tourism associated contamination Dumping of waste Washing of clothes
VI	Mannamvarambu	Non point pollution Sewage drains are opened to the lake
VII	Manamukku	Non point pollution Poultry unit Cleaning of vehicles

4.1.2. Dominant macrophytes

The details of dominant aquatic / terrestrial macrophytes collected from the selected sites of Vellayani wetland ecosystem during the pre monsoon period of 2014-15 are presented in Table 9. The dominant macrophytes comprised 14 aquatic

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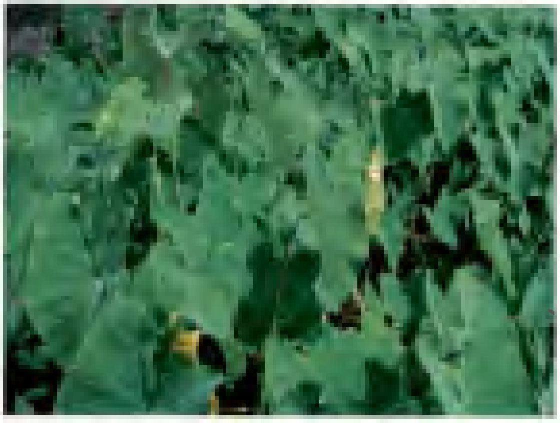
Table 9. Dominant macrophytes of Vellayani wetland ecosystem

Sl. No.	Botanical name	Family	Common name	Distribution
A	Aquatic			
a	Emergent			
1	*Colocasia esculenta* L.	Araceae	Kattu chembu	All sites
2	*Echinochloa colona* L.	Poaceae	Jungle rice	VI, VII
3	*Eleocharis dulcis* (Burm.f.)	Poaceae	Kuzhalpullu	All sites
4	*Limnocharis flava* L. (Buch.)	Alismataceae	Naagapola	All sites
5	*Monochoria vaginalis* (Burm.f.)	Pontederiaceae	Karinkoovalam	All sites
6	*Nelumbo nucifera* Gaertn. Fruct.	Nelumbonaceae	Chenthamara	I,III,IV, V,VI,VII
7	*Scirpus grossus*	Poaceae	Karadippullu	I,II,III,VI,VII
b	Floating			
1	*Eichhornea crassipes* Mart.	Pontederiaceae	Kulavazha	All sites
2	*Ipomoea aquatica* Forssk.	Convolvulaceae	Kozhuppa	I,V,VII
3	*Nymphaea nouchali* (Burm.f.)	Nymphaeaceae	Neerambal	I,II,IV,VI
4	*Nymphoides indica* L.	Gentianaceae	Chinnambal	I,III,V,VI,VII
5	*Pistia stratiotes* L.	Araceae	Water cabbage	All sites
6	*Salvinia molesta*	Salviniaceae	African payal	All sites
c	Submerged			
1	*Vallisneria natans* (Lour.)	Hydrocharitaceae	Eel grass	II,VII
B	Terrestrial			
1	*Acrostichum aureum* L.	Pteridaceae	Swamp fern	IV,V,VI
2	*Alternanthera sessilis* L.	Amaranthaceae	Kozhuppa Cheera	I,II
3	*Alternanthera tenella* Moq.	Amaranthaceae	Joy weed	All sites
4	*Blechnum orientale* L.	Blechnaceae	Centipede fern	All sites
5	*Brachearia mutica* (Forssk)	Poaceae	Parappullu	III,V,VII
6	*Buchloe dactyoides*	Poaceae	Buffalo grass	I,II,III,V, VI,VII
7	*Carex lindleyana* Nees.	Poaceae	Korapullu	II,III
8	*Cynadon dactylon* L.	Poaceae	Karuka	All sites
9	*Gymnopetalum tubiflorum* (Wight and Arn)	Cucurbitaceae		IV
10	*Isachne globosa* Thunb.	Poaceae	Swamp millet	IV
11	*Lindernia rotundifolia* L.	Scrophularaceae	Tsjanga puspam	I,II
12	*Pandanus kaida* Kurz.	Pandanaceae	Kaida	II,III,IV,V,VI
13	*Panicum repens*	Poaceae	Injipullu	III,IV,V, VI,VII
14	*Persicaria hydropiper* L.	Polygonaceae	Water pepper	IV,VII
15	*Sphagneticola trilobata* L.	Asteraceae	Manja kanjunni	All sites

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Plate 4. Dominant emergent aquatic macrophytes found in Vellayani wetland ecosystem



Colocasia esculenta

Nelumbo nucifera

Eleocharis dulcis

Limnocharis flava

Scirpus grossus

Monochoria vaginalis

and 15 terrestrial species belonging to 18 different families (Plates 4 to 6). Among the aquatic macrophytes, seven species were emergent type, six were floating type and one was submerged type.

Among the emergent aquatic macrophytes, *C. esculenta*, *E. dulcis*, *L. flava*, and *M. vaginalis* were present in all the seven sites. *N. nucifera* was found in all the sites except Pallichlthodu. *E. colona* was present in two sites (Mannamvarambu and Manamukku) and *S. grossus* in all the sites excluding Arattukadavu RB and Valiyavilagam.

The floating aquatic macrophytes *E. crassipes, P. stratiotes* and *S. molesta* were present in all the sampling sites while *N. indica* in all other sites except Pallichalthodu and Arattukadavu RB. *I. aquatica* was present in three sites (Palappoor, Valiyavilagam and Manamukku) and *N. nouchali* in four sites (Palappoor, Pallichalthodu, Valiyavilagam and Mannamvarambu). *V. natans*, submerged aquatic macrophyte, was found only at Pallichalthodu and Manamukku.

Among the terrestrial macrophytes, *A. tenella*, *B. orientale*, *C. dactylon* and *S. trilobata* were present in all the sites while *G. tubiflorum* and *I. globosa* were found only at Arattukadavu RB. *A. sessilis* and *L. rotundifolia* were present only at Palappoor and Pallichalthodu. *B. dactyoides* was found at six sites excluding Arattukadavu RB and *P. repens* at five sites excluding Palappoor and Pallichalthodu. Two species were present at three sites viz., *B. mutica* (Reservoir bund, Valiyavilagam and Manamukku) and *A. aureum* (Arattukadavu RB, Valiyavilagam and Mannamvarambu). *P. kaida* was present in all the other sites except Palappoor and Manamukku. *C. lindleyana* (Pallichalthodu and Reservoir bund) and *P. hydropiper* (Arattukadavu RB and Manamukku) were present in two sites only.

Plate 5. Dominant floating aquatic macrophytes found in Vellayani wetland ecosystem

Ipomoea aquatica

Eichhornea crassipes

Nympheae nouchali

Nymphoides indica

Salvinia molesta

Pistia stratiotes

4.1.2.1. Density of dominant macrophytes

Average density (number per square metre or No. m^{-2}) of the dominant macrophytes found in the experimental sites of Vellayani wetland ecosystem is furnished in Table 10. Out of the native macrophytes, *C. dactylon*, the terrestrial macrophyte had the highest average density (34.70) while the floating aquatic macrophyte, *N. nouchali* recorded the lowest (0.80).

Among the emergent aquatic macrophytes, the highest density was recorded by *E. dulcis* (17.99) and the lowest by *N. nucifera* (1.40). Mannamvarambu recorded the highest density for four macrophytes (*E. colona*, *L. flava*, *M. vaginalis* and *N. nucifera*) and one each at Pallichalthodu (*S. grossus*), Reservoir bund (*E. dulcis*) and Arattukdavu RB (*C. esculenta*). Manamukku (*E. colona* and *E. dulcis*) and Reservoir bund (*L. flava* and *N. nucifera*) had the lowest density for two macrophytes each. The density of *C. esculenta* was lowest at Pallichalthodu, *M. vaginalis* at Valiyavilagam and *S. grossus* at Mannamvarambu.

Pallichalthodu (*E. crassipes* and *I. aquatica*) and Arattukadavu RB (*P. stratiotes* and *S. molesta*) recorded the highest density for two floating macrophytes each, while Reservoir bund (*N. nouchali*) and Mannamvarambu (*N. indica*) for one each. The lowest density for *E. crassipes* and *N. indica* was recorded at Palappoor, *P. stratiotes* at Pallichalthodu, *N. nouchali* at Arattukadavu RB, *S. molesta* at Valiyavilagam and *I. aquatica* at Manamukku. *E. crassipes* (15.06) had the highest density among the floating macrophytes. The submerged macrophyte, *V. natans* had the highest density at Manamukku.

Among the terrestrial macrophytes, *C. dactylon* had the highest density and *P. hydropiper* (1.11) recorded the lowest density. Four species had the highest density at Manamukku (*A. tenella*, *B. mutica*, *C. dactylon* and *P. repens*) and three at Mannamvarambu (*A. aureum*, *B. orientale* and *P. kaida*). Palappoor (*A. sessilis* and *L. rotundifolia*), Pallichalthodu (*C. lindleyana* and *S. trilobata*) and Valiyavilagam

Plate 6. Dominant terrestrial macrophytes found in Vellayani wetland ecosystem

Alternanthera tenella

Buchloe dactyoides

Blechnum orientale

Pandanus kaida

Panicum repens

Sphagneticola trilobata

Table 10. Density of dominant macrophytes in Vellayani wetland ecosystem

Sl. No.	Macrophytes	Sites							
		I	II	III	IV	V	VI	VII	Mean
		Density (No. m^{-2})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	2.33	1.73	2.67	4.43	4.00	3.33	3.73	3.17
2	*E. colona*	-	-	-	-	-	3.67	0.87	2.27
3	*E. dulcis*	15.50	24.51	26.93	9.32	18.50	25.34	5.87	17.99
4	*L. flava*	1.87	2.25	1.53	3.47	2.80	5.07	4.32	3.04
5	*M. vaginalis*	1.73	1.27	2.27	2.50	1.07	2.97	2.73	2.08
6	*N. nucifera*	1.60	-	0.53	1.13	1.73	2.27	1.13	1.40
7	*S. grossus*	15.67	8.00	6.27	-	-	4.87	5.67	8.09
b	Floating								
1	*E. crassipes*	4.50	26.07	7.53	9.97	11.50	20.58	25.30	15.06
2	*I. aquatica*	3.07	-	-	-	1.4	-	0.73	1.73
3	*N. nouchali*	0.78	1.60	-	0.35	-	0.46	-	0.80
4	*N. indica*	0.47	-	0.75	-	0.98	1.53	1.20	0.99
5	*P. stratiotes*	6.47	4.17	7.17	11.47	8.67	7.58	7.12	7.52
6	*S. molesta*	10.53	16.67	20.93	25.33	5.85	10.53	7.40	13.89
c	Submerged								
1	*V. natans*	-	2.40	-	-	-	-	3.00	2.70
B	Terrestrial								
1	*A. aureum*	-	-	-	1.40	1.10	2.93	-	1.81
2	*A. sessilis*	3.47	1.27	-	-	-	-	-	2.37
3	*A. tenella*	4.13	4.87	2.60	3.87	5.33	5.73	6.13	4.66
4	*B. orientale*	0.58	1.07	1.13	1.55	0.93	2.20	1.83	1.33
5	*B. mutica*	-	-	1.20	-	1.67	-	3.93	2.27
6	*B. dactyoides*	2.43	3.13	3.00	-	6.87	3.33	3.50	3.71
7	*C. lindleyana*	-	1.8	1.1	-	-	-	-	1.45
8	*C. dactylon*	25.30	17.33	37.70	35.62	50.24	14.36	62.33	34.70
9	*G. tubiflorum*	-	-	-	2.93	-	-	-	2.93
10	*I. globosa*	-	-	-	1.93	-	-	-	1.93
11	*L. rotundifolia*	1.47	1.27	-	-	-	-	-	1.37
12	*P. kaida*	-	1.18	0.60	1.07	1.73	1.73	-	1.26
13	*P. repens*	-	-	2.5	1.8	2.80	3.21	4.47	2.95
14	*P. hydropiper*	-	-	-	1.30	-	-	0.93	1.11
15	*S. trilobata*	4.48	5.27	3.80	4.05	2.40	3.16	3.80	3.85

- *absence of macrophyte in the site*

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

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(*B. dactyoides* and *P. kaida*) recorded the highest density for two macrophytes each. *P. hydropiper* had the highest density at Arattukadavu RB. Reservoir bund had the lowest density for four macrophytes (*A. tenella, B. mutica, C. linleyana* and *P. kaida*) while Palappoor (*B. orientale* and *B. dactyoides*), Pallichalthodu (*A. sessilis* and *L. rotundifolia*) and Valiyavilagam (*A. aureum* and *S. trilobata*) for two each. *P. repens* recorded the lowest density at Arattukadavu RB, *C. dactylon* at Mannamvarambu and *P. hydropiper* at Manamukku. In general, the density was higher at Mannamvarambu compared to other sites.

4.1.3. Water

Physical, chemical and biological properties of lake water collected from five sampling points of the seven identified sites of Vellayani wetland ecosystem during pre and post monsoon periods are furnished in Tables 11 to 15.

4.1.3.1. Physical properties

Perusal of the data on physical properties revealed that all the physical properties except temperature were significantly influenced by the sampling sites (Table 11) while the interaction of sites and sampling points was not statistically significant except in the case of suspended solids. The values were compared with ISI / WHO standards of maximum permissible limits for evaluation.

Water temperature was lower during the post monsoon period compared to pre monsoon. Colour of lake water was significantly influenced by the sampling sites during both the seasons, while sampling points had significant influence only during pre monsoon season. Reservoir bund recorded the highest colour index during both pre and post monsoon seasons (13.40 and 13.47 respectively). The colour index was slightly higher during the post monsoon season. The colour of lake water was above the MPL at all the sites.

Turbidity and suspended solids of water were significantly influenced by sites and sampling points during both the seasons. The highest value for turbidity was

Table 11. Physical properties of water from Vellayani wetland ecosystem

Locations	Temperature °C		Colour Hazen units		Turbidity NTU		Suspended solids mg L^{-1}	
	Pre-M	Post-M	Pre-M	Post-M	Pre-M	Post-M	Pre-M	Post-M
Sites								
Palappoor	29.87	29.05	12.13^{bc}	12.40^{bc}	35.75^{a}	32.05^{a}	537.00^{a}	485.33^{a}
Pallichalthodu	29.96	29.05	12.87^{ab}	12.93^{ab}	30.59^{d}	20.22^{b}	552.00^{a}	464.66^{b}
Reservoir bund	29.96	29.00	13.40^{a}	13.47^{a}	32.54^{c}	21.99^{bc}	508.00^{bc}	463.33^{b}
ArattukadavuRB	29.91	28.91	12.80^{ab}	12.67^{ab}	34.14^{b}	18.88^{bc}	518.66^{b}	468.66^{b}
Valiyavilagam	29.88	28.87	11.53^{cd}	11.67^{c}	33.32^{bc}	21.39^{cd}	516.00^{b}	454.66^{b}
Mannamvarambu	30.12	28.98	11.67^{cd}	11.73^{c}	26.10^{e}	17.72^{e}	424.00^{d}	421.33^{c}
Manamukku	29.86	28.91	11.20^{d}	11.47^{c}	29.65^{d}	18.66^{de}	495.00^{c}	460.00^{b}
CD(0.05) Site	NS	NS	0.904	0.987	1.231	2.876	16.889	16.300
Sampling points (SP)								
SP_1	29.93	28.92	11.62^{b}	11.47	30.43^{c}	19.49^{c}	492.38^{c}	421.90^{d}
SP_2	29.92	29.01	11.67^{b}	12.24	30.28^{b}	20.59^{bc}	488.10^{c}	440.00^{c}
SP_3	29.93	28.97	12.57^{a}	12.62	31.71^{c}	22.29^{b}	497.14^{c}	464.29^{b}
SP_4	29.85	28.97	12.76^{a}	12.62	32.92^{a}	25.77^{a}	517.62^{b}	477.14^{b}
SP_5	30.06	28.95	12.52^{a}	12.71	33.30^{a}	27.22^{a}	541.19^{a}	495.24^{a}
CD (0.05) SP	NS	NS	0.764	NS	1.040	2.430	14.274	13.776
Site X SP	NS	NS	NS	NS	NS	NS	37.767	NS
MPL	20-35		5		10		500	

MPL: Maximum permissible limit Pre-M: Pre monsoon Post-M: Post monsoon

recorded at Palappoor and the lowest at Mannamvarambu during both pre and post monsoon seasons. The site and sampling point interaction was significant only for suspended solids during pre-monsoon season. Suspended solids were highest at Pallichalthodu (552 mg L^{-1}) during pre monsoon and at Palappoor (485.33 mg L^{-1}) during the post monsoon season. The lowest value for suspended solids was recorded at Mannamvarambu during both the seasons. The turbidity of lake water was above the MPL at all the sites during both the seasons while the suspended solids were above the MPL during pre-monsoon season only, except at Mannamvarambu and Manamukku. In general, the colour, turbidity and suspended solids decreased with increasing distance from the source of contamination (SP_5 to SP_1).

4.1.3.2. Chemical properties

pH and EC of water (Table 12) were significantly influenced by sampling sites during both the seasons. But the influence of sampling points and its interaction with site was significant for EC only, during pre monsoon period. Mannamvarambu and Palappoor recorded the highest values for pH and EC while Arattukadavu RB and Manamukku registered the lowest values for pH and EC, respectively. Both pH and EC were found to be below the MPL at all the sites. pH increased with distance from the source of contamination while EC followed the reverse pattern.

Table 12. pH, EC, BOD and COD of water from Vellayani wetland ecosystem

Locations	pH		EC (dS m^{-1})		BOD (mg L^{-1})		COD (mg L^{-1})	
	Pre-M	Post-M	Pre-M	Post-M	Pre-M	Post-	Pre-M	Post-M
Sites								
Palappoor	5.87^{bc}	6.01^{bc}	0.23^{a}	0.21^{a}	8.49^{a}	5.30^{a}	212.66	179.33^{ab}
Pallichalthodu	5.89^{bc}	6.10^{ab}	0.20^{c}	0.19^{ab}	8.21^{a}	4.33^{b}	231.33	189.33^{a}
Reservoir bund	5.90^{bc}	5.98^{c}	0.21^{bc}	0.18^{ab}	8.31^{a}	5.12^{a}	213.33	173.33^{b}
Arattukadavu RB	5.84^{c}	5.87^{d}	0.22^{b}	0.18^{ab}	8.62^{a}	5.18^{a}	233.33	179.33^{ab}
Valiyavilagam	6.06^{a}	6.07^{abc}	0.20^{bc}	0.19^{ab}	7.33^{b}	4.24^{b}	208.00	142.66^{c}
Mannamvarambu	6.08^{a}	6.13^{a}	0.21^{bc}	0.18^{b}	6.58^{c}	4.24^{b}	224.00	138.00^{c}
Manamukku	5.95^{b}	6.09^{ab}	0.17^{d}	0.15^{c}	6.24^{c}	3.66^{c}	197.33	128.66^{c}
CD(0.05) Site	0.101	0.112	0.013	0.026	0.711	0.474	NS	15.812
Sampling points (SP)								
SP_1	6.18^{a}	6.19^{a}	0.19^{c}	0.17	6.15^{e}	3.88^{d}	191.90^{c}	129.05^{d}
SP_2	6.06^{b}	6.14^{ab}	0.20^{b}	0.18	7.01^{d}	4.06^{cd}	218.33^{ab}	152.38^{c}
SP_3	5.93^{c}	6.07^{b}	0.21^{b}	0.19	7.70^{c}	4.40^{bc}	235.24^{a}	168.09^{b}
SP_4	5.81^{d}	5.96^{c}	0.21^{b}	0.19	8.38^{b}	4.33^{b}	212.86^{b}	172.38^{ab}
SP_5	5.73^{d}	5.80^{d}	0.22^{a}	0.19	9.20^{a}	5.79^{a}	227.38^{ab}	185.71^{a}
CD (0.05) SP	0.085	0.095	0.012	NS	0.601	0.400	19.60	13.36
Site X SP	NS	NS	0.030	NS	NS	NS	NS	NS
MPL	6.5 – 8.5		1 – 2		5		250	

MPL: Maximum permissible limit Pre-M: Pre monsoon Post-M: Post monsoon

BOD of water (Table 12) was significantly influenced by the sites and sampling points during both the seasons. All the sites recorded values above MPL during pre monsoon period and there was a decline in BOD towards the post monsoon season. Highest BOD was recorded by Arattukadavu RB (8.62 mg L^{-1}) in the pre-monsoon season and Palappoor (5.30 mg L^{-1}) in the post-monsoon period.

Manamukku recorded the lowest BOD values during both the seasons. COD was significantly influenced by the sites only during the post monsoon season. Arattukadavu RB recorded the highest value (233.33 mg L^{-1}) during the pre monsoon season and Pallichalthodu during post monsoon season (189.33 mg L^{-1}). COD was lowest at Manamukku during both the seasons. The COD values were below MPL for all the sites during both the seasons.

Data on NO_3-N, NH_4-N and P contents of water are furnished in Table 13 and all the three parameters were significantly influenced by the sites. But the effect of sampling points was significant only for NO_3-N (both seasons) and P (post monsoon). The highest NO_3-N was recorded at Arattukadavu RB during pre-monsoon season (0.131 mg L^{-1}) and at Palappoor during post-monsoon season (0.128 mg L^{-1}). The interaction between sites and sampling points was significant only during the post monsoon season. Arattukadavu RB recorded the highest values for NH_4-N and P during both the seasons.

Table 13. NO_3-N, NH_4-N and P contents of lake water

Locations	NO_3-N (mg L^{-1})		NH_4-N (mg L^{-1})		P (mg L^{-1})	
	Pre-M	Post-M	Pre-M	Post-M	Pre-M	Post-M
Sites						
Palappoor	0.124^{ab}	0.128^{a}	0.106^{b}	0.133^{bc}	0.097^{bc}	0.060^{b}
Pallichalthodu	0.108^{d}	0.110^{bc}	0.102^{b}	0.123^{bc}	0.085^{c}	0.040^{c}
Reservoir bund	0.122^{bc}	0.119^{ab}	0.107^{b}	0.135^{b}	0.103^{b}	0.046^{c}
Arattukadavu RB	0.131^{a}	0.124^{a}	0.142^{a}	0.155^{a}	0.118^{a}	0.072^{a}
Valiyavilagam	0.111^{cd}	0.104^{c}	0.132^{a}	0.117^{d}	0.094^{bc}	0.066^{ab}
Mannamvarambu	0.113^{cd}	0.120^{ab}	0.116^{b}	0.102^{cd}	0.106^{ab}	0.058^{b}
Manamukku	0.119^{bc}	0.108^{bc}	0.132^{a}	0.126^{bc}	0.103^{b}	0.059^{b}
CD(0.05) Site	0.008	0.012	0.014	0.016	0.013	0.009
Sampling points						
SP 1	0.106^{d}	0.112^{b}	0.110	0.130	0.099	0.044^{d}
SP 2	0.114^{c}	0.107^{b}	0.121	0.124	0.091	0.052^{cd}
SP 3	0.119^{bc}	0.117^{b}	0.126	0.125	0.103	0.062^{ab}
SP 4	0.123^{ab}	0.128^{a}	0.122	0.124	0.100	0.059^{bc}
SP 5	0.129^{a}	0.116^{b}	0.121	0.134	0.112	0.068^{a}
CD (0.05) SP	0.006	0.009	NS	NS	NS	0.008
Site X SP	NS	0.0263	NS	NS	NS	NS
MPL	10		16		0.025	

MPL: Maximum permissible limit Pre-M: Pre monsoon Post-M: Post monsoon

NH_4-N content was lowest at Pallichalthodu during the pre monsoon season and at Mannamvarambu during the post monsoon season. P content was lowest at Pallichalthodu during both the seasons. Both NO_3-N and NH_4-N were much below the MPL at all the sites during both the seasons while P was above the MPL specified for domestic use.

Fe, Al and Pb contents of lake water (Table 14) were significantly influenced by the sites during both the seasons while Cd was significantly influenced only during pre monsoon season. Sampling points had significant influence on Al content during both the seasons while it was significant for Fe, Pb and Cd during pre monsoon season only. Fe content was highest at Pallichalthodu (0.125 mg L^{-1}) during pre-monsoon period and at Arattukadavu RB during post-monsoon season (0.117 mg L^{-1}). The lowest value (0.072 mg L^{-1}) was recorded at Manamukku during pre monsoon season and at Mannamvarambu during post monsoon season.

Table 14. Fe, Al, Pb and Cd contents of water from Vellayani wetland ecosystem

Locations	Fe (mg L^{-1})		Al (mg L^{-1})		Pb (mg L^{-1})		Cd (mg L^{-1})	
	Pre-M	Post-M	Pre-M	Post-M	Pre-M	Post-M	Pre-M	Post-M
Sites								
Palappoor	0.110^{b}	0.084^{bc}	0.075^{b}	0.038^{b}	0.020^{bc}	0.016^{a}	0.0057^{b}	0.0008
Pallichalthodu	0.125^{a}	0.092^{b}	0.066^{c}	0.042^{a}	0.020^{c}	0.015^{ab}	0.0027^{c}	0.0008
Reservoir bund	0.114^{b}	0.061^{d}	0.060^{cd}	0.032^{c}	0.015^{d}	0.011^{bc}	0.0063^{b}	0.0011
Arattukadavu RB	0.112^{b}	0.117^{a}	0.104^{a}	0.045^{a}	0.023^{ab}	0.018^{a}	0.0112^{a}	0.0011
Valiyavilagam	0.074^{c}	0.069^{cd}	0.053^{e}	0.024^{d}	0.010^{e}	0.009^{c}	0.0026^{c}	0.0006
Mannamvarambu	0.074^{c}	0.021^{de}	0.046^{f}	0.028^{cd}	0.023^{ab}	0.018^{a}	0.0027^{c}	0.0007
Manamukku	0.072^{c}	0.067^{d}	0.059^{d}	0.035^{b}	0.019^{c}	0.019^{a}	0.0022^{c}	0.0006
CD(0.05) Site	0.009	0.015	0.007	0.004	0.002	0.005	0.0009	NS
Sampling points								
SP_1	0.088^{c}	0.068	0.058^{c}	0.029^{c}	0.015^{d}	0.012	0.0041^{b}	0.0008
SP_2	0.095^{b}	0.072	0.062^{bc}	0.032^{bc}	0.017^{c}	0.016	0.0043^{b}	0.0008
SP_3	0.098^{ab}	0.075	0.064^{b}	0.034^{b}	0.019^{bc}	0.016	0.0043^{b}	0.0009
SP_4	0.104^{a}	0.075	0.072^{a}	0.036^{b}	0.020^{ab}	0.016	0.0053^{a}	0.0007
SP_5	0.102^{ab}	0.074	0.076^{a}	0.044^{a}	0.022^{a}	0.015	0.0060^{a}	0.0009
CD (0.05) SP	0.008	NS	0.005	0.003	0.002	NS	0.0008	NS
Site X SP	NS	NS	NS	NS	NS	NS	NS	NS
MPL	0.30		0.2		0.10		0.01	

MPL- Maximum permissible limit Pre-M: Pre monsoon Post-M: Post monsoon

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Arattukadavu RB recorded the highest Al content during both the seasons while the lowest content was noticed at Mannamvarambu (0.046 mg L^{-1}) during pre monsoon and at Valiyavilagam (0.024 mg L^{-1}) during post monsoon season.

Pb content was highest (0.023 mg L^{-1}) at Arattukadavu RB and Mannamvarambu during pre monsoon season and at Manamukku (0.019 mg L^{-1}) during post monsoon season. Valiyavilagam recorded the lowest Pb content during both the seasons. The highest value for Cd (0.0112 mg L^{-1}) was recorded at Arattukadavu RB during pre monsoon season and at Reservoir bund and Arattukadavu RB during post monsoon season (0.0011 mg L^{-1}). The lowest Cd level was detected at Manamukku during pre monsoon season and at Manamukku and Valiyavilagam during post monsoon season.

Fe and Pb contents were within MPL limits. As per ISI standard, the desirable limit of Al is 0.03 mg L^{-1} and in all sites except Valiyavilagam and Mannamvarambu, the content was found to be above the desirable limit, but much below the MPL. Al content in water ranged from 0.024 to 0.104 mg L^{-1}. Cd content was below MPL at all sites except Arattukadavu RB during pre monsoon season.

4.1.3.3. Biological properties

The coliform population (Table 15) was not influenced by sites during both the seasons. The count was highest at Arattukadavu RB during both the seasons while Mannamvarambu recorded the lowest count during pre-monsoon season and Palappoor during the post-monsoon season. The influence of sampling points on coliform count was significant only during the pre monsoon season. Coliform count was much above the MPL at all sites and it gradually decreased with distance from the source of contamination.

Table 15. Coliform population in lake water

Locations	Coliform count MPN index per ml	
	Pre monsoon	Post monsoon
Sites		
Palappoor	2.318	1.683
Pallichalthodu	2.527	1.872
Reservoir bund	2.803	2.625
Arattukadavu RB	4.040	3.675
Valiyavilagam	2.198	2.265
Mannamvarambu	1.319	1.799
Manamukku	1.853	2.296
CD(0.05) Site	NS	NS
Sampling points		
SP_1	1.355^b	1.440
SP_2	1.873^b	3.019
SP_3	2.042^b	2.549
SP_4	2.421^b	1.579
SP_5	4.495^a	3.001
CD (0.05) SP	1.108	NS
Site X SP	NS	NS
MPL	0.1 coliform per ml	

MPL: Maximum permissible limit

Scrutiny of the data on physical, chemical and biological qualities of water revealed that the contamination was highest at Arattukadavu RB (10 parameters viz., BOD, COD, NH_4-N, NO_3-N, P, Fe, Al, Pb, Cd and coliforms) followed by Palappoor and lowest at Manamukku preceded by Mannamvarmabu and Valiyavilagam.

4.1.4. Sediment

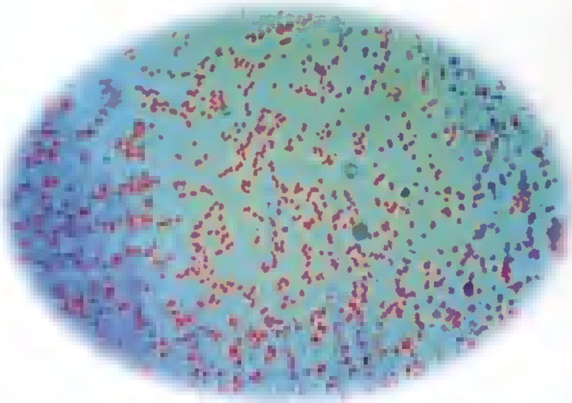
Texture and chemical properties of sediment collected from the five sampling points of the seven identified sites of Vellayani wetland ecosystem, during the pre and post monsoon periods of 2014-15 are furnished in Tables 16 to 19.

Table 7 . Estimation of coliform bacteria in lake water

Incubation of samples in lactose broth

Presence of lactose fermenting bacteria

Metallic sheen nucleated colonies



Gram negative *E. coli* bacteria

4.1.4.1. Sediment texture

Textural class ranged from sandy clay to sandy clay loam with sandy clay texture at Valiyavilagam; sandy loam at Palappoor, Reservoir bund, Arattukadavu RB and Mannamvarambu and sandy clay loam at Pallichalthodu and Manamukku.

Table 16. Textural class of sediments collected from selected sites of Vellayani lake

Location	Sand (%)	Silt (%)	Clay (%)	Textural class
Palappoor	86.84	3.25	9.91	Sandy loam
Pallichalthodu	69.09	9.27	21.64	Sandy clay loam
Reservoir bund	88.26	1.38	10.36	Sandy loam
Arattukadavu RB	80.15	8.13	11.72	Sandy loam
Valiyavilagam	68.34	1.02	30.64	Sandy clay
Mannamvarambu	85.90	2.54	11.56	Sandy loam
Manamukku	72.25	7.19	20.55	Sandy clay loam

4.1.4.2. Chemical properties of sediment

pH and EC of sediments (Table 17) were significantly influenced by sites and sampling points during both the seasons. It was observed that pH was slightly higher during post monsoon season. The highest pH was recorded at Mannamvarambu during both the seasons. pH was lowest at Palappoor during the pre-monsoon season and at Reservoir bund and Arattukadavu RB during the post-monsoon season. The site and sampling point interaction had significantly influenced pH during the pre monsoon season only. EC was highest at Reservoir bund during the pre monsoon period (0.134 dS m^{-1}) and at Arattukadvu RB (0.125 dS m^{-1}) during the post monsoon season. Mannamvarambu recorded the lowest value during both the seasons.

OC content of sediment (Table 17) was significantly influenced only by the sites during both the seasons. Arattukadavu RB recorded the highest value during both the seasons while Mannamvarambu recorded the lowest. There was a reduction in OC content in the post monsoon season compared to the pre monsoon period. The sampling points had no significant influence on OC content of the sediment during both the seasons. OC ranged from 22.9 to 29.9 g kg^{-1} during the pre monsoon season and from 22.3 to 27.8 g kg^{-1} during the post monsoon season.

Table 17. pH, EC and OC of sediment from Vellayani wetland ecosystem

Locations	pH		EC (dS m^{-1})		OC (g kg^{-1})	
	Pre- M	Post-M	Pre- M	Post- M	Pre- M	Post- M
Sites						
Palappoor	5.37^{d}	5.61^{bc}	0.126^{c}	0.117^{b}	26.5^{b}	24.2^{bc}
Pallichalthodu	5.44^{c}	5.56^{c}	0.131^{ab}	0.124^{a}	24.2^{c}	22.9^{cd}
Reservoir bund	5.40^{cd}	5.55^{c}	0.134^{a}	0.122^{ab}	26.4^{b}	24.7^{b}
Arattukadavu RB	5.38^{d}	5.55^{c}	0.131^{a}	0.125^{a}	29.9^{a}	27.8^{a}
Valiyavilagam	5.59^{b}	5.70^{ab}	0.123^{c}	0.115^{c}	24.3^{c}	22.9^{cd}
Mannamvarambu	5.65^{a}	5.76^{a}	0.111^{d}	0.105^{d}	22.9^{c}	22.3^{d}
Manamukku	5.46^{c}	5.61^{bc}	0.116^{d}	0.110^{cd}	26.1^{b}	24.6^{b}
CD(0.05) Sites	0.061	0.104	0.006	0.007	1.39	1.47
Sampling points						
SP_1	5.72^{a}	5.82^{a}	0.104^{e}	0.094^{c}	24.6	22.9
SP_2	5.53^{b}	5.60^{b}	0.115^{d}	0.112^{b}	25.4	24.3
SP_3	5.46^{c}	5.55^{b}	0.128^{c}	0.123^{a}	26.0	24.1
SP_4	5.35^{d}	5.54^{b}	0.133^{b}	0.128^{a}	26.3	24.6
SP_5	5.29^{e}	5.60^{b}	0.142^{a}	0.129^{a}	26.4	25.1
CD (0.05) SP	0.052	0.088	0.005	0.006	NS	NS
Site X SP	0.137	NS	NS	NS	NS	NS

Pre-M: Pre monsoon Post-M: Post monsoon

The sites and sampling points had significant influence on NO_3-N and available P contents (Table 18) of sediment during both the seasons while NH_4-N content was significantly influenced during the post monsoon season only. NO_3-N content was highest at Reservoir bund during both the seasons. Palappoor recorded the highest value for NH_4-N during the pre monsoon period (62.55 mg kg^{-1}) while it was at Arattukadavu RB during the post monsoon season (74.42 mg kg^{-1}). NO_3-N and NH_4-N contents of sediment decreased with increasing distance from the source of contamination. Also the contents were higher during the post monsoon period compared to the pre monsoon season.

Table 18. NO_3-N, NH_4-N and available P contents of sediment

Locations	NO_3-N (mg kg^{-1})		NH_4-N (mg kg^{-1})		P (mg kg^{-1})	
	Pre- M	Post- M	Pre- M	Post- M	Pre- M	Post- M
Sites						
Palappoor	9.87^{bc}	10.56^{c}	62.55	71.19^{bcd}	9.35^{bc}	9.54^{cd}
Pallichalthodu	10.13^{b}	11.21^{b}	60.33	71.34^{bc}	8.76^{c}	8.39^{e}
Reservoir bund	10.89^{a}	12.04^{a}	59.99	72.09^{b}	8.06^{d}	9.73^{bc}
ArattukadavuRB	9.75^{bc}	10.44^{c}	61.06	74.42^{a}	10.46^{a}	11.07^{a}
Valiyavilagam	9.29^{c}	9.62^{d}	59.80	69.01^{de}	9.13^{c}	9.63^{bc}
Mannamvarambu	10.01^{b}	10.19^{cd}	61.05	69.57^{cde}	8.04^{d}	8.90^{de}
Manamukku	9.89^{bc}	10.34^{bc}	62.11	68.26^{e}	9.97^{ab}	10.23^{b}
CD (0.05)	0.634	0.579	NS	2.187	0.637	0.675
Sampling points						
SP_1	8.65^{d}	8.74^{d}	59.53	66.99^{d}	8.46^{b}	8.09^{d}
SP_2	9.73^{c}	10.22^{c}	60.92	69.41^{c}	9.09^{a}	9.09^{c}
SP_3	10.18^{bc}	10.80^{b}	61.66	71.14^{bc}	9.29^{a}	9.83^{b}
SP_4	10.54^{ab}	11.57^{a}	61.28	71.75^{b}	9.30^{a}	10.23^{b}
SP_5	10.79^{a}	11.80^{a}	61.53	74.91^{a}	9.39^{a}	10.95^{a}
CD (0.05) SP	0.536	0.489	NS	1.848	0.538	0.570
Site X SP	NS	NS	NS	NS	NS	NS

Pre-M: Pre monsoon Post-M: Post monsoon

Available P content was highest at Arattukadavu RB during both the seasons. The lowest available P content was recorded at Mannamvarambu (8.04 mg kg^{-1}) during the pre monsoon season and at Pallichalthodu (8.39 mg kg^{-1}) during the post monsoon season. The site and sampling point interaction had no influence on the above three parameters during both the seasons.

Available Fe, Al, Pb and Cd contents of sediment (Table 19) were significantly influenced by sites and sampling points. Available Fe and Al contents were comparatively higher during the pre monsoon season. Available Fe content was highest at Palappoor during pre monsoon season and at Arattukadavu RB during post monsoon season. Mannamvarambu recorded the lowest available Fe content during both the seasons. The site and sampling point interaction had significant influence only on available Fe content during the post monsoon season. Available Al content was highest at Palappoor during both the seasons (693.13 and 673.33 mg kg^{-1},

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respectively). Valiyavilagam recorded the lowest Al content during the pre monsoon period and at Mannamvarambu during the post monsoon period.

Table 19. Available Fe, Al, Pb and Cd contents of sediment

Locations	Fe (mg kg^{-1})		Al (mg kg^{-1})		Pb (mg kg^{-1})		Cd (mg kg^{-1})	
	Pre- M	Post- M	Pre- M	Post- M	Pre- M	Post- M	Pre- M	Post- M
Sites								
Palappoor	850.93^{a}	800.13^{a}	693.13^{c}	673.33^{a}	4.82^{a}	5.28^{ab}	0.0057^{b}	0.0091^{bc}
Pallichalthodu	789.13^{b}	761.33^{b}	666.80^{b}	654.00^{ab}	4.79^{a}	5.69^{a}	0.0032^{c}	0.0093^{b}
Reservoir bund	725.87^{c}	705.60^{c}	637.80^{c}	639.00^{bc}	3.51^{b}	4.58^{b}	0.0063^{b}	0.0088^{bc}
ArattukadavuRB	844.07^{a}	804.93^{a}	676.53^{ab}	665.93^{a}	4.42^{a}	5.18^{ab}	0.0093^{a}	0.0119^{a}
Valiyavilagam	665.67^{d}	672.00^{d}	622.40^{c}	619.93^{cd}	2.98^{bc}	3.53^{c}	0.0029^{cd}	0.0079^{cd}
Mannamvarambu	642.47^{d}	637.40^{e}	623.73^{c}	612.00^{d}	2.93^{c}	2.64^{d}	0.0027^{cd}	0.0069^{de}
Manamukku	751.73^{c}	725.33^{c}	645.66^{bc}	633.06^{bcd}	2.66^{c}	3.25^{cd}	0.0022^{d}	0.0060^{de}
CD(0.05) Sites	37.190	27.958	28.265	25.880	0.564	0.751	0.0009	0.0013
Sampling points								
SP_1	690.90^{c}	680.76^{d}	617.67^{c}	615.38^{c}	3.06^{d}	3.68^{b}	0.0041^{c}	0.0096^{a}
SP_2	743.66^{b}	714.90^{bc}	646.05^{b}	629.57^{bc}	3.37^{cd}	3.79^{b}	0.0043^{bc}	0.0089^{ab}
SP_3	754.71^{b}	738.33^{bc}	661.62^{ab}	658.33^{a}	3.77^{bc}	4.55^{a}	0.0044^{bc}	0.0080^{bc}
SP_4	770.47^{b}	749.85^{a}	664.71^{ab}	649.62^{ab}	4.50^{a}	4.67^{a}	0.0049^{ab}	0.0075^{c}
SP_5	803.43^{a}	763.81^{a}	671.43^{a}	659.43^{a}	3.95^{b}	4.83^{a}	0.0054^{a}	0.0088^{ab}
CD (0.05) SP	31.431	23.629	23.888	21.872	0.477	0.635	0.0007	0.0011
Site X SP	NS	62.517	NS	NS	NS	NS	NS	NS

Pre-M: Pre monsoon Post-M: Post monsoon

Available Pb and Cd contents were higher during the post monsoon period. The available Pb content was highest at Palappoor in the pre monsoon season and at Pallichalthodu in the post monsoon season. Manamukku and Mannamvarambu recorded the lowest value for available Pb during the pre and post monsoon seasons, respectively. Available Cd was detected only in very small quantities at all the sites and Arattukadavu RB recorded the highest value (0.0093 and 0.0119 mg kg^{-1}, respectively) during both the seasons. The lowest available Cd content was recorded at Manamukku during both the seasons. The concentration of metals decreased with increase in distance from the source of contamination.

Perusal of the data on sediment properties revealed that the inorganic contamination was comparatively higher at Palappoor and Arattukadavu RB and lower at Manamukku and Mannamvarmabu.

4.1.4 Pesticide residues

Water samples collected from the selected sites of Vellayani wetland ecosystem were analysed for the presence of pesticide residues using GC-MS. Detectable limit of the GC-MS used was 0.005 mg kg^{-1}. Results are presented in Table 20 and none of the samples detected the presence of pesticide residue above the detectable limit of the instrument.

Table 20. Pesticide residue analysis of lake water

Sl.No.	Pesticide tested	Result	Sl. No.	Pesticide tested	Result
1	Phorate	*BDL	17	PP DDD	BDL
2	Alpha HCH	BDL	18	Ethion	BDL
3	Dimethoate	BDL	19	Endosulphan sulphate	BDL
4	Beta HCH	BDL	20	PP DDT	BDL
5	Lindane	BDL	21	Bifenthrin	BDL
6	Fluchloralin	BDL	22	Fenpropathrin	BDL
7	Delta HCH	BDL	23	Phosalone	BDL
8	Methyl parathion	BDL	24	Lambda cyhalothrin	BDL
9	Malathion	BDL	25	Cyfluthrin 1	BDL
10	Chlorpyriphos	BDL	26	Cyfluthrin 2	BDL
11	Pendimethalin	BDL	27	Cypermethrin 1	BDL
12	Quinalphos	BDL	28	Cypermethrin 2	BDL
13	Alpha Endosulphan	BDL	29	Cypermethrin 3	BDL
14	Profenphos	BDL	30	Cypermethrin 4	BDL
15	PP DDE	BDL	31	Fenvalerate 1	BDL
16	Beta Endosulphan	BDL	32	Fenvalerate 2	BDL

*BDL- Below detectable limit

The sediment samples collected from the selected sites were analysed using both GC- MS and LC- MS / MS and none of the samples could detect the presence of any of the pesticide residues above the detectable limit of 0.005 mg kg^{-1}.

Table 21. Pesticide residue analysis of lake sediment

Sl. No.	Pesticide tested	Result	Sl. No.	Pesticide tested	Result
1	Alpha HCH	*BDL	24	Fenpropathrin	BDL
2	Beta HCH	BDL	25	Spirotetramat	BDL
3	Gamma HCH	BDL	26	Novaluron	BDL
4	Delta HCH	BDL	27	Chlorantraniliprole	BDL
5	Alpha Endosulfan	BDL	28	Emamectin benzoate	BDL
6	Beta Endosulfan	BDL	29	Fipronil	BDL
7	Endosulfan sulphate	BDL	30	Fenpyroximate	BDL
8	p.p'DDD	BDL	31	Spinosad	BDL
9	p.p'DDE	BDL	32	Indoxacarb	BDL
10	p.p'DDT	BDL	33	Imidacloprid	BDL
11	Phorate	BDL	34	Thiamethoxam	BDL
12	Malathion	BDL	35	Carbaryl	BDL
13	Methyl parathion	BDL	36	Thiodicarb	BDL
14	Chlorpyriphos	BDL	37	Carbofuran	BDL
15	Quinalphos	BDL	38	Acetamiprid	BDL
16	Profenphos	BDL	39	Monocrotophos	BDL
17	Acephate	BDL	40	Trifloxystrobin	BDL
18	Triazophos	BDL	41	Spiromesifen	BDL
19	Ethion	BDL	42	Fenitrothion	BDL
20	Lamda Cyhalothrin	BDL	43	Tebuconazole	BDL
21	Beta Cyflutrin	BDL	44	Dimethoate	BDL
22	Cypermethrin	BDL	45	Flubendiamide	BDL
23	Fenvalarate	BDL	46	Carbofuran-3-hydroxy	BDL

*BDL- Below detectable limit

Results of the various analysis carried out under this section revealed that the Vellayani wetland ecosystem is experiencing contamination mainly from suspended solids, nutrient elements, toxic metals and coliform bacteria. The level of contamination was higher at Arattukadavu RB (Site IV) and lower at Mannamvarabu (Site VI). The macrophyte population was more dominant at Mannamvarambu and Manamukku and most of the selected sites were present in both the sites.

Part II

4.2. Evaluation of native macrophytes for possible hyper accumulation capacity for Fe, Al, Pb and Cd

4.2.1. Biomass production by macrophytes

The biomass (dry) per unit area (g m^{-2}) of the selected dominant macrophytes is furnished in Table 22. The highest biomass was recorded by *L. flava* (352.43) and the lowest by *C.lindleyana* (18.00). The highest biomass for three emergent aquatic macrophytes (*E. colona*, *L. flava* and *N. nucifera*) was at Mannamvarambu and one each at Palappoor (*S. grossus*), Reservoir bund (*E. dulcis*), Arattukadavu RB (*C. esculenta*) and Manamukku (*M. vaginalis*). *E. colona* (59.00) had the lowest biomass among the emergent aquatic macrophytes. Pallichalthodu recorded the lowest biomass for *C. esculenta*, Reservoir bund for *L. flava* and *N. nucifera*, Valiyavilagam for *M. vaginalis*, Mannamvarambu for *S. grossus* and Manamukku for *E. colona* and *E. dulcis*.

Among the floating macrophytes, *E. crassipes* had the highest biomass (335.57) and the lowest for *N. indica* (43.80). Pallichalthodu (*E. crassipes* and *N. nouchali*) and Arattukadavu RB (*P. stratiotes* and *S. molesta*) recorded the highest biomass for two macrophytes each. *I. aquatica* had the highest biomass at Palappoor and *N. indica* at Mannamvarambu. Two macrophytes noticed the lowest biomass at Palappoor (*E. crassipes* and *N. indica*) and one each at Pallichalthodu (*P. stratiotes*), Arattukadavu RB (*N. nouchali*), Valiyavilagam (*S. molesta*) and Manamukku (*I. aquatica*). *V. natans*, the submerged macrophyte, had the highest biomass at Manamukku and the lowest at Pallichalthodu.

Among the terrestrial macrophytes, the highest biomass was obtained for *P. kaida* (260.00) and the lowest for *C. lindleyana* (18.00). Four macrophytes (*A. tenella*, *B. mutica*, *C. dactylon* and *P. repens*) had the highest biomass at Manamukku and three at Mannamvarambu (*A. aureum, B. oreintale* and *P. kaida*). Two sites

Table 22. Biomass (dry) of dominant macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Biomass (g m^{-2})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	204	151	234	388	350	291	326	277.71
2	*E. colona*	-	-	-	-	-	94	24	59.00
3	*E. dulcis*	189	299	328	114	225	309	71	219.29
4	*L. flava*	216	261	177	402	324	587	500	352.43
5	*M. vaginalis*	88	65	116	128	55	152	140	106.29
6	*N. nucifera*	78	-	25	55	84	111	55	68.00
7	*S. grossus*	626	319	251	-	-	194	225	323.00
b	Floating								
1	*E. crassipes*	100	580	168	222	256	459	564	335.57
2	*I. aquatica*	105	-	-	-	47	-	25	59.00
3	*N. indica*	21	-	34	-	43	68	53	43.80
4	*N. nouchali*	165	338	-	76	-	99	-	169.50
5	*P. stratiotes*	120	77	133	213	161	141	133	139.71
6	*S. molesta*	186	295	370	448	103	186	131	245.57
c	Submerged								
1	*V. natans*	-	43	-	-	-	-	56	49.50
B	Terrestrial								
1	*A. aureum*	-	-	-	88	69	179	-	112.00
2	*A. sessilis*	49	19	-	-	-	-	-	34.00
3	*A. tenella*	91	107	57	85	117	126	135	102.57
4	*B. orientale*	26	44	48	66	39	93	74	55.71
5	*B. mutica*	-	-	54	-	75	-	177	102.00
6	*B. dactyoides*	27	35	33	-	76	38	39	41.33
7	*C. lindleyana*	-	23	13	-	-	-	-	18.00
8	*C. dactylon*	18	12	27	25	35	10	44	24.43
9	*G. tubiflorum*	-	-	-	194	-	-	-	194.00
10	*I. globosa*	-	-	-	188	-	-	-	188.00
11	*L. rotundifolia*	30	28	-	-	-	-	-	29.00
12	*P. repens*	-	-	211	152	236	271	377	249.40
13	*P. kaida*	-	236	145	218	346	355	-	260.00
14	*P. hydropiper*	-	-	-	144	-	-	106	125.00
15	*S. trilobata*	127	149	108	115	68	89	107	109.00

- absence of macrophyte in the site

I- Palappoor   II - Pallichalthodu   III – Reservoir bund   IV- Arattukadavu RB
V - Valiyavilagam   VI - Mannamvarambu   VII - Manamukku

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recorded the highest biomass for two macrophytes viz., Palappoor for *A. sessilis* and *L. rotundifolia* and Pallichalthodu for *C. lindleyana* and *S. trilobata*.

Valiyavilagam recorded the highest biomass for *B. dactyoides* and Arattukadavu RB for *P. hydropiper*. Reservoir bund registered the lowest biomass for four macrophytes (*A. tenella, B. mutica, C. lindleyana* and *P. kaida*). Four sites recorded the lowest biomass for two macrophytes each viz., Palappoor for *B. oreintale* and *B. dactyoides*; Pallichalthodu for *A. sessilis* and *L. rotundifolia*; Reservoir bund for *A. aureum* and *P. repens* and Mannamvarambu for *C. dactylon* and *P. hydropiper*.

4.2.2. Metal accumulation by selected macrophytes

The metal accumulation by selected macrophytes was evaluated by analyzing the metal content in their shoot and root. Data on Fe, Al, Pb and Cd content of shoot and root portions of the selected macrophytes are presented in Tables 23 to 30.

4.2.2.1. Iron (Fe) accumulation

4.2.2.1.1. Shoot

Among the emergent aquatic macrophytes, *M. vaginalis* showed the highest Fe in shoot and *E. colona*, the lowest (Table 23). Comparing the site wise accumulation, *M. vaginalis* and *S. grossus* recorded the highest Fe content in shoot at Pallichalthodu, *E. colona* and *N. nucifera* at Mannamvarambu, *E. dulcis at* Reservoir bund, *L. flava* at Valiyavilagam and *C. esculenta* at Manamukku. Three species (*E. dulcis, M. vaginalis* and *N. nucifera*) had their lowest Fe content at Palappoor and two (*E. colona* and *S. grossus*) at Manamukku and one each at Reservoir bund (*L. flava*) and Mannamvarambu (*C. esculenta*).

Among the floating aquatic macrophytes, shoot Fe content was highest for *N. nouchali* and the lowest for *I. aquatica*. Comparing their site wise accumulation of Fe, it was observed that three species (*E. crassipes, N. indica* and *S. molesta*) had the highest Fe content at Mannamvarambu and one each at Pallichalthodu (*N. nouchali*), Valiyavilagam (*P. stratiotes*) and Manamukku (*I. aquatica*).

Table 23. Shoot Fe content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Shoot Fe content (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	2810	2800	2769	2754	2725	2531	3214	2800
2	*E. colona*	-	-	-	-	-	1969	1910	1939
3	*E. dulcis*	6225	7634	7950	7656	6570	6878	6786	7100
4	*L. flava*	7450	7607	6974	7056	8175	7752	7250	7466
5	*M. vaginalis*	11224	12267	12075	12100	12059	11960	12050	11962
6	*N. nucifera*	3620	-	5045	5247	3980	5570	5095	4759
7	*S. grossus*	4481	4675	4328	-	-	4083	4015	4316
b	Floating								
1	*E. crassipes*	3440	3825	3675	3526	3551	4055	3850	3703
2	*I. aquatica*	3250	-	-	-	3129	-	3310	3230
3	*N. nouchali*	6562	8498	-	7185	-	6447	-	7173
4	*N. indica*	3040	-	3756	-	3765	3852	3500	3582
5	*P. stratiotes*	3549	4180	3549	4246	4768	4267	4452	4144
6	*S. molesta*	4831	5146	5236	5028	4372	5510	5109	5033
c	Submerged								
1	*V. natans*	-	13038	-	-	-	-	9854	11446
B	Terrestrial								
1	*A. aureum*	-	-	-	2350	2025	2115	-	2185
2	*A. sessilis*	2165	2195	-	-	-	-	-	2180
3	*A. tenella*	6317	6350	7058	6204	7040	7000	7630	6800
4	*B. orientale*	2485	2471	2511	2150	2485	2753	2875	2533
5	*B. mutica*	-	-	2873	-	3125	-	3157	3052
6	*B. dactyoides*	2950	3256	3064	-	3486	3112	3386	3209
7	*C. lindleyana*	-	1041	978	-	-	-	-	1009
8	*C. dactylon*	4058	4792	5152	5235	5833	3970	4480	4788
9	*G. tubiflorum*	-	-	-	3486	-	-	-	3486
10	*I. globosa*	-	-	-	1982	-	-	-	1982
11	*L. rotundifolia*	1235	1480	-	-	-	-	-	1357
12	*P. kaida*	-	3561	2559	3574	3420	3275	-	3278
13	*P. repens*	-	-	3237	3135	2356	3025	3887	3128
14	*P. hydropiper*	-	-	-	7894	-	-	8076	7985
15	*S. trilobata*	8975	8665	8652	8170	9275	8672	9408	8831

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

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Two species (*E. crassipes* and *N. indica*) had the lowest Fe content at Palappoor and one each at Reservoir bund (*P. stratiotes*) and Mannamvarambu (*N. nouchali*). Valiyavilagam recorded the lowest Fe content in shoot for both *I. aquatica* and *S. molesta*. *P. stratiotes* showed the same Fe content both at Palappoor and Reservoir bund and was the lowest value for that species.

The only submerged aquatic macrophyte, *V. natans* had the highest shoot Fe content at Pallichalthodu (13038 mg kg^{-1}) and the lowest at Mannamvarambu (9854 mg kg^{-1}).

Among the terrestrial macrophytes, shoot Fe content was highest for *S. trilobata* (8831 mg kg^{-1}) and the lowest for *C. lindleyana* (1009 mg kg^{-1}). On comparing the site wise accumulation, six species (*A. tenella*, *B. orientale*, *B. mutica*, *P. repens*, *P. hydropiper* and *S. trilobata*) had their highest Fe content at Manamukku, three (*A. sessilis*, *C. lindleyana* and *L. rotundifolia*) at Pallichalthodu and two each at Arattukadavu RB (*A. aureum* and *P. kaida)* and Valiyavilagam (*B. dactyoides* and *C. dactylon*).

Three species each had their lowest shoot Fe content at Palappoor (*A. sessilis*, *B. dactyoides* and *L. rotundifolia*), Reservoir bund (*B. mutica*, *C. lindleyana* and *P. kaida*) and Arattukadavu RB (*A. tenella*, *P. hydropiper* and *S. trilobata*). *A. aureum* and *P. repens* recorded the lowest Fe content in the shoot at Valiyavilagam and one each at Pallichalthodu (*B. orientale*) and Mannamvarmbu (*C. dactylon*) respectively.

Considering the entire native flora, the emergent aquatic macrophyte, *M. vaginalis* showed the highest Fe content (11962 mg kg^{-1}) in shoot (Table 23) and the lowest by the terrestrial macrophyte, *C. lindleyana* (1009 mg kg^{-1}).

4.2.2.1.2. Root

Among the emergent aquatic macrophytes, the highest quantity of Fe (Table 24) was accumulated by *M. vaginalis* (16463 mg kg^{-1}) and the lowest by *E. colona* (2463 mg kg^{-1}). Comparing the site wise accumulation of Fe in roots, three species (*E. colona*, *M. vaginalis* and *N. nucifera*) had their highest values at Mannamvarambu and one each at Pallichalthodu (*S. grossus*), Reservoir bund (*E. dulcis*), Valiyavilagam (*L. flava*) and Manamukku (*C. esculenta*).

Two species each had their lowest root Fe content at Palappoor (*E. dulcis* and *M. vaginalis*), Mannamvarambu (*C. esculenta* and *L. flava*) and Manamukku (*E. colona* and *S. grossus*) and one at Reservoir bund (*N. nucifera*).

Among the floating aquatic macrophytes, root Fe content was highest for *E. crassipes* (19041 mg kg^{-1}) and the lowest for *I. aquatica* (3823 mg kg^{-1}). On comparing the site wise accumulation, it was observed that three species (*E. crassipes*, *N. indica* and *S. molesta*) had their highest root Fe content at Mannamvarambu and one each at Reservoir bund (*N. nouchali)*, Valiyavilagam (*P. stratiotes*) and Manamukku (*I. aquatica*). Two species had their lowest root Fe content at Valiyavilagam (*I. aquatica* and *S. molesta*) and one at Mannamvarambu (*N. nouchali*). *P. stratiotes* showed the same root Fe content both at Palappoor and Reservoir bund and was the lowest for that species. Palappoor recorded the lowest Fe content for both *E. crassipes* and *N. indica*.

The only submerged macrophyte, *V. natans* had the highest root Fe content at Pallichalthodu (14965 mg kg^{-1}) and the lowest at Manamukku (11286 mg kg^{-1}).

The terrestrial macrophytes showed very wide variation in Fe content of root ranging from the highest value of 17317 mg kg^{-1} for *C. dactylon* to the lowest value of 1678 mg kg^{-1} for *A. aureum*. Four species (*A. tenella*, *B. orientale*, *P. repens* and *P. hydropiper*) recorded the highest Fe content at Manamukku, three each at Pallichalthodu (*C. lindleyana*, *L. rotundifolia* and *S. trilobata*) and Valiyavilagam (*B. mutica*, *B. dactyoides* and *C. dactylon*), two at Arattukadavu RB (*A. aureum* and *P. kaida*) and one at Palappoor (*A. sessilis*).

