Phytotechnologies for Ecodevelopment of Loktak Lake, Manipur

Thesis submitted to the University of Hyderabad for the Degree of

DOCTOR OF PHILOSOPHY

By

MAIBAM DHANARAJ MEITEI

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad - 500046

Enrolment No. 10LPPH21
June 2015

University of Hyderabad
School of Life Sciences
Department of Plant Sciences
Hyderabad - 500046

DECLARATION

I, **Maibam Dhanaraj Meitei**, hereby declare that the thesis entitled **"Phytotechnologies for Ecodevelopment of Loktak Lake, Manipur"** submitted to University of Hyderabad has been carried out by me under the supervision of **Prof. M.N.V. Prasad**, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad- 500046, India. I also declare that the work has not been submitted for any other degree or diploma to any University or Institute and is free from plagiarism.

Maibam Dhanaraj Meitei
Enrolment no. 10LPPH21

University of Hyderabad
School of Life Sciences
Department of Plant Sciences
Hyderabad - 500046

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Prof. M.N.V. Prasad
Supervisor

Head
Department of Plant Sciences
University of Hyderabad

Dean in-charge
School of Life Sciences
University of Hyderabad

University of Hyderabad
School of Life Sciences
Department of Plant Sciences
Hyderabad - 500046

CERTIFICATE

This is to certify that the thesis entitled "**Phytotechnologies for Ecodevelopment of Loktak Lake, Manipur**" submitted by **Mr. Maibam Dhanaraj Meitei,** Enrolment no. 10LPPH21**,** for the award of Doctor of Philosophy in Plant Sciences is **free from plagiarism** (22% similarity index; 31% total found by turnitin; anti-plagiarism software scan, including 9% from the authors original online papers published). The thesis can be deposited in Shodganga/INFLIBNET.

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Prof. M.N.V. Prasad
Department of Plant Sciences
University of Hyderabad

University of Hyderabad
School of Life Sciences
Department of Plant Sciences
Hyderabad - 500046

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Head
Department of Plant Sciences
University of Hyderabad

Dedicated to

my beloved parents and family members

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Maibam Dhanaraj Meitei

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Abbreviations

µs	Micro Seconds
AAS	Atomic Adsorption Spectrophotometer
BCF	Bioconcentration Factors
cum	Cubic Meters
CW	Constructed Wetlands
EC	Electrical Conductivity
EDI	Estimated Daily Intake
EDX	Energy Dispersive X-Ray Analysis
E_d	Exposure Duration
E_f	Exposure Frequency
FCC	Face Cubic Centre
FD	Forest Department
FTIR	Fourier Transform Infra-Red Spectroscopy
JECFA	Joint FAO/WHO expert committee on food additives
K	Kelvin
KLNP	Keibul Lamjao National Park
Km	Kilometers
LDA	Loktak Development Authority
Mcum	Million Cubic Meters
MW	Mega Watt
NGOs	Non-Governmental Organizations
NHPC	National Hydro Power Corporation
nm	Nanometers
PMTDI	Provisional Maximum Tolerable Daily Intake
PTDI	Provisional Tolerable Daily Intake
RfD	Oral Reference Dose
SAED	Selective Area Electron Diffraction
SEM	Scanning Electron Microscope
SPR	Surface Plasmon Resonance
TEM	Transmission Electron Microscopy
TF	Translocation Factors
THQ	Target Health Quotient
USEPA	United States Environmental Protection Agency
WAPCOS	Water and Power Consultancy Services
WHO	World Health Organisation
WISA	Wetlands International South Asia
XRD	X-Ray Diffraction

General introduction

Introduction

Wetlands can be found in all the climates, from tropical to tundra. Approximately 6% of the Earth's surface that equals to 2 billion acres (800 million ha), is covered by wetlands (Reddy and DeLaune, 2008). The Ramsar Convention (Ramsar, Iran, 1971) is "an intergovernmental treaty which provides the framework for International cooperation for the conservation of the wetland habitats across the world". The Convention defines wetlands as " areas of marshes, fen, peatland, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or saline, including areas of marine water, the depth of which at low tides does not exceed six metres". Later, Cowardin et al., (1979) gave a more cryptic but comprehensive definition of wetlands as "land transitional between terrestrial and aquatic systems when the water table is usually at or near the surface or the land is covered by shallow water".

India, with its annual rainfall of over 130 cm, varied topography and climatic regimes support and sustain diverse and unique wetland habitats. It is estimated that freshwater wetlands alone support 20% of the known range of biodiversity in India (Trishal and Manihar, 2002). India has 4.1 million ha of wetlands (excluding paddy fields and mangroves), of which 1.5 million ha are natural and 2.6 million ha are artificial. It has 26 wetlands designated as Internationally Important by the Ramsar Convention, **Fig.1a**. The country's wetlands are generally differentiated by region into eight categories viz. the lagoons and other wetlands of the South-West coast (eg. Ashtamudi); the vast saline expanses of Rajasthan, Gujarat and the Gulf of Kutch (eg. Nalsarovar bird sanctuary); freshwater wetland and reservoirs from Gujarat eastwards through Rajasthan (eg. Keoladeo National Park) and Madhya Pradesh (eg. Bhoj); the delta wetland and lagoons of Indian East coast (eg. Chilka); freshwater wetlands, marshes, swamps and rivers of North-East India (eg. Loktak) and the Himalayan foot hills (eg. Deepor Beel); wetlands and rivers of the montane region of Kashmir and Ladakh (eg.Tsomoriri); and mangroves and other wetlands of the island arcs of the Andaman and Nicobar islands (Trishal and Manihar, 2004).

Manipur because of its peculiar situation in the eastern Himalayas is blessed with an enormously rich and diverse heritage of wetlands or *pats*. The state is drained by two major river systems, the Barack drainage system and the Chindwin-Irrawaddy-Manipur river system, along with various wetlands in the central valley. Covering an area of 524.51 km^2 (Laiba, 1992), the wetlands share 2.35% of the total geographical area of Manipur. Loktak is the largest *pat* within the Manipur river basin covering 61% of the total identified wetlands of Manipur, **Fig.1b**. Likewise, there are several shallow wetlands or marshes in the interfluvial areas of the Imphal river. Thus, there is Lamphel *pat* between the rivers Nambul and Imphal; Waithou *pat* between the rivers Iril and Thoubal; Ikop, Kharung and Lousi *pats* between Thoubal and Sekmai rivers; Khoidum, Lamjao and Pumlen *pats* between Imphal and Sekmai rivers. On the west, between the rivers Manipur and Khuga, is Loktak, comprising of near to 20 small and large *pats*. Among them, Takmu, Ungamen, Laphu, Khulak, Yena and Tharopokpi *pats* are fairly large (more than 80 ha). During the rainy season, most of these *pats* become contiguous and merge under a large sheet of water forming the vast expanse of Loktak, but can be distinguished separately during the dry season. Several small rivers and streams rising in the surrounding hills drain into these *pats* on both the sides of the Manipur river. The Nambul and Nambol river are two major streams from the north flowing into Loktak basin. Likewise, there are over 34 small streams

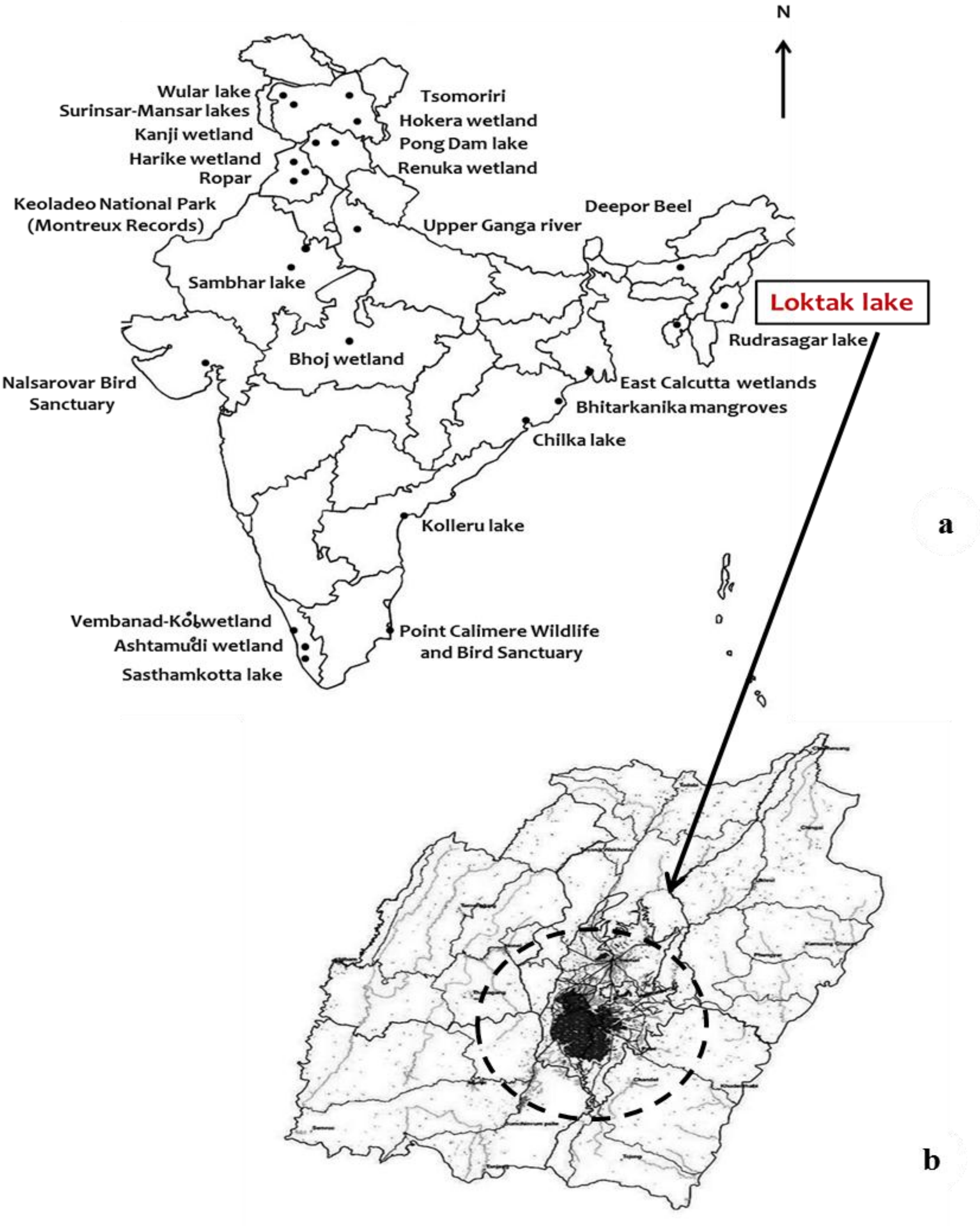


Fig. 1 (**a**) Ramsar sites in India; and (**b**) Location of Loktak and its morphometry.

1. Area: 287 km^2	6. Streams: 36	11. Open area: 73.5 km^2
2. Length: 32 km	7. Rainfall: 1500-1700 mm	12. Phoomdi area: 107 km^2
3. Breadth: 13 km	8. Humidity: 75-89%	13. Island area: 17.2 km^2
4. Depth: 2.7m	9. Min. temp: 2-21°C	14. Enchroached area: 89.3 km^2
5. Catchment: 8247 km^2	10 Max. temp: 23-26°C	

draining from the hills on the west into the Loktak basin. Loktak is connected to the Manipur river by a small channel called Khordak cut which drains into the river during the dry period. However, when the river is in spate, it backflows into the wetland through the channel.

Loktak (93°46' to 93°55' E and 24°22' to 24°42' N) is oval shaped with maximum length and width of 32 km and 13 km, **Fig.2a**. The depth varies between 0.5 and 4.6 m with average recorded at 2.7 m. It covers an area of 287 km^2, maintained at 768.5 m above mean sea level (MSL) by Ithai barrage. The wetland has a catchment area of 8247 km^2. The catchment elevates from 780 to 2068 m above mean sea level (Trishal and Manihar, 2002) and is characterized by a tropical to semi-tropical climate, with semi-temperate to temperate at higher altitude. The western catchment area directly draining into the wetland has 342 km^2 under agriculture, 133 km^2 under habitation; 262 km^2 under forests, 22 km^2 under waterlogged and 287 km^2 being the wetland area itself. Loktak watershed can be broadly divided into four physiographic units viz. high hills (900-1940 MSL), medium hills (760-900 MSL), plains (very gentle slope) and marshy lands (shallow water with the presence of thick growth of floating and submerged plants). The soils in the higher slopes of both high and medium hills are prone to excessive erosion. About 5.9% of the Loktak watershed falls under the very severe erosion class, 14.6% under severe and 7.2% under moderately severe. It records an annual rainfall of 1500 to 1700 mm with minimum temperature ranging from 2^0 to 21^0 C and maximum between 23^0 to 36^0 C. Relative humidity recorded varied from 75% in February to over 89% during the rainy season. Rainy season starts with the onset of monsoon in June and continues till September, and accounts for 63% of the annual average catchment precipitation. There are 14 hills located in the wetland varying in size and elevation appearing as islands in the southern part of the wetland. The most prominent among these are Sendra, Ithing and Thanga islands.

Loktak is an important source of water, fisheries and vegetation providing sustenance to a large population dependent of the wetland resources. The wetland water is used for irrigation, domestic purposes and power generation. The vegetation is harvested for use as food, fodder, fiber, fuel, handicrafts and medicinal purposes. National Hydro Electric Power Corporation (NHPC) is an important beneficiary using the wetland water for power generation with a total installed capacity of 105 MW. Loktak with its several islands located inside the wetland and surrounded by floating *phoomdi* of different geometrical shapes makes it a unique destination for tourism. The wetland has got a rich biodiversity inhabiting the water, *phoomdi* and islands; 233 species of plants that belong to emergent, submerged, free floating and rooted types; and 425 species of animals (249 vertebrates and 176 invertebrates), (Singh and Singh, 1994). It provides refuge to thousands of birds which belong to 116 species. Of these, 21 species of waterfowl are migratory, most migrating from different parts of the northern hemisphere beyond the Himalayas. Likewise, the fish fauna of Loktak comprises of 64 species (Singh and Singh, 1994). Two of these species *Monopterus albus* and *Osteobroma belangeri*, are restricted in their distribution to the Yunan state of China, Myanmar and Manipur only. In addition, the wetland serve as the breeding ground for several species of migratory fish such as *Labeo dero*, *Cirrhinus reba* and *Osteobroma belangeri* that migrate from the Chindwin-Irrawaddy river system of Myanmar to the upstream areas of Manipur river and breed in the shallow wetlands of the valley (Singh, 1996).

Keibul Lamjao National Park (KLNP), a unique floating wildlife reserve in the world is composed of a continuous mass of floating *phoomdi*. It is the only natural habitat of the endangered species of Elds deer, *Rucervus eldi eldi* McClelland, with a population of near to 200 heads in 2000 (FDI, 2011). *Phoomdi* are “heterogenous mass of soil, vegetation and organic matter in different stages of decay” (Singh and Singh, 1994), **Fig.2b-c**. They occur in various sizes, small or large with thickness varying from a few centimeters to above 2.5 m. They occupy nearly two-thirds of the surface area of the wetland. Various species of plants take part in the formation of *phoomdi* but those of primary importance are certain large grasses and sedges and other emergent that give out long floating rhizomes and runners with upright shoots. Free floating plants and partly decomposed roots and rhizomes contribute greatly to their development. A *phoomdi* may be initiated with a small mass of un-decomposed organic matter or dense growth of floating plants which accumulate suspended silts and are gradually colonized by grasses and other herbaceous plants. Later, as more and more organic matter is added from the death and decay of vegetation on and around the *phoomdi*, and the mineral soil accumulates from the suspended silt load, the *phoomdi* continue to grow in thickness and size, and supports increasingly more vegetation of different kinds. The high proportion of vegetable matter in the *phoomdi* gives it a low specific gravity and high buoyancy to keep it afloat. They float on the wetland water with about one fifth of their thickness above and four fifths under the water surface. About one meter thick *phoomdi* easily supports the weight of animals and human beings, with more than 1,500 hutments have been found on the *phoomdi* of Loktak. The core of the *phoomdi* is composed of detritus that is black in colour and highly spongy. *Phoomdi* is a habitat of a large variety of aquatic, semi-aquatic and terrestrial plants, comprising of 128 species from 46 families (Devi, 1993). Among

Fig. 2 (**a**) Aerial view of Loktak; (**b**) *Athaphoom* or Traditional fishing ponds; and (**c**) A newly formed *phoomdi*.

the dominant plants are several species of grasses and sedges. The reeds (*Phragmites karka*) alone constitute about half the vegetation of Keibul Lamjao National Park.

At present, “wetlands have become the *cause celebre* for conservationists, serving as symptoms of dismantled water resources and their disappearance represents a recognizable loss of natural areas to economic growth” (Mitsch and Gooselink, 2007). Wetlands across the world are threatened by eutrophication and heavy metal pollution from anthropogenic origins via industrial, sewage and agricultural runoff (Muhammad et al, 2011; Xu et al., 2014). Likewise, Loktak is threatened by excessive loading of silts, nutrients, pesticides and metals from various anthropogenic sources. Deforestation and shifting cultivation, uncontrolled use of fertilizers in the agricultural lands, discharge of domestic wastes and sewage from the human settlements including those on *phoomdi*, all contribute to the input of silts, nutrients and biogenic salts into the wetland, **Fig. 3a-b**. It accelerates the ageing of the wetland by rapid siltation and excessive biomass production. A major change in the ecological character of the wetland has been its conversion into a reservoir by the construction of the Ithai barrage for hydel power generation, **Fig. 3c**. Before 1983, *phoomdi* had a characteristic annual cycle of floating in the rainy season with the rise in water level and settling down on the wetland bed during the dry season. Whereas the decomposition of the detritus would be accelerated and the growth of vegetation retarded during the dry period, the vegetation growing on *phoomdi* used nutrients from the sediment. After the rain starts, the *phoomdi* rises and floats due to the buoyancy as the gases formed during decomposition. The raised *phoomdi* carried lots of sediment with them from the wetland bed through the roots that helped them grow

Fig. 3 Ecological degradation of Loktak; (**a**) Catchment degeneration for *jhoom* cultivation; (**b**) Major tributaries discharging various waste; and (**c**) Ithai barrage for Hydel power production.

in size and thickness. This annual cycle has now come to a halt and the *phoomdi* remain floating throughout the year. Ithai barrage has compounded the wetland with no excess for the silts, nutrients and metal ions to flow downstream. In addition, Loktak suffers from both natural and cultural eutrophication. The wetland basin receives 137.74 tons of P and 1152.14 tons of N km^{-2} $year^{-2}$ from its catchment (Shyamananda, 1991). The rate of fertilizer application in the surrounding fields is estimated between 100 to 200 kg ha^{-1}, making the wetland a heavily eutrophic one. Besides the nutrients from the runoffs, domestic sewage, small scale industrial effluents, organochlorine pesticides and various trace metal ions finds its way into Loktak, **Fig. 4a-c**. The consequences are loss of biodiversity, weed infestation, decreased fish production and proliferation of *phoomdi*.

Problems and prospects

The prolific growth of *phoomdi* in Loktak has reached an alarming situation, and these have been a great concern for the communities, **Fig. 5**. The enormous growth has severe impact on the wetland ecosystem processes and functions. *Phoomdi* float mostly in suspended state and displaces water due to buoyancy. This affects the elevation-area-storage capacity relationship. The observed volume of water displaced by 1 cum of *phoomdi* is 0.75 cum of water and *phoomdi* occupies near to 107 km^2 of the wetland surface. At present an estimated 45.96 Mcum of water is displaced by *phoomdi* (LDA, 2011). Therefore, increased *phoomdi* area will reduce the storage capacity of the wetland and eventually affect the water resources and wetland environment. The vast area of wetland covered by *phoomdi* (107 km^2) is responsible for heavy loss of water through evapotranspiration.

Fig. 4 Ecological degradation of Loktak; (**a**) Agricultural activities inside the wetland; (**b**) Enchroachment by locals; and (**c**) Discharge of large number of piggery wastes.

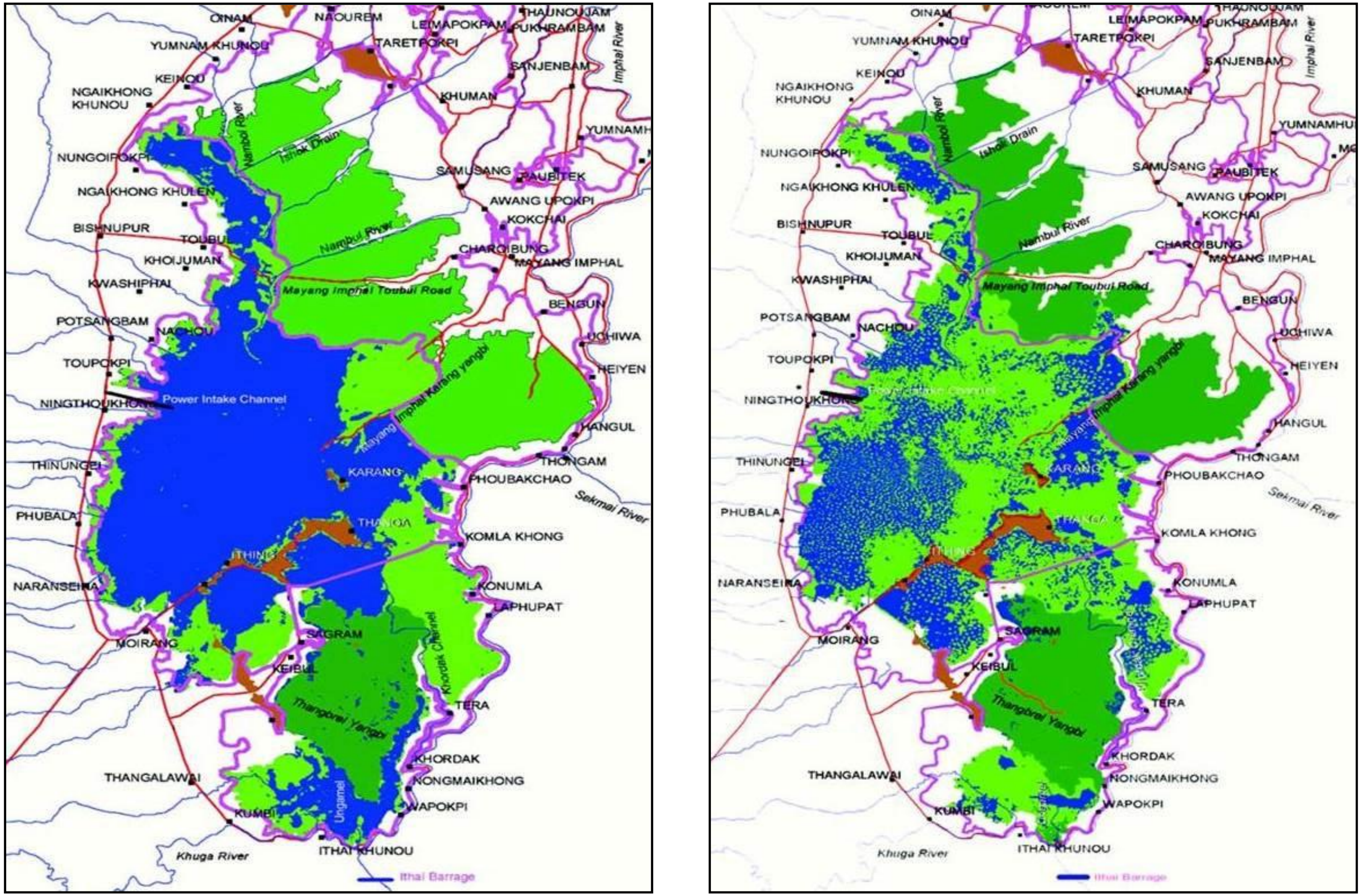


Fig. 5 *Phoomdi* proliferation in Loktak; a comparison of 1989 (left) and 2002 (right). (Source: Trishal and Manihar, 2002)

It has been estimated that water loss due to evaporation from the open water area of 73.5 km^2 is 75.3 Mcum and the loss due to evapotranspiration from *phoomdi* covered area of 107 km^2 is 136.4 Mcum. The presence of *phoomdi* in the channels and the channel mouth chokes and interferes with the flow regime. This results in prolonged stagnation and impoundment in the upstream courses and is one of the causative factors for flooding. As the *phoomdi* floats around the year, decaying of *phoomdi* plants and sinking in the wetland bottom has increased the eutrophication level of Loktak.

Realizing the problems of Loktak, the Government of Manipur constituted Loktak Development Authority (LDA) in 1986 for "overall improvement and management of the wetland". The objective of the authority is "to check deteriorating conditions of Loktak and to bring about improvement of the wetland along with the development in the fields of fisheries, agriculture, tourism and afforestation in consultation with the concerned departments of the state Government" (Singh and Singh, 1994). Initially, management of Loktak was mainly focused on removal of *phoomdi*, desiltation and availability of water to ensure sufficient generation of power, **Fig. 6a-b**. The master plan submitted by Water and Power Consultancy Services (India) Ltd (WAPCOS), a Delhi based Government of India's consultancy organization focused mainly on development of fisheries, tourism and agriculture etc, with the construction of a 126 km long dyke encircling the wetland, dredging of a major portion of the wetland bottom, a large number of sluice gates, culverts, bridges and canals etc. with the removal of *phoomdi* by mechanical approach (Trishal and Manihar, 2004). The measures have not taken into account the problem of eutrophication, metal and organics pollution which is a physiological ailment of the wetland. This cannot be

Fig. 6 Short Term Action Plan (STAP) of Loktak Development Authoirty (LDA); (**a**) *Phoomdi* removal using mechanical equipment's; and (**b**) Disposal on the bank of the wetland.

cured without understanding the ecosystem dynamics of the wetland which the authorities have failed to realize as relevant in their programme. The LDA and WAPCOS while aiming at the enhancement of economic utility of the wetland and its beautification have ignored the problems of ecological health of the wetland. Dredging is meaningless if there is no adequate arrangement for the disposal of dredge spoils. Likewise, in depth baseline data need to be gathered before the removal of the *phoomdi* cover from the wetland, **Fig. 7**.

The nutrient regime of the wetland should be so managed that the removal of *phoomdi* may not induce phytoplankton blooms in the wetland and cause water quality deterioration. Nutrient management can be achieved through a combination of different techniques like checking of nutrients inflow and enhancement of its outflow, changing the catchment land use and biotic harvesting. The application of various techniques of phytotechnologies, which are plant based technologies for environmental protection and conservation can be proposed, **Fig. 8**. Phytoremediation, ecosystem services and various eco-friendly crops for the protection of the catchment, watershed management and useful by-product generation is a part of the emerging field. Phytotechnology is used for the remediation of nutrients, metal ions, pesticides, aromatic hydrocarbons, explosives and petroleum hydrocarbons etc. In Loktak, the biotic harvesting and plantation of *phoomdi* macrophytes capable of stripping nutrients will be very effective as many aquatic plants are high biomass yielding and used as food and fodder by the locals that help in the economic development of the region, **Fig. 9**. Besides, there is a large scope for biotic community manipulation in the wetland so that a favourable food chain or food

Phoomdi Management, Water Management, Catchment Conservation, Biodiversity Conservation (Keibul Lamjao National Park management), Sustainable Resource Development and Livelihood Improvement

Issues and Challenges

Opportunities ?

Utilization of the unused floating biomass and its application for Ecodevelopment of Loktak

Fig. 7 Issues and Challenges in relation with *phoomdi* removal and degradation of Loktak, and the opportunities to harvest.

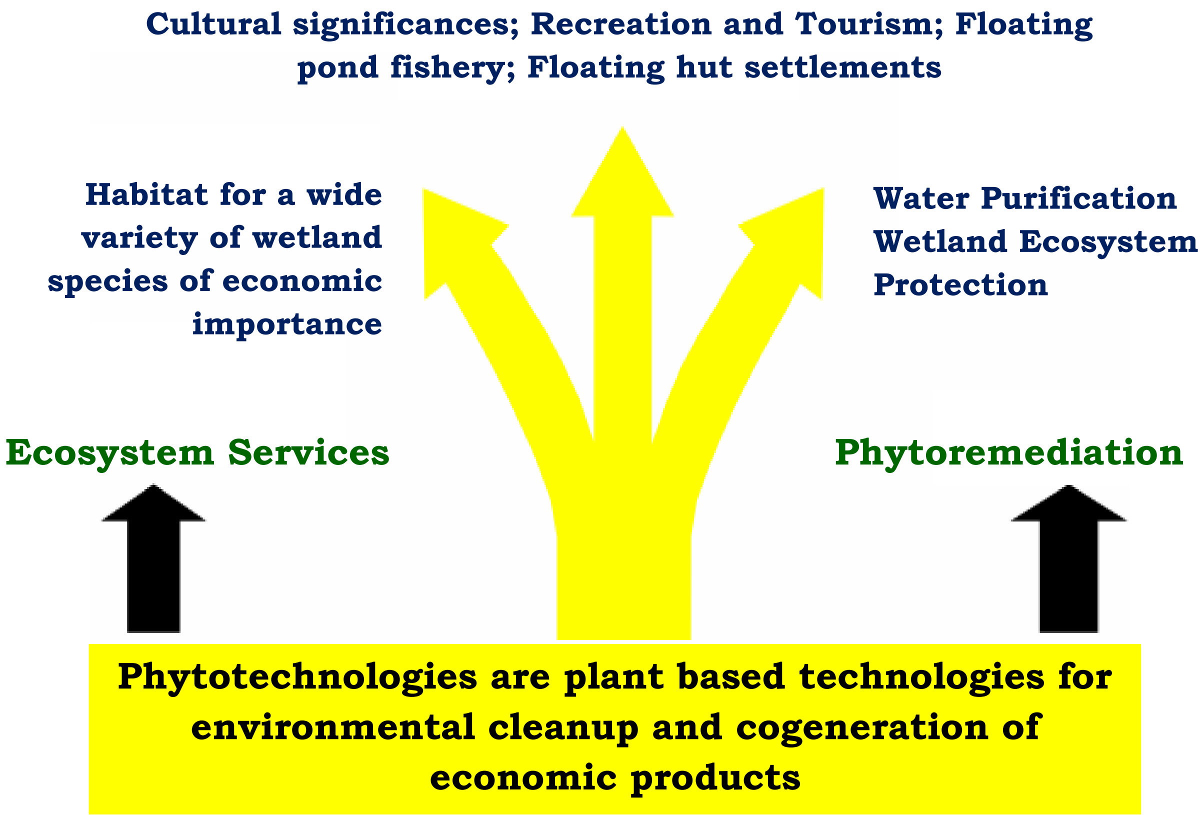


Fig. 8 Phytotechnologies for Ecodevelopment of Loktak.

(Ecosystem services provided by the application of various plant based techniques in Loktak will better the ecological health, via phytoremediation; promote the preservation of *phoomdi* habitat; will provide a sustainable source of resources; increase the aesthetic appeal and life of the wetland) Fig. adapted from Lalhruaitluanga, (2011).

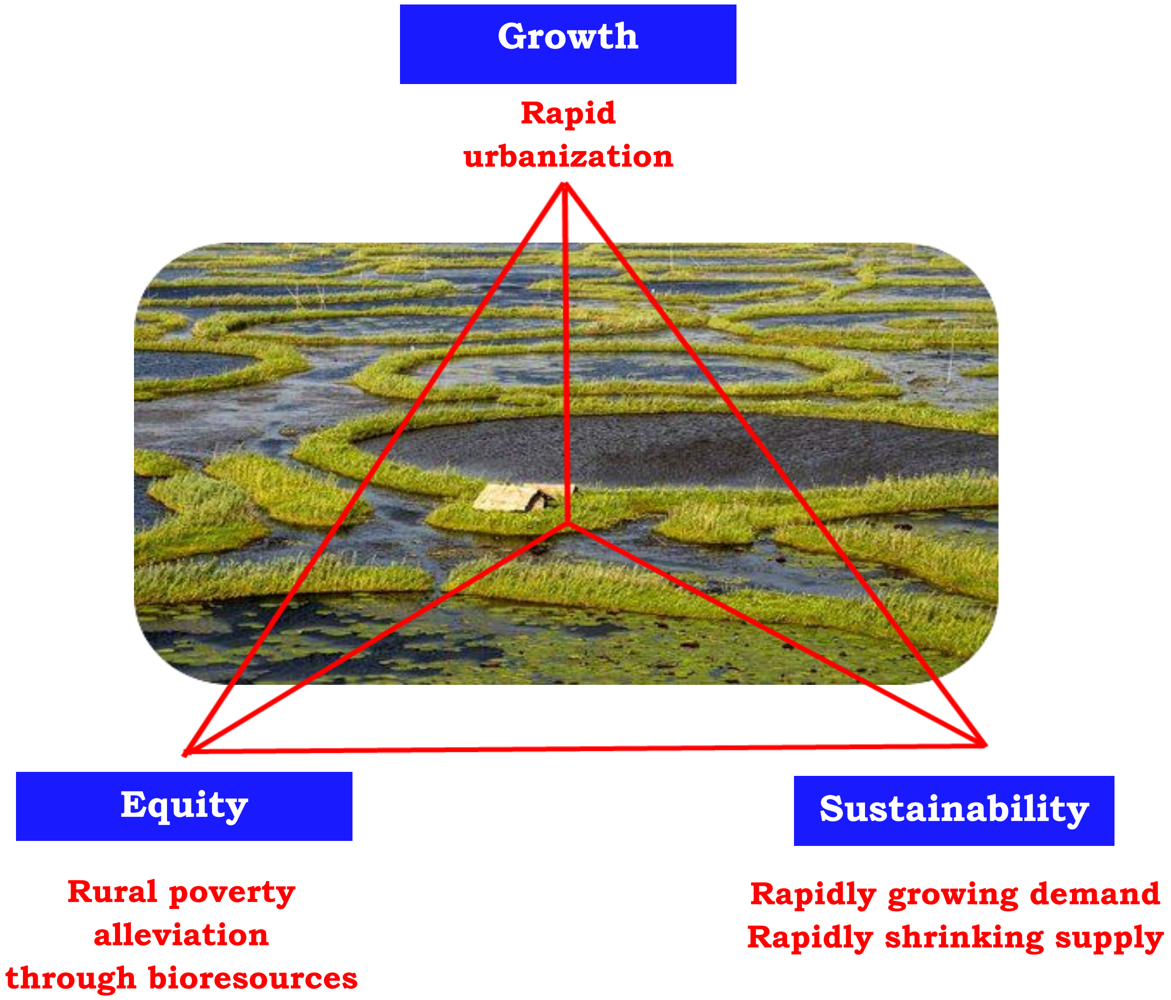


Fig. 9 Ecodevelopment parameters for sustainable utilization of Loktak.

web may control the level of nutrients and metals and lead to ecological health improvement of the wetland. Current short and long term action plans focused on several issues are in place but an overall integrated management action plan considering ecological, social, economic and cultural features of the wetland is lacking. Based on the information mentioned *supra vide* we framed the following objectives with the utilization of the unused floating biomass and its application for ecodevelopment of Loktak.