Table 24. Root Fe content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Root Fe content (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	9876	9824	9773	9610	9385	9283	11085	9834
2	*E. colona*	-	-	-	-	-	2500	2426	2463
3	*E. dulcis*	9985	11860	12593	12057	10386	10867	10349	11156
4	*L. flava*	14690	15115	15270	14325	16335	14115	14337	14884
5	*M. vaginalis*	15245	17156	16369	16400	16322	17495	16258	16463
6	*N. nucifera*	4834	-	5423	5678	4526	6079	5583	5354
7	*S. grossus*	5560	5687	5443	-	-	5245	5090	5405
b	Floating								
1	*E. crassipes*	17865	19258	18850	18194	18225	21042	19853	19041
2	*I. aquatica*	3795	-	-	-	3655	-	4021	3823
3	*N. nouchali*	7875	9820	-	8573	-	7758	-	8506
4	*N. indica*	7145	-	8910	-	8835	8965	8137	8398
5	*P. stratiotes*	12853	12885	12853	13256	14290	13325	13860	13332
6	*S. molesta*	5279	6225	5628	6165	5185	6770	5320	5796
c	Submerged								
1	*V.natans*	-	14965	-	-	-	-	11286	13125
B	Terrestrial								
1	*A. aureum*	-	-	-	1977	1427	1632	-	1678
2	*A. sessilis*	4850	4038	-	-	-	-	-	4444
3	*A. tenella*	8195	9156	9215	9117	8259	8075	9915	8847
4	*B. orientale*	3486	3857	3567	3981	3045	3218	4092	3606
5	*B. mutica*	-	-	3348	-	3843	-	3775	3655
6	*B. dactyoides*	12365	13752	13225	-	14880	13075	14575	13645
7	*C. lindleyana*	-	2575	2448	-	-	-	-	2511
8	*C. dactylon*	15750	17865	17235	18850	20070	15092	16358	17317
9	*G. tubiflorum*	-	-	-	3059	-	-	-	3059
10	*I. globosa*	-	-	-	2200	-	-	-	2200
11	*L. rotundifolia*	3321	3480	-	-	-	-	-	3400
12	*P. kaida*	-	4075	3586	4690	4213	3886	-	4090
13	*P. repens*	-	-	5510	5428	4890	4861	6155	5369
14	*P. hydropiper*	-	-	-	9438	-	-	9970	9704
15	*S. trilobata*	8346	8720	7960	8059	8593	8040	7549	8181

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Two sites recorded the lowest root Fe content for three species each Pallichalthodu (*B. mutica*, *C. lindleyana* and *P. kaida*) and Valiyavilagam (*A. aureum*, *B. orientale* and *C. dactylon*) and two species each at Palappoor (*B. dactyoides* and *L. rotundifolia*) and Mannamvarambu (*P. repens* and *A. tenella*). *A. sessilis*, *P. hydropiper* and *S. trilobata* had their lowest root Fe content at Palichalthodu, Arattukadavu RB and Manamukku, respectively.

The spatial variation was also there for each species for shoot Fe and root Fe and but not as conspicuous as the species difference. Roots were found to accumulate more Fe than shoots (Table 24), particularly for aquatic macrophytes. With regard to the entire native flora, the highest Fe content (19041 mg kg^{-1}) in the root was observed for the emergent aquatic macrophyte, *E. crassipes* and the lowest (1678 mg kg^{-1}) for *A. aureum*, the terrestrial macrophyte.

4.2.2.2. Aluminium (Al) accumulation

4.2.2.2.1. Shoot

Among the emergent aquatic macrophytes, shoot Al content was highest for *M. vaginalis* (4716 mg kg^{-1}, Table 25) and the lowest for *E. dulcis* (2167 mg kg^{-1}).

Comparing the site wise accumulation, *N. nucifera* recorded the highest shoot Al content at Palappoor, *E. dulcis* at Reservoir bund, *L. flava* and *M. vaginalis* at Arattukadavu RB, *S. grossus* at Mannamvarambu and *C. esculenta* and *E. colona* at Manamukku. Three species (*E. colona*, *N. nucifera* and *S. grossus*) recorded the lowest shoot Al content at Mannamvarambu and one each at Palappoor (*L. flava*), Pallichalthodu (*C. esculenta*), Arattukadavu RB (*E. dulcis*) and Valiyavilagam (*M. vaginalis*).

Among the floating aquatic macrophytes, *N. nouchali* (5687 mg kg^{-1}) recorded the highest shoot Al content and *I. aquatica* (913 mg kg^{-1}), the lowest. Comparing the site wise accumulation, it was observed that two species (*E. crassipes* and *N. indica*) had their highest Al content at Manamukku and one each at Palappoor (*I. aquatica*), Pallichalthodu (*N. nouchali*), Arattukadavu RB (*P. stratiotes*) and

Table 25. Shoot Al content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Shoot Al content (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	2985	1890	2825	2873	2359	3156	3215	2757
2	*E. colona*	-	-	-	-	-	2259	2621	2440
3	*E. dulcis*	2118	2276	2324	1987	2078	2095	2290	2167
4	*L. flava*	4105	4230	4278	4471	4138	4451	4418	4298
5	*M. vaginalis*	4410	4289	4875	5150	5037	4237	5015	4716
6	*N. nucifera*	2891	-	2700	2825	2890	2675	2830	2802
7	*S. grossus*	4008	3920	4290	-	-	4375	3685	4055
b	Floating								
1	*E. crassipes*	1655	2000	1845	1679	2100	2045	2115	1920
2	*I. aquatica*	1000	-	-	-	855	-	883	913
3	*N. nouchali*	5750	5800	-	5585	-	5615	-	5687
4	*N. indica*	1345	-	1412	-	1327	1510	1586	1436
5	*P. stratiotes*	1787	1670	1590	1773	1665	1956	1934	1768
6	*S. molesta*	3944	3950	3976	4257	3894	3815	3911	3964
c	Submerged								
1	*V. natans*	-	6021	-	-	-	-	5860	5940
B	Terrestrial								
1	*A. aureum*	-	-	-	1724	1530	1495	-	1583
2	*A. sessilis*	2190	2214	-	-	-	-	-	2202
3	*A. tenella*	2614	2950	2820	2582	3105	3255	2815	2877
4	*B. orientale*	1880	1942	1820	1871	1825	1815	1875	1861
5	*B. mutica*	-	-	3320	-	3214	-	3225	3253
6	*B. dactyoides*	3702	3525	3550	-	3668	3675	3650	3628
7	*C. lindleyana*	-	750	728	-	-	-	-	739
8	*C. dactylon*	2600	2670	2821	2823	2730	2875	2734	2750
9	*G. tubiflorum*	-	-	-	2898	-	-	-	2898
10	*I. globosa*	-	-	-	3486	-	-	-	3486
11	*L. rotundifolia*	1490	1425	-	-	-	-	-	1457
12	*P. kaida*	-	3357	3455	3512	3625	3632	-	3516
13	*P. repens*	-	-	5485	5998	6660	6615	5620	6075
14	*P. hydropiper*	-	-	-	1092	-	-	1385	1238
15	*S. trilobata*	3187	3270	3121	3278	2889	2810	3015	3081

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Mannamvarambu (*S. molesta*). Two sites recorded the lowest shoot Al content for two species each at Arattukadavu RB (*E. crassipes* and *N. nouchali*) and Valiyavilagam (*I. aquatica* and *N. indica*) and one each at Reservoir bund (*P. stratiotes*) and Mannamvarambu (*S. molesta*).

The only submerged aquatic macrophyte, *V. natans* had the highest shoot Al content at Pallichalthodu (6021 mg kg^{-1}) and the lowest at Manamukku (5860 mg kg^{-1}). With regard to the aquatic macrophytes, wide variation in shoot Al content was noted for floating type compared to other groups.

Terrestrial macrophytes also showed wide variation in shoot Al content (Table 25) and the highest value was noted for *P. repens* (6075 mg kg^{-1}) and the lowest for *C. lindleyana* (739 mg kg^{-1}).

On comparing the site wise accumulation, three species each had their highest Al content at Pallichalthodu (*A. sessilis, B. orientale* and *C. lindleyana*) and Mannamvarambu (*A. tenella, C. dactylon* and *P. kaitha*), two each at Palappoor (*B. dactyoides* and *L. rotundifolia*) and Arattukadavu RB (*A. aureum* and *S. trilobata*) and one each at Reservoir bund (*B. mutica*), Valiyavilagam (*P. repens*) and Manamukku (*P. hydropiper*) respectively.

Three species each had their lowest shoot Al content at Pallichalthodu (*B. dactyoides L. rotundifolia* and *P. kaida*) and Mannamvarambu (*A. aureum, B. orientale* and *S. trilobata*), two each at Palappoor (*A. sessilis* and *C. dactylon*), Reservoir bund (*C. lindleyana* and *P. repens*) and Arattukadavu RB (*A. tenella* and P. hydropiper) and one at Valiyavilagam (*B. mutica*).

Out of the entire native flora, the highest shoot Al content was observed for *P. repens* (6075 mg kg^{-1}) and the lowest for *C. lindleyana* (739 mg kg^{-1}).

4.2.2.2.2. Root

Among the emergent aquatic macrophytes, *M. vaginalis* showed the highest value for Al in root (17507 mg kg^{-1}) and *E. colona* (1255 mg kg^{-1}), the lowest (Table

26). Two species each had the highest Al content at Mannamvarambu (*L. flava* and *S. grossus*) and Manamukku (*C. esculenta* and *E. colona*) and one species each at Reservoir bund (*E. dulcis*), Arattukadavu RB (*M. vaginalis*) and Valiyavilagam (*N. nucifera*).

Two species each showed the lowest root Al content at Valiyavilagam (*C. esculenta* and *L. flava*) and Mannamvarambu (*E. colona* and *M. vaginalis*) and one species each at Pallichalthodu (*S. grossus*), Reservoir bund (*N. nucifera*) and Arattukadavu RB (*E. dulcis*).

Among the floating type aquatic macrophytes, root Al content was highest for *N. nouchali* (11397 mg kg^{-1}) and the lowest for *I. aquatica* (1748 mg kg^{-1}). Palappoor recorded the highest root Al content for *I. aquatica*, Pallichalthodu for *N. nouchali*, Arattukadavu RB for *S. molesta*, Valiyavilagam for *E. crassipes*, Mannamvarambu for *P. stratiotes* and Manamukku for *N. indica*.

Two species had the lowest root Al content at Palappoor (*E. crassipes* and *N. indica*) and one each at Reservoir bund (*P. stratiotes*), Arattukadavu RB (*N.nouchali*), Valiyavilagam (*I. aquatica*) and Mannamvarambu (*S. molesta*).

V. natans, only submerged aquatic macrophyte, recorded the highest Al content (14780 mg kg^{-1}) in root at Manamukku and the lowest at Pallichalthodu (13658 mg kg^{-1}).

Among the terrestrial macrophytes, *C. dactylon* recorded the highest Al content in root (15066 mg kg^{-1}) and *B. mutica*, the lowest (2019 mg kg^{-1}). Two sites recorded the highest root Al content for three species each at Pallichalthodu (*A. sessilis*, *B. orientale* and *C. lindleyana*) and Mannamvarambu (*A. tenella*, *P. kaida* and *P. repens*) and two species at Arattukadavu RB (*A. aureum* and *S. trilobata*) and Valiyavilagam (*B. dactyoides* and *C. dactylon*). *L. rotundifolia*, *B. mutica* and *P. hydropiper* had their highest root Al content at Palappoor, Reservoir bund and Manamukku, respectively.

Table 26. Root Al content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Root Al content (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	8632	8930	8576	8894	8275	9125	9220	8807
2	*E. colona*	-	-	-	-	-	1175	1335	1255
3	*E. dulcis*	6645	6775	6920	6225	6388	6395	6558	6558
4	*L. flava*	14356	14861	14880	15611	14120	15560	15328	14959
5	*M. vaginalis*	16266	15871	17896	19125	18860	15776	18755	17507
6	*N. nucifera*	4985	-	4883	5110	5218	5038	5116	5058
7	*S. grossus*	5624	5489	6008	-	-	6109	5115	5669
B	Floating								
1	*E. crassipes*	9785	11985	10870	9980	12315	12250	12245	11347
2	*I. aquatica*	1860	-	-	-	1659	-	1725	1748
3	*N. nouchali*	11550	11725	-	10875	-	11440	-	11397
4	*N. indica*	7380	-	7665	-	7364	8579	9123	8022
5	*P. stratiotes*	2075	1912	1825	2011	1986	2520	2486	2116
6	*S. molesta*	8556	8537	8560	9015	8435	8259	8416	8540
c	Submerged								
1	*V. natans*	-	13658	-	-	-	-	14780	14219
B	Terrestrial								
1	*A. aureum*	-	-	-	3122	2886	2681	-	2897
2	*A. sessilis*	2815	2927	-	-	-	-	-	2871
3	*A. tenella*	5705	6455	6205	5673	6610	7136	5987	6253
4	*B. orientale*	3386	3475	3276	3355	3281	3270	3380	3346
5	*B. mutica*	-	-	2075	-	1975	-	2008	2019
6	*B. dactyoides*	8350	7983	7986	-	8415	8255	8226	8202
7	*C. lindleyana*	-	3300	3150	-	-	-	-	3225
8	*C. dactylon*	14186	14390	14867	15100	16510	15585	14827	15066
9	*G. tubiflorum*	-	-	-	8234	-	-	-	8234
10	*I. globosa*	-	-	-	2258	-	-	-	2258
11	*L. rotundifolia*	2470	2395	-	-	-	-	-	2432
12	*P. kaida*	-	2210	2330	2335	2374	2481	-	2346
13	*P. repens*	-	-	9875	10872	12320	12855	10118	11208
14	*P. hydropiper*	-	-	-	6070	-	-	6218	6144
15	*S. trilobata*	4961	5125	4870	5236	4655	4378	4705	4847

- *absence of macrophyte in the site*

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Three species each had their lowest Al content in root at Pallichalthodu (*C. dactylon*, *L. rotundifolia* and *P. kaida*) and Mannamvarambu (*A. aureum*, *B. orientale* and *S. trilobata*) and two species each at Palappoor (*A. sessilis* and *C. dactylon*), Reservoir bund (*C. lindleyana* and *P. repens*) and Arattukadavu RB (*A. tenella* and *P. hydropiper*). *B. mutica* had the lowest Al content at Valiyavilagam.

Evaluating the root Al content of all the macrophytes, both the highest and lowest values were showed by emerging type with *M. vaginalis* (17507 mg kg $^{-1}$), being the highest and *I. aquatica* the lowest (Table 26).

4.2.2.3. Lead (Pb) accumulation

4.2.2.3.1. Shoot

The entire macrophytes sampled showed the presence of Pb in their shoot and root. Among the emergent aquatic type, *L. flava* showed the highest value for Pb in shoot (6.03 mg kg $^{-1}$) and *E. colona* (0.72 mg kg $^{-1}$), the lowest (Table 27). Two species each had their highest shoot Pb content at Mannamvarambu (*E. colona* and *M. vaginalis*) and Manamukku (*N. nucifera* and *S. grossus*) and one each at Pallichalthodu (*E. dulcis*), Reservoir bund (*L. flava*) and Arattukadavu RB (*C. esculenta*).

Three species had the lowest Pb content at Pallichalthodu (*C. esculenta*, *M. vaginalis* and *S. grossus*) and one each at Palappoor (*L. flava*) and Arattukadavu RB (*N. nucifera*). Manamukku recorded the lowest Pb content in shoot for both *E. colona* and *E. dulcis*.

Among the floating aquatic macrophytes, shoot Pb content was highest for *E. crassipes* (9.09 mg kg $^{-1}$) and the lowest for *I. aquatica* (1.43 mg kg $^{-1}$). Two species had the highest Pb content in shoot at Mannamvarambu (*N. nouchali* and *N. indica*) and one each at Palappoor (*I. aquatica*), Arattukadavu RB (*P. stratiotes*), Valiyavilagam (*S. molesta*) and Manamukku (*E. crassipes*).

Table 27. Shoot Pb content of selected macrophytes of Vellayani wetland cosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Shoot Pb content (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	3.50	3.20	3.60	4.84	3.24	3.70	3.70	3.68
2	*E. colona*	-	-	-	-	-	0.73	0.70	0.72
3	*E. dulcis*	4.50	4.79	4.19	3.98	3.88	3.82	3.58	4.11
4	*L. flava*	4.65	4.85	8.06	7.32	6.24	5.69	5.42	6.03
5	*M. vaginalis*	2.04	1.22	1.25	2.07	1.55	2.16	1.85	1.73
6	*N. nucifera*	5.56	-	5.60	5.08	6.13	6.05	6.41	5.80
7	*S. grossus*	4.85	4.30	6.10	-	-	7.07	7.78	6.02
b	Floating								
1	*E. crassipes*	8.82	7.10	9.45	9.62	7.97	9.56	11.15	9.09
2	*I. aquatica*	2.11	-	-	-	1.34	-	0.84	1.43
3	*N. nouchali*	5.67	4.35	-	4.50	-	6.65	-	5.29
4	*N. indica*	5.45	-	5.33	-	5.64	6.25	5.80	5.78
5	*P. stratiotes*	6.48	6.58	6.15	7.10	6.25	5.85	5.30	6.24
6	*S. molesta*	3.05	3.15	2.88	5.48	4.90	3.94	3.55	3.85
c	Submerged								
1	*V. natans*	-	9.95	-	-	-	-	8.48	9.22
B	Terrestrial								
1	*A. aureum*	-	-	-	3.23	3.18	3.08	-	3.16
2	*A. sessilis*	1.12	1.04	-	-	-	-	-	1.08
3	*A. tenella*	3.05	3.71	3.27	2.95	3.21	4.25	3.85	3.47
4	*B. orientale*	4.48	4.30	4.56	4.70	4.30	4.59	4.75	4.53
5	*B. mutica*	-	-	2.41	-	2.37	-	2.88	2.55
6	*B. dactyoides*	3.91	3.78	4.18	-	4.89	5.40	5.10	4.54
7	*C. lindleyana*	-	3.68	3.15	-	-	-	-	3.41
8	*C. dactylon*	8.09	9.21	7.83	9.16	8.74	8.68	8.13	8.55
9	*G. tubiflorum*	-	-	-	4.89	-	-	-	4.89
10	*I. globosa*	-	-	-	7.40	-	-	-	7.40
11	*L. rotundifolia*	1.17	1.02	-	-	-	-	-	1.09
12	*P. kaida*	-	1.85	1.97	2.26	1.81	2.20	-	2.02
13	*P. repens*	-	-	3.05	3.23	3.55	3.20	3.18	3.24
14	*P. hydropiper*	-	-	-	3.73	-	-	4.15	3.94
15	*S. trilobata*	10.19	13.20	9.79	10.76	9.82	9.51	9.20	10.35

- absence of macrophyte in the site

I- Palappoor   II - Pallichalthodu   III – Reservoir bund   IV- Arattukadavu RB
V - Valiyavilagam   VI - Mannamvarambu   VII - Manamukku

Three sites recorded the lowest shoot Pb content for two species each at Pallichalthodu (*N. nouchali* and *E. crassipes*), Reservoir bund (*N. indica* and *S. molesta*) and Manamukku (*I. aquatica* and *P. stratiotes*).

The only submerged aquatic macrophyte, *V. natans* had the highest shoot Pb content at Pallichalthodu (9.95 mg kg $^{-1}$) and lowest at Manamukku (8.48 mg kg $^{-1}$).

Among the terrestrial macrophytes, *S. trilobata* recorded the highest Pb content in shoot (10.35 mg kg $^{-1}$) and *A. sessilis*, the lowest (1.08 mg kg $^{-1}$). Comparing the site wise accumulation, three species each had the highest Pb content in shoot at Pallichalthodu (*C. lindleyana, C. dactylon* and *S. trilobata*) and Manamukku (*B. orientale, B. mutica* and *P. hydropiper*) and two each at Palappoor (*A. sessilis* and *L. rotundifolia*), Arattukadavu RB (*A. aureum* and *P. kaida*) and Mannamvarambu (*A. tenella* and *C. dacylon*). *P. repens* had the highest Pb content at Valiyavilagam.

Three species each had the lowest Pb content at Pallichalthodu (*A. sessilis, B. dactyoides* and *L. rotundifolia*) and Reservoir bund (*C. lindleyana, C. dactylon* and *P. repens*), two each at Arattukadavu RB (*A. tenella* and *P. hydropiper*) and Valiyavilagam (*B. mutica* and *P. kaida*) and one each at Mannamvarambu (*A. aureum*) and Manamukku (*S. trilobata*). *B. orientale* showed the same shoot Pb content both at Pallichalthodu and Valiyavilagam.

On evaluating the entire macrophytes, *S. trilobata*, the terrestrial one recorded the highest Pb content in shoot (10.35 mg kg $^{-1}$) and *E. colona*, emergent aquatic macrophyte, the lowest (0.72 mg kg $^{-1}$).

4.2.2.3.2. Root

Among the emergent aquatic macrophytes, the highest value for Pb in root (Table 28) was recorded by *C. esculenta* (17.68 mg kg^{-1}) and *E. colona* (1.45 mg kg^{-1}), the lowest.

Table 28. Root Pb content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		Root Pb content (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	16.50	16.15	17.5	20.39	16.58	18.15	18.50	17.68
2	*E. colona*	-	-	-	-	-	1.50	1.4	1.45
3	*E. dulcis*	8.58	8.99	8.50	8.46	8.06	7.62	7.50	8.24
4	*L. flava*	10.45	9.21	12.30	13.16	15.47	14.27	9.31	12.02
5	*M. vaginalis*	3.8	2.87	2.90	3.95	3.14	4.12	3.65	3.49
6	*N. nucifera*	7.25	-	7.54	6.74	8.84	8.64	9.35	8.06
7	*S. grossus*	6.50	6.10	7.63	-	-	8.79	9.25	7.65
b	Floating								
1	*E. crassipes*	10.57	8.45	11.39	11.60	9.51	11.45	13.45	10.92
2	*I. aquatica*	2.83	-	-	-	1.92	-	1.54	2.10
3	*N. nouchali*	12.78	9.84	-	10.15	-	14.98	-	11.94
4	*N. indica*	13.45	-	12.65	-	12.77	16.50	14.55	13.98
5	*P. stratiotes*	14.76	14.94	14.5	15.80	14.85	13.55	13.25	14.52
6	*S. molesta*	8.25	9.25	8.75	12.75	11.65	10.79	11.50	10.42
c	Submerged								
1	*V. natans*	-	14.48	-	-	-	-	9.39	9.94
B	Terrestrial								
1	*A. aureum*	-	-	-	2.39	2.27	2.24		2.30
2	*A. sessilis*	2.11	1.91	-	-	-	-	-	2.01
3	*A. tenella*	8.25	9.70	8.53	8.10	8.41	10.62	9.25	8.98
4	*B. orientale*	10.78	9.85	10.96	11.47	9.75	10.50	11.50	10.67
5	*B. mutica*	-	-	2.19	-	1.84	-	2.66	2.23
6	*B. dactyoides*	9.89	9.77	11.47	-	12.39	13.48	12.82	11.64
7	*C. lindleyana*	-	2.23	1.93	-	-	-	-	2.08
8	*C. dactylon*	13.24	14.48	12.78	15.38	14.22	14.78	13.53	14.06
9	*G. tubiflorum*	-	-	-	5.85	-	-	-	5.85
10	*I. globosa*	-	-	-	6.48	-	-	-	6.48
11	*L. rotundifolia*	4.13	3.86	-	-	-	-	-	4.00
12	*P. kaida*	-	3.75	3.99	4.24	3.84	4.15	-	3.99
13	*P. repens*	-	-	3.64	3.85	4.25	3.77	3.71	3.84
14	*P. hydropiper*	-	-	-	3.49	-	-	4.20	3.85
15	*S. trilobata*	8.47	11.50	8.134	8.78	8.27	8.11	7.83	8.73

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Comparing the site wise accumulation, three species had their highest root Pb content at Manamukku (*C. esculenta*, *N. nucifera* and *S. grossus*), two species at Mannamvarambu (*E. colona* and *M. vaginalis*) and one each at Pallichalthodu (*E. dulcis*) and Valiyavilagam (*L. flava*). Four species recorded the lowest Pb content at Pallichalthodu (*C. esculenta*, *L. flava*, *M. vaginalis* and *S. grossus*) and two at Manamukku (*E. colona* and *E. dulcis*). *N. nucifera* had the lowest Pb content at Arattukadavu RB.

Among the floating aquatic macrophytes, *P. stratiotes* recorded the highest Pb content in the root (14.52 mg kg^{-1}) and *I. aquatica*, the lowest (2.10 mg kg^{-1}). With regard to the site wise accumulation, two sites recorded the highest root Pb content for two species each viz., Arattukadavu RB (*P. stratiotes* and *S. molesta*) and Mannamvarambu (*N. nouchali* and *N. indica*). *I. aquatica* and *E. crassipes* had the highest Pb content at Palappoor and Manamukku, respectively. Two species each had their lowest Pb content in root at Pallichalthodu (*N. nouchali* and *E. crassipes*) and Manamukku (*I. aquatica* and *P. stratiotes*) and one each at Palappoor (*N. indica*) and Pallichalthodu (*S. molesta*).

Much variation for root Pb content among sites was shown by *V. natans*, the only submerged aquatic macrophyte, with the highest at Pallichalthodu (14.48 mg kg^{-1}) and the lowest at Manamukku (9.39 mg kg^{-1}).

Among the terrestrial macrophytes, root Pb content was highest for *C. dactylon* (14.06 mg kg^{-1}) and the lowest for *A. sessilis* (2.01 mg kg^{-1}).

Comparing their site wise accumulation, three species each had the highest Pb content in root at Arattukadavu RB (*A. aureum*, *C.dactylon* and *P. kaida*) and Manamukku (*B. orientale*, *B. mutica* and *P. hydropiper*) and two species each at Palappoor (*A. sessilis* and *L. rotundifolia*), Pallichalthodu (*C. lindleyana* and *S. trilobata*) and Mannamvarambu (*A. tenella* and *B. dactyoides*). The highest Pb content for *P. repens* was recorded at Valiyavilagam.

Four species had their lowest Pb content in root at Pallichalthodu (*A. sessilis*, *B. dactyoides*, *L. rotundifolia* and *P. kaida*), three at Reservoir bund (*C. lindleyana*, *C. dactylon* and *P. repens*), two each at Arattukadavu RB (*A. tenella* and *P. hydropiper*) and Valiyavilagam (*B. orientale* and *B. mutica*) and one each at Mannamvarambu (*A. aureum*) and Manamukku (*S. trilobata*).

Comparing all the selected macrophytes for their Pb accumulation in root, the highest (17.68 mg kg^{-1}) was recorded by *C. esculenta* (emergent type) and the lowest by *E. colona* (1.45 mg kg^{-1}).

4.2.2.4. Cadmium (Cd) accumulation

4.2.2.4.1. Shoot

All the selected macrophytes had shown the presence of Cd in their shoots (Table 29) and roots (Table 30). Among the emergent aquatic macrophytes, shoot Cd content was highest for *C. esculenta* (2.181 mg kg^{-1}) and the lowest (0.007 mg kg^{-1}) for *E. colona*. Comparing the site wise accumulation, three species had the highest Cd content at Manamukku (*E. colona*, *N. nucifera* and *S. grossus*) and two at Arattukadavu RB (*C. esculenta* and *M. vaginalis*). *E. dulcis* showed the same Cd content both at Palappoor and Arattukadavu RB and was the highest for that species. *L. flava* had the highest shoot Cd content at Valiyavilagam.

Three species each had the lowest Cd content in shoot at Mannamvarambu (*C. esculenta*, *E. colona* and *S. grossus*) and Manamukku (*E. dulcis*, *L. flava* and *M. vaginalis*). *N. nucifera* recorded the lowest Cd content at Palappoor.

Among the floating aquatic macrophytes, *P. stratiotes* recorded the highest Cd content (1.208 mg kg^{-1}) in shoot and *I. aquatica*, the lowest value (0.004 mg kg^{-1}). Four sites had the highest Cd content for one species each viz., Palappoor (*N. indica*), Pallichalthodu (*N. nouchali*), Valiyavilagam (*I. aquatica*) and Mannamvarambu (*S.*

molesta). Both *E. crassipes* and *P. stratiotes* recorded the highest Cd content at Arattukadavu RB.

Table 29. Shoot Cd content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		(mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	2.25	2.70	2.00	2.83	1.80	1.76	1.93	2.181
2	*E. colona*	-	-	-	-	-	0.007	0.008	0.007
3	*E. dulcis*	0.98	0.96	0.69	0.98	0.60	0.54	0.44	0.741
4	*L. flava*	1.50	1.5	1.54	1.61	1.80	1.35	1.33	1.519
5	*M. vaginalis*	0.60	0.58	0.42	0.68	0.58	0.40	0.38	0.520
6	*N. nucifera*	1.08	-	1.10	1.15	1.50	1.35	1.65	1.305
7	*S. grossus*	0.94	0.80	0.92	-	-	0.75	1.15	0.912
b	Floating								
1	*E. crassipes*	1.08	1.10	0.85	1.32	0.90	1.30	0.94	1.070
2	*I. aquatica*	0.005	-	-	-	0.006	-	0.003	0.004
3	*N. nouchali*	0.45	0.48	-	0.40	-	0.30	-	0.408
4	*N. indica*	1.42	-	0.88	-	1.05	0.92	1.26	1.104
5	*P. stratiotes*	1.20	1.27	1.2	1.45	1.10	1.10	1.14	1.208
6	*S. molesta*	0.08	0.09	0.08	0.09	0.08	0.10	0.09	0.087
c	Submerged								
1	*V. natans*	-	1.05	-	-	-	-	0.85	0.950
B	Terrestrial								
1	*A. aureum*	-	-	-	0.018	0.016	0.015	-	0.016
2	*A. sessilis*	0.03	0.024	-	-	-	-	-	0.027
3	*A. tenella*	0.90	0.84	0.84	0.95	0.87	0.90	0.95	0.893
4	*B. orientale*	0.028	0.025	0.015	0.025	0.023	0.012	0.002	0.018
5	*B. mutica*	-	-	.012	-	0.008	-	0.022	0.014
6	*B. dactyoides*	1.17	1.22	1.20	-	1.25	1.40	1.55	1.298
7	*C. lindleyana*	-	.010	.020	-	-	-	-	0.015
8	*C. dactylon*	1.12	1.10	1.08	1.02	0.95	1.15	0.95	1.053
9	*G. tubiflorum*	-	-	-	0.008	-	-	-	0.008
10	*I. globosa*	-	-	-	0.003	-	-	-	0.003
11	*L. rotundifolia*	0.35	0.40	-	-	-	-	-	0.375
12	*P. kaida*	-	0.50	0.42	0.55	0.48	0.60	-	0.517
13	*P. repens*	-	-	0.061	0.068	0.040	0.032	0.028	0.046
14	*P. hydropiper*	-	-	-	1.08	-	-	0.94	1.010
15	*S. trilobata*	2.21	2.31	1.85	2.15	2.35	2.20	2.57	2.235

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Three sites (Palappoor, Reservoir bund and Valiyavilagam) recorded the lowest shoot Cd content for *S. molesta* and two for *P. stratiotes* (Valiyavilagam and Mannamvarambu). *E. crassipes* and *N. indica* had their lowest Cd content at Reservoir bund. *N. nouchali* and *I. aquatica* recorded the lowest Cd content in shoot at Mannamvarambu and Manamukku, respectively.

The submerged macrophyte, *V. natans* had the highest Cd content at Pallichalthodu (1.05 mg kg^{-1}) and the lowest (0.85 mg kg^{-1}) at Manamukku.

Among the terrestrial macrophytes, shoot Cd content was the highest (2.235 mg kg^{-1}) for *S. trilobata* and the lowest (0.003 mg kg^{-1}) for *I. globosa*. Comparing the site wise accumulation, three species each had their highest Cd content at Arattukadavu RB (*A. aureum*, *P. repens* and *P. hydropiper*) and Manamukku (*B. mutica*, *B. dactyoides* and *S. trilobata*), two each at Palappoor (*A. sessilis* and *B. orientale*) and Mannamvarambu (*C. dactylon* and *P. kaida*) and one each at Pallichalthodu (*L. rotundifolia*) and Reservoir bund (*C. lindleyana*). The highest shoot Cd content for *A. tenella* was recorded both at Arattukadavu RB and Manamukku.

Three species had their lowest Cd content in shoot at Manamukku (*B. orientale*, *P. repens* and *P. hydropiper*), two each at Palappoor (*B. dactyoides* and *L. rotundifolia*), Pallichalthodu (*A. sessilis* and *C. lindleyana*) and Reservoir bund (*P. kaida* and *S. trilobata*) and one each at Valiyavilagam (*B. mutica*) and Mannamvarambu (*A. aureum*). Two species viz., *A. tenella* at Pallichalthodu and Reservoir bund (0.84 mg kg^{-1}) and *C. dactylon* at Valiyavilagam and Manamukku (0.95 mg kg^{-1}) recorded their lowest shoot Cd content at two sites each.

Evaluating the entire samples, the terrestrial macrophyte *S. trilobata* showed the highest value of 2.235 mg Cd kg^{-1} and *I. globosa* the lowest value of 0.003 mg Cd kg^{-1} in shoot.

4.2.2.4.2. Root

Considering the Cd accumulation in the roots of emergent aquatic macrophytes, the highest value was recorded by *L. flava* (3.263 mg kg^{-1}) and the lowest by *E. colona* (0.004 mg kg^{-1}). On comparing their site wise accumulation, three species recorded the highest content at Manamukku (*E. colona*, *N. nucifera* and *S. grossus*), two at Arattukadavu RB (*C. esculenta* and *M. vaginalis*) and one each at Pallichalthodu (*E. dulcis*) and Valiyavilagam (*L. flava*). Three species had their lowest Cd content in root at Mannamvarambu (*C. esculenta*, *E. colona* and *M. vaginalis*), two at Manamukku (*E. dulcis* and *L. flava*) and one each at Reservoir bund (*N. nucifera*) and Pallichalthodu (*S. grossus*).

Among the floating aquatic macrophytes, *P. stratiotes* recorded the highest (2.251mg kg^{-1}) root Cd content and *I. aquatica*, the lowest (0.005 mg kg^{-1}). Two species had the highest Cd content at Arattukadavu RB (*E. crassipes* and *P. stratiotes*) and one each at Manamukku (*I. aquatica*), Pallichalthodu (*N. nouchali*), Palappoor (*N. indica*) and Mannamvarambu (*S. molesta*).

Three species recorded the lowest accumulation of Cd in root at Valiyavilagam (*E. crassipes*, *I. aquatica* and *P. stratiotes*) and two at Reservoirbund (*N. indica* and *S. molesta*). The lowest root Cd content for *N. nouchali* was recorded at Mannamvarambu.

V. natans, submerged aquatic macrophyte, had the highest root Cd content at Pallichalthodu (2.860 mg kg^{-1}) and lowest at Manamukku (1.940 mg kg^{-1}).

Among the terrestrial macrophytes, the highest Cd content in root was recorded by *S. trilobata* (3.408 mg kg^{-1}) and *A. aureum*, the lowest (0.006 mg kg^{-1}). Comparing the site wise accumulation, three species each had their highest root Cd content at Arattukadavu RB (*A. aureum*, *P. repens* and *P. hydropiper*) and Manamukku (*A. tenella*, *B. dactyoides* and *C. dactylon*) and two each at Palappoor (*A. sessilis* and *B. orientale*), Pallichalthodu (*L. rotundifolia* and *S. trilobata*) and

Reservoir bund (*B. mutica* and *C. lindleyana*). *P. kaida* recorded the highest Cd content at Valiyavilagam.

Table 30. Root Cd content of selected macrophytes of Vellayani wetland ecosystem

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean
		(mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	1.75	2.15	1.52	2.17	1.47	1.31	1.48	1.693
2	*E. colona*	-	-	-	-	-	0.004	0.005	0.004
3	*E. dulcis*	1.77	1.81	1.60	1.80	1.25	1.18	1.04	1.493
4	*L. flava*	3.35	3.40	3.25	3.40	3.65	2.94	2.85	3.263
5	*M. vaginalis*	1.15	1.07	0.95	1.20	1.09	0.86	0.87	1.027
6	*N. nucifera*	1.90	-	1.88	2.15	2.50	2.25	2.75	2.238
7	*S. grossus*	1.35	1.10	1.33	-	-	1.14	1.65	1.314
b	Floating								
1	*E. crassipes*	2.25	2.20	2.00	2.76	1.85	2.50	2.11	2.239
2	*I. aquatica*	0.005	-	-	-	0.004	-	0.006	0.005
3	*N. nouchali*	0.84	0.95	-	0.76	-	0.55	-	0.775
4	*N. indica*	2.78	-	1.92	-	2.17	2.00	2.35	2.244
5	*P. stratiotes*	2.15	2.37	2.14	2.73	2.00	2.17	2.20	2.251
6	*S. molesta*	0.115	0.120	0.109	0.125	0.110	0.130	0.120	0.118
c	Submerged								
1	*V. natans*	-	2.86	-	-	-	-	1.94	2.400
B	Terrestrial								
1	*A. aureum*	-	-	-	0.008	0.007	0.006	-	0.006
2	*A. sessilis*	0.055	0.043	-	-	-	-	-	0.049
3	*A. tenella*	2.18	2.11	2.10	2.25	2.02	2.50	2.75	2.273
4	*B. orientale*	0.057	0.044	0.031	0.055	0.044	0.030	0.018	0.040
5	*B. mutica*	-	-	0.022	-	0.018	-	0.018	0.019
6	*B. dactyoides*	1.75	1.88	1.80	-	2.00	2.10	2.25	1.963
7	*C. lindleyana*	-	0.004	0.008	-	-	-	-	0.006
8	*C. dactylon*	1.68	1.70	1.55	1.50	1.60	1.75	1.85	1.661
9	*G. tubiflorum*	-	-	-	0.005	-	-	-	0.005
10	*I. globosa*	-	-	-	0.006	-	-	-	0.006
11	*L. rotundifolia*	0.15	0.20	-	-	-	-	-	0.175
12	*P. kaida*	-	0.95	0.87	1.10	1.23	0.725	-	1.100
13	*P. repens*	-	-	0.027	0.030	0.018	0.014	0.011	0.020
14	*P. hydropiper*	-	-	-	1.28	-	-	1.25	1.265
15	*S. trilobata*	3.60	4.15	3.32	3.84	2.10	3.75	3.10	3.408

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Three species had the lowest root Cd content at Manamukku (*B. orientale*, *P. repens* and *P. hydropiper*) and two each at Palappoor (*B. dactyoides* and *L. rotundifolia*), Pallichalthodu (*A. sessilis* and *C. lindleyana*), Valiyavilagam (*A. tenella* and *S. trilobata*) and Mannamvarambu (*A. aureum* and *P. kaida*). *B. mutica* recorded the lowest Cd content both at Valiyavilagam and Manamukku. The lowest Cd content in the root for *C. dactylon* was recorded at Arattukadavu RB.

On evaluating all the selected macrophytes of the experimental site, the terrestrial macrophyte, *S. trilobata* showed the highest Cd content (3.408 mg kg^{-1}) in root (Table 30) and the lowest (0.004 mg kg^{-1}) by the emergent aquatic macrophyte, *E. colona*.

4.2.3. Chemical properties of rhizosphere sediment / soil

Sediment collected from the rhizosphere of aquatic macrophytes and soil samples from the rhizosphere of terrestrial macrophytes were analyzed for chemical and biological characteristics and the results are furnished in Tables 31 to 40.

4.2.3.1. Soil reaction (pH)

The pH of sediments collected from the study sites of the Vellayani lake and that of rhizosphere soils of the terrestrial macrophytes of the same ecosystem (Table 31) were strongly to moderately acidic in reaction. Site wise as well as species wise variation in pH was noticed, though it was not that much conspicuous.

Perusal of the data on rhizosphere pH of the emergent aquatic macrophytes revealed that the highest value of 5.80 was noticed for *M. vaginalis* from Manamukku. But the same species showed lower pH for other sites. The lowest pH of 5.00 was recorded by *C. esculenta* from Pallichalthodu and *E. dulcis* from Arattukadavu RB. As in the case of highest value, here also the plants showed higher values at other sites. Among the emergent aquatic macrophytes, though there was not much variation in the mean pH of rhizosphere, *M. vaginalis* had the highest (5.43) mean pH and *C. esculenta*, the lowest (5.26).

Table 31. pH of rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere pH							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	5.11	5.00	5.35	5.05	5.44	5.35	5.50	5.26
2	*E. colona*	-	-	-	-	-	5.40	5.33	5.36
3	*E. dulcis*	5.11	5.48	5.27	5.00	5.41	5.50	5.59	5.34
4	*L. flava*	5.22	5.15	5.37	5.11	5.35	5.53	5.60	5.33
5	*M. vaginalis*	5.11	5.30	5.37	5.22	5.65	5.59	5.80	5.43
6	*N. nucifera*	5.05	-	5.24	5.22	5.44	5.59	5.65	5.36
7	*S. grossus*	5.20	5.22	5.30	-	-	5.62	5.77	5.42
b	Floating								
1	*E. crassipes*	5.22	5.25	5.30	5.25	5.41	5.89	5.65	5.42
2	*I. aquatica*	5.17	-	-	-	5.35	-	5.37	5.29
3	*N. nouchali*	5.07	5.22	-	4.97	-	5.55	-	5.20
4	*N. indica*	5.20	-	5.48	-	5.65	5.82	5.75	5.58
5	*P. stratiotes*	5.10	5.25	5.48	5.00	5.62	5.75	5.80	5.43
6	*S. molesta*	4.99	5.30	5.45	5.10	5.52	5.67	5.54	5.36
c	Submerged								
1	*V. natans*	-	5.51	-	-	-	-	5.67	5.59
B	Terrestrial								
1	*A. aureum*	-	-	-	5.11	5.22	5.22	-	5.18
2	*A. sessilis*	5.04	5.20	-	-	-	-	-	5.12
3	*A. tenella*	5.27	5.20	5.21	5.15	5.22	5.46	5.52	5.29
4	*B. orientale*	5.05	5.40	5.12	5.22	5.45	5.50	5.67	5.34
5	*B. mutica*	-	-	5.45	-	5.48	-	5.57	5.50
6	*B. dactyoides*	5.22	5.49	5.42	-	5.71	5.86	5.72	5.57
7	*C. lindleyana*	-	5.39	5.15	-	-	-	-	5.27
8	*C. dactylon*	5.44	5.30	5.21	5.11	5.65	5.73	5.63	5.44
9	*G. tubiflorum*	-	-	-	5.12	-	-	-	5.12
10	*I. globosa*	-	-	-	5.34	-	-	-	5.34
11	*L. rotundifolia*	5.06	5.25	-	-	-	-	-	5.15
12	*P. kaida*	-	5.38	5.12	5.11	5.52	5.43	-	5.31
13	*P. repens*	-	-	5.35	5.15	5.52	5.55	5.75	5.46
14	*P. hydropiper*	-	-	-	5.10	-	-	5.35	5.22
15	*S. trilobata*	5.14	5.20	5.05	5.05	5.31	5.53	5.45	5.24
Mean (Site wise)		5.14	5.28	5.30	5.12	5.47	5.57	5.60	

- *absence of macrophyte in the site*

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Comparing the site wise variation, rhizosphere sediment for all the species except *E. colona* recorded the highest pH at Manamukku. Sediment collected from the rhizosphere region of *E. colona* had the highest pH at Mannamvarambu. Rhizosphere sediment of three species (*M. vaginalis*, *N. nucifera* and *S. grossus*) had the lowest pH at Palappoor and two (*E. dulcis* and *L. flava*) at Arattukadavu RB. Sediment of *C. esculenta* and *E. colona* had the lowest pH at Pallichalthodu and Manamukku, respectively.

Among the floating aquatic macrophytes, sediment collected just beneath the macrophyte, *E. crassipes* at Mannamvarambu had the highest pH (5.89) and *N. nouchali* from Arattukadavu RB, the lowest (4.97). There was species wise variation in rhizosphere sediment pH and the highest mean value was recorded by *N. indica* (5.58) and *N. nouchali*, the lowest (5.20).

On comparing the site wise variation, four species (*E. crassipes*, *N. nouchali*, *N. indica* and *S. molesta*) recorded the highest pH at Mannamvarambu and two at Manamukku (*I.aquatica* and *P. stratiotes*). Palappoor had the lowest pH for sediment collected from the rhizosphere region of four species (*E. crassipes*, *I.aquatica*, *N.indica* and *S. molesta*) and Arattukadavu for two (*N. nouchali* and *P. stratiotes*).

The submerged aquatic macrophyte, *V. natans*, recorded the highest pH for sediment collected from Manamukku (5.67) and Pallichalthodu, the lowest (5.51).

On verifying the rhizosphere soil pH of terrestrial macrophytes, it was found that *B. dactyoides* recorded the highest pH for soil collected from Mannamvarambu (5.86) and *A. sessilis* (5.04) at Palappoor, the lowest. Comparing the mean rhizosphere soil pH, *B. dactyoides* had the highest mean pH value (5.57) and the lowest (5.12) by *A. sessilis* and *G. tubiflorum.*

Soil collected from the rhizosphere region of five species (*A. tenella, B. orientale, B. mutica, P. repens* and *P. hydropiper*) had the highest pH at Manamukku, three (*B. dactyoides*, *C. dactylon* and *S. trilobata*) at Mannamvarambu, two (*C.*

lindleyana and *L. rotundifolia*) at Pallichalthodu and one each at Palappoor (*A. sessilis*) and Valiyavilagam (*P. kaida*). Rhizosphere soil of *A. aureum* showed the same pH value both at Valiyavilagam and Mannamvarambu.

Six species (*A. aureum*, *A. tenella*, *C. dactylon*, *P. kaida*, *P. repens* and *P. hydropiper*) recorded the lowest rhizosphere soil pH at Arattukadavu RB, three (*B. orientale*, *C. dactylon* and *L. rotundifolia*) at Palappoor, two (*B. mutica* and *C. lindleyana*) at Reservoir bund and one (*A. sessilis*) at Pallichalthodu. *S. trilobata* had the lowest pH for rhizosphere soil collected from both Arattukadavu RB and Pallichalthodu.

Among the sites, the highest average pH was recorded at Manamukku (5.60) and the lowest at Arattukadavu RB (5.12).

4.2.3.2. Electrical conductivity (EC)

EC of sediments collected from the selected sites of the Vellayani lake and rhizosphere soils of the terrestrial macrophytes (Table 32) were well within the tolerable limit. Though not prominent, variation was noticed among the sites as well as the species.

Among the emergent aquatic macrophytes, the highest rhizosphere sediment EC was recorded by *E. dulcis* at Palappoor (0.148 dS m^{-1}) and the lowest by *N. nucifera* at Mannamvarambu (0.085 dS m^{-1}). Comparing the site wise variation, two species (*N. nucifera* and *S. grossus*) had obtained the highest sediment EC at Reservoir bund and one each at Palappoor (*E. dulcis*), Arattukadavu RB (*M. vaginalis*) and Mannamvarambu (*E. colona*). Two sites recorded the same EC for two species each viz., *C. esculenta* at Arattukadavu RB and Mannamvarambu and *L. flava* at Palappoor and Arattukadavu RB.

Table 32. EC of rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere EC (dS m^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	0.125	0.132	0.132	0.136	0.134	0.136	0.133	0.132
2	*E. colona*	-	-	-	-	-	0.142	0.130	0.136
3	*E. dulcis*	0.148	0.132	0.125	0.120	0.139	0.130	0.124	0.131
4	*L. flava*	0.137	0.124	0.125	0.137	0.120	0.118	0.115	0.125
5	*M. vaginalis*	0.135	0.124	0.125	0.138	0.122	0.114	0.118	0.125
6	*N. nucifera*	0.086	-	0.112	0.110	0.098	0.085	0.101	0.098
7	*S. grossus*	0.125	0.123	0.138	-	-	0.130	0.132	0.129
b	Floating								
1	*E. crassipes*	0.128	0.114	0.112	0.130	0.125	0.107	0.115	0.118
2	*I. aquatica*	0.135	-	-	-	0.124		0.120	0.126
3	*N. nouchali*	0.095	0.112	-	0.119		0.112	-	0.109
4	*N. indica*	0.108	-	0.109	-	0.098	0.107	0.098	0.104
5	*P. stratiotes*	0.115	0.118	0.109	0.112	0.098	0.107	0.101	0.108
6	*S. molesta*	0.133	0.105	0.115	0.109	0.107	0.092	0.101	0.108
c	Submerged								
1	*V. natans*	-	0.122	-	-	-	-	0.099	0.110
B	Terrestrial								
1	*A. aureum*	-	-	-	0.149	0.143	0.146	-	0.146
2	*A. sessilis*	0.132	0.151	-	-	-	-	-	0.141
3	*A. tenella*	0.156	0.132	0.128	0.154	0.125	0.135	0.127	0.136
4	*B. orientale*	0.137	0.143	0.152	0.149	0.143	0.146	0.147	0.145
5	*B. mutica*	-	-	0.140	-	0.139	-	0.132	0.137
6	*B. dactyoides*	0.148	0.139	0.147	-	0.117	0.126	0.120	0.132
7	*C.lindleyana*	-	0.124	0.132	-	-	-	-	0.128
8	*C. dactylon*	0.144	0.145	0.148	0.155	0.148	0.136	0.142	0.145
9	*G. tubiflorum*	-	-	-	0.123	-	-	-	0.123
10	*I. globosa*	-	-	-	0.123	-	-	-	0.123
11	*L. rotundifolia*	0.148	0.139	-	-	-	-	-	0.143
12	*P. kaida*	-	0.143	0.152	0.149	0.143	0.146	-	0.146
13	*P. repens*	-	-	0.144	0.138	0.115	0.128	0.125	0.130
14	*P. hydropiper*	-	-	-	0.123	-	-	0.130	0.126
15	*S. trilobata*	0.148	0.151	0.152	0.155	0.143	0.145	0.148	0.148
Mean (Site wise)		0.131	0.130	0.131	0.133	0.125	0.124	0.122	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Two species each had the lowest rhizosphere sediment EC at Mannamvarambu (*M. vaginalis* and *N. nucifera*) and Manamukku (*E. colona* and *L. flava*) and one each at Palappoor (*C. esculenta*), Pallichalthodu (*S. grossus*) and Arattukadavu RB (*E. dulcis*). On comparing the species wise variation, the highest mean EC for rhizosphere sediment was recorded by *E. colona* (0.136 dS m^{-1}) and *N. nucifera*, the lowest (0.098 dS m^{-1}).

On examining the data on sediment EC of floating aquatic macrophytes collected just beneath them, it was seen that *I. aquatica* at Palappoor recorded the highest (0.135 dS m^{-1}) value and *S. molesta* at Mannamvarambu, the lowest (0.092 dS m^{-1}).

Three species (*E. crassipes, I. aquatica* and *S. molesta*) had the highest rhizosphere sediment EC at Palappoor and one each at Pallichalthodu (*P. stratiotes*), Reservoir bund (*N. indica*) and Arattukadavu RB (*N. nouchali*). Mannamvarambu recorded the lowest sediment EC for *E. crassipes* and *S. molesta*, Palappoor for *N. nouchali*, Valiyavilagam for *P. stratiotes* and Manamukku for *I. aquatica*. The lowest sediment EC for *N. indica* was obtained at Mannamvarambu and Manamukku. It was observed that *I. aquatica* had the highest mean rhizosphere sediment EC (0.126 dS m^{-1}) and *N. indica*, the lowest (0.104 dS m^{-1}).

V. natans, the submerged aquatic macrophyte, recorded the highest EC for sediment collected from the rhizosphere region of Pallichalthodu and the lowest at Manamukku.

Among the terrestrial macrophytes, the highest rhizosphere soil EC was recorded for *A. tenella* collected from Palappoor (0.156 dS m^{-1}) and *P. repens* from Valiyavilagam, the lowest (0.115 dS m^{-1}). Comparing the species wise variation, *S. trilobata* had the highest mean EC (0.148 dS m^{-1}) and *G. tubiflorum* and *I. globosa*, the lowest (0.123 dS m^{-1}).

Five species (*B. orientale*, *B. mutica*, *C. lindleyana*, *P. kaida* and *P. repens*) had the highest rhizosphere soil EC at Reservoir bund, three each at Palappoor (*A. tenella*, *B. dactyoides* and *L. rotundifolia*) and Arattukadavu RB (*A. aureum*, *C. dactylon* and *S. trilobata*) and one each at Pallichlthodu (*A. sessilis*) and Manamukku (*P. hydropiper*). Soil collected from the rhizosphere region of five species (*A. aureum*, *A. tenella*, *B. dactyoides*, *P. repens* and *S. trilobata*) recorded the lowest EC value at Valiyavilagam, two each at Palappoor (*A. sessilis* and *B. orientale*) and Pallichalthodu (*C. lindleyana* and *L. rotundifolia*) and one each at Arattukadavu RB (*P. hydropiper*), Mannamvarambu (*C. dactylon*) and Manamukku (*B. mutica*).

The highest mean EC for rhizosphere sediment / soil was obtained at Arattukadavu RB (0.133 dS m^{-1}) and the lowest at Manamukku (0.122 dS m^{-1}).

4.2.3.3. Organic carbon (OC)

Among the emergent aquatic macrophytes, sediment OC was highest for *N. nucifera* at Reservoir bund (28.9 g kg^{-1}) and the lowest for *E. colona* at Mannamvarambu (19.5 g kg^{-1}). Comparing the species wise variation, *N. nucifera* recorded the highest mean rhizosphere sediment OC and *E. colona*, the lowest. On analysis of site wise variation, two species (*M. vaginalis* and *N. nucifera*) had their highest rhizosphere sediment OC at Arattukadavu RB and one species each at Pallichalthodu (*C. esculenta*), Reservoir bund (*L. flava*), Valiyavilagam (*E. dulcis*), Mannamvarambu (*S. grossus*) and Manamukku (*E. colona*).

Three species (*C. esculenta*, *E. colona* and *E. dulcis*) recorded the lowest sediment OC at Mannamvarambu, two at Palappoor (*L. flava* and *N. nucifera*) and one at Pallichalthodu (*S. grossus*). The lowest rhizosphere sediment OC for *M. vaginalis* was recorded at Mannamvarambu and Manamukku.

Comparing the species wise variation of sediment OC for floating aquatic macrophytes, it was observed that sediment from the rhizosphere region of *E. crassipes*, *I. aquatica* and *N. nouchali* recorded the highest OC (26.9) and *P.stratiotes*

Table 33. OC of rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere OC ($g\ kg^{-1}$)							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	25.2	25.9	24.0	25.2	23.8	23.0	24.8	24.5
2	*E. colona*	-	-	-	-	-	19.5	23.7	21.6
3	*E. dulcis*	22.7	22.5	22.8	23.3	23.7	22.0	22.5	22.7
4	*L. flava*	21.1	26.5	28.4	22.8	25.5	23.8	24.8	24.7
5	*M. vaginalis*	28.5	25.0	26.9	33.3	25.8	23.5	23.5	26.6
6	*N. nucifera*	23.4	-	28.9	30.5	26.7	25.6	25.2	26.7
7	*S. grossus*	26.1	25.4	26.5	-	-	27.0	26.3	26.2
b	Floating								
1	*E. crassipes*	26.2	25.7	26.3	31.4	26.1	27.2	25.4	26.9
2	*I. aquatica*	29.7	-	-	-	25.9	-	25.2	26.9
3	*N. nouchali*	22.9	30.4	-	31.5	-	22.8	-	26.9
4	*N. indica*	24.0	-	23.2	-	23.3	23.9	23.2	23.5
5	*P. stratiotes*	22.8	22.9	23.3	23.1	23.4	22.2	23.6	23.0
6	*S. molesta*	24.0	23.6	24.5	26.8	25.0	26.5	24.7	25.0
c	Submerged								
1	*V. natans*	-	28.5	-	-	-	-	27.8	28.1
B	Terrestrial								
1	*A. aureum*	-	-	-	24.4	26.1	23.5	-	24.6
2	*A. sessilis*	23.5	23.2	-	-	-	-	-	23.3
3	*A. tenella*	23.2	22.9	25.7	26.0	24.3	23.2	19.8	23.5
4	*B. orientale*	24.4	26.0	27.1	27.4	24.3	30.3	24.5	26.2
5	*B. mutica*	-	-	23.8	-	24.8	-		24.3
6	*B. dactyoides*	23.1	23.8	22.7	-	22.7	23.5	22.0	22.9
7	*C. lindleyana*	-	25.2	25.7	-	-	-	-	25.4
8	*C. dactylon*	27.8	25.7	25.2	23.8	22.4	22.8	24.7	24.6
9	*G. tubiflorum*	-	-	-	29.6	-	-	-	29.6
10	*I. globosa*	-	-	-	19.5	-	-	-	19.5
11	*L. rotundifolia*	20.4	22.7	-	-	-	-	-	21.5
12	*P. kaida*	-	26.8	25.1	2.75	26.2	25.3	-	26.1
13	*P. repens*	-	-	26.6	24.0	22.6	22.8	23.6	23.9
14	*P. hydropiper*	-	-	-	23.8	-	-	22.5	23.1
15	*S. trilobata*	27.0	26.1	28.5	31.2	27.2	28.6	29.8	28.3
Mean (Site wise)		24.5	25.2	25.5	26.6	24.7	24.4	24.5	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

the lowest (23.0). Three species (*E. crassipes*, *N. nouchali* and *S. molesta*) had their highest rhizosphere sediment OC at Arattukadavu RB, two (*I. aquatica* and *N. indica*) at Palappoor and one (*P. stratiotes*) at Manamukku.

Pallichalthodu (*E. crassipes* and *S. molesta*) and Mannamvarambu (*N. nouchali* and *P. stratiotes*) recorded the lowest sediment OC for two species each and Manamukku for *I. aquatica*. *N. indica* had its lowest sediment OC both at Reservoir bund and Manamukku. *N. nouchali* at Arattukadavu RB (31.5) obtained the highest sediment OC and *P. stratiotes*, the lowest (22.2).

The only submerged aquatic macrophyte, *V. natans,* had the highest sediment OC (2.85) at Pallichalthodu and the lowest at Manamukku (27.8).

Among the terrestrial macrophytes, *S. trilobata* at Pallichalthodu (31.2) recorded the highest soil OC while *I. globosa*, the lowest (19.5). On comparing the species wise variation, the highest mean rhizosphere soil OC was obtained for *S. trilobata* and the lowest for *I. globosa*. Four species at Arattukadavu RB (*A. tenella*, *P. kaida*, *P. hydropiper* and *S. trilobata*) and two each at Palappoor (*A. sessilis* and *C. dactylon*), Pallichalthodu (*B. dactyoides* and *L. rotundifolia*), Reservoir bund (*C. lindleyana* and *P. repens*) and Valiyavilagam (*A. aureum* and *B. mutica*) obtained the highest rhizosphere soil OC. *B. orientale* recorded the highest rhizosphere OC at Mannamvarambu.

Three species each recorded the lowest OC at Pallichalthodu (*A. sessilis*, *C. lindleyana* and *S. trilobata*), Valiyavilagam (*B. orientale*, *C. dactylon* and *P. repens*) and Manamukku (*A. tenella*, *B. dactyoides* and *P. hydropiper*), two at Reservoir bund (*B. mutica* and *P. kaida*) and one each at Palappoor (*L. rotundifolia*) and Mannamvarambu (*A. aureum*). Though there was only slight variation in the mean rhizosphere soil OC content, Arattukadavu RB (26.6) recorded the highest and Valiyavilagam, the lowest (24.4).

4.2.3.4 Metal contents of rhizosphere sediment / soil

Fe, Al, Pb and Cd contents of sediment collected from the rhizosphere region of aquatic macrophytes and soil from the rhizosphere region of terrestrial macrophytes are given in Tables 34 to 37.

4.2.3.4.1. Iron (Fe)

Among the emergent aquatic macrophytes, *S. grossus* at Palappoor recorded the highest sediment Fe content (840 mg kg^{-1}) while *E. colona* at Mannamvarambu (685 mg kg^{-1}), the lowest (Table 34). Comparing various species, it was revealed that the highest mean value for sediment Fe was recorded by *M. vaginalis* (799 mg kg^{-1}) and the lowest by *E. colona* (710 mg kg^{-1}). Three species (*C. esculenta*, *E. dulcis* and *L. flava*) had their highest Fe content at Arattukadavu RB and one each at Palappoor (*S. grossus*), Pallichalthodu (*M. vaginalis*), Reservoir bund (*N. nucifera*) and Manamukku (*E. colona*).

Four species (*E. colona*, *E. dulcis*, *L. flava* and *M. vaginalis*) recorded their lowest sediment Fe content at Mannamvarambu and three (*C. esculenta*, *N. nucifera* and *S. grossus*) at Manamukku.

Evaluation of sediment Fe content of floating aquatic macrophytes revealed that the highest value of 860 mg kg^{-1} was noticed for *E. crassipes* from Arattukadavu RB while *P. stratiotes* from Manamukku showed the lowest value of 700 mg kg^{-1}. Among the macrophytes, *I. aquatica* recorded the highest mean value (809 mg kg^{-1}) and the lowest by *N. indica* (741mg kg^{-1}).

Three species had their highest sediment Fe content at Palappoor (*E. crassipes*, *I. aquatica* and *N. indica*) and one each at Pallichalthodu (*N. nouchali*), Reservoir bund (*P. stratiotes*) and Arattukadavu RB (*S. molesta*). Excluding *I. aquatica*, all other floating macrophytes had their lowest sediment Fe content at Mannamvarambu. *I. aquatica* had its lowest Fe content at Valiyavilagam.

Table 34. Fe_{av} content of rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere available Fe (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	800	795	787	820	746	760	710	774
2	*E. colona*	-	-	-	-	-	685	735	710
3	*E. dulcis*	822	805	783	838	740	720	735	777
4	*L. flava*	815	793	808	833	775	762	755	791
5	*M. vaginalis*	825	835	790	805	810	750	778	799
6	*N. nucifera*	770	-	812	795	715	720	698	751
7	*S. grossus*	840	822	810	-	-	775	725	794
b	Floating								
1	*E. crassipes*	835	808	822	860	780	718	745	795
2	*I. aquatica*	825	-	-	-	787	-	815	809
3	*N. nouchali*	760	830	-	788	-	722	-	775
4	*N. indica*	766	-	740	-	760	711	730	741
5	*P. stratiotes*	783	779	780	772	715	700	717	749
6	*S. molesta*	820	790	806	835	800	735	788	796
c	Submerged								
1	*V. natans*	-	725	-	-	-	-	690	707
B	Terrestrial								
1	*A. aureum*	-	-	-	815	710	725	-	750
2	*A. sessilis*	760	775	-	-	-	-	-	767
3	*A. tenella*	755	740	715	795	685	698	711	728
4	*B. orientale*	815	830	800	840	789	765	778	802
5	*B. mutica*	-	-	755	-	725	-	740	740
6	*B. dactyoides*	743	722	765		625	675	640	695
7	*C. dactylon*	718	704	688	766	645	710	622	693
8	*C. lindleyana*	-	800	828	-	-	-	-	814
9	*G. tubiflorum*	-	-	-	780	-	-	-	780
10	*I. globosa*	-	-	-	811	-	-	-	811
11	*L. rotundifolia*	783	792	-	-	-	-	-	787
12	*P. kaida*	-	833	820	835	770	786	-	809
13	*P. repens*	-	-	735	803	693	678	640	710
14	*P. hydropiper*	-	-	-	775	-	-	685	730
15	*S. trilobata*	780	793	786	775	756	720	765	768
Mean (Site wise)		790	787	781	807	738	726	724	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

V. natans, the submerged aquatic macrophyte, had its highest sediment Fe content at Pallichalthodu (725 mg kg^{-1}) and lowest at Manamukku (690 mg kg^{-1}).

Among the terrestrial macrophytes, the highest value for rhizosphere soil Fe was noticed by *B. orientale* from Arattukadavu RB (840 mg kg^{-1}) and the lowest by *C. dactylon* (622 mg kg^{-1}) from Manamukku. Comparison of species wise variation, it was noticed that there was much variation in the rhizosphere Fe content and the highest mean value was obtained for *C. lindleyana* (814 mg kg^{-1}) and *C. dactylon*, the lowest (693 mg kg^{-1}).

Soil collected from the rhizosphere region of seven species (*A. aureum*, *A. tenella*, *B. orientale*, *C. dactylon*, *P. kaida*, *P. repens* and *P. hydropiper*) had their highest value for Fe at Arattukadavu RB and three each at Pallichalthodu (*A. sessilis*, *L. rotundifolia* and *S. trilobata*) and Reservoir bund (*B. mutica*, *B. dactyoides* and *C. lindleyana*).

Four species recorded their lowest values at Valiyavilagam (*A. tenella*, *B. mutica*, *B. dactyoides* and *L. rotundifolia*), three each at Mannamvarambu (*A. aureum B. orientale*, and *S. trilobata*) and Manamukku (*C. dactylon*, *P. repens* and *P. hydropiper*), two at Palappoor (*A. sessilis* and *L. rotundifolia*) and one at Pallichalthodu (*C. lindleyana*).

Comparison of site wise variation revealed that Arattukadavu RB recorded the highest mean value (807 mg kg^{-1}) and Manamukku, the lowest (724 mg kg^{-1}).

4.2.3.4.2. Aluminium (Al)

Perusal of the data on Al content of rhizosphere sediment of emergent aquatic macrophytes revealed that *E. dulcis* from Arattukadavu recorded the highest value (726 mg kg^{-1}) and *E. colona* from Manamukku (584 mg kg^{-1}), the lowest (Table 35). Comparing the different species, it was found that the highest mean value was recorded by *L. flava* (670 mg kg^{-1}) and the lowest (594 mg kg^{-1}) *by E. colona.* With regard to the site wise Al content, three species (*L. flava*, *N. nucifera* and *S. grossus*) had their highest content at Reservoir bund and one each at Pallichalthodu (*C. esculenta*), Arattukadavu RB (*E. dulcis*) and Manamukku (*E. colona*). *M. vaginalis* recorded the lowest rhizosphere Al content both at Palappoor and Reservoir bund.

Four species had the lowest Al content in sediment collected from the rhizosphere region of Mannamvarambu (*E. colona*, *E. dulcis*, *L. flava* and *N. nucifera*), two at Valiyavilagam (*C. esculenta* and *M. vaginalis*) and one at Manamukku (*S. grossus*).

Among the floating aquatic macrophytes, sediment collected just underneath the macrophyte, *P. stratiotes* from Pallichalthodu had the highest Al content (715 mg kg^{-1}) and the lowest (580 mg kg^{-1}) for *N. indica* from Mannamvarambu. On comparing the different species, it was observed that the highest mean value was obtained for *P. stratiotes* (670 mg kg^{-1}) and the lowest for *N. indica* (615 mg kg^{-1}). Sediment Al content of three species (*E. crassipes*, *N. nouchali* and *P. stratiotes*) had the highest value at Pallichalthodu, two (*I. aquatica* and *N. indica*) at Palappoor and one at Arattukadavu RB (*S. molesta*). Mannamvarambu recorded the lowest Al content for three species (*N. nouchali*, *N. indica* and *S. molesta*), Valiyavilagam for *E. crassipes* and Manamukku for *P. stratiotes*. *I. aquatica* showed the same Al content both at Valiyavilagam and Manamukku, which was the lowest content recorded for that species.

V. natans, the only submerged macrophyte, has recorded the highest rhizosphere Al content at Pallichalthodu (680 mg kg^{-1}) and the lowest at Manamukku (637 mg kg^{-1}).

Among the terrestrial macrophytes, *A. tenella* from Arattukadavu RB recorded the highest rhizosphere Al content (728 mg kg^{-1}) and *B. dactyoides* from Valiyavilagam, the lowest (587 mg kg^{-1}).

Table 35. Al_{av} content of rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere available Al (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	680	693	678	645	610	635	645	655
2	*E. colona*	-	-	-	-	-	584	605	594
3	*E. dulcis*	650	690	682	726	640	620	635	663
4	*L. flava*	680	688	695	685	645	640	660	670
5	*M. vaginalis*	670	660	670	658	636	595	625	645
6	*N. nucifera*	660	-	675	630	615	590	600	628
7	*S. grossus*	670	660	680	-	-	620	605	647
b	Floating								
1	*E. crassipes*	660	675	670	674	615	630	645	652
2	*I. aquatica*	690	-	-	-	640	-	640	656
3	*N. nouchali*	635	648	-	634	-	603	-	630
4	*N. indica*	640	-	633	-	605	580	615	615
5	*P. stratiotes*	690	715	710	670	655	640	615	670
6	*S. molesta*	650	640	640	670	631	610	618	637
c	Submerged								
1	*V. natans*	-	680	-	-	-	-	637	658
B	Terrestrial								
1	*A. aureum*	-	-	-	620	605	590	-	605
2	*A. sessilis*	635	620	-	-	-	-	-	627
3	*A. tenella*	665	648	675	728	630	620	612	654
4	*B. orientale*	655	665	643	660	640	615	650	647
5	*B. mutica*	-	-	620	-	620	-	630	623
6	*B. dactyoides*	643	625	658	-	587	600	592	617
7	*C. dactylon*	635	610	633	658	600	580	595	615
8	*C. lindleyana*	-	640	628	-	-	-	-	634
9	*G. tubiflorum*	-	-	-	660	-	-	-	660
10	*I. globosa*	-	-	-	650	-	-	-	650
11	*L. rotundifolia*	635	640	-	-	-	-	-	637
12	*P. kaida*	-	675	640	648	640	618	-	644
13	*P. repens*	-	-	665	695	605	611	596	634
14	*P. hydropiper*	-	-	-	652	-	-	593	622
15	*S. trilobata*	680	670	655	680	625	610	640	651
Mean (Site wise)		659	660	661	665	623	610	622	

- absence of macrophyte in the site

I- Palappoor   II - Pallichalthodu   III – Reservoir bund   IV- Arattukadavu RB
V - Valiyavilagam   VI - Mannamvarambu   VII - Manamukku

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Five terrestrial species (*A. aureum, A. tenella, C. dactylon, P. repens* and *P. hydropiper*) had their highest soil Al content at Arattukadavu RB, four (*B. orientale, C. lindleyana, L. rotundifolia* and *P. kaida*) at Pallichalthodu and one each at Palappoor (*A. sessilis*), Reservoir bund (*B. dactyoides*) and Manamukku (*B. mutica*). *S. trilobata* recorded the lowest Al content both at Palappoor and Arattukadavu RB.

Soil collected from the rhizosphere region of Mannamvarambu showed the lowest Al content for five species (*A. aureum, B. orientale, C. dactylon, P. kaida* and *S. trilobata*) and Manamukku for three (*A. tenella, P. repens* and *P. hydropiper*). Palappoor, Pallichalthodu, Reservoir bund and Valiyavilagam recorded the lowest Al content for *L. rotundifolia, A. sessilis, C. lindleyana* and *B. dactyoides* respectively. *B. mutica* recorded the same Al content both at Reservoir bund and Valiyavilagam which was the lowest value for that macrophyte.

Arattukadavu RB recorded the highest mean rhizosphere Al content (665 mg kg^{-1}) among the selected sites and Mannamvarambu, the lowest (610 mg kg^{-1}).

4.2.3.4.3. Lead (Pb)

Among the emergent aquatic macrophytes, the highest Pb content was noticed for *L. flava* from Palappoor (7.10 mg kg^{-1}) and the lowest by *E. colona* (2.80 mg kg^{-1}) from Manamukku (Table 36). Comparison of species wise variation, *L. flava* recorded the highest mean value of 5.64 mg kg^{-1} and the lowest (3.44 mg kg^{-1}) by *E. colona*.

Three species (*E. dulcis, L. flava* and *M. vaginalis*) had the highest mean Pb content in rhizosphere sediment from Palappoor, two (*C. esculenta* and *N. nucifera*) at Arattukadavu RB and one each at Pallichalthodu (*S. grossus)* and Mannamvarambu (*E. colona*). Rhizosphere sediment for all the species except *C. esculenta* and *M. vaginalis* recorded the lowest Pb content at Manamukku. Valiyavilagam and Mannamvarambu noticed the lowest rhizosphere sediment Pb content for *C. esculenta* and *M. vaginalis*, respectively.

Table 36. Pb_{av} content of rhizosphere sediment /soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere available Pb (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	5.75	4.85	5.11	6.07	4.21	4.87	4.35	5.03
2	*E. colona*	-	-	-	-	-	4.08	2.80	3.44
3	*E. dulcis*	4.25	4.00	4.15	4.11	3.68	3.80	3.45	3.92
4	*L. flava*	7.10	5.78	5.80	6.15	5.00	4.85	4.80	5.64
5	*M. vaginalis*	4.75	4.70	4.25	4.72	4.08	3.94	4.03	4.35
6	*N. nucifera*	4.68	-	4.65	4.96	3.76	3.83	3.37	4.20
7	*S. grossus*	4.25	5.90	5.09	-	-	3.85	3.55	4.52
b	Floating								
1	*E. crassipes*	5.09	4.45	5.11	5.20	3.76	4.05	3.98	4.52
2	*I. aquatica*	4.55	-	-	-	3.85	-	3.50	3.96
3	*N. nouchali*	3.76	4.30	-	4.46	-	2.98	-	3.87
4	*N. indica*	5.67	-	4.14	-	3.02	4.05	2.68	3.91
5	*P. stratiotes*	6.11	5.58	5.85	5.26	4.75	4.83	4.64	5.28
6	*S. molesta*	3.76	4.25	3.87	4.86	3.50	3.30	3.35	3.84
c	Submerged								
1	*V. natans*	-	5.13	-	-	-	-	3.38	4.25
B	Terrestrial								
1	*A. aureum*	-	-	-	4.20	3.25	3.80	-	3.75
2	*A. sessilis*	4.50	3.95	-	-	-	-	-	4.22
3	*A. tenella*	6.25	5.81	5.11	5.62	4.95	4.30	5.08	5.30
4	*B. orientale*	4.35	3.85	4.35	5.16	4.10	4.25	4.00	4.29
5	*B. mutica*	-	-	4.48	-	3.55	-	3.95	3.99
6	*B. dactyoides*	5.10	4.35	4.80	-	4.95	4.25	4.30	4.62
7	*C. dactylon*	4.52	4.25	4.65	4.90	4.50	4.27	4.65	4.53
8	*C. lindleyana*	-	4.08	4.22	-	-	-	-	4.14
9	*G. tubiflorum*	-	-	-	4.75	-	-	-	4.75
10	*I. globosa*	-	-	-	4.60	-	-	-	4.60
11	*L. rotundifolia*	3.90	3.25	-	-	-	-	-	3.57
12	*P. kaida*	-	4.80	4.30	4.72	4.23	4.10	-	4.43
13	*P. repens*	-	-	4.70	4.15	3.60	4.15	3.85	4.09
14	*P. hydropiper*	-	-	-	5.68	-	-	5.15	5.41
15	*S. trilobata*	4.30	4.75	4.15	4.67	4.20	4.10	3.95	4.29
Mean (Site wise)		4.87	4.63	4.67	4.96	4.05	4.08	3.95	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- ArattukadavuRB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

On evaluating the sediment Pb content of floating aquatic macrophytes, it was found that, *P. stratiotes* from Palappoor recorded the highest value (6.11 mg kg^{-1}) and *N. indica* from Manamukku, the lowest (2.68 mg kg^{-1}). Comparing the species mean, it was found that *P. stratiotes* recorded the highest mean Pb content (5.28 mg kg^{-1}) and the lowest by *S. molesta* (3.84 mg kg^{-1}). Three species each had their highest sediment Pb content at Palappoor (*I. aquatica*, *N. indica* and *P. stratiotes*) and Arattukadavu RB (*E. crassipes*, *N. nouchali* and *S. molesta*). Two species each recorded their lowest Pb content at Valiyavilagam (*E. crassipes* and *N. indica*), Mannamvarambu (*N. nouchali* and *S. molesta*) and Manamukku (*I. aquatica* and *P. stratiotes*).

V. natans (submerged aquatic macrophyte) recorded the highest sediment Pb content (5.13 mg kg^{-1}) at Pallichalthodu and lowest at Manamukku (3.38 mg kg^{-1}).

Among the terrestrial macrophytes, Pb content was highest for soil collected from the rhizosphere region of *A. tenella* (6.25 mg kg^{-1}). *A.aureum* from Valiyavilagam and *L. rotundifolia* from Pallichalthodu recorded the same Pb content (3.25 mg kg^{-1}), which was the lowest among these species. Comparing the species mean, *P. hydropiper* (5.41 mg kg^{-1}) showed the highest value and *L. rotundifolia*, the lowest (3.57 mg kg^{-1}).

Rhizosphere soil of four species each had the highest Pb content at Palappoor (*A. sessilis*, *A. tenella*, *B. dactyoides* and *L. rotundifolia*) and Valiyavilagam (*A. aureum*, *B. orientale*, *C. dactylon and P. hydropiper*). Three species (*B. mutica*, *C. lindleyana* and *P. repens*) recorded the highest Pb content at Reservoir bund and two (*P. kaida* and *S. trilobata*) at Pallichalthodu.

Soil collected from the rhizosphere region of five species (*A. sessilis*, *B. orientale*, *C. dactylon*, *C. lindleyana* and *L. rotundifolia*) had the lowest Pb content at Pallichalthodu, three each at Valiyavilagam (*A. aureum*, *B. mutica* and *P. repens*) and

Mannamvarambu (*A. tenella*, *B. dactyoides* and *P. repens*) and two at Manamukku (*P. hydropiper* and *S. trilobata*).

On comparing the site wise mean, the highest value for rhizosphere Pb content was recorded at Arattukadavu RB (4.96 mg kg^{-1}) and Mannamvarambu (3.95 mg kg^{-1}), the lowest.

4.2.3.4.4. Cadmium (Cd)

Cd content in the rhizosphere soil / sediment of selected macrophytes was very low (Table 37) but site wise as well as species wise variation was noticed.

Among the emergent aquatic macrophytes, the highest value of 0.013 mg kg^{-1} was obtained for *L. flava* at Reservoir bund. Comparison of species mean revealed that *N. nucifera* recorded the highest mean Cd content (0.009 mg kg^{-1}) and the lowest by *E. colona* (0.003 mg kg^{-1}). The highest Cd content for *E. dulcis* was recorded at Palappoor, *L. flava* at Reservoir bund, *C. esculenta* and *N. nucifera* at Arattukadavu RB and *E. colona* at Mannamvarambu. *M. vaginalis* had its highest Cd content both at Arattukadavu RB and Valiyavilagam while *S. grossus* at Pallichalthodu and Reservoir bund.

Three sites each noticed the same Cd content in the rhizosphere region for *C. esculenta* (Valiyavilagam, Mannamvarambu and Manamukku), *E. dulcis* (Pallichalthodu, Valiyavilagam and Mannamvarambu) and *M. vaginalis* (Pallichalthodu, Reservoir bund and Mannamvarambu) and it was the lowest content obtained for these species. *N. nucifera* recorded the lowest content both at Valiyavilagam and Manamukku. Two species (*E. colona* and *L. flava*) had the lowest rhizosphere Cd content at Manamukku and one at Palappoor (*S. grossus*).

On analyzing the Cd content of sediment collected just beneath the floating aquatic macrophytes, it was revealed that the highest value of 0.010 mg $kg^{-1}$was recorded by *N. indica* at Palappoor and *P. stratiotes* from Palappoor and Pallichalthodu.

Table 37. Cd_{av} content of rhizosphere sediment /soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere available Cd (mg kg^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	0.007	0.008	0.008	0.009	0.006	0.006	0.006	0.007
2	*E. colona*	-	-	-	-	-	0.004	0.003	0.003
3	*E. dulcis*	0.008	0.003	0.004	0.004	0.003	0.003	0.007	0.004
4	*L. flava*	0.011	0.010	0.013	0.012	0.010	0.008	0.007	0.010
5	*M. vaginalis*	0.004	0.003	0.003	0.005	0.005	0.003	0.004	0.004
6	*N. nucifera*	0.010	-	0.008	0.012	0.007	0.008	0.007	0.009
7	*S. grossus*	0.003	0.005	0.005	-	-	0.004	0.004	0.004
b	Floating								
1	*E.crassipes*	0.007	0.008	0.009	0.009	0.007	0.006	0.006	0.007
2	*I. aquatica*	0.004	-	-	-	0.004	-	-	0.004
3	*N. nouchali*	0.006	0.006	-	0.009	-	0.007	-	0.007
4	*N. indica*	0.010	-	0.008	-	0.007	0.007	0.007	0.008
5	*P. stratiotes*	0.010	0.010	0.009	0.009	0.007	0.009	0.008	0.009
6	*S. molesta*	0.005	0.004	0.005	0.006	0.006	0.004	0.005	0.005
c	Submerged								
1	*V. natans*	-	0.011	-	-	-	-	0.006	0.008
B	Terrestrial								
1	*A. aureum*	-	-	-	0.005	0.004	0.003	-	0.004
2	*A. sessilis*	0.003	0.002	-	-	-	-	-	0.002
3	*A. tenella*	0.010	0.009	0.009	0.011	0.009	0.008	0.007	0.009
4	*B. orientale*	0.005	0.006	0.005	0.006	0.007	0.006	0.007	0.006
5	*B. mutica*	-	-	0.004	-	0.004	-	0.004	0.004
6	*B. dactyoides*	0.008	0.007	0.009	-	0.010	0.007	0.006	0.008
7	*C. dactylon*	0.006	0.007	0.005	0.007	0.008	0.008	0.007	0.007
8	*C. lindleyana*	-	0.002	0.004	-	-	-	-	0.003
9	*G. tubiflorum*	-	-	-	0.009	-	-	-	0.009
10	*I. globosa*	-	-	-	0.009	-	-	-	0.009
11	*L. rotundifolia*	0.005	0.002	-	-	-	-	-	0.003
12	*P. kaida*	-	0.006	0.005	0.007	0.006	0.005	-	0.006
13	*P. repens*	-	-	0.004	0.004	0.003	0.005	0.004	0.004
14	*P. hydropiper*	-	-	-	0.010	-	-	0.008	0.009
15	*S. trilobata*	0.012	0.010	0.009	0.008	0.008	0.007	0.008	0.009
Mean (Site wise)		0.007	0.006	0.006	0.008	0.006	0.004	0.005	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- Arattukadavu RB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

I. aquatica from Palappoor and Valiyavilagam and *S. molesta* from Pallichalthodu and Mannamvarambu recorded the lowest value (0.004 mg kg^{-1}). Comparing the species wise variation, it was observed that *P. stratiotes* had the highest mean sediment Cd content (0.009) and *I. aquatica* the lowest (0.004).

Among the different sites from where sediment was collected for *E. crassipes,* the highest Cd content was noticed at Reservoir bund and Arattukadavu RB, both showing the same values and for *P. stratiotes* at Palappoor and Pallichalthodu; and for *S. molesta* at Arattukadavu RB and Valiyavilagam. *N. indica* and *N. nouchali* recorded the highest sediment Cd content at Palappoor and Arattukadavu RB, respectively. *N. indica* had the lowest sediment Cd at three sites (Valiyavilagam, Mannamvarambu and Manamukku) and *E. crassipes* (Mannamvarambu and Manamukku), *N. nouchali* (Palappoor and Pallichalthodu) and *S. molesta* (Pallichalthodu and Mannamvarambu) at two sites each. *P. stratiotes* recorded the lowest Cd content at Valiyavilagam.

V. natans, the only submerged aquatic macrophyte, had the highest Cd content at Pallichalthodu (0.011 mg kg^{-1}) and the lowest at Manamukku (0.006 mg kg^{-1}).

The terrestrial macrophytes had also showed wide variation in sediment Cd status. *S. trilobata* from Palappoor recorded the highest (0.012 mg kg^{-1}) rhizosphere soil Cd content and the lowest (0.002 mg kg^{-1}) by *A. sessilis*, *C. lindleyana* and *L. rotundifolia* from Pallichalthodu. Comparing the different species, the highest mean value of 0.009 mg kg^{-1} was recorded by five species (*A. tenella*, *G. tubiflorum*, *I. globosa*, *P. hydropiper* and *S. trilobata*) and the lowest mean value (0.002 mg kg^{-1}) by *A. sessilis*.

Three species each had the highest rhizosphere Cd content at Palappoor (*A. sessilis*, *L. rotundifolia* and *S. trilobata)* and Arattukadavu RB (*A. tenella*, *P. kaida* and *P. hydropiper*), two at Valiyavilagam (*A. aureum* and *B. dactyoides*) and one each at Reservoir bund (*C. lindleyana*) and Mannamvarambu (*P. repens*). The highest rhizosphere Cd content for *B. orientale* (Valiyavilagam and Manamukku) and *C. dactylon* (Valiyavilagam and Mannamvarambu) was recorded at two sites each. *B. mutica* recorded the same content at all the three sites (Reservoir bund, Valiyavilagam and Manamukku).

Soil collected from the rhizosphere region of three species each had the lowest Cd content at Pallichalthodu (*A. sessilis*, *C. lindleyana* and *L. rotundifolia*) and Manamukku (*A. tenella*, *B. dactyoides* and *P. hydropiper*), two at Mannamvarambu (*A. aureum* and *S. trilobata*) and one each at Reservoir bund (*C. dactylon*) and Valiyavilagam (*P. repens*). *B. orientale* (Palappoor and Reservoir bund) and *P. kaida* (Reservoir bund and Mannamvarambu) recorded the lowest soil Cd content at two sites each.

With regard to the site wise variation for rhizosphere soil Cd content, the highest mean value was noticed at Arattukadavu RB (0.008 mg kg^{-1}) and the lowest at Mannamvarambu (0.004 mg kg^{-1}).

4.2.3.5. Microbial load of rhizosphere sediment / soil

Bacterial, fungal and actinomycete population present in the sediment collected from the rhizosphere region of aquatic macrophytes and soil from the rhizosphere region of terrestrial macrophytes are given in Tables 38 to 40.

4.2.3.5.1. Bacteria

Perusal of the data on bacterial count in the sediment from rhizosphere of emergent aquatic macrophytes revealed that *E. colona* from Mannamvarambu (6.93 log cfu g^{-1}) recorded the highest value while *N. nucifera* from Arattukadavu RB (5.99), the lowest (Table 38). Comparing species wise variation, the highest mean value for bacterial count was noticed for *E. colona* (6.91) and the lowest for *N. nucifera* (6.27).

Two species each had their highest bacterial count at Palappoor (*L. flava* and *M. vaginalis*), Mannamvarambu (*E. colona* and *E. dulcis*) and Manamukku (*C.

esculenta and *N. nucifera*). *S. grossus* recorded the highest bacterial count both at Mannamvarambu and Manamukku. The lowest value for bacterial count was recorded at Arattukadavu RB for four species (*C. esculenta*, *E. dulcis*, *L. flava* and *N. nucifera*), Pallichalthodu for *M. vaginalis* and *S. grossus* and Manamukku for *E. colona*.

Among the floating aquatic macrophytes, the highest bacterial count in the sediment was obtained for *I. aquatica* from Manamukku (6.53). The lowest value was noticed for *N. nouchali* and *S. molesta*, both from Arattukadavu RB (5.98). On comparing the species, it was noticed that *I. aquatica* recorded the highest mean bacterial count (6.47) and *N. nouchali* and *S. molesta*, the lowest (6.12).

Sediment collected from the rhizosphere region of three species (*E. crassipes*, *P. stratiotes* and *S. molesta*) had the highest bacterial count at Valiyavilagam, two at Manamukku (*I. aquatica* and *N. indica*) and one at Mannamvarambu (*N. nouchali*). Three species recorded their lowest count for bacteria at Arattukadavu RB (*E. crassipes*, *N. nouchali* and *S. molesta*) and two at Palappoor (*I. aquatica* and *N. indica*). The lowest sediment bacterial count for *P. stratiotes* was noticed at Palappoor and Pallichalthodu.

V. natans, the only submerged aquatic macrophyte, had its highest sediment bacterial count at Pallichalthodu (5.91) and the lowest count at Manamukku (5.88).

On analyzing the data on bacterial count of soil collected from the rhizosphere region of terrestrial macrophytes, *C. dactylon* from Manamukku recorded the highest value (7.14) and *I. globosa*, the lowest (6.17). Five species (*A. tenella*, *B. orientale*, *C. dactylon*, *P. repens* and *P. hydropiper*) had the highest count at Manamukku, three at Valiyavilagam (*B. dactyoides*, *P. kaida* and *S. trilobata*), two at Palappoor (*A. sessilis* and *L. rotundifolia*) and one each at Pallichalthodu (*C. lindleyana*) and Mannamvarambu (*A. aureum*). For *B. mutica*, the highest bacterial count (6.98 log cfu g^{-1}) was noted at two sites viz., Valiyavilagam and Manamukku.

Table 38. Total bacteria in the rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere bacteria (log cfu g^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	6.57	6.49	6.50	6.45	6.55	6.53	6.63	6.53
2	*E. colona*	-	-	-	-	-	6.93	6.90	6.91
3	*E. dulcis*	6.52	6.40	6.43	6.20	6.57	6.60	6.59	6.47
4	*L. flava*	6.59	6.49	6.44	6.28	6.30	6.58	6.55	6.46
5	*M. vaginalis*	6.60	6.49	6.52	6.53	6.54	6.55	6.58	6.54
6	*N. nucifera*	6.28	-	6.34	5.99	6.30	6.32	6.41	6.27
7	*S. grossus*	6.54	6.47	6.60	-	-	6.63	6.63	6.57
b	Floating								
1	*E.crassipes*	6.40	6.32	6.34	6.25	6.43	6.41	6.40	6.36
2	*I. aquatica*	6.38	-	-	-	6.52	-	6.53	6.47
3	*N. nouchali*	6.14	6.04	-	5.98	-	6.32	-	6.12
4	*N. indica*	6.11	-	6.25	-	6.32	6.30	6.36	6.27
5	*P. stratiotes*	6.04	6.04	6.20	6.41	6.30	6.14	6.17	6.18
6	*S. molesta*	5.99	5.99	6.25	5.98	6.32	6.08	6.23	6.12
c	Submerged								
1	*V. natans*	-	5.91	-	-	-	-	5.88	5.89
B	Terrestrial								
1	*A. aureum*	-	-	-	6.84	6.96	6.98	-	6.92
2	*A. sessilis*	6.86	6.84	-	-	-	-	-	6.85
3	*A. tenella*	6.92	6.93	6.92	6.78	6.96	6.96	6.98	6.92
4	*B. orientale*	6.73	6.75	6.74	6.70	6.82	6.80	6.84	6.77
5	*B. mutica*	-	-	6.94	-	6.98	-	6.98	6.97
6	*B. dactyoides*	6.97	7.04	6.96	-	7.11	6.99	7.08	7.02
7	*C. lindleyana*	-	6.84	6.80	-	-		-	6.82
8	*C. dactylon*	6.98	6.99	7.08	6.99	7.08	7.11	7.14	7.05
9	*G. tubiflorum*	-	-	-	6.49	-	-	-	6.49
10	*I. globosa*	-	-	-	6.17	-	-	-	6.17
11	*L. rotundifolia*	6.47	6.46	-	-	-	-	-	6.46
12	*P. kaida*	-	6.65	6.62	6.64	6.67	6.62	-	6.64
13	*P. repens*	-	-	6.91	6.90	6.94	6.92	6.96	6.92
14	*P. hydropiper*	-	-	-	6.55	-	-	6.78	6.66
15	*S. trilobata*	6.70	6.72	6.70	6.67	6.90	6.85	6.86	6.77
Mean (Site wise)		6.52	6.52	6.61	6.46	6.66	6.63	6.64	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- ArattukadavuRB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

Six species (*A. aureum*, *A. tenella*, *B. orientale*, *P. repens*, *P. hydropiper* and *S. trilobata*) had the lowest count at Arattukadavu RB, three at Reservoir bund (*B. mutica*, *B. dactyoides* and *C. lindleyana*), two at Pallichalthodu (*A. sessilis* and *L. rotundifolia*) and one at Palappoor (*C. dactylon*). *P. kaida* recorded the same value both at Reservoir bund and Mannamvarambu, which was the lowest, for that species.

C. dactylon had the highest mean rhizosphere bacterial count (7.05) among the terrestrial macrophytes and the lowest (6.17) for *I. globosa*. Among the sites, the highest mean rhizosphere bacterial count (6.66) was recorded at Valiyavilagam and the lowest at Arattukadavu RB (6.46).

4.2.3.5.2. Fungi

Among the emergent aquatic macrophytes, highest value of 4.04 log cfu g^{-1} for the sediment rhizosphere fungal count (Table 39) was noticed for *L. flava* from Arattukadavu RB and the lowest for *N. nucifera* from Manamukku (2.88).

Rhizosphere sediment of four species (*C. esculenta*, *E. dulcis*, *L. flava* and *N. nucifera*) had the highest fungal count at Arattukadavu RB, two (*M. vaginalis* and *S. grossus*) at Pallichalthodu and one (*E. colona*) at Manamukku. Three species (*E. colona*, *E. dulcis* and *L. flava*) had the lowest fungal population in the rhizosphere region at Mannamvarambu, two (*N. nucifera* and *S. grossus*) at Manamukku and one (*M. vaginalis*) at Valiyavilagam. *C. esculenta* showed the same fungal population both at Reservoir bund and Manamukku, which was the lowest, recorded for that species.

Analysing the data on fungal count in the sediment collected just beneath the floating aquatic macrophytes, it was noticed that *E. crassipes* from Arattukadavu RB recorded the highest count (3.66) while *N. indica* (2.78) from Manamukku, the lowest. Arattukadavu RB had the highest fungal population in the rhizosphere sediment for three species (*E. crassipes*, *N. nouchali* and *S. molesta*) and Palappoor

Table 39. Total fungi in rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere fungi (log cfu g^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	3.63	3.58	3.46	3.66	3.61	3.64	3.46	3.58
2	*E. colona*	-	-	-	-	-	3.30	3.40	3.35
3	*E. dulcis*	3.68	3.70	3.66	3.76	3.47	3.38	3.41	3.58
4	*L. flava*	3.69	3.76	3.58	4.04	3.71	3.43	3.67	3.70
5	*M. vaginalis*	3.41	3.68	3.58	3.55	3.36	3.64	3.44	3.52
6	*N. nucifera*	2.95	-	2.92	3.25	2.92	2.89	2.88	2.97
7	*S. grossus*	3.14	3.23	3.04	-	-	2.98	2.97	3.07
b	Floating								
1	*E. crassipes*	3.17	3.25	3.23	3.66	3.50	3.30	3.20	3.33
2	*I. aquatica*	3.54	-	-	-	3.28	-	3.17	3.33
3	*N. nouchali*	2.96	2.98	-	3.30	-	2.96	-	3.05
4	*N. indica*	2.93	-	2.91	-	2.88	2.89	2.78	2.88
5	*P. stratiotes*	2.98	2.98	2.97	2.95	2.96	2.97	2.95	2.96
6	*S. molesta*	2.98	2.97	2.92	3.11	2.90	2.96	2.94	2.97
c	Submerged								
1	*V. natans*	-	3.11	-	-	-	-	3.23	3.17
B	Terrestrial								
1	*A. aureum*	-	-	-	3.41	3.20	3.04	-	3.22
2	*A. sessilis*	3.69	3.50	-	-	-	-	-	3.59
3	*A. tenella*	3.30	3.23	3.32	4.08	3.25	3.14	3.08	3.34
4	*B. orientale*	3.54	3.49	3.54	3.38	3.11	3.32	3.28	3.38
5	*B. mutica*	-	-	3.32	-	3.17	-	2.97	3.15
6	*B. dactyoides*	3.41	3.20	3.36	-	3.14	3.20	3.08	3.23
7	*C. lindleyana*	-	3.58	3.69	-	-	-	-	3.63
8	*C. dactylon*	3.28	3.11	2.98	3.20	2.97	2.96	2.97	3.06
9	*G. tubiflorum*	-	-	-	3.85	-	-	-	3.85
10	*I. globosa*	-	-	-	3.91	-	-	-	3.91
11	*L. rotundifolia*	3.14	3.28	-	-	-	-	-	3.21
12	*P. kaida*	-	3.17	3.30	3.43	3.28	3.40	-	3.31
13	*P. repens*	-	-	3.38	3.46	3.17	3.23	3.04	3.25
14	*P. hydropiper*	-	-	-	2.99	-	-	2.97	2.98
15	*S. trilobata*	3.85	3.81	3.78	3.92	3.58	3.52	3.49	3.71
Mean (Site wise)		3.33	3.35	3.31	3.52	3.24	3.21	3.16	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- ArattukadavuRB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

for two (*I. aquatica* and *N. indica*). Both Palappoor and Pallichalthodu recorded the highest fungal count for *P. stratiotes.*

Manamukku had the lowest fungal count for *I. aquatica* and *N. indica*, Valiyavilagam for *S. molesta* and Palappoor for *E. crassipes*. Rhizosphere sediment of *N. nouchali* (Palappoor and Mannamvarambu) and *P. stratiotes* (Arattukadavu RB and Manamukku) had the lowest fungal population at two sites each. The highest mean value of 3.33 for the fungal count in the rhizosphere region was noticed by *E. crassipes* and *I. aquatica* and the lowest value of 2.88 by *N. indica*.

The submerged aquatic macrophyte, *V. natans*, had the highest fungal count at Manamukku (3.23) and the lowest at Pallichalthodu (3.11).

Among the terrestrial macrophytes, *A. tenella* from Arattukadavu RB recorded the highest fungal count (4.08) in the rhizosphere soil and *C. dactylon* from Mannamvarambu, the lowest (2.96). On comparing the species wise variation, soil collected from the rhizosphere region of *I. globosa* showed the highest mean fungal population (3.91) and the lowest for *P. hydropiper* (2.98).

Six species had the highest fungal count at Arattukadavu RB (*A. aureum*, *A. tenella*, *P. kaida*, *P. repens*, *P. hydropiper* and *S. trilobata*), three at Palappoor (*A. sessilis*, *B. dactyoides* and *C. dactylon*), two at Reservoir bund (*B. mutica* and *C. lindleyana*) and one at Pallichalthodu (*L. rotundifolia*). *B. orientale* obtained the same value both at Palappoor and Pallichalthodu, which was the highest value recorded for that species.

Soil collected from the rhizosphere region of six species had the lowest fungal count at Manamukku (*A. tenella*, *B. dactyoides*, *B. mutica*, *P. repens*, *P. hydropiper* and *S. trilobata*), three at Pallichalthodu (*A. sessilis*, *C. lindleyana* and *P. kaida*), two at Mannamvarambu (*A. aureum* and *C. dactylon*) and one each at Palappoor (*L. rotundifolia*) and Valiyavilagam (*B. orientale*).

Comparing the site wise mean fungal count, the highest value was recorded at Arattukadavu RB (3.52) and the lowest at Manamukku (3.16).

4.2.3.5.3. Actinomycetes

Among the emergent aquatic macrophytes, sediment collected from the rhizosphere region of *E. colona* from Mannamvarambu recorded the highest value (4.43 log cfu g^{-1}) for acitnomycete population and *N. nucifera* from Arattukadavu RB (3.14), the lowest (Table 40). Excluding *E. colona* and *E. dulcis*, all other species had the highest actinomycete count at Manamukku. Mannamvarambu recorded the highest actinomycete count for sediment collected from the rhizosphere region of both *E. colona* and *E. dulcis*.

Four species (*C. esculenta*, *E. dulcis*, *L. flava* and *N. nucifera*) had the highest count at Arattukadavu RB, two at Pallichalthodu (*M. vaginalis* and *S. grossus*) and one at Manamukku (*E. colona*). Among the species, the highest mean count for actinomycetes in the rhizosphere region was noticed for *E. colona* and the lowest for *N. nucifera*.

On analyzing the data on actinomycete population of the sediments collected just beneath the floating macrophytes, the highest count was obtained for *P. stratiotes* (3.99) and the lowest for *N. nouchali* (3.11), both from Arattukadavu RB. Manamukku recorded the highest actinomycete count for four species (*E. crassipes*, *I. aquatica*, *N. indica* and *S. molesta*) and Arattukadavu RB (*P. stratiotes*) and Mannamvarambu (*N. nouchali*) for one each.

Three species had the lowest count at Arattukadavu RB (*E. crassipes*, *N. nouchali* and *S. molesta*), two at Palappoor (*I. aquatica* and *N. indica*) and Pallichalthodu for one (*P. stratiotes*). On comparing the species wise variation, the highest mean value for actinomycetes population was noticed by *P. stratiotes* (3.92) and the lowest by *N. nouchali* (3.38).

Table 40. Total actinomycetes in rhizosphere sediment / soil of selected macrophytes

Sl. No.	Macrophytes	I	II	III	IV	V	VI	VII	Mean (Species wise)
		Rhizosphere actinomycetes (log cfu g^{-1})							
A	Aquatic								
a	Emergent								
1	*C. esculenta*	3.98	3.95	3.94	3.86	4.08	3.98	4.28	4.01
2	*E. colona*	-	-	-	-	-	4.43	4.38	4.40
3	*E. dulcis*	3.54	3.49	3.52	3.25	3.70	3.73	3.72	3.56
4	*L. flava*	3.98	3.93	3.89	3.70	3.71	3.94	4.11	3.89
5	*M. vaginalis*	4.04	3.98	3.99	3.99	4.11	4.20	4.38	4.10
6	*N. nucifera*	3.53	-	3.55	3.14	3.53	3.58	3.62	3.49
7	*S. grossus*	3.96	3.94	3.98	-	-	4.11	4.17	4.03
b	Floating								
1	*E. crassipes*	3.55	3.43	3.47	3.20	3.62	3.57	3.67	3.50
2	*I. aquatica*	3.46	-	-	-	3.60	-	3.66	3.57
3	*N. nouchali*	3.46	3.40	-	3.11	-	3.57	-	3.38
4	*N. indica*	3.45	-	3.52	-	3.54	3.53	3.65	3.54
5	*P. stratiotes*	3.87	3.86	3.97	3.99	3.97	3.90	3.92	3.92
6	*S. molesta*	3.53	3.52	3.70	3.41	3.75	3.70	3.76	3.62
c	Submerged								
1	*V. natans*	-	3.28	-	-	-	-	3.41	3.34
B	Terrestrial								
1	*A. aureum*	-	-	-	3.96	4.08	4.11		4.05
2	*A. sessilis*	4.17	4.04						4.10
3	*A. tenella*	4.25	4.34	4.28	3.94	4.44	4.47	4.53	4.32
4	*B. orientale*	3.84	3.86	3.86	3.82	4.04	3.99	4.20	3.94
5	*B. mutica*	-	-	4.20	-	4.38	-	4.43	4.33
6	*B. dactyoides*	4.30	4.32	4.08	-	4.36	3.98	4.20	4.20
7	*C. lindleyana*	-	4.14	4.11	-	-	-	-	4.12
8	*C. dactylon*	4.23	4.28	4.32	4.11	4.36	4.40	4.45	4.30
9	*G. tubiflorum*	-	-	-	3.30	-	-	-	3.30
10	*I. globosa*	-	-	-	2.90	-	-	-	2.90
11	*L .rotundifolia*	3.88	3.87	-	-	-	-	-	3.87
12	*P. kaida*	-	4.38	4.36	4.36	4.55	4.44	-	4.41
13	*P. repens*	-	-	4.32	3.98	4.41	4.68	4.66	4.41
14	*P. hydropiper*	-	-	-	3.96	-	-	3.99	3.97
15	*S. trilobata*	4.17	4.30	4.04	3.99	4.30	4.22	4.28	4.18
Mean (Site wise)		3.85	3.91	3.95	3.68	4.03	4.03	4.07	

- absence of macrophyte in the site

I- Palappoor II - Pallichalthodu III – Reservoir bund IV- ArattukadavuRB
V - Valiyavilagam VI - Mannamvarambu VII - Manamukku

V. natans, the submerged aquatic macrophyte, recorded the highest actinomycete count for sediment collected from the rhizosphere region of Manamukku (3.41) and the lowest at Pallichalthodu (3.28).

Among the terrestrial macrophytes, *P. repens* from Mannamvarambu (4.68) showed the highest count for actinomycetes in the rhizosphere soil and *I. globosa* from Arattukadavu RB, the lowest (2.90). Five species had their highest count at Manamukku (*A. tenella*, *B. orientale*, *B. mutica*, *C. dactylon* and *P. hydropiper*) and two each at Palappoor (*A. sessilis* and *L. rotundifolia*), Valiyavilagam (*B. dactyoides* and *P. kaida*) and Mannamvarambu (*A. aureum* and *P. repens*). *C. lindleyana* recorded the highest count at Pallichalthodu while *S. trilobata* both at Pallichalthodu and Valiyavilagam.

Soil collected from the rhizosphere region of seven species (*A. aureum*, *A. tenella*, *B. orientale*, *C. dactylon*, *P. repens*, *P. hydropiper* and *S. trilobata*) had the highest value for actinomycete population at Arattukadavu RB, two each at Pallichalthodu (*A. sessilis* and *L. rotundifolia*) and Reservoir bund (*B. mutica* and *C. lindleyana*) and one at Mannamvarambu (*B. dactyoides*). *P. kaida* recorded the same value both at Reservoir bund and Arattukadavu RB, which was the lowest value, noticed for that species.

Comparing the sites, the highest mean value of 4.07 log cfu g^{-1} for rhizosphere actinomycete count was recorded at Manamukku and the lowest value of 3.68 log cfu g^{-1} at Arattukadavu RB.

4.2.4. Correlation studies

4.2.4.1. Rhizosphere metal content and their uptake by macrophytes

Correlation studies with Fe, Al, Pb and Cd contents of soil / sediment and their concentration in plant parts, and uptake were carried out and presented in Table 41. All the above metals in soil showed a positive correlation with metal accumulation both in the shoot and root of plants. But a significant correlation was obtained only for rhizosphere Cd content and Cd accumulation in the plant parts (shoot and root).

Significant correlations were obtained for plant uptake by roots as well as total uptake for all the elements excluding Fe. For shoot uptake, only Al showed a significant positive correlation with corresponding rhizosphere Al content at 5 % level.

Table 41. Correlation between rhizosphere metal content and plant uptake

Metals	Correlation coefficients				
	Concentration in plant		Uptake by plant		
	Shoot	Root	Shoot	Root	Total
Fe	0.016	0.076	0.275	0.242	0.269
Al	0.194	0.264	0.381**	0.417**	0.425**
Pb	0.257	0.347*	0.376*	0.449**	0.439**
Cd	0.506**	0.587**	0.358*	0.415**	0.398**

**significant at 5 % level *significant at 1 % level

4.2.4.2. Rhizosphere metal content and microbial count

Table 42 represents the correlation between rhizosphere Fe, Al, Pb and Cd with bacteria, fungi and actinomycete population. No significant correlations were obtained. Fungal count in the rhizosphere was found to be positively correlated with all the metals studied while bacteria showed a negative correlation.

Table 42. Correlation between rhizosphere metals and microbial population

Metals	Correlation coefficients		
	Microbial population		
	Bacteria	Fungi	Actinomycetes
Fe	-0.251	0.196	-0.293
Al	-0.443	0.338	-0.339
Pb	-0.032	0.224	0.031
Cd	-0.378	0.126	-0.375

The major source of contamination in the area was non-point type. Hence correlation between the metal concentrations in plant parts with the distance from the source of contamination could not be estimated and not presented in the session.

4.2.5. Bioremediation capacity of macrophytes for Fe, Al, Pb and Cd

Bioconcentration factors (BCF) for Fe, Al, Pb and Cd were calculated for all the collected macrophytes (shoot and root portions) to estimate their bioremediation capacity and presented in Table 43. In general, the roots showed higher BCF values than the shoots, especially for aquatic macrophytes.

Among the emergent aquatic macrophytes, BCF for Fe and Al were highest for *M. vaginalis* for both shoot and root. With respect to Pb, *N. nucifera* had the highest BCF for shoot and *C. esculenta* for root. In case of Cd, *C. esculenta* showed the highest BCF for shoot and *E. dulcis* for root. Shoots of *E. colona* showed more translocation of Al and Cd as evidenced by the BCF. *E. colona* recorded the lowest BCF for all the elements except Al in shoot. *E. dulcis* noticed the lowest BCF for shoot Al. For Fe, Al and Pb, the BCF for root were higher compared to that of shoot for all the selected species. While for Cd, two species viz., *C. esculenta and E.colona* showed higher BCF for shoot.

All the floating aquatic macrophytes showed higher BCF for roots compared to shoots. With respect to Fe, *N. nouchali* had the highest BCF for shoot and *E. crassipes*, for root. For Al, the highest BCF for both shoot and root was noticed for *N. nouchali*. In case of Pb, the highest BCF for root was noted for *N. indica and lowest for E. crassipes*. With regard to Cd the highest BCF for both shoot and root was for *E. crassipes*. The lowest BCF for all the elements, among floating macrophytes, was recorded by *I. aquatica*. *V. natans*, the only submerged aquatic macrophyte, roots showed higher BCF for all the elements compared to shoot.

Table 43. Bioconcentration factors of selected macrophytes for Fe, Al, Pb and Cd

Sl. No.	Macrophyte	Fe		Al		Pb		Cd	
		Bio concentration factor (BCF)							
		S	R	S	R	S	R	S	R
A	Aquatic								
a	Emergent								
1	*C. esculenta*	3.62	12.70	4.21	13.44	0.73	3.51	305.34	237.02
2	*E. colona*	2.73	3.47	4.10	2.11	0.21	0.42	2.00	1.14
3	*E. dulcis*	9.13	14.35	3.27	9.89	1.05	2.10	162.09	326.59
4	*L. flava*	9.43	18.80	6.41	22.31	1.07	3.36	149.76	321.70
5	*M. vaginalis*	14.97	20.60	7.31	27.15	0.40	0.80	134.81	266.26
6	*N. nucifera*	6.33	7.12	4.46	8.05	1.38	1.91	150.58	258.23
7	*S. grossus*	5.43	6.80	6.26	8.76	1.33	1.69	217.14	312.85
b	Floating								
1	*E.crassipes*	4.65	23.94	2.94	17.38	2.01	2.41	144.03	301.40
2	*I. aquatica*	3.99	4.72	1.39	2.66	0.36	0.53	1.00	1.25
3	*N. nouchali*	9.25	10.98	9.03	18.09	1.36	3.08	58.28	110.71
4	*N. indica*	4.83	11.33	2.34	13.05	1.48	3.57	141.54	287.69
5	*P. stratiotes*	5.52	17.78	2.63	3.15	1.18	2.75	136.38	254.14
6	*S. molesta*	6.32	7.27	6.22	13.40	1.00	2.71	17.40	23.60
c	Submerged								
1	*V.natans*	16.17	18.55	9.02	21.59	2.17	2.34	111.76	282.35
B	Terrestrial								
1	*A. aureum*	2.91	2.24	2.62	4.79	0.84	0.61	4.00	1.50
2	*A. sessilis*	2.84	5.79	3.51	4.57	0.26	0.48	10.80	19.60
3	*A. tenella*	9.33	12.14	4.40	9.56	0.65	1.69	99.22	252.55
4	*B. orientale*	3.16	4.49	2.88	5.17	1.05	2.48	3.00	6.66
5	*B. mutica*	4.12	4.94	5.22	3.24	0.64	0.56	3.50	4.75
6	*B. dactyoides*	4.62	19.63	5.88	13.28	0.98	2.52	165.70	250.60
7	*C. lindleyana*	1.24	3.08	1.16	5.09	0.82	0.50	5.00	2.00
8	*C. dactylon*	6.91	24.98	4.46	24.46	1.88	3.10	153.56	242.23
9	*G. tubiflorum*	4.47	3.92	4.39	12.48	1.03	1.23	0.88	0.55
10	*I. globosa*	2.44	2.71	5.36	3.47	1.61	1.41	0.33	0.66
11	*L. rotundifolia*	1.72	4.32	2.28	3.81	0.30	0.71	107.14	50.00
12	*P. kaida*	4.05	5.05	5.46	3.64	0.46	0.90	89.14	189.65
13	*P. repens*	4.41	7.56	9.58	17.67	0.79	0.94	11.50	5.00
14	*P. hydropiper*	12.30	13.29	1.99	9.87	0.73	0.71	112.22	140.55
15	*S. trilobata*	11.50	10.65	4.73	7.44	2.41	2.03	252.34	384.77

S -shoot R- root

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Among the terrestrial macrophytes, the highest BCF for Fe in shoot was recorded by *P. hydropiper*. In the case of Al, *P. repens* and for Pb, *S. trilobata* showed the highest BCF for shoot. In general, the roots were found to have more BCF than shoots except for a few macrophytes. Roots of *C. dactylon* showed the highest BCF for Fe, Al and Pb.

Bioremediation capacity of macrophytes was evaluated based on their BCF value. Macrophytes showing higher BCF values either in shoot or root were selected for further studies. Six species under emergent aquatic macrophytes (*C. esculenta, E.dulcis, L. flava, M. vaginalis, N. nucifera and S. grossus*); five species under floating aquatic macrophytes (*E. crassipes, N. nouchali, N. indica, P. stratiotes and S. molesta*) and six species under terrestrial macrophytes (*A. tenella, B. dactyoides, C. dactylon, P. kaida, P. repens* and *S. trilobata*) were identified as bioremediators.

4.2.6 Screening trial for selection of macrophytes under Part III

The above macrophytes identified as hyperaccumulators / bioremediators were subjected to a screening trial under graded doses of Fe (500, 750 and 1000 mg kg^{-1}), Al (250, 500 and 750 mg kg^{-1}), Pb (10, 20 and 30 mg kg^{-1}) and Cd (10, 20 and 30 mg kg^{-1}). Extraction of metals by these macrophytes was assessed. Metal accumulation by these macrophytes at highest level of each metal (1000 mg kg^{-1} for Fe; 750 mg kg^{-1} for Al; and 30 mg kg^{-1} for Pb and Cd) is presented in Table 44.

Among the emergent macrophytes, *L. flava* recorded the highest content for Fe, Pb and Cd. *M. vaginalis* noticed the highest accumulation for Al and the lowest for Pb. *S. grossus* obtained the lowest concentration for Fe and Cd. *N. nucifera* showed the lowest accumulation for Al.

E. crassipes recorded the highest concentration for Fe among the floating macrophytes while it was *N. indica*, for Al. *P. stratiotes* obtained the highest concentration for Pb and Cd and the lowest for Al. *S. molesta* showed the lowest values for Fe and Pb while *N. nouchali*, for Cd.

Table 44. Metal accumulation by macrophytes under screening trial

Sl. No.	Macrophyte	Fe @ 1000	Al @750	Pb @ 30	Cd @ 30
		Metal accumulation (mg kg^{-1})			
A	Aquatic				
a	Emergent				
1	*C. esculenta*	13850	10655	21.60	20.50
2	*E. dulcis*	15795	8710	13.78	18.65
3	*L. flava*	16255	13625	32.80	26.44
4	*M. vaginalis*	15826	14820	13.66	15.50
5	*N. nucifera*	7784	5958	18.50	23.16
6	*S. grossus*	6780	6850	20.73	15.72
b	Floating				
1	*E. crassipes*	16685	8524	20.25	21.58
2	*N. nouchali*	9155	8076	14.88	14.50
3	*N. indica*	11880	10350	20.40	17.80
4	*P. stratiotes*	10205	5774	28.80	28.75
5	*S. molesta*	7150	9923	14.14	14.88
B	Terrestrial				
1	*A. tenella*	11500	6735	22.15	20.77
2	*B. dactyoides*	12165	8970	17.50	14.15
3	*C. dactylon*	13550	11455	14.28	13.88
4	*P. kaida*	6750	5640	14.61	12.75
5	*P. repens*	6355	11160	12.77	10.87
6	*S. trilobata*	10274	6118	13.50	21.85

In the case of terrestrial macrophytes, *C. dactylon* recorded the highest for Fe and Al accumulation, *A. tenella* for Pb and *S. trilobata* for Cd. *P. repens* showed the lowest for Fe, Pb and Cd. *P. kaida* recorded the lowest value for Al. Much varation was noticed in the metal accumulation potential of these macophytes.

Based on their survival and accumulation of metals, the best performing macrophytes were selected for continuing the experiment under Part III and Table 45 represents the list of macrophytes that were selected.

Table 45. Macrophytes selected for experiments under Part III

Sl. No.	Fe	Al	Pb	Cd
1	*C. esculenta*	*C. esculenta*	*C. esculenta*	*C. esculenta*
2	*E. dulcis*	*E. dulcis*	*L. flava*	*E. dulcis*
3	*L. flava*	*L. flava*	*N. nucifera*	*L. flava*
4	*M. vaginalis*	*M. vaginalis*	*S. grossus*	*N. nucifera*
5	*E. crassipes*	*N. indica*	*E. crassipes*	*E. crassipes*
6	*N. indica*	*S. molesta*	*N. indica*	*N. indica*
7	*A. tenella*	*B. dactyoides*	*P. stratiotes*	*P. stratiotes*
8	*B. dactyoides*	*C. dactylon*	*A. tenella*	*A. tenella*
9	*C. dactylon*	*P. repens*	*B. dactyoides*	*S. trilobata*

These macrophytes were further grown under three graded doses of each metal (Fe, Al, Pb and Cd) for a period of 45 days to select the best phytoextractor / bioremediator for each metal.

Part III

4.3. Performance of selected hyperaccumulators under graded doses of toxic metals to identify their selective retention sites

Four separate pot culture experiments were carried out with selected macrophytes under graded doses of each of the metals viz., Fe, Al, Pb and Cd at three levels and the results are presented in Tables 46 to 61.

4.3.1. Performance of hyperaccumulators under graded doses of Iron (Fe)

Nine macrophytes viz., *A. tenella, B. dactyoides, C. esculenta, C. dactylon, E. crassipes, E. dulcis, L. flava, M. vaginalis* and *N. indica* were grown in pots under graded doses of Fe viz., 1000, 2000 and 3000 mg kg^{-1} for 45 days. Data on pH, EC and Fe content of water, sediment and macrophytes are presented in Tables 46 to 49.

4.3.1.1. Water

The macrophytes and levels of Fe had shown significant influence on pH, EC and Fe content of water at 45 DAT (days after transplanting) (Table 46). Both main and interaction effects were significant. Water pH was found to decrease with levels of Fe while EC and Fe content of water increased. Among the tested macrophytes, *E. crassipes* recorded the highest pH and lowest EC and Fe content under all levels of Fe while the lowest pH and highest EC was shown by *E. dulcis*. Fe content in water was highest for *C. dactylon.*

Comparison of interaction effects revealed that *E. crassipes* treated with 1000 mg Fe kg^{-1} showed the highest pH and lowest EC and Fe content. Highest EC (1.03) was noticed for *E. dulcis* at 3000 mg Fe kg^{-1} while *C. dactylon* with 3000 mg Fe kg^{-1} showed the highest Fe content. Interaction of *A. tenella* with 3000 mg Fe kg^{-1} recorded the lowest pH.