Objectives

1. *Phoomdi* - a unique plant biosystem of Loktak: Traditional Knowledge.

2. Metals and nutrients in sediment, water and *phoomdi*: Health risk assessment and Opportunities for phytoremediation.

3. Phytofiltration of iron-contaminated waters by *Typha latifolia* L.: Laboratory studies.

4. Adsorption of Pb (II), Cd (II), Cu (II), Zn (II) and Mn (II) by *Spirodela polyrhiza* (L.) Schleiden: Application for wastewater treatment.

5. Silver nanoparticles synthesis mediated by *Hedychium coronarium* J. Koenig, *Spirodela polyrhiza* (L.) Schleiden and *Trapa natans* L. extracts: Application for dye photodegradation.

Objective 1

Phoomdi - a unique plant biosystem of Loktak: Traditional Knowledge

Introduction

Communities living in and around the wetland are directly or indirectly dependent upon the wetland resources for the sustenance. There is a significant population which harvests aquatic plants from Loktak for different purposes. It has been estimated that 33% of the wetland shore households harvest aquatic vegetation for use as fuel; 18% for use as vegetables; 2% for use as fodder and 1% for manufacturing handicrafts. Annually, 15,400 MT of plant biomass is harvested for use as fuel, 1,900 MT for use as vegetables, 230 MT for use as fodder and 40 MT for making handicrafts (Trishal and Manihar, 2002). Wetland degradation leading to decline in these resources has directly impacted the livelihoods of the local communities. Species composition, in general, has slightly changed due to modifications of hydrological regimes and human pressure. *Phragmites karka* that earlier formed thick and tall stands and provided shelter to various animals including Sangai, is now found stunted in growth. Similarly, several other species including *Cymbopogon nardus*, *Erianthus arundinaceous*, and *Imperata cylindrica*, which were earlier distributed widely, are now confined to some pockets of the wetland (Trishal and Manihar, 2002). Likewise, invasive species such as *Eichhornia crassipes*, *Salvinia cucullata*, and *Brachiaria mutica*, taking advantage of absence of competition and availability of free space has profusely grown in the wetland and has presently assumed nuisance proportions and is interfering with natural biodiversity (Shyamananda, 1991; Singh and Singh, 1994).

Therefore, proper understanding of the relationship between the wetland resources and community livelihoods is critical to management of the wetland. Human activities in the catchments are linked with the changes in the wetland ecosystem. Sustainable management of Loktak emphasizes on regulating human activities within the catchments to maintain the ecological processes, functions and attributes of the wetland ecosystem while ensuring economic stability of the communities. Keeping this in mind, a study was conducted on the relevance of *phoomdi* for the sustenance and commercial needs of locals. Survey includes interviews with locals focusing *phoomdi* for: (1) traditional knowledge, (2) commercially important *phoomdi* edibles, and (3) rare traded species.

Materials and methods

Study area

Loktak lake (93°46'-93°55' E, 24°22'-24°42' N) lies in the central part of Manipur, a hilly state in North-East India. It is situated at 768.5 m above MSL and has an area of 287 km^2 (Laiba, 1992). The catchment area is 8247 km^2 and is fed by 36 streams (Trishal and Manihar, 2004). It records an annual rainfall of 1500-1700 mm. Minimum temperature ranges between 2° to 21° C and maximum 23° to 36° C. Relative humidity varies from 75% in February to over 89% during Monsoon.

Data collection

The study was conducted from 2010 to 2011 in Loktak. Data were gathered through interviews of the locals, literature and field observations. Five villages, viz. Ningthoukhong, Moirang, Sendra, Thanga and Keibul Lamjao were chosen for data collection. In total, 83 locals were interviewed (45 men and 38 women between 42 and 85 years old). Informants were subdivided into four groups; (1) farmers, (2) fisherfolks (*ngamis*), (3) traders of wild edibles, and (4) traditional healers (*maiba*). Following informations were gathered through questionnaire based on; (1) bioresources obtained from *phoomdi*, (2) cultural significances, (3) common traded wild edibles, (4) Manipuri names of the plants, plant parts used, availability periods, processing of plants, and mode of consumption, (5) management of floating fishing pond or *athaphoom*, (6) fishing methods in *athaphoom*, and (7) raw material from *phoomdi* to build the floating huts. Voucher specimens collected were identified according to the checklist available in the published scientific literatures (Devi, 1993; Singh and Singh, 1994; Singh, 1996; Elangbam, 2002; Trishal and Manihar, 2002; Devi et al., 2010; Jain et al., 2011). Author names were standardized according to Brummitt and Powell (1992). Data on fish harvested from *athaphoom* and the number of huts on *phoomdi* was obtained from Loktak Development Authority (LDA) and Forest Department, Government of Manipur, India (FD, 2011).

Trade activity of wild edibles was collected from three primary bazaars (markets), viz. Moirang, Ningthoukhong and Bishnupur bazaars. A total of 60 women vendors (20 from each bazaar) have been interviewed. Questionnaire was based on, (1) species collected, (2) parts sold, (3) season for collection, (4) availability status on *phoomdi*, (5) local demands and (6) prevailing market price. Survey was conducted during the peak hours of the day, between 6-9 am and 2-5 pm. Demand and use value (including availability status of a particular plant species) was analysed based on questionnaire/observations. They were ranked as, (1) rarely distributed, (2) sparsely distributed, and (3) extensively distributed. This ranking gave an idea of the particular *phoomdi* species that is extensively exploited and needs conservation measures.

Results and discussion

A total of 47 *phoomdi* species from 17 different families were used traditionally by the locals, **Table 1**.

Table 1 Traditional knowledge of *phoomdi* plants and market prices of wild edibles (2010-2011, 1$ = 51.36), Loktak lake, India

Scientific name and family	**Local name (Manipuri)**	**Parts used and Price (Rs./Kg)**	**Use and preparation**
Ageratum conyzoides L. Asteraceae	Khongjainapi	Shoot	MD: Shoot paste is applied on cut and wounds.
Alisma plantago aquatica L. Alismataceae	Kaothum	Rhizome (30-40)	WE: Rhizome is eaten fresh.
Alocasia cucullata Schott Araceae	Singjupan	Rhizome (15-20)	WE: Rhizomes are used in *singju*.
Alpinia galanga Willd. Zingiberaceae	Kanghoo	Rhizome (40-50), flowers	MD: Rhizome paste is used to treat fever and rheumatism. WE: Rhizomes are used in *iromba*. CS: Flowers for God.
Alpinia nigra (Gaertn.) B.L. Burtt Zingiberaceae	Pullei	Rhizome (40-50)	WE: Rhizomes used in *iromba*.
Alternanthera philoxeroides Griseb. Amaranthaceae	Kabonapi	Tender shoot	MD: Leaf paste applied on cut and injuries. WE: Used in *utti*. FD: Whole plant as fodder.
Alternanthera sessilis (L.) R. Br. ex DC. Amaranthaceae	Phakchet	Tender shoot	WE: Shoots are used in *kangsoi*.
Argyreia nervosa (Burm.f.) Bojer Convolvulaceae	Uri tujombi	Root, leaves	MD: Root extract is tonic and used in treating rheumatism and nervous disorders. Leaf extract is applied on cut, wounds and skin diseases.
Arundo donax L. Araceae	Yenthou	Rhizome, culm, leaves	MD: Rhizome extracts are diuretic and stimulate menstrual discharge. HM: Culms as thatching material. FD: Leaves for cattles. FL: Culms as fuel.
Carex indica L. Cyperaceae	Hundung	Leaves	FD: For cattles.
Centella asiatica (L.) Urb. Apiaceae	Peruk	Whole plant (30-40)	MD: Whole plant extract is used as tonic and cures skin diseases and leprosy. WE: Whole plant is used for *kangsu*.

Coix lachryma L. Poaceae	Chaning	Seed	MD: Seed powder acts as tonic, diuretic and blood purifier.
Colocasia esculenta (L.) Schott Araceae	Lampal	Rhizome (20-30), leaves	MD: Petiole juice is applied on cut and wounds. WE: Rhizome in *iromba* and leaves in *utti*.
Crotalaria juncea L. Papilionaceae	U hawai maton	Leaves, seed	MD: Seed powder as blood purifier. WE: Leaves used in *kangsoi*.
Cymbopogon nardus (L.) Rendle Poaceae	Charot	Leaves	HM: Thatching material.
Cynodon dactylon (L.) Pers. Poaceae	Tingthou	Shoot, root	MD: Shoot paste is applied on cut, wounds and root extract in treating dropsy and epilepsy. CS: Twigs are offered to God.
Cyperus rotundus L. Cyperaceae	Sembang kaothum	Tuber	MD: Paste of the tuber is diuretic, stimulant and useful in treating stomach disorders.
Desmodium motorium (Houtt.) Merr. Papilionaceae	Lam Hawai	Root	MD: Root extract is used in asthma and fever.
Dioscorea alata L. Dioscoraceae	Ha	Tuber	WE: Tubers are steamed and eaten as a rice substitute.
Echinochloa stagnina (Retz.) P. Beauv. Poaceae	Hoop	Shoot	FD: Shoot for cattles and fish. CS: Twigs offered to God.
Enhydra fluctuans Lour. Asteraceae	Komprek tujombi	Shoot	MD: Shoot extract is laxative, cures skin and nervous diseases. WE: Tender shoots used in *singju*.
Erianthus arundinaceous (Retz.) Jeswiet. Poaceae	Singnang	Shoot	FD: Shoots. HM: Thatching material.
Gynura cusimbua S. Moore Asteraceae	Terapaibi	Shoot	MD: Shoot paste is applied on cut and wounds. WE: Shoot cooked and eaten occasionally.
Hedychium coronarium J. Koenig. Zingiberaceae	Loklei	Rhizome (40-50), tender shoot	MD: Paste of the rhizome and extract can cure stomach, liver trouble and is tonic. WE: Tender shoot and rhizome are used in *iromba*.
Hedychium spicatum Lodd. Zingiberaceae	Takhellei	Rhizome	MD: Rhizome extracts are used in stomach disorders, liver troubles etc. tonic and stimulant.

Imperata cylindrica Raeusch. Pocaeae	Ee	Root, shoot	MD: Root paste and extract are used to treat piles. HM: Shoots as thatching material.
Ipomoea aquatica Forssk. Convolvulaceae	Kolamani	Shoot (20-30)	MD: Shoot extract is purgative and emetic. WE: Tender shoots are used in the preparation of *shak*.
Jussiaea repens L. Onagranaceae	Ishing kundo	Tender shoot	MD: Leaf paste is applied on cut and wounds. WE: Tender shoots are used to make *kangsoi*.
Leersia hexandra Sw. Poaceae	Choura	Shoot twigs	CS: Twigs for God.
Ludwigia octovalvis ver. *sessiliflora* (Jacq.) P.H. Raven Onagranaceae	Devo	Fruits (30-50)	WE: Fresh Fruits are eaten.
Marsilea minuta L. Marsileaceae	Ishing yenshang	Leaves, petioles	WE: Leaves and petioles are used in *kangsoi*.
Mikania micrantha Kunth Asteraceae	Uri hingchabi	Tender shoot	WE: Tender shoot.
Monochoria hastifolia C. Presl. Pontederiaceae	Kakla	Shoot	MD: Shoot extract is diuretic and applied on boils.
Narenga porphyrocoma (Hance) Bor. Poaceae	Singhut	Culm	HM: Culms are used in house making. FL: Culms.
Neptunia oleracea Lour. Mimosaceae	Ishing - ikaithabi	Shoot (30-40)	WE: Shoots are eaten raw with *iromba* or *singju*.
Oenanthe javanica DC. Apiaceae	Komprek	Tender shoot (15-25)	WE: Tender shoots are used in *singju*. CS: Tender shoots.
Oxalis corniculata L. Oxalidaceae	Yensil	Shoot	WE: Tender shoots are used in *kangsoi*.
Persicaria chinensis (L.) H. Gross Polygonaceae	Yengkhuman	Shoot	WE: Shoots in *kangsoi*.
Persicaria posumbu (Buch-Ham ex. D. Don) H. Gross Polygonaceae	Ishing kengoi	Whole plant (30-40)	WE: Whole plants after removing roots are used for *kangsu* and *kangsoi*.
Phragmites karka (Retz.) Trin. ex Steud. Poaceae	Tou	Culm	HM: Culms are used in house making. FL: Culms.
Polygonum barbatum L. Polygonaceae	Yellang	Aerial parts (20-30)	MD: Shoot paste is used in stomach disorder. WE: Aerial parts for preparing *singju*.

Polygonum chinense L. Polygonaceae	Angom yensil	Shoot	WE: Shoots.
Polygonum orientale L. Polygonaceae	Chaokhong	Shoot	MD: Shoot extract is tonic.
Saccharum munja Roxb. Poaceae	Khoimom	Shoot	MD: Shoot extract is applied on burning sensations and helps in treating blood troubles.
Sagittaria sagittifolia L. Alismataceae	Koukha	Rhizome (50-60)	MD: Rhizome paste is useful in cough. WE: Rhizomes are eaten fresh.
Scirpus lacustris L. Cyperaceae	Kouna	Shoot	HM: Aerial parts are used for thatching. FL: Shoots. HC: *Kouna*, a handicraft material.
Zizania latifolia Turcz. Ex. Stapf. Poaceae	Ishing kambong	Tender shoot (20-30), inflorescence	MD: Inflorescence extracts to treat indigestion. WE: Tender shoot eaten fresh. HM: Aerial parts as thatching material.

Notes: CS, Culturally significant; FD, Fodder; FL, Fuel; HC, Handicrafts; HM, House making; MD, Medicinal; WE, Wild edible.

Phoomdi plants were classified as per the usage of the plant parts by the communities: (1) plants for food; (2) medicinal value; (3) plants of cultural significances; (4) plants for fodder and fuel; (5) raw materials for handicrafts; (6) plants in *athaphoom*; and (7) plants for house building material.

Wild edibles and their trade

A total of 27 species from 15 families were recorded from *phoomdi*. All the edible species are mainly herbaceous in nature with different life forms, viz. creeping, prostrate, slender, free floating or rhizomatous types. The species are consumed for their shoots (13 sp.), roots/rhizome/tuber (8 sp.), whole plant (3 sp.), leaves (2 sp.), and fruits (1 sp.). *Alocasia cucullata, C. asiatica, H. coronarium, N. oleracea, O. javanica*, and *P. barbatum* are common consumed species. Fresh vegetables are used to prepare Manipuri traditional delicacies, viz. *iromba, kangsoi, kangsu, shak, singju, and utti. Iromba* is prepared by boiling *A. nigra, C. esculenta*, *H. coronarium,* chilli and potato, **Fig. 10a**. Boiled vegetables are mashed and mixed with fermented fish. *Crotalaria juncea*, *J. repens* and *P. chinensis* shoots are used in *kangsoi*. Vegetables are boiled with potato, onions, chillies, fermented fish and spices. *Kangsu* is prepared by boiling *C. asiatica, P. posumbu*, potato, chilli and then mashed with fermented fish. Tender shoots of *I. aquatica* are boiled with potato, fish and spices in the preparation of *shak*. *Singju* is a salad made by mixing *A. cucullata, I. aquatica, N. oleracea, O. javanica*, and *P. barbatum*, with fermented fish and chilli, **Fig 10b**. *Alternanthera philoxeroides* and *C. esculenta* leaves are used in *utti*. They are boiled with green peas, chillies, spices, fermented fish and sodium bicarbonate. Atleast an item mentioned above is a common constituent of the local meal every day.

Common staple diet of the people is rice. Locals normally have their meal with a vegetable prepared from *phoomdi* edibles. Local dishes, viz. *iromba, kangsoi, kangsu, shak, singju* and *utti,* thus form an important part of the traditional meal. Collection of the wild edibles from *phoomdi* is mainly done by womenfolk of the local communities. Survey in the 3 local bazaars revealed a total of 16 edible species traded by the communities. Womenfolks occupy the major stakeholders in the trade, **Fig.10c**. Price of the *phoomdi* species varied with season, demand and bazaar to bazaar. *Alocasia cucullata, C. asiatica, H. coronarium, N. oleracea, O. javanica,* and *P. barbatum* were most preferred traded species. Price of the species varied from Rs. 15-20 and 50-60 / kg. Edible parts sold are rhizome/tubers/roots, corm, whole plant, fruit and shoot. Low cost of many species is attributed to its abundance in *phoomdi* and not so preferred by the communities. At the same time, high cost of some species is due to their limited distribution in *phoomdi* and availability during the growing season only. However, in recent years, the vendors revealed that the trade of wild edible plant is on sharp decline, due to destruction and human pressure mounting on the wetland ecosystem.

Medicinal value

Of the total 47 species recorded, 25 species were used for medicinal purpose by *maiba,* **Fig.10d-f**. Reported *phoomdi* species were found to cure a total of 18 diseases and ailments. Various parts used were shoots (13 sp.), rhizomes/roots/tubers (9 sp.), seeds (2 sp.), inflorescence (1 sp.) and whole plant (1 sp.). Common practices for the use of these species were in the form of paste, extracts, powder or to eat raw. The ways of preparation and applications for a *phoomdi* species differ from the other: *Argyreia nervosa* root extract is used in treating rheumatism and nervous disorders;

Fig. 10 Wild edibles and their trade, (**a**) *Iromba*; (**b**) *Singju*; and (**c**) Bishnupur bazaar women vendors; and Medicinal plants, (**d**) *Hedychium coronarium*, (**e**) *Gynura cusimbua*, and (**f**) *Alocasia esculenta*.

C. dactylon shoot paste is applied on cut, wounds and treating dropsy and epilepsy; and *Z. latifolia* inflorescence extract is used in treating indigestion. *Maiba* in their traditional practices have used *phoomdi* species to treat cut and wounds (7 sp.), stomach disorders (5 sp.), blood troubles (3 sp.), skin diseases (3 sp.), liver troubles (2 sp.), nervous disorders (2 sp.), fever (2 sp.), rheumatism (2 sp.), boils (2 sp.), and menstrual problems, leprosy, cough, dropsy, epilepsy, asthma and piles (1sp. each). But, *phoomdi* medicinal plants are not commercially exploited and traded in the local bazaars. However, they served as the most effective means of curing various diseases by the communities for generations. Many of *phoomdi* plants have multiple usage. The phenomenon can be interpretated as an optimization of natural resources due to a tight connection of the people with the local environment (Pieroni et al. 2002; Scherrer et al. 2005). During the study, we got 13 edible species with medicinal values. Local *maiba* have treated, cured and saved lives of many people using the wetland plants. However, the traditional practice of medications by *maiba* is disappearing, as modern day drugs are easily available and people now rely on advanced methods of treatment.

Cultural significances

Phoomdi species are closely related to the custom and tradition of Manipur society, **Fig. 11a-c**. *Echinochloa stagnina* shoots are used during Govardhan puja, a Hindu festival. During the ritual, shoots are collected and fed to the cows, a Holy animal for the Hindus. Twigs of *C. dactylon* are commonly used in many ritual practices by *maiba*; either ill-possessed by spirits or birth of children. Tender shoots of *O. javanica* are offered to deities during *Cheirauba* or *Imoinu puja*, Manipuri festivals.

Fodder and fuel

Phoomdi of Loktak lake provides luxuriant fodder for domestic animals, **Fig. 11d**. As a common practice, animals, viz. cows, pigs, buffaloes and goats are let free to graze in the *phoomdi*. Preferred fodders for the animals include *E. stagnina, E. fluctuans, I. cylindrica, O. javanica*, and *Z. latifolia*. In recent years, numbers of piggery stalls around the lake, maintained by the communities have increased. Pigs are fed with cooked rice or fresh vegetables, viz. *A. cucullata* or *A. philoxeroides*. Four plants were commonly used as fuel by the communities, viz. culms of *A. donax*, *N. porphyrocoma* and *P. karka*, **Fig. 11e**, and shoots of *S. lacustris* in local households.

Handicrafts

Weaving of mats is one of the most important and ancient handicrafts. *Scirpus lacustris*, known as *kouna* is used to prepare handicrafts of various applications. The plant material is flexible, strong and durable. Mats or *phak* woven mainly by womenfolks serves as a source of income for many households, **Fig.11f**. The plant material is used to make cushions, pillows and floor mats. It is used to make baskets of various sizes and shapes, different carrying capacities, depending on the applications. People normally use baskets every day, either for gathering wild edibles, fish, ornamentals, fodders, and fruits. In addition, *kouna* is used to make decorative items such as flower pots, hanging mats for walls, and bags of various shapes and sizes. *Phak* or mats, made by the womenfolks are sold at the rate of Rs. 200-300 per item. Handicrafts made from *kouna* helps in shaping the economy of many households devoted to the industry. Commercial exploitation of the *phoomdi* plant raises its conservation needs and sustainable way of harvesting from *phoomdi*, so the

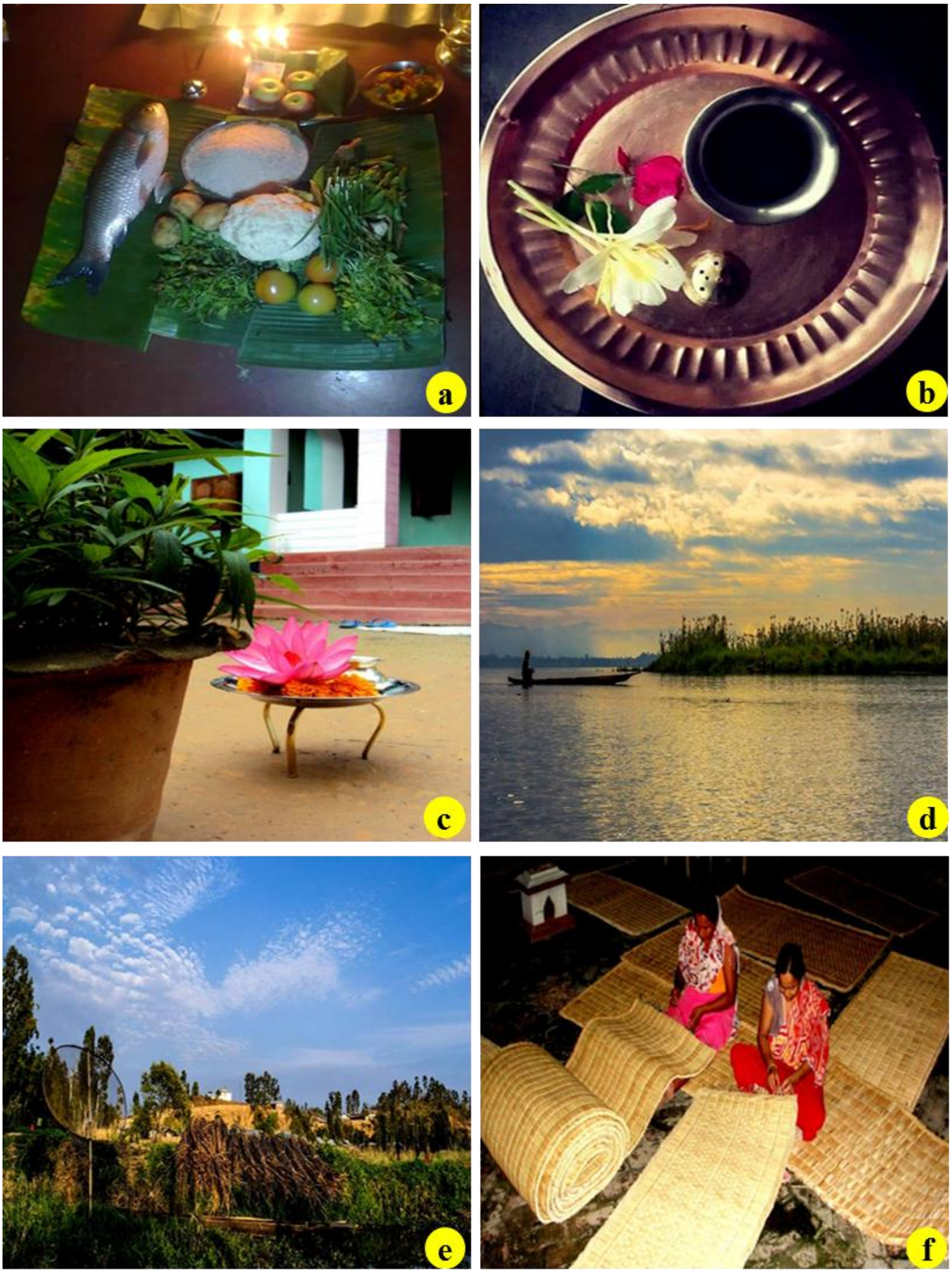


Fig. 11 Cultural significance of various *phoomdi* species (**a-c**); and Fodder and fuel plants; (**d**) collection of fodder using wooden canoes, (**e**) dried *Phragmites karka* on the bank of the wetland and (**f**) handicrafts (*phak*) from *Scirpus lacustris*.

plants can regenerate in the natural habitat. In addition, measures to cultivate *S. lacustris* in private farms can serve as a source to meet the demand of the market in the near future.

Fishing

Phoomdi of Loktak lake provides shelter to 54 species of fish from 17 families (Singh and Singh, 1994). Floating pond or *athaphoom* fishing in the lake involved preparation of an enclosure in a circular form, **Fig.12a**. Fishing in such ponds involved two phases. First phase (*phoom thaba*): During the initial stage, large circular *phoom* (2m wide and 1 m thick) is prepared and kept floating in the wetland. The floating *phoomdi* are cut into long strips (2m wide and 1 m thick) and then transplanted maintaining a circumference of 300-350 m. Fishermen put either cut pieces of *phoomdi* or aquatic plants such as *E. crassipes* or *S. cucullata* in the enclosed circle. The fishing pond is kept undisturbed for few months. In the second phase (*phoom namba*): harvesting is done. The process involves a team of 30-40 fishermen and women in 15-20 canoes. The enclosure is surrounded with dip nets touching the lake bed, leaving no room for the fish to escape. Then, *phoomdi* plants are removed from the enclosure for free operation of the fishing gears. Simultaneously, the bottom sediment of enclosed circle is disturbed by bamboo poles or canoes. It results in the deoxygenation of water due to suspended silts and organic materials from the lake bottom. Fish in need of oxygen turns upwards to the surface of the water, and then the fishermen catch them with dip nets. *Athaphoom* or floating pond fishing of Loktak lake, serves as an important means of livelihood for the communities. Estimated yield from the *athaphoom* varied from Rs. 50-500 per kg. More than 19,000 tons of fish was harvested form Loktak and adjoining lake of

Manipur during 2010-11, with 39% of the harvest from *athaphoom* fishing (Fisheries, 2011). It explains the importance of *phoomdi* in the economic life of the communities and their dependency on the wetland.

House building material

Eight *phoomdi* plants were used in the building of floating huts. They serve as temporary camps for the fishermen during the harvesting season. The huts are built on the floating *phoomdi* and move from one place to another responding to the change in water level, **Fig. 12b-c**. They are made of light materials such as bamboo, wooden planks and reeds commonly available on *phoomdi*. Floors are made of wooden planks or bamboo and made thicker (2-3m) by addition of *phoomdi* layers. Frames are made of bamboos or light wooden pillar, which are pushed down the *phoomdi* to give the support required. Walls are covered with straw or reeds such as *A. donax, N. porphyrocoma, P. karka,* or *S. lacustris,* to give light weight to the hut. *Cymbopogon nardus, E. arundinaceous, I. cylindrica* and *Z. latifolia* are used to cover the top. The huts are then anchored at a convenient place for fishing with the help of bamboo poles on all sides to avoid its displacement by wind. During the study, more than 700 floating huts were present, with more than 80% of the people not having a home in the mainland (LDA, 2011).

Usage pattern and availability status

As far as the use value of *phoomdi* species are concerned, six species are commonly preferred while three species are not so preferred, **Table 2**. Commonly preferred species includes *A. cucullata, C. asiatica, H. coronarium, N. oleracea, O. javanica,* and *P. barbatum*. Use value corresponds with the demand of market for the

Fig. 12 (**a**) Floating ponds and (**b-c**) huts of Loktak.

Table 2 Use value, market demand and availability status ranking of traded *phoomdi* plants

Scientific name	Local name	Use value	Demand	Status	Total
Alisma plantago aquatica	Kaothum	1	2	2	5
Alocasia cucullata	Singjupan	3	3	3	9
Alpinia nigra	Pullei	2	2	2	6
Alpinia galanga	Kanghoo	2	2	2	6
Centella asiatica	Peruk	3	3	3	9
Colocasia esculenta	Lampal	2	2	2	6
Dioscorea alata	Ha	2	1	2	5
Hedychium coronarium	Loklei	3	3	2	8
Ipomoea aquatica	Kolamani	2	2	3	7
Ludwigia octovalvis	Devo	1	2	2	5
Neptunia oleracea	Ishing ikaithibi	3	3	2	8
Oenanthe javanica	Komprek	3	3	3	9
Persicaria posumbu	Kengoi	1	2	2	5
Polygonum barbatum	Yellang	3	3	3	9
Sagittaria sagittifolia	Koukha	2	2	3	7
Zizania latifolia	Ishing kambong	3	3	2	8

Notes: Use value, (1) not so preferred, (2) occasionally preferred, and (3) commonly preferred; Demand in market, (1) occasionally, (2) commonly, and (3) most preferred; Availability status, (1) rarely distributed, (2) sparsely distributed, and (3) extensively distributed.

most preferred species. On the other hand, availability status showed that six species are commonly distributed and 10 species are sparsely distributed on *phoomdi*. During the study, there was no report of rare traded species from *phoomdi*. Threat to the wetlands from human due to industrialization and commercialization of the habitats is a common phenomenon (Croce et.al, 2012).Wetlands of Manipur, including Loktak lake is facing serious ecological problems. The problems in turn affect the *phoomdi*, on which the inhabitants rely for their sustenance and livelihood. Use value, demand and availability status ranking of the traded *phoomdi* species revealed the most preferred *phoomdi* plants as *A. cucullata, C. asiatica, H. coronarium, O. javanica, P. barbatum* and *Z. latifolia*. Dependency of the communities on the plants suggests the need for conservation measures. Conservation measures such as cultivation of the important traded species and their harvest from *phoomdi* keeping in mind their regeneration capacity in nature should be taken up. Management of *phoomdi* in Loktak lake requires awareness education programme among the communities and users dependent on *phoomdi*.

Conclusions

At present, measures are needed to control and manage the proliferating *phoomdi* by physical methods to a level where the lake can sustain its presence without degrading the ecosystem. At the same time, sustainable management practices including conservation measures of *phoomdi*, by providing education to local communities and proposing income generation options for the locals need to be investigated in detailed for this fascinating renewable resources of *phoomdi*.

Objective 2

Metals and nutrients in sediment, water and *phoomdi*:

Health risk assessment and opportunities for phytoremediation

Introduction

Wetlands as unique ecological habitats serve to promote and sustain biota of many forms, and act as living filters that process pollutants from terrestrial runoff and atmospheric deposition (Sharma et al., 2004). Wetlands across the world are threatened by eutrophication and metal pollution via industrial, sewage and agricultural runoff (Muhammad et al, 2011; Xu et al., 2014). Metals reduce sediment and water quality, and affects the organisms of the particular ecosystem because of their persistence, toxic nature and their bioaccumulation ability in the food chain (Zhang et al., 2009). Likewise, eutrophication (excess load of N and P) leads to harmful phytoplankton bloom, their decomposition leading to anoxia and hypoxia and subsequent mortality, reduced growth rates and altered distributions and behaviors of fish (Todd et al., 2010). Wetland plants are reported to concentrate higher metals and nutrient than the surrounding environment (Hadad and Maine, 2006). They serve as ideal *in situ* biomonitors for metal contamination (Bonanno and Giudice, 2010), increase environmental diversity in the root zone and promote a series of chemical and biochemical reactions within various biogeochemical cycles (Prasad, 2004).

At present, Loktak is marked by nutrient enrichment, metal pollution, loss of biodiversity, siltation and an alarming proliferation of floating plants. Lack of adequate sewerage and solid waste management systems in the urban areas led to high amount of wastes drained to the wetland. Nearly, 0.28 million people living within the Nambul river catchment generate on a daily basis, an estimated 72.23 million tons of solid waste and 31,027 m^3 of sewage. Similarly 4.9 million tons of solid waste and 2,121 m^3 of sewage are drained from the Nambol river annually (Trishal and Manihar, 2002). Singh et al., (2013) reported that the discharge of various metals by the

Nambul river play a significant role in the pollution of the wetland. Loktak catchments are degraded mainly due to the practice of *jhoom* or slash - burn cultivation by the local population, logging, and landslides. Annual average sediment input into the wetland has been 650,000 metric tons (Trishal and Manihar, 2002). Rapid siltation reduces the overall water carrying capacity of the wetland and increases eutrophication leading to uncontrolled growth of *phoomdi*. Further, Loktak Multipurpose Project (1983) for hydel power and irrigation generation by construction of a barrage at Ithai converted the natural wetland with fluctuating water level into a reservoir with more or less constant water level. Ithai barrage has compounded the deposition of nutrients, heavy metals and silts by interfering with the wetland hydro-dynamics and flushing of pollutants.

Hence, to tackle the problems associated, LDA has been searching for ways to combat the task to clean *phoomdi* from Loktak as their short term action plan (LDA, 2011). Based on the information collected on the wetland and its catchments as well as implementation of demonstration projects, we framed objectives related with the utilization of *phoomdi* in the protection of Loktak. The objectives were (1) to investigate nutrients and heavy metal distribution in sediment, water and *phoomdi*, (2) to study the differences among *phoomdi* species in nutrients and metal distribution between aboveground and underground parts, (3) to assess the health risk of metals in the local inhabitants *via* the consumption of *phoomdi* wild edibles, and (4) to discuss the potential role of *phoomdi* species in phytoremediation and the possible use in constructed wetlands.

Materials and methods

Study site and sampling

The study was conducted from 2012 to 2013 in Loktak at five sites; Moirang (MR), Ningthoukhong (NK), Thanga (TG), Keibul Lamjao (KL) and Mayang Imphal (MI). Sediment, water and *phoomdi* species were collected during the month of April 2012 and March 2013 (winter), and November 2012 and October 2013 (summer). Sediment, water and *phoomdi* species were randomly collected at three positions in each site. pH and EC values for sediments and water were analysed *in situ* using a pH meter and an EC meter (Soil : water = 1:5). Approximately 1 kg of sediment was collected at a depth of 0-15 cm at each site. Likewise, water samples were collected at a depth of 0.5 cm below the surface water and stored in 1000 mL polyethylene bottles, precleaned with HNO_3 and rinsed with double deionised water. Eleven dominant *phoomdi* species were collected with a square frame of 50 cm in three replicates at each site, representing the plants in the wetland (Singh and Singh, 1994, Trishal and Manihar, 2002). *Phoomdi* species collected, consisted of 11 genera and 10 families, with the dominant being Poaceae, **Table 3**. They were stored in polyethylene bags after species identification (Brummitt and Powell, 1992; Singh and Singh, 1994; Trishal and Manihar, 2002).