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Plate 8. Macrophytes grown under graded doses of metal

Table 46. pH, EC and Fe content of water under graded doses of Fe

Treatments Main effects	pH	EC (dS m^{-1})	Fe (mg L^{-1})
Graded doses of Fe			
Fe-1000 mg kg^{-1} (F_1)	4.20^{a}	0.62^{c}	3.10^{c}
Fe-2000 mg kg^{-1} (F_2)	3.52^{b}	0.78^{b}	3.86^{b}
Fe-3000 mg kg^{-1} (F_3)	3.09^{c}	0.90^{a}	4.16^{a}
CD (0.05)	0.045	0.013	0.031
Macrophytes			
A. tenella (M_1)	3.45^{e}	0.78^{d}	3.91^{c}
B. dactyoides (M_2)	3.50^{e}	0.82^{c}	3.88^{c}
C. esculenta (M_3)	3.64^{cd}	0.78^{d}	3.78^{d}
C. dactylon (M_4)	3.62^{d}	0.83^{c}	4.13^{a}
E. crassipes (M_5)	3.90^{a}	0.58^{f}	3.22^{h}
E. dulcis (M_6)	3.43^{e}	0.91^{a}	3.98^{b}
L. flava (M_7)	3.70^{bc}	0.67^{e}	3.37^{g}
M. vaginalis (M_8)	3.73^{b}	0.68^{e}	3.46^{f}
N. indica (M_9)	3.48^{e}	0.86^{b}	3.63^{e}
CD (0.05)	0.046	0.023	0.054
Interaction effects			
F_1xM_1	4.20^{a}	0.59^{k}	3.30^{l}
F_1xM_2	4.28^{a}	0.58^{k}	3.25^{l}
F_1xM_3	4.19^{a}	0.65^{j}	3.27^{l}
F_1xM_4	4.05^{b}	0.70^{i}	3.26^{l}
F_1xM_5	4.32^{a}	0.45^{m}	2.71^{o}
F_1xM_6	4.29^{a}	0.80^{gh}	3.22^{l}
F_1xM_7	4.22^{a}	0.47^{m}	2.82^{n}
F_1xM_8	4.29^{a}	0.52^{l}	2.91^{n}
F_1xM_9	3.98^{bc}	0.78^{h}	3.12^{m}
F_2xM_1	3.46^{ef}	0.82^{fg}	4.09^{f}
F_2xM_2	3.38^{fg}	0.85^{ef}	4.09^{f}
F_2xM_3	3.45^{ef}	0.85^{ef}	3.90^{gh}
F_2xM_4	3.60^{d}	0.77^{h}	4.21^{de}
F_2xM_5	3.86^{c}	0.59^{k}	3.28^{l}
F_2xM_6	3.25^{gh}	0.91^{cd}	4.27^{cd}
F_2xM_7	3.55^{de}	0.69^{ij}	3.49^{k}
F_2xM_8	3.65^{d}	0.67^{ij}	3.60^{j}
F_2xM_9	3.53^{de}	0.88^{de}	3.79^{i}
F_3xM_1	2.71^{k}	0.92^{c}	4.34^{c}
F_3xM_2	2.85^{ij}	1.01^{ab}	4.29^{cd}
F_3xM_3	3.28^{gh}	0.99^{b}	4.17^{ef}
F_3xM_4	3.21^{h}	0.88^{ef}	4.92^{a}
F_3xM_5	3.52^{de}	0.70^{i}	3.68^{j}
F_3xM_6	2.75^{jk}	1.03^{a}	4.44^{b}
F_3xM_7	3.33^{fgh}	0.83^{ef}	3.81^{i}
F_3xM_8	3.24^{gh}	0.83^{ef}	3.86^{hi}
F_3xM_9	2.93^{i}	0.94^{c}	3.97^{g}
CD (0.05)	0.138	0.040	0.095

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Table 47. pH, EC, OC and total Fe of sediment under graded doses of Fe

Treatments Main effects	pH	EC (dS m^{-1})	OC (g kg^{-1})	Fe (mg kg^{-1})
Graded doses of Fe				
Fe-1000 mg kg^{-1}(F_1)	4.96^{a}	0.44^{c}	28.4^{c}	12180^{c}
Fe-2000 mg kg^{-1}(F_2)	4.39^{b}	0.65^{b}	31.7^{b}	13115^{b}
Fe-3000 mg kg^{-1}(F_3)	4.07^{c}	0.75^{a}	32.4^{a}	14133^{a}
CD (0.05)	0.028	0.011	0.26	6.741
Macrophytes				
A. tenella (M_1)	4.44^{c}	0.67^{bc}	32.6^{a}	13174^{c}
B. dactyoides (M_2)	4.50^{b}	0.65^{cd}	31.1^{d}	13190^{b}
C. esculenta (M_3)	4.43^{c}	0.67^{b}	29.9^{e}	13191^{b}
C. dactylon (M_4)	4.33^{d}	0.61^{e}	30.9^{d}	13151^{d}
E. crassipes (M_5)	4.91^{a}	0.48^{h}	27.4^{f}	12945^{f}
E. dulcis (M_6)	4.26^{e}	0.74^{a}	30.8^{d}	13231^{a}
L. flava (M_7)	4.53^{b}	0.52^{g}	31.6^{c}	13118^{e}
M. vaginalis (M_8)	4.44^{c}	0.55^{f}	32.1^{b}	13110^{e}
N. indica (M_9)	4.40^{c}	0.65^{d}	31.3^{c}	13174^{c}
CD (0.05)	0.049	0.019	0.45	11.676
Interaction effects				
F_1xM_1	5.02^{b}	0.43^{mn}	31.1^{hij}	12143^{p}
F_1xM_2	5.08^{b}	0.40^{n}	28.9^{k}	12197^{o}
F_1xM_3	5.05^{b}	0.45^{lm}	26.4^{lm}	12199^{o}
F_1xM_4	4.71^{d}	0.49^{l}	26.0^{m}	12224^{n}
F_1xM_5	5.20^{a}	0.34^{o}	24.7^{n}	12114^{q}
F_1xM_6	4.66^{d}	0.53^{k}	27.0^{l}	12215no
F_1xM_7	5.17^{a}	0.36^{o}	30.4^{j}	12093^{r}
F_1xM_8	4.84^{c}	0.46^{lm}	31.0^{ij}	12199^{o}
F_1xM_9	4.86^{c}	0.53^{k}	30.6^{j}	12235^{n}
F_2xM_1	4.32^{fg}	0.74^{ef}	32.7^{c}	13155^{i}
F_2xM_2	4.38^{f}	0.74^{ef}	31.7^{fghi}	13159^{i}
F_2xM_3	4.26^{g}	0.71^{efg}	31.5^{ghi}	13141^{ij}
F_2xM_4	4.25^{g}	0.64^{ij}	33.0^{bc}	13121^{k}
F_2xM_5	5.02^{b}	0.47^{l}	28.4^{h}	12908^{m}
F_2xM_6	4.16^{h}	0.79^{d}	32.5^{cde}	13205^{h}
F_2xM_7	4.34^{fg}	0.56^{k}	32.0^{defg}	13121^{k}
F_2xM_8	4.37^{f}	0.54^{k}	32.4^{cdef}	13099^{l}
F_2xM_9	4.39^{f}	0.67^{gh}	31.4^{ghi}	13123^{jk}
F_3xM_1	3.98^{kl}	0.83^{bc}	34.1^{a}	14223^{b}
F_3xM_2	4.05^{ijk}	0.80^{cd}	32.7^{cd}	14215^{b}
F_3xM_3	3.98^{kl}	0.83^{bc}	31.7^{fghi}	14233^{b}
F_3xM_4	4.01^{jkl}	0.71^{efg}	33.6^{ab}	14107^{e}
F_3xM_5	4.49^{e}	0.62^{j}	29.2^{k}	13813^{g}
F_3xM_6	3.95^{l}	0.89^{a}	32.9^{bc}	14271^{a}
F_3xM_7	4.09^{hij}	0.64^{hij}	32.6^{cde}	14140^{d}
F_3xM_8	4.11^{hi}	0.65^{hij}	32.9^{bc}	14032^{f}
F_3xM_9	3.97^{kl}	0.73^{ef}	31.8^{efgh}	14164^{c}
CD (0.05)	0.086	0.033	0.770	20.223

4.3.1.2. Sediment

Main as well as interaction effects of macrophytes with levels of Fe had significant influence on pH, EC, OC and Fe content of sediment (Table 47). Among the macrophytes, *E. crassipes* recorded the highest pH (4.91) and lowest EC (0.48), OC (27.4) and Fe content (12945 mg kg^{-1}) and *E. dulcis,* the highest EC and Fe content and lowest pH. Highest OC content was noticed for *A. tenella* (32.6).

Comparison of interaction effects revealed that combination of *E. crassipes* and 1000 mg Fe kg^{-1} showed the highest pH and lowest EC and OC. Highest EC and Fe content were obtained for *E. dulcis* at 3000 mg Fe kg^{-1}. OC was highest for *A. tenella* treated with 3000 mg Fe kg^{-1} (34.1). A general increase in OC content was noticed with levels of Fe.

4.3.1.3. Rhizosphere microbial count

Table 48 represents the effect of graded doses of Fe, macrophytes and their interactions on bacterial, fungal and actinomycete count of sediments. Main effects showed significant variation on the microbial count while interaction effect was significant only for actinomycete population. In general, the fungal population increased with levels of Fe while bacterial and actinomycete population followed a reverse pattern.

Comparing the microbial count for different species, it was observed that the bacteria and actinomycete count was highest for *C. dactylon* and the fungal count for *E. dulcis*. Lowest count for bacteria, fungi and actinomycete was recorded by *N. indica*, *E. crassipes* and *C. esculenta*, respectively.

With regard to the interaction effects, *C. dactylon* at 1000 mg Fe kg^{-1} recorded the highest bacteria (6.78 log cfu g^{-1}) and actinomycete (4.81 log cfu g^{-1}) count and *A. tenella* at 2000 mg Fe kg^{-1}, the highest fungal count (3.46 log cfu g^{-1}). Fungal count was lowest for *E. crassipes* at 1000 mg Fe kg^{-1}, bacteria for *N. indica* at 3000 mg Fe kg^{-1}, and actinomycete for *M. vaginalis* at 3000 mg Fe kg^{-1}.

Table 48. Microbial count of sediment under graded doses of Fe

Treatments Main effects	Bacteria	Fungi	Actinomycetes
	(log cfu g^{-1})		
Graded doses of Fe			
Fe-1000 mg kg^{-1}(F_1)	6.16^{a}	3.00^{c}	4.14^{a}
Fe-2000 mg kg^{-1}(F_2)	5.71^{b}	3.22^{a}	3.80^{b}
Fe-3000 mg kg^{-1}(F_3)	5.24^{c}	3.08^{b}	3.46^{c}
CD (0.05)	0.075	0.069	0.012
Macrophytes			
A. tenella (M_1)	6.15^{a}	3.28^{ab}	3.96^{c}
B. dactyoides (M_2)	6.10^{a}	3.16^{b}	4.02^{b}
C. esculenta (M_3)	5.37^{e}	3.00^{cd}	3.49^{i}
C. dactylon (M_4)	6.19^{a}	3.06^{cd}	4.48^{a}
E. crassipes (M_5)	5.70^{b}	2.78^{e}	3.87^{d}
E. dulcis (M_6)	5.57^{cd}	3.32^{a}	3.67^{e}
L. flava (M_7)	5.68^{bc}	3.09^{cd}	3.62^{f}
M. vaginalis (M_8)	5.52^{d}	3.08^{cd}	3.51^{h}
N. indica (M_9)	5.05^{f}	3.15^{c}	3.59^{g}
CD (0.05)	0.13	0.119	0.021
Interaction effects			
F_1xM_1	6.48	3.20	4.45^{d}
F_1xM_2	6.43	3.14	4.70^{b}
F_1xM_3	5.71	2.92	3.80^{kl}
F_1xM_4	6.78	3.06	4.81^{a}
F_1xM_5	6.27	2.54	4.04^{f}
F_1xM_6	6.09	3.11	3.83^{jkl}
F_1xM_7	6.21	2.93	3.87^{hi}
F_1xM_8	6.02	2.99	3.92^{g}
F_1xM_9	5.43	3.13	3.84^{ij}
F_2xM_1	6.16	3.46	3.93^{g}
F_2xM_2	6.05	3.31	3.82^{jk}
F_2xM_3	5.35	3.17	3.42^{p}
F_2xM_4	6.11	3.14	4.53^{c}
F_2xM_5	5.72	2.88	3.88^{h}
F_2xM_6	5.58	3.39	3.77^{l}
F_2xM_7	5.70	3.23	3.70^{m}
F_2xM_8	5.58	3.20	3.41^{p}
F_2xM_9	5.09	3.26	3.69^{m}
F_3xM_1	5.79	3.17	3.48^{o}
F_3xM_2	5.80	3.04	3.53^{n}
F_3xM_3	5.03	2.91	3.23^{r}
F_3xM_4	5.67	2.99	4.09^{e}
F_3xM_5	5.11	2.93	3.67^{m}
F_3xM_6	5.02	3.45	3.40^{p}
F_3xM_7	5.14	3.09	3.29^{q}
F_3xM_8	4.99	3.07	3.20^{r}
F_3xM_9	4.62	3.07	3.23^{r}
CD (0.05)	NS	NS	0.036

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4.3.1.4. Macrophytes

The main effects of macrophytes and / graded doses of Fe and their interactions showed significant influence on shoot and root biomass, Fe concentration and total quantity of Fe extracted (Table 49). *E. crassipes* recorded the highest shoot biomass and *N. indica*, the root biomass. Shoot and root biomass was lowest for *E. dulcis*. Fe concentration in shoot and root was highest for *E. crassipes* and lowest in shoot for *E. dulcis* and root for *A. tenella*. *E. crassipes* had extracted highest quantity of Fe by both shoot and root. Fe extraction by shoot was lowest for *E. dulcis* and *B. dactyoides* (0.86 g) in root.

Comparison of interaction effects revealed that application of 3000 mg Fe kg^{-1} to *E. crassipes* resulted in the highest shoot and root Fe concentration and extraction and was found to be significantly superior to all others. *C. esculenta* applied with 1000 mg Fe kg^{-1} resulted in the highest shoot biomass. Highest root biomass was obtained for *N. indica* receiving 2000 mg Fe kg^{-1}. *E. dulcis* treated with 3000 mg Fe kg^{-1} had produced the lowest shoot and root biomass and lowest shoot Fe extraction. *N. indica* receiving 1000 mg Fe kg^{-1} showed the lowest shoot Fe concentration and *A. tenella* at 1000 mg Fe kg^{-1}, the root Fe concentration.

E. crassipes can be selected as the best bioremediator (rhizofilter) for Fe. *M. vaginalis* and *L. flava* (phytoextractors) showed better performance next to *E. crassipes*.

Table 49. Biomass and Fe content of macrophytes under graded doses of Fe

Treatments Main effects	Biomass (dry) ($g\ pot^{-1}$)		Fe concentration ($mg\ kg^{-1}$)		Fe extraction ($g\ pot^{-1}$)	
	Shoot	Root	Shoot	Root	Shoot	Root
Graded doses of Fe						
Fe-1000 mg kg^{-1}(F_1)	66.35^{a}	58.02^{b}	16015^{c}	18784^{c}	1.08^{c}	1.06^{b}
Fe-2000 mg kg^{-1}(F_2)	63.78^{b}	67.55^{a}	20265^{b}	22016^{b}	1.31^{a}	1.49^{a}
Fe-3000 mg kg^{-1}(F_3)	48.58^{c}	59.55^{b}	22089^{a}	23987^{a}	1.17^{b}	1.45^{a}
CD (0.05)	1.571	1.575	113.953	132.083	0.037	0.039
Macrophytes						
A. tenella (M_1)	51.33^{d}	70.26^{d}	21320^{d}	16144^{h}	1.09^{e}	1.12^{ef}
B. dactyoides (M_2)	64.37^{c}	42.86^{fg}	18441^{f}	20442^{e}	1.18^{d}	0.86^{h}
C. esculenta (M_3)	62.00^{c}	60.73^{e}	15872^{g}	18706^{g}	0.89^{f}	1.14e
C. dactylon (M_4)	62.80^{c}	45.33^{f}	22407^{c}	22009^{d}	1.44^{c}	1.00^{g}
E. crassipes (M_5)	84.27^{a}	78.46^{b}	25275^{a}	28978^{a}	2.15^{a}	2.34^{a}
E. dulcis (M_6)	43.93^{f}	41.40^{g}	13257^{i}	25979^{b}	0.57^{h}	1.06^{fg}
L. flava (M_7)	48.27e	73.93^{c}	23341^{b}	22363^{c}	1.10^{e}	1.66^{b}
M. vaginalis (M_8)	74.80^{b}	61.06^{e}	20771^{e}	20421^{e}	1.59^{b}	1.25^{d}
N. indica (M_9)	44.47^{f}	81.33^{a}	14425^{h}	19321^{f}	0.64^{g}	1.56^{c}
CD (0.05)	2.721	2.728	197.372	228.775	0.065	0.067
Interaction effects						
F_1xM_1	68.60^{i}	78.80^{de}	20750^{j}	13837^{r}	1.42^{h}	1.12^{ij}
F_1xM_2	67.60^{i}	52.60^{ij}	15350^{o}	17885^{n}	1.03^{ij}	0.94^{klm}
F_1xM_3	92.60^{a}	50.00^{j}	11855^{q}	17210^{o}	1.10^{i}	0.86^{mn}
F_1xM_4	52.40^{k}	40.60^{kl}	16880^{m}	20217^{l}	0.88^{kl}	0.82^{no}
F_1xM_5	73.80^{gh}	59.20^{gh}	19850^{k}	22576^{i}	1.46^{h}	1.33^{ef}
F_1xM_6	54.00^{k}	48.60^{j}	11827^{q}	23762^{f}	0.64^{n}	1.15^{ghi}
F_1xM_7	82.00^{de}	60.40^{g}	21700^{h}	20400^{kl}	1.78^{e}	1.23^{fgh}
F_1xM_8	62.00^{j}	52.20^{ij}	15708^{n}	18825^{m}	0.97^{jk}	0.98^{jk}
F_1xM_9	44.20^{lm}	79.80^{de}	10220^{r}	14350^{q}	0.45^{op}	1.14^{ghi}
F_2xM_1	53.20^{k}	76.60^{e}	21230^{i}	16475^{p}	1.13^{i}	1.26^{fg}
F_2xM_2	80.60^{ef}	38.80^{kl}	20125^{k}	19011^{m}	1.62^{fg}	0.74^{o}
F_2xM_3	68.40^{i}	76.00^{e}	16030^{n}	18877^{m}	1.09^{i}	1.43^{e}
F_2xM_4	65.80^{ij}	53.00^{ij}	23330^{f}	22665^{i}	1.53^{gh}	1.20^{ghi}
F_2xM_5	91.20^{ab}	86.00^{bc}	25180^{c}	29930^{b}	2.30^{b}	2.57^{b}
F_2xM_6	53.40^{k}	43.40^{k}	13815^{p}	26650^{d}	0.74^{mn}	1.15^{ghi}
F_2xM_7	35.00^{n}	80.00^{de}	24460^{d}	23510^{fg}	0.85^{l}	1.88^{c}
F_2xM_8	77.00^{fg}	60.60^{g}	22342^{g}	20380^{kl}	1.72^{ef}	1.23^{fghi}
F_2xM_9	49.40^{k}	93.60^{a}	15875^{n}	20650^{k}	0.78^{lm}	1.93^{c}
F_3xM_1	32.20^{no}	55.40^{hi}	21980^{h}	18120^{n}	0.71^{mn}	1.00^{jk}
F_3xM_2	44.60^{l}	37.20^{l}	19849^{k}	24430^{e}	0.88^{kl}	0.90^{klmn}
F_3xM_3	25.00^{p}	56.20^{ghi}	19732^{l}	20032^{l}	0.49^{o}	1.12^{hi}
F_3xM_4	70.20^{h}	42.40^{k}	27013^{b}	23145^{gh}	1.89^{d}	0.98^{jkl}
F_3xM_5	87.80^{bc}	90.20^{ab}	30795^{a}	34430^{a}	2.70^{a}	3.10^{a}
F_3xM_6	24.40^{p}	32.20^{m}	14130^{p}	27525^{c}	0.34^{p}	0.88^{lmn}
F_3xM_7	27.80^{op}	81.40^{cd}	23865^{e}	23180^{gh}	0.66^{n}	1.88^{c}
F_3xM_8	85.40^{cd}	70.40^{f}	24265^{d}	22060^{j}	2.07^{c}	1.55^{d}
F_3xM_9	39.80^{m}	70.60^{f}	17180^{m}	22965^{hi}	0.68^{mn}	1.62^{d}
CD (0.05)	4.714	4.726	341.859	396.250	0.112	0.117

4.3.2 Performance of hyperaccumulators under graded doses of Aluminium (Al)

Nine macrophytes viz., *N. indica, S. molesta, C. esculenta, E. dulcis, L. flava, M. vaginalis, B. dactyoides, P. repens* and *C. dactylon* were grown in pots under three graded doses of Al viz., 750, 1000 and 1250 mg kg^{-1}. Data on pH, EC and Al content of water, sediment and macrophytes are presented in Tables 50 to 53.

4.3.2.1. Water

pH, EC and Al content of water at 45 DAT (Table 50) were significantly influenced by the main as well as interaction effects of macrophytes and levels of Al. Among the macrophyes, highest pH (4.28) was recorded by *M. vaginalis* and lowest by *E. dulcis*. EC was highest for *S. molesta* and the lowest for both *M. vaginalis* and *P. repens* (0.65 dS m^{-1}). The Al content of water was highest for *S. molesta* and lowest for *M. vaginalis.*

On comparing the interaction effects, it was observed that interaction of *M. vaginalis* and Al @ 750 mg Al kg^{-1} resulted in the highest pH and lowest EC and Al content while S. *molesta* with 1250 mg Al kg^{-1} recorded the lowest pH, highest EC and Al content.

4.3.2.2. Sediment

pH, EC, OC and Al content of sediments were significantly influenced by the macrophytes, graded levels of Al and their interactions (Table 51). Highest pH and lowest EC and Al content were recorded by *M. vaginalis* while the lowest pH by *S. molesta*. *N. indica* recorded the highest OC and *C. dactylon*, the lowest. The highest sediment Al content was also noticed for *C. dactylon.*

On comparing the interaction effects, it was observed that *M. vaginalis* treated with 750 mg Al kg^{-1} produced the highest pH and lowest EC and Al content. *B. dactyoides* treated with 1250 mg Al kg^{-1} produced the lowest pH.

Table 50. pH, EC and Al content of water under graded doses of Al

Treatments Main effects	pH	EC (dS m^{-1})	Al (mg L^{-1})
Graded doses of Al			
Al- 750 mg kg^{-1} (A_1)	4.11^{a}	0.57^{c}	0.97^{c}
Al-1000 mg kg^{-1}(A_2)	3.66^{b}	0.71^{b}	2.49^{b}
Al-1250 mg kg^{-1}(A_3)	3.08^{c}	1.02^{a}	3.45^{a}
CD (0.05)	0.045	0.021	0.020
Macrophytes			
N. indica (M_1)	3.38^{de}	0.89^{b}	2.62^{c}
S. molesta (M_2)	3.30^{ef}	0.95^{a}	3.27^{a}
C. esculenta (M_3)	3.68^{c}	0.72^{d}	1.35^{h}
E. dulcis (M_4)	3.28^{f}	0.81^{c}	3.04^{b}
L. flava (M_5)	3.80^{b}	0.74^{d}	1.88^{g}
M. vaginalis (M_6)	4.28^{a}	0.65^{e}	1.31^{i}
B. dactyoides(M_7)	3.39^{d}	0.74^{d}	2.57^{d}
P. repens (M_8)	3.81^{b}	0.65^{e}	2.25^{f}
C. dactylon (M_9)	3.66^{c}	0.74^{d}	2.44^{e}
CD (0.05)	0.078	0.036	0.035
Interaction effects			
A_1xM_1	3.66^{i}	0.63^{klm}	1.84^{m}
A_1xM_2	3.85^{f}	0.60^{m}	1.73^{n}
A_1xM_3	4.15^{c}	0.54^{nop}	0.78^{r}
A_1xM_4	3.81^{fg}	0.60^{m}	1.63^{o}
A_1xM_5	4.08^{cd}	0.53^{nop}	0.55^{t}
A_1xM_6	5.09^{a}	0.49^{p}	0.28^{u}
A_1xM_7	4.03^{cde}	0.62^{lm}	0.67^{s}
A_1xM_8	4.35^{b}	0.52^{op}	0.62^{s}
A_1xM_9	3.99^{de}	0.57^{mno}	0.68^{s}
A_2xM_1	3.40^{ij}	0.77^{hi}	2.11^{k}
A_2xM_2	3.40^{ij}	0.80^{gh}	3.43^{f}
A_2xM_3	3.99^{de}	0.68^{jkl}	1.56^{p}
A_2xM_4	$3.32i^{jk}$	0.79^{hi}	3.10^{h}
A_2xM_5	3.94^{ef}	0.73^{ij}	1.94^{l}
A_2xM_6	4.30^{b}	0.61^{lm}	1.47^{q}
A_2xM_7	3.24^{k}	0.69^{jk}	3.27^{g}
A_2xM_8	3.68^{g}	0.59^{mn}	2.62^{j}
A_2xM_9	3.69^{g}	0.77^{hi}	2.87^{i}
A_3xM_1	3.091	1.28^{b}	3.90^{c}
A_3xM_2	2.67^{n}	1.43^{a}	4.65^{a}
A_3xM_3	2.90^{m}	0.94^{de}	1.72^{n}
A_3xM_4	2.70^{n}	1.03^{c}	4.40^{b}
A_3xM_5	3.37^{ijk}	0.96^{d}	3.16^{h}
A_3xM_6	3.45^{i}	0.87^{fg}	2.17^{k}
A_3xM_7	2.90^{m}	0.91^{def}	3.77^{d}
A_3xM_8	3.39^{ij}	0.85^{efg}	3.51^{e}
A_3xM_9	3.29^{jk}	0.88^{ef}	3.79^{d}
CD (0.05)	0.136	0.063	0.060

Table 51. pH, EC, OC and total Al of sediment under graded doses of Al

Treatments Main effects	pH	EC (dS m^{-1})	OC (g kg^{-1})	Al (mg kg^{-1})
Graded doses of Al				
Al- 750 mg kg^{-1} (A_1)	4.25^{a}	0.47^{c}	25.1^{c}	11848^{c}
Al-1000 mg kg^{-1}(A_2)	3.90^{b}	0.64^{b}	27.9^{b}	12062^{b}
Al-1250 mg kg^{-1}(A_3)	3.52^{c}	0.90^{a}	30.1^{a}	12354^{a}
CD (0.05)	0.036	0.004	0.11	6281
Macrophytes				
N. indica (M_1)	3.85^{c}	0.75^{b}	31.2^{a}	12046^{e}
S. molesta (M_2)	3.68^{e}	0.77^{a}	31.1^{a}	12136^{c}
C. esculenta (M_3)	4.08^{b}	0.60^{g}	24.9^{g}	11974^{f}
E. dulcis (M_4)	3.70^{de}	0.74^{b}	30.7^{b}	12185^{b}
L. flava (M_5)	3.88^{c}	0.66^{d}	27.4^{d}	12043^{e}
M. vaginalis (M_6)	4.15^{a}	0.55^{h}	25.4^{f}	11887^{g}
B. dactyoides(M_7)	3.76^{d}	0.69^{c}	27.8^{c}	12191^{b}
P. repens (M_8)	4.04^{b}	0.61^{f}	26.2^{e}	12103^{d}
C. dactylon (M_9)	3.88^{c}	0.63^{e}	24.5^{h}	12231^{a}
CD (0.05)	0.062	0.007	0.20	10.88
Interaction effects				
A_1xM_1	4.11^{fgh}	0.48^{o}	28.8^{g}	11831^{lm}
A_1xM_2	4.05^{ghi}	0.47^{op}	29.2^{f}	11831^{lm}
A_1xM_3	4.32^{b}	0.45^{q}	23.1^{q}	11781^{o}
A_1xM_4	4.16^{defg}	0.50^{n}	28.1^{i}	11915^{k}
A_1xM_5	4.30^{bc}	0.46^{p}	23.4^{q}	11805^{n}
A_1xM_6	4.56^{a}	0.41^{s}	22.2^{r}	11740^{p}
A_1xM_7	4.25^{bcd}	0.58^{k}	25.4^{n}	11929^{jk}
A_1xM_8	4.30^{bc}	0.42^{rs}	24.1^{p}	11818^{mn}
A_1xM_9	4.23^{bcd}	0.43^{r}	21.7^{s}	11985^{i}
A_2xM_1	3.84^{j}	0.66^{h}	30.5^{d}	11993^{i}
A_2xM_2	3.65^{lm}	0.73^{f}	31.0^{c}	12138^{g}
A_2xM_3	4.13^{fg}	0.56^{l}	24.6^{o}	11936^{j}
A_2xM_4	3.61^{m}	0.70^{g}	31.2^{c}	12183^{f}
A_2xM_5	3.95^{i}	0.64^{i}	28.7^{g}	11993^{i}
A_2xM_6	4.20^{cdef}	0.54^{m}	26.4^{l}	11841^{l}
A_2xM_7	3.75^{jkl}	0.65^{i}	28.2^{hi}	12197^{ef}
A_2xM_8	4.02^{hi}	0.62^{j}	26.0^{m}	12087^{h}
A_2xM_9	3.98^{i}	0.63^{j}	24.3^{op}	12194^{ef}
A_3xM_1	3.60^{m}	1.10^{a}	34.3^{a}	12313^{d}
A_3xM_2	3.35no	1.12^{a}	33.0^{b}	12436^{b}
A_3xM_3	3.80^{jk}	0.78^{e}	27.0^{k}	12205^{e}
A_3xM_4	3.33^{no}	1.02^{b}	32.9^{b}	12455^{b}
A_3xM_5	3.40^{n}	0.88^{c}	30.0^{e}	12330^{d}
A_3xM_6	3.69^{klm}	0.71^{g}	27.5^{j}	12080^{h}
A_3xM_7	3.27^{o}	0.83^{d}	29.9^{e}	12447^{b}
A_3xM_8	3.80^{j}	0.80^{e}	28.5^{gh}	12404^{c}
A_3xM_9	3.42^{n}	0.82^{d}	27.5^{j}	12512^{a}
CD (0.05)	0.108	0.013	0.34	18.845

EC was highest for the interaction between *S. molesta* and 1250 mg Al kg^{-1}. Highest OC and Al content was recorded by *N. indica* and *C. dactylon*, respectively under 1250 mg Al kg^{-1}. *C. dactylon* treated with 750 mg Al kg^{-1} produced the lowest organic carbon.

4.3.2.3. Rhizosphere microbial count

Macrophytes and graded doses of Al showed significant variation on bacteria, fungi and actinomycete population while the interactions had significant influence only on bacterial and actinomycete count (Table 52).

Among the macrophytes, *P. repens* recorded the highest bacterial count and *N. indica* the lowest. Highest fungal count was noticed in the rhizosphere sediment of *B. dactyoides* and lowest in *N. indica*. *C. dactylon* recorded the highest actinomycete population and *S. molesta*, the lowest.

Comparing the interaction effects it was noted that *P. repens* treated with 750 mg Al kg^{-1} showed the highest bacterial count and *N. indica* with 1250 mg Al kg^{-1}, the lowest. *B. dactyoides* at 1250 mg Al kg^{-1} interaction obtained highest fungal count and *M. vaginalis* at 750 mg Al kg^{-1}, the lowest. Highest count for actinomycete population was observed for *C. dactylon* treated with 750 mg kg^{-1} Al and *S. molesta* with 1250 mg kg^{-1} Al, the lowest.

4.3.2.4. Macrophytes

The main and interaction effects between macrophytes and graded doses of Al had significantly influenced plant biomass (dry) and Al content (Table 53). *M. vaginalis* recorded the highest shoot biomass and *N. indica*, the lowest while highest root biomass was obtained for *L. flava* and lowest for *S. molesta*. Al concentration in both shoot and root were highest for *M. vaginalis*. *E. dulcis* recorded the lowest shoot Al concentration while *B. dactyoides*, the lowest root Al concentration.

Table 52. Microbial count of sediment under graded doses of Al

Treatments	Bacteria	Fungi	Actinomycetes
Main effects	(log cfu g^{-1})		
Graded doses of Al			
Al- 750 mg kg^{-1} (A_1)	5.87^{a}	2.61^{c}	2.99^{a}
Al-1000 mg kg^{-1}(A_2)	5.70^{b}	2.79^{b}	2.70^{b}
Al-1250 mg kg^{-1}(A_3)	5.50^{c}	2.90^{a}	2.59^{c}
CD (0.05)	0.031	0.021	0.018
Macrophytes			
N. indica (M_1)	5.29^{g}	2.36^{h}	2.52^{g}
S. molesta (M_2)	5.30^{g}	2.45^{g}	2.49^{g}
C. esculenta (M_3)	5.49^{e}	2.65^{e}	2.69^{e}
E. dulcis (M_4)	5.41^{f}	3.06^{a}	2.64^{f}
L. flava (M_5)	5.53^{e}	2.85^{d}	2.73^{d}
M. vaginalis (M_6)	5.89^{d}	2.53^{f}	2.91^{c}
B. dactyoides(M_7)	6.03^{c}	3.09^{a}	2.94^{c}
P. repens (M_8)	6.19^{a}	2.91^{c}	3.02^{b}
C. dactylon (M_9)	6.11^{b}	2.98^{b}	3.11^{a}
CD (0.05)	0.054	0.037	0.032
Interaction effects			
A_1xM_1	5.48^{i}	2.24	2.69^{h}
A_1xM_2	5.48^{i}	2.28	2.68^{h}
A_1xM_3	5.67^{fg}	2.53	2.96^{d}
A_1xM_4	5.60^{gh}	2.91	2.83^{g}
A_1xM_5	5.72^{f}	2.70	2.93^{def}
A_1xM_6	5.98^{de}	2.20	3.05^{c}
A_1xM_7	6.17^{c}	2.86	3.07^{c}
A_1xM_8	6.45^{a}	2.74	3.31^{b}
A_1xM_9	6.30^{b}	2.77	3.39^{a}
A_2xM_1	5.34^{j}	2.36	2.55^{j}
A_2xM_2	5.32^{j}	2.49	2.52^{jk}
A_2xM_3	5.53^{hi}	2.67	2.68^{h}
A_2xM_4	5.46^{i}	3.06	2.62^{i}
A_2xM_5	5.59^{gh}	2.90	2.70^{h}
A_2xM_6	5.93^{e}	2.53	2.89^{ef}
A_2xM_7	5.97^{e}	3.13	2.94^{de}
A_2xM_8	6.14^{c}	2.95	2.97^{d}
A_2xM_9	6.04^{d}	3.03	3.04^{c}
A_3xM_1	5.05^{m}	2.48	2.31^{m}
A_3xM_2	5.09^{lm}	2.58	2.27^{m}
A_3xM_3	5.26^{jk}	2.76	2.43^{l}
A_3xM_4	5.17^{kl}	3.21	2.48^{kl}
A_3xM_5	5.29^{j}	2.96	2.55^{d}
A_3xM_6	5.75^{f}	2.63	2.80^{g}
A_3xM_7	5.94^{e}	3.28	2.82^{g}
A_3xM_8	5.96^{de}	3.04	2.78^{g}
A_3xM_9	5.99^{de}	3.14	2.89^{f}
CD (0.05)	0.095	NS	0.055

M. vaginalis recorded the highest quantity of Al extraction both by shoots and roots followed by *C. esculenta* and *N. indica*. The lowest Al extraction was noticed for *E. dulcis*, both by shoot and root.

Evaluating the interaction effects, it was observed that *M. vaginalis* treated with 1000 mg Al kg^{-1} produced the highest shoot biomass and lowest by *N. indica* treated with 1250 mg Al kg^{-1}. *L. flava* at1000 mg Al kg^{-1} produced the highest root biomass and lowest by *S. molesta* at 1250 mg Al kg^{-1} interaction. Application of 1250 mg Al kg^{-1} on *C. esculenta* resulted in the highest shoot Al concentration and *C. dactylon* at 750 mg Al kg^{-1}, lowest. Similarly for root, the highest concentration was noted for *M. vaginalis* and lowest for *E. dulcis*, both treated with 1250 mg Al kg^{-1}. The highest extraction of Al by shoot was noticed for *M. vaginalis* at 1250 mg Al kg^{-1} and *E. dulcis* at 1250 mg Al kg^{-1}, lowest. *M. vaginalis* and *S. molesta*, both treated with 1250 mg Al kg^{-1}, recorded the highest and lowest root Al extraction, respectively.

With all the three graded doses of Al (750, 1000 and 1250), *M. vaginalis* produced the highest Al concentration and extraction, both by shoots and roots except for shoot Al concentration at 1250 mg Al kg^{-1}. C. esculenta resulted in the highest shoot Al concentration when treated with 1250 mg Al kg^{-1}.

M. vaginalis can be selected as the best bioremediator for Al, which was followed by C. *esculenta* and *L. flava* in their performance.

Table 53. Biomass and Al content of macrophytes under graded doses of Al

Treatmets Main effects	Biomass (dry) ($g\ pot^{-1}$)		Al concentration ($mg\ kg^{-1}$)		Al extraction ($g\ pot^{-1}$)	
Graded doses of Al	Shoot	Root	Shoot	Root	Shoot	Root
Al- 750 $mg\ kg^{-1}$ (A_1)	76.31^{a}	56.31^{b}	12099^{c}	15562^{c}	0.93^{b}	0.90^{c}
Al-1000 $mg\ kg^{-1}$(A_2)	68.89^{b}	59.75^{a}	15127^{b}	18065^{b}	1.09^{a}	1.14^{a}
Al-1250 $mg\ kg^{-1}$(A_3)	48.84^{c}	45.11^{c}	17027^{a}	18820^{a}	0.89^{b}	0.94^{b}
CD (0.05)	2.592	1.683	7.898	4.974	0.043	0.031
Macrophytes						
N. indica (M_1)	36.33^{f}	69.13^{c}	18221^{b}	19361^{c}	0.64^{f}	1.33^{c}
S. molesta (M_2)	57.06^{cd}	19.93^{h}	15227^{e}	13676^{g}	0.83^{e}	0.27^{h}
C. esculenta (M_3)	87.33^{b}	66.00^{d}	18175^{c}	19818^{b}	1.41^{b}	1.32^{c}
E. dulcis (M_4)	41.73^{e}	31.67^{g}	9832^{i}	11161^{i}	0.40^{g}	0.35^{g}
L. flava (M_5)	60.00^{c}	93.93^{a}	16235^{d}	16917^{f}	0.97^{d}	1.58^{b}
M. vaginalis (M_6)	113.26^{a}	72.67^{b}	20319^{a}	27482^{a}	2.32^{a}	2.00^{a}
B. dactyoides(M_7)	54.00^{d}	46.87^{e}	11519^{g}	11999^{h}	0.62^{f}	0.56^{f}
P. repens (M_8)	86.53^{b}	44.33^{e}	13180^{f}	18973^{d}	1.01^{c}	0.81^{d}
C. dactylon (M_9)	45.86^{e}	39.00^{f}	10049^{h}	18163^{e}	0.46^{g}	0.71^{e}
CD (0.05)	4.490	2.915	23.694	14.923	0.075	0.054
Interaction effects						
A_1xM_1	36.00^{lm}	68.00^{d}	13365^{n}	15350^{q}	0.48^{nop}	1.04^{h}
A_1xM_2	90.00^{d}	36.00^{ij}	13068^{o}	12869^{u}	1.17^{f}	0.46^{mn}
A_1xM_3	120.00^{ab}	60.00^{f}	10110^{x}	15269^{r}	1.21^{ef}	0.92^{i}
A_1xM_4	49.80^{ghi}	39.80^{i}	8658^{y}	10865^{z}	0.43^{nop}	0.43^{mn}
A_1xM_5	60.00^{f}	89.00^{b}	17226^{g}	16031^{p}	1.03^{gh}	1.43^{f}
A_1xM_6	102.40^{c}	67.00^{d}	15882^{k}	24560^{c}	1.62^{c}	1.64^{d}
A_1xM_7	68.40^{e}	53.20^{g}	10874^{t}	11227^{y}	0.74^{jk}	0.60^{jkl}
A_1xM_8	116.00^{ab}	56.00^{fg}	11327^{s}	16115^{o}	1.31^{de}	0.90^{i}
A_1xM_9	44.20^{ijk}	37.80^{i}	8377^{z}	17770^{m}	0.37^{op}	0.67^{j}
A_2xM_1	49.00^{hijk}	80.00^{c}	18676^{e}	20560^{h}	0.91^{hi}	1.64^{d}
A_2xM_2	50.00^{ghij}	20.00^{k}	16272^{i}	14385^{s}	0.81^{ij}	0.29^{op}
A_2xM_3	88.00^{d}	72.00^{d}	18550^{f}	20835^{g}	1.63^{c}	1.50^{ef}
A_2xM_4	42.00^{kl}	32.00^{j}	10270^{v}	11769^{x}	0.43^{nop}	0.38^{no}
A_2xM_5	73.60^{e}	112.80^{a}	15462^{l}	16691^{n}	1.14^{fg}	1.88^{c}
A_2xM_6	123.40^{a}	81.00^{c}	20431^{d}	28165^{b}	2.52^{b}	2.28^{a}
A_2xM_7	42.40^{jkl}	47.60^{h}	11583^{q}	12220^{w}	0.49^{mn}	0.58^{jkl}
A_2xM_8	94.20^{d}	45.00^{h}	13342^{n}	19109^{i}	1.25^{ef}	0.86^{i}
A_2xM_9	57.40^{fg}	47.40^{h}	11559^{r}	18849^{j}	0.66^{kl}	0.89^{i}
A_3xM_1	24.00^{n}	59.40^{f}	22623^{c}	22174^{e}	0.54^{lmn}	1.32^{g}
A_3xM_2	31.20^{mn}	3.80^{l}	16343^{h}	13774^{t}	0.51^{mn}	0.05^{q}
A_3xM_3	54.00^{fgh}	66.00^{e}	25865^{a}	23350^{d}	1.39^{d}	1.54^{e}
A_3xM_4	33.40^{m}	23.20^{k}	10567^{u}	10849^{aa}	0.35^{op}	0.25^{p}
A_3xM_5	46.40^{hijk}	80.00^{c}	16019^{j}	18029^{k}	0.74^{jk}	1.44^{f}
A_3xM_6	114.00^{b}	70.00^{e}	24644^{b}	29721^{a}	2.81^{a}	2.08^{b}
A_3xM_7	33.00^{m}	39.80^{i}	12102^{p}	12550^{v}	0.40^{mn}	0.50^{lm}
A_3xM_8	49.40^{hijk}	32.00^{j}	14871^{m}	21064^{f}	0.73^{jk}	0.67^{j}
A_3xM_9	36.00^{lm}	31.80^{j}	10212^{w}	17870^{l}	0.37^{op}	0.57^{kl}
CD (0.05)	7.777	5.049	23.694	14.923	0.131	0.093

4.3.3. Performance of hyperaccumulators under graded doses of lead (Pb)

Under graded doses of Pb (50, 75 and 100 mg kg^{-1}), nine macrophytes viz., *N. nucifera, N.indica, P. stratiotes, E. crassipes, L. flava, S. grossus, C. esculenta, , B. dactyloides* and *A. tenella* were grown in pots for 45 days. Data on pH, EC and Pb content of water, sediment and macrophytes are presented in Tables 54 to 57.

4.3.3.1. Water

pH, EC and Pb content of water at 45 DAT (Table 54) were significantly influenced by the main as well as interaction effects of macrophytes and levels of Pb. With regard to the main effect of different levels of Pb on water properties, 50 mg Pb kg^{-1} recorded the highest pH and lowest EC and Pb content while 100 mg Pb kg^{-1} showed lowest pH and highest EC and Pb content. In general pH decreased with levels of Pb while EC and Pb content of water showed a reverse trend.

Comparing the main effect of macrophytes on water quality, *L. flava* recorded the highest pH and lowest EC. pH was lowest for *C. esculenta*. Pb content was highest for *E. crassipes* and lowest for *A. tenella. C. esculenta, E. crassipes* and *P. stratiotes* recorded the highest EC.

Among the interaction effects, *L. flava* treated with 50 mg Pb kg^{-1} recorded the highest water pH and lowest Pb content. *C. esculenta* at 100 mg Pb kg^{-1} noticed the lowest pH. Application of 100 mg Pb kg^{-1} to *P. stratiotes* resulted in the highest EC and *E. crassipes*, the highest Pb content. EC was lowest for *A. tenella* and *L. flava* receiving 50 mg Pb kg^{-1}.

Table 54. pH, EC and Pb content of water under graded doses of Pb

Treatments Main effects	pH	EC (dS m^{-1})	Pb (mg L^{-1})
Graded doses of Pb			
Pb-50 mg kg^{-1} (P_1)	4.62^{a}	0.32^{c}	0.043^{c}
Pb-75 mg kg^{-1} (P_2)	4.25^{b}	0.36^{b}	0.087^{b}
Pb-100 mg kg^{-1} (P_3)	3.85^{c}	0.41^{a}	0.151^{a}
CD (0.05)	0.039	0.003	0.003
Macrophytes			
N. nucifera (M_1)	4.32^{c}	0.38^{c}	0.121^{b}
N. indica (M_2)	4.37^{c}	0.39^{b}	0.092^{d}
P. stratiotes (M_3)	4.04^{d}	0.43^{a}	0.092^{d}
E. crassipes (M_4)	3.88^{e}	0.43^{a}	0.130^{a}
L. flava (M_5)	4.79^{a}	0.27^{f}	0.051^{h}
S. grossus (M_6)	4.10^{d}	0.38^{bc}	0.086^{e}
C. esculenta (M_7)	3.79^{f}	0.43^{a}	0.102^{c}
B. dactyoides (M_8)	4.36^{c}	0.32^{d}	0.096^{d}
A. tenella (M_9)	4.50^{b}	0.28^{e}	0.072^{f}
CD (0.05)	0.067	0.005	0.005
Interaction effects			
P_1xM_1	4.70^{d}	0.34^{h}	0.062^{jk}
P_1xM_2	4.80^{cd}	0.34^{h}	0.054^{kl}
P_1xM_3	4.31^{fg}	0.38^{fg}	0.045^{lm}
P_1xM_4	4.25^{fgh}	0.39^{ef}	0.049^{lm}
P_1xM_5	5.14^{a}	0.23^{m}	0.019^{p}
P_1xM_6	4.36^{efg}	0.34^{h}	0.043^{mn}
P_1xM_7	4.19^{h}	0.39^{e}	0.048^{lm}
P_1xM_8	4.83^{c}	0.25^{l}	0.038^{no}
P_1xM_9	5.01^{b}	0.23^{m}	0.032^{o}
P_2xM_1	4.27^{fgh}	0.37^{g}	0.115^{ef}
P_2xM_2	4.37^{e}	0.39^{e}	0.086^{h}
P_2xM_3	4.05^{i}	0.41^{d}	0.081^{hi}
P_2xM_4	3.95^{ijk}	0.42^{c}	0.105^{g}
P_2xM_5	4.87^{c}	0.27^{k}	0.032^{o}
P_2xM_6	4.18^{h}	0.39^{ef}	0.076^{i}
P_2xM_7	3.85^{kl}	0.43^{c}	0.108^{fg}
P_2xM_8	4.25^{fgh}	0.31^{i}	0.114^{ef}
P_2xM_9	4.45^{e}	0.28^{j}	0.064^{j}
P_3xM_1	4.00^{ij}	0.41^{d}	0.186^{b}
P_3xM_2	3.93^{jk}	0.42^{c}	0.136^{d}
P_3xM_3	3.75^{l}	0.48^{a}	0.147^{c}
P_3xM_4	3.43^{m}	0.46^{b}	0.235^{a}
P_3xM_5	4.36^{efg}	0.32^{i}	0.102^{g}
P_3xM_6	3.76^{l}	0.41^{d}	0.138^{d}
P_3xM_7	3.34^{m}	0.47^{b}	0.151^{c}
P_3xM_8	3.99^{ij}	0.37^{g}	0.137^{d}
P_3xM_9	4.04^{ij}	0.32^{i}	0.120^{e}
CD (0.05)	0.117	0.009	0.0089

Table 55. pH, EC, OC and total Pb of sediment under graded doses of Pb

Treatments Main effects	pH	EC (dS m^{-1})	OC (g kg^{-1})	Pb (mg kg^{-1})
Graded doses of Pb				
Pb-50 mg kg^{-1} (P_1)	5.10^{a}	0.252^{c}	28.8^{c}	62.91^{c}
Pb-75 mg kg^{-1} (P_2)	4.96^{b}	0.266^{b}	31.3^{b}	87.53^{b}
Pb-100 mg kg^{-1} (P_3)	4.81^{c}	0.286^{a}	31.7^{a}	112.50^{c}
CD (0.05)	0.026	0.0026	0.56	0.012
Macrophytes				
N. nucifera (M_1)	5.07^{c}	0.261^{e}	29.9^{d}	88.22^{a}
N. indica (M_2)	5.14^{b}	0.249^{f}	29.5^{e}	87.83^{c}
P. stratiotes (M_3)	4.64^{f}	0.295^{b}	28.8^{g}	87.65^{c}
E. crassipes (M_4)	4.71^{e}	0.310^{a}	29.3^{f}	87.69^{d}
L. flava (M_5)	5.35^{a}	0.227^{g}	29.3^{f}	87.06^{i}
S. grossus (M_6)	4.88^{d}	0.257^{e}	30.8^{c}	87.51^{g}
C. esculenta (M_7)	4.68^{ef}	0.278^{c}	32.9^{a}	87.61^{f}
B. dactyoides (M_8)	5.04^{c}	0.269^{d}	32.0^{b}	87.96^{b}
A. tenella (M_9)	5.13^{b}	0.270^{d}	32.8^{a}	87.29^{h}
CD (0.05)	0.046	0.0046	0.09	0.066
Interaction effects				
P_1xM_1	5.22^{cd}	0.241^{jkl}	28.1^{m}	63.19^{q}
P_1xM_2	5.26^{c}	0.239^{kl}	27.1^{o}	63.08^{s}
P_1xM_3	4.78^{lm}	0.286^{def}	26.3^{p}	62.82^{v}
P_1xM_4	4.80^{lm}	0.280^{f}	27.0^{o}	63.03^{t}
P_1xM_5	5.53^{a}	0.210^{n}	27.5^{n}	62.47^{x}
P_1xM_6	5.11^{f}	0.259^{h}	28.7^{l}	62.78^{w}
P_1xM_7	4.85^{kl}	0.269^{g}	32.7^{c}	62.92^{u}
P_1xM_8	5.15^{d}	0.251^{i}	30.7^{h}	63.13^{r}
P_1xM_9	5.25^{c}	0.233^{l}	31.2^{f}	62.78^{w}
P_2xM_1	5.02^{gh}	0.260^{h}	30.6^{h}	88.20^{i}
P_2xM_2	5.16^{d}	0.241^{jkl}	30.1^{i}	87.75^{k}
P_2xM_3	4.64^{op}	0.290^{cd}	29.8^{j}	87.37^{n}
P_2xM_4	4.74^{mn}	0.309^{b}	30.2^{i}	87.60^{l}
P_2xM_5	5.35^{b}	0.224^{m}	29.3^{k}	86.96^{p}
P_2xM_6	4.91^{jk}	0.245^{ijk}	31.8^{e}	87.38^{n}
P_2xM_7	4.70^{no}	0.282^{ef}	33.5^{b}	87.51^{m}
P_2xM_8	5.04^{fg}	0.268^{g}	32.5^{d}	87.91^{j}
P_2xM_9	5.11^{f}	0.280^{f}	33.8^{a}	87.06^{o}
P_3xM_1	4.95^{hij}	0.282^{ef}	31.1^{f}	113.27^{a}
P_3xM_2	5.01^{ghi}	0.267^{gh}	31.2^{f}	112.66^{d}
P_3xM_3	4.50^{q}	0.308^{b}	30.1^{i}	112.74^{c}
P_3xM_4	4.59^{p}	0.339^{a}	30.8^{g}	112.45^{e}
P_3xM_5	5.16^{e}	0.248^{ij}	31.2^{f}	111.74^{h}
P_3xM_6	4.62^{op}	0.267^{gh}	32.0^{e}	112.36^{f}
P_3xM_7	4.49^{q}	0.283^{def}	32.6^{cd}	112.39^{f}
P_3xM_8	4.93^{ij}	0.289^{cde}	32.8^{c}	112.82^{b}
P_3xM_9	5.03^{g}	0.295^{c}	33.5^{b}	112.02^{b}
CD (0.05)	0.079	0.008	0.17	0.115

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4.3.3.2. Sediment

pH, EC, OC and sediment Pb content were found to be significantly influenced by the main effects and their interactions (Table 55). As in the case of water, pH was found to decrease with levels of Pb while EC and Pb content of sediment also increased with levels of Pb. Highest pH was recorded by *L. flava* under all the treatment doses and the lowest by *C. esculenta*. Lowest EC and Pb content were also noticed for *L. flava*. *E. crassipes* and *N. nucifera* recorded the highest EC and sediment Pb content, respectively. OC was highest for *A. tenella* and lowest for *P. stratiotes*.

With regard to the interaction between treatments, *L. flava* treated with 50 mg $kg^{-1}$of Pb had resulted in the highest pH and lowest EC and Pb content. *E. crassipes* treated with 100 mg Pb kg^{-1} produced the highest EC while *N. nucifera* with 100 mg Pb kg^{-1} obtained the highest sediment Pb content. Application of 75 mg Pb kg^{-1} to *A. tenella* produced the highest OC and to *P. stratiotes*, the lowest.

4.3.3.3. Rhizosphere microbial count

Population of bacteria and actinomycetes found to decrease with levels of Pb while the fungal count increased with it (Table 56). Among the macrophytes, *L. flava* recorded the highest count for bacteria, *C. esculenta* for fungi and *B. dactyoides* for actinomycetes. The lowest count was showed by *E. crassipes* for bacteria; *A. tenella* for fungi and *E. crassipes* for actinomycetes.

On evaluating the interaction effects, *A. tenella* at 50 mg Pb kg^{-1} resulted in the highest bacterial count and *P. stratiotes* and *E. crassipes* with 100 mg Pb kg^{-1} the lowest. Fungal count was highest for *C. esculenta* treated at 100 mg Pb kg^{-1} and lowest for *A. tenella* with 50 mg Pb kg^{-1}. Highest actinomycete population was recorded by *B. dactyoides* at 50 mg Pb kg^{-1} and the lowest for *C. esculenta* at 100 mg Pb kg^{-1}.

Table 56. Microbial count of sediment under graded doses of Pb

Treatments Main effects	Bacteria	Fungi	Actinomycetes
	(log cfu g^{-1})		
Graded doses of Pb			
Pb-50 mg kg^{-1} (P_1)	7.50^{a}	3.40^{c}	3.78^{a}
Pb-75 mg kg^{-1} (P_2)	7.09^{b}	3.46^{b}	3.71^{b}
Pb-100 mg kg^{-1} (P_3)	6.96^{c}	3.53^{a}	3.49^{c}
CD (0.05)	0.011	0.008	0.011
Macrophytes			
N. nucifera (M_1)	7.14^{c}	3.20^{fg}	3.63^{d}
N. indica (M_2)	7.12^{f}	3.21^{f}	3.58^{f}
P. stratiotes (M_3)	7.00^{h}	3.65^{c}	3.56^{g}
E. crassipes (M_4)	6.99^{h}	3.67^{b}	3.54^{h}
L. flava (M_5)	7.44^{a}	3.44^{d}	3.78^{c}
S. grossus (M_6)	7.06^{g}	3.67^{b}	3.60^{e}
C. esculenta (M_7)	7.23^{d}	3.75^{a}	3.48^{i}
B. dactyoides (M_8)	7.31^{c}	3.38^{e}	3.91^{a}
A. tenella (M_9)	7.36^{b}	3.19^{g}	3.86^{b}
CD (0.05)	0.018	0.015	0.019
Interaction effects			
P_1xM_1	7.44^{d}	3.13^{mn}	3.76^{f}
P_1xM_2	7.44^{d}	3.11^{op}	3.75^{f}
P_1xM_3	7.25^{efg}	3.63^{f}	3.68^{ghi}
P_1xM_4	7.24^{fg}	3.59^{g}	3.66^{hij}
P_1xM_5	7.65^{b}	3.32^{k}	3.85^{d}
P_1xM_6	7.27^{ef}	3.64^{e}	3.71^{g}
P_1xM_7	7.59^{c}	3.74^{b}	3.64^{ij}
P_1xM_8	7.81^{a}	3.30^{kl}	4.01^{a}
P_1xM_9	7.82^{a}	3.10^{p}	3.98^{ab}
P_2xM_1	7.03^{j}	3.17^{mn}	3.63^{jk}
P_2xM_2	6.99^{kl}	3.20^{m}	3.64^{jk}
P_2xM_3	6.93^{no}	3.64^{ef}	3.64jk
P_2xM_4	6.92^{o}	3.67^{d}	3.61^{k}
P_2xM_5	7.44^{d}	3.47^{i}	3.81^{e}
P_2xM_6	6.98^{kl}	3.66^{e}	3.66^{hij}
P_2xM_7	7.09^{i}	3.74^{b}	3.56^{l}
P_2xM_8	7.17^{h}	3.36^{i}	3.97^{b}
P_2xM_9	7.28^{e}	3.17^{n}	3.92^{c}
P_3xM_1	6.96^{lmn}	3.28^{l}	3.49^{m}
P_3xM_2	6.92^{o}	3.30^{kl}	3.36^{o}
P_3xM_3	6.82^{p}	3.66^{de}	3.38^{o}
P_3xM_4	6.82^{p}	3.73^{b}	3.36^{o}
P_3xM_5	7.23^{g}	3.53^{h}	3.66^{hij}
P_3xM_6	6.93^{no}	3.70^{c}	3.45^{n}
P_3xM_7	7.01^{jk}	3.78^{a}	3.25^{p}
P_3xM_8	6.94^{mno}	3.46^{i}	3.78^{f}
P_3xM_9	6.97^{lmn}	3.30^{kl}	3.68^{gh}
CD (0.05)	0.032	0.026	0.032

4.3.3.4. Macrophytes

Table 57 represents the shoot and root biomass, Pb concentration in plant parts and total quantity of Pb extracted. The main as well as the interaction effects of treatments were significant. Both shoot and root biomass were found to increase up to 75 mg Pb kg^{-1} and then decreased. Comparing the macrophytes, *A. tenella* produced the highest shoot biomass and *L. flava*, highest root biomass. Among the interaction effects, *A. tenella* treated with 75 mg Pb kg^{-1} produced the highest shoot biomass and *N. nucifera* at 100 mg Pb kg^{-1}, the lowest. Root biomass was highest for *L. flava* at 75 mg Pb kg^{-1} and the lowest for *N. nucifera* at 100 mg Pb kg^{-1}.

Pb concentration in both shoot and root increased with levels of Pb. Among the macrophytes, both shoot and root concentration was highest for *L. flava* and lowest for *N. nucifera*. With regard to the interaction, application of 100 mg Pb kg^{-1} to *P. stratiotes* resulted the highest shoot Pb concentration while *L. flava* at 100 mg Pb kg^{-1}, the highest root Pb concentration. *E. crassipes* at 50 mg Pb kg^{-1} resulted in the lowest shoot Pb concentration and *N. nucifera* at 50 mg Pb kg^{-1}, lowest root concentration.

P. stratiotes recorded the highest quantity of Pb extraction by shoots and *L. flava*, by roots. *N. nucifera* noticed the lowest value for both shoots and roots. Evaluating the interaction effects, application of 75 mg Pb kg^{-1} on *P. stratiotes* obtained the highest extraction of Pb by shoots and *L. flava* with 100 mg Pb kg^{-1}, root Pb extraction. *N. nucifera* X 100 mg Pb kg^{-1} recorded the lowest Pb extraction by both shoots and roots.

L. flava can be selected as the best bioremediator and is followed by *A. tenella* and *S. grossus* based on the phytoextraction of Pb.