Analysis of nutrients and metals

Sediments were brought to laboratory, air dried at room temperature for 3 days and sieved through a 2 mm nylon sieve to remove coarse debris. The samples were ground using a mortar and sieved to get fine powders. To determine Total P (TP), sediment samples were digested with HNO_3: $HClO_4$ (3:1, v/v) mixture and measured as soluble

Table 3 *Phoomdi* species from Loktak

Family	Plant	Habit	Local name	Usage
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	FF	Kabokang	Weed
Salviniaceae	*Salvinia cucullata* Bory	FF	Kangjao	Weed
Araceae	*Spirodela polyrhiza* (L.) Schleiden	FF	Kangkup	Weed
Amaranthaceae	*Alternanthera philoxeroides* Griseb.	EG	Kabonapi	Edible
Convolvulaceae	*Ipomoea aquatica* Forssk.	EG	Kolamani	Edible
Araceae	*Colocasia esculenta* (L.) Schott	EG	Lampan	Edible
Lythraceae	*Trapa natans* L.	RF	Heikak	Edible
Poaceae	*Phragmites karka* (Retz.) Trin. ex Steud.	EG	Tou	Fuel
Poaceae	*Zizania latifolia* Turcz. Ex. Stapf.	EG	Ishing-Kambong	Edible / Medicinal
Apiaceae	*Oenanthe javanica* DC.	EG	Komprek	Edible / Medicinal
Polygonaceae	*Polygonum barbatum* L.	EG	Yellang	Edible

Notes: FF, free floating; EG, Emergent; RF, Rooted floating.

reactive phosphorous (SRP) following Murphy and Riley (1962). Estimation of Total nitrogen (TN) was done following Gupta (2002). Total metal content was determined by digesting the sediment samples with conc. HNO_3: $HClO_4$ (3:1, v/v). The residue was filtered with Whatman filter papers and diluted with double deionised water to 50 mL. Water samples were filtered through 0.45 µm Millipore membranes, acidified with conc. HNO_3 to pH < 2 and stored in the dark at 4ºC. Acid digestion of the water samples were carried out with conc. HNO_3: $HClO_4$ (3:1, v/v). The digested samples were refiltered and stored prior to analysis. TP and TN were determined by the calorimetric molybdenum blue method (Murphy and Riley, 1962) and Gupta (2002). *Phoomdi* samples were thoroughly washed with tap water and rinsed with deionised water to remove unwanted materials. Aboveground and underground parts were then separated and oven dried (70ºC) for 24 hours to get a constant weight. Plant samples were ground using a Clotech Powder Mill and sieved to get fine powders. The samples were then acid digested: 10 mL of HNO_3:$HClO_4$, 3:1, v/v added to samples of 1.00 g. After cooling at room temperature, the residue was diluted with double deionized water to 30 mL (Deng et al., 2004). TP and TN in *phoomdi* samples were determined following the protocols used in sediment analysis. Heavy metal (Fe, Mn, Zn and Cu) concentrations were determined using Atomic Adsorption Spectrometry (GBC-932, Australia) for sediment, water and *phoomdi* samples. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run to calibrate the instruments.

Data analysis

Experiments were performed in triplicates and the results represent the mean ± s.e values. Estimated daily intake (EDI) of metals depends on both the metal concentrations in in the wild edible *phoomdi* species and their consuming amounts (Song et al., 2009; Li et al., 2013).

$$EDI = C_{metal} \times W_{wild\ edibles} / B_w \tag{1}$$

where *EDI* represents the estimated daily intake of metals via the wild edibles (µg^{-1} kg^{-1} day^{-1}), C_{metal} is the concentration of metals in the wild edibles, $W_{wild\ edibles}$ is the daily consumption of wild edibles (assumed to be a single serving or 100 g wet weight per day per adult) (Falinski et al., 2014), and B_w is the body weight (assumed to be 70 kg for an adult) (Adhikari et al., 2009). Provisional upper tolerable daily intake (PTDI) of Fe, Mn, Zn and Cu are 45, 11, 40, and 10 mg kg^{-1} body weight d^{-1} (FDA, 2001). Risk assessment of metals through *phoomdi* wild edible consumption was done by calculating the target hazard quotients (THQ), proposed by the US Environmental Protection Agency (US EPA 2011; Fang et al., 2014).

$$THQ = (E_f \times E_d \times W_{wild\ edibles} \times C_{metal} / RfD \times B_w \times T_n) \times 10^{-3} \tag{2}$$

where E_f is the exposure frequency (182.5 days year^{-1}, considering locals collect the wild edibles for 6 months year^{-1}), E_d is the exposure duration (65.5 years, equivalent to life expectancy), $W_{wild\ edibles}$ is the daily consumption of *phoomdi* edibles (g d^{-1}), C_{metal} is the metal concentration in *phoomdi* species (mg kg^{-1}), *RfD* is the oral reference dose (mg kg^{-1} d^{-1}), B_w is the body weight of the individuals, T_n is the average exposure time for noncarcinogens ($E_d \times 365$ days year^{-1}), and 10^{-3} is the unit conversion factor. The values of *RfD* are as follows: Fe = 0.7 µg kg^{-1} d^{-1}, Mn = 0.14 µg kg^{-1} d^{-1}, Zn = 0.3 µg kg^{-1} d^{-1}, and Cu = 0.04 µg kg^{-1} d^{-1} (US EPA, 2011). Relative amount of nutrient and metals accumulated between *phoomdi* species and water was compared by calculating the bioconcentration factors (BCF). BCF was selected as an indicator of the tendency for nutrients and metals to accumulate in plants. It is the ratio of nutrients and metal concentrations in the plant to that of the water. The larger the BCF, the more is the ability of the plant to absorb nutrients and metals from water (Ha et al., 2011; Favas et al., 2012). The translocation factors (TF) were expressed as:

$$TF = [\text{Metal/Nutrient}]_{\text{Shoot}} / [\text{Metal/Nutrient}]_{\text{Root}} \quad (3)$$

It shows nutrients and metal translocation properties from underground to aboveground parts of *phoomdi* species (Deng et al., 2004; Mirza et al., 2011). All statistical analysis of data was performed using Microsoft Excel 2010 ver. and SPSS 10.0 for windows.

Results and discussion

Sediment characteristics

Higher sediment pH and conductivity were found during summer than winter at all the sampling locations which can be attributed to the more inflow of water from the river, **Table 4**. They ranged from 7.2 to 7.8 and 80.5 to 319.4 μScm^{-1} for summer and 7.0 to 7.6 and 66.6 to 274.7 μScm^{-1} for winter. Highest nitrogen content in the sediment ranged upto 924.2 to 982.3 mg kg^{-1} at KL and the lowest of 280.4 to 308.2 mg kg^{-1} at MI. Similarly, highest P content of 619.7 to 687.2 mg kg^{-1} was found at KL and lowest of 281.2 to 321.1 mg kg^{-1} at MI. Concentrations of Fe, Mn, Zn and Cu ranged upto 81.8 to 253.1, 2.6 to 71.9, 0.13 to 0.43 and 0.02 to 0.04 mg kg^{-1}. Higher concentrations were recorded for Fe at all the locations, followed by Mn at MI, Zn at MR and Cu at MI. It was observed that in natural water bodies most of the P amount is deposited in the sediment where the adsorption to metallic oxides was identified as one of the main reactions (Lijklema, 1977).

Table 4 Sediment characteristics (mean ± s.e) of Loktak during the study period: 2012-2013

Parameters	Season / Metal	MR	NK	TG	KL	MI
pH	**S**	7.9 ± 0.01	7.2 ± 0.04	7.4 ± 0.02	7.8 ± 0.03	7.8 ± 0.02
	W	7.4 ± 0.02	7.0 ± 0.03	7. 2 ± 0.04	7. 6 ± 0.02	7.2 ± 0.02
EC	**S**	90.9 ± 0.68	218.8 ± 1.52	319.5 ± 1.13	119.5 ± 0.84	80.5 ± 1.23
(μS cm^{-1})	**W**	66.6 ± 0.90	169.9 ± 2.04	274.1 ± 1.58	109.1 ± 1.58	70.7 ± 0.42
N	**S**	586.1 ± 0.03	461. 1 ± 0.09	377.5 ± 0.35	982.3 ± 0.05	308.2 ± 0.04
(mg kg^{-1})	**W**	560.8 ± 0.25	420.3 ± 0.11	340.5 ± 0.30	924.2 ± 0.10	280.4 ± 0.30
P	**S**	381.1 ± 0.02	357.2 ± 0.06	331.2 ± 0.08	687.2 ± 0.03	321.1 ± 0.04
(mg kg^{-1})	**W**	347.2 ± 0.03	324.5 ± 0.14	302.2 ± 0.05	619.7 ± 0.08	281.2 ± 0.02
Metals	**Fe**	209.5 ± 1.22	130.9 ± 0.75	253.1 ± 0.51	217.6 ± 0.56	81.8 ± 0.45
(mg kg^{-1})	**Mn**	9.9 ± 0.20	71.9 ± 0.34	15.4 ± 0.42	2.6 ± 0.06	57.2 ± 0.05
	Zn	0.43 ± 0.107	0.40 ± 0.017	0.22 ± 0.011	0.13 ± 0.006	0.13 ± 0.005
	Cu	0.08 ± 0.002	0.09 ± 0.002	0.02 ± 0.003	0.02 ± 0.001	0.43 ± 0.003

Notes: MR, Moirang; NK, Ningthoukhong; TG, Thanga; KL, Keibul Lamjao; MI, Mayang Imphal.

Sediment characteristics reflect the historic accumulation of the nutrient input and its storage over a long period of time, and do not signify the snap shot value of its accumulation over the study period. Metal content in the selected locations was ordered as Fe > Mn > Zn > Cu. Iron concentrations differed significantly according to the sites. Maximum Fe content (253.1 mg kg^{-1}) was found at TG and the lowest at MI (81.8 mg kg^{-1}). High Fe concentrations in the wetland sediment may be attributed to its solubilization from bed rocks of the catchment hills, and industrial and sewage effluents. Likewise, wetland sediment enriched with organic matter serve as a potential good sink for various metals compared to surface water, as the humic substances present in sediment create various metal complexes (Rognerud and Fjeld, 2011). Manganese concentrations were found highest at NK (71.7 mg kg^{-1}) and lowest at KL (2.6 mg kg^{-1}). Similarly, relative low concentrations of Zn and Cu were found at the range of 0.13 to 0.43 mg kg^{-1} and 0.02 to 0.43 mg kg^{-1}. Low Zn and Cu concentrations in the sediment may be because of continuous metal uptake by the wetland macrophytes during their growth and development. Variations in metal content could be because of physico-chemical characteristics of sediment (pH, organic matter etc.), microbial population and level of contamination of the particular environment (Adhikari et al., 2009).

Water characteristics

Similar to the sediment characteristics, water pH and EC were higher in the summer than winter, **Table 5**. pH and EC values ranged upto 6.6 to 7.0 and 105.2 to 152.5 μScm^{-1} for summer and 6.5 to 6.8 and 82.4 to 141.7 μScm^{-1} for winter. Higher conductivity during summer revealed the presence of many dissolved inorganic materials in water that is enhanced by the inflows of various rivers. In addition, significant variation in TN and TP content was observed in the studied locations. Highest TN value was found at MR (39.2 to 43.8 mg L^{-1}) and lowest at MI (22.4 to 28.3 mg L^{-1}). Total phosphorus concentrations ranged from 0.06 to 0.09 mg L^{-1} in summer and 0.03 to 0.07 mg L^{-1} in winter. For summer, the macrophyte growth accumulates organic matter in bottom sediments, which decreases dissolved oxygen and redox potential of the sediment releasing TP in the water column (Hadad and Maine, 2007). In addition, high TN and TP content might be because of excessive nitrogenous fertilizer and pesticides used in the nearby agricultural areas and their leaching. Awang Khujairok, Ningthoukhong, Potsangbam, Merakhong and Irumbi draining the agricultural fields subjected to high amount of fertilizers, in general, have higher concentrations of TN and TP (Trishal and Manihar, 2004). Singh et al., 2013 showed that the Nambul river was the main source for metal pollution in Loktak. Among the 36 rivers, few big rivers viz. Nambol, Awang Khujairok, and Merakhong pass through highly polluted urban settlements of the Imphal valley. In our work, water samples at different locations of Loktak exceeded the threshold limit set by WHO (drinking water) for Fe (0.37 to 0.57 mg L^{-1}) (WHO, 1996). Peak Fe content might be related to the leached Fe complexes from bed rocks and release of effluents enriched in Fe from the industrial areas located upstream. Further, domestic wastewater might increase metal concentrations in the wetland. A wide variety of domestic household products, such as toothpaste, cosmetics, batteries, cleaning materials contain trace concentrations of Fe, Mn, Zn and Cu. Likewise, the urban habitats in the Imphal valley do not have proper sewage treatment plants. Run-off from the agricultural areas surrounding the wetland could be a possible reason for Fe, Mn, Zn and Cu release, as these metals are present in fertilizers and pesticides.

Table 5 Water characteristics (mean ± s.e) of Loktak during the study period: 2012-2013

Parameters	Season / Metal	MR	NK	TG	KL	MI
pH	**S**	6.9 ± 0.03	6.6 ± 0.02	7.0 ± 0.03	6.9 ± 0.04	6.9 ± 0.02
	W	6.7 ± 0.01	6.5 ± 0.03	6.8 ± 0.03	6.8 ± 0.05	6.7 ± 0.04
EC	**S**	105.2 ± 0.08	127.5 ± 0.30	132.5 ± 0.24	152.5 ± 0.33	137.2 ± 0.08
(μS cm^{-1})	**W**	82.4 ± 0.30	98.6 ± 0.11	110.7 ± 0.35	141.7 ± 0.42	112.6 ± 0.50
N	**S**	43.8 ± 0.07	39.4 ± 0.32	31.5 ± 0.38	35.7 ± 0.07	28.3 ± 0.13
(mg kg^{-1})	**W**	39.2 ± 0.03	36.8 ± 0.02	24.7 ± 0.07	28.1 ± 0.14	22.4 ± 0.06
P	**S**	0.09 ± 0.004	0.06 ± 0.001	0.09 ± 0.002	0.06 ± 0.003	0.08 ± 0.003
(mg kg^{-1})	**W**	0.06 ± 0.002	0.03 ± 0.003	0.07 ± 0.003	0.04 ± 0.003	0.05 ± 0.002
Metals	**Fe**	0.39 ± 0.760	0.44 ± 1.237	0.37 ± 0.697	0.57 ± 1.010	0.38 ± 0.412
(mg kg^{-1})	**Mn**	0.13 ± 1.250	0.12 ± 0.640	0.19 ± 0.739	0.21 ± 0.590	0.11 ± 0.833
	Zn	0.03 ± 0.774	0.02 ± 0.680	0.02 ± 0.834	0.03 ± 0.739	0.03± 0.802
	Cu	0.02 ± 0.116	0.01 ± 0.065	0.01 ± 0.041	0.02 ± 0.035	0.02 ± 0.040

Notes: MR, Moirang; NK, Ningthoukhong; TG, Thanga; KL, Keibul Lamjao; MI, Mayang Imphal.

Adding the problem is the traditional practice of floating pond fishing called *athaphoom*. Copper sulphate is found commonly used in aquaculture practices. During the study, more than 700 huts were present floating in *phoomdi* of Loktak that releases their domestic waste directly into the wetland (LDA, 2011). Highest Mn concentration (0.21 mg L^{-1}) was found at KL and lowest (0.11 mg L^{-1}) at MI. Similarly, Zn concentration ranged from 0.02 to 0.03 mg L^{-1} and Cu concentration from 0.001 to 0.002 mg L^{-1}. High Mn concentration at KL might be due to the coverage of the entire fresh water by the thick floating weeds that lead to anaerobic action of the microorganisms with the soil at the wetland bottom (Singh et al., 2013). Low metal concentrations in water compared to sediment and *phoomdi* may be attributed to the continuous uptake of metals by the floating plants. This is supported by the data of metal concentrations in the tissues of dominant *phoomdi* species selected during the study. Water characteristics represented a snapshot of metal content, as concentrations will change after prolonged drought or a heavy monsoon.

Nutrients in *phoomdi*

Seasonal patterns of N and P concentrations in underground and aboveground parts of *phoomdi* species are shown in **Fig. 13(a-d)**. Higher nutrient concentrations were recorded for summer compared to winter. Highest nitrogen concentration of 16511.4 mg kg^{-1} was recorded in the aboveground tissues (AG) of *Spirodela polyrhiza* and 19308.7 mg kg^{-1} in the underground tissues (UG) of *Oenanthe javanica*. In summer, concentrations of N and P in the AG and UG tissues ranged upto 6411.2 to 16511.4 mg kg^{-1} and 6717.8 to 19308.7 mg kg^{-1}, and 537.8 to 1175.8 mg kg^{-1} and 717.1 to 1660.9 mg kg^{-1}.

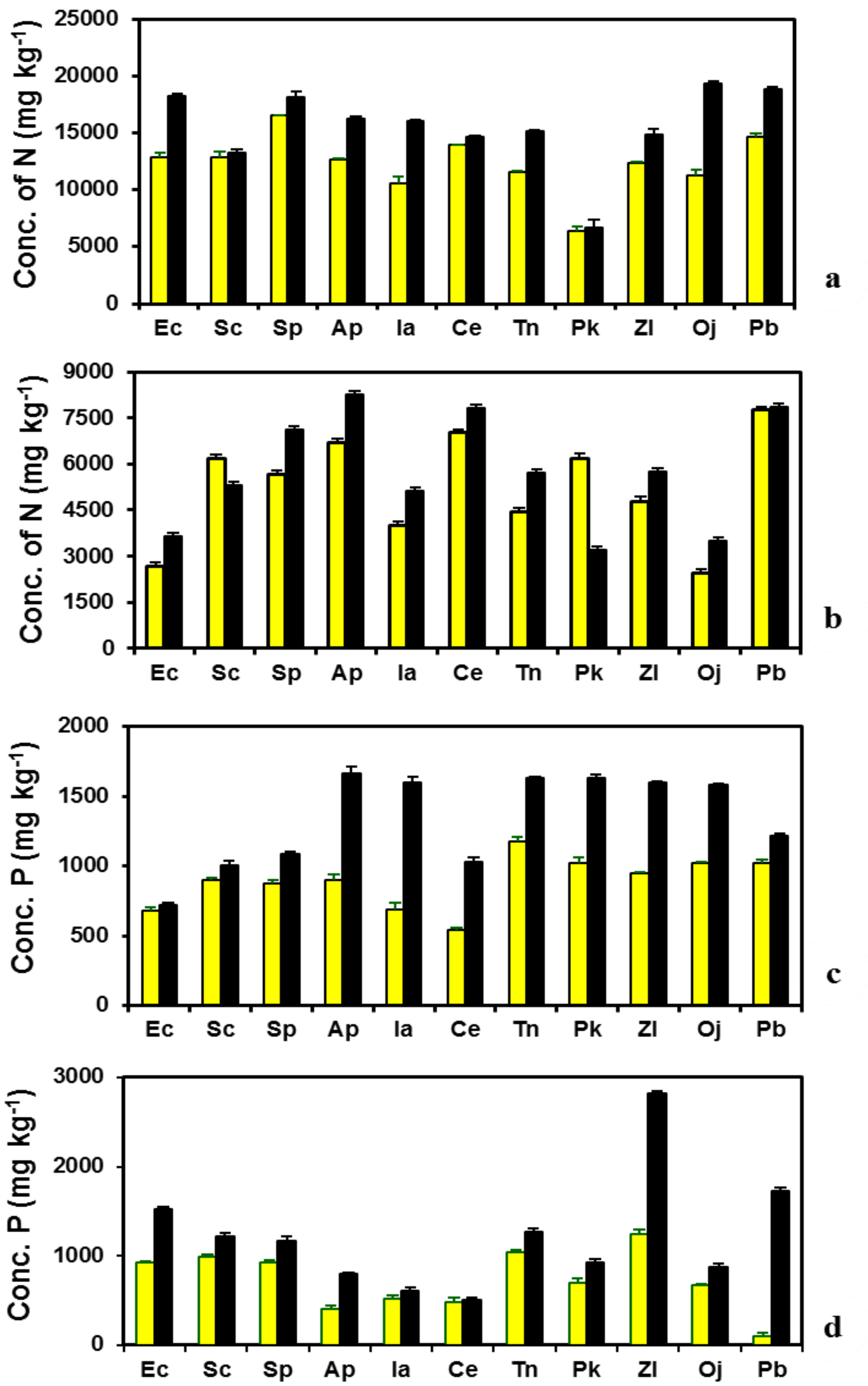


Fig. 13 Concentrations of nutrients (N and P) in aboveground (**yellow**) and underground (**black**) parts of *phoomdi* species (**a-c**; summer; **b-d**; winter [*E. crassipes* (Ea), *S. cucullata* (Sc), *S. polyrhiza* (Sp), *A. philoxeroides* (Ap), *I. aquatica* (Ia), *C. asiatica* (Ca), *T. natans* (Tn), *P. karka* (Pk), *Z. latifolia* (Zl), *O. javanica* (Oj), and *P. barbatum* (Pb)].

Likewise, for winter, N and P concentrations ranged from 2453.4 to 7746.1 mg kg^{-1} and 3221.4 to 8254.7 mg kg^{-1}, and 430.9 to 1042.8 mg kg^{-1} and 513.2 to 2820.9 mg kg^{-1}. *Phragmites karka* (1.91) and *Colocasia esculenta* (0.95) showed maximum translocation of N and P. Further, highest BCFs of 9.7×10^4 and 7.9×10^4 for N and P were observed in *Spirodela polyrhiza* and *Zizania latifolia*. *Phoomdi* species assimilated higher TN during the growing season (summer). Nutrient concentrations were significantly more in underground parts, and in general, translocation values were below 1. For summer, highest TN concentrations were found in underground parts of *O. javanica*, *P. barbatum*, *E. crassipes*, *S. polyrhiza*, *A. philoxeroides* and *I. aquatica*. Similarly, *S. polyrhiza*, *P. barbatum*, *C. esculenta*, *S. cucullata* and *E. crassipes* showed highest TN values in the aboveground parts. Likewise, for winter, high TN content were observed in underground parts of *A. philoxeroides*, *P. barbatum*, *C. esculenta* and *S. polyrhiza*. In aboveground parts, *P. barbatum*, *C. esculenta*, *A. philoxeroides*, *P. karka* and *S. cucullata* showed high TN content. Among several species, accumulation of nutrient was observed at a magnitude much higher than their concentrations in surrounding water. *Phoomdi* species, *S. polyrhiza*, *P. barbatum*, *E. crassipes*, *O. javanica* and *A. philoxeroides*, showed average BCFs of 9.7×10^2, 9.4×10^2, 8.7×10^2, 8.6×10^2 and 8.1×10^2 during summer and 4.2×10^2, 5.2×10^2, 2.1×10^2, 1.9×10^2 and 4.9×10^2 in winter, revealing a good phytoextraction ability of TN from the wetland. *Eichhornia crassipes*, *S. cucullata*, *S. polyrhiza* and *Z. latifolia* showed higher TP accumulation in winter. Highest TP content was found in underground parts of *A. philoxeroides*, *P. karka*, *T. natans*, *Z. latifolia* and *I. aquatica*, and in aboveground parts of *T. natans*, *O. javanica*, *P. karka* and *P. barbatum* during summer. *Trapa natans*, *O. javanica*, *P. karka*, *A. philoxeroides* and *Z. latifolia* showed average BCFs of 3.6×10^4, 3.4×10^4, 3.3×10^4,

3.3×10^4, and 3.3×10^4 during summer and 4.5×10^4, 3.0×10^4, 3.2×10^4, 2.4×10^4, 7.9×10^4 for winter. Total nitrogen and total phosphorous uptake from the surface water and their sequestration in *phoomdi* tissues indicate nutrient retention and storage. Thus, regular harvesting of *phoomdi* aboveground parts could serve as a useful practical approach for the control and management of eutrophication associated with Loktak. Eutrophication represents the aging process of the wetland - where the wetland becomes rich in nutrient and therefore supports dense plant population which kills animal life by depriving its oxygen (Chathrath, 1992). Nutrient stripping mechanism of *phoomdi* may slow down the process of Loktak - aging.

Metals in *phoomdi*

Macrophytes growing in the polluted environment accumulate considerable amount of metals at high concentrations depending on their availability in the particular environment, viz. their concentration in the sediment and water. Many plants have indispensable property of metal tolerance, which is achieved by preventing particular metal uptake through the roots and / or by accumulating the metal that can be sequestered in the plant tissue level (Chatterjee et al., 2011). Metal accumulation in the plant tissues are reported to be influenced by various factors, viz. competition between the available metal ions, availability of various organic substances at the rhizospheric region, metal-metal interactions etc.

Phoomdi species of the wetland showed varied metal concentrations depending on their accumulation abilities and physico chemical characteristics of the wetland water column, **Fig. 14(a-d)**. Metal concentrations in *phoomdi* tissues followed the order: Fe > Mn > Zn > Cu. Highest Fe accumulation was found in UG and AG parts of S. cucullata (9461.4 and 6131.5 mg kg^{-1} dw) followed by *E. crassipes* (2220.3 and

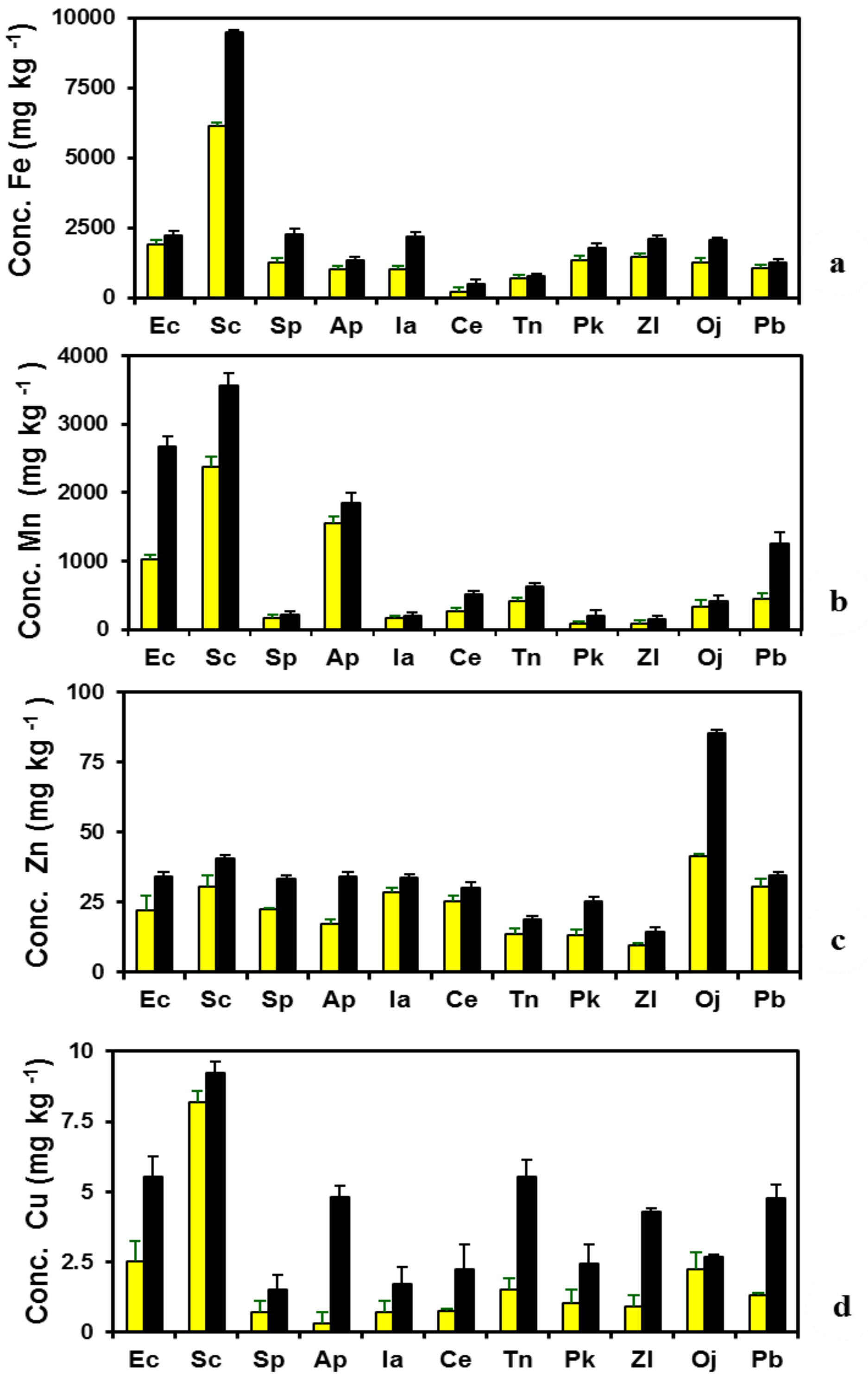


Fig. 14 Concentrations of (**a**) Iron, (**b**) Manganese, (**c**) Zinc and (**d**) Copper in aboveground (**yellow**) and underground (**black**) parts of *phoomdi* species [*E. crassipes* (Ea), *S. cucullata* (Sc), *S. polyrhiza* (Sp), *A. philoxeroides* (Ap), *I. aquatica* (Ia), *C. asiatica* (Ca), *T. natans* (Tn), *P. karka* (Pk), *Z. latifolia* (Zl), *O. javanica* (Oj), and *P. barbatum* (Pb)].

1899.5 mg kg^{-1} dw), *S. polyrhiza* (2262.1 and 1267.8 mg kg^{-1} dw) and *I. aquatica* (2193.7 and 1008.2 mg kg^{-1} dw). Likewise, *S. cucullata* (3565.1 and 2383.4 mg kg^{-1} dw), *E. crassipes* (2673.3 and 1030.7 mg kg^{-1} dw) and *A. philoxeroides* (1858.2 and 1546.3 mg kg^{-1} dw) accumulated largest Mn in UG and AG parts. The highest amount of Zn was accumulated in UG and AG parts of *O. javanica* (85.3 and 41.4 mg kg^{-1} dw), *S. cucullata* (40.6 and 30.6 mg kg^{-1} dw) and *P. barbatum* (34.5 and 30.4 mg kg^{-1} dw). While the highest amount of Cu was accumulated by *S. cucullata* (9.2 and 8.1 mg kg^{-1} dw), *E. crassipes* (5.6 and 2.5 mg kg^{-1} dw) and *T. natans* (5.5 and 1.5 mg kg^{-1} dw) in UG and AG parts. Generally, metal concentrations in *phoomdi* tissues ordered as underground parts > aboveground parts. Higher metal content in underground tissues depicts the tolerance of the macrophytes to the particular metal and the internal metal detoxification strategies existing within the plants. Although, Fe represents one of the vital elements for humans and various life forms on Earth, it might be a toxic contaminant under acidic environments in sulfide deposits (Mazumdar and Das, 2014). High doses of Fe are reported to cause hemorrhagic necrosis, tissue damage by catalyzing the conversion of H_2O_2 to free radical ions and can activate oncogenes (Gurzau et al., 2003). In our work, the dominant *phoomdi* species were found to accumulate high Fe concentrations in the underground parts as well as aboveground parts. Similarly, Mn concentrations in *phoomdi* species were considerably high compared to various wetland macrophytes reported in literature (Rai et al., 2009). Rai et al., (2009) showed Mn accumulation in the roots and shoots of *E. crassipes* upto 880 ± 28.5 and 123 ± 11.3 mg kg^{-1} dw compared to our results of 2673.3 and 1030.7 mg kg^{-1} dw in the underground and aboveground parts from Loktak. Zinc concentrations in *phoomdi* species ranged from 9.6 to 41.4 mg kg^{-1} dw in the aboveground parts and 14.6 to 85.3 mg kg^{-1} dw in the underground parts. Copper is an essential element for plant growth, but causes toxic effects when the concentrations accumulated in the aboveground tissues exceeds 20 mg kg^{-1} dw (Borkert et al., 1998). Average Cu concentrations in *phoomdi* species ranged upto 0.7 to 8.2 mg kg^{-1} dw and 1.5 to 9.2 mg kg^{-1} dw in the aboveground and underground parts.

Health risk assessment

Table 6 showed the comparison of EDI and THQ values for the studied metals Fe, Mn, Zn and Cu *via* the consumption of various *phoomdi* wild edibles. For Fe, EDI levels were found highest for *Z. latifolia* (2056 µg kg^{-1} day^{-1}) and the lowest for *C. esculenta* (303.3µg kg^{-1} day^{-1}). The EDI values were far below the PTDI values of 45,000 µg kg^{-1} day^{-1} for Fe. Similarly, the EDI levels of Mn for the *phoomdi* wild edibles ranged between 122.4 to 2209 µg kg^{-1} day^{-1}. *Alternanthera philoxeroides* accounted for 0.2% of the corresponding PTDI value of 11,000 µg kg^{-1} day^{-1} for Mn. Daily intake of Zn in the present study is 13.7 to 59.1 µg kg^{-1} day^{-1} for the *phoomdi* wild edibles. Likewise, EDI values of Cu ranged from 0.4 to 2.2 µg kg^{-1} day^{-1} and were far below the PTDI value of 10,000 µg kg^{-1} day^{-1} for Cu. Generally, the EDI levels of the studied metals Fe, Mn, Zn and Cu from the *phoomdi* wild edibles were below the tolerable limits during the study. THQ, proposed by the US Environmental Protection Agency (US EPA) has been recognized as one of the reasonable indexes for the health risk evaluation associated with the intake of heavy metals by consuming contaminated food (Li et al., 2013).