Table 57. Biomass and Pb content of macrophytes under graded doses of Pb

Treatments Main effects	Biomass (dry) (g pot^{-1})		Pb concentration (mg kg^{-1})		Pb extraction (mg pot^{-1})	
Graded doses of Pb	Shoot	Root	Shoot	Root	Shoot	Root
Pb-50 mg kg^{-1} (P_1)	66.80^{b}	54.51^{c}	45.50^{c}	60.20^{c}	3.06^{b}	3.31^{c}
Pb-75 mg kg^{-1} (P_2)	69.02^{a}	63.53^{a}	67.83^{b}	77.12^{b}	4.77^{a}	5.01^{b}
Pb-100 mg kg^{-1} (P_3)	60.20^{c}	57.00^{b}	80.97^{a}	87.70^{a}	4.80^{a}	5.15^{a}
CD (0.05)	1.443	1.217	0.482	0.663	0.087	0.101
Macrophytes						
N. nucifera (M_1)	34.93^{h}	37.06^{g}	39.72^{h}	46.74^{h}	1.36^{f}	1.70^{g}
N. indica (M_2)	43.66^{g}	53.73^{d}	73.12^{d}	63.62^{f}	3.17^{d}	3.44^{e}
P. stratiotes (M_3)	67.00^{e}	30.86^{h}	89.00^{a}	91.43^{b}	5.73^{a}	2.81^{f}
E. crassipes (M_4)	65.13^{e}	48.60^{f}	69.94^{e}	79.64^{d}	4.45^{c}	3.97^{d}
L. flava (M_5)	70.13^{d}	89.80^{a}	83.61^{b}	101.50^{a}	5.83^{a}	9.17^{a}
S. grossus (M_6)	84.40^{b}	80.20^{b}	56.72^{g}	70.00^{e}	4.81^{b}	5.65^{c}
C. esculenta (M_7)	59.46^{f}	51.46^{e}	74.22^{c}	80.21^{d}	4.38^{c}	4.11^{d}
B. dactyoides (M_8)	73.80^{c}	65.80^{c}	30.87^{i}	54.37^{g}	2.33^{e}	3.59^{e}
A. tenella (M_9)	89.50^{a}	67.60^{c}	65.70^{f}	87.54^{c}	5.82^{a}	5.97^{b}
CD (0.05)	2.499	2.108	0.835	1.148	0.151	0.175
Interaction effects						
P_1xM_1	44.20^{mn}	45.20^{jk}	35.71^{p}	42.30^{o}	1.58^{o}	1.91^{m}
P_1xM_2	42.00^{n}	50.00^{i}	48.75^{l}	50.35^{m}	2.05^{n}	2.52^{kl}
P_1xM_3	78.20^{def}	30.00^{n}	60.60^{j}	75.33^{h}	4.74^{g}	2.56^{l}
P_1xM_4	71.00^{ghi}	39.60^{l}	40.51^{o}	60.82^{k}	2.87^{l}	2.41^{l}
P_1xM_5	70.40^{h}	82.80^{c}	62.91^{i}	80.51^{g}	4.42^{h}	6.66^{c}
P_1xM_6	80.00^{de}	72.41^{d}	45.48^{m}	56.69^{l}	3.64^{ij}	4.11^{g}
P_1xM_7	59.20^{d}	50.40^{i}	54.36^{k}	60.65^{k}	3.21^{k}	3.05^{j}
P_1xM_8	64.80^{c}	60.00^{g}	20.65^{r}	47.50^{n}	1.34^{op}	2.85^{j}
P_1xM_9	91.40^{b}	60.20^{g}	40.55^{o}	67.61^{j}	3.70^{ij}	4.07^{g}
P_2xM_1	31.20^{o}	41.60^{kl}	40.77^{o}	46.69^{n}	1.27^{p}	1.94^{m}
P_2xM_2	49.00^{l}	55.20^{h}	73.46^{g}	67.55^{j}	3.59^{j}	3.73^{h}
P_2xM_3	75.40^{f}	35.80^{m}	95.78^{c}	94.15^{d}	7.22^{a}	3.37^{i}
P_2xM_4	65.00^{i}	50.40^{i}	75.80^{f}	80.43^{g}	4.93^{g}	4.05^{g}
P_2xM_5	75.20^{fg}	97.60^{a}	85.36^{e}	105.52^{b}	6.42^{c}	10.30^{a}
P_2xM_6	87.60^{bc}	88.20^{b}	60.95^{j}	72.64^{i}	5.34^{de}	6.40^{c}
P_2xM_7	65.40^{i}	58.00^{gh}	75.40^{f}	83.00^{f}	4.93^{g}	4.81^{e}
P_2xM_8	76.40^{ef}	70.80^{de}	31.42^{q}	55.40^{l}	2.40^{m}	3.92^{gh}
P_2xM_9	96.00^{a}	74.20^{d}	71.53^{h}	88.70^{e}	6.86^{b}	6.58^{c}
P_3xM_1	29.40^{o}	24.40^{o}	42.68^{n}	51.22^{m}	1.25^{p}	1.25^{n}
P_3xM_2	40.00^{n}	56.00^{h}	97.15^{c}	72.96^{i}	3.88^{i}	4.08^{g}
P_3xM_3	47.40^{lm}	26.80^{no}	110.65^{a}	104.81^{b}	5.23^{ef}	2.81^{jk}
P_3xM_4	59.40^{j}	55.80^{h}	93.48^{d}	97.68^{c}	5.55^{d}	5.45^{d}
P_3xM_5	64.80^{i}	89.00^{b}	102.57^{b}	118.46^{a}	6.64^{bc}	10.54^{a}
P_3xM_6	85.60^{c}	80.00^{c}	63.75^{i}	80.77^{g}	5.45^{de}	6.46^{c}
P_3xM_7	53.80^{k}	46.00^{j}	92.92^{d}	96.96^{c}	5.00^{fg}	4.46^{f}
P_3xM_8	80.20^{de}	66.60^{f}	40.55^{o}	60.21^{k}	3.25^{k}	4.01^{gh}
P_3xM_9	81.20^{d}	68.40^{ef}	85.00^{e}	106.3^{b}	6.90^{b}	7.27^{b}
CD (0.05)	4.329	3.652	1.446	1.989	0.261	0.304

4.3.4. Performance of hyperaccumulators under graded doses of Cadmium (Cd)

Nine macrophytes viz., *N. nucifera*, *N. indica*, *P. stratiotes*, *E. crassipes*, *L. flava*, *E. dulcis*, *C. esculenta*, *A. tenella* and *S. trilobata* were grown in pots under three graded doses of Cd (50, 75 and 100 mg kg^{-1}) for a period of 45 days. Data on pH, EC and Cd content of water, sediment and macrophytes at 45 DAP are presented in Tables 58 to 61.

4.3.4.1. Water

Main effects as well as interaction effects of treatment had significantly influenced the pH, EC and Cd content (Table 58) of water. As in the case of Fe, Al and Pb, Cd content also showed a negative relation with pH and positive relation with EC and Cd content of water. Among the macrophytes, *L. flava* recorded the highest pH and the lowest by *N. nucifera* and *A. tenella.* Highest EC was recorded by *A. tenella*. Highest Cd content in water was obtained for *N. nucifera* and the lowest for *C. esculenta*.

On evaluating the interaction effects, it was observed that *L. flava* at 50 mg Cd kg^{-1} recorded the highest pH and lowest EC and Cd content. *N. nucifera* with 100 mg Cd kg^{-1} resulted in the lowest pH and highest Cd content for water.

4.3.4.2 Sediment

Table 59 represents the pH, EC, OC and Cd content of sediment as influenced by the levels of Cd, macrophytes and their interactions. The main effects and interaction effects except sediment pH were significant. As in the case of other elements, pH maintained a negative relationship and EC, OC and Cd content of sediment a positive relationship. *L. flava* recorded the highest pH and lowest EC and sediment Cd content. *N. nucifera* resulted in the lowest pH and highest EC and OC content. *E. crassipes* obtained the lowest OC content while the highest sediment Cd content by both *N. nucifera* and *N. indica*.

Table 58. pH, EC and Cd content of water under graded doses of Cd

Treatments Main effects	pH	EC (dS m^{-1})	Cd (mg L^{-1})
Graded doses of Cd			
Cd-50 mg kg^{-1} (C_1)	4.32^{a}	0.34^{c}	0.25^{c}
Cd-75 mg kg^{-1} (C_2)	4.11^{b}	0.37^{b}	0.30^{b}
Cd-100 mg kg^{-1} (C_3)	3.90^{c}	0.48^{a}	0.35^{a}
CD (0.05)	0.037	0.0035	0.0035
Macrophytes			
N. nucifera (M_1)	3.92^{e}	0.42^{b}	0.47^{a}
N. indica (M_2)	4.03^{d}	0.40^{c}	0.36^{b}
P. stratiotes (M_3)	4.19^{b}	0.37^{e}	0.24^{f}
E. crassipes (M_4)	4.17^{bc}	0.38^{d}	0.26^{e}
L. flava (M_5)	4.43^{a}	0.36^{f}	0.24^{f}
E. dulcis (M_6)	4.03^{d}	0.41^{b}	0.32^{d}
C. esculenta (M_7)	4.12^{c}	0.40^{c}	0.23^{f}
A. tenella (M_8)	3.92^{e}	0.44^{a}	0.33^{c}
S. trilobata (M_9)	4.19^{bc}	0.38^{d}	0.26^{e}
CD (0.05)	0.065	0.0061	0.0058
Interaction effects			
C_1xM_1	4.20^{de}	0.34^{mn}	0.40^{c}
C_1xM_2	4.15^{e}	0.35^{jkl}	0.31^{f}
C_1xM_3	4.35^{bc}	0.32^{op}	0.21^{i}
C_1xM_4	4.40^{b}	0.32^{op}	0.21^{i}
C_1xM_5	4.65^{a}	0.31^{op}	0.20^{j}
C_1xM_6	4.25^{cde}	0.33^{o}	0.26^{g}
C_1xM_7	4.30^{bcd}	0.35^{jkl}	0.21^{i}
C_1xM_8	4.20^{de}	0.36^{j}	0.24^{h}
C_1xM_9	4.35^{bc}	0.34^{lmn}	0.21^{i}
C_2xM_1	3.96^{fg}	0.39^{hi}	0.44^{b}
C_2xM_2	4.03^{f}	0.40^{gh}	0.36^{e}
C_2xM_3	4.22^{de}	0.34^{klmn}	0.24^{h}
C_2xM_4	4.20^{de}	0.34^{klmn}	0.26^{g}
C_2xM_5	4.40^{b}	0.35^{jkl}	0.24^{h}
C_2xM_6	4.01^{fg}	0.40^{gh}	0.31^{f}
C_2xM_7	4.03^{f}	0.39^{hi}	0.23^{h}
C_2xM_8	3.96^{fg}	0.40^{gh}	0.36^{e}
C_2xM_9	4.21^{de}	0.35^{jkl}	0.26^{g}
C_3xM_1	3.59^{i}	0.52^{b}	0.56^{a}
C_3xM_2	3.92^{fgh}	0.47^{e}	0.40^{c}
C_3xM_3	3.99^{fg}	0.46^{f}	0.26^{g}
C_3xM_4	3.90^{gh}	0.48^{d}	0.30^{f}
C_3xM_5	4.25^{cde}	0.41^{g}	0.26^{g}
C_3xM_6	3.83^{h}	0.50^{c}	0.38^{d}
C_3xM_7	4.03^{f}	0.47^{c}	0.26^{g}
C_3xM_8	3.60i	0.55^{a}	0.39^{d}
C_3xM_9	4.00^{fg}	0.45^{f}	0.30^{f}
CD (0.05)	0.112	0.011	0.0102

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Table 59. pH, EC, OC and total Cd of sediment under graded doses of Cd

Treatments Main effects	pH	EC (dS m^{-1})	OC (g kg^{-1})	Cd (mg kg^{-1})
Graded doses of Cd				
Cd-50 mg kg^{-1} (C_1)	5.19^{a}	0.24^{c}	25.6^{c}	52.33^{c}
Cd-75 mg kg^{-1} (C_2)	4.97^{b}	0.28^{b}	29.0^{b}	76.92^{b}
Cd-100 mg kg^{-1} (C_3)	4.64^{c}	0.32^{a}	31.8^{a}	101.77^{a}
CD (0.05)	0.028	0.003	0.05	0.017
Macrophytes				
N. nucifera (M_1)	4.59^{f}	0.33^{a}	31.0^{a}	77.40^{a}
N. indica (M_2)	4.75^{e}	0.31^{b}	28.2^{e}	77.40^{a}
P. stratiotes (M_3)	4.98^{cd}	0.27^{e}	27.6^{f}	76.77^{f}
E. crassipes (M_4)	5.03^{bc}	0.27^{e}	26.5^{h}	76.92^{e}
L. flava (M_5)	5.14^{a}	0.25^{f}	26.6^{g}	76.11^{g}
E. dulcis (M_6)	4.94^{d}	0.28^{d}	30.1^{d}	77.36^{b}
C. esculenta (M_7)	4.95^{d}	0.27^{e}	30.9^{b}	77.15^{d}
A. tenella (M_8)	4.96^{d}	0.29^{c}	30.2^{c}	76.77^{f}
S. trilobata (M_9)	5.04^{b}	0.27^{e}	28.2^{e}	77.19^{c}
CD (0.05)	0.049	0.006	0.09	0.030
Interaction effects				
C_1xM_1	4.87	0.27^{gh}	28.0^{l}	52.36^{q}
C_1xM_2	5.06	0.25^{jk}	25.3^{r}	52.58^{o}
C_1xM_3	5.22	0.23^{m}	24.2^{t}	52.11^{s}
C_1xM_4	5.30	0.22^{n}	23.5^{v}	52.46^{p}
C_1xM_5	5.40	0.21^{o}	23.8^{u}	51.87^{t}
C_1xM_6	5.15	0.24^{lm}	27.1^{n}	52.53^{o}
C_1xM_7	5.18	0.24^{lm}	27.0^{n}	52.34^{qr}
C_1xM_8	5.17	0.25^{kl}	26.5^{p}	52.35^{qr}
C_1xM_9	5.35	0.22^{no}	24.7^{s}	52.31^{r}
C_2xM_1	4.57	0.33^{b}	31.5^{e}	77.33^{i}
C_2xM_2	4.79	0.31^{d}	28.0^{l}	77.32^{i}
C_2xM_3	5.02	0.27^{gh}	27.7^{m}	76.61^{m}
C_2xM_4	5.07	0.28^{fgh}	26.2^{q}	76.91^{l}
C_2xM_5	5.14	0.24^{lm}	26.8^{o}	75.99^{n}
C_2xM_6	4.97	0.28^{fgh}	30.5^{h}	77.31^{i}
C_2xM_7	5.00	0.27^{ghi}	31.1^{f}	77.05^{k}
C_2xM_8	5.05	0.29^{f}	30.5^{h}	76.57^{m}
C_2xM_9	5.08	0.26^{ij}	28.6^{k}	77.15^{j}
C_3xM_1	4.34	0.36^{a}	33.4^{c}	102.50^{a}
C_3xM_2	4.42	0.34^{b}	31.2^{f}	102.28^{b}
C_3xM_3	4.71	0.31^{d}	30.8^{g}	101.60^{f}
C_3xM_4	4.72	0.31^{d}	29.8^{i}	101.37^{g}
C_3xM_5	4.88	0.28^{fg}	29.2^{j}	100.47^{h}
C_3xM_6	4.70	0.32^{cd}	32.5^{d}	102.22^{c}
C_3xM_7	4.66	0.31^{d}	34.5^{a}	102.03^{e}
C_3xM_8	4.64	0.32^{cd}	33.7^{b}	101.37^{g}
C_3xM_9	4.70	0.32^{cd}	31.2^{f}	102.10^{d}
CD (0.05)	NS	0.010	0.16	0.052

Among the interaction effects, *N. nucifera* treated with 100 mg Cd kg^{-1} produced the highest EC and Cd content while *L. flava* treated with 50 mg Cd kg^{-1} recorded the lowest. OC was highest for *C. esculenta* at 100 mg Cd kg^{-1} and the lowest for *E. crassipes* treated with 50 mg Cd kg^{-1}.

4.3.4.3. Rhizosphere microbial count

The main as well as interaction effects have significantly influenced the bacteria, fungi and actinomycete population (Table 60). With increasing levels of Cd, the fungal population increased and both bacteria and actinomycetes decreased. Among the macrophytes, the bacterial count was highest for *S. trilobata* but it showed lowest fungal population. Highest count of fungi was for *N. nucifera* and lowest for *S. trilobata*. With regards to actinomycetes, *L. flava* showed the highest values and *C. esculenta,* the lowest.

On evaluating the interaction effects, *S. trilobata* treated with 50 mg Cd kg^{-1} resulted in the highest bacterial count and *N. nucifera* supplied with 100 mg Cd kg^{-1}, the lowest. A reverse pattern was shown by these macrophytes with regard to fungal count. *L. flava* receiving 50 mg Cd kg^{-1} produced the highest actinomycete count and *C. esculenta* treated with 100 mg Cd kg^{-1}, the lowest. Under all the three graded doses of Cd, *N. nucifera* obtained the highest fungal count and *S. trilobata*, the lowest. With 100 mg Cd kg^{-1}, *L. flava* recorded the highest bacterial count and A. tenella, the highest actinomycete count.

4.3.4.4. Macrophytes

Table 61 represents the shoot and root biomass of macrophytes, Cd concentration and Cd extracted by shoot and root. Both main effects and interaction effects were significant. All the above parameters except biomass maintained a positive relation with levels of Cd. Both shoot and root biomass showed an increase up to 75 mg Cd kg^{-1}.

Table 60. Microbial count of sediment under graded doses of Cd

Treatments Main effects	Bacteria	Fungi	Actinomycetes
Graded doses of Cd	(log cfu g^{-1})		
Cd-50 mg kg^{-1} (C_1)	7.14^{a}	3.31^{c}	3.66^{a}
Cd-75 mg kg^{-1} (C_2)	6.81^{b}	3.48^{b}	3.48^{b}
Cd-100 mg kg^{-1} (C_3)	6.25^{c}	3.57^{a}	3.26^{c}
CD(0.05)	0.015	0.010	0.013
Macrophytes			
N. nucifera (M_1)	6.53^{g}	3.64^{a}	3.45^{ef}
N. indica (M_2)	6.59^{f}	3.59^{b}	3.42^{g}
P. stratiotes (M_3)	6.59^{f}	3.51^{d}	3.51^{bc}
E. crassipes (M_4)	6.66^{e}	3.47^{e}	3.52^{b}
L. flava (M_5)	6.90^{b}	3.46^{e}	3.55^{a}
E. dulcis (M_6)	6.80^{c}	3.57^{c}	3.43^{fg}
C. esculenta (M_7)	6.75^{d}	3.42^{f}	3.36^{h}
A. tenella (M_8)	6.74^{d}	3.28^{g}	3.49^{cd}
S. trilobata (M_9)	7.02^{a}	3.13^{h}	3.47^{de}
CD(0.05)	0.026	0.017	0.023
Interaction effects			
C_1xM_1	6.95^{fg}	3.50^{fg}	3.65^{cde}
C_1xM_2	6.94^{fg}	3.50^{fg}	3.63^{ef}
C_1xM_3	6.96^{f}	3.36^{j}	3.68^{bcd}
C_1xM_4	6.98^{f}	3.28^{l}	3.70^{ab}
C_1xM_5	7.29^{b}	3.23^{m}	3.72^{a}
C_1xM_6	7.17^{c}	3.33^{jk}	3.62^{ef}
C_1xM_7	7.12^{d}	3.32^{k}	3.60^{f}
C_1xM_8	7.10^{d}	3.16^{n}	3.65^{de}
C_1xM_9	7.73^{a}	3.06^{o}	3.69^{abc}
C_2xM_1	6.56^{k}	3.70^{bc}	3.46^{hi}
C_2xM_2	6.62^{j}	3.57^{e}	3.43^{ij}
C_2xM_3	6.62^{j}	3.51^{f}	3.54^{g}
C_2xM_4	6.72^{i}	3.46^{hi}	3.61^{ef}
C_2xM_5	6.97^{f}	3.48^{gh}	3.63^{ef}
C_2xM_6	6.93^{fg}	3.65^{d}	3.49^{h}
C_2xM_7	6.88^{h}	3.44^{i}	3.39^{k}
C_2xM_8	6.91^{gh}	3.33^{k}	3.40^{jk}
C_2xM_9	7.04^{e}	3.20m	3.39^{k}
C_3xM_1	6.08^{p}	3.73^{a}	3.25^{n}
C_3xM_2	6.21^{no}	3.70^{bc}	3.19^{o}
C_3xM_3	6.17^{o}	3.65^{d}	3.32^{lm}
C_3xM_4	6.27^{m}	3.66^{d}	3.25^{n}
C_3xM_5	6.44^{l}	3.67^{cd}	3.29^{m}
C_3xM_6	6.29^{m}	3.72^{ab}	3.18^{o}
C_3xM_7	6.25^{mn}	3.49^{fgh}	3.11^{p}
C_3xM_8	6.21^{no}	3.34^{jk}	3.43^{ij}
C_3xM_9	6.29^{m}	3.14^{n}	3.34^{l}
CD (0.05)	0.041	0.030	0.040

Table 61. Biomass and Cd content of macrophytes under graded doses of Cd

Treatments Main effects	Biomass (dry) (g pot^{-1})		Cd concentration (mg kg^{-1})		Cd extraction (mg pot^{-1})	
Graded doses of Cd	Shoot	Root	Shoot	Root	Shoot	Root
Cd-50 mg kg^{-1} (C_1)	66.86^{b}	48.31^{c}	44.95^{c}	73.55^{c}	3.06^{c}	3.62^{c}
Cd-75 mg kg^{-1} (C_2)	68.40^{a}	56.04^{a}	65.07^{b}	109.67^{b}	4.48^{b}	6.30^{b}
Cd-100 mg kg^{-1} (C_3)	61.64^{c}	51.06^{b}	79.19^{a}	137.82^{a}	4.89^{a}	7.35^{a}
CD (0.05)	1.265	1.172	0.401	0.595	0.092	0.141
Macrophytes						
N. nucifera (M_1)	48.46^{g}	40.13^{e}	39.25^{g}	101.62^{e}	1.89^{g}	4.07^{e}
N. indica (M_2)	34.00^{h}	55.40^{c}	36.68^{h}	86.40^{g}	1.27^{h}	4.73^{d}
P. stratiotes (M_3)	63.00^{f}	35.00^{f}	145.31^{a}	90.62^{f}	9.03^{a}	3.20^{g}
E. crassipes (M_4)	75.73^{b}	43.93^{d}	56.64^{e}	140.35^{b}	4.35^{d}	6.45^{c}
L. flava (M_5)	70.13^{d}	77.00^{a}	64.65^{c}	182.19^{a}	4.55^{c}	14.25^{a}
E. dulcis (M_6)	61.13^{f}	54.00^{c}	52.97^{f}	59.03^{h}	3.20^{f}	3.18^{g}
C. esculenta (M_7)	72.33^{c}	63.00^{b}	68.81^{b}	55.83^{i}	4.91^{b}	3.61^{f}
A. tenella (M_8)	66.66^{e}	61.40^{b}	63.47^{d}	128.21^{c}	4.22^{d}	8.07^{b}
S. trilobata (M_9)	99.26^{a}	36.40^{f}	39.85^{g}	118.87^{d}	3.89^{e}	4.25^{e}
CD (0.05)	2.192	2.031	0.694	1.030	0.160	0.245
Interaction effects						
C_1xM_1	52.60^{n}	48.40^{i}	30.30^{r}	95.60^{m}	1.59^{n}	4.62^{jk}
C_1xM_2	30.00^{r}	56.20^{e}	20.72^{s}	63.14^{s}	0.62^{o}	3.55^{no}
C_1xM_3	65.40^{jkl}	30.60^{m}	105.20^{c}	65.00^{r}	6.88^{c}	1.98^{r}
C_1xM_4	70.00^{hi}	34.20^{kl}	41.03^{p}	75.20^{p}	2.87^{kl}	2.57^{p}
C_1xM_5	65.40^{jkl}	68.00^{c}	45.52^{o}	119.85^{i}	2.97^{k}	8.15^{f}
C_1xM_6	62.20^{l}	53.40^{efgh}	35.20^{q}	45.24^{u}	2.18^{m}	2.41^{pq}
C_1xM_7	80.00^{de}	50.40^{ghi}	55.61^{k}	40.70^{v}	4.45^{h}	2.05^{qr}
C_1xM_8	66.00^{jk}	53.60^{efg}	40.48^{p}	70.98^{q}	2.67^{l}	3.80^{mn}
C_1xM_9	110.20^{a}	40.00^{j}	30.50^{r}	86.29^{o}	3.36^{i}	3.45^{no}
C_2xM_1	48.80^{o}	40.00^{j}	46.76^{n}	110.76^{k}	2.28^{m}	4.43^{jk}
C_2xM_2	40.00^{q}	60.00^{d}	40.82^{p}	85.80^{o}	1.63^{n}	5.15^{hi}
C_2xM_3	68.60^{ij}	40.00^{j}	150.00^{b}	90.88^{n}	10.29^{a}	3.63^{mn}
C_2xM_4	76.60^{ef}	50.00^{hi}	56.50^{k}	130.35^{g}	4.32^{h}	6.52^{g}
C_2xM_5	75.20^{fg}	84.00^{a}	60.65^{j}	183.70^{c}	4.56^{gh}	15.43^{b}
C_2xM_6	64.20^{kl}	56.00^{ef}	52.77^{l}	60.96^{t}	3.39^{j}	3.41^{no}
C_2xM_7	72.40^{gh}	70.80^{c}	70.33^{h}	60.50^{t}	5.09f	4.28^{kl}
C_2xM_8	70.40^{hi}	67.60^{c}	67.31^{i}	138.60^{f}	4.74^{g}	9.37^{e}
C_2xM_9	99.40^{b}	36.00^{k}	40.48^{p}	125.48^{h}	4.02^{i}	4.52^{jk}
C_3xM_1	44.00^{p}	32.00^{lm}	40.70^{p}	98.50^{l}	1.79^{n}	3.15^{o}
C_3xM_2	32.00^{r}	50.00^{hi}	48.50^{m}	110.28^{k}	1.55^{n}	5.51^{h}
C_3xM_3	55.00^{mn}	34.40^{kl}	180.70^{a}	116.00^{j}	9.93^{b}	3.99^{lm}
C_3xM_4	80.60^{d}	47.60^{i}	72.40^{g}	215.00^{b}	5.83^{e}	10.25^{d}
C_3xM_5	69.80^{hi}	79.00^{b}	87.78^{d}	243.00^{a}	6.12^{d}	19.20^{a}
C_3xM_6	57.00^{m}	52.60^{fgh}	70.94^{h}	70.90^{q}	4.04^{i}	3.73^{mn}
C_3xM_7	64.60^{kl}	67.80^{c}	80.50^{f}	66.31^{r}	5.20^{f}	4.50^{jk}
C_3xM_8	63.60^{kl}	63.00^{d}	82.63^{e}	175.00^{d}	5.25^{f}	11.02^{c}
C_3xM_9	88.20^{c}	33.20^{klm}	48.58^{m}	144.85^{e}	4.28^{hi}	4.81^{ij}
CD (0.05)	3.797	3.518	1.202	1.784	0.278	0.425

Among the macrophytes, shoot biomass was highest for *S. trilobata* and lowest for *N. indica*. *L. flava* recorded the highest root biomass and lowest by *P. stratiotes.* Cd concentration in shoot was highest for *P. stratiotes* and in root it was for *L. flava.* Cd extraction by shoot and root followed the same trend. Cd concentration in the shoot was the lowest for *N. indica* and in root for *C. esculenta.*

Among the interaction effects, *S. trilobata* treated with 50 mg Cd kg^{-1} produced the highest shoot biomass and *L. flava* treated with 75 mg Cd kg^{-1}, the highest root biomass. Highest shoot Cd concentration was recorded for *P. stratiotes* and root Cd for *L. flava*, both receiving 100 mg Cd kg^{-1}.

P. stratiotes treated with 75 mg Cd kg^{-1} recorded the highest Cd extraction by shoot while *L. flava* with 100 mg Cd kg^{-1}, root Cd extraction. *N. indica* at 50 mg Cd kg^{-1} observed the lowest shoot biomass, shoot Cd concentration and extraction. Root Cd concentration and extraction were lowest for *C. esculenta* treated with 50 mg Cd kg^{-1}.

L. flava can be selected as the best bioremediator for Cd followed by *A. tenella* (phytoextractors) and *P. stratiotes* (rhizofilter).

Among the macrophytes treated with the varied doses of metal viz., Fe, Al, Pb and Cd, *E. crassipes* was observed as the best hyperaccumulator for Fe, *M. vaginalis* for Al and *L. flava* for both Pb and Cd and they were also identified as the best phytoextractors / bioremediators for the above metals. The major site of metal retention was identified as root for these phytoextractors.

Part IV

4.4. Bioavailability of phytoextracted metals and comparison of common disposal methods

Macrophytes identified as hyperaccumulator / phytoextractor for individual metals were raised in pots spiked with corresponding metal at specified concentration. The plants were grown for 60 days and biomass were subjected to processing methods viz., ordinary composting, vermicomposting, ashing and biochar production (Plate 8). Metal concentration of materials used in the pot culture study is given in Table 62.

Table 62. Metal content of materials used in pot studies

Source material	Fe	Al	Pb	Cd
	mg kg^{-1}			
Compost	15065	11250	36.50	58.50
Vermicompost	12050	8990	29.20	46.80
Ash	60490	44975	146.00	234.00
Biochar	20125	14990	48.65	78.00
Soil	6850	4364	0.130	0.007
FYM	2450	1025	0.027	0.001

Using the processed materials four separate pot culture experiments were carried out each for Fe, Al, Pb and Cd using amaranthus, var. Arun as the test crop. Amaranthus was grown for 30 days, uprooted and processed for analysis. Plant and growing medium were analysed for the metal content and the data are presented in Tables 63 to 66.

4.4.1. Iron (Fe)

The processed biomass generated from *E. crassipes*, the bioremediator for Fe (based on Part III) was used for raising amaranthus and grown for a period of 30 days. Plant as well as growth medium were analysed for Fe concentration and the data are presented in Table 63.

Plate 9. Processed materials prepared from phytoextracted (Fe) biomass

Vermicompost

Compost

Biochar

Ash

Table 63. Biomass (dry) production and Iron (Fe) content of plant and soil at 30 DAP

Treatments	Plant			Total soil Fe mg kg^{-1}
	Biomass (dry) g plant^{-1}	Fe concentration mg kg^{-1}	Fe extraction mg plant^{-1}	
T1 (FYM)	1.18^{c}	909.00^{d}	1.08^{e}	6908.00^{d}
T2 (compost)	1.34^{b}	1495.33^{b}	2.00^{c}	7446.33^{ab}
T3 (vermicompost)	1.50^{a}	1584.66^{b}	2.38^{b}	7446.00^{b}
T4 (ash)	0.93^{e}	4386.00^{a}	4.09^{a}	7443.00^{c}
T5 (biochar)	1.39^{b}	1240.00^{c}	1.73^{d}	7448.66^{a}
T6 (control)	1.02^{d}	572.33^{e}	0.58^{f}	6847.00^{e}
CD (0.05)	0.066	137.07	0.141	2.371

The treatments *ie.* the disposal methods had significantly influenced the biomass production, Fe extraction and plant and soil Fe concentration. Among the treatments, vermicomposting had produced highest biomass and was significantly superior to all other treatments. Ashing resulted in the lowest biomass production.

With regard to the influence of disposal methods on Fe concentration in plant, ashing recorded the highest value and biochar, the lowest. While considering the total Fe content of soil, reverse was the case. Phytoextraction of Fe by amaranthus followed the same pattern as that of Fe concentration in plant. In both soil and plant, lowest value was recorded by the control treatment *ie.*, without any phytoextracted material followed by FYM treatment. Thus biochar retained highest quantity of Fe in soil and ash facilitated highest translocation to the plant.

4.4.2. Aluminium (Al)

Phytoextracted Al through *M. vaginalis* was subjected to four disposal methods and the resultant processed biomass was used to raise amaranthus. Data on plant biomass and soil Al content are presented in Table 64. Various disposal methods had significantly influenced the production of biomass, soil Al content and Al extraction.

Plate 10. Amaranthus grown using processed phytoextracted biomass

As in the case of Fe, vermicomposting resulted in the highest biomass production and the lowest by ashing. Among the disposal methods, Al concentration in amaranthus was highest for ashing and lowest for biochar. Plant Al extraction also followed the same trend as that of concentration. Soil Al content was highest for ordinary composting which was on par with vermicomposting. Treatment without any phytoextracted material (control) resulted in lowest Al extraction by amaranthus followed by FYM. Al content of soil also behaved in a similar manner.

Table 64. Biomass (dry) production and Al content of plant and soil at 30 DAP

Treatments	Plant			Total soil Al mg kg^{-1}
	Biomass (dry) g $plant^{-1}$	Al concentration mg kg^{-1}	Al extraction mg $plant^{-1}$	
T1 (FYM)	1.173^{d}	1059.456^{b}	1.243^{c}	4389.94^{c}
T2 (compost)	1.511^{c}	1045.409^{b}	1.576^{b}	4814.10^{a}
T3 (vermicompost)	1.860^{a}	820.191^{c}	1.526^{b}	4813.44^{a}
T4 (ash)	0.829^{f}	4406.437^{a}	3.653^{a}	4808.94^{b}
T5 (biochar)	1.567^{b}	589.491^{e}	0.924^{d}	4812.38^{ab}
T6 (control)	1.033^{e}	711.083^{d}	0.736^{e}	4362.85^{d}
CD (0.05)	0.026	44.716	0.086	3.493

On comparing the disposal treatments, biochar was found to retain the highest quantity of Al in soil and least in plant while ash showed the reverse.

4.4.3. Lead (Pb)

The processed biomass generated from *L. flava*, the phytoextractor for Pb (based on Part III) was used for raising amaranthus and grown for a period of 30 days. Plant as well as growth medium were analysed for Pb concentration and the data are presented in Table 65. Disposal treatments had significantly influenced the biomass production and Pb concentration in soil and plant.

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Table 65. Biomass (dry) production and Pb content of plant and soil at 30 DAP

Treatments	Plant			Total soil Pb mg kg^{-1}
	Biomass (dry) g plant^{-1}	Pb concentration mg kg^{-1}	Pb extraction µg plant^{-1}	
T1 (FYM)	1.24^{c}	0.004^{b}	0.008^{b}	0.126^{c}
T2 (compost)	1.39^{b}	0.024^{b}	0.034^{b}	1.589^{a}
T3 (vermicompost)	1.54^{a}	0.035^{b}	0.048^{b}	1.589^{a}
T4 (ash)	1.16^{d}	15.239^{a}	17.75^{a}	1.544^{b}
T5 (biochar)	1.55^{a}	0.014^{b}	0.012^{b}	1.589^{a}
T6 (control)	1.11^{d}	0.005^{b}	0.007^{b}	0.129^{c}
CD (0.05)	0.064	1.572	1.967	0.009

Biochar application had resulted in the highest biomass production which was on par with vermicompost treatment. Control treatment without any phytoextracted material produced the lowest biomass followed by FYM. Among the disposal methods, ashing recorded the highest Pb concentration in amaranthus and lowest by biochar which was statistically on par with other treatments. Pb extracted by amaranthus was in line with concentration.

In general, total content of Pb was very low (0.130 mg kg^{-1}) in soil. However the treatment with phytoextracted material had increased the value above 1.5 mg kg^{-1}. Among the treatments, only ash had shown a slight decrease in soil Pb content while composting, vermicomposting and biochar maintained same Pb content in soil (1.589 mg kg^{-}1).

Comparing the different disposal methods, biochar extracted the lowest quantity of Pb from growth medium and ash, the highest.

4.4.4. Cadmium (Cd)

L. flava, the phytoextractor for Cd was processed as per treatments and applied to amaranthus which was raised for 30 days. Though the Cd content of soil

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was very low, disposal treatments had shown significant effects on biomass production, Cd extraction by amaranthus and soil Cd content (Table 66).

Table 66. Biomass (dry) production and Cd content of plant and soil at 30 DAP

Treatments	Plant			Total Soil Cd (mg kg^{-1})
	Biomass (dry) (g plant^{-1})	Cd concentration (mg kg^{-1})	Cd extraction (µg plant^{-1})	
T1 (FYM)	1.450^e	0.004^b	0.005^b	0.006^c
T2 (compost)	1.960^b	0.037^b	0.063^b	2.348^a
T3 (vermicompost)	2.360^a	0.031^b	0.080^b	2.348^a
T4 (ash)	1.596^d	12.829^a	20.48^a	2.300^b
T5 (biochar)	1.796^c	0.026^b	0.042^b	2.349^a
T6 (control)	1.230^f	0.002^b	0.002^b	0.009^c
CD (0.05)	0.145	0.765	1.257	0.005

Biomass production was highest for vermicompost and was significantly superior to all other treatments. Comparing the different disposal methods ashing resulted in lowest biomass. With regard to Cd concentration in plant, ash recorded the highest value and was significantly superior to all other treatments. Cd extraction by plants showed the same pattern as that of concentration. In the case of Cd content of soil, the lowest quantity was retained by ash. Compost, vermicompost and biochar were on par with each other for soil Cd content and were significantly superior to ash.

In general, conversion of phytoextracted biomass to biochar showed the lowest metal extraction by amaranthus and highest metal retention in soil while ash showed a reverse trend. Hence biochar is the safest method among the tested disposal methods of phytoextracted materials.

Discussion

5. DISCUSSION

5.1. Vellayani wetland ecosystem – Level of contamination

Half of the world's wetlands have been disappeared since 1900. Development and conversion continue to pose major threats to wetland, despite their value and importance. Vellayani lake, the major drinking water source for the southern parts of Thiruvananthapuram district is under the threat of pollution, encroachment and sand mining. A major portion of the lake was reclaimed since 1926 and reclaimed areas were put to agriculture / commercial development / residential purposes / tourism / small scale industries etc. All these had contributed towards the degradation of the lake. An Environment Impact Assessment by CESS (2003) had pointed out the impact of laterite quarrying from Thiruvallom hills on the ecology and habitat change of Vellayani wetlands. A study carried out by Kamal (2011) revealed the presence of several inorganic contaminants including heavy metals in the lake water, sediment and native macrophytes. Taking all these into consideration, an investigation was planned to find out the sources of contamination and their extent and how phytoremediation can be employed for decontaminating the system.

5.1.1. Sources of contamination

The Vellayani fresh water lake had an expansion of 750 ha during 1926. Later various developmental activities and encroachment had reduced its area to 243.39 ha in 2014 (Job *et al.*, 2014) which had adversely affected the recharge capacity. The peripatetic survey carried out revealed that the major sources of contamination at Vellayani wetland ecosystem are mainly non-point type. Agriculture, commercial and residential developments, roads and bridges, fishing, water sports, non native plants and animals, waste disposal, wastewater and storm water, tourism, toxic pollutants from workshops / small industries contributed to the non point pollution. Katip and Karaer (2013) were also of the same opinion. In order to verify the information gathered from the survey, water and sediment samples collected from the study area during pre and post monsoon periods were subjected to analysis for physical, chemical and biological properties. The samples were also tested for the presence of pesticide residues. Based on the analytical results of water and sediment, the levels and extent of contamination in Vellayani wetland ecosystem were evaluated.

The `Grow More Food' programme launched by the Government of India in the early 1950s had led to the reclamation of vast areas of the lake for paddy cultivation. It was a common practice to dewater the lake twice annually for paddy cultivation by the farmers. Though the paddy cultivation was stopped by the year 2005, the area became a hub for vegetable cultivation. The practicing agriculture around the year had resulted in the release of several agrochemicals to the lake. In the present study also it was observed that the lake water and sediments contained even heavy metals (Tables 14 and 19) and had favoured the growth of non native weeds like *E. crassipes*, *P. stratiotes*, *S. molesta*, *L. flava*, *M. vaginalis* etc. (Table 9) and even facilitated eutrophication.

Sand mining which is very common in the area was another major threat to the life of the ecosystem. Removal of sand layer is bound to affect the water table in the region. The resultant decrease in hydraulic pressure will also lead to salinity intrusion beyond the spillway. Sand mining was also posing a threat of decrease of groundwater recharge and increase of turbidity by intervening with the natural filtering system. Turbidity and suspended solids of the study area were higher during pre monsoon season when the lake experienced a decrease in water table (Table 11). The water quality was also poor and water is getting more and more acidic. Evaluating the electro chemical properties of water it was found that water was more acidic and saline during pre monsoon season (Table 12).

Developmental activities in terms of roads, commercial / residential buildings and parking lots have enhanced the amount of impervious surface. Impervious surfaces decrease ground water recharge within a watershed and can reduce water flow into wetlands (USEPA, 1993). Impervious surfaces also prevent rainfall from percolating into the soil. Rainfall carries sediments, organic matter, animal wastes, pesticide and fertilizer residues from crop fields, heavy metals and debris, and deposits them into the lake. Excessive inputs of nutrients can lead to eutrophication or result in the release of pollutants from a wetland into adjacent water resources (USEPA, 1993) and decreased the pollutant removal efficiency. Nitrogen load of water was higher during post monsoon season, might be the result of better mineralization of the organic materials in response to enhanced aeration during this period. The lower OC content of sediment during post monsoon period supports the behaviour of N (Table 17). P, Fe, Al, Pb and Cd contents were higher during pre monsoon compared to post monsoon season, might be due to the reduction in volume of water during pre monsoon, and contributions from the agrochemicals, detergents and other degradable / non degradable wastes to the lake. John *et al.* (2014) reported that the Vellayani lake is undergoing a steady deterioration in water quality due to pollution from urban and agricultural sources. Fertilizer-intensive farming in the adjoining crop fields and the exponential rise in the quantity of N-P-K fertilizer use in Thiruvananthapuram district from 1961 to 2010 are cited as reasons. A substantial portion of the unused nutrients in the catchment area would reach the lake through surface and groundwater pathways, especially during monsoon. This may change the nutrient dynamics and wetland hydrology of the ecosystem. These changes may adversely affect spawning, migration and species composition and thus the food web in a wetland as well as in associated ecosystems as reported by USEPA (1993).

The disturbance and habitat degradation experienced by the wetlands resulted in the invasion by aggressive and highly tolerant non native vegetation. The Vellayani lake had experienced the invasion by non native aggressive species like *L. flava, E. crassipes, S. molesta, P. stratiotes, A. sessilis, S. trilobata* etc. which are very prominent. Invasion by native species like *S. grossus, E. dulcis, N. indica, A. tenella* and *N. nucifera* was much wide spread and heavy (Table 10). As per local opinion, invasion by *N. nucifera* (lotus) was most troublesome and is becoming highly noxious also, as it adversely affects the habitat of native fish though it provides livelihood income to a section of society. Non-native and tolerant native species may outcompete with other species leading to a reduction in species diversity and in due course less adaptive / tolerant species may get vanished or become extinct from the system.

Many of the houses in the area do not have proper sanitation facilities and the sewage is released to the lake, which had reduced the biological quality of the water (Chippy *et al.*, 2011) as indicated especially by enhanced BOD and *E.coli* count (Table 12 and 15). The poor lake water quality experienced during pre monsoon season was due to the increased levels of nutrients. The pre monsoon season coincides with the peak vegetable cultivation in the catchment of Vellayani wetland ecosystem and the drainage water from the agricultural fields, though in small quantities might be responsible for this.

Dumping of solid wastes of varied nature ranging from degradable to non degradable types (battery, tubes, CFL lamps, low quality pet bottles, carry bags etc.) were observed in all the selected sites during both the seasons. These had deteriorated the water quality and the aesthetic value of the lake. Though this was originally a fresh water lake, the dumping of all these wastes and release of metals / salts increased the electrical conductivity of the water (Table 12) which may finally lead to the loss of fresh water status of the lake. Among the inorganic contaminants, P was very much above the MPL and Al above desirable limits while N, Fe, Pb and Cd were within the specified limits for drinking water. Measures should be taken to curtail the reach of chemical fertilizers / pesticides and their residues and other contaminants to lake water so as to protect the water quality. Action for this has to be initiated from the public side by resorting to less inorganic intensive agriculture and resorting to proper treatment of solid waste and sewerage. Kamal (2011) also reported the deterioration of water quality in Vellayani lake.

Fish culture by the Fisheries Department had also affected the natural biota of the lake causing pollution (Reenamole *et al.*, 2016). Another factor degrading the lake was constructional aspects and developmental works in the name of tourism. Controlled fish farming and restricted tourism activities are only to be allowed in the lake to maintain water quality. Anti-pollution signboards should be placed around the lake and organic farming should be promoted in the catchment area of lake and allow only ecotourism. Bio fencing around the lake with phytoremediators will be an ideal choice to prevent contamination of lake water from outside sources.

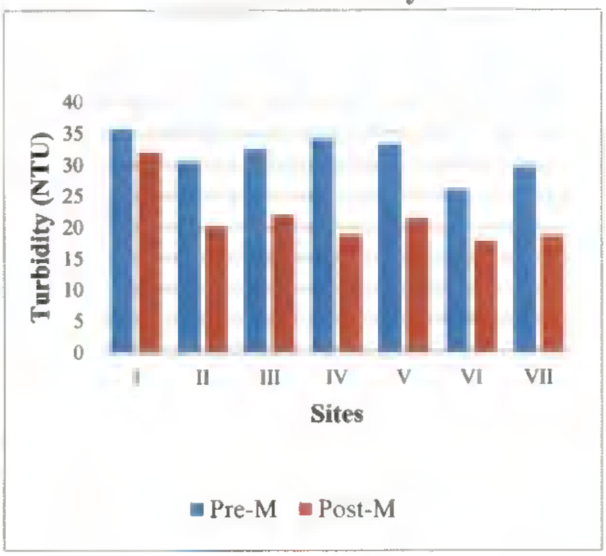
5.1.2. Extent of contamination /degradation

5.1.2.1. Physical degradation

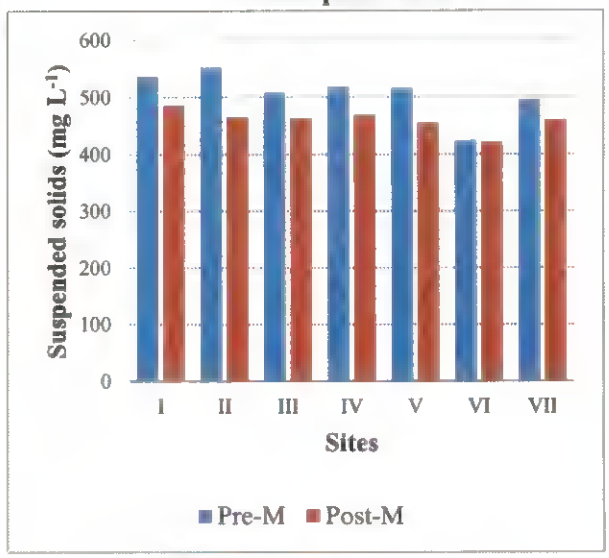
The non point pollution in a watershed or catchment results the physical deterioration of water quality by allowing the reach of fine soil particles and remnants of organic residues in the water bodies. With regard to the physical properties of lake water within the study area, it was observed that except temperature, the other properties like colour, turbidity and suspended solids exceeded the maximum permissible limits during both pre and post monsoon seasons, clearly revealing the existence of physical degradation of lake water in the selected sites viz., Palappoor, Pallichalthodu, Resevoir bund, Arattukadavu RB, Valiyavilagam, Mannamvarambu and Manamukku. During post monsoon season, the suspended solids were just below the MPL. This had thrown an inference that the lake suffers physical degradation especially during pre monsoon period. The water quality was poorer during the pre monsoon period. The excessive evaporation during that months and the receipt of occasional pre-monsoon showers and the resultant muddy runoff water have increased the turbidity. Among the different sites, the turbidity was highest at Palappoor. This might be due to the receipt of drainage water from agricultural fields containing fine soil particles and organic manure residues, where cultivation is at its peak during this season. The turbidity and suspended solids (Fig. 1a and 1b) were at their lowest at Mannamvarambu where agriculture is practiced to a lesser extent. In

Fig. 1 Water quality parameters of Vellayani lake during pre and post monsoon seasons of 2014

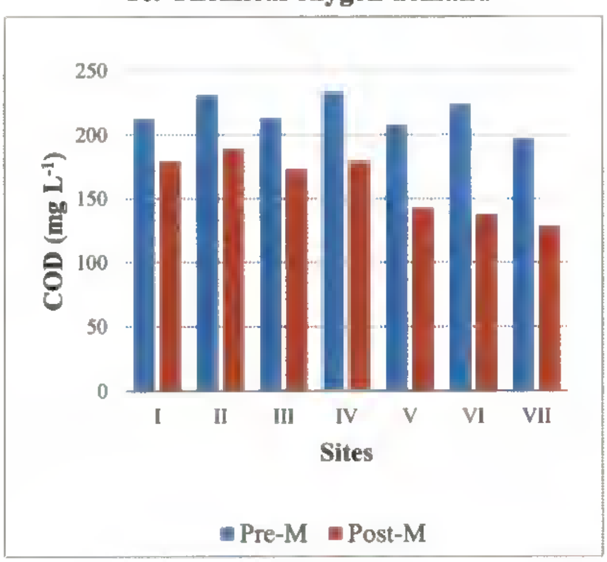
1a. Turbidity



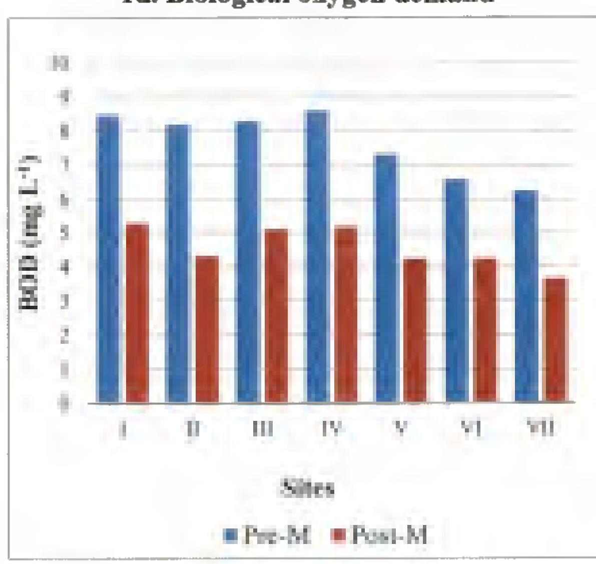
1b.Suspended solids



1c. Chemical oxygen demand



1d. Biological oxygen demand



I- Palappoor	II - Pallichalthodu	III – Reservoir bund	IV- Arattukadavu RB
V - Valiyavilagam	VI - Mannamvarambu	VII - Manamukku	

general, Manamukku was lowest in terms of physical degradation and Arattukadavu RB, the highest. The rivulets as they approach the lake, a decrease in above properties were observed indicating that as the distance from sources of contamination increases, the water quality improved. This was in accordance with the observation of Rowe (2014).

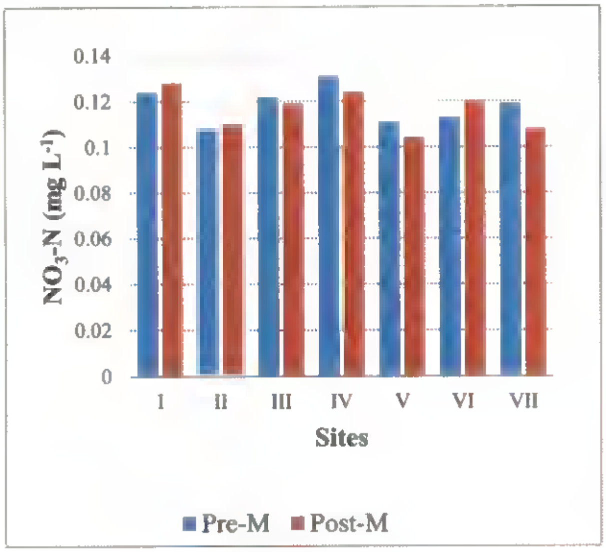
5.1.2.2. Chemical degradation

Chemical deterioration of lake water quality is very common in most of the human inhabited areas due to the release and reach of various chemicals in water. Among the chemical properties of water, pH, EC, COD, NO_3-N, NH_4-N, Fe, Pb and Cd contents were within the MPL. But BOD and P content of water were well above the MPL. Al content of water was above the desirable limit though it was below MPL. The values of pH and EC during both seasons (Table 12) indicated that the lake water is comparatively safe for use and it contains only very little quantities of inorganic contaminants. The COD value (Fig. 1c) also supports this, though COD was more or less near to MPL during pre monsoon season. But the high values of BOD (Fig. 1d) indicated the presence of organic contamination mainly during pre monsoon season and in selected sites during post monsoon season also. Rahul *et al.* (2013) also reported the enhanced deterioration of water quality during pre monsoon season.

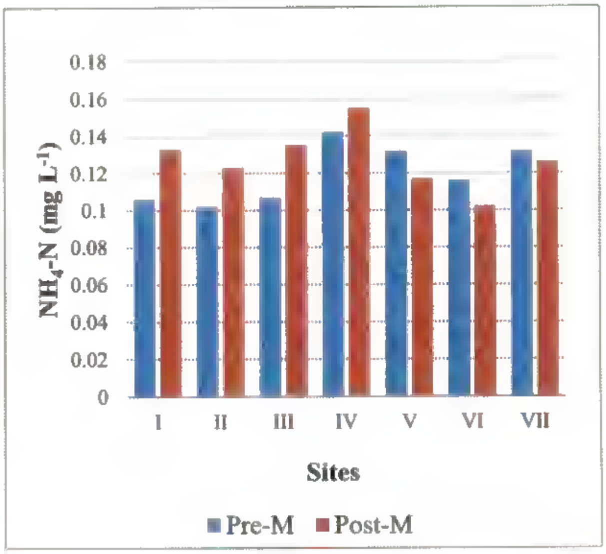
The concentration of nitrate and ammoniacal N in water were also very low and hence there is no problem of N contamination during these two seasons (Fig. 1e and 1f). But the high values of P indicated the chances of eutrophication. For P also the values were higher during the pre monsoon season compared to post monsoon indicating poor water quality during pre monsoon season. In general, water quality was poor during pre monsoon season compared to post monsoon season, as reported by Radhika *et al.* (2004) for Vellayani lake.

Fig. 1 Water quality parameters of Vellayani lake during pre and post monsoon seasons of 2014 (continued)

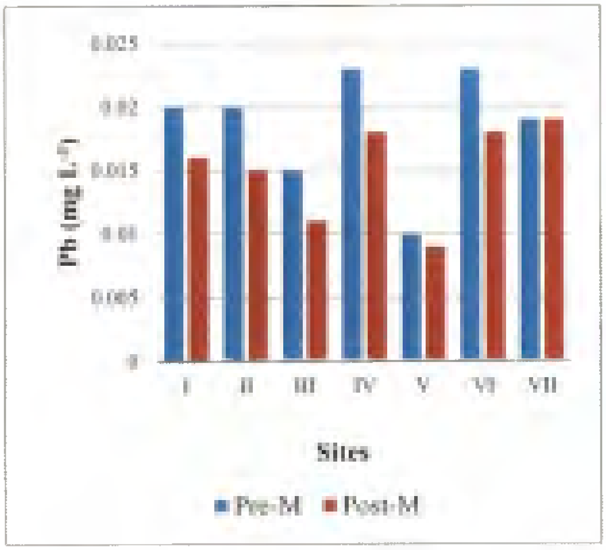
1e. Nitrate -N



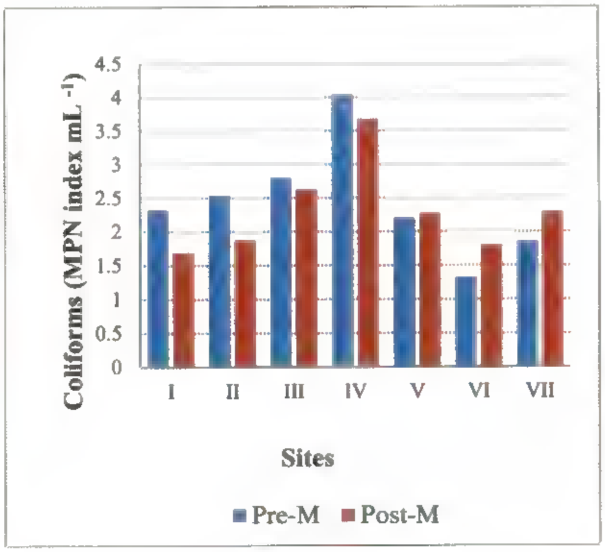
1f. Ammoniacal-N



1g. Lead



1h. Coliform count



I- Palappoor	II - Pallichalthodu	III – Reservoir bund	IV- Arattukadavu RB
V - Valiyavilagam	VI - Mannamvarambu	VII - Manamukku	

The heavy metals and Al content of water were also well below the MPL. This was in contrast to the earlier reports of Kamal (2011) and John *et al.* (2014). Either the precautionary measures taken from the part of Government or the efforts of Save Vellayani Fresh Water Lake Campaign and Vellayani Jagratha Samithi might have created much awareness among the public from throwing the metal containing wastes into the lake or may be due to the dilution effect of the heavy rainfall that Kerala received during the monsoon season of 2013. Devi and Yadav (2014) reported about the heavy monsoon showers during 2013 in Kerala.

Al content of water was much below the MPL, but it was above the desirable limit for drinking water. This is not a good indication, since the element is associated with Alzheimer's disease (Rondeau *et al.*, 2000). The values were higher during pre monsoon period. Lower pH during this season might have maintained more of Al in soluble form. Hence measures should be taken to prevent reaching of Al to the lake or to make Al insoluble. Though in trace quantities, Pb was also detected in all the sites selected for study (Fig. 1g).

For all the above parameters, the values were found to decrease as they deviate from the sources of contamination. As per the investigation, the ecosystem experiences chemical degradation also though the extent is small and varied among the sites. In general, Manamukku was lowest in terms of chemical / organic contamination and Arattukadavu RB, the highest.

5.1.2.3. Biological degradation

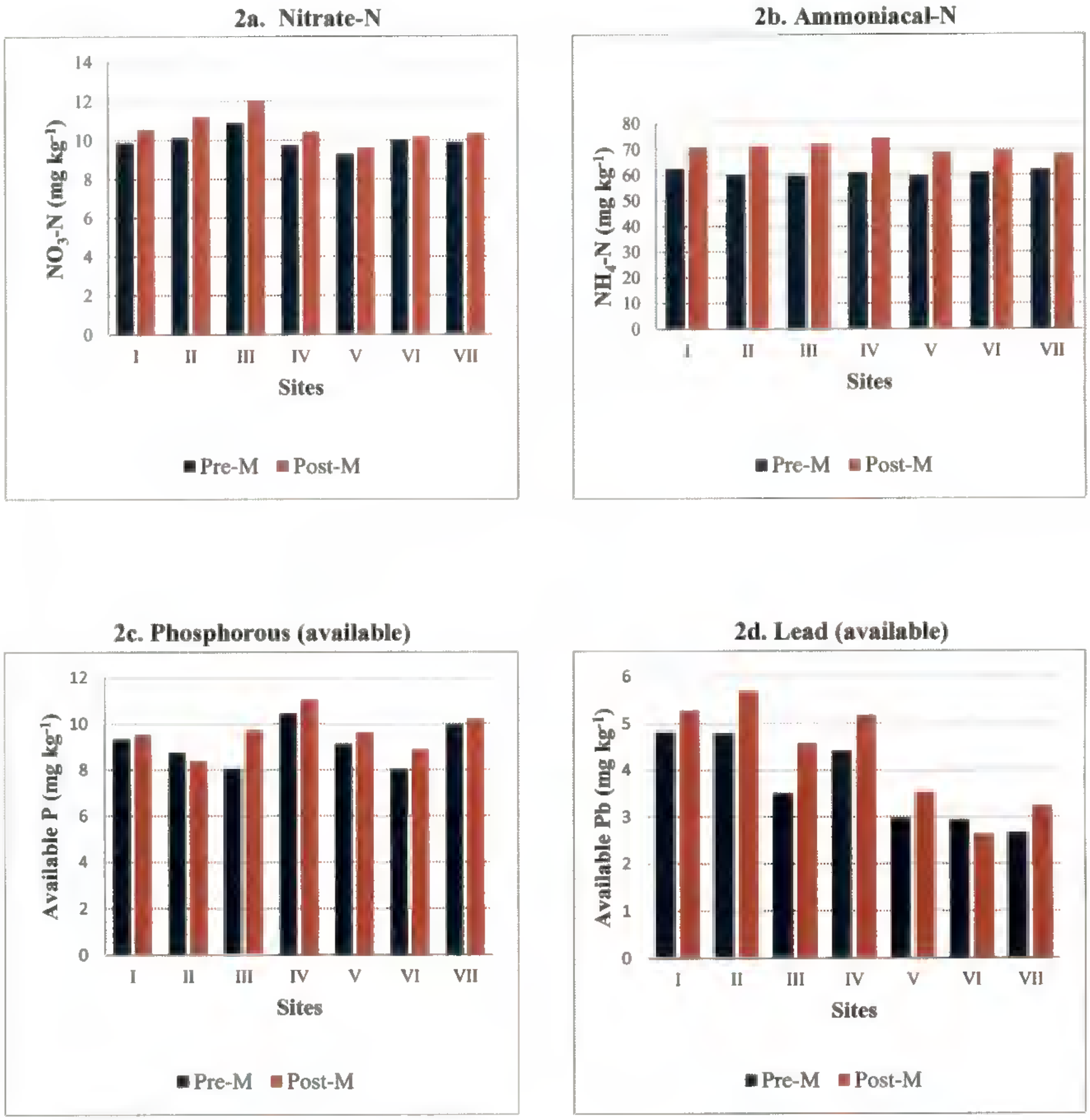
Among the biological properties, only the coliform count is discussed here while BOD and COD have been discussed under section 5.1.2.2. The coliform count was above the permissible limit, indicating the water quality deterioration and chances of sewage entry into the lake water (COA, 2009; Girijakumari *et al.*, 2006). The coliform count was also highest at Arattukadavu RB during both the seasons (Fig. 1h). The count was lower during post monsoon season. During the monsoon season most of the contaminants might have washed out from the ecosystem by the rain water and this might have led the ecosystem to be comparatively healthy during the post monsoon season. In general biological degradation was also highest at Arattukadavu RB and lowest at Manamukku.