Table 6 Estimated daily intake (EDI) of metals by locals and target hazard quotients (THQ) via *phoomdi* wild edibles consumption during the study period: 2012-2013

Phoomdi wild edibles	Metals								C-THQ
	Fe		Mn		Zn		Cu		
	EDI	THQ	EDI	THQ	EDI	THQ	EDI	THQ	
Alternanthera philoxeroides	1433.3	1.0	2209.0	7.9	24.9	0.04	0.4	0.01	**8.95**
Ipomoea aquatica	1440.3	1.0	237.4	0.8	41.0	0.07	1.0	0.01	**1.88**
Colocasia Esculenta	303.3	0.2	380.6	1.3	36.4	0.06	1.1	0.01	**1.57**
Trapa natans	994.6	0.7	586.7	2.1	19.7	0.03	2.2	0.02	**2.84**
Zizania latifolia	2056.0	1.5	122.4	0.4	13.7	0.02	1.3	0.01	**1.93**
Oenanthe javanica	1818.4	1.3	467.6	1.7	59.1	0.09	3.1	0.04	**2.13**
Polygonum barbatum	1507.0	1.1	647.3	2.3	43.4	0.07	1.9	0.02	**3.49**
PTDI values (μg kg^{-1} day^{-1}) (FDA, 2001)	**45,000**		**11,000**		**40,000**		**10,000**		

Notes: Provisional upper tolerable daily intake (PTDI) represents the permissible human exposure to a particular contaminant; due to their natural occurrence in food and drinking water (FDI, 2001).

THQ is defined as the ratio of consumed dose of a toxic metal *via* as oral reference dose (RfD). THQ < 1 suggests that the exposed population is safe from the harmful effects via the consumption of the contaminated food and 1 < THQ < 5 means that the exposed population is in a level of concern interval (Harmanescu et al., 2011). It was observed that the THQ values of Mn were comparatively higher than Fe, Zn and Cu. THQ values of Zn and Cu for the *phoomdi* wild edibles were far below 1, suggesting that consumption of the wild edibles are safe from the toxic effects of the metals. For Fe, THQ values ranged from 0.2 to 1. 5, with higher values found for *Z. latifolia* (1.5), *O. javanica* (1.3) and *P. barbatum* (1.1). It suggests the need for attention to be paid while consuming the wild edibles. Similarly, the highest THQ value of Mn is 7.9 for *A. philoxeroides*, followed by 2.3 for *P. barbatum* and 2.1 for *T. natans*. The relative high THQ values of Mn found in the present study indicated that the contamination of the metal in the *phoomdi* wild edibles might pose a potential health hazard on human body. The situation was observed in sites with sediments especially rich in Mn, where the *phoomdi* species accumulated the metals more in the tissues. Combined THQ values ranged from 1.57 to 8.95 for the *phoomdi* wild edibles. *Alternanthera philoxeroides* showed the highest combined THQ value (8.95), which might be due to its better absorption capacity for Mn compared to other species. THQ values between 1 and 5 during the study showed the level of concern to the health of the local population that might arise due to the consumption of the *phoomdi* wild edibles. Manganese contributed mostly in the combined THQ values, followed by Fe, Zn and Cu.

Phytoremediation potential of *phoomdi*

Phoomdi species showed high BCFs for the metals compared to the concentrations in the surrounding water of the wetland, **Table 7**. *Salvinia cucullata*, *E. crassipes*, *S. polyrhiza*, *Z. latifolia*, *O. javanica*, *I. aquatica* and *P. karka* showed a significant Fe accumulation in the tissues and high BCFs that averages 5.8×10^4, 1.5×10^4, 1.3×10^4, 1.3×10^4, 1.2×10^4, 1.2×10^4 and 1.2×10^4, respectively. While the species, *S. cucullata*, *E. crassipes* and *A. philoxeroides* showed average BCFs of 3.9×10^4, 2.5×10^4 and 2.3×10^4, revealing good phytoextracting ability of Mn from Loktak. For Zn, high BCFs of 4.9×10^3, 2.7×10^3, 2.5×10^3, 2.4×10^3, and 2.3×10^3 were found in *O. javanica*, *S. cucullata*, *P. barbatum*, *I. aquatica* and *A. philoxeroides*. Similarly, the species *S. cucullata* showed a good ability to accumulate Cu with average BCF of 1.7×10^4. It showed the ability of *phoomdi* species to sequester high metal concentrations and their help in the purification Loktak by acting as sinks for the metals. As the wetland serve as a source of drinking water for the locals, low metal concentrations maintained by the *phoomdi* species acts as a secure protection for the health of the locals and environment of Loktak. **Table 8** showed restricted metal translocation from underground parts to aboveground parts of *phoomdi* species. Normal ratio of AG / UG metal concentrations is > 1 in hyperaccumulators of the specific metal. Maximum TF of Fe was 0.90 by *T. natans*. *Eichhornia crassipes*, *P. barbatum*, *P. karka* and *A. philoxeroides* showed high TFs of 0.85, 0.84, 0.76 and 0.75 for Fe, respectively. Highest TFs of Mn was 0.83 by *A. philoxeroides*. While the species *A. philoxeroides*, *I. aquatica* and *O. javanica* showed high TFs of Mn upto 0.83, 0.81 and 0.77. Maximum TFs obtained for Zn and Cu were 0.88 by *P. barbatum* and *S. cucullata*.

Table 7 Bioconcentration factors (BCF = [Metals or Nutrients] $_{Plant}$ / [Metals or Nutrients] $_{Water}$) in *phoomdi* species

Phoomdi species	Nutrients and Metals							
	N		P		Fe	Mn	Zn	Cu
	S	W	S	W				
Eichhornia crassipes	8.7×10^{2}	2.1×10^{2}	1.8×10^{4}	4.8×10^{4}	1.5×10^{4}	2.5×10^{4}	2.2×10^{3}	8.1×10^{3}
Salvinia cucullata	7.3×10^{2}	3.8×10^{2}	2.5×10^{4}	4.3×10^{4}	5.8×10^{4}	3.9×10^{4}	2.7×10^{3}	1.7×10^{4}
Spirodela polyrhiza	9.7×10^{2}	4.2×10^{2}	2.5×10^{4}	4.1×10^{4}	1.3×10^{4}	2.6×10^{3}	2.1×10^{3}	2.2×10^{3}
Alternanthera philoxeroides	8.1×10^{2}	4.9×10^{2}	3.3×10^{4}	2.4×10^{4}	8.6×10^{3}	2.3×10^{4}	2.3×10^{3}	5.1×10^{3}
Ipomoea aquatica	7.5×10^{2}	3.0×10^{2}	2.9×10^{4}	2.2×10^{4}	1.2×10^{4}	2.5×10^{3}	2.4×10^{3}	2.4×10^{3}
Colocasia esculenta	7.9×10^{2}	4.9×10^{2}	2.0×10^{4}	1.9×10^{4}	2.7×10^{3}	5.2×10^{3}	2.1×10^{3}	2.9×10^{3}
Trapa natans	7.5×10^{2}	3.4×10^{2}	3.6×10^{4}	4.5×10^{4}	5.4×10^{3}	6.9×10^{3}	1.3×10^{3}	7.0×10^{3}
Phragmites karka	3.7×10^{2}	3.1×10^{2}	3.3×10^{4}	3.2×10^{4}	1.2×10^{4}	1.9×10^{3}	1.5×10^{3}	3.4×10^{3}
Zizania latifolia	7.6×10^{2}	3.5×10^{2}	3.3×10^{4}	7.9×10^{4}	1.3×10^{4}	8.9×10^{3}	0.9×10^{3}	5.2×10^{3}
Oenanthe javanica	8.6×10^{2}	1.9×10^{2}	3.4×10^{4}	3.0×10^{4}	1.2×10^{4}	4.9×10^{3}	4.9×10^{3}	4.9×10^{3}
Polygonum barbatum	9.4×10^{2}	5.2×10^{2}	2.9×10^{4}	3.6×10^{4}	8.6×10^{3}	7.4×10^{3}	2.5×10^{3}	6.1×10^{3}

Table 8 Translocation factors (TF = [Metals or Nutrients] $_{Shoot}$ / [Metals or Nutrients] $_{Root}$) in *phoomdi* species

Phoomdi species	Nutrients and Metals							
	N		P		Fe	Mn	Zn	Cu
	S	W	S	W				
Eichhornia crassipes	0.70	0.73	0.94	0.60	0.85	0.38	0.64	0.45
Salvinia cucullata	0.97	1.15	0.88	0.80	0.64	0.66	0.76	0.88
Spirodela polyrhiza	0.91	0.79	0.80	0.79	0.56	0.76	0.67	0.46
Alternanthera philoxeroides	0.78	0.81	0.53	0.50	0.75	0.83	0.50	0.06
Ipomoea aquatica	0.66	0.78	0.43	0.85	0.45	0.81	0.84	0.42
Colocasia esculenta	0.95	0.89	0.52	0.95	0.41	0.51	0.84	0.33
Trapa natans	0.76	0.77	0.72	0.82	0.90	0.65	0.72	0.27
Phragmites karka	0.95	1.91	0.62	0.75	0.76	0.43	0.52	0.41
Zizania latifolia	0.83	0.82	0.59	0.44	0.68	0.58	0.65	0.21
Oenanthe javanica	0.58	0.69	0.64	0.76	0.62	0.77	0.48	0.83
Polygonum barbatum	0.77	0.98	0.83	0.06	0.84	0.68	0.88	0.27

The results showed metal immobilization in the underground parts and the rhizospheric zones by the *phoomdi* plants. Depending on the ability of *phoomdi* species to uptake metals in aboveground tissues, the candidate species could be used for phytoextraction of the particular metal. They can be introduced in constructed wetlands for the treatment of wastewater contaminated with various metals. For instance, *P. barbatum, C. esculenta, I. aquatica*, *S. cucullata* and *T. natans* with high TFs of 0.88, 0.84, 0.84, 0.76 and 0.72 for Zn, could be used for phytoremediation of wastewater contaminated with Zn. While the high TFs of 0.88 and 0.83 for *S. cucullata* and *O. javanica* indicates the ability of the plants to pytoextract Cu from the surface water of Loktak and store in the aboveground tissues. On the contrary, *phoomdi* species with the ability to reduce metal translocation from the underground parts to the aboveground parts could be suitable for their use as phytostabilizers for the re-vegetation and metal removal from waterlogged contaminated sites. *Phoomdi* species *A. philoxeroides*, *Z. latifolia*, *P. barbatum* and *T. natans* might be suitable for stabilization of Cu contaminated waterlogged environments. They grow well and propagate quickly in the environment that is low in nutrient, adding an advantage for its use in re-vegetation of waterlogged metal contaminated sites. Rai et al., 2013 demonstrated the use of six wetland species in constructed wetlands for the removal of Cr, Mn, Co, Ni, Cu, Zn, As and Pb from the river water of Ganga. Likewise, *phoomdi* species of Loktak with the ability to accumulate high metal concentrations in the aboveground tissues could be used for the treatment of the untreated rivers and sewage water of the Imphal valley before the water reaches Loktak.

Conclusions

The present field study showed that in the water column of Loktak, nutrients and metals, except for Fe (0.57 mg L^{-1}), were found present in low levels. It suggests the need to treat the freshwater for domestic consumption and agricultural applications (for Fe). Sediment characteristics showed retention of nutrients and metals with higher values of Fe (178.6 mg kg^{-1}) compared to Mn, Zn and Cu. *Salvinia cucullata* showed highest Fe (15,592.9 mg kg^{-1}), Mn (5948.5 mg kg^{-1}) and Cu (18.1 mg kg^{-1}) accumulation and 126.7 mg kg^{-1} Zn by *O. javanica*. Estimated daily intakes of Fe, Mn, Zn and Cu were from 303.3 to 2056, 122.4 to 2209, 13.7 to 59.1 and 0.4 to 3.1 µg kg^{-1} day^{-1}, respectively. THQ values calculated for the metals showed that the consumption of the *phoomdi* wild edibles is not free of risks. Relative high BCFs obtained depict the probable use of *phoomdi* in phytoextraction of nutrients and metals from the wetland and use in re-vegetation of waterlogged contaminated sites and constructed wetlands. The results suggest the need of proper and in-depth scientific investigation by the concerned bodies (LDA and Forest Department), if they planned to remove *phoomdi* from the surface of Loktak. Further, the study reports the level of concern to the human health via the consumption of the *phoomdi* wild edibles due to metal contamination. Further research in this area is required for the interests of public health of the locals. Degradation of the wetland will lead to several impacts on the livelihoods of a large population depending on various Loktak resources for sustenance, viz. increased poverty, health hazards, and changes in occupational structure and decreased agricultural yield around the wetland.

Objective 3

Phytofiltration of Iron contaminated wastewaters by *Typha latifolia* L.: Laboratory studies

Introduction

Wetlands are known to be efficient regulators of water quality (Mitsch and Gooselink, 2007). The luxuriant growth of various macrophytes, especially free floating and emergent forms contribute significantly to nutrient and metal stripping from the eutrophic waters and also help in regulating the quality of water. However, in Loktak, the nutrient and metal loading from both external and internal sources far exceeds the assimilative capacity of the macrophyte species on the surrounding wetland and the inside *phoomdi* biomass. Since the wetland has become a closed system with no outlet, the nutrients and metals remain within the ecosystem and their concentration tends to increase year to year (Trishal and Manihar, 2004). The wetland is surrounded by the tertiary hills, dominantly constituting of sedimentary sequences belonging to Indo Myanmar range of the Eastern Himalaya that are rich in iron ore deposits (Laiba, 1992). It leads to the deposition of iron into the wetland through various rivers draining into Loktak as the iron deposits leaches and falls into the tributaries joining the main river system of Imphal valley. The sewage and other waste products from various small scale industries in the Imphal are drained into Nambul river and thus help directly in its dumping into the wetland. With a population of 0.28 million living along the Nambul river catchments generates near to 31,207 m^3 of sewage daily (FD, 2011). The pollution of the wetland is again enhanced by the increased use of excessive amounts of fertilizers, pesticides, insecticides and fungicides by the agricultural folks nearby the wetland.

In our previous study (Objective 2), water samples at different locations of Loktak at the period of collection (for 2 years) exceeded the threshold limit set by WHO (drinking water) for Fe (0.37 to 0.57 mg L^{-1}) (WHO, 1996). Likewise, Singh et al., 2013 reported high Fe concentrations in Nambul river (upto 1486.51 ppb), a main tributary and at various places of the wetland with concentration that range from 105.70 to 646.17 ppb, respectively. Iron is the second most abundant element in the earth crust that account for near to 5%. Iron in water usually exists in ferrous, however it is readily oxidized to ferric form and the ferric salts are precipitated as rust colour deposits. The salts impart a bitter sweet stringent taste to water. Though iron is an essential element in human nutrition, high intake of iron above the lethal dose of 40 mg kg^{-1} of body weight can cause death of the human (WHO, 2011). Chronic iron overload results into haemochromatosis, haemorrhagic necrosis, sloughing of areas of mucosa in the stomach with extension in the sub mucosa, and tissue damage to a variety of organs by catalyzing the conversion of H_2O_2 to free radical ions that attack cell membranes, proteins and break the DNA strands and cause oncogene activation (Guanawardhana et al., 2002; Gurzau et al., 2003). Iron toxicity further leads to diabetes mellitus, atherosclerosis and related cardiovascular diseases, hormonal abnormalities and a dysfunctional immune system (Gurzau et al., 2003). Inorder to serve as a precaution against excessive storage of iron in human body, JECFA has set a provisional maximum tolerable daily intake (PMTDI) of 0.8 mg kg^{-1} of body weight, which is applicable to iron accumulating from various sources such as coloring agents, food supplements and water (JECFA, 1983).

Various conventional treatment techniques that include precipitation, reduction, artificial membranes and ion exchange, have been employed for the removal of toxic metals from industrial effluents (Qdaisa and Moussa, 2004). However, the techniques proved to be expensive, generate a huge amount of waste, leading to disposal problems and ineffective to remove the metals when present in low concentrations.

There arises the need to look for different water management techniques that are cost effective, have low environmental impact and public acceptance is not a problem to be discussed for the decision makers. Phytoremediation techniques, the use of plants to reduce, remove and degrade pollutants are being described in the years as a sustainable option for long term decontamination of polluted environments. Among the various phytoremediation techniques, phytofiltration and rhizofiltration that involve the removal of pollutants, especially the metals by the roots with their transport into the aerial portions of the plant shows the highest potential (Gomes et al., 2014). For the phytoremediation of effluents contaminated with metals, plant species with high pollutant uptake capacity and high growth rate is a must. Constructed wetlands (CW) are engineered wastewater treatment systems that uses a number of treatment principles including biological, chemical and physical processes, which are all alike to processes occurring in the natural treatment wetlands (Rai et al., 2013). The constructed wetlands have been used for the treatment of a wide variety of wastewaters including that of industrial effluents, urban and agricultural, animal wastewaters, leachates, sludges, medical wastes and mine drainage (Zhang et al., 2012).

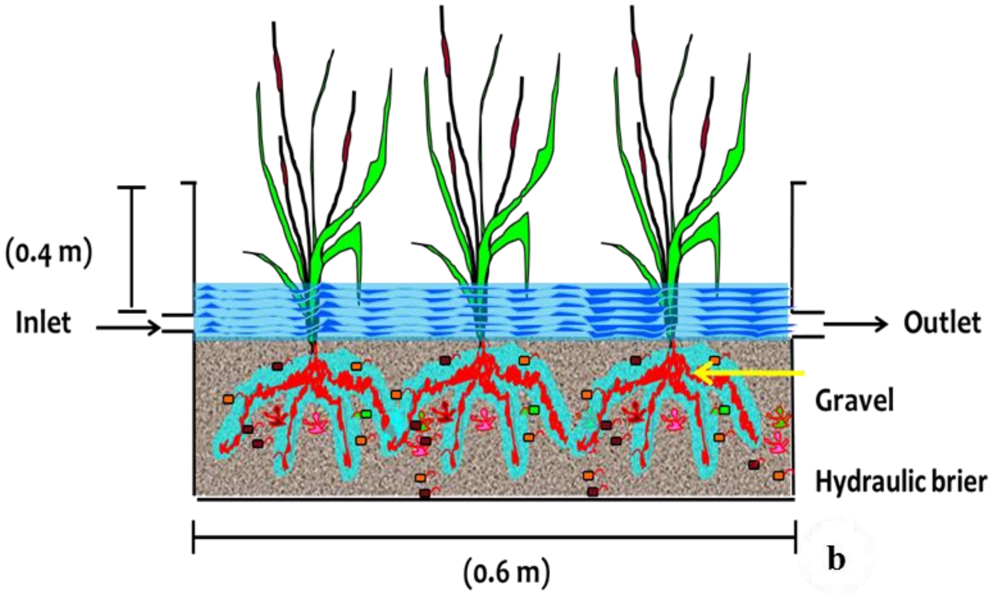
Typha is one of the cosmopolitan distributed species of eleven species of flowering plants that belong to Typhaceae family (Akkol et al., 2011). *Typha latifolia* L. is an emergent aquatic macrophyte that commonly grows in all tropical and temperate climatic regions (Eid et al., 2012). *Typha* species has been extensively exploited for its use in constructed wetlands to improve the quality of wastewater in various treatment systems (Hegazy et al., 2011). It grows naturally in floodplains, marshes, dams, drainage channels, wetlands and dump sites. The species shows high tolerance and high uptake capacity of various pollutants from degraded environments (Dordio et al., 2009; Park et al., 2009; Gomes et al., 2014). In addition, the plant species is abundant in the *phoomdi* of Loktak. As metal concentrations in the water column of Loktak, particularly Fe is relatively high; there is an urgent need to develop cost effective new technologies to treat the water. The plant based water management technologies can be considered as a means to remediate the wastewaters pouring into Loktak and its tributary rivers. Construction of artificial wetlands along the bank of the river Nambul and Loktak may act as biofilter and can remove high loads of nutrients and other pollutants including metals from the river water and the wetland. In laboratory pilot scale, the present study was done to evaluate potential of the *phoomdi* macrophyte *T. latifolia* to treat iron contaminated wastewaters and its Fe uptake aggravation by Cu supplementation.

Materials and methods

Pilot scale microcosm design and preparation

Two replicate lab-scale constructed microcosms (0.6 m × 0.4 m × 0.4 m) were set in a greenhouse located in University of Hyderabad with controlled environmental conditions of 18 h light length, temperature of 25±2°C, and relative humidity of 70%, **Fig. 15(a-b)**. Each microcosm was filled to a depth of 30 cm with a mixture of sand and gravel (1:1, v/v). Afterwards, in the microcosm set up 1, 20 L of wastewater with iron concentrations of 30 mg L^{-1} was transferred. To the second set up, 20 L of wastewater with iron and copper concentrations of 30 and 15 mg L^{-1} were transferred. In both set ups, aquatic macrophytes buds of *T. latifolia* species of about 15 cm long were cultivated. The microcosm, made through the action of the gravity in the

a



b

Fig. 15 (**a**) Growing *Typha latifolia* plants in laboratory; and (**b**) Schematic diagram showing rhizosphere responsible for Fe removal.

inferior part of the set up allowed the direct contact of the contaminated water with the bottom substrates and the roots of *T. latifolia*.

Transplant and acclimation of *T. latifolia*

The healthy young buds of *T. latifolia* were collected from the surrounding wetlands located inside the University of Hyderabad. They were brought to laboratory, washed with tap water and rinsed with distilled water and immediately transplanted and cultivated with a density of 20 buds per square meter, for 30 days, enough for the individuals to reach 30 cm in height. The water used during the acclimation phase was normal tap water.

Sampling

The microcosm set ups were monitored for 2 weeks. Water samples were collected for all the 14 days from the outlet and conserved in polyethylene bottles previously decontaminated. Sediment samples were collected on the 1st, 7th and 14th day of the experimental exposure. The *T. latifolia* vegetal materials consisting of aerial parts were collected at about 5 cm above the water level on 1st, 7th and 14th day of the exposure time.

Analysis of metals

Water samples were filtered through 0.45 µm Millipore membranes, acidified with conc. HNO_3 to pH < 2 and stored in the dark at 4ºC. Acid digestion of the water samples were carried out with conc. HNO_3: $HClO_4$ (3:1, v/v). The digested samples were refiltered stored prior to analysis. Sediments were brought to laboratory, air dried at room temperature for 3 days and sieved through a 2 mm nylon sieve to remove coarse debris. The samples were ground using a mortar and sieved to get fine powders. Total metal content was determined by digesting the sediment samples with conc. HNO_3: $HClO_4$ (3:1, v/v). The aerial parts of *T. latifolia* were thoroughly washed with tap water and rinsed with deionised water to remove unwanted materials. The parts were oven dried (70ºC) for 24 hours to get a constant weight and were ground using a Clotech Powder Mill and sieved to get fine powders. The samples were then acid digested: 10 mL of HNO_3:$HClO_4$, 3:1, v/v added to samples of 1.00 g. After cooling at room temperature, the residue was diluted with double deionized water to 30 mL (Deng et al., 2004). Heavy metals (Fe and Cu) concentrations were determined using Atomic Adsorption Spectrometry (GBC-932, Australia) for sediment, water and *T. latifolia* samples. Blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run to calibrate the instruments.

Data analysis

The data represents the mean of experiments performed in triplicates and were statistically evaluated using functions of Microsoft Excel 2010 (version Office Windows 7, Microsoft Corporation, USA).

Results and discussion

Variation of Fe and Cu concentration in the water

The performance of the constructed microcosm for Fe removal in the 2 set ups are shown in **Table 9**. Concentrations of Fe were reduced from the initial 30 ± 0.0 mg L^{-1} to 1.67 ± 0.076 mg L^{-1} and 0.87 ± 0.013 mg L^{-1}, respectively in the set ups 1 and 2. The removal rates of Fe exceeded 95% and remained stable throughout the 336 h (14 day) study period.

Table 9 Concentrations of Iron in water				
Exposure time (Hours)	**Contaminated water (Fe)**		**Contaminated water (Fe – Cu)**	
	Concentration of Fe (mg L^{-1})	**Remaining Fe (%)**	**Concentration of Fe (mg L^{-1})**	**Remaining Fe (%)**
0	30.00 ± 0.00	100.0 ± 0.0	30.00 ± 0.00	100 ± 0.00
24	26.87 ± 0.043	89.57 ± 1.3	24.11 ± 0.012	80.37 ± 2.1
48	21.09 ± 0.021	70.30 ± 2.1	19.98 ± 0.048	66.60 ± 1.7
72	17.76 ± 0.010	59.17 ± 3.4	13.12 ± 0.021	43.73 ± 2.0
96	11.56 ± 0.097	38.53 ± 1.7	9.36 ± 0.013	31.20 ± 1.8
120	7.09 ± 0.036	23.63 ± 2.8	6.12 ± 0.089	20.40 ± 1.6
144	5.43 ± 0.061	18.10 ± 1.8	4.78 ± 0.038	15.93 ± 2.6
168	4.68 ± 0.054	15.60 ± 1.9	4.11 ± 0.011	13.70 ± 2.3
192	4.23 ± 0.082	14.10 ± 2.1	3.89 ± 0.054	12.97 ± 1.9
216	3.83 ± 0.097	12.77 ± 2.6	3.56 ± 0.040	11.86 ± 3.5
240	3.11 ± 0.011	10.37 ± 1.6	2.87 ± 0.022	9.57 ± 1.6
264	2.76 ± 0.051	9.20 ± 0.9	2.67 ± 0.072	8.90 ± 1.2
288	2.25 ± 0.046	7.50 ± 1.2	1.23 ± 0.078	4.10 ± 1.8
312	2.01 ± 0.031	6.70 ± 2.3	1.01 ± 0.098	3.37 ± 2.7
336	1.67 ± 0.076	5.57 ± 1.1	0.87 ± 0.013	2.90 ± 1.8

Likewise, the concentration of copper in set up 2 got reduced to 0.014 ± 0.076 mg L^{-1} during 144 h (6 days) from the intital concentration of 15 ± 0.0 mg L^{-1}. It showed 100% removal of the copper in the set-up 2 microcosm, **Table 10**. Removal of the heavy metals was mainly governed through the processes of sedimentation, adsorption in the trickling filters, phytofiltration and phytoextraction by *T. latifolia*. In addition, formation of milky white, cloudy appearances were noticed on the surface of the wastewater in both the set ups, suggesting the formation of Fe rich colloidal (precipitates).

Table 10 Concentrations of Cu in water

Exposure time (Hours)	**Contaminated water (Fe-Cu)**	
	Concentration of Cu (mg L^{-1})	**Remaining Cu (%)**
0	15.00 ± 0.00	100.00 ±0.00
24	8.17 ± 0.012	54.45 ± 1.8
48	3.76 ± 0.062	25.07 ± 2.6
72	1.17 ± 0.202	7.80 ± 1.5
96	1.02 ± 0.069	6.80 ± 1.8
120	0.087 ± 0.041	0.58 ± 2.5
144	0.014 ± 0.076	0.09 ± 1.7

In most of the cases, the precipitation of Fe occurs following either atmospheric (abiotic) and bacterial mediated oxidation (*Thiobacillus ferrooxidans*, *Sphaerotillus* sp., *Metallogenium* sp., *Crenothrix* sp.) of Fe (II) to Fe (III) (Groudeva et al., 2005). Likewise, chemical precipitation of Fe added as hydrous oxides (Fe_2O_3) and hydroxides or oxyhydroxides ($Fe (OH)_3$ or FeOOH) after the diffusion of sufficient oxygen at the air-ware interface was observed. The initial pH of the wastewater was between 7.41 to 7.96 (data not shown) in both the set up 1 and 2. It is important to mention that chemical precipitation of Fe occurs in the pH range of 3.5-9.0 (Metcalf et al., 2003; Potgeiter et al., 2005) compared to bacterial mediated oxidation of Fe (II) and subsequent precipitation is not that likely to be observed at low pH conditions (Vyzamal, 1995). The facts justifies that any direct involvement of microorganisms in the oxidation of Fe (II) and its subsequent precipitation was an insignificant process in our study.

Variation of Fe and Cu concentration in the sediment

The concentrations of Fe and Cu significantly increased compared with the initial concentration, depicting that the substantial amount of both the metal ions were accumulated in the substrates of set up 1 and 2, respectively, **Fig. 16(a-b)**. Iron concentration in the substrate increased from 345.5 ± 15.23 mg kg^{-1} to 765.75 ± 53.82 mg kg^{-1} during 1 and 14 days of exposure in set up 1. Likewise, iron in set up 2 increased from 329.25 ± 14.23 mg kg^{-1} to 915.75 ± 25.13 mg kg^{-1} during 1 and 14 days exposure. Similarly, copper concentrations in the substrate of set up 2 changed from 329.5 ± 15.23 mg kg^{-1} to 1065.45 ± 17.81 mg kg^{-1} and 614.85 ± 35.00 mg kg^{-1} during 1^{st}, 7^{th} and 14^{th} exposure days. Since the Fe concentrations change in the substrate occurred gradually with time, apart from slow flocculation rates and subsequent sedimentation of Fe-rich colloidals, senescing plants too tend to produce Fe rich organic detritus (mostly the underground tissues). As a result, the organic detritus may complex with the settled precipitates of Fe_2O_3 and Fe $(OH)_3$ through adsorptive mechanisms. Therefore, the detritus from the aboveground tissues of *T. latifolia* may also serve as an additional adsorption site for Fe (II) that remains in the wastewater (O'Sullivan et al., 2004). The decrease in Cu concentrations by 14 days

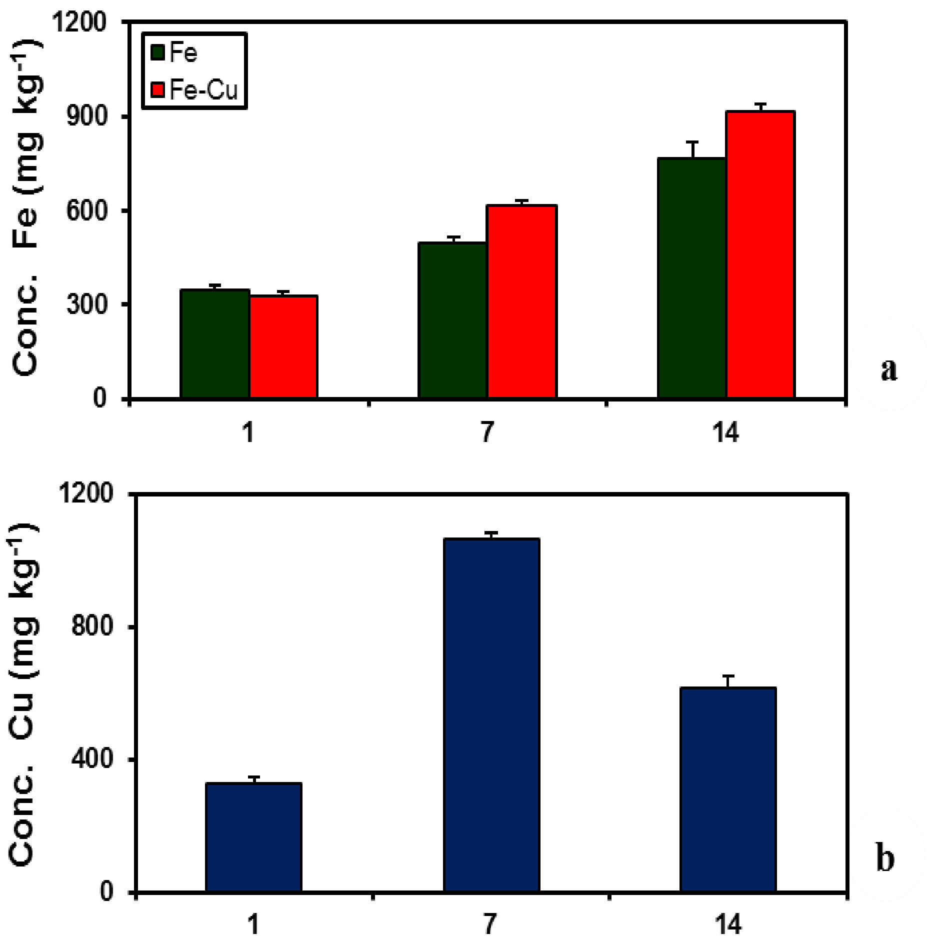


Fig. 16 Concentrations of (**a**) Fe and (**b**) Cu in sediments in set up 1 and 2.

may be attributed to the uptake of more copper from the substrate by the roots of *T. latifolia*, as 100% removal of Cu in set up 2 from the water is achieved within 7 days exposure period. The result shows that substrate is a primary sink for the retention of the contaminants, viz. Fe and Cu within the constructed microcosm (Ye et al., 2003). Therefore, the fate of the heavy metals, viz. Fe and Cu in the substrates is important in assessing the permanent and safe removal of the metal ions using constructed microcosm treatment techniques.

Fe and Cu accumulation in *T. latifolia*

In addition to chemical precipitation and subsequent sedimentation of the flocculated Fe-rich colloidals, phytoextraction of Fe by *T. latifolia* play an important role in the removal of Fe from the wastewaters. Concentrations of Fe and Cu in the aboveground *T. latifolia* tissues are presented in **Fig. 17(a-b)**. The average Fe concentration in the shoots increased from 348 ± 41.23 mg kg^{-1} to 1446 ± 36.01 mg kg^{-1} during 1^{st} and 14^{th} day of exposure in set up 1 microcosm. It was observed that rhizofiltration is the prominent mechanism involved during the phytoremediation of Fe and its subsequent transfer to the aboveground tissues. The adsorption of Fe to the anionic sites viz. phosphate and carboxyl groups in the cell walls and the precipitation of Fe_2O_3 and $Fe(OH)_3$ within the cell walls can be the reason for high rhizofiltration (Soltan and Rashed, 2003). In addition, some of the Fe-rich colloidals formed in the wastewater were absorbed by the underground rhizomatic tissues of *T. latifolia* possibly for solubilization and subsequent assimilation through the secretion of organic acids. The plants cultured in set up 1 and 2 showed significant active effluxing of Fe and with time (7 to 14^{th} days) probably to prevent any phytotoxic

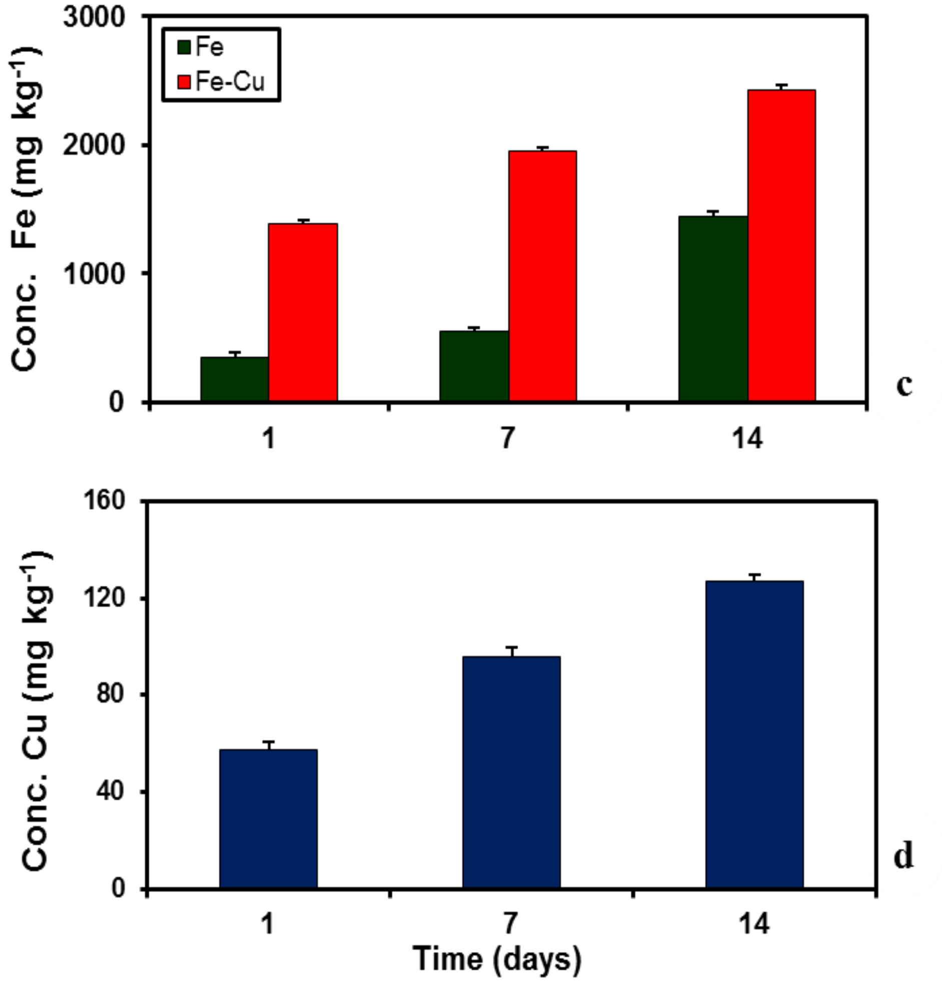


Fig. 17 Concentrations of (**a**) Fe and (**b**) Cu in *T. latifolia* in set up 1 and 2.

levels of Fe being accumulated in the *T. latifolia* tissues. Likewise, root mediated precipitation (Fe as $FePO_4$ and $FeCO_3$) and sedimentation of the Fe flocs was noticed during most stages of active effluxing to avoid Fe-phytotoxicity (Jayaweera et al., 2008). Most probably, the Fe colloidals were formed inside the root cells and involved a series of root exudates, involving some peptides for the flocculation of the colloidal particles. The above mechanism of root mediated precipitation was identified by Brennan and Shelley (1999) as an approach to stimulate the uptake and translocation of Pb (II) in maize plants.