5.1.2.4. Sediment

The sediment from all sampling sites was moderately acidic with slight salt content and high in OC. The nutrient retention property of sediment might have helped to retain most of the nutrient ions on its matrix and thus showing very small EC values (Table 17) for water. The high OC content of sediment favoured the metal retention on its exchange sites (Sheela *et al.*, 2012; Fernandes and Poleto, 2017).

N content of sediment, both nitrate and ammoniacal forms were higher during post monsoon season as in the case of water and highest at Reservoir bund (Fig. 2a and 2b). P was also higher during post monsoon season (Fig. 2c). This was in contrary to the behaviour of water. Among the toxic metals Fe and Al were higher during the pre monsoon period. The more acidic pH of sediment during pre monsoon might have enhanced their availability during this season, while Pb (Fig. 2d) and Cd were more during post monsoon period. The very high contents of Fe and Al might have enforced an antagonistic effect on the cationic elements, Pb and Cd, rendering them less available. Perusal of the data on toxic metals, Cd content was much lower compared to others. Available Fe, Al and Cd contents were highest at Arattukadavu RB during both the seasons. Available Pb content was highest at Palappoor during both the seasons. Arattukadavu RB is having more human settlement and hence more the associated contamination while Palappoor is mainly concentrating on agriculture. Open waste disposal contributed to soil contamination and results in accumulation of heavy metals, especially, Pb content (Ali *et al.*, 2014).

Fig. 2 Sediment quality parameters of Vellayanilake during pre and post monsoon seasons of 2014



I- Palappoor	II – Pallichalthodu	III – Reservoir bund	IV- Arattukadavu RB
V - Valiyavilagam	VI - Mannamvarambu	VII - Manamukku	

5.1.2.5. Pesticide residues

Dharmapalan (2014) stated that the Vellayani lake water and sediment have been contaminated with pesticides. But the present study revealed that neither water nor sediment contains any pesticide residue (Tables 20 and 21) based on the analysis for common pesticides used in agriculture. This was in conformity with the findings of COA (2009). Thus the quality degradation of Vellayani ecosystem based on the contamination with pesticide residues is nil. Even though cultivation practices are being carried out in a large scale in the catchment area of Vellayani lake, it is not reflected in the pesticide residue content of the system. This may be due to the high adsorption capacity of soil and the adsorbed pesticide residues get degraded by various biotic and abiotic factors before reaching the lake. Traces of pesticide residue that may reach the lake along with surface runoff get diluted in the entire water body. As soon as it reaches the water body, either it may get adsorbed on clay / silt particles or other suspended organisms in water which undergo further degradation leading to their disappearance. Sediment particles adsorb the pesticides of organic nature because of their lipophilic nature and get deposited. Even if it reaches the aquatic system, there are minor chances of getting detected as revealed by the present study due to high dilution of residues reaching the lake.

Degradation due to agriculture is also very small since only P had contributed to ecodegradation. But the source of P was mainly detergents, and the contribution of P from commonly applied insoluble rock phosphate was very negligible. Kundu *et al.* (2015) also reported the influence of over use of detergents in increasing the P status of water bodies. Thus the extent of degradation of Vellayani wetland ecosystem can be ranked slight which is in conformity with the findings of Radhika *et al.* (2004) and Abhijna (2016).

5.2. Natural vegetation/ dominant macrophytes

Natural vegetation of an ecosystem is a phenological indicator of seasonal development, climatic effect and soil properties. They truly reflect the soil conditions. Contaminated soils always have predominance of some typical species that can thrive the extreme situations. Hence the biodiversity assessment is always done to evaluate the ecodegradation in an area. The vegetation of the study area includes aquatic as well as terrestrial macrophytes. All together 29 dominant species were identified along the rivulets. Out of these, 15 were terrestrial in nature. Out of the 14 aquatic species, six were emergent type, one submerged type and six were floating type. The most dominant species under each category were *A. tenella* and *S. trilobata* (terrestrial), *L. flava*, *N. nucifera* and *M. vaginalis* (aquatic emergent) and *E. crassipes* and *S. molesta* (floating). At Pallichalthodu, excessive growth of *E. crassipes* and *N. nouchali* was noted compared to other sites. This might be due to accumulation of organic debris and water stagnation resulted due to the closure of the nearby barrage at Madhupalam. Stagnated water with organic debris promotes luxuriant growth of aquatic species especially *E. crassipes* (Qin, 2009).

The profuse growth of macrophytes was confirmed by their higher biomass production and plant density (Table 10). The biomass (fresh) production (Fig. 4) by, both emergent and floating aquatic macrophytes was higher compared to the terrestrial ones. The higher water content and the succulent nature of aquatic macrohytes resulted in higher fresh biomass production. The ability of aquatic species to cover an area is more compared to terrestrial plants due to availability of free area. Nutrients can enter the system through both shoots and roots resulting in higher biomass production for aquatic macrophytes (Denny, 1972). Also the aquatic ecosystem provides less competition with a strong food-web interaction leading to more flourishing nature of these macrophytes (Chase, 2000).

Among the emergent types, biomass production was highest for *L. flava* followed by *S. grossus* and *C. esculenta*. *E. crassipes* recorded the highest value

Fig. 3 Number of macrophytes found in the selected sites of Vellayani wetland ecosystem

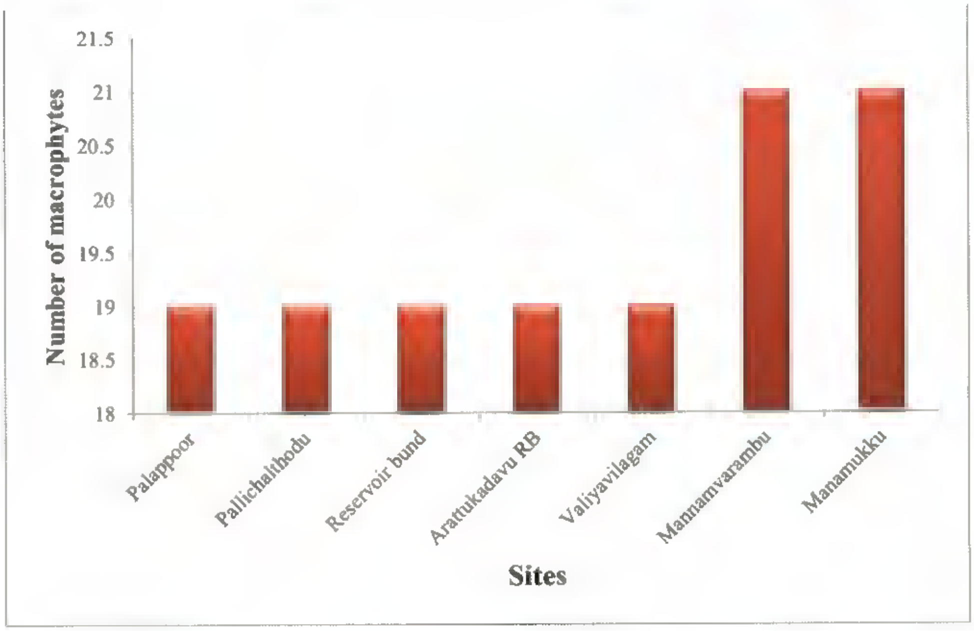
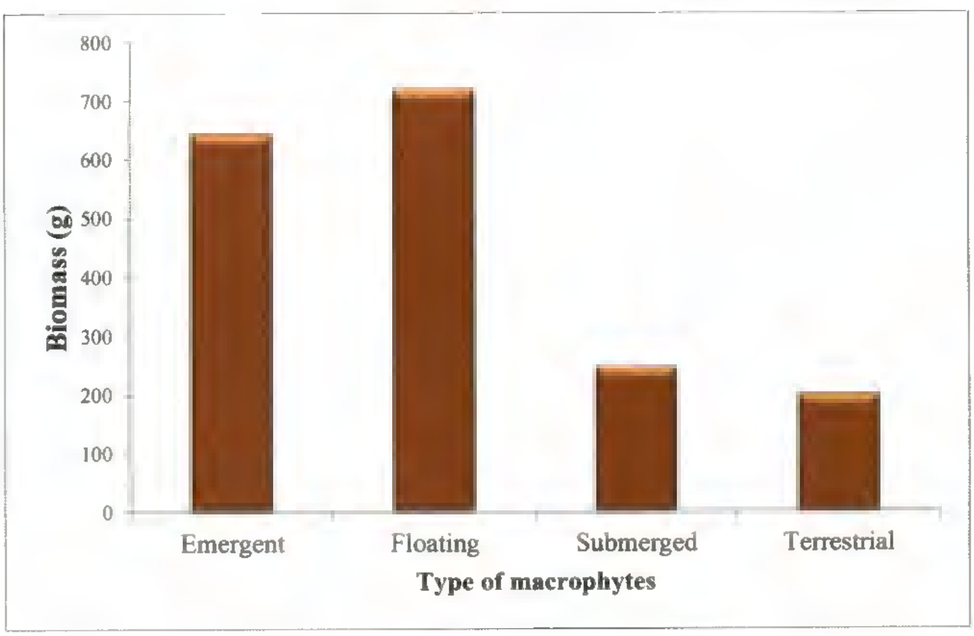


Fig. 4 Biomass (fresh) of different types of macrophytes in Vellayani wetland ecosystem



followed by *S. molesta* and *N. nouchali* in the case of floating macrophytes. For terrestrial group, highest biomass was produced by *P. kaida* surely due to large plant size, followed by *P. repens* (Table 22). The higher biomass production especially, that of shoot which can be easily removed, is an ideal character for phytoremediators. Definitely the concentration of toxic metal within the plant should also be higher to label it as a good phytoremediator.

Among the sites, Mannavarmbu and Manamukku, which were less contaminated, had shown the presence of 21 dominant macrophytes while all other sites have 19 only (Fig. 3). Comparing the biomass production by the most dominant species of each category, highest was from Mannavarmbu for emergent, Pallichalthodu and Arattukadavu for floating and Manamukku for submerged and terrestrial types, indicating the prominence of these sites (Table 22). But a direct relation with the contaminant level in soil / sediment and dominance of particular macrophytes was not observed. This might be due to the non point nature of contamination. The density and biomass production followed the same pattern since both are directly related. However, the predominance of *E. crassipes, L. flava, S. grossus*, *C. esculenta* and *N. nucifera* was observed. (Plates 4 to 6).

5.2.1. Phytoaccumulation potential of native macrophytes for heavy metals

The ability of native macrophytes to hyperaccumulate heavy metals makes them ideal candidates for phytoremediation. The role of aquatic macrophytes in the cleanup of water bodies is very high since they play an important role in the structural and functional aspects of aquatic ecosystems by altering the water movement regimes, providing shelter to fish and aquatic invertebrates, serving as a food source, and altering water quality by regulating oxygen balance, nutrient cycles, and accumulating heavy metals. The ability to hyperaccumulate heavy metals makes them interesting research candidates, especially for the treatment of industrial effluents and sewage waste water. The use of aquatic macrophytes as phytoremediators is known to be an environmentally friendly option to restore polluted aquatic resources. Among various water pollutants, heavy metals are of major concern because of their persistent and bio-accumulative nature (Lokeshwari and Chandrappa, 2006; Yadav *et al.*, 2010). The phytoaccumulation potential of macrophytes for toxic metals in comparison with rhizosphere soil / sediment properties is discussed.

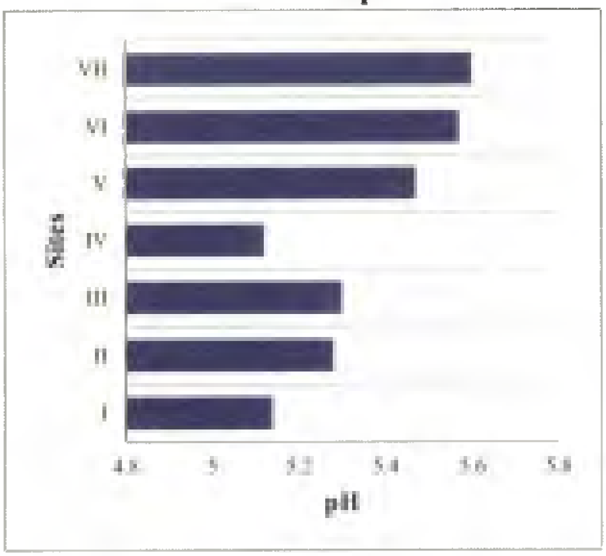
5.2.2. Electrochemical properties and metal load of rhizosphere

The metal load of rhizosphere soil / sediment plays most important role in phytoextraction of metals by macrophytes. Even though the soil's metal load is high, its availability is directly controlled by soil pH. Hence soil pH is the major factor that regulates the metal absorption by plant roots. The absorbed metals are further translocated to plant parts. In the case of hyperaccumulators, the metals get deposited / sequestered in vacuoles or cell membranes or chelated in cytosol with the aid of phytochelatin synthetase. The sediment / soil pH is discussed below.

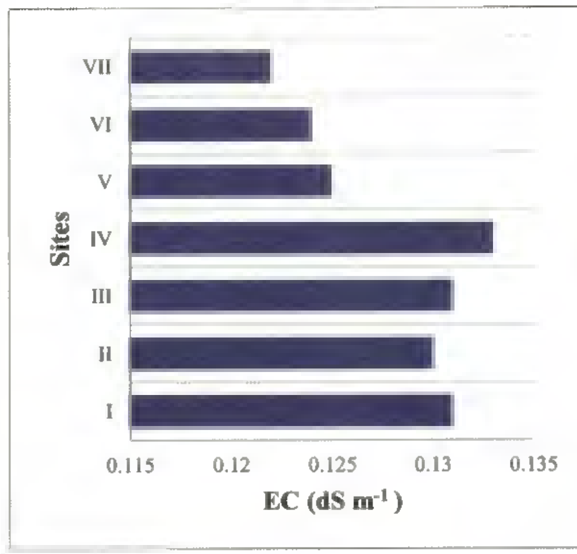
The rhizosphere pH at all sites was acidic in reaction. In the case of aquatic macrophytes the sediment pH ranged from 4.99 to 5.89 and for terrestrial group, soil pH ranged from 5.04 to 5.86 (Table 31). Acidic pH promotes the availability of cationic metals like Fe, Al, Pb and Cd. When the site wise mean pH was compared, the variation was comparatively small ranging from 5.12 to 5.60 (Fig. 5a). Since all the sites are closely located, naturally site wise variation will be less. The species wise pH ranged from 5.12 to 5.59. Thus both site wise and species wise variation of rhizosphere pH was marginal. Hence rather than the species / soil factors, pH is mainly controlled by the geogenic properties of soil (Stewart *et al.*, 2003). Among the sites, pH was highest at Manamukku and lowest at Arattukadavu RB. At Arattukadavu RB human interference was more compared to other sites. When the macrophytes were compared, among the emerging types *M. vaginalis* (5.43) recorded the highest and *C. esculenta*, the lowest rhizosphere pH (5.26). In the case of floating types, *N. indica* (5.58) recorded the highest and *N. nouchali*, the lowest (5.20). For

Fig. 5 pH, EC and organic carbon content of rhizosphere sediment /soil (mean values)

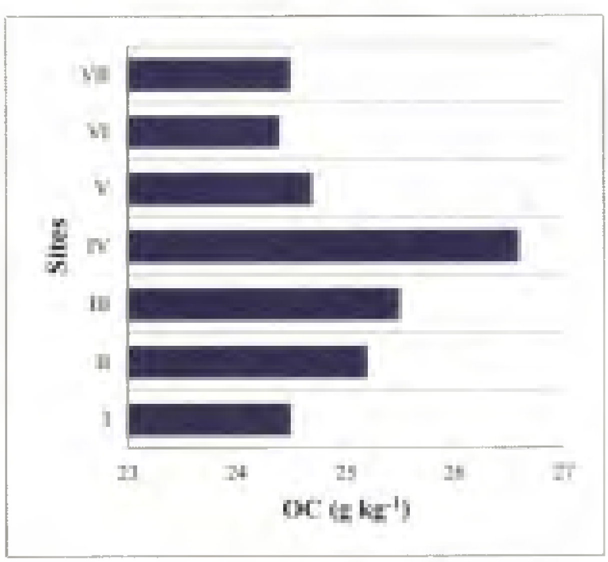
5a. pH



5b. EC



5c. Organic carbon



I- Palappoor	II - Pallichalthodu	III – Reservoir bund	IV- Arattukadavu RB
V - Valiyavilagam	VI - Mannamvarambu	VII - Manamukku	

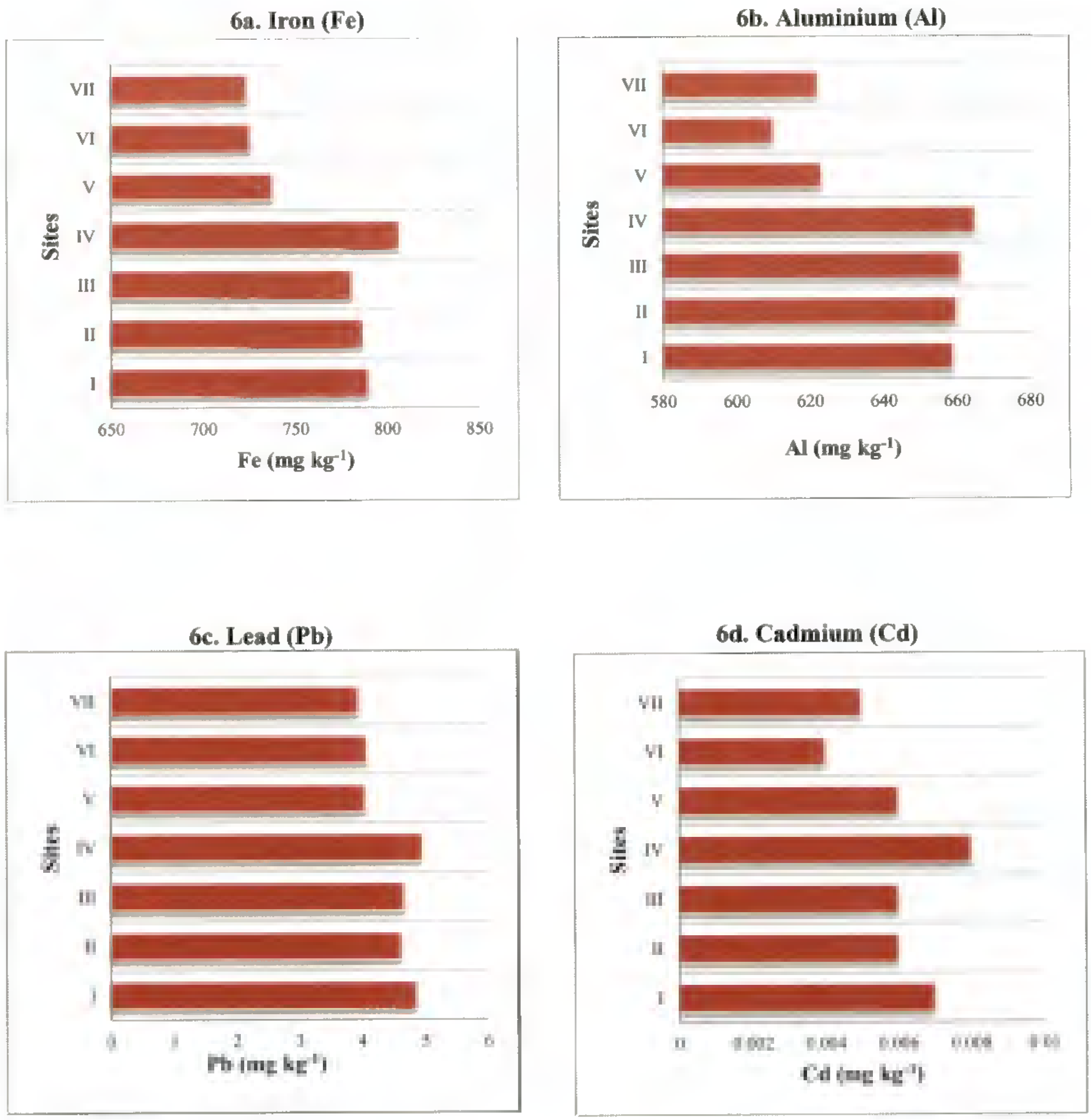
terrestrial types, the highest pH was obtained for *B. dactyoides* (5.57) and both *A. sessilis* and *G. tubiflorum*, the lowest (5.12).

EC of the sediment / soil which is an indicator of the soluble salts, showed the highest value at Arattukadavu RB and the lowest at Manamukku. Rhizosphere EC ranged from 0.122 to 0.133 dS m^{-1}. Sitewise variation was very marginal. As in the case of lake water (Fig. 5b) behavior of sediment EC was just reverse of pH. More human interventions at Arattukadavu RB resulted in a higher waste load and this might have caused an increase in EC and a decrease in pH. When the species wise variation was compared, it ranged from 0.104 to 0.146 dS m^{-1}. EC was comparatively higher for terrestrial group (Table 32). *E. colona, I. aquatica* and *S. trilobata* recorded the highest values among the emergent, floating and terrestrial groups, respectively. The lower values of EC indicated lower inorganic contamination (Kiziloglu *et al.*, 2008).

The rhizosphere OC was also highest at Arattukadavu RB and lowest at Mannamvarambu, confirming the presence of more waste dumping at Arattukadavu RB. Among emerging types, it ranged from 21.6 to 26.7 g kg^{-1}, for floating 23.0 to 26.9 and for terrestrials 19.5 to 29.6 g kg^{-1} (Table 33). All sites were rated as high in OC and the site wise variation was only marginal (Fig. 5c) ranging from 24.4 to 25.2. High OC will help to bind the heavy metals in soil and their availability will be reduced (Davis *et al.*, 1998; Bentivegna *et al.*, 2004) allowing the plants to thrive under high contamination.

Among the tested heavy metals, available Fe content was very high at all sites. Since the ecosystem has been located in a lateritic belt, naturally the Fe content will be high and the wetland situation keep it in available form rating the status high. The response of Fe under submerged conditions has been well explained by Sahrawat (2003). The rhizosphere Fe_{av} of the study area ranged from 724 mg kg^{-1} at Manamukku to 807 mg kg^{-1} at Arattukadavu RB (Fig. 6a). As the pH decreases, the availability of Fe increases. The lowest pH and highest OC at Arattukadavu RB have

Fig. 6 Available Fe, Al, Pb and Cd contents of rhizosphere sediment / soil (mean values)



I- Palappoor	II - Pallichalthodu	III – Reservoir bund	IV- Arattukadavu RB
V - Valiyavilagam	VI - Mannamvarambu	VII - Manamukku	

promoted higher Fe solubilisation and bioavailability. In general, the cationic elements like Fe, Al, Pb and Cd increases with decrease in pH (Tyler and Olsson, 2001). Among the species, it ranged from 710 to 794 mg kg^{-1} for emergent types, 741 to 809 mg kg^{-1} for floating and 693 to 814 mg kg^{-1} for terrestrials. Since the sediment is continuously under submerged condition, the sediment Fe might have reached equilibrium with Fe content of water. This might be the reason for lesser difference between highest and lowest for aquatic species. Even under such a high level of Fe_{av}, the macrophytes performed satisfactorily, indicating their ability to thrive under higher Fe_{av} concentration and their suitability for phytoremediation (Table 34). Similar ability of aquatic plants to tolerate high concentration of Fe was reported by Mazumdar and Das (2015).

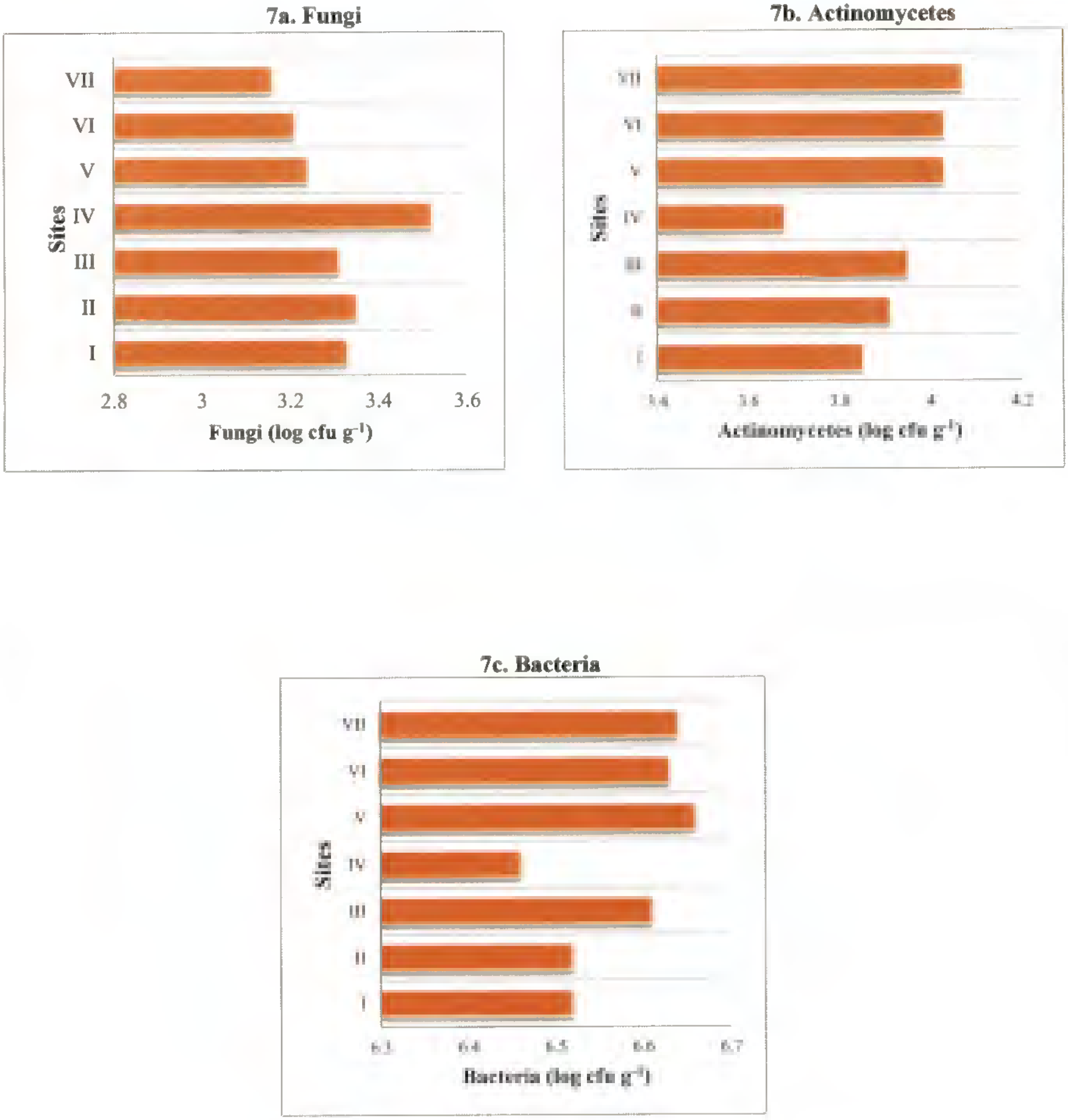
The rhizosphere Al_{av} content of the study area was high due to its lateritic origin and Arattukadavu RB recorded the highest value and lowest by Manamukku (Table 35). The availability of Al is following a negative relation with pH where the availability increases with decreasing pH (Melakeberhan *et al.*, 1995). Rhizosphere Al_{av} of macrophytes ranged from 594 to 670 mg kg^{-1} for emergent type, 615 to 670 mg kg^{-1} for floating and 605 to 660 mg kg^{-1} for terrestrials. The site wise variation was from 610 to 665 mg kg^{-1}. A striking difference in Al_{av} of rhizosphere among the species or sites was not observed (Fig. 6b). Since all the sampling sites are closely located, general soil characteristics might be controlling the availability of most of the metals.

The study area showed the presence of small quantities of Pb in all the sampling sites as reported by Kumar (2005), indicating its susceptibility to Pb contamination. This might be mainly due to dumping of solid wastes including package / storage containers and nearness to automobile servicing centres. The rhizosphere Pb_{av} was also highest at Arattukadavu RB and lowest at Manamukku. Here also the availability is following a negative relation with pH as reported by Zeng *et al.* (2011). Among the species, the mean values ranged from 4.20 to 5.64 mg kg^{-1} for emergent type, 3.84 to 5.28 mg kg^{-1} for floating, and 3.57 to 5.41 mg kg^{-1} for terrestrials (Table 36). Mean values of rhizosphere Pb_{av} of sites varied from 3.95 to 4.96 mg kg^{-1}. An average difference of 1.01 mg kg^{-1} was noticed for the rhizosphere Pb_{av} among the sites (Fig. 6c) and may be due to the difference in Pb contamination sources. Pb being highly toxic metal, proper measures has to be taken to control the increasing presence of Pb in soil / sediment.

The available Cd content in rhizosphere was the smallest, compared to Fe, Al and Pb. Among the sites, it was highest at Arattukadavu RB and lowest at Mannamvarambu. In general Cd_{av} was very low in the study area. Since the organic carbon content was high at all the sites, Cd might have strongly adsorbed to the carboxylic or phenolic groups of OC, reducing its availability (Fig. 6d). Giesy (1983) observed such a phenomenon for Cd in acid soils. Among the macrophytes, it ranged from 0.003 to 0.10 mg kg^{-1} for emergent type, 0.004 to 0.009 mg kg^{-1} for floating and 0.002 to 0.009 mg kg^{-1} for terrestrials (Table 37). The site wise variation was very much negligible. A difference of 0.004 mg kg^{-1} was noticed for the Cd_{av} among the sites. Cd contamination in the study area might be from agriculture or through unsegregated solid wastes like corroded pipes, e-waste, battery etc. Precautionary measures have to be advocated to protect the area from Cd contamination.

In general, Fe and Al are mainly responsible for the degradation of the ecosystem. Pb and Cd are contributing to it, creating further aggravated conditions. Hence lake water has to be protected from the entry of such contaminants. Pollution through heavy metals is a major hazard to the aquatic ecosystem and to the society as a whole, when it comes to a fresh water entity. This is where the role of phytoremediators becomes important where they are able to decontaminate a system causing minimum pressure to the ecosystem (Verma *et al.*, 2016).

Fig. 7 Microbial population of rhizosphere sediment / soil of selected sites



I- Palappoor	II - Pallichalthodu	III – Reservoir bund	IV- Arattukadavu RB
V - Valiyavilagam	VI - Mannamvarambu	VII - Manamukku	

5.2.3. Microbial load of rhizosphere soil / sediment

The presence of microbes in a soil is mainly controlled by pH. In general, fungi predominate in the acidic range and bacteria and actinomycetes in neutral range. This contrasting nature of soil microflora was reported by Rousk *et al.* (2009) and here also the same trend was observed. Arattukadavu RB having the lowest pH showed the predominance of fungal population and Manamukku having the highest pH had the dominance of bacteria and actinomycetes (Tables 38 to 40). Fungal count varied from 3.16 log cfu g^{-1} at Manamukku to 3.52 log cfu g^{-1} at Arattukadavu RB (Fig. 7a). Comparing the macrophyte wise variation for fungal count, it was observed that the count was lowest for *N. nucifera* (2.97 log cfu g^{-1}) and highest for *L. flava* (3.70 log cfu g^{-1}) among the emergent types. For floating macrophytes, fungal count ranged from 2.88 to 3.33 log cfu $g^{-1}$and for terrestrial from 3.06 to 3.91 log cfu g^{-1}.

Comparing the bacterial population among different sites, the values ranged from 6.46 log cfu g^{-1} at Arattukadavu RB to 6.66 log cfu g^{-1} at Valiyavilagam (Fig. 7c). The species wise variation was from 5.89 log cfu g^{-1} for *V. natans* to 7.05 for *C. dactylon*. The actinomycete population was lowest at Arattukadavu RB and highest at Manamukku (Fig. 7b). The mean values ranged from 3.68 to 4.07 log cfu g^{-1}. The species wise variation was from 3.56 to 4.40 log cfu g^{-1} for emergent types, 3.5 to 3.92 log cfu g^{-1} for floating and 2.92 to 4.41 log cfu g^{-1} for terrestrial. According to Hiroki (1992), fungi are the least affected by heavy metals while actinomycetes, the most.

The bacterial population showed negative correlation with rhizosphere metal content and the highest negative correlation was with Al (-0.443) and Pb, the lowest (-0.032). Actinomycetes behaved in a similar manner except that it showed a positive correlation with Pb (0.031). Fungi exhibited a positive correlation with sediment Fe, Al, Pb and Cd and the highest correlation was with rhizosphere Al content (0.338). Lenart and Wolny-Koladka (2013) also obtained a weak negative correlation between bacterial and actinomycete population and heavy metal content of soils.

5.2.4. Phytoextraction and accumulation of metals by native macrophytes

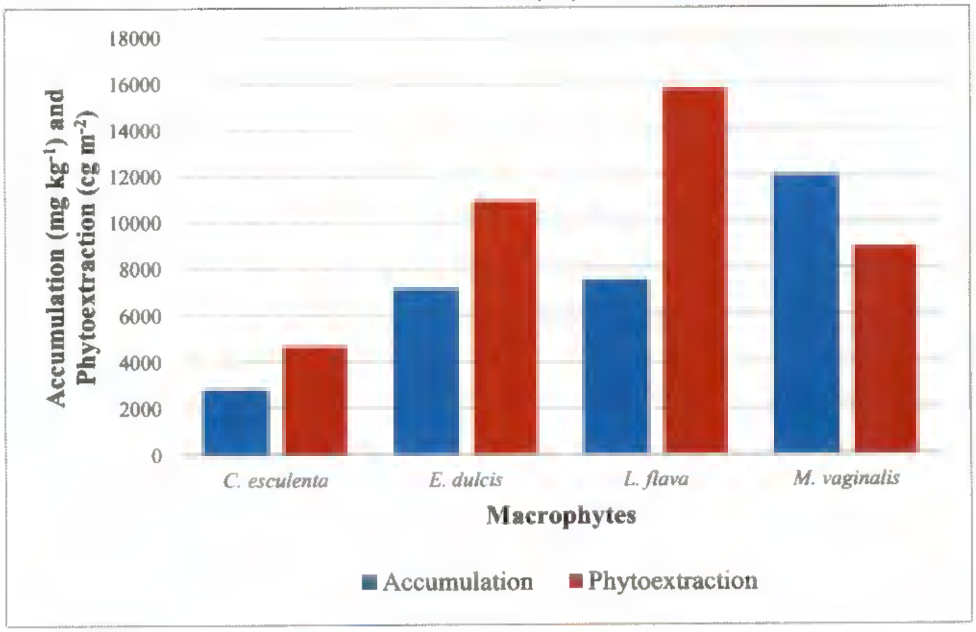
The phytoextraction of metals by macrophytes is a function of its biomass production and metal extraction from the growing medium and its accumulation in plant parts. Hence phytoextraction ability is controlled by biomass production, metal concentration and accumulation in plant parts. A setback in any of the above will decrease the phytoremediation capacity. A macrophyte that possesses a high rating for all the three characters could really perform as an excellent phytoextractor.

Looking into the phyoextracting ability of emerging macrophytes for Fe, it was observed that the Fe concentration in shoot was highest for *M. vaginalis* under emergent type followed by *L. flava* and *E. dulcis* (Table 23). For root also, Fe concentration followed the same trend (Table 24). But Fe removal per square metre was highest for *L. flava*, followed by *E. dulcis*, which was second in shoot and root Fe concentration (Fig. 8a and 8b). Higher biomass production by *L. flava* resulted in the highest Fe extraction. The phytoremediation potential of *L. flava* was reported by several workers (Abhilash *et al.*, 2009; Kamrudzaman *et al.*, 2012; Anning *et al.*, 2013). But the highest BCF for both shoot and root was for *M. vaginalis* indicating its high potential to extract Fe from soil (Table 43). Several authors support the heavy metal accumulation potential of *M. vaginalis* (Mahmud *et al.*, 2008; Hariyadi *et al.*, 2013).

Under floating type, *N. nouchali* had the highest shoot Fe concentration followed by *S. molesta* and *E. crassipes*. For root, highest value was recorded by *E. crassipes* followed by *P.stratiotes*. The highest quantity was removed by *E. crassipes* followed by *S. molesta* (Fig. 12a and 12b). The phytoremediation potential of *E. crassipes* was reported by several workers (Gamage and Yapa, 2001; Kulkarni *et al.*, 2007; Jayaweera *et al.*, 2008; Aldae and Ojoawo, 2009; Govindaswamy *et al.*, 2011; Ajayi and Ogunbayo, 2012; Padmapriya and Murugesan, 2012; Razak *et al.*, 2013; Sukumaran, 2013; Yang *et al.*, 2014; Rai and Singh, 2016). *N. nouchali* had the highest BCF for shoot and *E. crassipes* for root.

Fig. 8 Accumulation and phytoextraction of iron by dominant emergent macrophytes of Vellayani wetland ecosystem

8a. Shoot Iron (Fe)



8b. Root Iron (Fe)

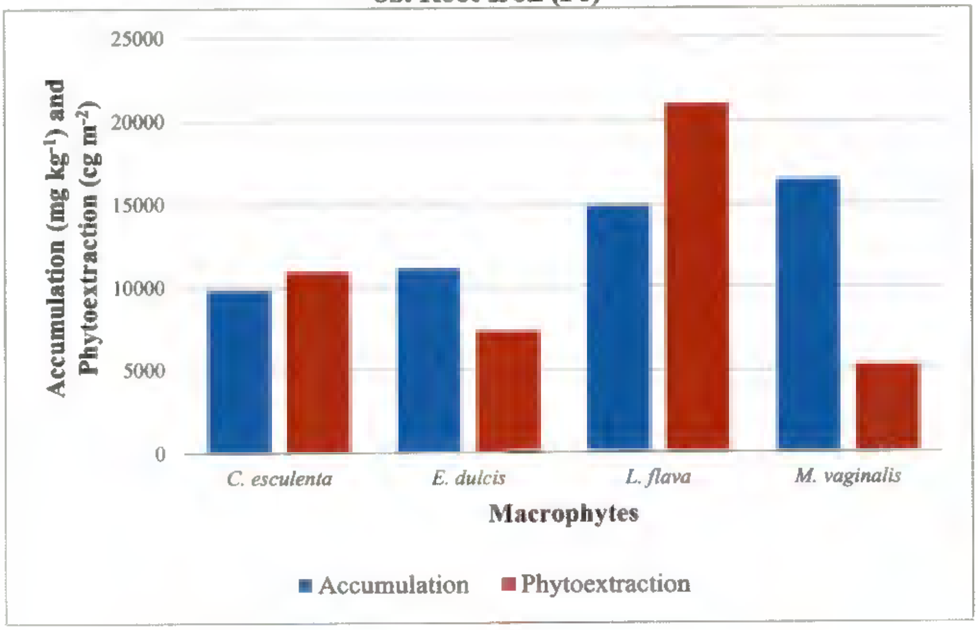
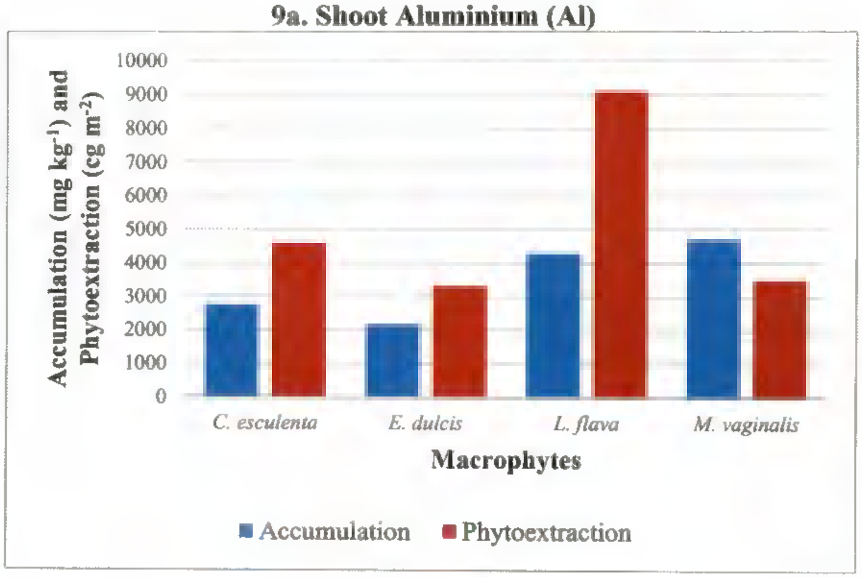


Fig. 9 Accumulation and phytoextraction of aluminium by dominant emergent macrophytes of Vellayani wetland ecosystem



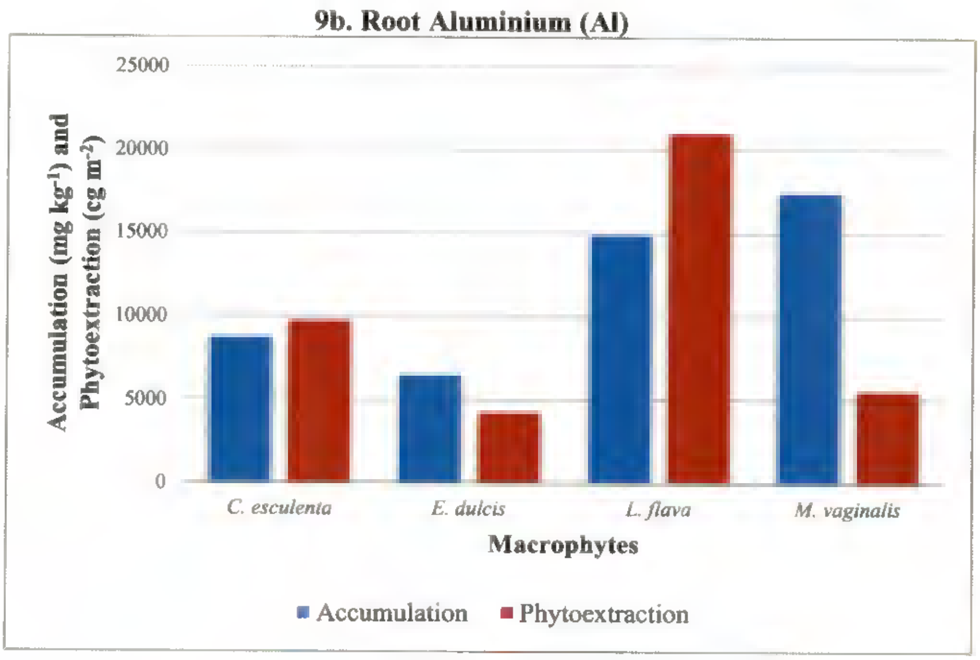
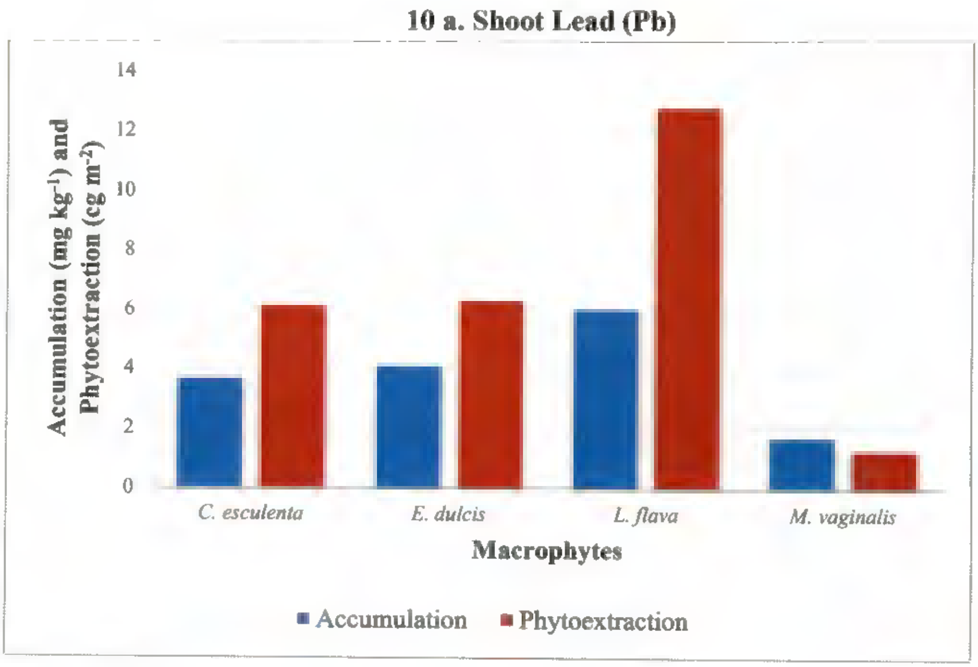


Fig. 10 Accumulation and phytoextraction of lead by dominant emergent macrophytes of Vellayani wetland ecosystem



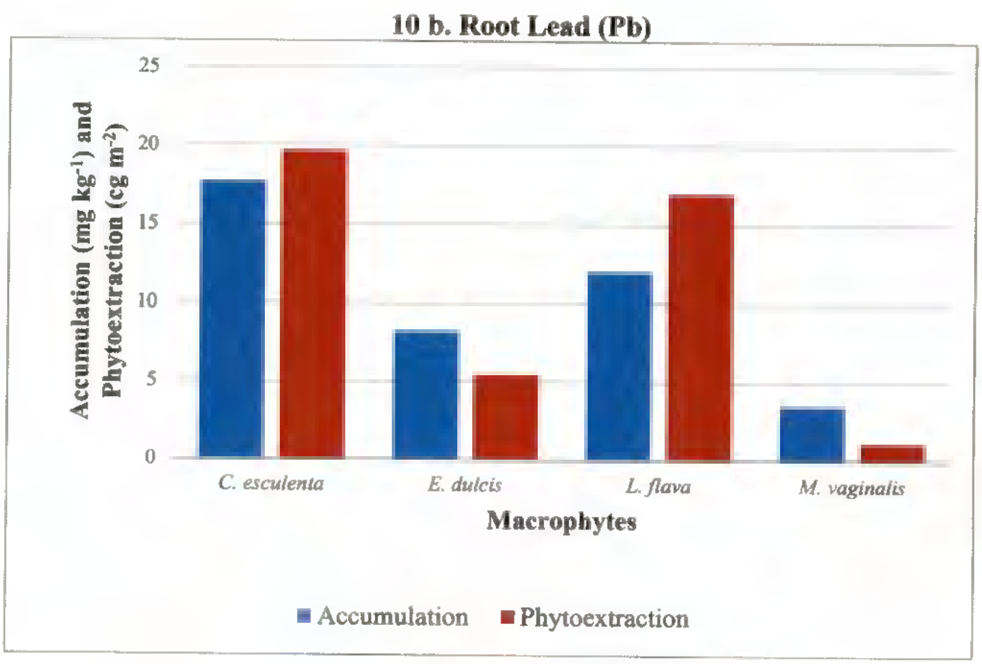
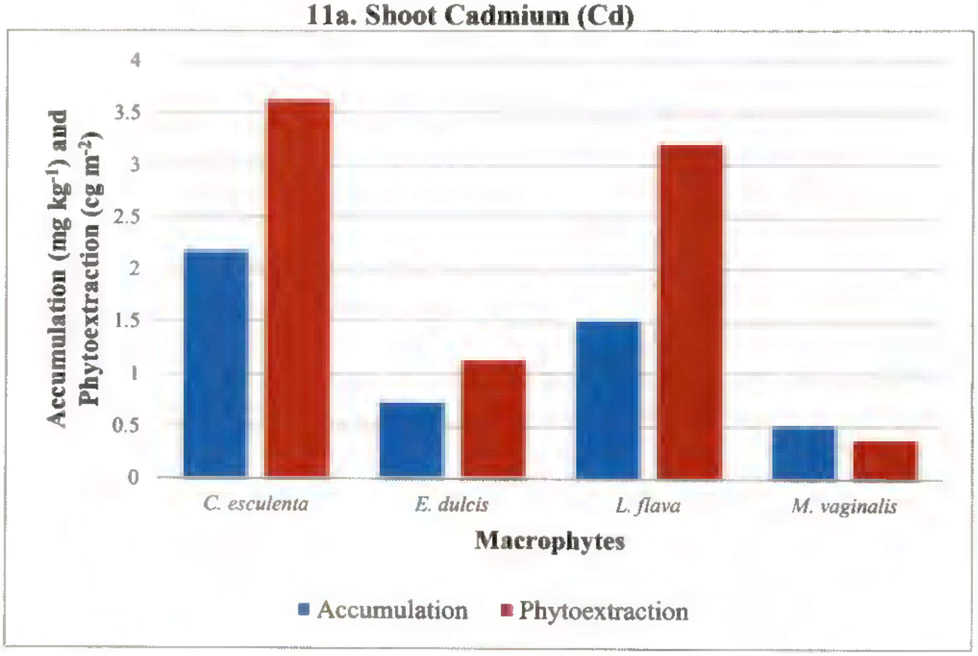
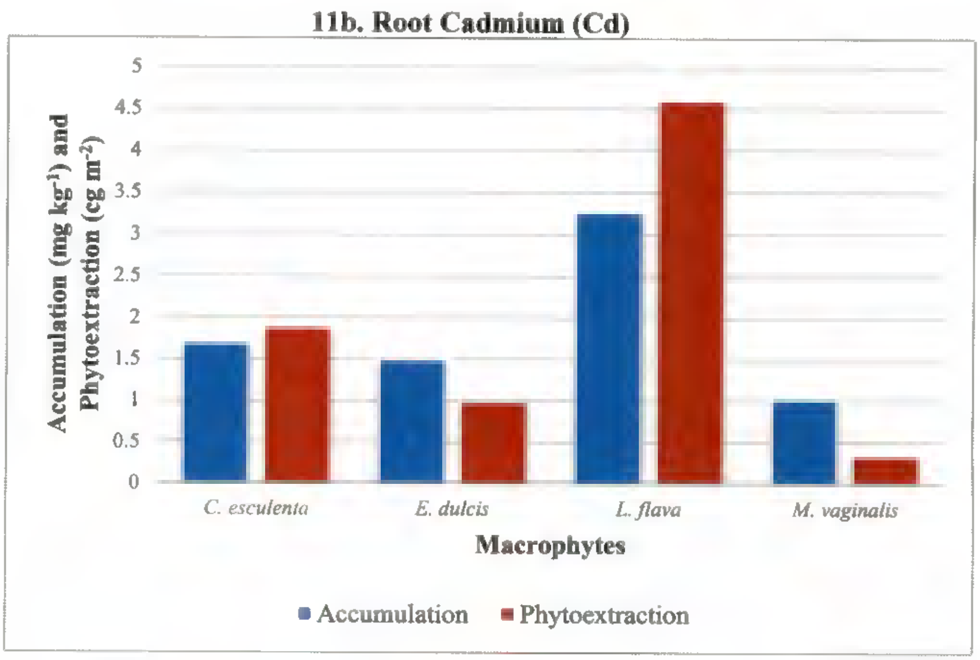


Fig. 11 Accumulation and phytoextraction of cadmium by dominant emergent macrophytes of Vellayani wetland ecosystem





Among the terrestrials, *S. trilobata* recorded the highest Fe concentration in shoot followed by *P. hydropiper* and *A. tenella*. For root, the highest value was for *C. dactylon* followed by *B. dactyoides*. But the highest quantity was extracted by *P. hydropiper*, followed by *P. repens* clearly due to higher biomass production (Fig. 16a and 16b). The ability of *C. dactylon* to accumulate heavy metals in plant parts have already been reported by Shu *et al.* (2002), Soleimani *et al.* (2009), Singh *et al.* (2013) and Maiti *et al.* (2015), but the low biomass production never allowed it to be an good or excellent phytoremediatior. Among the terrestrial group shoot BCF was highest for *P. hydropiper* and root BCF for *C. dactylon* (Table 43). High BCF values in shoot and root of *C. dactylon* for heavy metals was reported by Maiti *et al.* (2015).

The phytoextracting ability for Al was evaluated based on the Al concentration in plant parts and the total quantity of Al extracted from the soil / sediment. Among the emergent types, highest concentration in shoot was recorded by *M. vaginalis*, followed by *L. flava* and *S. grossus*. *M. vaginalis* recorded the highest value in root, followed by *L. flava* (Fig. 9a and 9b). When the total quantity removed was compared, *L. flava* removed the highest quantity (3112 mg $m\text{-}^2$) followed by *S. grossus* whose concentration in shoot and root were not that much high, but the higher biomass production favoured better Al extraction. When BCF values were compared, the highest BCF for both shoot and root was recorded by *M. vaginalis*.

Among the floating type, highest concentration in shoot was for *N. nouchali* followed by *S. molesta* and *E. crassipes*. For root also, highest value was for *N. nouchali* followed by *E. crassipes* and *S. molesta*. Total Al extracted was highest for *E. crassipes* followed by *N.nouchali* and *S. molesta*. The difference in biomass production accounted this behaviour (Fig. 13a and 13b). When BCF values were compared, it was also highest for *N. nouchali* for both shoot and root indicating its high extraction ability. Since *E. crassipes* had higher biomass it was able to phytoextract more Al from soil / sediment.

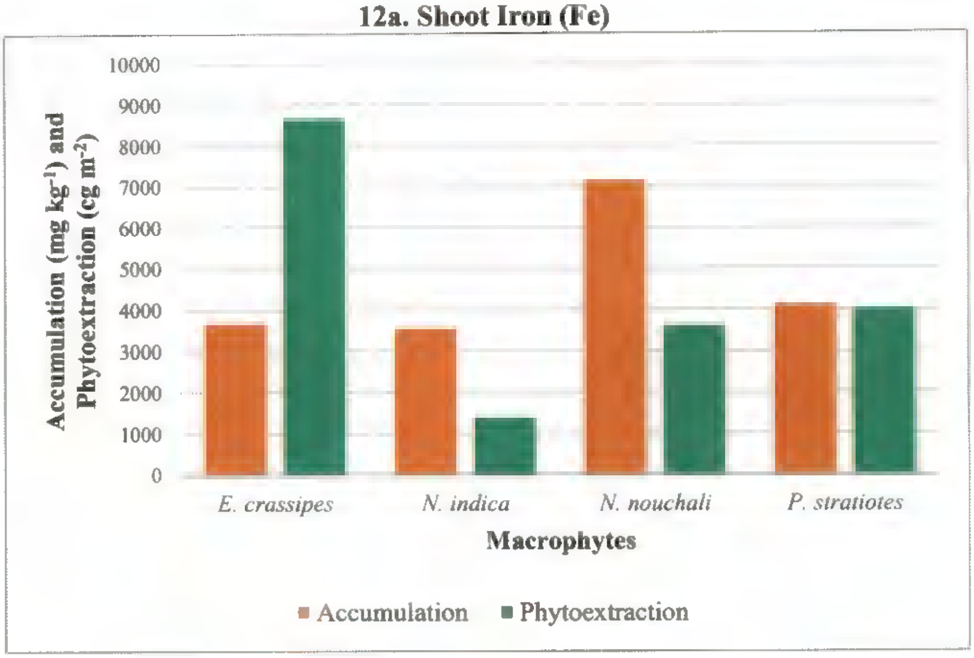
In the case of terrestrial macrophytes, the Al concentration in shoot was highest for *P. repens* followed by *B. dactyoides* and *P. kaida*. For root, *C. dactylon* showed the highest concentration followed by *P. repens* and *B. dactyoides*. Al concentration in *C. dactylon* was very high but the biomass production being very low, the quantity removed was very small compared to the macrophytes having lower concentration (Fig. 17a and 17b). *P. repens* had removed the highest quantity of Al from soil followed by *P. kaida*. According to Yetneberk *et al.* (2013) wide variation in Al accumulation was noticed even within closely related species indicating the species as well as site wise preference in phytoextraction of Al. The BCF values were highest for *P. repens* and *C. dactylon,* respectively for shoot and root.

All the selected macrophytes had shown the presence of Pb in shoot ranging from 0.72 mg kg^{-1} for *E. colona* to 10.35 mg kg^{-1} for *S. trilobata* (Tables 27 and 28). The macrophytes showed much variation in their shoot concentration evidently due to the difference in phytoextraction ability of different species.

Among the emergent types, highest shoot Pb concentration was recorded by *L. flava* followed by *S. grossus* and *M. vaginalis*. For root, the highest value was recorded by *C. esculenta* followed by *L. flava* and *N. nucifera*. But the highest quantity was extracted by *C. esculenta* followed by *S. grossus* (Fig. 10a and 10b). For *C. esculenta*, the shoot BCF for Pb was less than one while root BCF was 3.51 and its shoot concentration was very low compared to root. However, it had removed highest quantity of Pb from unit area. For *S. grossus*, both shoot and root BCF were above one.

Among the floating types, highest concentration in shoot was for *E. crassipes* followed by *P. stratiotes* and *N. indica*. In the case of root, concentration was highest for *P. stratiotes* followed by *N. indica* and *N. nouchali*. But the highest quantity was removed by *E. crassipes* followed by *N. nouchali* and *P. stratiotes*. This is also a true reflection of biomass production (Fig. 14a and 14b).