In the presence of Cu in the set-up 2 microcosm, the uptake of Fe in the aboveground tissues increases upto 1390.36 ± 23.56 mg kg^{-1} and 2425.65 ± 41.01 mg kg^{-1} during day 1 and 14 exposure period. Likewise, the concentration of Cu in *T. latifolia* increased from 57.15 ± 3.4 mg kg^{-1} to 127.05 ± 2.7 mg kg^{-1} during the exposure period of 1 to 14 days. La Fontaine et al, (2002) reported that the photosynthetic algae *Chlamydomonas reinhardtii* possesses both Cu dependent (orthologue to Fet_3) and Cu independent pathways for Fe acquisition. However, it is indicated that Fe acquisition by roots does not require copper, but they instead depend on Cu independent transporters. Sancenon et al., (2003) reported that the plasma membrane Cu and Fe-chelate reductase activities are inextricably linked. The root plasma membrane Cu and Fe reductase activities were significantly induced upon both Cu and Fe depletion in pea plants (Cohen et al., 1997). In addition, the induction of ferric reductase activity by simultaneous Fe and Cu deficiency is synergetic rather than additive, which is consistent with a single gene responding to both Fe and Cu deficiency (Romera et al., 2003). Wu et al., (2005) reported that Ferric reductase oxidase 2 (FRO2) and FRO3 as the main components responsible for Fe acquisition and metabolism in *Arabidopsis* roots. FRO2 reductase also exhibits Cu reductase activity under Fe deficiency and FRO3 expression increases in *Arabidopsis* roots upon both Fe and Cu limitation suggesting its involvement in Fe and Cu acquisition.

The development of sustainable phytoremediation techniques depends on the ability of the plants for (hyper) accumulation of various metals, their environment and the biogeochemical cycles. The acquired informations and knowledge can be applied in man-made systems that resemble the natural environment of the studied plants, in the form of constructed wetlands. The CW has been employed for the remediation of mine wastewater, sewage water and industrial effluents since 1980s in across the countries (Gerth et al., 2005). The CW systems can be combined with various other components of hydraulic, geochemical and biological polishing processes that will based on the continuous removal of metals by the use of plants growth and microorganisms activity in the wetland bottom sediments (Kalin et al., 2005). Thus, there arises the need for further research concentrating on various metals and macrophyte species across worldwide range. However, phytofiltration and rhizofiltration have its own disadvantages, viz. some of these methods have failed due to various factors that governs the plant growth (temperature), nutrient balance maintenance in the solution and the hindrance during reproducing the laboratory results in the field and when foreign plan species are used (Prasad and Freitas, 2003). The results of our preliminary study in laboratory conditions showed that *T. latifolia* can play an important role in the immobilization of Fe in natural aquatic environment by sequestering the metal in their tissues and thereby reducing the dispersion of Fe in the ecosystem. Further, the species showed high accumulation of Fe when treated alone and supplemented with Cu in the microcosm. The scope of improvement of the phytoremediation system need to address a better understanding of the metal uptake mechanism, retention time in the plant tissues and its decomposition as plant litter in the sediment. A frequent harvesting of the aboveground parts of *T. latifolia* is recommended for the stripping of Fe from the contaminated wastewater environment. The understanding of the basic interactions between plants, sediment, microorganisms and physical properties of water in the natural wetland system will help in designing long term remediation approaches based on natural models.

Transfer coefficient of Fe in *T. latifolia*

The water-plant transfer coefficient (TC) was calculated as the relation between the metal ion concentrations in the aerial parts of *T. latifolia* and in the contaminated water as a proper way to express the relative metal absorption by *T. latifolia*, **Table 11**.

Table 11 Transfer co-efficient of Iron

Exposure time (Hours)	**Transfer coefficient (L kg-1)**	
	Iron	**Iron-Copper**
24	12.95 ± 0.013	57.67 ±0.032
168	117.62 ± 0.011	474.34 ± 0.018
336	865.87 ± 0.034	2788.10 ± 0.024

The concentration of Fe was 348 ± 41.23 mg kg^{-1}, attaining 1446 ± 36.01 mg kg^{-1} during the exposure of 14 days, and corresponds to the maximum TC of 865.87 ± 0.034 L kg^{-1} in set up 1. For set up 2, when supplemented with Cu, the TC of iron increased from 1390.65 ± 23.56 L kg^{-1} to 2425.65 ± 41.01 L kg^{-1}, respectively in 14 days. The high values of water plant transfer coefficient explain the high uptake rate of the heavy metal by *T. latifolia* in the microcosm constructed. Cardwell et al., (2002) have found metal magnification from 0.00 to 0.40 mg kg^{-1} for cadmium; 3.13 to 16.40 mg kg^{-1} for copper; 1.35 to 5.39 mg kg^{-1} for lead; 19.8 to 90.02 mg kg^{-1} for zinc, in the aerial parts of *T. domingensis* collected from Southeast of Queensland. Hadad et al., (2006) evaluated the growth of various macrophytes in a constructed wetland in a pilot scale study for treating industrial wastewater and found out that *Typha* sp. proved to be the most dominant species accumulating 36.0, 32.0 and 39.0 mg kg^{-1} of chromium, nickel and zinc in the aerial tissues.

Conclusions

The present study aimed to develop a laboratory scale bioassay set up to treat iron contaminated waters using *Typha latifolia* L. a *phoomdi* species (set up 1) and the aggravation of Fe uptake by Cu supplementation (set up 2). The initial concentration of iron in set up 1 and 2 water samples was 30.00 ± 0.00 mg L^{-1}, decreasing to 1.67 ± 0.076 mg L^{-1} and 0.087 ± 0.013 mg L^{-1} during 336 hours (14 days). Copper addition increased the uptake of iron in *T. latifolia* upto 2425.65 mg g^{-1} (set up 2) compared with 1446.00 mg g^{-1} in set up 1. The water plant transfer coefficient (TC) values increased from 12.95 ± 0.013 L kg^{-1} (1 day) to 865.87 ± 0.034 L kg^{-1} (14 days) in set up 1 plant compared to 57.67 ± 0.032 L kg^{-1} (1 day) and 2788.10 ± 0.024 L kg^{-1} (14 days) in set up 2 plant. The present work showed the effectiveness of *T. latifolia* in removing Fe and Cu from wastewaters. Thus, plant based technology of CW can be used to treat the sewage and industrial effluents before it is discharged into Loktak.

Objective 4

Adsorption of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) by *Spirodela polyrhiza* (L.) Schleiden: Application for wastewater treatment

Introduction

Heavy metals are released into the wastewater environment from various sources such as mining, electroplating, coal burning, steel production, petroleum refining, batteries, plating, ceramic and glass industries (Fu and Wang, 2011). Heavy metals are persistent environmental contaminants, because they are non-biodegradable and tend to accumulate in living organisms through food chain (Meneghel et al., 2013). Heavy metals like Cu (II), Mn (II) and Zn (II) ions are required for the growth of plants and animals. They are highly toxic if present above permissible limits and causes negative health effects (Garcia-Mendieta et al., 2012; Gorgievski et al., 2013). The permissible limits of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) in drinking water as set by the World Health Organisation (WHO) is 0.010, 0.005, 2.0, 0.4 and 4.0 mg L^{-1} (WHO, 2011), respectively. Lead poisoning causes damage to the nervous system, reproductive system, kidney and brain (Lawal et al., 2010). Similarly, cadmium toxicity leads to hypertension, lung damage and dysfunction of the kidney (Srivastava et al., 2006). Copper toxicity causes skin irritation, stomach disorder, kidney damage, anemia, hepatic damage, and gastrointestinal irritation (Rahman and Islam, 2009; Wan Ngah and Hanafiah, 2008). Manganese poisoning leads to neurological disorders and brain damage (Guzel et al., 2008) and zinc causes muscular stiffness, loss of appetite, nausea and irritation (Areco et al., 2012). Due to the given reasons, there is a great interest evolved for the removal of the heavy metal ions from wastewaters seing their toxic nature towards human health and ecosystems (Sener et al., 2014).

In order to eliminate the heavy metals from contaminated aquatic environment, various treatment technologies such as ion exchange, chemical precipitation, membrane filtration, reverse osmosis, evaporation and solvent extraction have been used (Fu and Wang, 2011). They are generally expensive, generate huge amount of waste by-products and ineffective especially when contamination of heavy metals persist in low concentrations (Li et al., 2013; Lalhruaitluanga and Prasad, 2015). Biosorption technique using biological materials as adsorbents emerged as a promising method because of its effectiveness in metal removal to low level, abundant, low price (since the bio-materials are waste or by products of food, fiber or agricultural industry with low or no price value) and ease of handling (Norton et al., 2004; Ullah et al., 2013). Due to the reasons, number of biological materials has been widely employed as adsorbents for the removal of heavy metals from wastewaters (Schneider and Rubio, 1999; Wang and Qin, 2006; Romera et al., 2007; Nagpal et al., 2011; Zuo et al., 2012; Bozic et al., 2013; Gorgievski et al., 2013; Li et al., 2013; Das et al., 2014; Weng et al., 2014).

Realizing the prolific growth and its impacts on the wetland ecosystem, workshops have been organized by LDA inviting all the stakeholders, scientists, policy makers, NGOs and community groups to deliberate on the issues related to the *phoomdi* management. However, the management strategies got concentrated on the removal of the biomass rather than adding its utility by further application for various purposes. Keeping this in mind, a study was conducted for the utilization of a dominant *phoomdi* species of Loktak for its use as an adsorbent to cleanup aquatic environments contaminated with various heavy metal ions. *Spirodela polyrhiza* (L.) Schleiden (Greater duckweed) helps in the initial formation of *phoomdi* and is abundant in Loktak (Singh and Singh, 1994). *S. polyrhiza* is a free floating aquatic macrophyte of the family Araceae, distributed worldwide in the freshwater habitats

(Davidson and Simon, 1981). The plant body is not differentiated into a stem or leaf. It is reduced to a fleshy or a thallus like ovoid or flattened structure bearing one to several roots (without root hairs) on the underside. The daughter plants are produced in a budding pouch at the basal end or along the 2 lateral margins of parent plant, often remaining attached to parent plant by a short stipe. They are among the smallest and structurally simplest of all angiosperms, with greatly reduced vascular tissues (Henssen, 1954). The plant is perennial, grows in dense colonies and forms a mat like cover. They are highly productive and fast growing macrophyte (Davidson and Simon, 1981).

In the study, the potential of *S. polyrhiza* for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions removal from aqueous solutions were investigated. As per literature review, there are no reference to the work involving the use of *S. polyrhiza* biomass to remove Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions from aqueous solution and their simultaneous removal. Batch adsorption experiments with varying pH, adsorbent dosage, contact time, initial concentration of metal ions and temperature was conducted to study the equilibrium, kinetics and thermodynamics of the adsorption process. The study aimed to explore a new phytotechnological application of the *phoomdi* biomass obtained from *S. polyrhiza* (as an effective, low-cost adsorbent for removal of copper, manganese and zinc from wastewaters), that otherwise is treated as a biological material with no usage. The findings will open a horizon towards sustainable harvesting and utilization of the *phoomdi* biomass of Loktak and its use in future applications for the restoration of degraded aquatic environments contaminated with heavy metals.

Materials and methods

Reagents

The chemicals used during the experiments were of analytical pure grades. The stock solutions (1000 mg L^{-1}) of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) were prepared by dissolving appropriate amounts of $Pb(NO_3)_2$, $CdCl_2$, $CuSO_4.5H_2O$, $MnSO_4.H_2O$ and $ZnSO_4.7H_2O$ in deionised water. The working solutions of different concentrations were prepared by diluting the stock solutions with deionised water.

Biomass collection and preparation

The aquatic macrophyte, *S. polyrhiza* was collected from the *phoomdi* of Loktak lake (93°46'-93°55' E; 24°22'-24°42' N), Manipur, North-East India, **Fig. 18a-b**. The plant material was brought to laboratory, rinsed with deionized water to remove unwanted materials and dried in hot air oven for 48 hours at 60^o C, until a constant weight was achieved (Hanif et al., 2009). The dried sample was powdered in Clotech 1093 sample mill, then sieved to give a fraction of 100 mesh screen and used as an adsorbent.

Batch adsorption experiment

The adsorption experiments were carried out in 150 mL conical Erlenmeyer flasks containing 100 mL of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) solutions. One N NaOH or HNO_3 solutions were used to adjust pH of the heavy metal solutions before the adsorbent was added. The flasks with adsorbent dosage of 0.1 g were incubated at 20^o C (for Pb (II) and Cd (II)), 30^o C (for Cu (II), Mn (II), and Zn (II)) with 180 rpm for 120 min.

Fig.18 (**a-b**) *Spirodela polyrhiza* in stock cultures.

They were filtered using Whatmann 32 filter paper and the metal concentrations were determined. The experiments were performed at different pH values from 2.0 to 6.0 for Pb (II), 2.0 to 5.0 for Cu (II) and 2.0-7.0 for Cd (II), Mn (II) and Zn (II), contact time from 5-120 min, adsorbent dosage from 0.05-0.25 g, temperature from 20-40^{o} C, and initial metal concentrations from 100-160 mg L^{-1} for Pb (II), 10-40 mg L^{-1} for Cd (II), 60-120 mg L^{-1} for Cu (II), 30-90 mg L^{-1} for Mn (II) and 5-35 mg L^{-1} for Zn (II), respectively. Each experiment was carried out in triplicates. The amount of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorbed at the equilibrium, q_e (mg g^{-1}) was calculated using Eq. (1):

$$q_e = (C_i - C_e)\, V / x \qquad (4)$$

where C_i and C_e are the initial and equilibrium concentrations of the metal, V is the volume of the metal solution, q_e is the amount of metal adsorbed at equilibrium (mg of Pb g^{-1} or mg of Cd g^{-1}or mg of Cu g^{-1} or mg of Mn g^{-1}or mg of Zn $g^{-1}$of the adsorbent), and x is weight of adsorbent (g).

Multi-metal ion adsorption experiment

In each of the experiment, 0.1 g of *S. polyrhiza* biomass was contacted with 100 mL of the metal solution containing equimolar concentrations of each cation for the metal combinations: Pb+Cd, Pb+Cu, Pb+Mn, Pb+Zn, Cd+Cu, Cd+Mn, Cd+Zn, Cu+Mn, Mn+Zn, Zn+Cu and Pb+Cd+Cu+Mn+Zn. The analysis of the heavy metal ion was done after 120 min incubation.

Desorption experiment

S. polyrhiza biomass (0.1 g) was saturated with 100 mg L^{-1} Pb (II), 10 mg L^{-1} Cd (II), 60 mg L^{-1} Cu (II), 30 mg L^{-1} Mn (II) and 5 mg L^{-1} Zn (II), respectively in different 150 mL Erlenmeyer flasks for 120 min, at temperature 20^{o} C (for Pb (II) and Cd (II)) and 30^{o} C (for Cu (II), Mn (II) and Zn (II)) and pH 4.0, 6.0, 5.0, 7.0 and 6.0, respectively. The biomass was washed with deionised water for several times to remove the heavy metal ions adsorbed. Further, it was treated with 100 mL of 0.1 M HCl each and incubated for 120 min, temperature 20^{o} C (for Pb (II) and Cd (II)) 30^{o} C (for Cu (II), Mn (II) and Zn (II)) at 180 rpm. The supernatants were collected and metal analysis was done.

Metal ions determination

The concentrations of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) were analysed using Flame Atomic Adsorption Spectrometer (GBC 932 Plus, Australia). The wavelengths of the heavy metals ions were 283.3 nm for Pb (II), 228.8 nm for Cd (II), 217.9 nm for Cu (II), 403.1 nm for Mn (II) and 213.9 nm for Zn (II), with split width of 0.5 nm for Pb (II), Cd (II) and Zn (II) and 0.2 nm for Cu (II) and Mn (II) and air-C_2H_2 flame type.

SEM, EDX and FTIR analysis

The characterization of the surface structure and elemental composition of *S. polyrhiza* biomass, before and after adsorption with Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions was done using SEM / EDX (XL30 ESEM, Philips, USA). The analysis was done by coating the samples with gold particles to increase the electrical conductivity and protect it from electron beam damage and dehydration in the vacuum (Ullah et al., 2013). The determination of the functional groups involved in the process, before and after adsorption biomass was done using FTIR Spectrometer (NICOLET 5700-FTIR) in the range of 400-4000 cm^{-1}. The metal treated biomass was prepared by contacting *S. polyrhiza* (0.1 g) with 100 mg L^{-1} Pb (II), 10 mg L^{-1} Cd (II), 60 mg L^{-1} Cu (II), 30 mg L^{-1} Mn (II) and 5 mg L^{-1} Zn (II), pH 4.0, 6.0, 5.0, 7.0 and 6.0, respectively.

Data analysis

The data represents the mean of experiments performed in triplicates. The correlation coefficient (R^2) values of Pseudo-first order, Pseudo-second order, Freundlich and Langmuir isotherm models were determined using statistical functions of Microsoft Excel 2010 (version Office Windows 7, Microsoft Corporation, USA).

Results and discussion

Effect of pH on metal adsorption

pH is an important parameter controlling adsorption of the metal ions on solids. This may be because of the fact that hydrogen ions themselves are strongly competing with the adsorbate. The increased in the sorption of metal ions with increased in pH value can be explained by the reaction of metal hydrolysis and that between binding sites of the adsorbent and the metal, where the bond for hydrogen is broken and hydrogen ions are released, which is then substituted by the metal (Tipping, 2002). The adsorption experiments were carried out at the pH range of 2.0-6.0 for Pb (II), 2.0-5.0 for Cu (II) and 2.0-7.0 for Cd (II), Mn (II) and Zn (II), solution concentrations of 100 mg L^{-1} Pb (II), 10 mg L^{-1} Cd (II), 60 mg L^{-1} Cu (II), 30 mg L^{-1} Mn (II) and 5 mg L^{-1} Zn (II), adsorbent dosage of 0.1 g, contact time of 120 min and temperature at 20^{o} C and 30^{o} C. The removal of the metal ions was pH dependent. The response of metal uptake by the biomass is shown in **Fig.19a**. It is observed that a gradual rise in the adsorption of the metal ions took place with increase of pH from 2.0-4.0 for Pb

(II), 2.0-6.0 for Cd (II), 2.0-5.0 for Cu (II), 2.0-7.0 for Mn (II) and 2.0-6.0 for Zn (II), respectively. The low biomass-metal binding capacity in lower pH is due to the competition of metal ions with protons for active sites, as the functional groups on *S. polyrhiza* biomass surface are protonated. It is also because of the electrostatic repulsion between the protonated surface and the metal ions. The rate of adsorption increased from 57.19-91.77% for Pb (II), 22.39-96.76% for Cd (II), 7.04-57.81% for Cu (II), 22.06-61.66% for Mn (II) and 37.68-86.44% for Zn (II), as the higher pH weakens the competition between the metal ions and H^+ ions bond to the biomass, and H^+ ions got replaced by the metal ions (Azouaou et al., 2010). The maximum adsorption was found at pH 4.0, 6.0, 5.0, 7.0 and 6.0 for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II), respectively. Further increase of pH causes reduction of metal adsorption due to metal-hydroxide ions formation. The reason for the adsorption of metal ions is attributed to the presence of different functional groups on the surface of *S. polyrhiza* biomass. For further study, the optimum pH values obtained were used.

Effect of adsorbent dosage on metal adsorption

The adsorption experiments were carried out with adsorbent dosage 0.05-0.25 g, and fixed initial concentrations of metal ions, 100 mg L^{-1} Pb (II), 10 mg L^{-1} Cd (II), 60 mg L^{-1} Cu (II), 30 mg L^{-1} Mn (II) and 5 mg L^{-1} Zn (II), respectively. The percentage of adsorption increased from 80.05-93.28% for Pb (II), 95.39-97.97% for Cd (II), 50.10-59.11% for Cu (II), 39.80-66.41% for Mn (II) and 80.68-93.24% for Zn (II) (**Fig.19b**), respectively. This behaviour is attributed to increase in surface area for binding of the metal ions with increasing adsorbent dosage. It eventually increases the number of adsorption sites / functional groups for metal ions adsorption. Similar findings for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption using

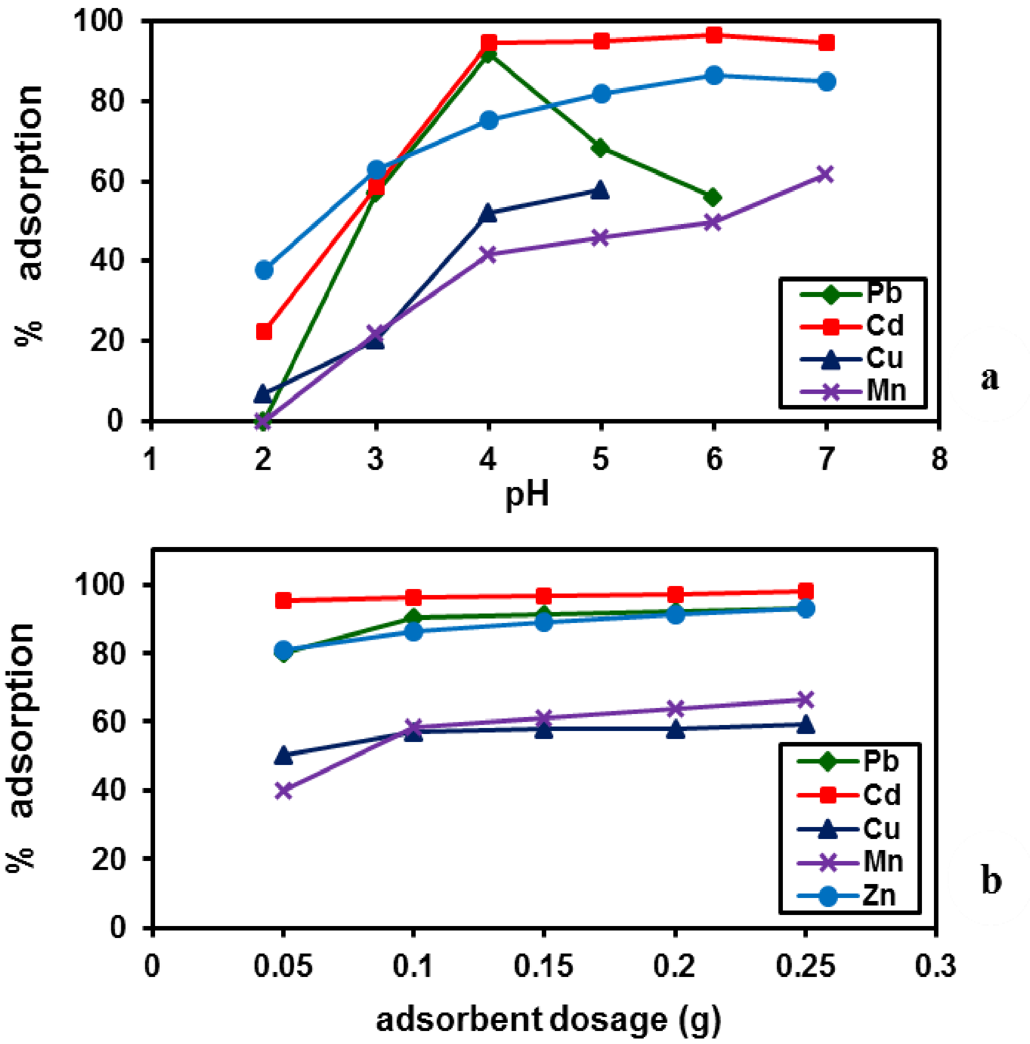


Fig. 19 Effect of (**a**) pH, and (**b**) adsorbent dosage on Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) adsorption by *S. polyrhiza*.

Ulva lactuca (Areco et al., 2012), *Saccharomyces cerevisiae* (Parvathi et al., 2007), *Tectona grandis* (Kumar et al., 2006a), and *Eichhornia crassipes* (Schneider and Rubio, 1999) were reported in literature.

Effect of contact time on metal adsorption

Fig. 20a shows the effect of contact time on Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions removal by *S. polyrhiza* biomass. The results showed the increase of adsorption efficiency with initial contact of 5-30 min. The process became slower with no further significant adsorption beyond 120 min. 80.27% of Pb (II), 94.08% of Cd (II), 50.84% of Cu (II), 48.97% of Mn (II) and 81.66% of Zn (II) were removed within 5 min of the adsorption experiment. The adsorption process involved two steps: an initial fast step for a short duration of 5-30 min and slower second step that lasts until the equilibrium was attained (120 min). The rapid initial uptake of the heavy metal ions occurred through physical adsorption to the biomass surface, since the adsorption phenomenon tend to attain instantaneous equilibrium (Acheampong et al., 2012). As the biomass has fixed active sites adsorbing only a metal ion forming a monolayer, the uptake at initial stages was rapid and then decreases with time. The equilibrium adsorption for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions was attained at 120 min, with no further significant change in the adsorption. Similar trends have been reported in literature using *Daucus carota* (Guzel et al., 2008), *Myriophyllum spicatum* (Keskinkan et al., 2003), *Ceratophyllum demersum* (Keskinkan et al., 2004), and *Cymbopogon schoenanthus* (Zuo et al., 2012). The metal removal rate is of great importance for developing adsorbent based water technology in the future (Hegazy et al., 2011).

Effect of temperature on metal adsorption

The effect of temperature on metal adsorption was investigated within the range of 20-40^{o} C, adsorbent dosage of 0.1 g, contact time of 120 min, pH 4.0, 6.0, 5.0, 7.0 and 6.0 for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions, respectively. It was observed that the maximum adsorption was found at 20^{o} C (for Pb (II) and Cd (II)) and 30^{o} C (for (Cu (II), Mn (II) and Zn (II)), with 91.31, 96.76, 57.81, 60.08 and 86.44% removal of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions from the aqueous solution, **Fig. 20b**. The results showed decrease in the adsorption with rising temperature, supporting that low temperature favoured adsorption of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions by *S. polyrhiza* biomass. It is due to the fact that at higher temperature, the kinetic energy of the metal ions is low; therefore the contact between the metal ions and active sites of the biomass is not sufficient, leading to decrease adsorption efficiency. According to the adsorption theory, adsorption decreases with an increase in temperature and the molecules adsorbed earlier on the surface tend to desorb from the surface of the biomass at increasing temperature (Aksu and Kutsal, 1991; Javed et al., 2007).

Effect of initial concentration of metal ions

The initial concentration is an important factor to be considered for effective adsorption of the metal ions, **Fig. 21(a-d)**. The adsorption experiments with varying initial concentrations of 100-160 mg L^{-1} for Pb (II), 10-40 mg L^{-1} for Cd (II), 60-120 mg L^{-1} for Cu (II), 30-90 mg L^{-1} for Mn (II) and 3-35 mg L^{-1} for Zn (II), with fixed adsorbent dosage (0.1 g / 100 mL) at pH 4.0. 6.0, 5.0, 7.0 and 6.0 were performed. The adsorption capacity of the biomass increases with increasing concentration of the

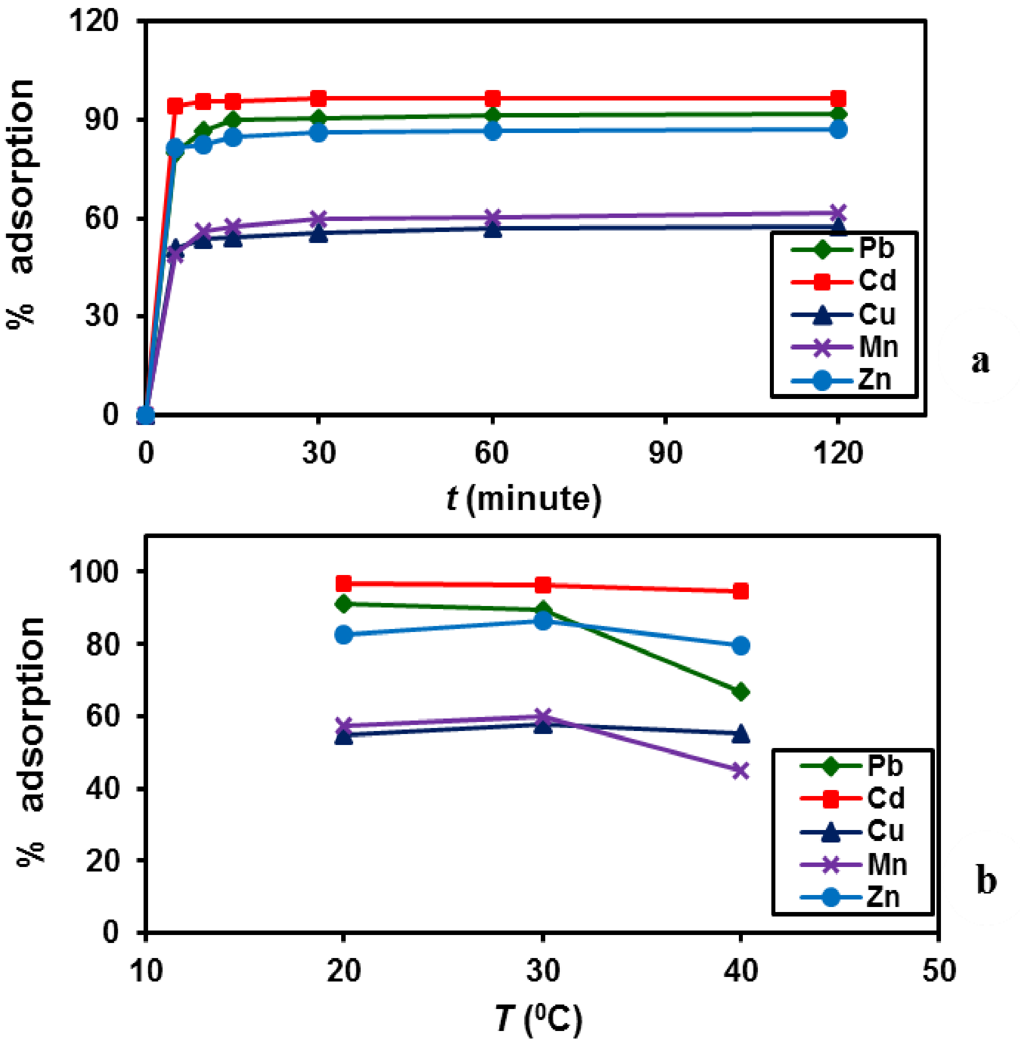


Fig. 20 Effect of (**a**) contact time and (**b**) temperature on Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) adsorption by *S. polyrhiza*.