Fig.12 Accumulation and phytoextraction of iron by dominant floating macrophytes of Vellayani wetland ecosystem



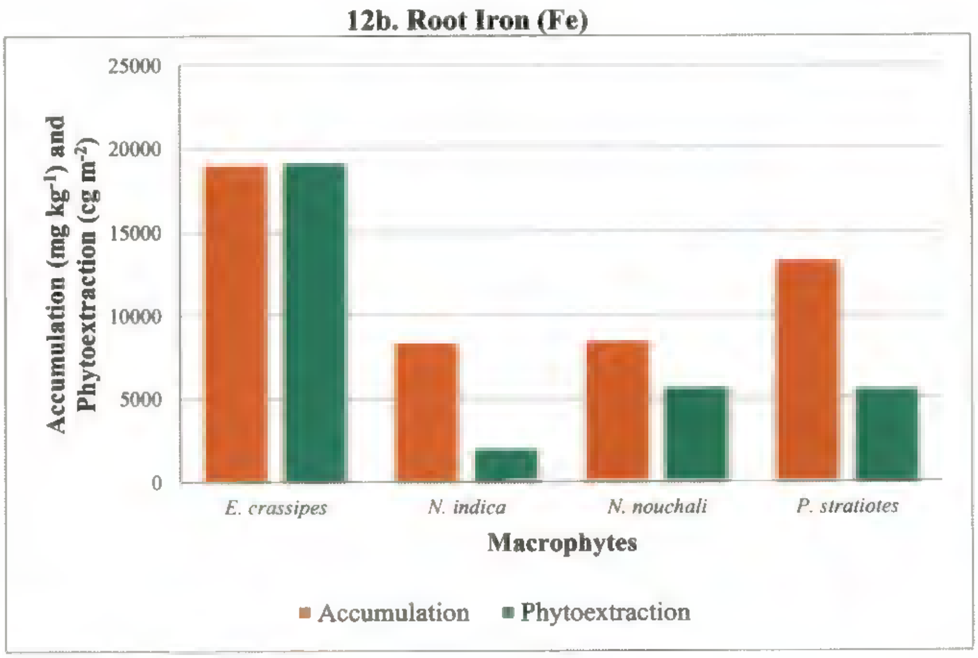
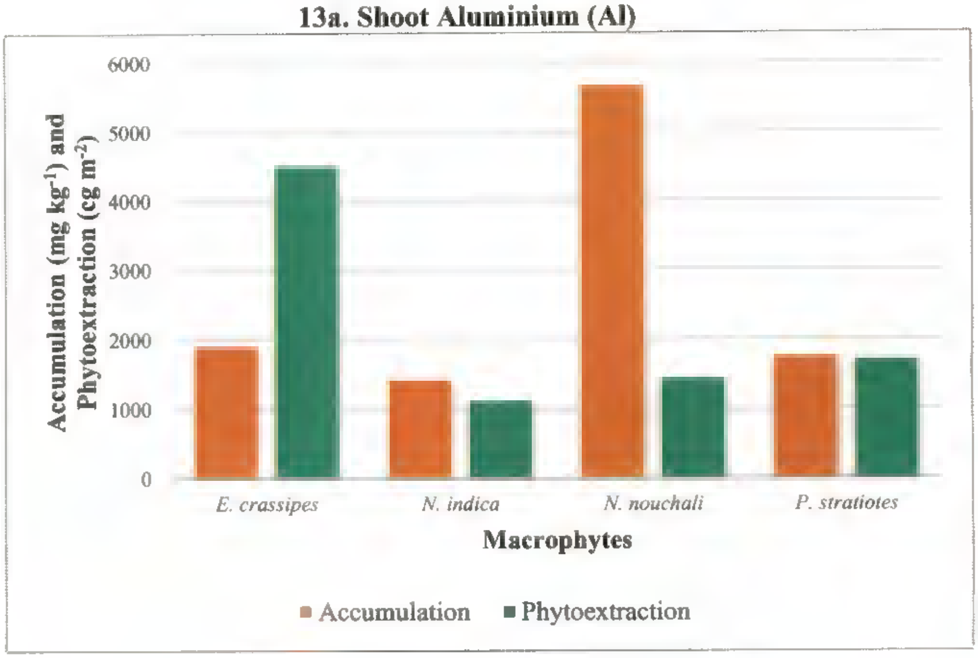


Fig.13 Accumulation and phytoextraction of aluminium by dominant floating macrophytes of Vellayani wetland ecosystem



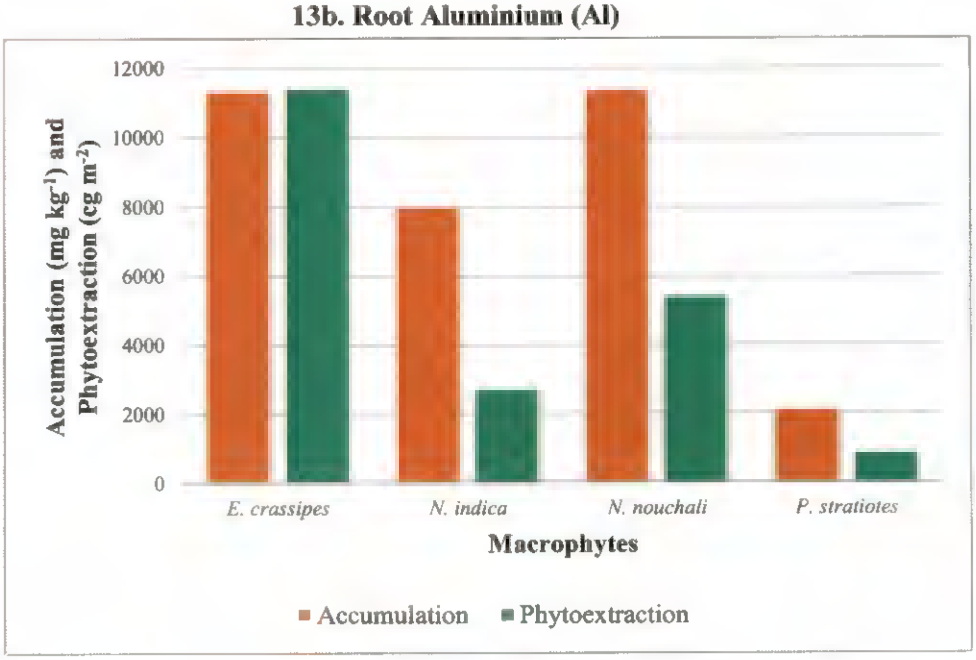
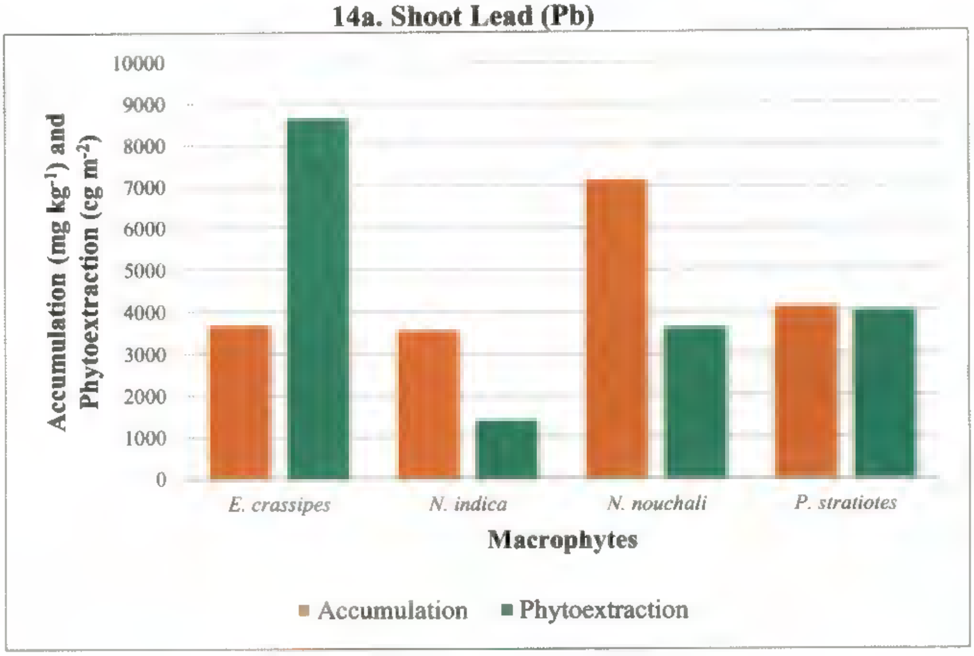


Fig.14 Accumulation and phytoextraction of lead by dominant floating macrophytes of Vellayani wetland ecosystem



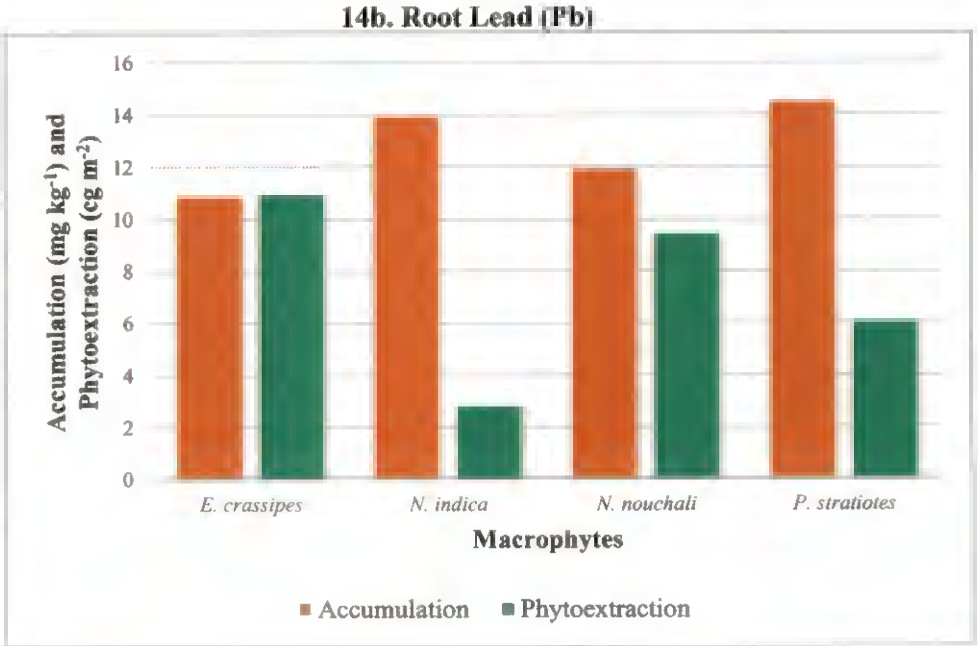
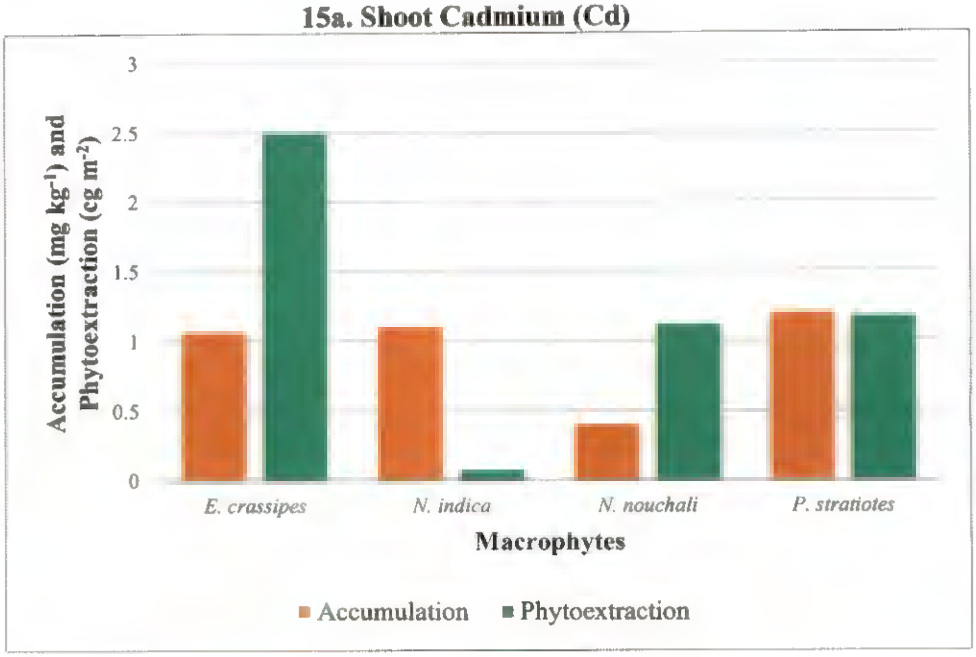
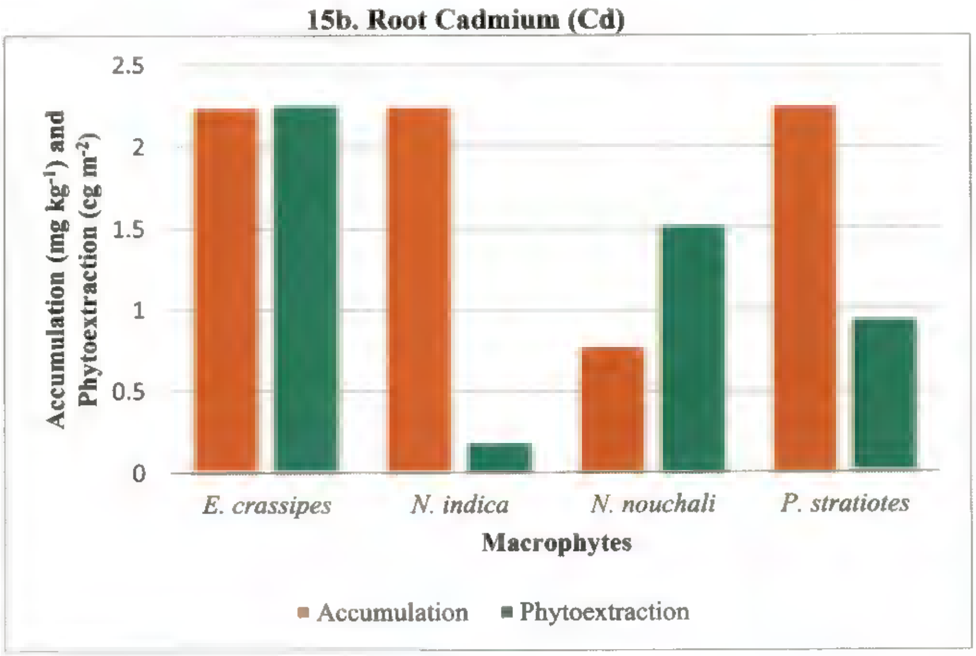


Fig.15 Accumulation and phytoextraction of cadmium by dominant floating macrophytes of Vellayani wetland ecosystem





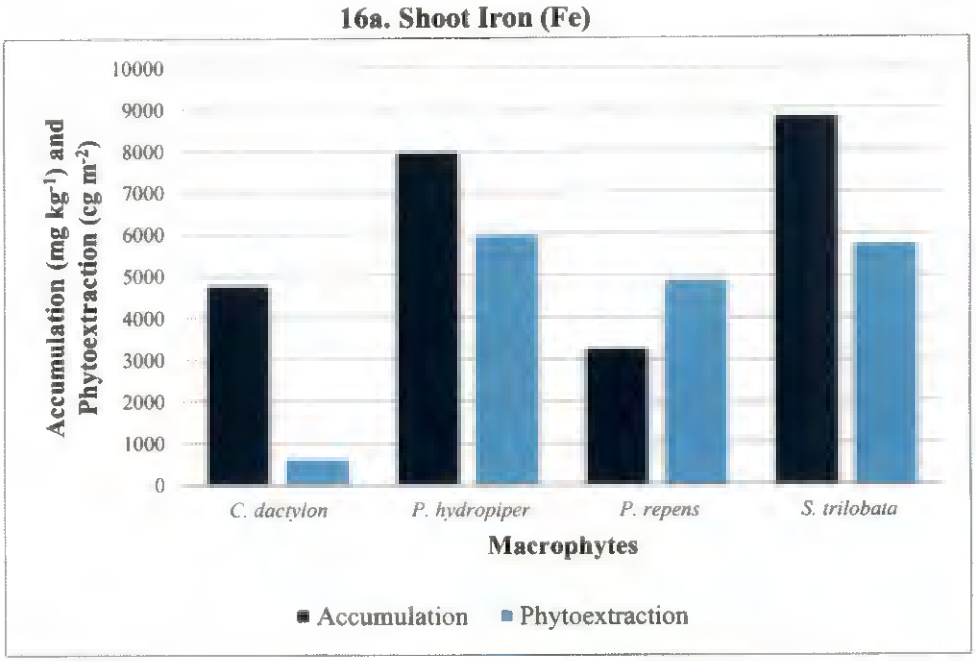
Among the terrestrial types, the highest concentration of shoot Pb was for *S. trilobata* followed by *C. dactylon* and *I. globosa*. For root, *C. dactylon* recorded the highest value followed by *B. dactyoides* and *B. orientale*. The highest quantity was removed by *S. trilobata*. Compared to this, the quantity of Pb extracted by other macrophytes was very small (Fig. 18a and 18b). The low content of Pb in soil and low extracting ability as evidenced by low BCF values (Table 43) might have created such a situation. When BCF values for Pb were compared, it was much lower to that for Fe and Al.

The plant availability of Cd was very low in soil / sediment but all the selected macrophytes had shown its presence in both shoot and root (Tables 29 and 30). Among the emergent types, highest shoot concentration was recorded by *C. esculenta* followed by *L. flava* and *N. nucifera*. For root, it was highest for *L. flava* followed by *N. nucifera* and *C. esculenta*. Highest quantity of Cd was extracted by *L. flava* followed by *C. esculenta*, (Fig. 11a and 11b). Abhilash *et al.* (2009) reported that *L. flava* could be used as a phytoremediator in filtering the low level Cd contamination in water bodies and it showed a very high BCF and TF for Cd. The highest BCF for shoot was reported for *N. nucifera* and the lowest for *E. colona*. For root, *C. esculenta* and *E. colona* respectively. The highest quantity was extracted by *C. esculenta* and *E. dulcis* for shoot and root respectively.

Among the floating types, highest concentration in shoot was for *P. stratiotes* followed by *N. indica* and *E. crassipes* and root also followed the same trend (Fig. 15a and 15b). The highest quantity was extracted by *E. crassipes* followed by *P. stratiotes*. However, the BCF was highest for *E. crassipes* for both shoot and root (Table 43). It had higher accumulation in shoot compared to root.

Among the terrestrial types, the highest concentration in shoot was for *S. trilobata* followed by *B. dactyoides* and *C. dactylon*. For root also, *S. trilobata* recorded the highest concentration followed by *A. tenella* and *B. dactyoides*. The highest quantity was removed by *S. trilobata,* which was in line with Cd

Fig. 16 Accumulation and phytoextraction of iron by dominant terrestrial macrophytes of Vellayani wetland ecosystem



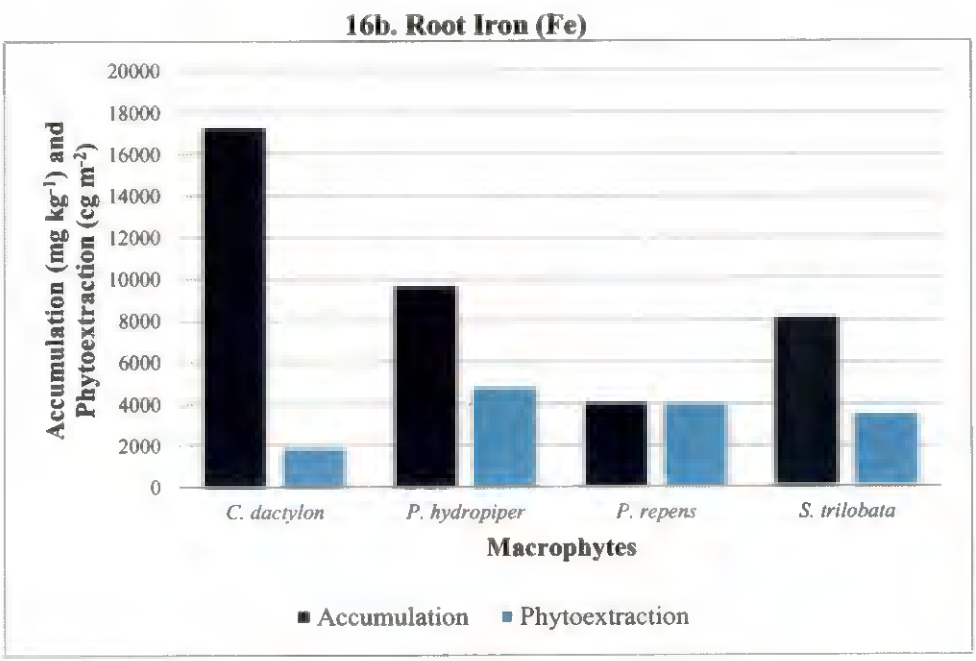
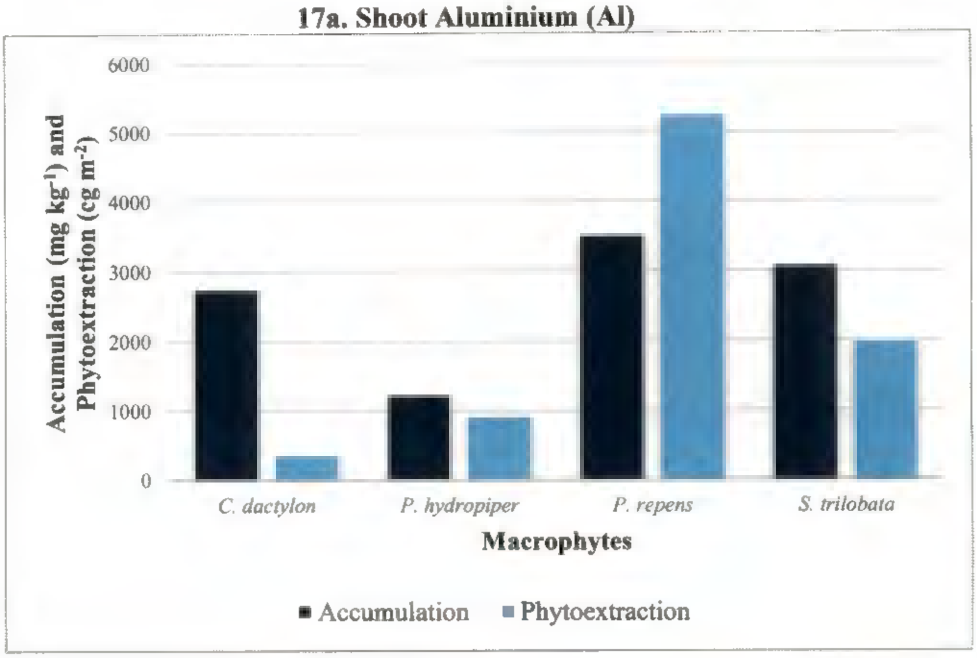


Fig. 17 Accumulation and phytoextraction of aluminium by dominant terrestrial macrophytes of Vellayani wetland ecosystem



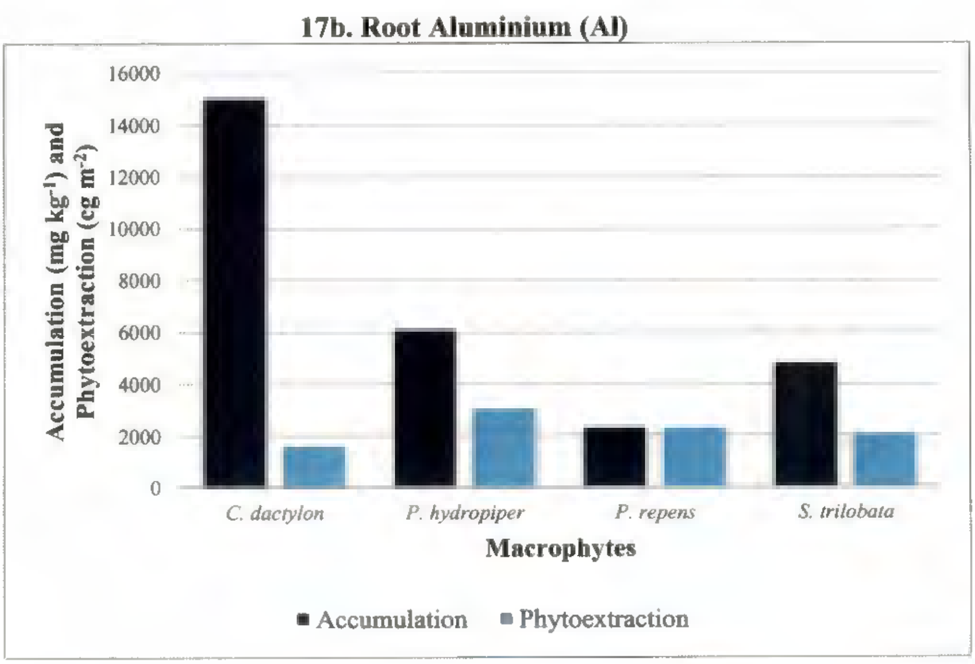
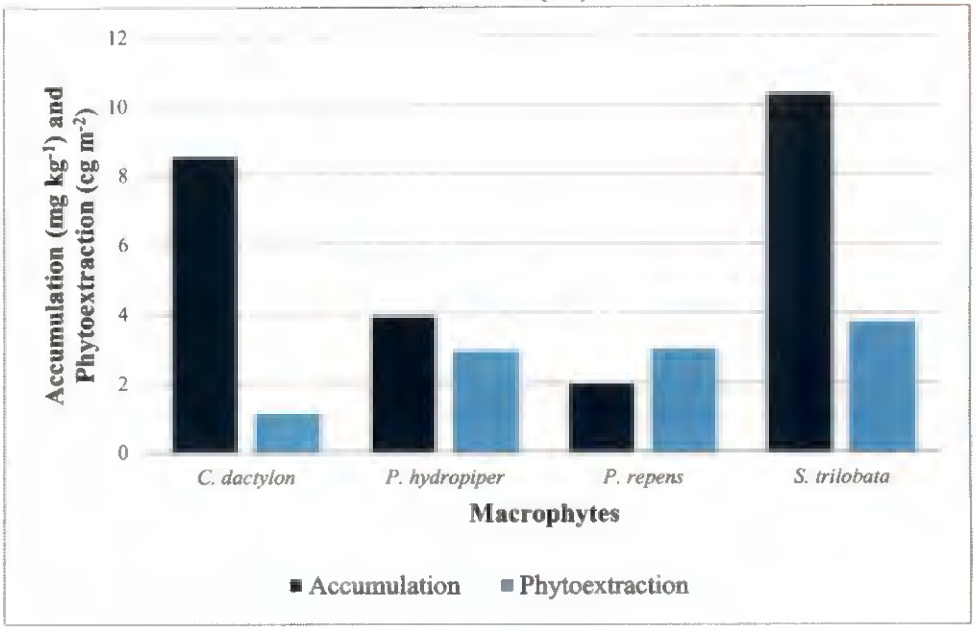


Fig. 18 Accumulation and phytoextraction of lead by dominant terrestrial macrophytes of Vellayani wetland ecosystem

18a. Shoot lead (Pb)



18b. Root lead (Pb)

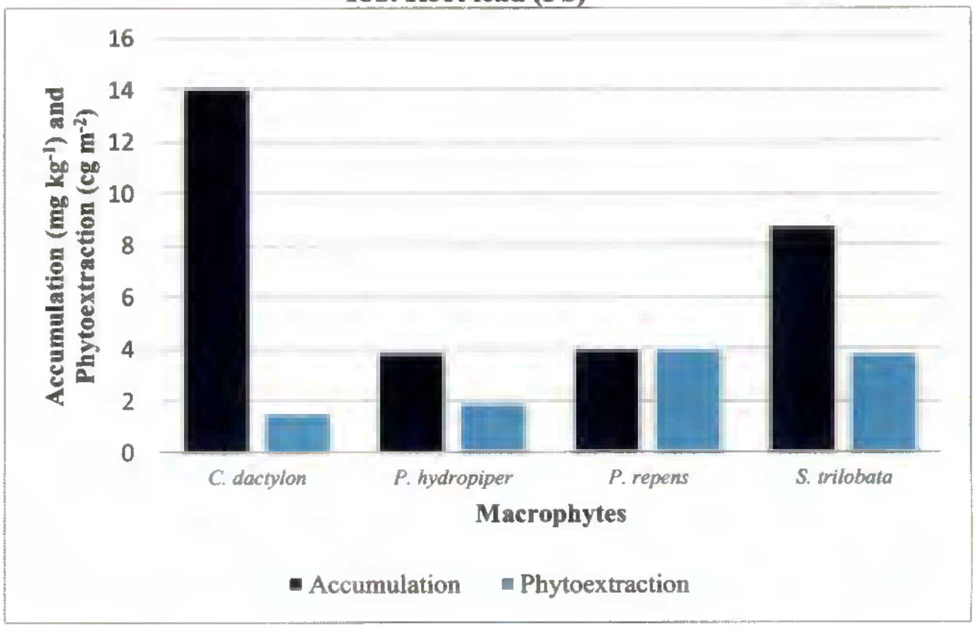
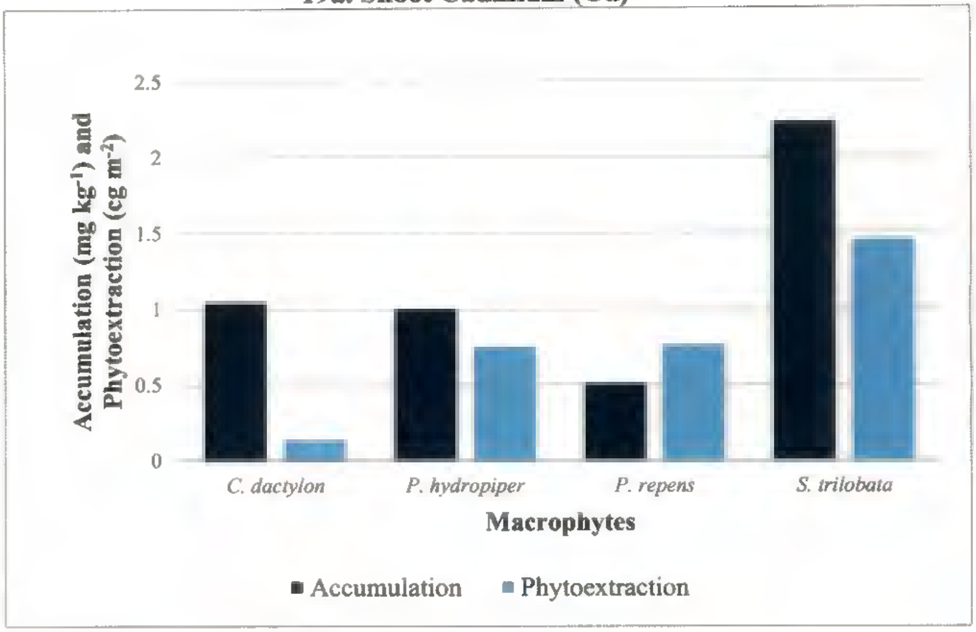
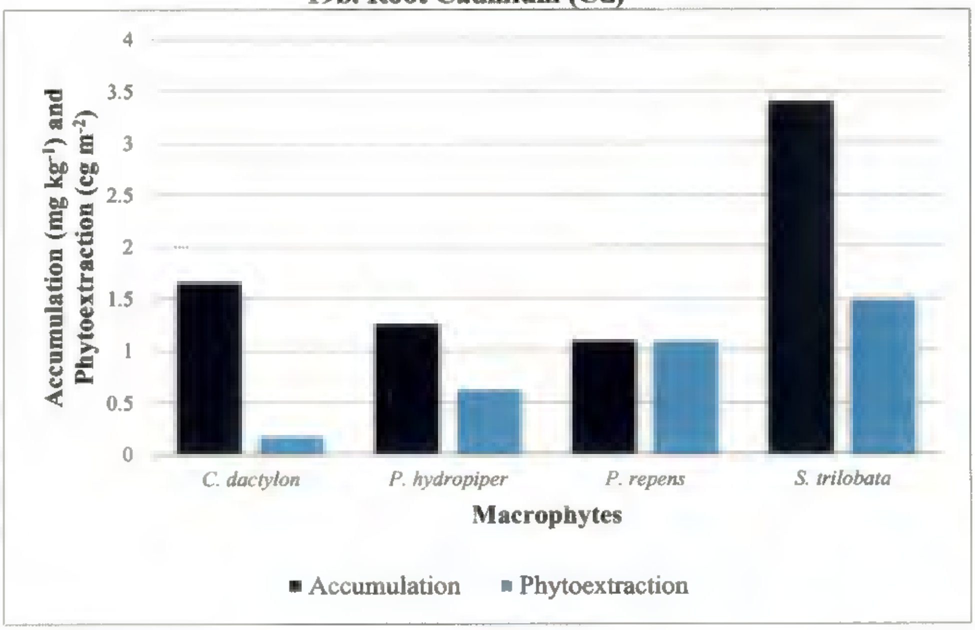


Fig. 19 Accumulation and phytoextraction of cadmium by dominant terrestrial macrophytes of Vellayani wetland ecosystem

19a. Shoot Cadmium (Cd)



19b. Root Cadmium (Cd)



concentration. BCF values for Cd were the highest compared to Fe, Al and Pb. Though the Cd content was very small, macrophytes were able to extract it efficiently and translocate to upper parts (Fig. 19a and 19b). There are several reports indicating the Cd accumulation of aquatic macrophytes (Eid *et al.*, 2012; Das *et al.*, 2014; Liu *et al.*, 2014; Chayapan *et al.*, 2015).

For all the selected macrophytes, BCF values were above one except a few, which made these macrophytes to be rated as phytoaccumulators of Fe, Al, Pb and Cd. BCF values were highest for Cd and lowest for Pb. Even from the very low Cd_{av}, macrophytes were able to extract and translocate to the plant parts giving very high BCF values.

Correlation between the metal load in sediment / soil, plant accumulation and plant uptake by macrophytes was calculated and presented in Table 41. For Fe, the correlation for shoot / and root accumulation and soil metal content were not significant and the coefficients showed very small values. Even though the soil is high in Fe_{av}, in most of the cases it was not reflected on plant concentration. Fe extracted by different plant organs and the total extraction were also not significant, but the values were higher compared to that of concentration. This was due to the effect of biomass (Liu *et al.*, 2014).

The behaviour of Al was slightly different from that of Fe. Here also, there was no significant relation between soil Al and plant accumulation. But the uptake by root and shoot and total uptake showed significant correlation with soil Al. The correlation was higher for root compared to shoot, showing a better positive relation with root uptake. In the case of plant accumulation also, correlation coefficients were higher for root compared to shoot. Hegazy *et al.* (2013) had also reported significantly higher positive correlations with metal content of root and growth medium.

In the case of Pb, both shoot and root accumulation was not significantly correlated with soil metal content. But the root accumulation showed a significant correlation at 1% level. Uptake being the product of accumulation and biomass, its behaviour is controlled by these two and hence higher values were obtained than that of accumulation. Though the soil Pb content was very low, macrophytes were able to extract and translocate it to different plant parts.

For Cd, both shoot and root accumulation and extraction were significant. The values of correlation coefficients were higher for accumulation rather than uptake. So Cd had showed a better correlation with distribution of Cd within the plant parts compared to the uptake (Mishra and Tripathi, 2008).

The native macrophytes under various categories were evaluated for their phytoremediation potential based on the total quantity of metal extracted, metal accumulation and BCF for shoot and root.

With regard to the phytoremediatin potential of emergent aquatic macrophytes, mainly three species viz., *L. flava, M. vaginalis* and *C. esculenta* dominated in metal extraction and accumulation (Table 67). *L. flava* phytoextraced highest quantity of Fe and Al while it was *C. esculenta* that had removed highest quantity of Pb and Cd from the natural ecosystem.

In the case of floating aquatic macrophytes, four species *E. crassipes, P. stratiotes, N. nouchali* and *N. indica* were the dominant ones. *E. crassipes* extracted the highest quantity of all the four metals studied though it had showed highest concentration for Fe in root and Pb in shoot only. The high biomass production made *E. crassipes* to be rated as the best phytoextractor under natural conditions.

Among terrestrial macrophytes, four species *P. hydropiper, P.repens* and *S. trilobata* and *C. dactylon* were the dominant species of Vellayani wetland ecosystem. Highest quantity of Fe was extracted by *P. hydropiper* and Al by *P. repens*. *S.*

trilobata extracted highest quantity of Pb and Cd. It also showed highest BCF for Pb (shoot) and Cd (both shoot and root).

Table 67. Evaluation of macrophytes for remediation potential for Fe, Al, Pb and Cd

Metal	Macrophyte with highest phytoextraction	Macrophyte with highest shoot concentration	Macrophyte with highest root concentration	Macrophyte with highest shoot BCF	Macrophyte with highest root BCF
Emergent					
Fe	*L. flava*	*M. vaginalis*	*M. vaginalis*	*M. vaginalis*	*M. vaginalis*
Al	*L. flava*	*M. vaginalis*	*M. vaginalis*	*M. vaginalis*	*M. vaginalis*
Pb	*C. esculenta*	*L. flava*	*C. esculenta*	*C. esculenta*	*C. esculenta*
Cd	*C. esculenta*	*C. esculenta*	*L. flava*	*C. esculenta*	*E.dulcis*
Floating					
Fe	*E. crassipes*	*N. nouchali*	*E. crassipes*	*N. nouchali*	*N. nouchali*
Al	*E. crassipes*	*N. nouchali*	*N. nouchali*	*N. nouchali*	*N. nouchali*
Pb	*E. crassipes*	*E. crassipes*	*P. stratiotes*	*E. crassipes*	*N. indica*
Cd	*E. crassipes*	*P. stratiotes*	*P. stratiotes*	*E. crassipes*	*E. crassipes*
Terrestrial					
Fe	*P. hydropiper*	*S. trilobata*	*C. dactylon*	*P. hydropiper*	*C. dactylon*
Al	*P. repens*	*P. repens*	*C. dactylon*	*P. repens*	*C. dactylon*
Pb	*S. trilobata*	*S. trilobata*	*C. dactylon*	*S. trilobata*	*C. dactylon*
Cd	*S. trilobata*	*S. trilobata*	*S. trilobata*	*S. trilobata*	*S. trilobata*

While evaluating the phytoextraction ability of native macrophytes, *L. flava, M. vaginalis, C. esculenta, E. crassipes, P. stratiotes, P. hydropiper*, *P. repens* and *S. trilobata* can be considered as good phytoremediators.

5.3. Performance of selected hyperaccumulators / phytoremediators under graded doses of toxic metals

Identified phytoremediators (from Part II) for Fe, Al, Pb and Cd were grown under graded doses of each metal and their growth, metal accumulation and extraction are discussed here.

5.3.1. Growth performance and phytoextraction of Fe by macrophytes

Nine macrophytes viz., *A. tenella, B. dactyoides, C. esculenta, C. dactylon, E. crassipes, E. dulcis, L. flava, M. vaginalis* and *N. indica* were grown in pots under graded doses of Fe viz., 1000, 2000 and 3000 mg kg^{-1} of soil for 45 days.

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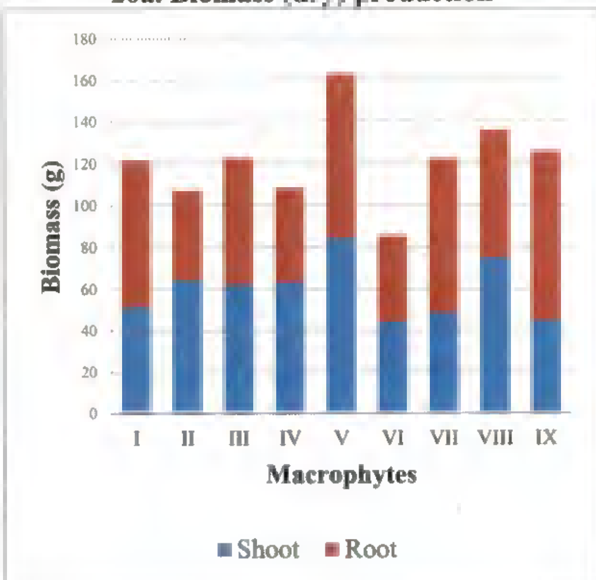
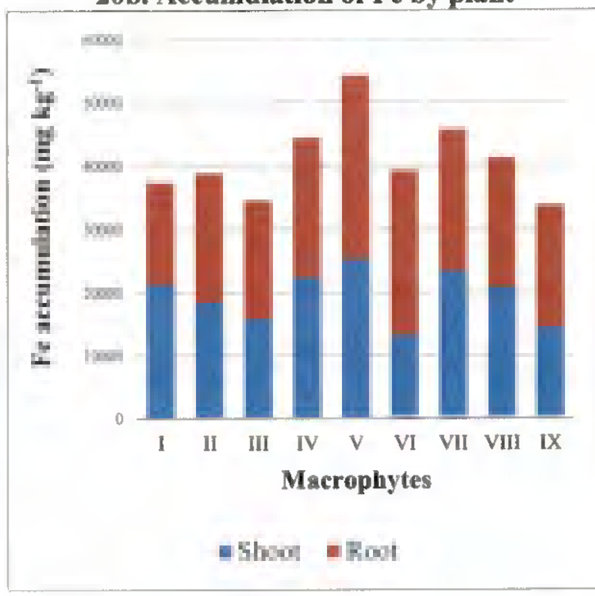
Fig. 20 Biomass (dry) production, iron accumulation and uptake by selected macrophytes under graded doses of Fe

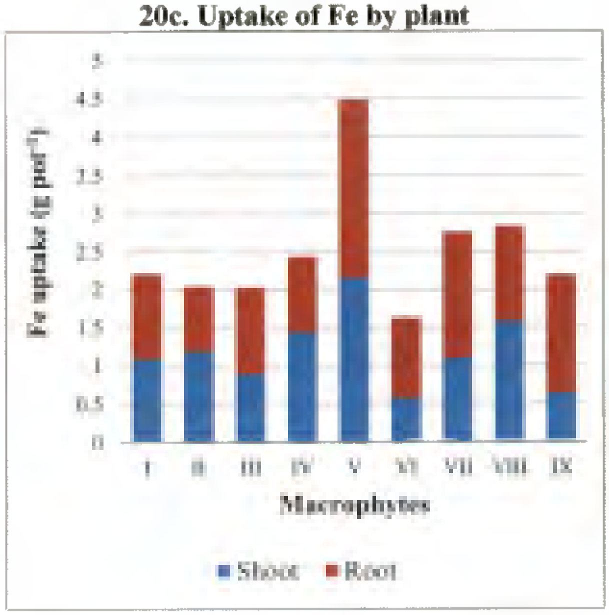
20a. Biomass (dry) production



20b. Accumulation of Fe by plant



20c. Uptake of Fe by plant



I – *A. tenella* II – *B. dactyoides* III – *C. esculenta* IV – *C. dactylon* V – *E. crassipes*
VI – *E. dulcis* VII – *L. flava* VIII – *M. vaginalis* IX – *N. indica*

The macrophytes and graded doses of Fe and their interactions had significant influence on shoot and root biomass, Fe accumulation and total quantity extracted (Table 49). Among the macrophytes, *E. crassipes* recorded the highest shoot biomass and *N. indica*, the root biomass (Fig. 20a). However, the total biomass production was highest for *E. crassipes.* Fe accumulation in shoot and root was also highest for it (Fig. 20b). Looking in to the interaction between graded doses of Fe and biomass production by *E. crassipes,* it was found that the shoot biomass increased up to 2000 mg kg^{-1} while root and total biomass up to the highest level tested i.e., 3000 mg kg^{-1}. Thus *E. crassipes* could tolerate the higher levels of Fe. In general, shoot biomass production decreased with graded levels of Fe but root biomass increased up to the second level and in the case of *E. crassipes*, it preferred a higher level of Fe compared to other macrophytes as evidenced by the total biomass production. Similarly for *M. vaginalis* also, biomass production increased with levels of Fe but the total biomass was lower than that of *E. crassipes.* According to Lema *et al.* (2014) macrophytes differ widely in their biomass production and affinity towards heavy metal, resulting in varied metal accumulation capacity and thus efficiency in cleaning up mechanism.

Fe accumulation in both shoot and root increased up to 3000 mg kg^{-1} while the quantity extracted increased only up to 2000 mg kg^{-1} and then decreased which is in tune with biomass production. In the case of *E. crassipes*, both concentration and extraction increased with levels of Fe indicating the suitability of it as a phytoremediator. But the corresponding increase in Fe extraction decreased with increasing Fe levels (Fig. 20c). The hyperaccumulators possess certain physiological mechanisms to outcome the extreme conditions of metal toxicity. According to Chinmayee *et al.* (2014), accumulation of heavy metals inside the plants stimulates the production of various antioxidant enzymes that help to withstand the stress induced by these metals without affecting their growth performance. Also some physiological changes can happen inside the plant with variation in gas exchange and net respiration rate effecting the sequestration of metals inside (Prasad, 2001). Fe was mainly concentrated in roots as evidenced by very high values (Khankhane *et al.*, 2014; Ndimele *et al.*, 2014).

M. vaginalis also behaved in a similar manner but the quantity extracted was lower than that of *E. crassipes*. At higher levels, *M. vaginalis* accumulated more Fe in the shoot compared to root while *E. crassipes* always showed higher accumulation in root. The third dominant macrophyte, *L. flava* showed an increase in Fe accumulation in both shoot and root only up to 2000 mg kg^{-1}. Here more of Fe was accumulated in the shoot but the extraction by shoot was highest at 1000 mg kg^{-1} and with increasing Fe levels, it decreases. Shoot biomass was highest at 1000 mg kg^{-1} but the root biomass increased with higher levels of Fe. For root, the same quantity (1.88) was extracted at both 2000 and 3000 mg kg^{-1}.

Comparing the BCF values, (Table 68) it was highest for *E. crassipes* (2.37) and it increased up to 2000 mg kg^{-1}. This had indicated that the Fe extraction by *E. crassipes* in relation with soil Fe content increased up to 2000 mg kg^{-1} and was not able to produce a corresponding increase in extraction at the highest level of Fe compared to the increase in soil Fe. The same has been reported by Ndimele *et al.* (2014). Though *M. vaginalis* was second in Fe extraction, all the BCF values were less than one except under second dose of Fe while under natural condition its BCF was higher than one. Hence *M. vaginalis* is having more of phytostabilisation nature.

For *L. flava*, BCF was highest at 1000 mg kg^{-1} (2.60) and decreased to below one with increasing levels. The lowest value was noted at highest Fe level. This had revealed that its phytoextraction ability is best at 1000 mg kg^{-1}. Suitability of *L. flava* in phytoremediation works had been explained by Anning *et al.* (2013) and Kamrudzaman *et al.* (2012).

Table 68. BCF and TF of macrophytes under graded doses of Fe

Macrophytes	Graded doses of Fe (mg kg^{-1})					
	1000		2000		3000	
	BCF	TF	BCF	TF	BCF	TF
A. tenella	2.34	1.50	0.90	1.29	0.32	1.21
B. dactyoides	1.37	0.86	0.84	1.05	0.32	0.81
C. esculenta	1.63	0.69	1.07	0.85	0.34	0.98
C. dactylon	0.99	0.83	1.07	1.03	0.73	1.16
E. crassipes	2.37	0.88	2.50	0.84	1.95	0.89
E. dulcis	1.25	0.49	0.62	0.52	1.15	0.51
L. flava	2.60	1.06	0.89	1.04	0.49	1.03
M. vaginalis	0.89	0.83	1.01	1.09	0.90	1.10
N. indica	0.99	0.71	1.13	0.77	0.55	0.75

Most of the tested macrophytes showed higher accumulation of Fe in root compared to shoot. This was very much conspicuous in the case of *E. crassipes* while *M. vaginalis*, *L. flava*, and *A. tenella* accumulated more Fe in shoot (Table 68). Thus among the selected phytoremediators, *M. vaginalis*, *L. flava*, and *A. tenella* have the site of Fe retention as root and for *E. crassipes, B. dactyiodes, C. esculenta* and *E. dulcis* as shoot. *C. dactylon* showed a differential response for Fe accumulation. As the levels of Fe increases, it showed higher accumulation in shoot indicating more translocation of Fe from root to shoot. TF increases from 0.83 at 1000 mg Fe kg^{-1} and 1.16 at 3000 mg Fe kg^{-1} (Fig. 24).

E. crassipes and *L. flava* could be used as phytoextractors while *M. vaginalis* performed best as a phytostabiliser. Hence these macrophytes could be used as phytoremediators for Fe. Among the macrophytes, performance as a phytoremediator was the poorest for *E. dulcis* as evidenced by the lowest shoot and root biomass and Fe extraction, though it is a native species of most of the acidic wetlands.

The increasing levels of Fe had increased the EC and Fe concentration in water and sediment and decreased the pH. Fe sources used are acidic in nature and hence pH had decreased and the salts being soluble in water naturally increased the EC and Fe concentration (Table 46).

The microbial count was significantly influenced by macrophytes and graded doses of Fe and in general, the fungal population increased with levels of Fe up to 2000 mg kg^{-1} while bacterial and actinomycete population followed a decrease. This differential response of microorganisms to elevated levels of heavy metal was explained by Rajapaksha *et al.* (2004). This was very much in tune with decreasing values of pH and increasing values of EC. Fungi can make use of the additional carbon resulted with the decay of bacterial mass and has resulted in their hike even under adverse conditions (Alden *et. al.*, 2001). The fugal population was highest for *B. dactyoides* and lowest for *E. crassipes. C. dactylon* recorded the highest value for both bacteria and actinomycetes and the lowest by *M. vaginalis.* Any specific influence of the treatments on microbial load of individual macrophytes was not observed (Table 48).

5.3.2. Growth performance and phytoextraction of Al by selected macrophytes

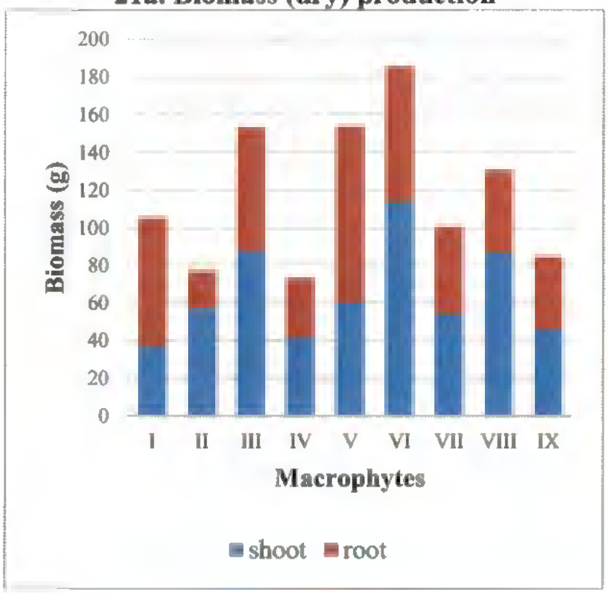
Nine macrophytes viz., *N. indica, S. molesta, C. esculenta, E. dulcis, L. flava, M. vaginalis, B. dactyoides, P. repens* and *C. dactylon* were grown in pots under three graded doses of Al viz., 750, 1000 and 1250 mg kg^{-1} of soil for 45 days.

The macrophytes, graded doses of Al and their interactions had significantly influenced plant biomass and Al concentration and quantity extracted (Table 53). *M. vaginalis* recorded the highest shoot and total biomass, and Al accumulation in both shoot and root. Hence the highest quantity of Al was extracted by it. Root biomass was highest for *L. flava.* Kassaye *et al.* (2013) had reported on the significantly higher accumulation of Al in *Cynadon* sp. without any toxicity symptoms as revealed by the absence of any reactive oxygen species (ROS) induction or cell damage.

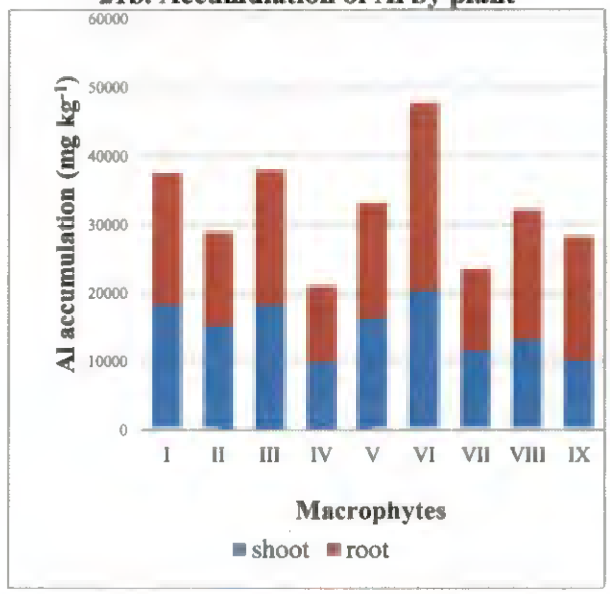
With increasing level of Al, shoot and total biomass decreased while root showed an increase up to 1000 mg Al kg^{-1}. Hence the selected macrophytes find it

Fig. 21 Biomass (dry) production, aluminium (Al) accumulation and uptake by selected macrophytes under graded doses of Al

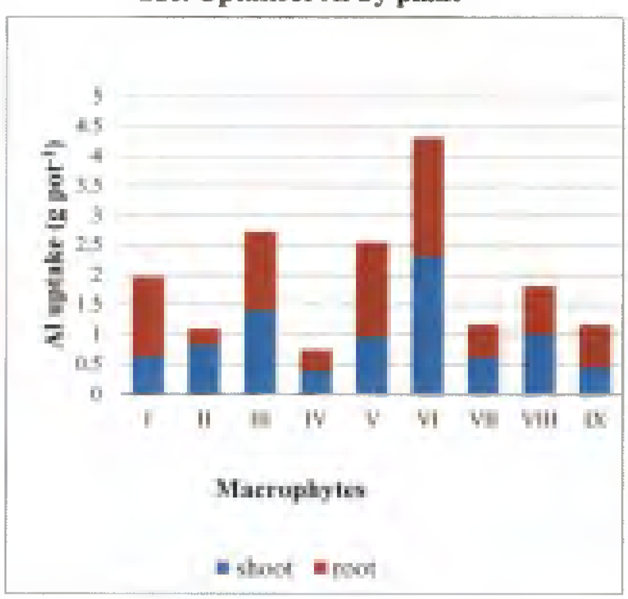
21a. Biomass (dry) production



21b. Accumulation of Al by plant



21c. Uptakeof Al by plant



I – *N. indica*	II – *S. molesta*	III – *C. esculenta*	IV – *E. dulcis*	V – *L. flava*
VI – *M. vaginalis*	VII – *B. dactyoides*	VIII – *P. repens*	IX – *C. dactylon*	

difficult to survive under increased levels of Al (Fig. 21a). When Al distribution within the plant parts were compared, both root and shoot maintained a positive relationship with increased levels of Al, but the proportionate increase was comparatively low. Thus the higher quantity of Al might have been sequestered in vacuoles or get bonded at cell membranes without much interference with plant growth. Even then it might have affected some of the growth promoting factors (Ajoy *et al*., 1998) which finally resulted in reduced biomass.

When the biomass production by individual macrophytes were considered, *M. vaginalis* showed an increasing pattern for both shoot and root biomass up to 1000 mg Al kg^{-1} while the concentration increased with higher levels of Al. Shoot extraction increased up to 1250 mg Al kg^{-1} and root only up to 1000 mg Al kg^{-1} (Fig. 21b). This had indicated that *M. vaginalis* could be a good bioremediator for Al. Heavy metal extraction potential of *M. vaginalis* had already been reported by Liu *et al.* (2014). But as the concentration increased, growth decreased after an initial hike. Some of the plant metabolic activities might have been interrupted.

The quantity of Al extracted increased up to the second level (1000 mg kg^{-1}) and then decreased. *M. vaginalis* extracted the highest quantity of Al by shoot and root followed by *C. esculenta*. Rather than accumulation, the influence of biomass was more dominant in deciding the total extraction. For both of these species the site of Al retention was root while for *S. molesta* it was shoot (Fig. 21c). In the case of *L. fava*, it retained more Al in shoot at lowest dose of Al (750 mg kg^{-1}) but as the levels of Al increased, the site of retention changed to root while for *N. indica* and *C. esculenta* reverse was true. This might be due to the difference in location where the extracted metals are deposited safely either in vacuoles or cell membrane or on the root tissues. Hegazy *et al.* (2011) had reported on the highest level of Al accumulation in the roots and rhizomes of aquatic plants indicating the rhizofiltration ability of the macrophyte in removing contaminants. Most of the macrophytes obtained a low TF in the present study (Table 69).

To assess the phytoextraction ability, BCF was calculated and highest BCF was observed for *M. vaginalis* at all the three levels of Al. BCF decreased with higher levels of Al. *C. esculenta* showed second highest BCF but it decreased with higher levels. Its use as a phytoremediator at very high levels of Al was not advisable. Hence *M. vaginalis* was found to be the best phytoextractor for Al. Shoot uptake by *S. molesta* was much higher compared to root uptake and its phytoextraction ability was evident from the increase in TF with higher doses of Al (Fig. 25).

Table 69. BCF and TF of macrophytes under graded doses of Al

Macrophytes	Graded doses of Al (mg kg^{-1})					
	750		1000		1250	
	BCF	TF	BCF	TF	BCF	TF
N. indica	1.15	0.87	1.08	0.90	0.47	1.02
S. molesta	1.26	1.01	0.36	1.13	0.05	1.18
C. esculenta	1.60	0.66	1.33	0.89	0.78	1.10
E. dulcis	0.54	0.80	0.26	0.87	0.09	0.97
L. flava	1.71	1.07	1.24	0.93	0.48	0.88
M. vaginalis	1.98	0.65	1.76	0.72	1.17	0.83
B. dactyoides	0.84	0.97	0.24	0.95	0.10	0.96
P. repens	1.15	0.70	0.55	0.70	0.11	0.70
C. dactylon	0.44	0.47	0.51	0.61	0.12	0.57

Similar response of many aquatic plants in sequestering Al without causing any deleterious effects was reported by Farid *et al.* (2014). Here also the increasing levels of Al had increased the EC and Al concentration in water and sediment and decreased the pH. Al sources used are acidic in nature and hence decrease in pH and the salts being soluble in water, naturally increased the EC and Al concentration (Table 50).

As in the case of Fe, the behaviour of microbial population showed preponderance of fungi with increasing levels of Al and a decrease of bacteria and actinomycetes (Table 52). Prasad *et al.* (2014) also noticed this differential response by fungi and bacteria with the addition of heavy metals. Here also a specific trend for individual macrophytes with microbial population under different levels of Al was not observed.

5.3. Growth performance and Phytoextraction of Pb by selected macrophytes

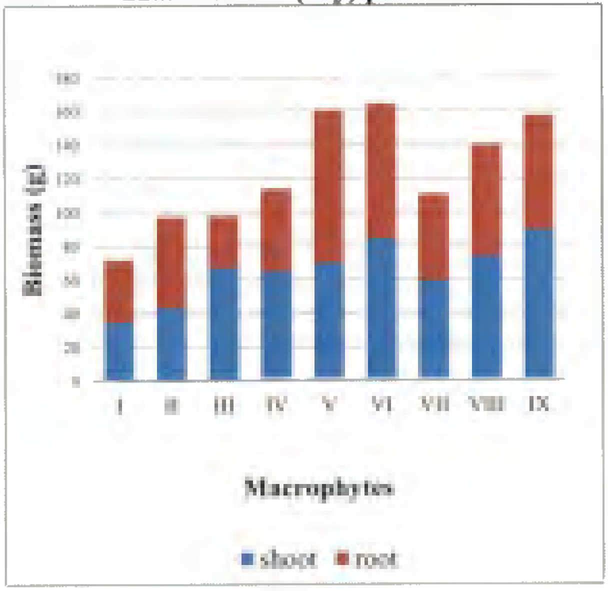
Under graded doses of Pb (50, 75 and 100 mg kg^{-1} of soil), nine macrophytes viz., *N. nucifera, N. indica, P. stratiotes, E. crassipes, L. flava, S. grossus, C. esculenta, , B. dactyloides* and *A. tenella* were grown in pots for 45 days.

The macrophytes, graded doses of Pb and their interaction effects were significant. Both shoot and root biomass was found to increase up to 75 mg Pb kg^{-1} and then decreased (Table 57). Shoot as well as root concentration and extraction of Pb were highest at 100 mg Pb kg^{-1}. Though there was an increase in total extraction with increase in dose from 75 to 100 mg Pb kg^{-1}, the rate of enhancement was comparatively very low.