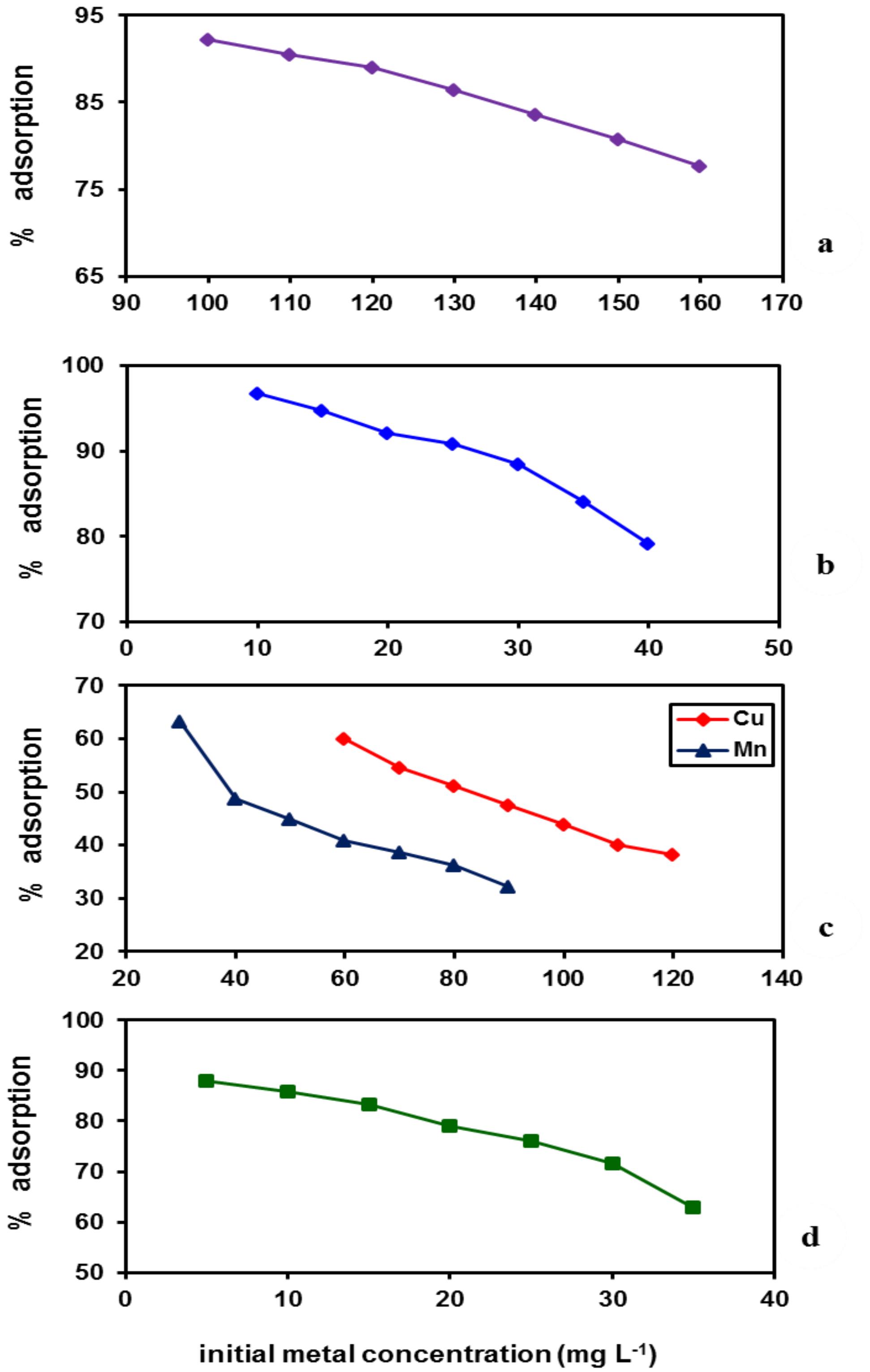


Fig. 21 Effect of initial concentrations of (**a**) Pb (II), (**b**) Cd (II), (**c**) Cu (II) and Mn (II) and (**d**) Zn (II) adsorption by *S. polyrhiza*.

the metal ions, however, percentage removal decreased from 92.25-77.61% for Pb (II), 96.76-79.16% for Cd (II), 59.95 to 38.31% for Cu (II), 63.16-32.24% for Mn (II) and 87.96-62.96% for Zn (II), respectively. The phenomenon showed that *S. polyrhiza* biomass has fixed number of active sites that become saturated at a certain concentration of the metal ions. Further, the results obtained showed that the adsorbent can be efficiently used for removal of metal ions from wastewater with low metal ion concentrations. Similar reports using *Daucus carota* (Guzel et al., 2008), *Alternanthera philoxeroides* (Wang and Qin, 2006), *Laminaria hyperborea* (Freitas et al., 2007), and *Ulva lactuca* (Areco et al., 2012) have been reported in literature. The data obtained were used to determine the isotherms of Cu (II), Mn (II) and Zn (II) ions adsorption.

Adsorption kinetics

The determination of the mechanism involved in the adsorption of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions onto *S. polyrhiza* biomass was done using two kinetic models (pseudo first and pseudo second order).

The Pseudo first order or Lagergren model states that the rate is proportional to the number of unoccupied sites (Liu and Liu, 2007).

It is represented by the Eq. (5):

$$dq / dt = K_1 (q_e - q_t) \quad (5)$$

Integrating Eq. (5) in the conditions (t = 0, q = 0) and (t = t, q = q_t) gives the linear formula:

$$\log (q_e - q_t) = \log q_e - K_1\ t / 2.303 \qquad (6)$$

where q_e and q_t are the amount of metal ions adsorbed (mg g^{-1}) onto the biomass at equilibrium and at any time t, K_1 (min^{-1}) is the rate constant of pseudo first order model and t is the time (minute). The value of K_1 was calculated from the plot of log ($q_e - q_t$) versus t for the metal ions (**Fig.22a**).

The Pseudo second order assumes that the rate of sorption is proportional to the square of the number of unoccupied sites (Ho, 2006).

It is described by Eq. (7):

$$dq / dt = K_2 (q_e - q)^2 \qquad (7)$$

Equation (7) when integrated in the conditions (t = 0, q = 0) and (t = t, q = q_t) gives:

$$t / q_t = 1 / K_2 q_e^2 + t / q_e \qquad (8)$$

where K_2 is the equilibrium rate constant of pseudo second order model (g mg^{-1} min^{-1}). The slopes and intercepts of plots from t / q_t versus t were used to calculate q_e and K_2 (**Fig.22b**).

Table 12 gives the comparison of pseudo first and pseudo second order parameters for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption. The pseudo-second order correlation coefficient (R^2) value was 1.00 for the metal ions adsorption and experimental q_e value was also agreed well with the calculated value.

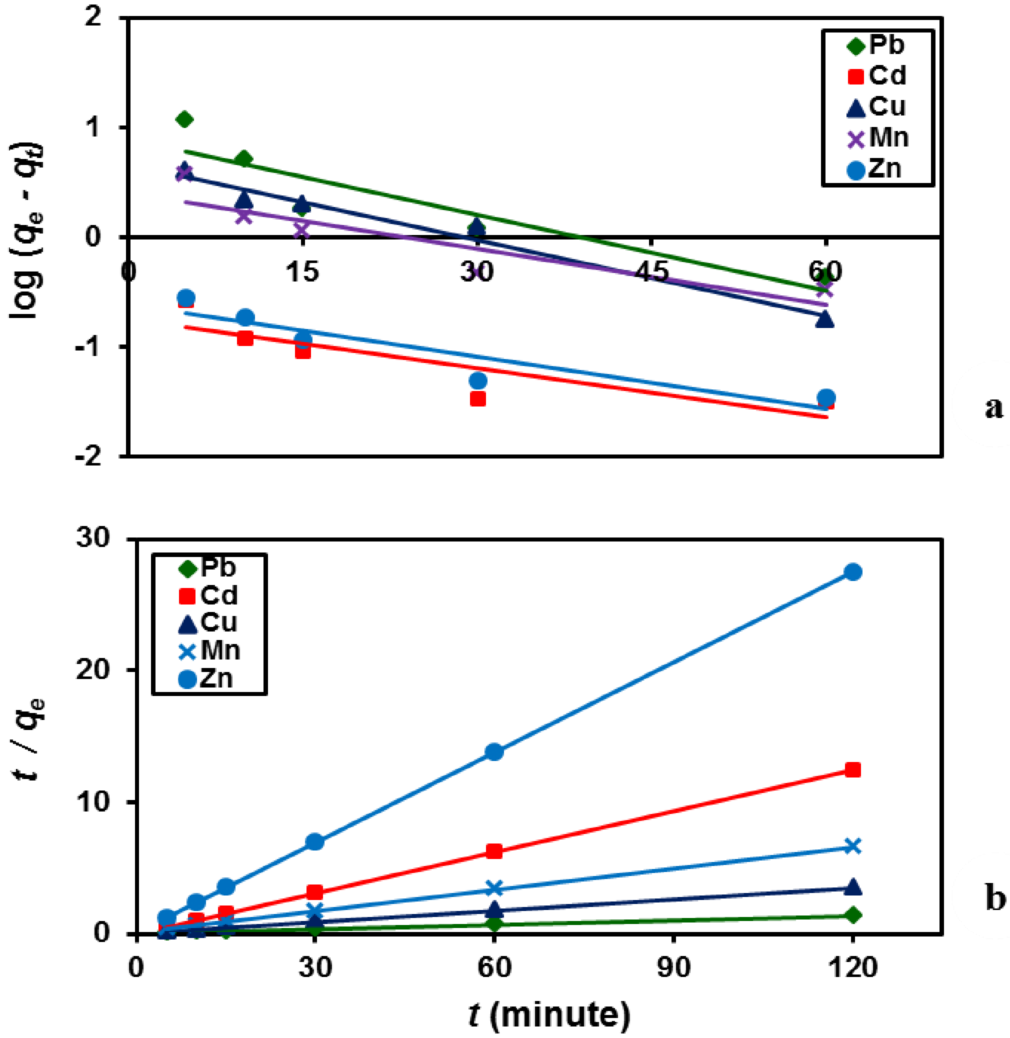


Fig. 22 Pseudo (**a**) first and (**b**) second order kinetics plot for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) adsorption by *S. polyrhiza*.

Table 12 Comparison of pseudo-first and pseudo-second order kinetic parameters for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption

Kinetic parameters	Metal ions				
	Pb (II)	**Cd (II)**	**Cu (II)**	**Mn (II)**	**Zn (II)**
Experimental value, q_e (mg g^{-1})	91.7	9.67	34.5	18.4	4.36
Pseudo first order model					
q_e cal.(mg g^{-1})	1.05	5.47	1.05	1.03	1.03
K_1 (min^{-1})	2.16	0.03	1.52	0.91	1.41
R^2	0.846	0.530	0.973	0.798	0.845
Pseudo second order model					
q_e cal. (mg g^{-1})	92.6	9.68	35.7	18.5	4.38
K_2 (g mg^{-1} min^{-1})	0.02	0.64	1.12	0.90	1.94
R^2	1.00	1.00	1.00	1.00	1.00

The efficiency of metal removal increased with contact time until equilibrium was obtained (120 min). The removal process involved two steps: a fast initial short phase (5-30 min) and a second longer step with slow adsorption of the metal ions until the equilibrium. The behaviour can be attributed to intercellular space adsorption of the heavy metal ions initially and onto the surface at the later stage of the adsorption process (Bhatti et al., 2009). Similarly, the correlation coefficient (R^2) values of the pseudo-first order were 0.846, 0.730, 0.973, 0.798 and 0.845 for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption. Also, the experimental q_e value was not agreed with the calculated value from the rate equation, supporting that the adsorption process followed a pseudo second order reaction. The results suggest that the rate limiting step for the adsorption process is probably chemisorption, involving valence forces through the sharing or exchange of electrons between the metal ions and the adsorbent, chelation, complexation and/or coordination (Febrianto et al., 2009). Further, it refers that both of the species, metal ions and adsorbent were involved in the adsorption phenomenon.

Adsorption isotherms

The equilibrium relationship between adsorbent and metal ions in the solution was explained using two isotherm models (Freundlich and Langmuir). The equilibrium studies were carried with concentrations of 100-160 mg L^{-1} for Pb (II), 10-40 mg L^{-1} for Cd (II), Cu (II) concentrations from 60-120 mg L^{-1}, 30-90 mg L^{-1} for Mn (II) and 5-35 mg L^{-1} for Zn (II), with fixed adsorbent dosage (0.1 g / 100 mL) at pH 4.0, 6.0, 5.0, 7.0 and 6.0, respectively.

The Langmuir isotherm assumes the uptake of metal ions on a homogenous surface by monolayer adsorption without any interaction between adsorbed ions (Ho and McKay, 2000).

The linear form of the equation is:

$$C_e / q_e = 1 / b\, q_{max} + C_e / q_{max} \quad (9)$$

where C_e (mg L^{-1}) is the equilibrium concentration, q_e is the amount of metal ion adsorbed at equilibrium, q_{max} is the maximum adsorption capacity and b is the equilibrium Langmuir constant.

The plots of C_e / q_e versus C_e gave straight lines with correlation coefficients (R^2) of 0.999, 0.995, 0.991, 0.977 and 0.995 for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) adsorption (**Fig.23** and **24**). The slopes and intercepts of the graphs were used to calculate the values of b and q_{max}. The calculated q_{max} agreed well with the experimental q_e values for the metal ions adsorption, indicating that the equilibrium data fitted well with the Langmuir model (**Table 13**). The results obtained conclude that Langmuir isotherm explains the equilibrium relationship of the adsorption process for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions better than Freundlich isotherm. The maximum adsorption capacities onto *S. polyrhiza* biomass were 137 for Pb (II), 36.0 mg g^{-1} for Cd (II), 52.6 mg g^{-1} for Cu (II), 35.7 mg g^{-1} for Mn (II) and 28.5 mg g^{-1} for Zn (II), at optimum parameters, pH 4.0, 6.0, 5.0, 7.0 and 6.0, temperature at 20^0 C and 30^0 C, biomass dosage of 0.1 g and 100, 10, 60, 30 and 5 mg L^{-1} initial concentrations of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions, respectively. The fitness of the data onto Langmuir model implies that the biomass has smooth surface with uniform energies and monolayer adsorption took place without any interaction between the adsorbed ions.

The Freundlich isotherm assumes a monolayer sorption with a heterogenous energetic distribution of active sites accompanied by interaction between adsorbed molecules (Liu and Liu, 2007).

The linear form of the equation is described by:

$$\log q_e = \log K_F + (1/n) \log C_e \tag{10}$$

where q_e (mg g^{-1}) is the amount of metal ion adsorbed at equilibrium by the adsorbent, C_e (mg L^{-1}) is the equilibrium concentration, K_F (mg g^{-1}) and n are constants representing the adsorption capacity and intensity of adsorption.

The graphs plotted with log q_e versus log C_e gave straight lines with correlation coefficients (R^2) of 0.976, 0.982, 0.969, 0.915 and 0.955 for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption (**Fig. 25** and **26**). The slopes and intercepts obtained were used to calculate n and K_F values, **Table 13**. The n values obtained from Freundlich model suggest heterogeneity of the adsorbent surface and indicate a favourable adsorption of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions onto *S. polyrhiza* biomass. The K_F values suggest that the metal binding affinity was in the order, Pb (II) > Cd (II) > Cu (II) > Mn (II) > Zn (II). However, the values of K_F when compared with the experimental q_e and the lower R^2 indicate that Freundlich model was not so adequate to describe the equilibrium relationship between the amount of sorbed metal ions and their equilibrium concentration in the solution.

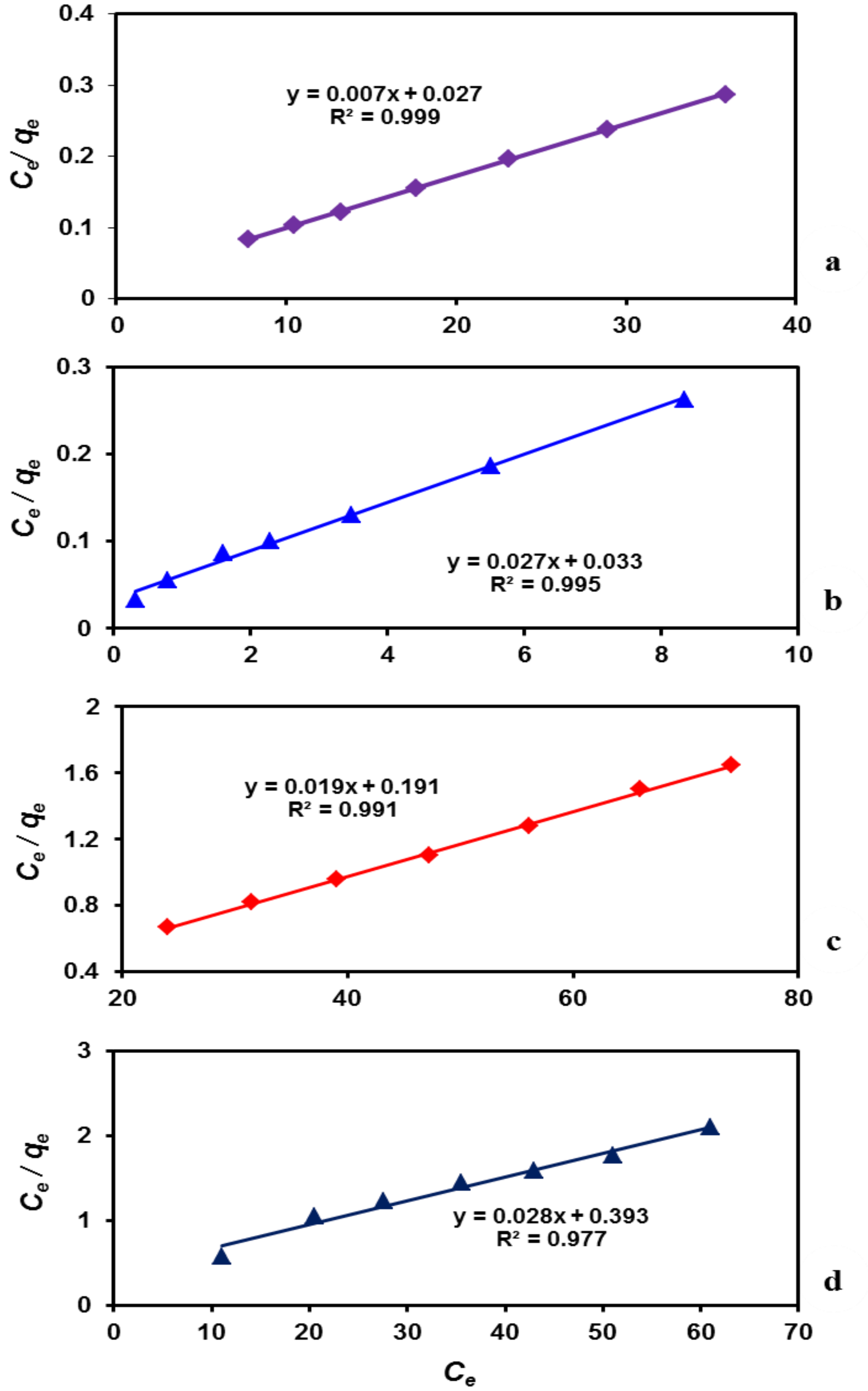


Fig. 23 Linearized Langmuir isotherm plots for (**a**) Pb (II), (**b**) Cd (II), (**c**) Cu (II) and (**d**) Mn (II) adsorption by *S. polyrhiza*.

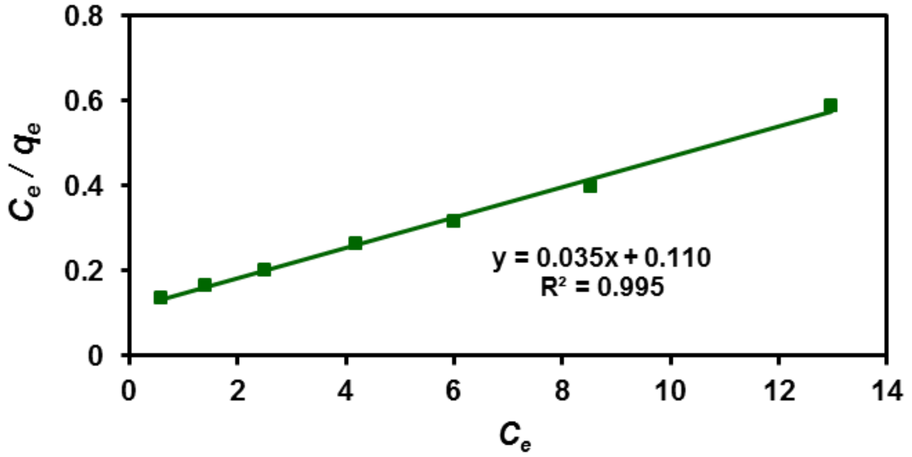


Fig. 24 Linearized Langmuir isotherm plots for Zn (II) adsorption by *Spirodela polyrhiza*.

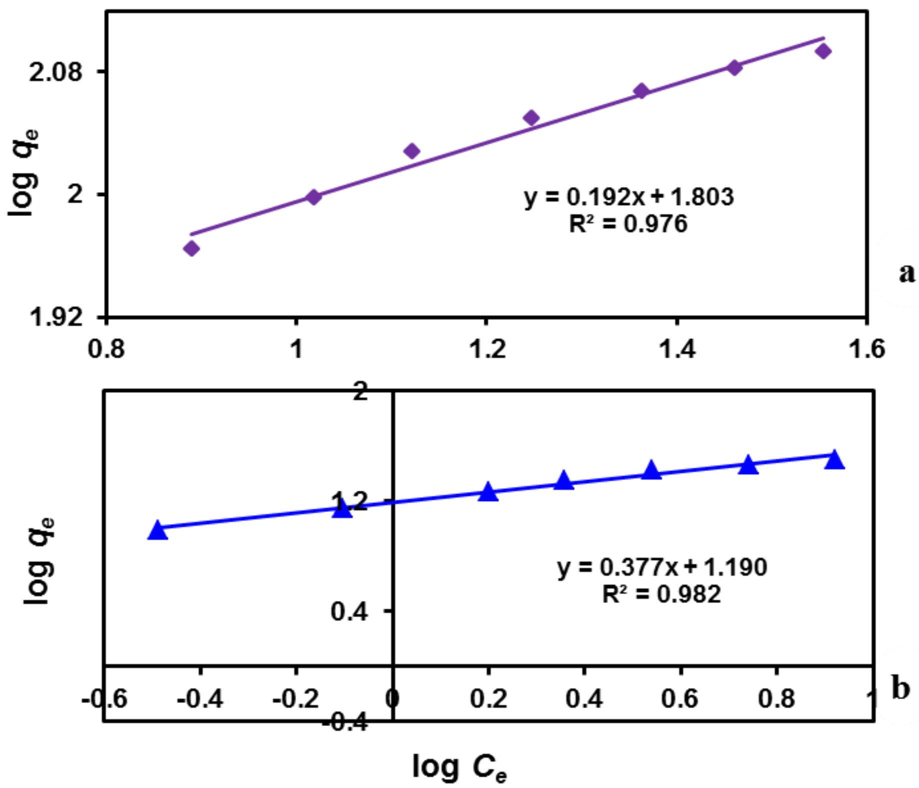


Fig. 25 Linearized Freundlich isotherm plots for (**a**) Pb (II) and (**b**) Cd (II) adsorption by *S. polyrhiza*.

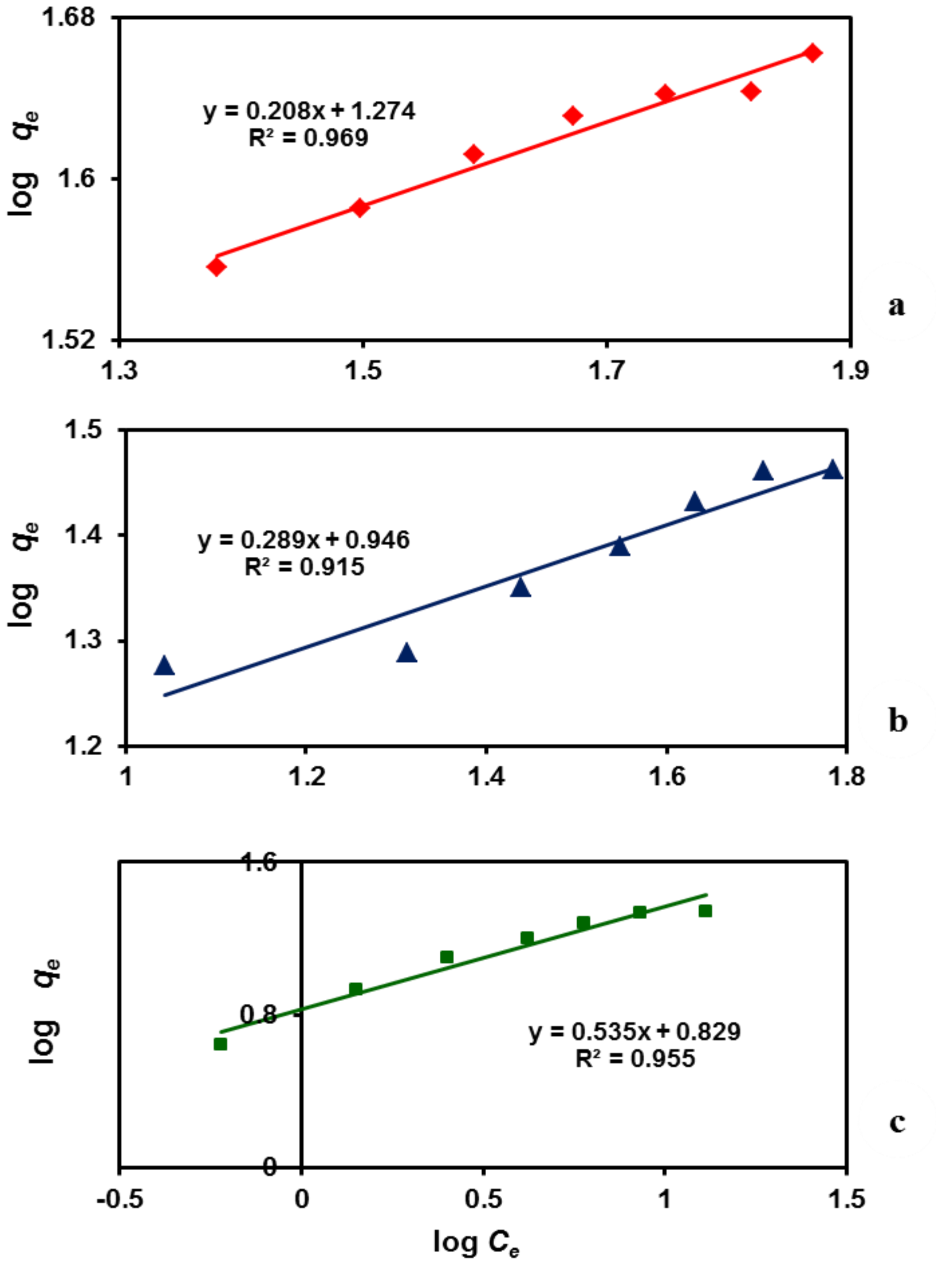


Fig. 26 Linearized Freundlich isotherm plots for (**a**) Cu (II), (**b**) Mn (II) and (**c**) Zn (II) adsorption by *S. polyrhiza*.

Table 13 Comparison of Freundlich and Langmuir isotherm parameters for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption

Isotherm parameters	Metal ions				
	Pb (II)	Cd (II)	Cu (II)	Mn (II)	Zn (II)
Experimental value, q_e (mg g^{-1})	124	31.7	64.5	29.0	22.0
Freundlich isotherm					
K_f (mg g^{-1})	63.6	15.5	52.6	35.7	28.5
n	5.20	2.6	0.09	0.06	0.31
R^2	0.976	0.982	0.991	0.977	0.995
Langmuir isotherm					
q_{max} (mg g^{-1})	137	36.0	52.6	35.7	28.5
b (mg^{-1})	0.26	0.82	0.09	0.06	0.31
R^2	0.999	0.995	0.991	0.977	0.995

The Langmuir isotherm was used to calculate a dimensionless constant called separation factor (R_L) describing the essential characteristics of the isotherm, **Fig. 27(a-d)**.

Separation factor (R_L) is calculated using the formula:

$$R_L = 1 / (1 + b\, C_i) \qquad (11)$$

where R_L is a dimensionless constant called separation factor, b is the Langmuir equilibrium constant and C_i (mg L^{-1}) is the initial concentrations of the metal ion.

The R_L values were used to predict the nature of the sorption system as given below:

R_L **value**	**Type of isotherm**
$R_L = 0$	Irreversible isotherm
$R_L = 1$	Linear isotherm
$R_L > 1$	Unfavourable isotherm
$0 < R_L < 1$	Favourable isotherm

The separation factor values decreased from 0.331-0096, 0.789-0.114, 0.289-0.116, 0.560-0.187 and 0.837-0.192 as the initial concentrations of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions increased from 100-160 mg L^{-1}, 10-40 mg L^{-1}, 60-120 mg L^{-1}, 30-90 mg L^{-1} for Mn (II) and 3-35 mg L^{-1}. The results suggest that adsorption of the metal ions increased with the increase of initial concentrations. Moreover, calculated R_L at different concentrations of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions fall within the range, $0 < R_L < 1$, revealing that the biosorption process is favourable at all the metal concentrations investigated.

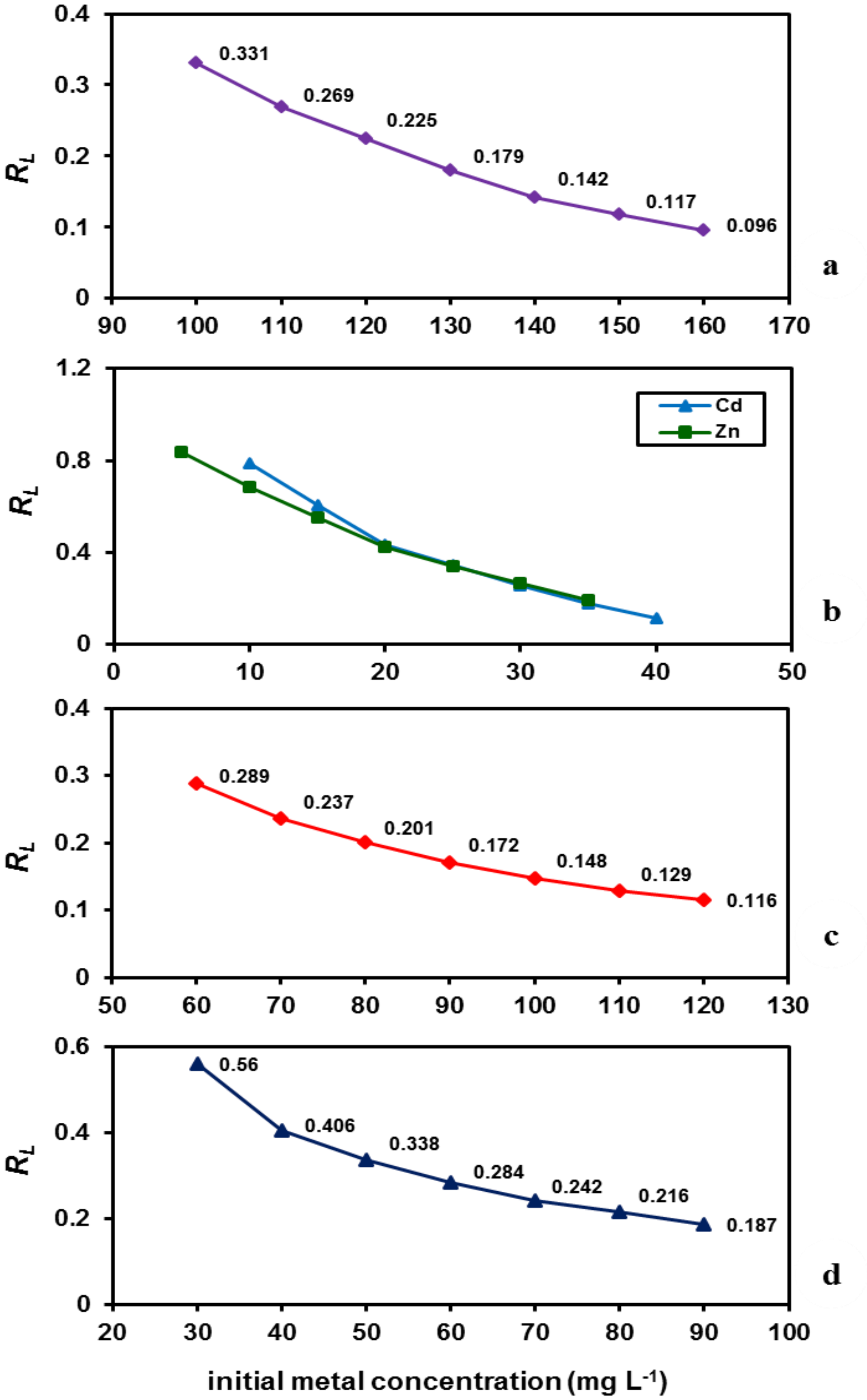


Fig. 27 Calculated separation factor for (a) Pb (II), (b) Cd and Zn (II), (c) Cu (II), and (d) Mn (II) adsorption by *S. polyrhiza*.

Adsorption thermodynamics

The thermodynamic parameters such as equilibrium constant K (q_e / C_e), change in Gibbs free energy (ΔG^o), enthalpy change (ΔH^o) and entropy change (ΔS^o) were calculated to determine the feasibility and rate of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) adsorption onto *S. polyrhiza* biomass, **Table 14**.

The equations are described by:

$$\Delta G^o = - RT \ln K \quad (12)$$

$$\ln (K_2 / K_1) = - \Delta H^o / R (1 / T_2 - 1 / T_1) \quad (13)$$

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad (14)$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is temperature (K).

The Gibbs free energy values were between - 5.729, - 5.232 and - 1.680 kJ mol^{-1} for Pb (II), - 8.272, - 7.909 and - 7.008 kJ mol^{-1} for Cd (II), - 0.199, - 0.342 and - 0.229 kJ mol^{-1} for Cu (II), - 0.316, - 0.445 and - 0.221 kJ mol^{-1} for Mn (II) and - 1.640, - 2.026 and - 1.542 kJ mol^{-1} for Zn (II) at 20, 30 and 40^o C, respectively. The negative values of Gibbs free energy change indicate that the thermodynamic process was spontaneous and feasible. The entropy change values were - 0.035, - 0.032 and - 0.020 J $mol^{-1}K^{-1}$ for Pb (II), - 0.034, - 0.033 and - 0.029 J $mol^{-1}K^{-1}$ for Cd (II), - 0.002, - 0.002 and - 0.002 J $mol^{-1}K^{-1}$ for Cu (II), - 0.030, - 0.029 and - 0.028 J $mol^{-1}K^{-1}$ for Mn (II) and - 0.005, - 0.017 and - 0.005 J $mol^{-1}K^{-1}$ for Zn (II) at 20, 30 and 40^o C, respectively. The negative values reveal decreased of randomness at the interface of the solid-solution during the adsorption of the metal ions on the active sites of the biosorbent.

Further, the negative value of enthalpy change, - 4.678 kJ mol^{-1} for Pb (II), - 1.978 kJ mol^{-1} for Cd (II), - 0.239 $kJmol^{-1}$ for Cu (II), - 8.550 kJ mol^{-1} for Mn (II) and - 3.189 $kJmol^{-1}$ for Zn (II) confirms that the metal adsorption process was exothermic in nature (Kumar et al., 2006b). The behaviour indicates that the adsorption of the heavy metal ions onto *S. polyrhiza* preferred lower temperature.

Table 14 Comparison of thermodynamic parameters such as change in Gibbs free energy (ΔG^o), enthalpy change (ΔH^o) and entropy change (ΔS^o) for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) adsorption

Thermodynamic parameters	Temperature	Metal ions				
		Pb (II)	Cd (II)	Cu (II)	Mn (II)	Zn (II)
ΔG^o (kJ mol^{-1})	293	-5.729	-8.272	-0.199	-0.316	-1.640
	303	-5.232	-7.909	-0.342	-0.445	-2.026
	313	-1.680	-7.008	-0.229	-0.221	-1.542
ΔS^o (J $mol^{-1}K^{-1}$)	293	-0.035	-0.034	-0.002	0.030	-0.005
	303	-0.032	-0.033	-0.002	-0.029	-0.017
	313	-0.020	-0.029	-0.002	-0.028	-0.005
ΔH^o ($kJmol^{-1}$)		-4.678	-1.978	-0.239	-8.550	-3.189

SEM, EDX and FTIR study of *S. polyrhiza* biomass

Fig.28 and **29** (inset) shows the SEM images of *S. polyrhiza* biomass before and after adsorption with Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions. The biomass has homogenous surface structure with deep pores, as observed in both control and treated biomass. The elemental composition analysis of the adsorbent and confirmation of the metal ions adsorption was done using EDX analysis, **Fig.28** and **29**. The spectra showed the presence of C (61.57 %), O (33.31 %), Ca (3.30 %), Mg (0.73 %), Si (0.36 %), K (0.27 %), Cl (0.25 %) and Al (0.21 %). The presence of the metal ions was revealed in the EDX spectra of the biomass after adsorption. On the contrary, the before adsorption biomass spectra did not show the characteristics Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions signal.

The functional groups involved in the adsorption of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions onto *S. polyrhiza* biomass were confirmed by FTIR spectral comparison of the adsorbent, before and after adsorption with the metal ions. The significant bands obtained from the spectra are presented in **Table 15**. The FTIR spectra were recorded in the range of 400-4000 cm^{-1} to find out the information regarding the bending vibrations and the stretching of the functional groups which are responsible for the adsorption process. The broad O-H band at 3391.65 cm^{-1} was shifted to 3402.47, 3394, 3386.17, 3375.34 and 3419.04 cm^{-1}, respectively in Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions treated biomass. It reveals the possible involvement of hydroxyl groups in the metal ions adsorption. The bands due to C-H, C=O and N-H stretching vibrations got shifted after metal adsorption.

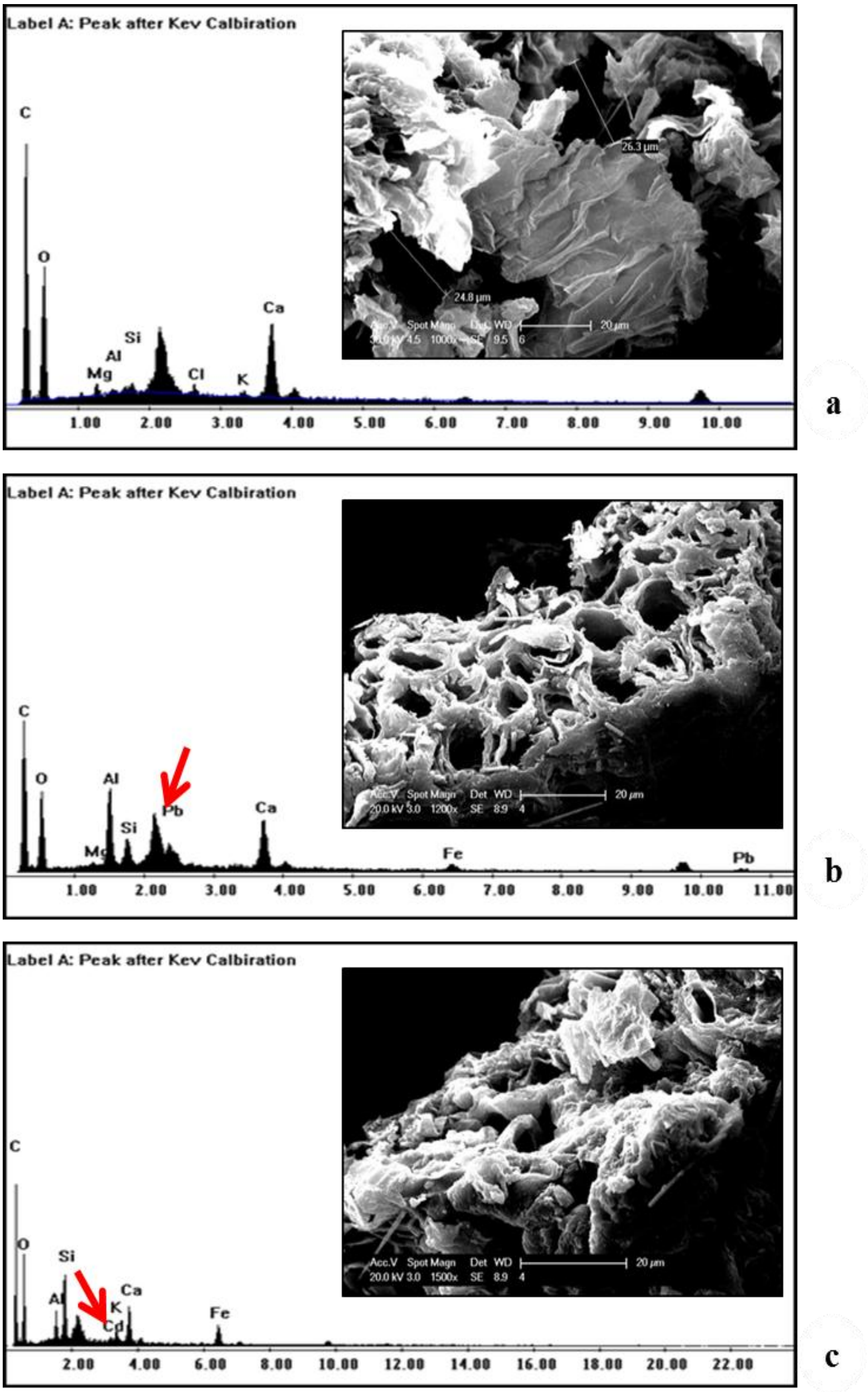


Fig. 28 SEM and EDX spectra of *S. polyrhiza* biomass, (**a**) before, and after adsorption with (**b**) Pb (II), and (c) Cd (II).

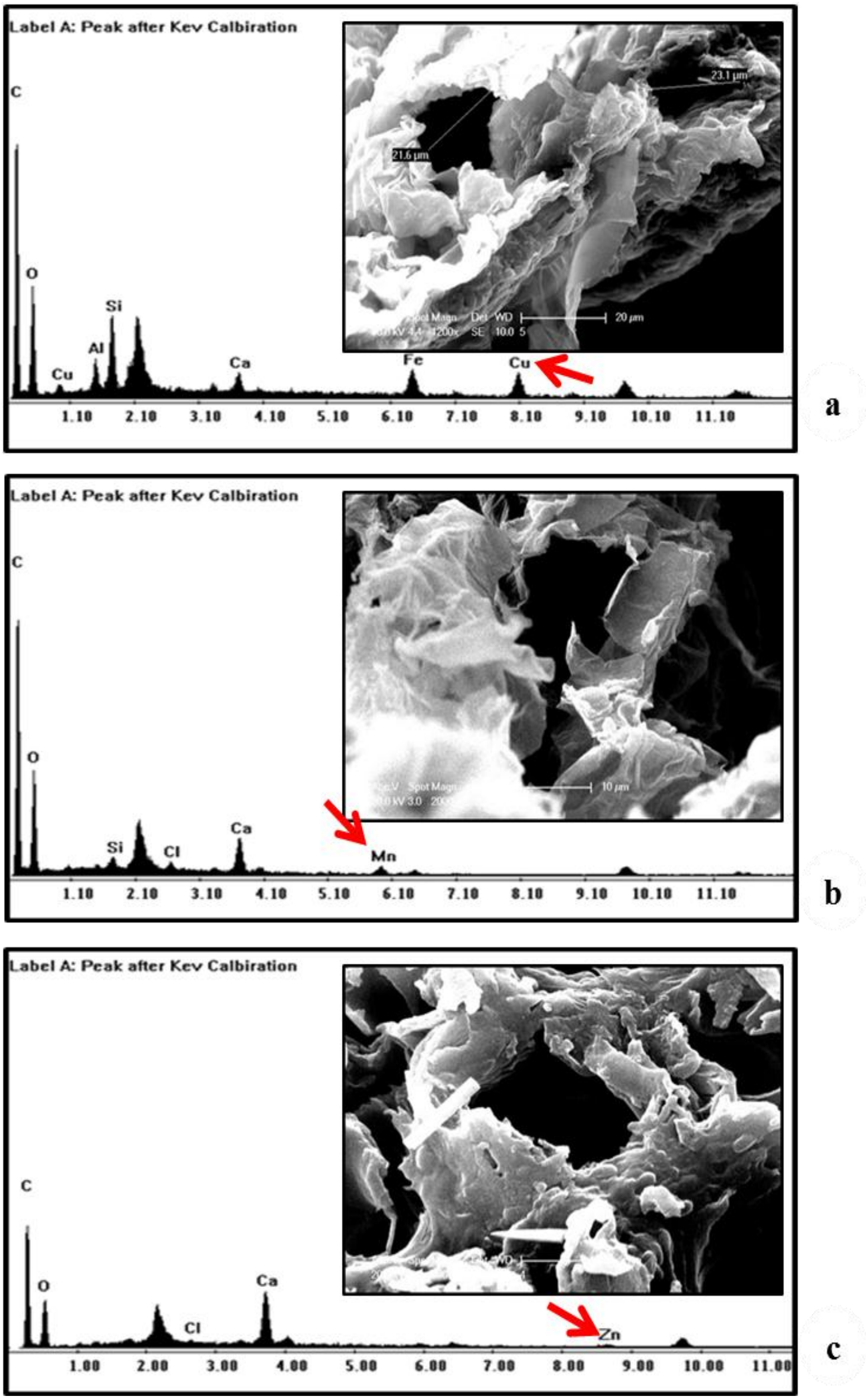


Fig. 29 SEM and EDX spectra of *S. polyrhiza* biomass after adsorption with (**a**) Cu (II), (**b**) Mn (II), and (**c**) Zn (II).

The peak located at 1320.51 cm^{-1} could be assigned to C-N stretching vibration in aromatic amine (Li et al., 2007). It has been shifted to 1314.44, 1319.30, 1320.51, 1315.07 and 1325.99 cm^{-1} after Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption. It reveals the possible involvement of aromatic amine in the metal adsorption. The FTIR spectra showed peaks at 3391.65, 2920.43, 1638.30, 1534.20, 1320.51, 1161.61 and 1046.55 cm^{-1} had shifted, respectively to 3402.47, 2920.20, 1648.74, 1539.14, 1314.44, 1155.51 and 1051.38 cm^{-1} and 3394.39, 2918.31, 1634.96, 1541.81, 1319.30, 1153.70 and 1060.56 cm^{-1} and 3386.17, 2920.43. 1638.30, 1534.20, 1320.51, 1156.13 and 1052.03 cm^{-1}, 3375.34, 2931.51, 1643.84, 1534.25, 1315.07, 1161.64 and 1035.62 cm^{-1} and 3419.04, 2920.44, 1649.26, 1539.68, 1325.99 and 1041.07 cm^{-1} in Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorbed biomass compared to control.

Table 15 Wave numbers (cm^{-1}) of possible functional groups obtained from FTIR analysis of *S. polyrhiza* biomass

Functional groups	***S. polyrhiza*, before and after adsorption**					
	Before	**Pb (II)**	**Cd (II)**	**Cu (II)**	**Mn (II)**	**Zn (II)**
-OH stretching vibration	3391.65	3402.47	3394.39	3386.17	3375.34	3419.04
C-H stretching vibration	2920.43	-	-	2920.43	2931.51	2920.44
C=O stretching vibration	1638.30	1648.74	1634.96	1638.30	1643.84	1649.26
-NH stretching vibration	1534.20	1539.14	1541.81	1534.20	1534.25	1539.68
C-N stretching vibration	1320.51	1314.44	1319.30	1320.51	1315.07	1325.99
C-O stretching vibration	1161.61	1155.51	1153.70	1156.13	1161.64	-
C-O stretching vibration	1046.55	1051.38	1060.56	1052.03	1035.62	1041.07

Metal ion adsorption from binary and ternary system

The wastewaters often comprises of more than a single metal ion. Presence of various metal ions is bound to cause interactive effects on each other, depending on the metal ion concentrations, competition of the metal ions for binding sites and the nature and amount of adsorbent dosage in the solution. **Figs. 30** and **31** shows the results for the adsorption of the metal ions from binary and ternary systems onto *S. polyrhiza* biomass. The adsorption of a metal was suppressed by the presence of the other metal ions in the binary and ternary solution. Pb (II) ion adsorption got reduced from 91.74% to 88.26, 86.8, 87.47 and 87.61% in the presence of Cd (II), Cu (II), Mn (II) and Zn (II), respectively in the binary system. Likewise, the adsorption was further reduced to 82.1% when all the 5 metals were present (Ternary system). Similarly, for Cd (II), 89.32, 90.15, 90.5 and 91.2% adsorption was found in the binary system, when compared to 96.76% adsorption for the single metal ion. 87.62% adsorption was observed in the ternary metal solution system. Cu (II) ion adsorption was reduced from 57.45 to 50.01, 53.6, 56.97 and 51.93% in the presence of Pb (II), Cd (II), Mn (II) and Zn (II) ions in the binary system. In the ternary system, Cu (II) adsorption was reduced to 46.54%. The values indicate that there was no significant suppression in the magnitude of Pb (II), Cd (II) and Cu (II) ion uptake ions in the binary system. However, suppression was more in the ternary system, when all the 5 metal ions were available. Similar trend was observed in Zn (II) ions adsorption in the binary system with Pb (II), Cd (II), Mn (II) and Cu (II) ions. The adsorption rate reduced to 78.46, 79.96, 79.74 and 77.86% compared to 87.30% in single metal solution. It reveals the inhibitory effect of one metal ion binding on the other metal ion.

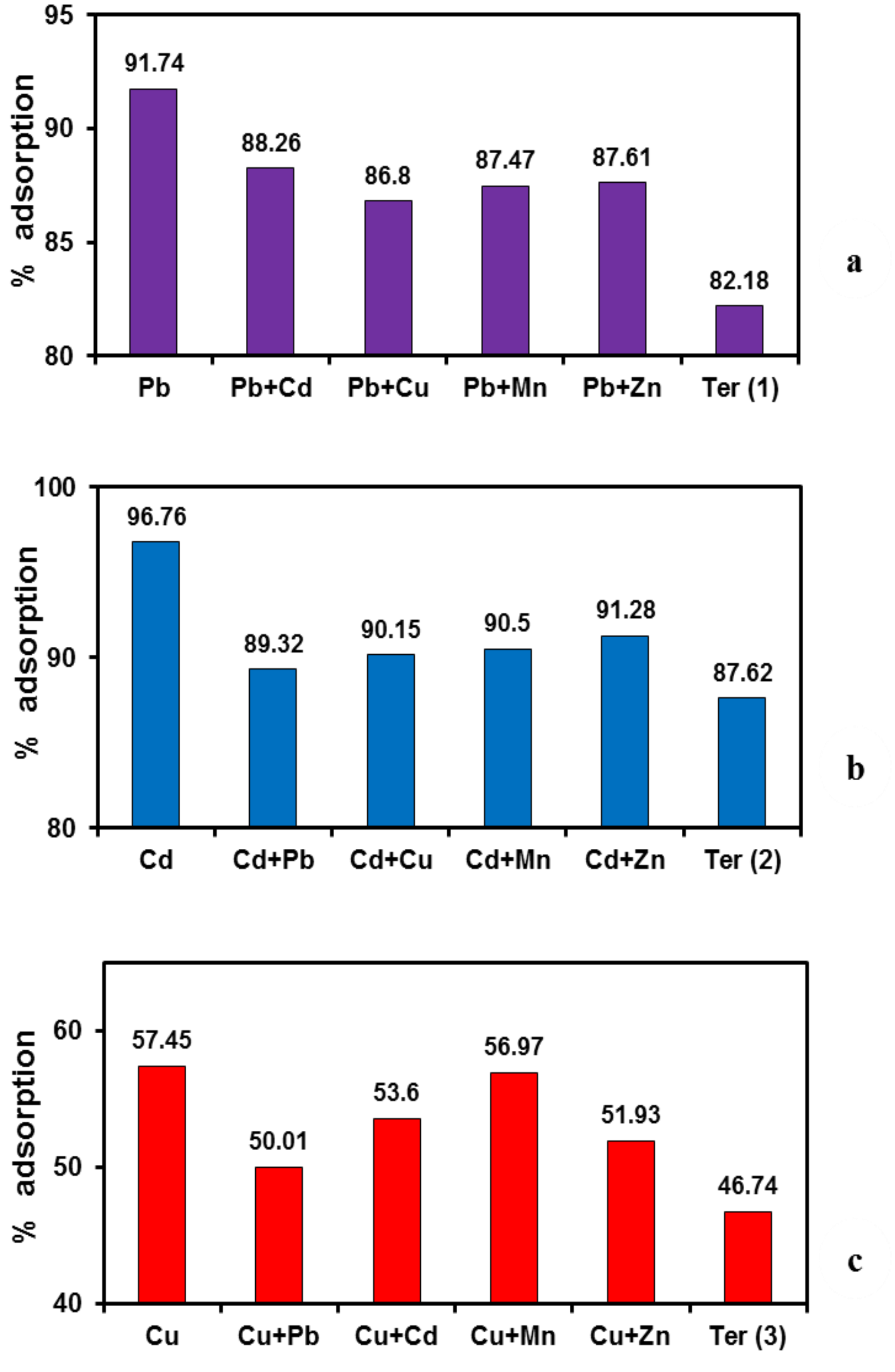


Fig 30 (**a**) Pb (II), (**b**) Cd (II) and (**c**) Cu (II) removal from binary and ternary system by *S. polyrhiza* biomass. (Notes: Ter (1): Pb+Cd+Cu+Mn+Zn; Ter (2): Cd+Pb+Cu+Mn+Zn; and Ter (3): Cu+Pb+Cd+Mn+Zn)

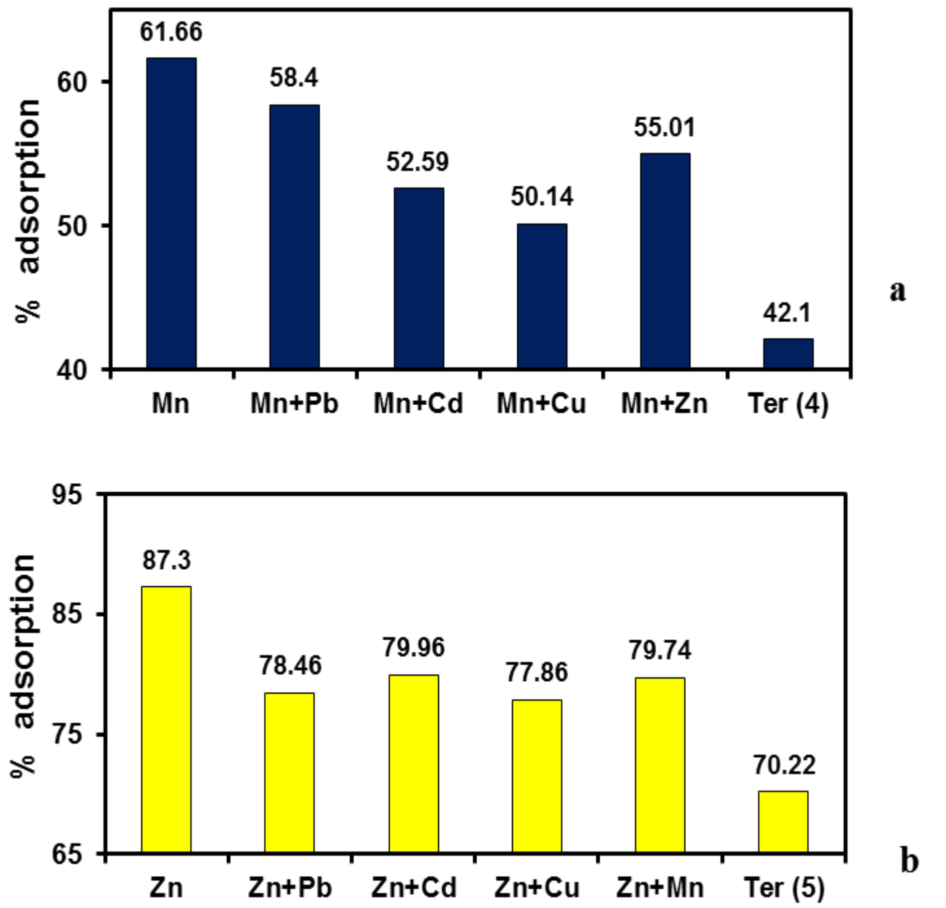


Fig. 31 (**a**) Mn (II), and (**b**) Zn (II) removal from binary and ternary system by *S. polyrhiza* biomass. (Notes: Ter (4): Mn+Pb+Cd+CuZn; and Ter (5): Zn+Pb+Cd+Cu+Mn)

The maximum inhibition was seen in ternary system with 70.22% adsorption of Zn (II) ions. Similarly, there was greater suppression of metal uptake in the ternary system for Mn (II), with 42.10% adsorption compared to 58.4, 52.59, 50.14 and 55.01% in the binary system (Cu+Pb, Cu+Cd, Cu+Mn and Mn+Zn). The decreased adsorption of the heavy metal ions in binary and ternary systems is due to increase in the electrostatic repulsion (screening effect) among the cations that would limit the adsorption of the metal ions of our interest. The interactions between the metal ions and that between the biomass resulted into the competition for the active sites for adsorption onto the surface of *S. polyrhiza* biomass. All of the above reasons led to lower efficiency of the specific metal ions to adsorb onto the biomass in the binary and ternary combinations compared to single metal solution. Further, the experimental findings suggest that *S. polyrhiza* biomass has the potential of application when simultaneous removal of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions is to be done at low initial concentrations of the desired metal ions.

Metal ions desorption

The adsorption phenomenon may be due to physical bonding, ion-exchange or combinations of both. If the mechanism is ion-exchange or chemical bonding, desorption of the metal ions from the adsorbent could be done using strong adsorbents like acid or alkali solutions. On the other hand, if it is physical bonding then the metal ions can be desorbed by deionised water. During the desorption of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions from *S. polyrhiza* biomass using 0.1 M HCl, 73.56, 52.83, 80.53, 42.88 and 93.28% recovery were achieved for the metals compared to 13.21, 11.72, 12.11, 8.70 and 15.72% using deionised water (DW). The results indicate that the adsorption process is due to chemical or ion-exchange mechanism between the metal ions and adsorbent.

Comparison with other low-cost adsorbents and cost-effectiveness of S. polyrhiza biomass

The adsorption performance of *S. polyrhiza* biomass for Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions were compared with other low-cost adsorbents, **Table 16**. The comparison shows that *S. polyrhiza* biomass is an effective adsorbent for the removal of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions from aqueous solutions. The shift and reduction of the peaks explain the interaction of the metal ions with the functional groups on the biomass during the adsorption process. The main functional groups involved are hydroxyl, amino and carboxyl groups. The functional groups mentioned are highly reactive and deprotonated during the adsorption of the metal ions, Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions. The maximum adsorption capacities obtained clearly depict its superiority when compared to some of the adsorbents reported, such as *Spirogyra insignis* (Romera et al., 2007), *Alternanthera philoxeroides* (Wang and Qin, 2006), *Botrytis cinerea* (Tunali and Akar, 2006), *Ceratophyllum demersum* (Keskinkan et al., 2004), *Fontinalis antipyretica* (Martins et al., 2004), *Myriophyllum spicatum* (Keskinkan et al., 2003), *Salvinia herzogii* (Schneider and Rubio, 1999), and *Eichhornia crassipes* (Schneider and Rubio, 1999) etc. The variations in the metal uptake by the adsorbents are attributed to its different structure, functional groups and surface area. Other than the properties of the adsorbents, the parameters such as pH, temperature, and the presence of competing cations (binary and ternary solution system) would also influence the degree of adsorption onto an adsorbent to a certain extent.

Table 16 Pb (II), Cd (II) , Cu (II), Mn (II) and Zn (II) ions sorption capacities of various adsorbents reported in literature and *Spirodela polyrhiza* biomass (Present study)

Adsorbent	Metal ion	pH	q_{max} (mg g^{-1})	References
Eichhornia crassipes	Pb	-	26.32	Mahamadi et al., (2010)
Ceratophyllum demersum	Pb	-	44.80	Keskinkan et al., (2004)
Myriophyllum spicatum	Pb	-	46.40	Keskinkan et al., (2003)
Laminaria hyperborea	Pb	5.0	50.30	Freitas et al., (2008)
Ulva lactuca	Pb	5.5	54.30	Areco et al., (2012)
Spirodela polyrhiza	**Pb**	**4.0**	**137.0**	**Present study**
Eichhornia crassipes	Cd	-	12.60	Mahamadi et al., (2010)
Cystoreia indica	Cd	5.5	19.56	Montazer-Rahmati et al., (2011)
Fontinalis antipyretica	Cd	5.0	28.00	Martins et al., (2004)
Laminaria hyperborea	Cd	5.0	31.30	Freitas et al., (2008)
Spirodela polyrhiza	**Cd**	**6.0**	**36.0**	**Present study**
Ceratophyllum demersum	Cu	6.0	6.17	Keskinkan et al., (2004)
Myriophyllum spicatum	Cu	-	10.37	Keskinkan et al., (2003)
Salvinia herzogii	Cu	-	19.70	Schneider and Rubio, (1999)
Eichhornia crassipes	Cu	-	23.10	Schneider and Rubio, (1999)
Spirodela polyrhiza	**Cu**	**5.0**	**52.60**	**Present study**
Oryza sativa	Mn	-	7.70	Krishnani et al., (2008)
Physalis philadelphica	Mn	6.0	15.22	Garcia-Mendieta et al., (2012)
Saccharomyces cerevisiae	Mn	5.0	18.95	Parvathi et al., (2007)
Aspergillus niger	Mn	11.0	19.34	Parvathi et al., (2007)
Spirodela polyrhiza	**Mn**	**7.0**	**35.70**	**Present study**
Botrytis cinerea	Zn	-	12.98	Tunali and Akar, (2006)
Salvinia herzogii	Zn	-	18.10	Schneider and Rubio, (1999)
Alternanthera philoxeroides	Zn	6.0	18.57	Wang and Qin, (2006)
Eichhornia crassipes	Zn	-	19.20	Schneider and Rubio, (1999)
Spirodela polyrhiza	**Zn**	**6.0**	**27.50**	**Present study**

In addition, the total amount of metal ions anchored onto the biomass surface will depend on the number of active sites present and how easily they can be assessed (Hasim and Chu, 2004). The cost of a particular adsorbent is an important parameter to be discussed while choosing an adsorbent. *S. polyrhiza* biomass is abundant in the *phoomdi* of Loktak lake, India and distributed worldwide in the freshwater habitats (Davidson and Simon, 1981), making the biomass freely available for future practical applications (No data on economic exploitation of *S. polyrhiza*).

Conclusions

The *phoomdi* macrophyte *S. polyrhiza* possessed a good Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption quality. The adsorption efficiency of the heavy metal ions was found to be significantly higher as compared to reported biomasses (Table 5). Maximum adsorption capacities of 137 mg g^{-1} for Pb (II), 52.6 mg g^{-1} for Cu (II), 36.0 mg g^{-1} for Cd (II), 35.7 mg g^{-1} for Mn (II), and 28.5 mg g^{-1} for Zn (II), respectively were achieved at the optimized conditions. Hydroxyl, amino and carboxyl groups were involved in the adsorption of the metal ions. The sequence of affinities between the biomass and the metal ions was as follows: lead > copper > cadmium > manganese > zinc. The multi-metal effect on the adsorption process showed antagonistic nature due to screening effect and competition of the metal ions for the active sites on the biomass. The results provided a new, low-cost and effective adsorbent of Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions. The study explored a new phytotechnological application of the *phoomdi* macrophyte, *S. polyrhiza*. It paved a way towards sustainable utilization of the biomass, floating in Loktak, and led to the generation of a useful by-product in the form of an adsorbent for the restoration of degraded aquatic environments contaminated with heavy metal ions.

Objective 5

Silver nanoparticle synthesis mediated by *Hedychium coronarium* (L.) Koenig., *Spirodela polyrhiza* (L.) Schleiden and *Trapa natans* L. extracts: Application for dye photodegradation

Introduction

Nanotechnology can be defined as “the understanding and control of matter at dimensions that are near to 1 to 100 nanometers, where the unique phenomena enable novel applications” (NNI, 2007). The technology has been applied in electronics, food industry, textiles, cosmetics, bio sensing, material sciences, pharmaceuticals, biomedical, agriculture, energy and environmental pollution control (Chen et al., 2006; Maynard et al., 2006). At present, the nanoparticles are manufactured by physical and chemical methods. The chemical methods uses reducing agents (sodium borohydride, hydroxylamine methoxypolyethylene glycol and potassium bitartarate), protecting agents (polyvinyl pyrollidone), stabilizers (2-vinylpyridine telomers and citric acid) (Metlzer et al., 2001; Tan et al., 2003; Fukuoka et al., 2004; Mallick et al., 2004; Liu et al., 2009). Physical methods involve sonication, laser ablation, chemical vapor deposition, ultra violet irradiation and aerosol technology (Kammler et al., 2001; Kim et al., 2007; Yu et al., 2007). These methods, however, use toxic and dangerous solvents that are to some extent sources of health and environmental concerns. These arise, the need for eco-friendly methods for the synthesis of nanoparticles that are of potential industrial applications.

At present, a variety of research has been carried out to investigate the bioreduction potential of various biochemicals in natural and renewable materials such as microbes (Shankar et al., 2003; Yang et al., 2010) and plant extracts (Mukherjee et al., 2001; Yong et al., 2002; Liu et al., 2004) to reduce metals ions (e.g. Ag, Au, Pd and Pt) into zero valence nanoparticles. The biological process involves the three main steps, viz. solvent medium selection, environmental benign reducing agent selection and non-toxic substances for nanoparticle stability selection (El-

Shishtawy et al., 2011). The bioreduction solutions consist of natural capping agents that act to hinder the aggregation of synthesized metal nanoparticles and help controlling the particle sizes. The components in the plant extracts such as reducing sugars, terpenoids, proteins and other phenolic compounds were found responsible for the reduction of the metal ions into nanoparticles. The reduction mechanism, however, is very complicated and the contribution and availability of the reducing agents vary from one species to the other. At present, there has been an extensive exploitation of various plant parts such as leaf, flower, bark or tuber for the synthesis of silver nanoparticles (Sathishkumar et al., 2009; Ghosh et al., 2012; Gude et al., 2012; Hong et al., 2013). The use of various plant parts extracts can be advantageous over other biological processes such as microbial route, as it eliminates the elaborate process of cell culture maintenance and can be suitably scaled for large scale synthesis of nanoparticles. Silver nanoparticles possess unique electrical, optical as well as biological properties and therefore, are applied in bio sensing, catalysis, drug delivery, nano device fabrications and in medicine (Jain et al., 2008).

Dyes are synthetic organic compounds released from various paper, plastic, rubber, leather, food, textile, cosmetics and pharmaceutical industries (Kulkarni et al., 1985) and contribute significantly in environmental pollution. Azo dyes are considered as potent carcinogens and as per records, 2% of dyes that are produced from various industries are directly discharged in aqueous effluent (Rauf et al., 2009). There arise the need to develop novel treatment methods that effectively remove dyes from wastewater. The dye effluents are normally resistant to the actions of the microorganisms, rendering the reduction using conventional biological treatment ineffective and in addition during high effluent concentration, they are resistant to destruction by physico-chemical treatments (Vidhu and Philip, 2014). In recent years, the filed application of nanotechnology has been extended to wastewater treatments as high surface of the nanoparticles exhibits an enhanced activity (Kang et al., 2010).

In 2005-2006, Imphal bazaar received a total volume of 68.63 tons of edible wetland plants involving a business of more than Rs.9,07,778, while Bishnupur bazaar received 20.18 tons with a trade of Rs. 2,41,000 (Jain et al., 2011). Of the total, *Hedychium coronarium* J. Koenig and *Trapa natans* L. accounted for 2.5 and 1.35 tons with revenues of Rs. 60,020 and Rs.13,475 respectively. The wild edibles were harvested mainly from Loktak, with the primary sources being the *phoomdi*. *Hedychium coronarium* rhizomes are edible and have healing properties while *T. natans* fruits are commonly consumed for its rich carbohydrate content. However, the shoot of *H. coronarium* and fruit coat of *T. natans* does not serve any economic purpose and are being treated as agricultural waste. In addition, *Spirodela polyrhiza*, an abundant *phoomdi* species does not have any applications for the locals. Huge quantity of these waste are being discarded without making good use of it. In this study, as a part of the quest for green synthesis of AgNPs and to get an economical and hazard free method of disposing the waste generated from *H. coronarium*, *S. polyrhiza* and *T. Natans,* we report the first synthesis of AgNPs using the shoot, the whole plant and fruit coat extracts as both the reducing and stabilizing agent, **Fig. 32(a-c)**. The present study highlights: (1) the methods employed in the synthesis of Ag nanoparticles using the three *phoomdi* species extracts; (2) characterization of the nanoparticles synthesised; and (3) application of the nanoparticles in photocatalytic degradation of dyes (methylene blue, amido black, methyl orange and brilliant blue green) under visible light irradiation.

Fig. 32 (**a**) *Hedychium coronarium* shoot, (**b**) *Spirodela polyrhiza* whole plants and (**c**) *Trapa natans* fruit coat.

Materials and methods

Materials

Silver nitrate, sodium borohydride, methylene blue, amido black, methyl orange and brilliant blue green used in the study were of analytical grades and were purchased from Aldrich chemicals. Healthy, disease free shoots of *Hedychium coronarium* J. Koenig, *Spirodela polyrhiza* (L.) Schleiden whole plants, and *Trapa natans* L. fruit coats were collected during the month of November in 2013 from the *phoomdi* of Loktak lake, Manipur.

Extract preparation

The collected plant parts were washed thoroughly in tap water and then rinsed with distilled water until no foreign materials remained. They were oven dried for 48 hours at 60°C until a constant weight was achieved. The dried samples were powdered in Clotech 1093 sample mill, and then sieved to get a fraction of 100 mesh screen and stored in air tight containers. The plant extracts were prepared by taking 5 g of the plant powder in 500 mL Erlenmeyer flask along with 100 mL of distilled water and then boiling the mixture for 20 min. Further, the extracts were filtered with Whatman No.1 filter paper. The filtered extracts were stored in refrigerator at 4°C for further studies.

Reduction of Ag ions

In order to investigate the reduction potential of the plant extracts, 5 mL of *H. coronarium* shoot, *S. polyrhiza* whole plant and *T. natans* fruit coat broths were added to 100 mL of 10^{-3} M silver nitrate solution. The solutions were incubated for 36 hours (for *H. coronarium* and *S. polyrhiza*) and 2 hours (for *T. natans*) at room temperature at dark. The bioreduction of silver ions was monitored at 15 min intervals by sampling aliquots of the reaction mixture. Reduction of the silver nanoparticles was observed by change in the colour of the reaction mixture during the treatment. The experiments were carried out at different pH values 2.0 to 9.0, extract concentrations from 1 to 5 mL, salt concentrations from 0.5 to 4 10^{-3} M and temperature from 5 to 45°C, respectively.