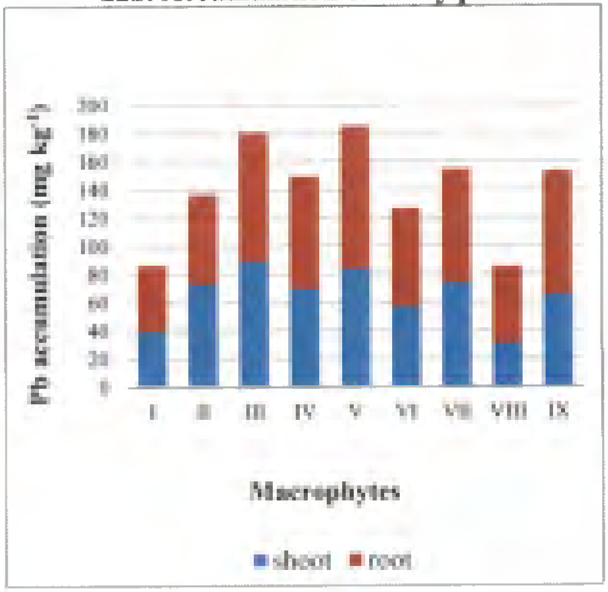
Comparing the macrophytes, *A. tenella* produced the highest shoot biomass (89.50 g) and *L. flava*, highest root biomass (89.80 g). Total biomass (Fig. 22a) was highest for *S. grossus* (164.60 g). Pb accumulation in shoot was highest for *P. stratiotes* and for root, *L. flava* recorded the highest. Among the tested species, only *N. indica* accumulated more Pb in shoot compared to root (Fig. 22b). Except *N. indica* and *P. stratiotes*, all others obtained a TF below one at all the doses of Pb. This clearly indicates the rhizofiltration ability of macrophytes which prevents the metal from getting translocated to aerial parts. Macrophytes in general showed an increase in TF with increased levels of metal application. For *N. indica*, *S. grossus* and *A. tenella*, TF decreased with the higher level of Pb application. Emergent macrophytes were found to accumulate more Pb in the roots and increased with metal concentration in the growing medium. Emergent aquatic macrophytes found to accumulate more Pb with increasing levels of metal in the medium. This made possible by the higher biomass produced by these macrophytes and more Pb was

Fig. 22 Biomass (dry) production, lead (Pb) accumulation and uptake by selected macrophytes under graded doses of Pb

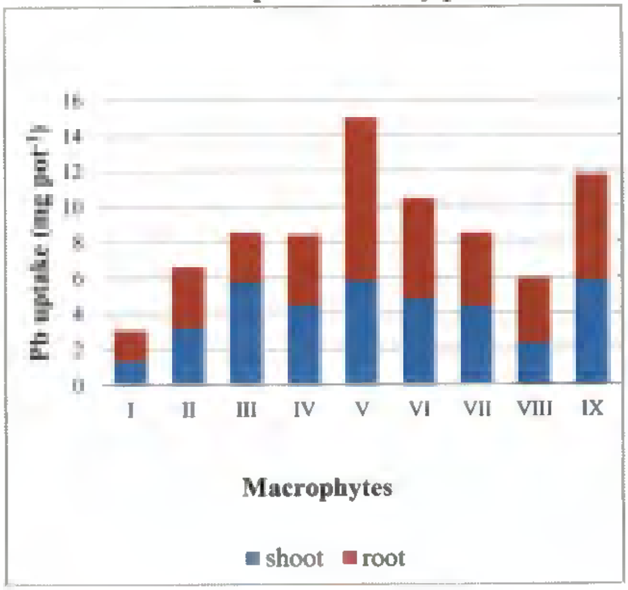
22a. Biomass (dry) production



22b. Accumulation of Pb by plant



22c. Uptake of Pb by plant



I – *N. nucifera*	II – *N. indica*	III – *P. stratiotes*	IV – *E. crassipes*	V – *L. flava*
VI – *S. grossus*	VII – *C. esculenta*	VIII – *B. dactyoides*	IX – *A. tenella*	

retained in their roots (Deng *et al.*, 2004). According to Marbaniang and Chaturvedi (2014), Pb accumulation in plant parts increases with concentration and time.

For *L. flava*, shoot and root biomass increased up to 75 mg Pb kg^{-1} and then decreased. But the concentration as well as extraction by plant parts increased with levels of Pb indicating the better phytoremediation capacity of it. *L. flava* showed the highest Pb extraction. Pb was mainly concentrated in the roots which showed the rhizofiltration ability of the macrophyte. Next to *L. flava*, the largest quantity of Pb was extracted by *A. tenella* followed by *S. grossus* (Fig. 22c). Both behaved in a manner similar to that of *L. flava* and produced the highest extraction at 100 mg Pb kg^{-1}. Tangahu *et al.* (2013) reported the suitability of *S. grossus* as a hyperaccumulator for Pb. *N. nucifera* recorded the lowest shoot biomass and *P. stratiotes*, lowest root biomass. Because of low biomass production, these macrophytes cannot perform well for Pb extraction.

BCF values (Table 70) were very low (less than one) for all the macrophytes. Among them, *L. flava* had the highest BCF and it increased up to 75 mg Pb kg^{-1}. *A. tenella* and *S. grossus* had the same BCF value at 50 mg Pb kg^{-1} but with increasing

Table 70. BCF and TF of macrophytes under graded doses of Pb

Macrophytes	Graded doses of Pb (mg kg^{-1})					
	50		75		100	
	BCF	TF	BCF	TF	BCF	TF
N. nucifera	0.06	0.84	0.04	0.87	0.02	0.83
N. indica	0.08	0.97	0.09	1.08	0.07	1.33
P. stratiotes	0.14	0.80	0.14	1.02	0.08	1.05
E. crassipes	0.09	0.66	0.11	0.94	0.10	0.95
L. flava	0.20	0.78	0.21	0.81	0.16	0.86
S. grossus	0.14	0.80	0.15	0.84	0.11	0.79
C. esculenta	0.11	0.89	0.12	0.91	0.08	0.96
B. dactyoides	0.07	0.43	0.07	0.56	0.06	0.67
A. tenella	0.14	0.60	0.17	0.81	0.13	0.80

levels of Pb, *A. tenella* had higher BCF compared to *S. grossus.* Thus, *A. tenella* had higher phytoremediation potential for Pb. Increase in TF with increasing levels of Pb for *P. stratiotes* was not reflected in the uptake because of the lower biomass production (Fig. 26). The poorest performance was for *N. nucifera* as indicated by the lowest Pb extraction. Several workers have reported on the phytoremediation potential of both *L. flava* and *A. tenella* for Pb (Anning *et al.*, 2013; Chinmayee *et al.*, 2014; Rachmadiarti *et al.*, 2012).

Here also, pH of water and sediment decreased and EC increased with levels of Pb (Tables 54 and 55). Specific effect of individual macrophytes on microbial count was not observed. In general, fungal population increased with increasing levels of Pb and bacterial and actinomycete count declined (Table 56). *C. esculenta* obtained the highest fungal count and the lowest actinomycete population. Bacterial count was the highest for *L. flava*. Greater removal of the heavy metal by *L. flava* might have created a situation conducive for the development of bacteria. Wang *et al.* (2007) reported on the adverse influence of heavy metals on bacterial and actinomycete population as indicated by reduction in microbial biomass and enzyme activity with heavy metal pollution.

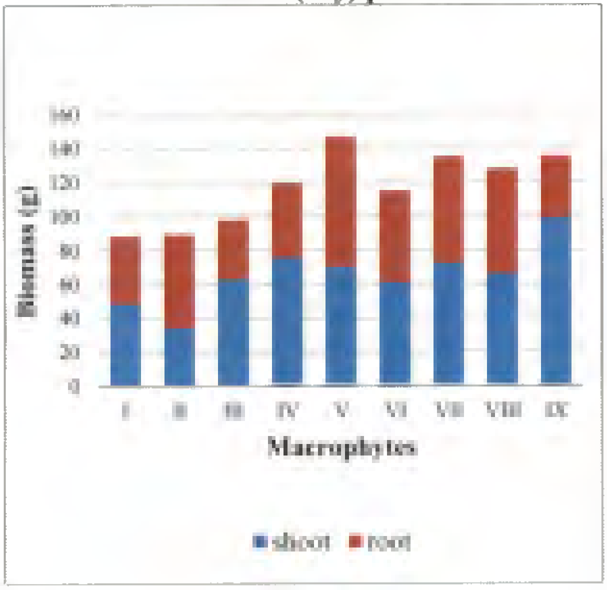
5.3. 4. Growth performance and Phytoextraction of Cd by macrophytes

Nine macrophytes viz., *N. nucifera, N. indica, P. stratiotes, E. crassipes, L. flava, E. dulcis, C. esculenta, A. tenella* and *S. trilobata* were grown in pots under three graded doses of Cd (50, 75 and 100 mg Cd kg^{-1} of soil) for a period of 45 days.

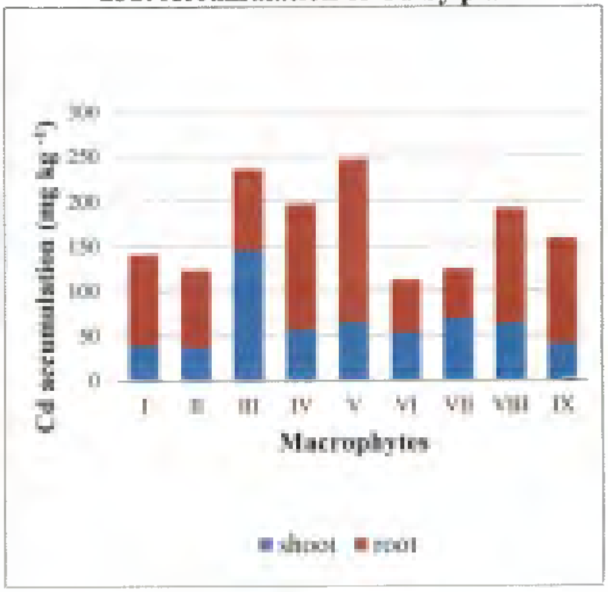
The macrophytes, graded doses of Cd and their interaction effects had significantly influenced the biomass, metal accumulation and the extraction by plant parts (Table 61). Shoot and root biomass were highest at 75 mg Cd kg^{-1} (Fig. 23a). Metal concentration as well as extraction by both shoot and root increased with levels of Cd. Among the macrophytes, *S. trilobata* recorded the highest shoot biomass and *L. flava*, the root and total biomass. Cd accumulation in shoot was highest for *P.*

Fig. 23 Biomass (dry) production, cadmium (Cd) accumulation and uptake by selected macrophytes under graded doses of Cd

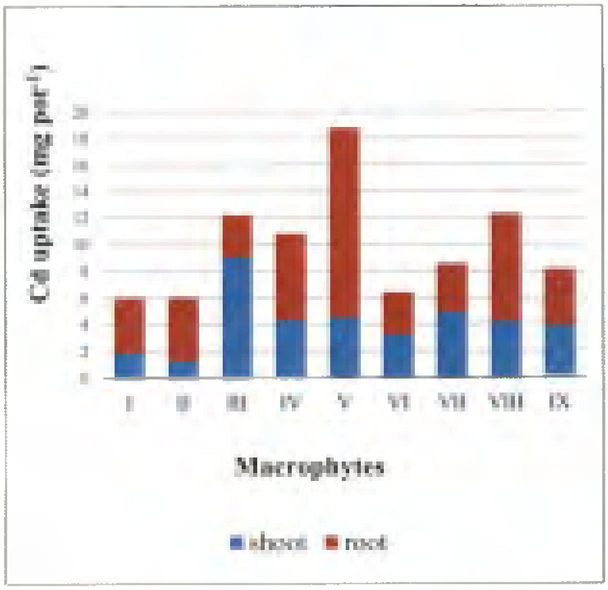
23a. Biomass (dry) production



23b. Accumulation of Cd by plant



23c. Uptake of Cd by plant



I – *N. nucifera*	II – *N. indica*	III – *P. stratiotes*	IV – *E. crassipes*	V – *L. flava*
VI – *E. dulcis*	VII – *C. esculenta*	VIII – *A. tenella*	IX – *S. trilobata*	

Fig. 24 Translocation factor for promising macrophytes under graded doses of Fe

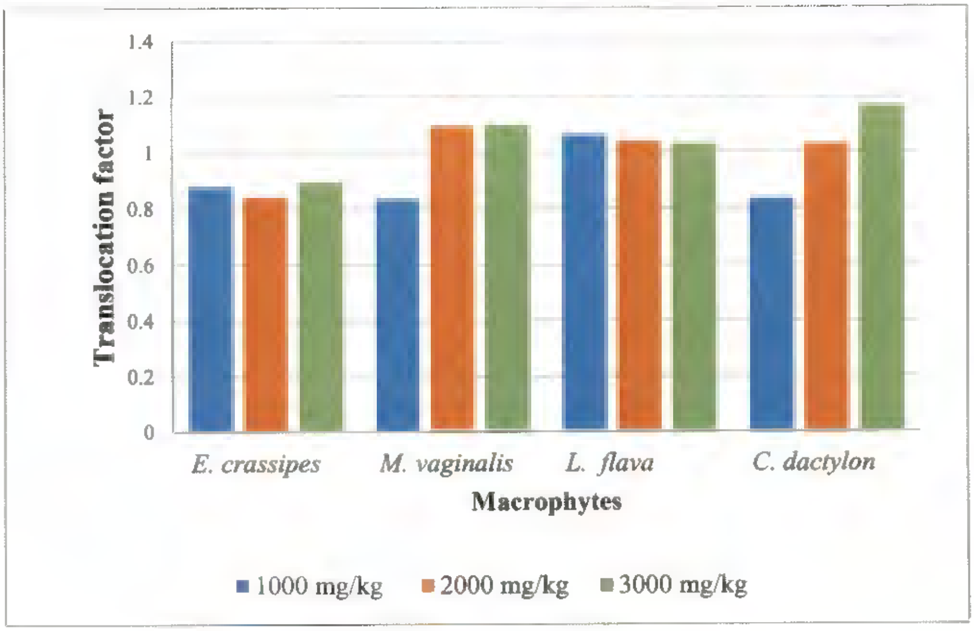
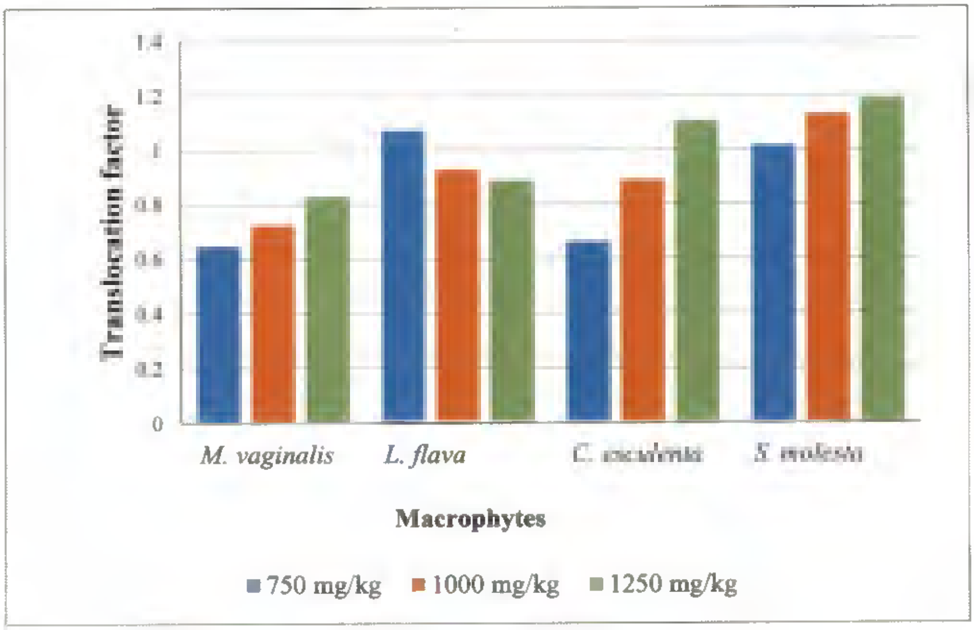


Fig. 25 Translocation factor for promising macrophytes under graded doses of Al



stratiotes and *L. flava* for root (Fig. 23b). Highest quantity of Cd was extracted by *L. flava* followed by *A. tenella* which is an indication of their phytoremediation potential. Though the biomass production for *S. trilobata* was high, Fe extracted was much lower due to its low accumulation in plant (Fig. 23c). Phytoremediation potential of *S. trilobata* had already been reported by Patel *et al.* (2014). The macrophytes showed a wide variation in their Cd accumulation capacity.

Among the tested species, only *P. stratiotes* and *C. esculenta* showed higher Cd accumulation in shoot revealing shoot as their major Cd retention site. This is clearly evident from translocation factor (Table 27). For all other tested macrophytes, Cd retention site was root itself. Highest accumulation of Cd in the root portions of *Typha angustifolia* which is capable of producing very high biomass was reported by Chayapan *et al.* (2015). Next to *L. flava*, total Cd extraction was highest for *A. tenella*. It followed the same pattern as that of *L. flava* in biomass production, Cd concentration and extraction (Abhilash *et al.*, 2009).

Table 71. BCF and TF of macrophytes under graded doses of Cd

Macrophytes	Graded doses of Cd (mg kg^{-1})					
	50		75		100	
	BCF	TF	BCF	TF	BCF	TF
N. nucifera	0.12	0.32	0.09	0.42	0.05	0.41
N. indica	0.08	0.33	0.08	0.47	0.07	0.44
P. stratiotes	0.18	1.62	0.18	1.65	0.14	1.56
E. crassipes	0.11	0.54	0.14	0.43	0.16	0.33
L. flava	0.22	0.38	0.27	0.33	0.25	0.36
E. dulcis	0.090	0.78	0.09	0.86	0.07	1.00
C. esculenta	0.126	1.36	0.12	1.16	0.09	1.21
A. tenella	0.128	0.57	0.18	0.48	0.16	0.47
S. trilobata	0.133	0.35	0.11	0.32	0.08	0.33

BCF values were computed to assess the phytoextraction ability of the macrophytes and it was observed that all the macrophytes, in general, noticed low BCF (less than one). *L. flava* recorded the highest BCF at all levels of Cd and it

Fig. 26 Translocation factor for promising macrophytes under graded doses of Pb

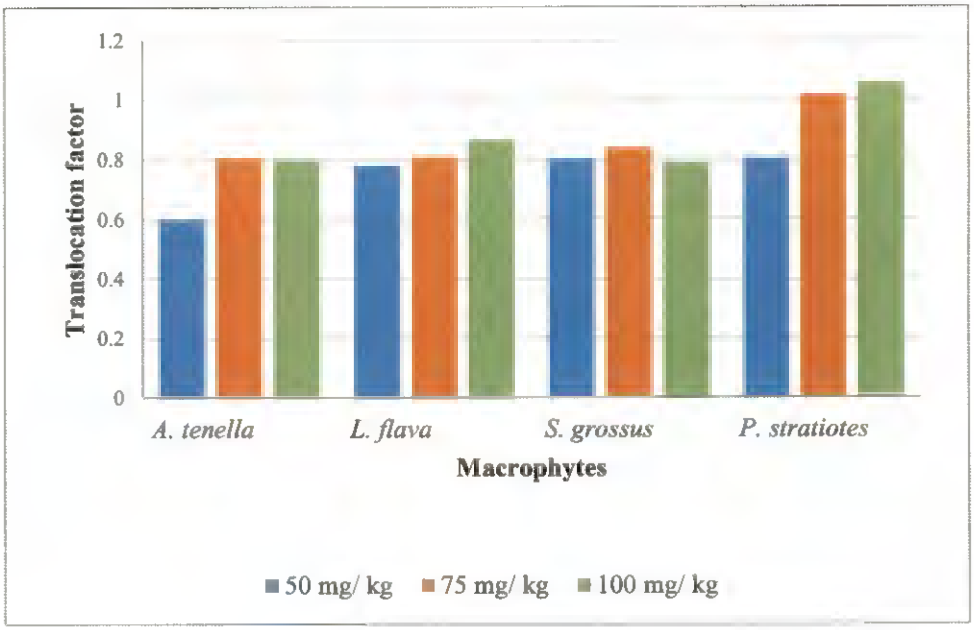
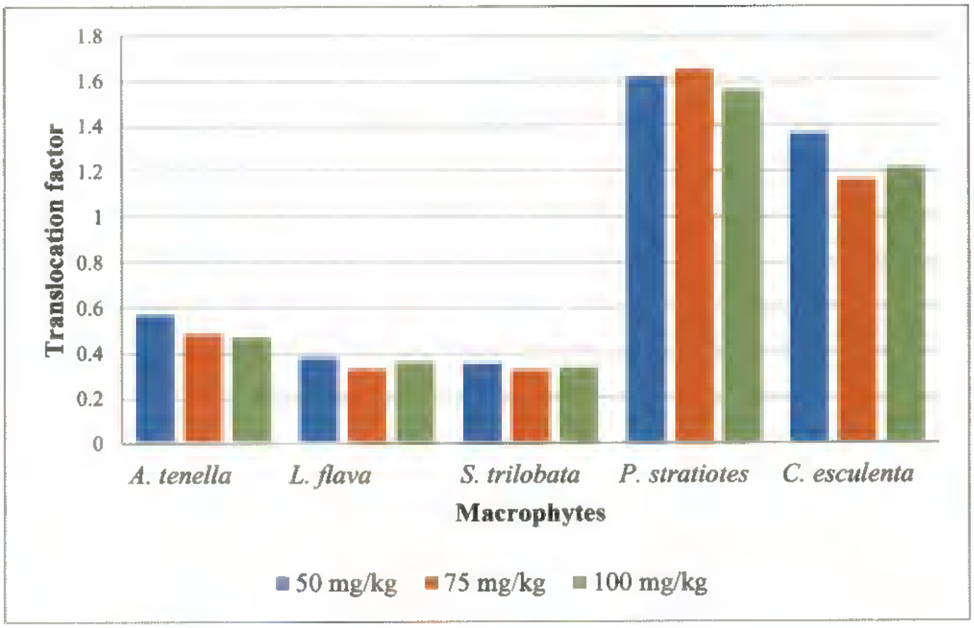


Fig. 27 Translocation factor for promising macrophytes under graded doses of Cd



increased up to 75 mg Cd kg^{-1} indicating comparatively high phytoextraction up to 75 mg Cd kg^{-1}. On comparing the BCF values of *A. tenella* and *S. trilobata*, *S. trilobata* (0.133) recorded a higher value at 50 mg kg^{-1} and showed a declining trend with further higher levels of Cd, indicating that the macrophyte could exhibit its phytoextraction potential only at lower levels. For *A. tenella*, there was an increase in BCF value from 50 mg kg^{-1} (0.128) to 75 mg Cd kg^{-1} (0.189) and then it decreased.

As in the case of Fe, Al and Pb, increasing levels of Cd had resulted in decrease in pH and increase in EC and Cd concentration of water and sediment (Tables 58 and 59).

There was no specific pattern of relation with levels of Cd and the individual macrophytes except that the highest bacterial and lowest fungal count was obtained for *S. trilobata* and the reverse for *N. nucifera* (Table 60). In general, there was dominance of fungi at increasing levels of Cd and a decrease of bacteria and actinomycetes.

The pot culture experiments with graded doses of metals viz., Fe, Al, Pb and Cd revealed the following:

E. crassipes was the best phytoextractor for Fe and its major site of retention was root followed by *M. vaginalis* and *L. flava* where the major site of retention was shoot. For Al, the best phytoremediators were *M. vaginalis* and *C. esculenta*, where the major site of retention was root. For both Pb and Cd, the best phytoremediators were *L. flava* and *A. tenella* with their major site of retention as root.

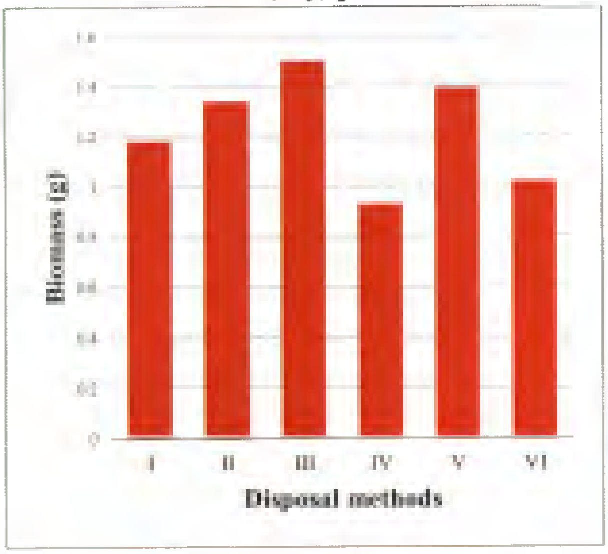
5.4. Comparison of disposal techniques of phytoextracted biomass and bioavailability of metals

5.4.1. Iron

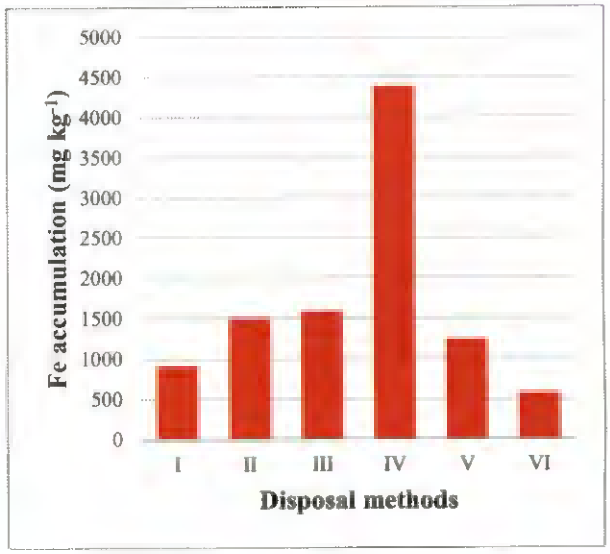
Phytoremediation technology is regarded as a simple and efficient way to remove heavy metals from contaminated soil. But the safe disposal of the

Fig. 28 Effect of different disposal methods of phytoextracted biomass (Fe) on biomass production, Fe accumulation and uptake by amaranthus and soil retention

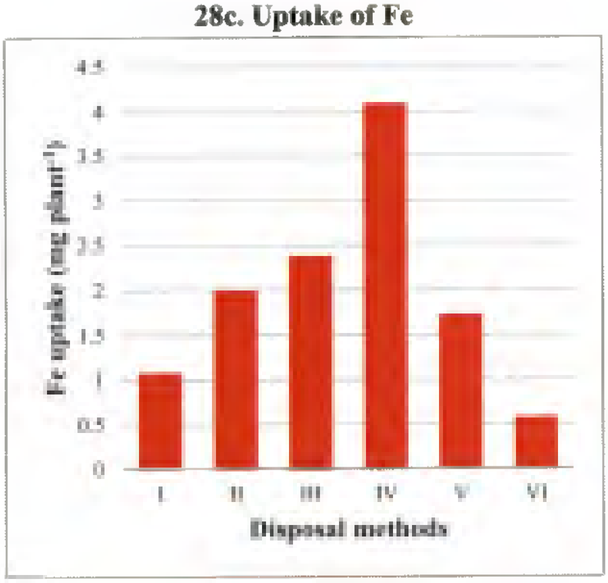
28a. Biomass (dry) production



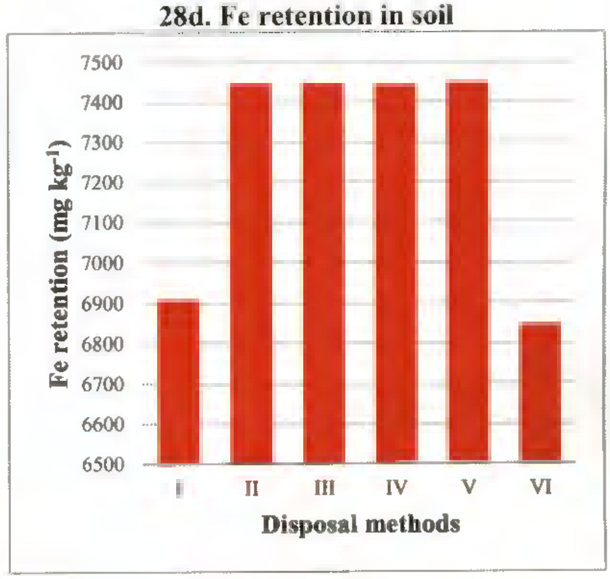
28b. Accumulation of Fe



28c. Uptake of Fe



28d. Fe retention in soil



I – FYM	II – Compost	III – Vermicompost
IV – Ash	V – Biochar	VI - Control

phytoextracted biomass is always a major issue in its reuse. Several technologies including thermal and biological methods are attempted worldwide for the safe disposal of the phytoextracted biomass or the hyperaccumulators. The thermal methods include gasification (Singhal and Rai, 2003), incineration (Keller *et al.*, 2005), phytomining and pyrolysis (Brendova *et al.*, 2015). Composting (Cao *et al.*, 2010) and vermicomposting (Sasidharan *et al.*, 2013) are the commonly employed biological methods. Here, two biological methods viz., composting and vermicomposting and two thermal methods viz., ashing and biochar production were evaluated to assess the uptake of the contaminant (metal) by the amaranthus crop grown in the medium containing the treated or processed biomass.

The best phytoremediator for each metal selected based on the results of Part III was subjected separately to different processing techniques viz., ordinary composting, vermicomposting, ashing and biochar production. The phytoremediators, *E. crassipes* and *M. vaginalis* were used for the phytoextraction of Fe and Al respectively. *L. flava* was used for the phytoextraction of Pb and Cd. The processed materials were used for raising amaranthus in pots. For each metal, separate experiment was carried out and the bioavailability and uptake were estimated.

Evaluating the performance of amaranthus receiving Fe in the form of compost, vermicompost, ash and biochar, it was observed that the biomass production (Fig. 28a) was highest for vermicompost treatment and lowest for ash. Compost, vermicompost and biochar were able to produce significantly higher quantity of biomass compared to FYM. It might be due to the additional quantity of organic carbon supplied by them. The contribution from processed phytoextracted biomass per pot varied from 301.0 mg in compost to 302.5 mg Fe in ash while the contribution from FYM was only 30.63 mg Fe kg^{-1}. Higher Fe content of the processed biomass did not adversely affect the biomass production by amaranthus.

Looking in to Fe concentration in plant (Fig. 28b), the highest concentration was for ash, where the metal was present in free form which speeds up the uptake.

This was followed by vermicompost > compost > and biochar. The total quantity of Fe extracted by amaranthus (Fig. 28c) showed the same trend as that of concentration. Though the biomass production by ash was the lowest, very high Fe concentration in plant biomass compensated it, showing highest extraction. The metal availability from ash was higher compared to composts (Lau *et al*., 2001) and biochar (Park *et al*., 2011). Fe retained within the soil after harvest of amaranthus confirmed it. Comparing the total Fe content after harvest to that at the time of planting, the lowest value was recorded for ash and highest for biochar (Table 63). Comparing the loss of Fe from soil, it was also highest for ash and lowest for biochar among the processed biomass (Fig. 28d).

Fe extraction by all the four disposal methods was significantly superior to FYM indicating the phytoextraction of Fe from the processed materials. Among the methods, biochar maintained the lowest concentration in plant and highest in soil ensuring lowest bioavailability to the plant (Table 63). Leaching loss was also lowest for biochar. Hence the conversion of phytoextracted biomass to biochar was the best among the tested methods. .

Table 72. Budgeting of phytoextracted Fe under different disposal techniques

Treatment	Initial Fe content of medium (mg pot^{-1})	Final Fe content of medium (mg pot^{-1})	Plant Fe uptake (mg pot^{-1})	Leaching loss of Fe (mg pot^{-1})	Extraction efficiency
T1 (FYM)	3455.6	3454.0	1.08	0.52	1.632
T2 (Compost)	3726.0	3723.1	2.00	0.90	0.471
T3 (Vermicompost)	3726.5	3723.0	2.38	1.12	0.595
T4 (Ash)	3727.5	3721.5	4.09	1.91	1.159
T5 (Biochar)	3726.9	3724.3	1.73	0.84	0.380
T6 (Control)	3425.0	3423.5	0.58	0.92	

The adsorption of metals to biochar might have prevented its easy removal by amaranthus. The higher surface area of biochar renders better adsorption of heavy metals and makes them less available. The retention efficiency for Fe in growth medium was also highest for biochar compared with that of compost, vermicompost and ash. Amaranthus had extracted 1.364 (biochar) to 4.825 (ash) times more Fe compared to FYMFe content of FYM is about four times lower compared to that of compost, but Fe extraction from FYM was not comparable to this. The high Fe extraction efficiency of FYM is due to the presence of more water soluble OC which helped the movement of Fe to the plant roots and increased availability of Fe with manure / compost application. Lower pH might have encouraged better crop removal, resulting in Fe extraction of this magnitude.

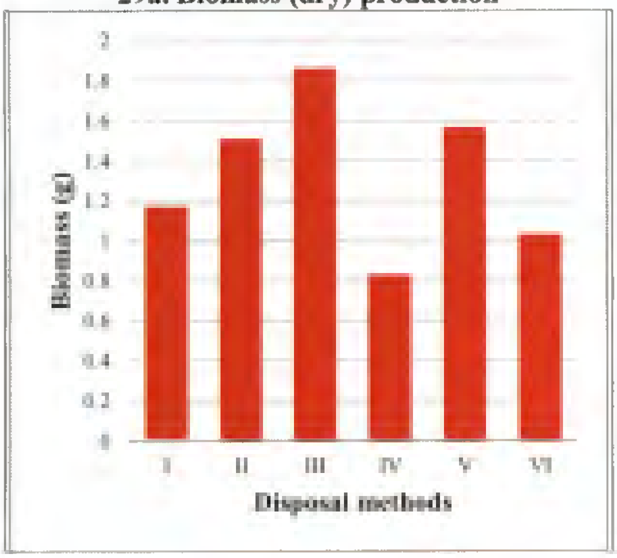
5.4.2. Aluminium

Al phytoextracted through *M. vaginalis* was processed as per treatments viz., compost, vermicompost, ash and biochar and used for raising amaranthus. Evaluating the performance of amaranthus receiving the above materials, it was observed that vermicompost had produced highest biomass and ash, the lowest as in the case of Fe. Compost, vermicompost and biochar were able to produce significantly higher quantity of biomass compared to FYM. The processed biomass added to each pot contained 225 mg Al, while the contribution from FYM was only 12.81 mg Al. But Al extraction by FYM treatment was comparable to other treatments, even though it was not spiked with Al. The difference between FYM treatment and that received 225 mg Al per pot was not able to produce a drastic increase in Al extraction as evidenced from Fig. 29a. Again the lower pH of FYM might have also favoured better Al uptake by amaranthus. Hence the higher Al content of the processed phytoextracted biomass had not adversely affected the biomass production by amaranthus.

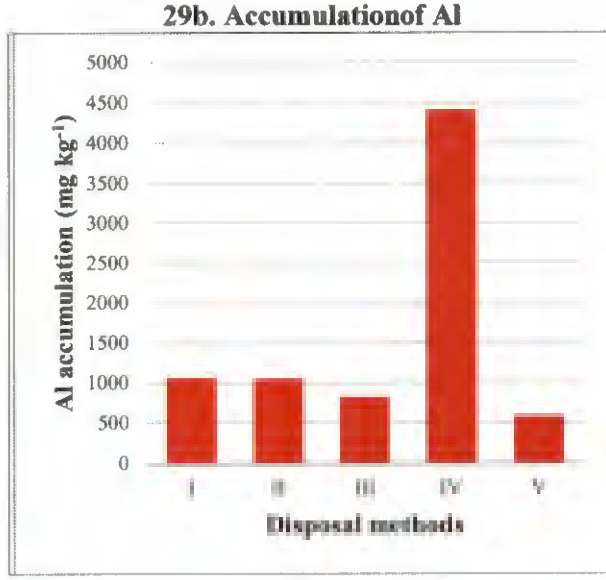
With regard to Al concentration in plant, the highest concentration was for ash, where the metal was present in free form which might have speeded up the uptake (Fig. 29b). It was followed by FYM > compost > vermicompost > and biochar. The Al concentration presented a slightly different picture from that of Fe. When it comes to the total quantity of Al extracted by amaranthus it followed the

Fig. 29 Effect of different disposal methods of phytoextracted biomass (Al) on biomass production, Al accumulation and uptake by amaranthus and soil retention

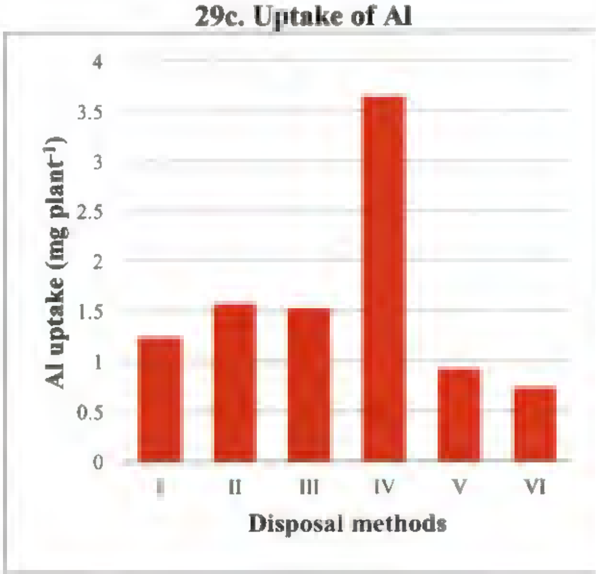
29a. Biomass (dry) production



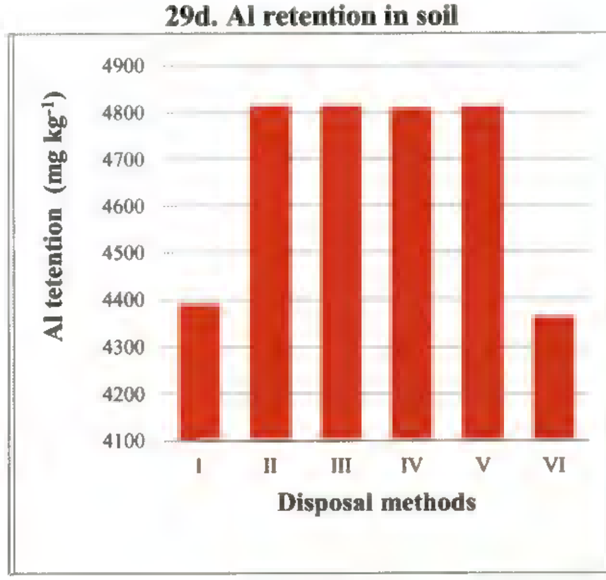
29b. Accumulationof Al



29c. Uptake of Al



29d. Al retention in soil



I – FYM	II – Compost	III – Vermicompost
IV – Ash	V – Biochar	VI - Control

same trend as that of Fe showing highest extraction for ash and lowest for biochar among the treatments receiving processed biomass (Fig. 29c). Ash had produced lowest biomass, but the very high Al concentration in plant biomass compensated this showing highest extraction. The metal availability from ash was higher compared to composts and biochar (Lucchini *et al.*, 2014). Al retained within the soil after harvest of amaranthus (Fig. 29d) confirmed the above observation.

Table 73. Budgeting of phytoextracted Al under different disposal techniques

Treatment	Initial Al content of medium (mg pot^{-1})	Final Al content of medium (mg pot^{-1})	Plant Al uptake (mg pot^{-1})	Leaching loss of Al (mg pot^{-1})	Extraction efficiency
T1 (FYM)	2196.8	2194.70	1.240	0.860	3.957
T2 (Compost)	2409.0	2407.00	1.570	0.430	0.373
T3 (Vermicompost)	2409.0	2406.70	1.526	0.774	0.351
T4 (Ash)	2409.0	2404.47	3.653	0.877	1.296
T5 (Biochar)	2409.0	2407.34	0.924	0.736	0.083
T6 (Control)	2184.0	2181.4	0.736	1.864	

Comparing the total Al content of soil after harvest, the lowest value was recorded for ash and highest for biochar (Table 64) among the treatments receiving processed biomass. Comparing the loss of Al from soil, it was also highest for ash and lowest for biochar further confirming the firm adsorption of heavy metals in soil with the incorporation of biochar. Al extraction by FYM was significantly superior to biochar. Thus there is every chance for Al uptake by the plants receiving FYM, though it was lower than that of compost, vermicompost and ash. Comparing the extent of extraction, it was also highest for FYM compared to others. Biochar maintained lowest concentration within the plant, and highest in soil ensuring lowest bioavailability. Leaching loss was also lowest for biochar. Park *et al.* (2011) reported that biochar because of high specific surface area is capable of firmly holding the heavy metals causing their immobilization and making availability extremely difficult. High cation binding capacity of biochar (Liang *et al.*, 2006) might have resulted in firm binding of Al ions making it less bioavailable. Hence the production of biochar from phytoextracted biomass was the best among the tested methods. The adsorption of metals to biochar might have prevented its easy removal by amaranthus. However, Houben *et al.* (2013) reported an increase in biomass production with biochar incorporation for rapeseed cultivated in a heavy metal contaminated soil. The retention efficiency for Al in growth medium was also highest for biochar compared with that of compost, vermicompost and ash. Amaranthus had extracted 0.743 (biochar) to 2.938 (ash) times Al compared to FYM.

54.3. Lead

The amount of Pb present in soil and that extracted by plants was very small compared to that of Fe and Al. Since Pb being a toxic heavy metal and not essential for plants and being carcinogenic makes its presence undesirable. However, the increasing soil and water pollution had led its entry to the food chain. This has to be taken seriously, since its biomagnification power in animal systems is very high (Lokeshwari and Chandrappa, 2006). Hence its dynamics within soil, water and in plant systems need further research.

Pb phytoextracted through *L. flava* was processed as per treatments and used for pot culture with amaranthus. Among the treatments, biochar had produced highest biomass which was statistically on par with vermicompost and lowest for control. Biomass production showed quite a different trend from that of Fe and Al. Compost, vermicompost and biochar were able to produce significantly higher quantity of biomass compared to FYM. The processed biomass contained 0.730 mg Pb per pot while the contribution from FYM was almost nil (0.00035 mg). Ash treatment was significantly superior to control. Pb content of ash, though the highest, was very minute compared to total volume of the growth medium and because of this it might not have affected the biomass production adversely.

Pb concentration in plant was very high for ash compared to other treatments and was significantly superior to all. All the other treatments were statistically on par with each other. Among the processed biomass, Pb concentration and extraction were lowest for biochar but at the same time Pb retention in soil was highest for it. The very high surface area and adsorbing power (Park *et al.*, 2011) of biochar had retained Pb in the medium rendering it less bioavailable to the plant.

Pb extraction by ash was significantly superior to all other treatments while all other treatments were statistically on par with each other. Except ashing, all other processing methods were almost equal to the common practice of FYM addition in relation to Pb uptake. The processed materials from 100 g biomass contained only 730 μg of Pb and its entry to amaranthus was quite low. Hence the processing methods except ashing is almost equal with regard to the bioavailability of Pb and can be considered safe.

Among the tested disposal methods, biochar had lowest Pb concentration in plant and highest in soil ensuring lowest bioavailability. Only ash treatment had shown extraction efficiency above one while it was lowest for biochar. Leaching loss was also lowest for biochar. Hence biochar production from phytoextracted biomass was the best among the tested disposal methods.

5.4.4. Cadmium

Among the tested metals, Cd was present in smallest quantity in soil and FYM. But the quantity present in processed materials was higher than that of Pb and hence the contamination risk from Cd is much higher compared to Pb. However, the amount of Cd present in soil and that extracted was very small compared to that of Fe and Al. Since the biomagnification potential of Cd is very high, its entry to food chain has to be curtailed in order to prevent further health hazards. (Majed *et al.*, 2016). Hence its movement through soil, water and plant systems needs further research.

Cd was also phytoextracted through *L. flava*, processed as per treatments and used for pot culture experiment with amaranthus. Among the treatments, vermicompost had produced the highest biomass which was significantly superior to all others. Control recorded the lowest biomass since no organic matter / nutrients were supplied to it. Ash had produced significantly higher quantity of biomass compared to FYM. The nutrient availability from ash had favoured the growth and biomass production. Since the amount of Cd present being very small it might not have affected the plant growth adversely. The processed biomass added to each pot contained 1.170 mg Cd while the contribution from FYM was almost nil (0.0000125 mg Cd). Cd extraction by ash was significantly superior to all other treatments, which were statistically on par with each other, similar to that of Pb. Except ashing all other processing methods were almost equal to the common practice of FYM addition when Cd concentration within the plant and uptake by amaranthus were considered. The processed materials from 100 g phytoextracted biomass contained only 1170 μg of Cd and its entry to amaranthus was quite low. Hence the processing methods except ashing were almost equal in their performance with regard to the bioavailability of Cd.

Cd concentration in plant was highest for ash and lowest for control. Here also ash was significantly superior to all others, which were on par with each other. Plant Cd concentration for ash was not at all comparable to other processing methods since ash contained substantial amount of Cd compared to others. The same was true for Cd extraction also.

Comparing the extraction efficiency of disposal methods, ashing alone gave a value more than one indicating it as the least preferred method for safe disposal of hyperaccumulators while all the other methods were more or less equal. Among that biochar showed lowest extraction and highest retention, though it was on par with others.

The total quantity of toxic metal extracted by the crop can be considered as the most crucial parameter to assess the contamination potential of disposal methods for biomass containing toxic metals. Among the tested methods viz., composting, vermicomposting, ashing and biochar production, ashing was found to be least desirable or more fatal since it extracted highest quantity of metal and facilitated its translocation to plant parts. The lowest quantity of toxic metal was extracted by biochar and it had the highest retention in soil. Thus the toxic metal contamination will be least from biochar compared to other methods. Hence the safest method of disposal of phytoextracted (with toxic metals) biomass was the conversion to biochar. Raising of phytoremediators / hyperaccumultors in contaminated sites and their conversion to biochar can be considered as the most viable technology for metal decontamination.

Summary

6. SUMMARY

The project entitled "Phytoremediation of inorganic contaminants in Vellayani wetland ecosystem" was carried out at the College of Agriculture, Vellayani during 2013 –16 with the objectives to track the potential sources of contaminants in Vellayani wetland ecosystem and suggest viable phytoremediation technologies for decontamination. The study comprised of four parts which include a (i) peripatetic survey in the catchment area of Vellayani wetland ecosystem covering the seven contaminated sites viz., Palappoor, Pallichalthodu, Reservoir bund, Arattukadavu RB, Valiyavilagam, Mannamvarambu and Manamukku; (ii) evaluation of native macrophytes for their hyperaccumulation capacity for Fe, Al, Pb and Cd; (iii) performance of selected hyperaccumulators under graded doses of toxic metals and (iv) comparison of common disposal methods of selected phytoextractors based on the bioavailability of phytoextracted metals.

Part I

Based on the peripatetic survey, it was observed that the potential sources of contaminants that threatens the Vellayani wetland ecosystem were discharge from automobile workshops and servicing centers, domestic wastes from hotels / houses /other solid wastes like battery, tubes, CFL lamps, low quality pet bottles, carry bags etc., sewage, cleaning of vehicles and animals, washing of clothes and drainage from agricultural fields.

The dominant macrophytes identified in the study area include 14 aquatic and 15 terrestrial species belonging to 18 different families. Among the aquatic macrophytes, seven species were emergent type, six were floating type and one was submerged type. *C. esculenta. E. dulcis, L. flava, M. vaginalis, S. grossus, E. crassipes, P. stratiotes, N. nucifera, A. tenella, C. dactylon, S. trilobata* were the predominant species.

The Vellayani lake had experienced the invasion by non native aggressive species like *L. flava, E. crassipes, S. molesta, P. stratiotes, A. sessilis, S. trilobata* etc. which are very prominent. Invasion by native species like *S. grossus, E.dulcis, N. indica, A. tenella* and *N. nucifera* was much wide spread and heavy.

The sampling sites varied widely in density of macrophytes and Mannamvarambu had higher density compared to other sites. *C. dactylon*, the terrestrial macrophyte had the highest average density (34.70) while the floating aquatic macrophyte, *N. nouchali* recorded the lowest (0.80).

Evaluating the physical, chemical and biological qualities of water, it was observed that water quality was poorest at Arattukadavu RB, might be due to contamination as evidenced by highest values for parameters viz., NH_4-N, NO_3-N, P, BOD, COD, Fe, Al, Pb, Cd and *E. coli*, followed by Palappoor. The level of contamination was lowest at Manamukku preceded by Mannamvarmabu and Valiyavilagam.

The level of inorganic contamination of sediment was comparatively higher at Palappoor and Arattukadavu RB and lower at Manamukku and Mannamvarmabu.

The lake water as well as sediment was free from any of the pesticide residues.

The water quality was poorer during the pre monsoon period. The excessive evaporation during that months and the receipt of occasional pre-monsoon showers and the resultant muddy runoff water have increased the turbidity. The lake water experienced physical degradation as evidenced by the values of colour, turbidity and suspended solids that exceeded the maximum permissible limits.

Among the chemical properties of water, pH, EC, COD, NO_3-N, NH_4-N, Fe, Pb and Cd contents were within the MPL. But BOD and P content of water were well above the MPL. Al content of water was above the desirable limit though it was below MPL.

The coliform count was above the permissible limit, indicating water quality deterioration and chances of sewage entry in the lake water and count was highest at Arattukadavu RB during both the seasons.

The rivulets as they approach the lake, a decrease in above properties were observed indicating as the distance from sources of contamination increases the water quality improved.

Part II

The highest biomass was recorded by *L. flava* (emergent), *E. crassipes* (floating) and *P. kaida* (terrestrial) among the dominant macrophytes.

The entire study area showed moderate acidity and slight salinity. Among the sites, pH was highest at Manamukku and lowest at Arattukadavu RB while EC was just reverse to that. OC content also followed the same trend as that of EC.

Among the tested heavy metals, available Fe and Al contents were very high at all sites. Arattukadavu RB recorded the highest value and lowest by Manamukku. A striking difference in their availability in rhizosphere soil / sediment for different species or sites was not observed. Also noted contamination with small quantities of Pb and Cd. This might be mainly due to dumping of solid wastes including package / storage containers and nearness to automobile servicing centers. Their availability was also highest at Arattukadavu RB.

Fungi predominated in the acidic range and bacteria and actinomycetes in neutral range. Arattukadavu RB having the lowest pH showed the predominance of fungal population and Manamukku having the highest pH had the dominance of bacteria and actinomycetes.

Out of the emergent macrophytes, it was observed that *L. flava* had the highest Fe extraction ability followed by *E. dulcis*. The phytoextraction ability was highest for *E. crassipes* followed by *S. molesta* under floating types and *P. hydropiper*, followed by *P. repens* among terrestrials.

For Al, the phytoextraction ability was highest for *L. flava* followed by *S. grossus* (emergent); *E. crassipes* followed by *N. nouchali* and *S. molesta* (floating); and *P. repens* followed by *P. kaida* (terrestrial).

C. esculenta had the highest phytoextraction ability for Pb followed by *S. grossus* among the emergent types; *E. crassipes* followed by *P. stratiotes* and *N. indica* (floating) and *S. trilobata* followed by *B. dactyoides* and *C. dactylon* (terrestrial).

For Cd, phytoextraction ability was highest for *L. flava* followed by *C. esculenta* and *E. dulcis* (emergent); *E. crassipes* followed by *P. stratiotes* (floating) and *S. trilobata* followed by *A. tenella* (terrestrial).

Correlation between the metal load in sediment / soil, plant accumulation and plant uptake by macrophytes revealed a significant positive correlation for Cd only.

The native macrophytes under various categories were evaluated for their phytoremediation potential based on the total quantity of metal extracted, metal accumulation and BCF for shoot and root.

With regard to the phytoremediatin potential of emergent aquatic macrophytes, three species viz., *L. flava, M. vaginalis* and *C. esculenta* dominated in metal extraction and accumulation. In the case of floating aquatic macrophytes, *E. crassipes* extracted the highest quantity of all the four metals studied. Among the terrestrial macrophytes, highest quantity of Fe was extracted by *P. hydropiper* and Al by *P. repens*. *S. trilobata* extracted highest quantity of Pb and Cd.

Part III

Among the tested macrophytes for Fe, *E. crassipes, L. flava* and *M. vaginalis* performed best as phytoremediators. Major site of Fe retention for *E. crassipes* was root and for others it was shoot.

For Al, the best phytoextractor was *M. vaginalis* followed by *C. esculenta* . For both of these species the major site of Al retention was root.

The phytoextraction ability for Pb was highest for *L. flava* followed by *A. tenella* and *S. grossus*. For all, the major site of retention was root.

With regard to Cd, highest phytoextraction ability was exhibited by *L. flava* followed by *A. tenella*, where the major site of retention was root.

Part IV

The best phytoremediator for each metal selected based on the results of Part III was subjected separately to different processing techniques viz., ordinary composting, vermicomposting, ashing and biochar production. The phytoremediators, *E. crassies* and *M. vaginalis* were used for the phytoextraction of Fe and Al, respectively while *L. flava* for Pb and Cd. The processed materials were used for raising amaranthus in pots.

Among the disposal methods, biochar maintained the lowest concentration of Fe, Al, Pb and Cd in plant and highest retention in soil ensuring lowest bioavailability. Leaching loss was also lowest for biochar.

Ashing was found to be least desirable or more fatal since it extracted highest quantity of metal and facilitated its translocation to plant parts. The lowest quantity of toxic metal was extracted from biochar and it had the highest retention in soil. Hence the safest method of disposal of phytoextracted (with toxic metals) biomass was the conversion to biochar.

Raising of phytoremediators / hyperaccumultors in contaminated sites and their conversion to biochar can be considered as the most viable technology for metal decontamination among the tested methods viz., composting, vermicomposting, ashing and biochar production.

Conclusion

The potential sources of contaminants threatening Vellayani wetland ecosystem were mainly non-point type. Lake water was more contaminated by organic residues with BOD, coliforms and P above the MPL. Al content was above the DL in all the selected sites. The catchment area experienced contamination with Pb and Cd, but in traces only. Hence strict measures should be undertaken to maintain the quality of lake water. The entire area was found to be heavily infested with aggressive tolerant macrophytes viz., *L. flava, E. crassipes, S. molesta, P. stratiotes,* etc. showing effective bioremediation potential for the metals studied.

E. crassipes was observed as the best phytoextractor for Fe under treatment with varied doses of the metal; *M. vaginalis* for Al and *L. flava* for Pb and Cd. The major site of retention of metals was identified as root. Biochar resulted in the lowest concentration of metals (Fe, Al, Pb and Cd) in the plant and highest retention in soil. Hence conversion to biochar can be advocated as the safest method for disposal of phytoextracted biomass ensuring lowest metal bioavailability.

Future line of work

The present study revealed the extent of contamination in Vellayani wetland ecosystem and potential of biochar in reducing the bioavailability of metals and their availability in soil. Also the macrophytes exhibited wide variability in their bioremediation capacity and site of metal retention. Hence to explore the full possibility of phytoremediation mechanism in decontaminating a system, the following future line of work is being proposed.

a) Thorough understanding of the genes involved in hyperaccumulation so as to provide a genetic engineering approach to improve the biomass and growth habit of natural hyperaccumulators.

b) Further studies to assess the long term effect of biochar prepared from phytoextracted biomass in maintaining the mobility of heavy / toxic metals within and their release into soil. Actual mechanism involved in the adsorption of metals and the fate of metals with ageing of biochar.

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Appendices

APPENDIX I

I Media composition

a. Nutrient agar medium

Peptone	5 g
Sodium chloride	5 g
Beef extract	3 g
Distilled water	1 L
pH	7.0

b. Martin's Rose Bengal agar medium

Dextrose	1.0 g
KH_2PO_4	0.1 g
$NaNO_3$	0.1 g
KCl	0.1 g
$MgSO_4.7H_2O$	0.1 g
Distilled water	1 L

c. Kenknight's medium

Glucose	10 g
Peptone	5.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Distilled water	1 L

d. EMB medium

EMB (Eosine Methylene Blue) agar	35.96 g
Distilled water	1 L

APPENDIX II

II Florel–Ule Colour scale

Colour scale	Colour
1 – 5	Pale blue
6 – 10	Greenish blue
11 – 16	Pale green
17 – 21	Yellowish brown
22	Brown

PHYTOREMEDIATION OF INORGANIC CONTAMINANTS IN VELLAYANI WETLAND ECOSYSTEM

by

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ABSTRACT

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ABSTRACT

An investigation entitled "Phytoremediation of inorganic contaminants in Vellayani wetland ecosystem" was carried out at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani, during 2013-16. The objective of the study was to track the potential sources of contaminants threatening the Vellayani wetland ecosystem and suggest a viable phytoremediation technology. The experiment comprised of four parts.

The first part involved a peripatetic survey in the catchment of Vellayani lake along the rivulets that contribute water to Palappoor, Pallichalthodu, Reservoir bund, Arattukadavu RB, Valiyavilagam, Mannamvarambu and Manamukku sites. Three rivulets per site were identified, and geocoded water and sediment samples were drawn from five sampling points under each rivulet during pre and post monsoon seasons of 2014-15. Among the physical properties of water colour, turbidity and suspended solids were above the maximum permissible limit (MPL). Chemical properties viz., pH, EC, NO_3-N, NH_4-N, P, Fe, Al, Pb, Cd and BOD of water showed significant difference among the sites during both the seasons but COD was significant only for post monsoon. P content and BOD exceeded the MPL and Al the desirable limit. Coliforms were detected at all sites during both seasons and were above the MPL. Texture of the sediment varied from sandy clay to sandy clay loam. EC, OC, NO_3-N, NH_4-N and P contents of sediment showed a decreasing trend during the post monsoon season. Arattukadavu RB was the most contaminated site followed by Palppoor and Manamukku the least contaminated site preceeded by Mannamvarambu and Valiyavilagam with respect to water and sediment quality. No pesticide residue was detected in water and sediment. The highest plant density was noticed for *Cynadon dactylon* L.

In the second part, potential sources of contaminants were identified as automobile workshops/servicing centres, domestic wastes from hotels/houses and sewage. Out of the 29 species of dominant macrophytes, highest biomass was

recorded by *Limnocharis flava* L. (Buch.). In shoot, the highest concentration for Fe was recorded by *M. vaginalis, Panicum repens* L. for Al and *S. trilobata* for Pb and Cd. In root, the highest concentration for Fe, Al, Pb and Cd was showed by *Eichhornea crassipes* Mart., *Monochoria vaginalis* (Burm.f.), *Colacasia esculenta* L. and *Sphagneticola trilobata* L. respectively. Sediment from Arattukadavu recorded the highest contents for Fe, Al, Pb and Cd and count for bacteria, fungi and actinomycetes. It was observed from the study that concentration of Fe, Al, Pb and Cd decreased with the distance from the source of contamination. Higher quantities of Fe was extracted by *E.crassipes* and *M.vaginalis*; Al by *M. vaginalis* and *L. flava* and Pb and Cd by *L.flava* and *E.crassipes* from the wetland ecosystem.

In the third part, four pot culture experiments were carried out with graded doses of Fe (1000, 2000 and 3000 mg kg^{-1}), Al (750, 1000 and 1250 mg kg^{-1}) and Pb and Cd (50, 75 and 100 mg kg^{-1}) to determine the hyperaccumulation ability of selected macrophytes based on a screening trial. The macrophytes were grown in sediment and water collected from the Vellayani wetland ecosystem for a period of 45 days. From the study it was observed that *E. crassipes* was the best hyperaccumulator for Fe, *M. vaginalis* for Al and *L. flava* for both Pb and Cd and can be identified as the best phytoextractors for the same. Root was the major retention site for all the metals. Among the rhizosphere microbes, fungi maintained a positive relation with levels of of Fe, Al, Pb and Cd and a negative relation by bacteria and actinomycetes.

In the fourth part, the macrophytes showing highest hyperaccumulation ability for each metal (based on part III) were raised in pots containing the respective metals (2000 mg Fe kg^{-1}, 1000 mg Al kg^{-1} and 75 mg Pb /Cd kg^{-1}) for 60 days. The plants were harvested and the biomass was put to different disposal methods viz., composting, vermicomposting, ashing and production of biochar and was used for the pot culture experiments with amaranthus. The treatment effects were significant for biomass production and metal extraction. Among the four disposal methods, vermicomposting had resulted the highest biomass production for all except Pb. Regarding the metal extraction by amaranthus, application of ash (T4) showed the highest removal and the least by the biochar (T5). The metal retention in soil was highest for biochar and least for ash. Loss of metals from the processed materials was also lowest for biochar. Thus the best disposal technique of phytoextractors /hyperaccumulators is conversion to biochar.

The viable phytoremediation technology is to raise suitable phytoextractors / hyperaccumulators in the contaminated area and dispose them through biochar production.