Characterization of nanoparticles

The bioreduction of silver ions into Ag nanoparticles by the plant extracts were characterized using a UV-vis spectrophotometer (GBC Cintra 5, Australia) operated between 300 to 650 nm and 10^{-3} M $AgNO_3$ solution was used for the baseline correction. For EDX analysis, the fully reduced solution was centrifuged at 10,000 rpm for 10 min, the supernatant liquid was discarded and the pellet obtained was redispersed in deionized water. The centrifugation process was repeated 3 to 4 times to wash off any adsorbed substances on the surface of the silver nanoparticles. The purified AgNPs were analysed for the elemental composition using a scanning electron microscope (XL30 ESEM, Philips, USA). For XRD studies, the dried nanoparticles were coated on XRD grid and the spectra were recorded using Philips PW 1830 X-ray generator operated at a voltage of 40 kV and a current of 30 mA with Cu Kα1 radiation in θ-2θ configurations. The FTIR spectra of the aqueous plant extracts and the purified AgNPs were analyzed by a FTIR spectrophotometer (NICOLET 5700-FTIR) within the range of 400-4000 cm^{-1}. Further, the size of the nanoparticles is confirmed by using TEM analysis (TECNAI G^2-STWIN 5021/20).

Photodegradation of dyes

The photocatalytic activity of the AgNPs was investigated by measuring the ability of the synthesised NPs to degrade the dyes (methylene blue at 665nm, amido black at 620 nm, methyl orange at 465 nm and brilliant blue green at 610 nm) under visible light. Five reactions were carried out 100 mL Erlenmeyer flasks and the absorbance values were recorded using UV-vis spectrophotometer. In the first flask, 1 mL of dye (1×10^{-4} M) was mixed with 9 mL of distilled water. In second reaction, 1 mL of dye (1×10^{-4} M) was mixed with 1 mL of extracts and 8 mL of distilled water. In third reaction, 1 mL of dye (1×10^{-4} M) was mixed with 1 mL of $NaBH_4$ and 8 mL of distilled water. In fourth reaction, 1 mL of dye (1×10^{-4} M) was mixed with 1 mL of AgNPs and 8 mL of distilled water. In the fifth reaction, 1 mL of dye (1×10^{-4} M) was mixed with 1 mL of AgNPs, 1 mL of $NaBH_4$ and 7 mL of distilled water. One mL of the reaction mixture was removed and analysed using UV-vis at different time intervals viz., 5, 15, 30, 45, 60 and 120 min and then returned to the solution. The percentage degradation was calculated by following the equation:

$$\text{Degradation (\%)} = (A_i - A_t) / A_i \times 100 \qquad (15)$$

where A_i is the absorbance of blank (control, only dye solution exposed to visible light) and A_t is the absorbance of test (dye solution with NPs exposed to visible light). Similarly, another set of the reaction mixture five was kept at dark condition to analyse the adsorption of dyes onto the NPs synthesised.

Data analysis

All the experiments were carried out in triplicates and mean data is presented. The data obtained were statistically evaluated using functions of Microsoft Excel 2010 (version Office Windows 7, Microsoft Corporation, USA).

Results and discussion

Reduction of Ag ions into AgNPs by the plant extracts

As shown in **Fig. 33(a-c)**, the reaction mixture consisting of silver nitrate and *H. coronarium*, *S. polyrhiza* and *T. natans* extracts showed change in the colour range from clear to deep red as the reaction proceeds. Within 30-60 min for *H. coronarium* and *S. polyrhiza* and 15-30 min for *T. natans*, an orange colour was observed that changed to deep red by 2160 min for *H. coronarium* and *S. polyrhiza* and 120 min for *T. natans*. The colour of a particular silver nanoparticle solution indicates the particle size due to the changes in the surface plasmon resonance (SPR) band of the synthesised particles. The SPR resulted because of the interaction of the metal nanoparticles with light resulting in the collective oscillation of the conduction electrons on the metal surface (Vidhu and Philip, 2014). **Fig. 34(a-c)** confirms the change in the SPR band through UV-vis spectroscopy. The SPR bands are influenced by shape, size, morphology, composition and dielectric environment of the reduced nanoparticles (Kelly et al., 2003). From **Fig. 34(a-c)**, it is observed that the characteristics SPR band of the silver nanoparticles synthesised using the plant extracts is at 420 nm. As per literature, the band at 420 nm is due to the longitudinal plasmon vibrations (Shankar et al., 2003), with the broad band indicating a wider particle size distribution (Prathna et al., 2011). With time, the intensity of the band at

Fig. 33 Reduction of silver by (**a**) *H. coronarium* shoot, (**b**) *S. polyrhiza* whole plants and (**c**) *T. natans* fruit coat extracts.

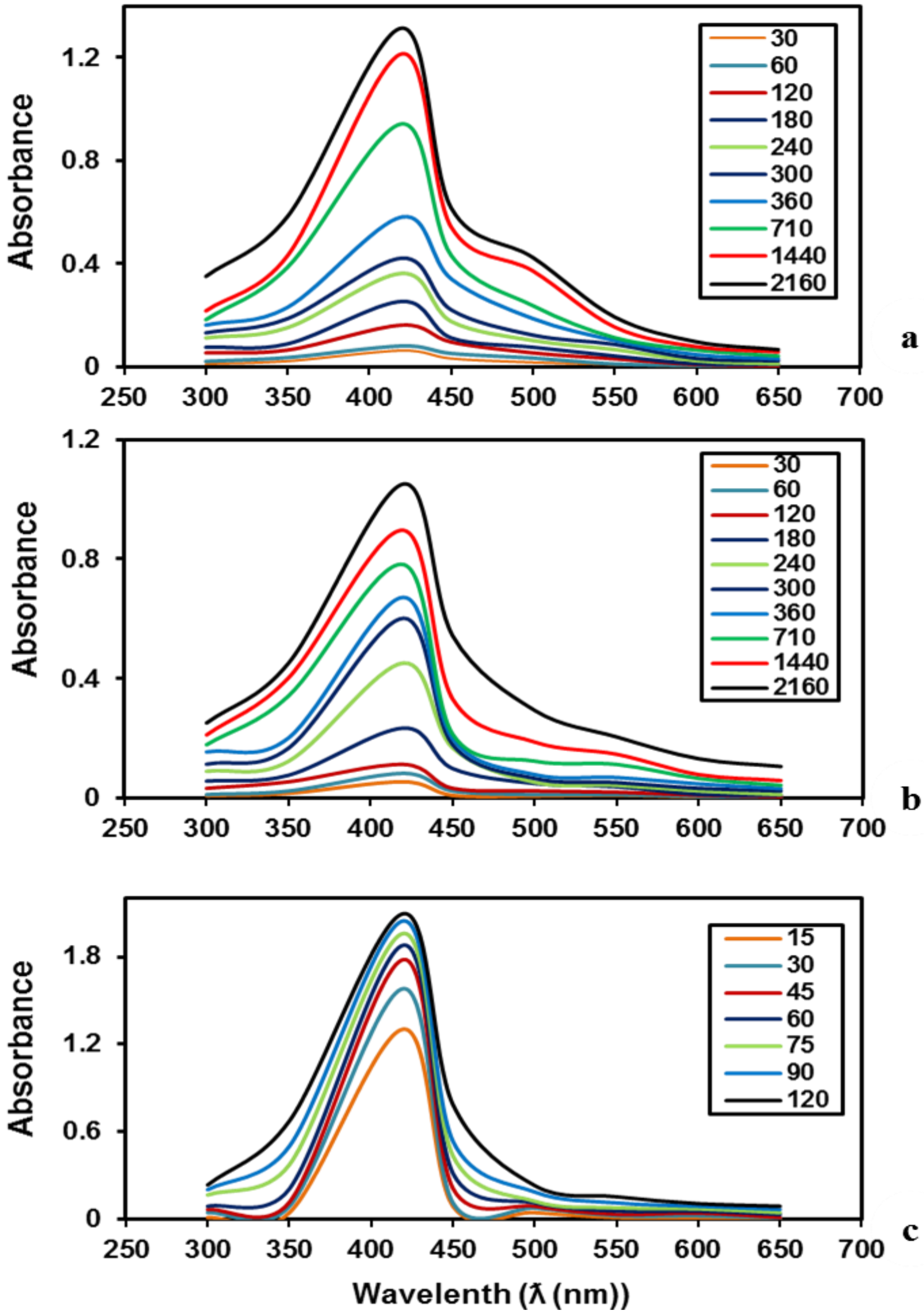


Fig. 34 Time course of silver nanoparticles formation with 1 mM $AgNO_3$ using, (**a**) *H. coronarium*, (**b**) *S. polyrhiz*a, and (**c**) *T. natans* at pH 5.0; temperature, 25 $^{\circ}$c; extract conc., 5 mL.

420 nm increases showing the rapid nature of reduction by the plant extracts. This shows that the plant extracts used are efficient for reduction of the silver ions.

Fig. 35(a-c) shows the effect of initial pH on the silver nanoparticles formation by UV-vis spectroscopy, with pH ranged from 2.0 to 11.0. At higher pH (5-11), intense dark red colour solution mixture with intense peaks having higher absorbance values were measured (data not shown). The peaks got shifted to shorter wavelengths and became narrower with increased pH of the reaction mixture. The reason may be due to decreased size or anisotropy degree of the silver particles (Mie, 1908; Noguez, 2007). These can be due to the dissociated state for capping functional groups of the plant extracts got influenced by the pH of the reaction mixture. As the pH increases, the deprotonation of the capping functional groups are more strengthened. The deprotonated functional groups carrying negative charges bind with the silver nanoparticles and enhanced the stability due to the electrostatic repulsion. Thus, high pH values, viz. 11.0 is referred for the formation of mono-dispersed and spherical nanoparticles while no characteristics peak formation at lower pH may be due to the inactivation of the reducing functional groups under high acidic conditions (Yang and Li, 2013). The effect of different extract concentrations can be seen in **Fig. 36(a-c)**. It was observed that with the increased in the concentrations of the extracts from 1.0 to 5.0 mL, the absorption peaks got shifted from shorter to longer wavelengths, the peaks got broadened up and the absorbance increased. It showed the characteristics nature of increased in particle size which is resulted because of reducing agents bound to the surface of preformed nuclei that intensifies the secondary reduction of silver ions on the surface of the nuclei. This will lead to increased growth rate of nanoparticles and leading to larger nanoparticles. At the same time, the presence of

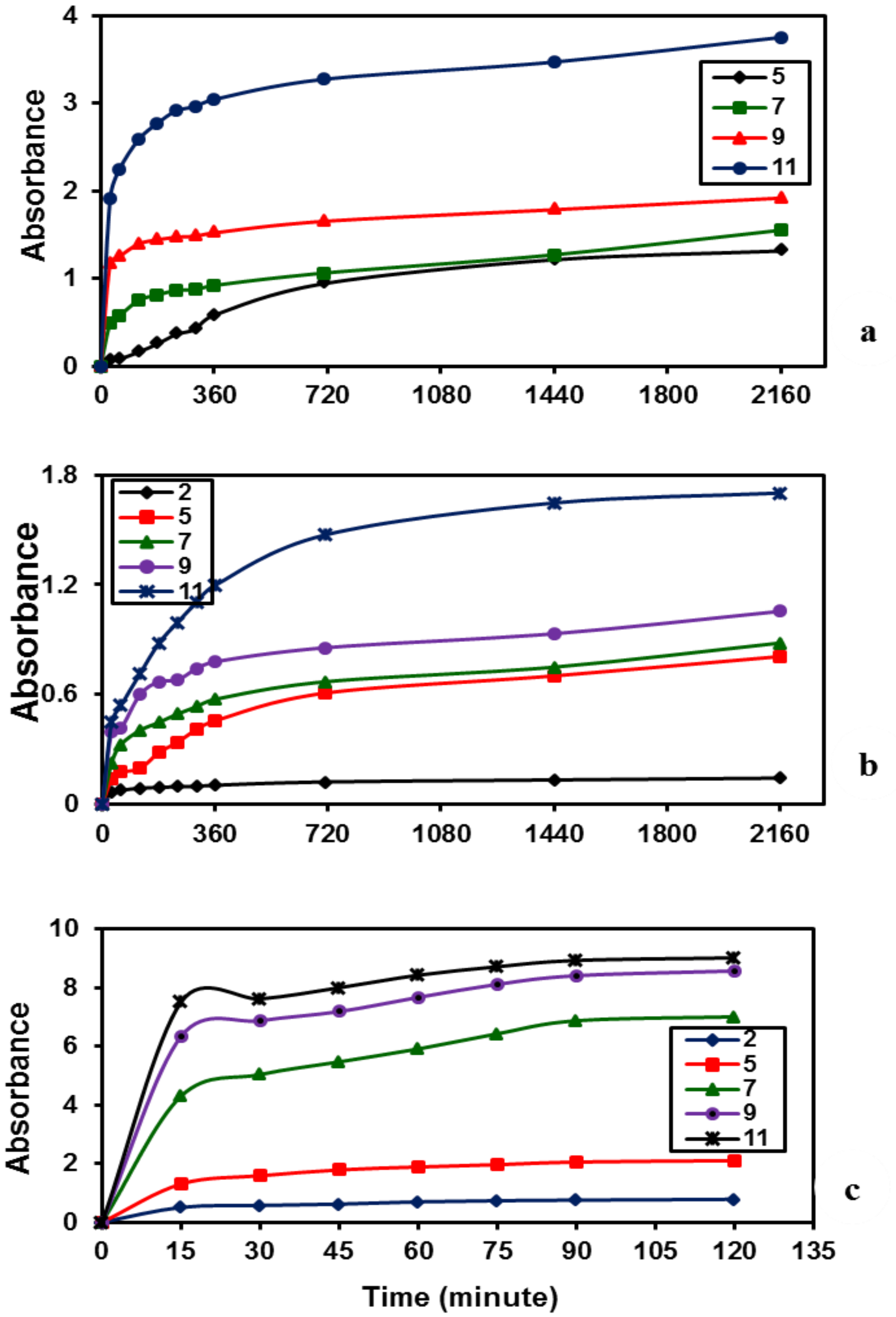


Fig. 35 Time course of silver nanoparticles formation with 1 mM $AgNO_3$ using, (**a**) *H. coronarium*, (**b**) *S. polyrhiz*a, and (**c**) *T. natans* at different pH.

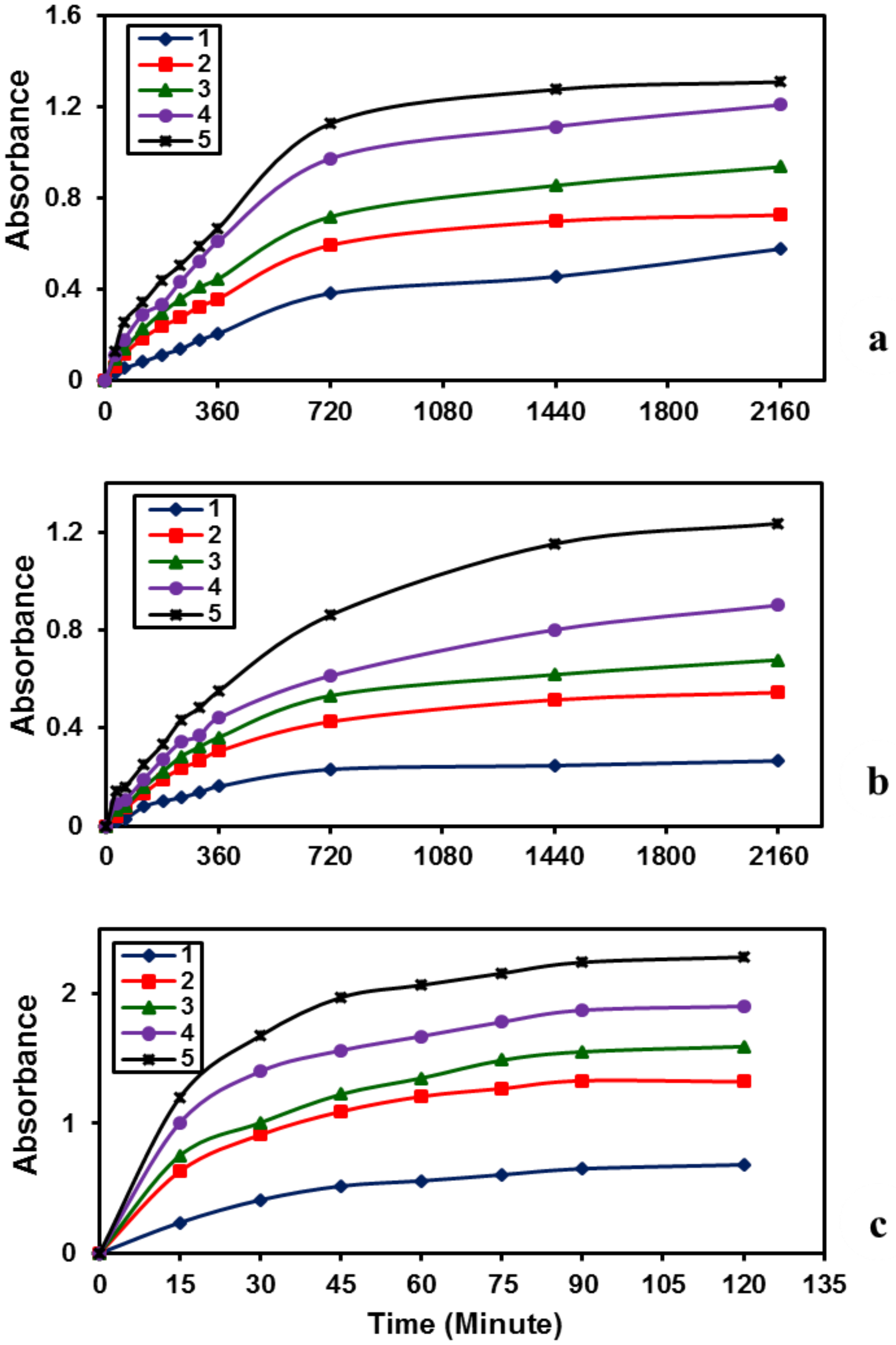


Fig. 36 Time course of silver nanoparticles formation with 1 mM $AgNO_3$ using, (**a**) *H. coronarium*, (**b**) *S. polyrhiz*a, and (**c**) *T. natans* at different extract concentrations.

many reducing agents can enhance the bridging effects among the formed nanoparticles, resulting in the aggregation of the nanoparticles (Song and Kim, 2009).

Fig. 37 (a-c) shows the effect of silver nitrate concentration on the formation of nanoparticles. The peak intensity shiftet to higher wavelength and the absorbance increased with an increase of the salt concentration from 0.5 to 4.0 mL. The reason can be because of too many silver ions absorbed on the surface of the performed nuclei, where the secondary reduction process occurred and it leads to the formation of larger nanoparticles.

Similarly, **Fig. 38 (a-c)** shows the nanoparticles production at different temperature range from 5 to 45°C. The rise in the temperature increased the absorbance values while the peaks got shifter to shorter wavelengths, signifying a decrease in the size of the nanoparticles. It can be explained as, with increased temperature, the reduction rate increases and most of the silver ions are consumed in the formation of the nuclei thereby blocking the secondary reduction process on the surface of the preformed nuclei (Song and Kim, 2009).

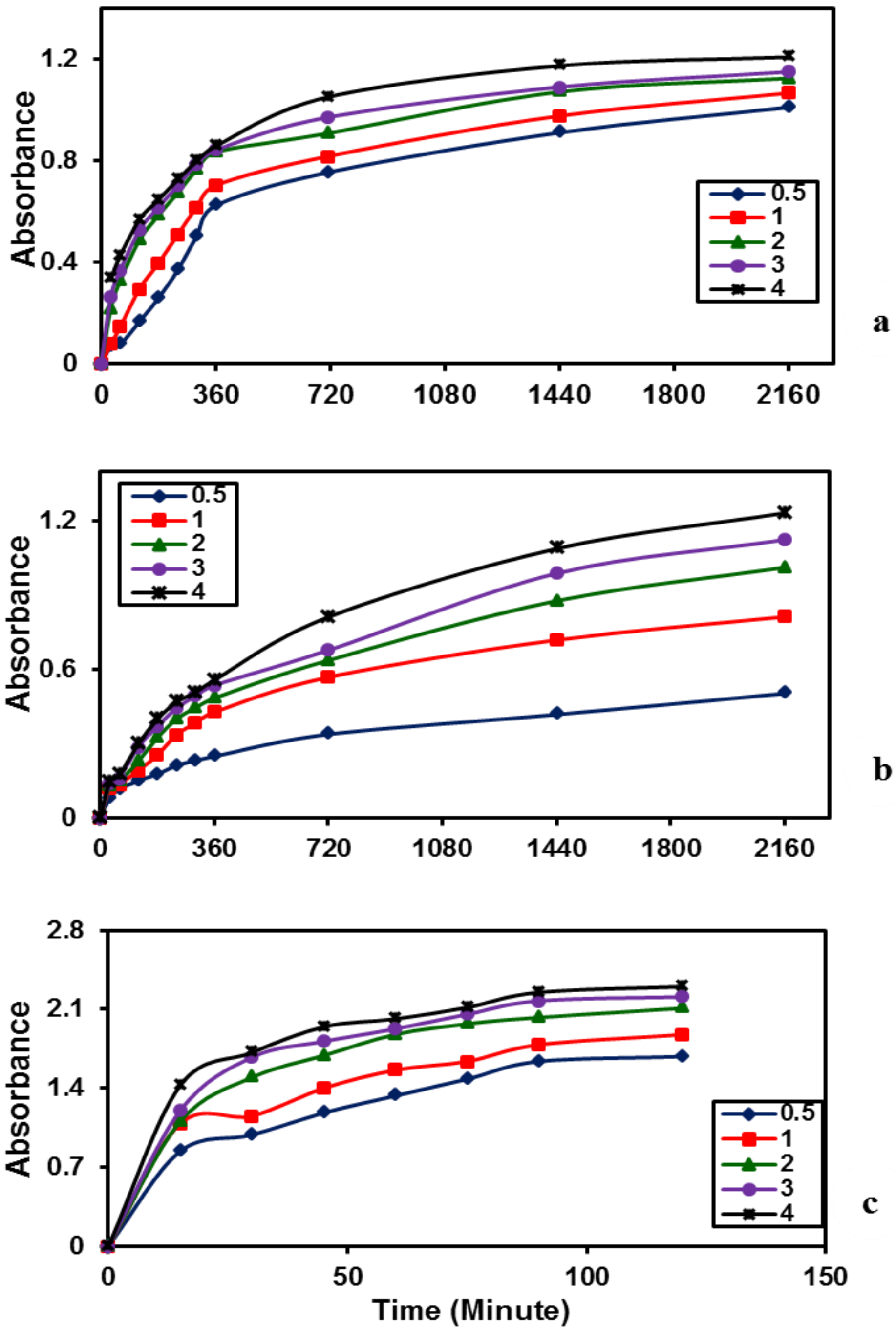


Fig. 37 Time course of silver nanoparticles formation using (**a**) *H. coronarium*, (**b**) *S. polyrhiza*, and (**c**) *T. natans* extracts at different salt concentrations.

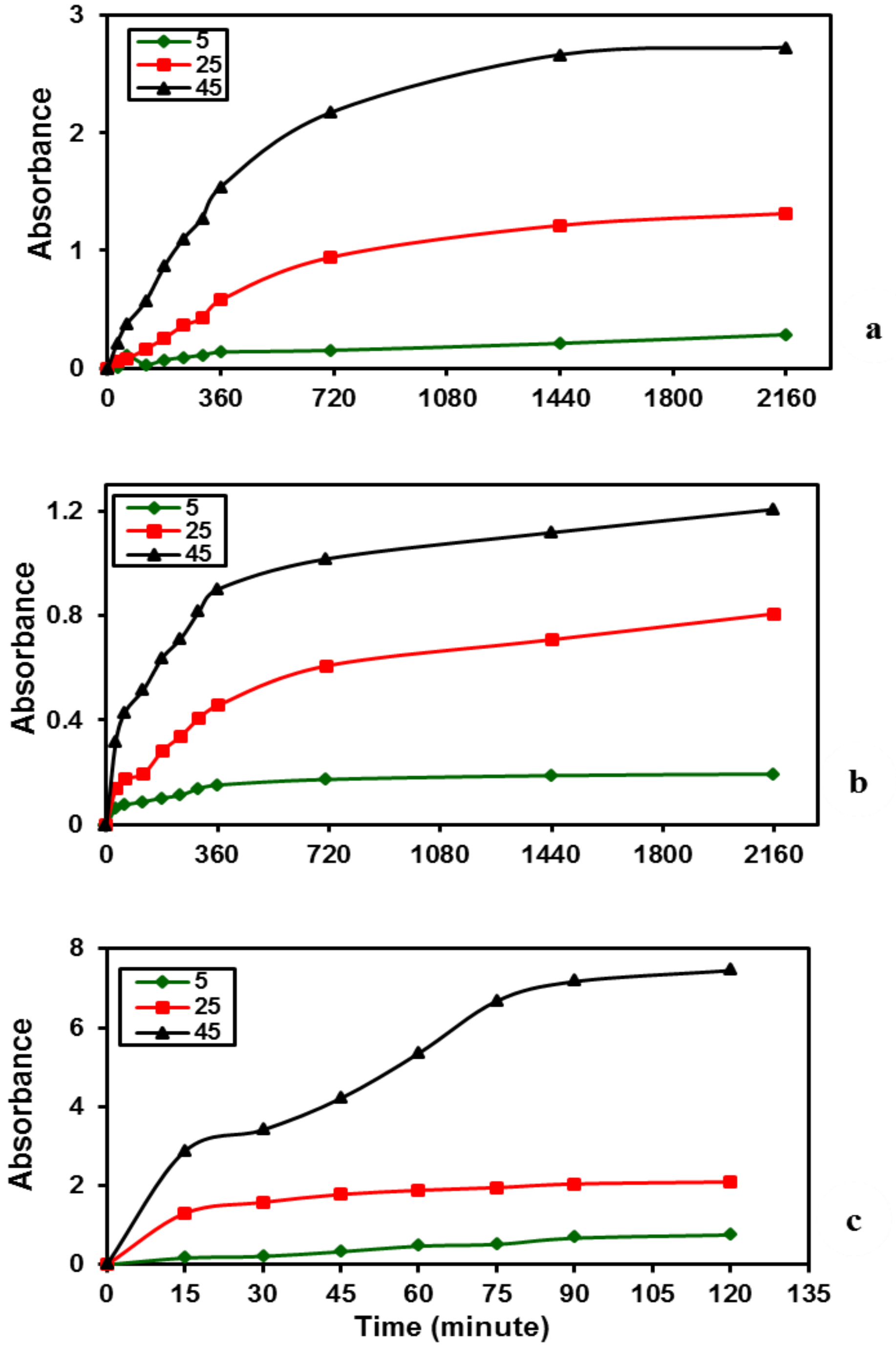


Fig. 38 Time course of silver nanoparticles formation with 1 mM $AgNO_3$ using, (**a**) *H. coronarium*, (**b**) *S. polyrhiza*, and (**c**) *T. natans* at different temperature.

Characterization of AgNPs

The elemental composition of the purified nanoparticles was determined using SEM instrument equipped with an EDX detector. The EDX spectra shown in **Fig. 39(a-c)** revealed the strong signal in the Ag region and confirm the formation of the silver nanoparticles and clearly show the crystalline nature of the nanoparticles. This confirms that the weight % of the Ag is 56.24 for *H. coronarium*, 42.33 for *S. polyrhiza* and 34.21 for *T. natans* mediated reduced nanoparticles. The spectra also showed strong signals of oxygen and carbon indicating that the extracellular organic groups were adsorbed on the surface or in the nearby of the metallic nanoparticles.

FTIR spectroscopy analysis was done to characterize and identify the functional groups or the biomolecules that were bound on the synthesised AgNPs, **Table 17**. The spectra of control plant extracts and synthesised nanoparticles showed shift in peaks: 2360.9 (C-H stretching vibrations), 1219.5 (C-N stretching vibrations) and 1015.4 cm^{-1} (C-O stretching vibrations) for *H. coronarium*; 2360.1 (C-H stretching vibrations), 1219.5 (C-N stretching vibrations) and 1034 cm^{-1} (C-O stretching vibrations) for *S. polyrhiza* and 3310.3 (-OH stretching vibrations), 2883.8 (C-H stretching vibrations, alkanes), 1647.1 (C=O stretching vibrations), 1339.8 (C-N stretching vibrations), 1149.4 (C-O stretching vibrations) and 1014.4 cm^{-1} (C-O stretching vibrations) for *T. natans* mediated reduced AgNPs. The results showed the involvement of hydroxyl, carboxyl, amino groups and amino acid residues present in the extracts of *H. coronarium*, *S. polyrhiza* and *T. natans* in the reduction and capping of AgNPs. It reveals that the presence of phenolic compounds along with the flavonoids present in the *T. natans* fruit coat extract may be suggested as responsible for the reduction process (Raut et al., 2009).

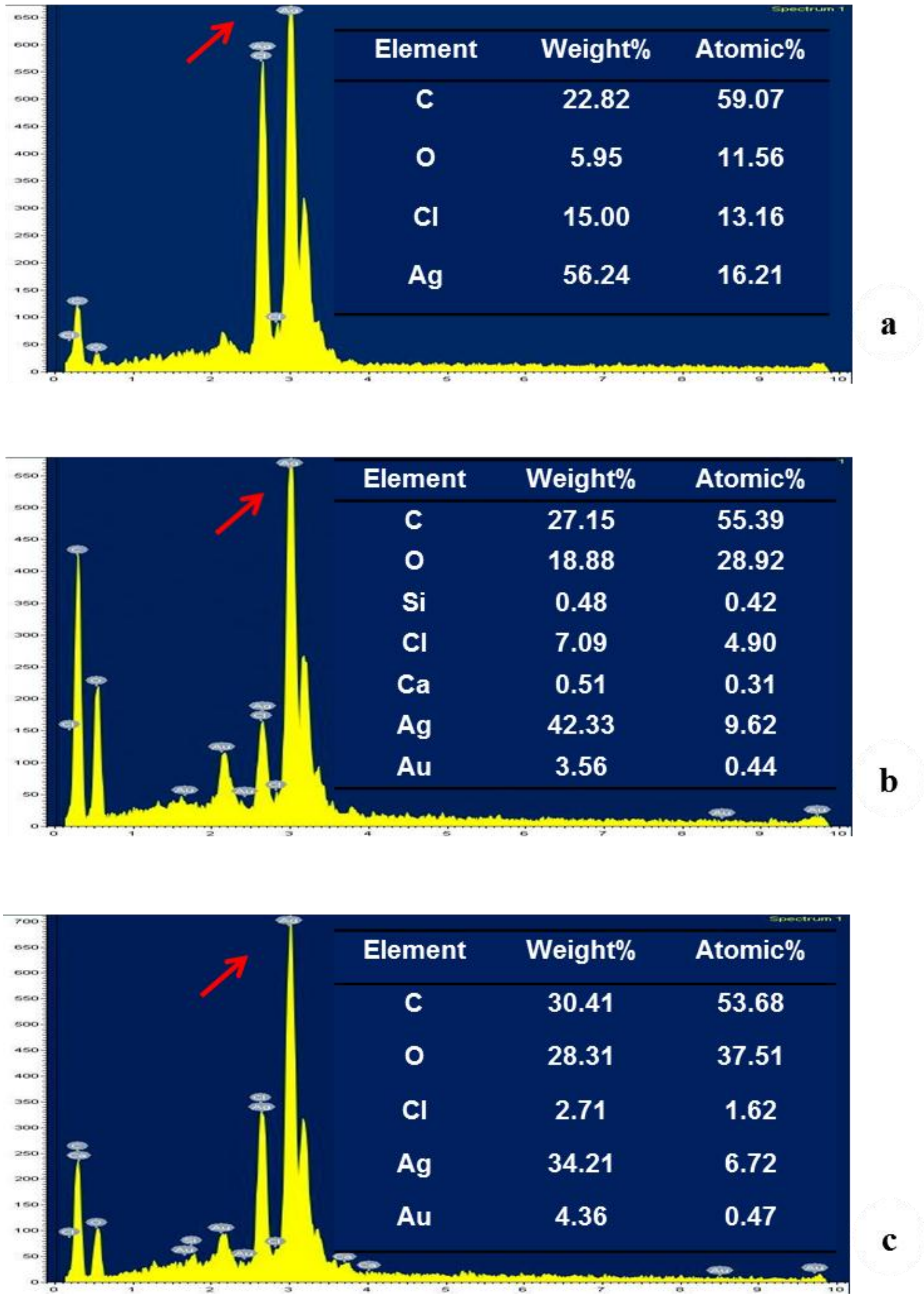


Fig. 39 EDX analysis of Ag nanoparticles synthesised from aqueous extracts of; (**a**) *H. coronarium*, (**b**) *S. polyrhiz*a and (**c**) *T. natans*.

For *H. coronarium* and *S. polyrhiza*, the process is attributed to either free amine groups or cysteine residues in the proteins bound to the AgNPs and therefore stabilizing the nanoparticles (Gole et al., 2001; Bar et al., 2009; Bankar et al., 2010). The FTIR analysis clearly depict that the capping and reduction of NPs by the biomolecules present in the aqueous extracts of the three plant species could be responsible for the prolonged stability.

Table 17 FTIR analysis of Ag nanoparticles synthesised from aqueous extracts of *H. coronarium*, *S. polyrhiz*a, and *T. natans*

Functional groups	***H. coronarium***	***S. polyrhiza***	***T. natans***
-OH stretching vibration	-	-	3310.3
C-H stretching vibration	2360.9	2360.1	2883.8
C=O stretching vibration	-	-	1647.1
C-N stretching vibration	1219.5	1219.5	1339.8
C-O stretching vibration	-	-	1149.4
C-O stretching vibration	1015.4	1034.1	1014.4

Fig. 40(a-c) shows XRD pattern of the biosynthesized silver nanoparticle structures. The observed peaks in the whole spectrum of 2θ value that range from 20 to 90 confirm the crystalline phase occurrence of the nanoparticles. The peaks at 38.55°, 44.65° and 73.25° (*H. coronarium*), 38.35°, 44.65° and 73.25° (*S. polyrhiza*) and 38.85°, 44.75° and 73.25° (*T. natans*) can be indexed to the (111), (200) and (311) Braggs reflections of face centered cubic (FCC) crystal structure of metallic Ag respectively. The additional peaks in the XRD pattern are mainly due to crystalline components of the plant extracts that acts as the capping agents for the silver nanoparticles.

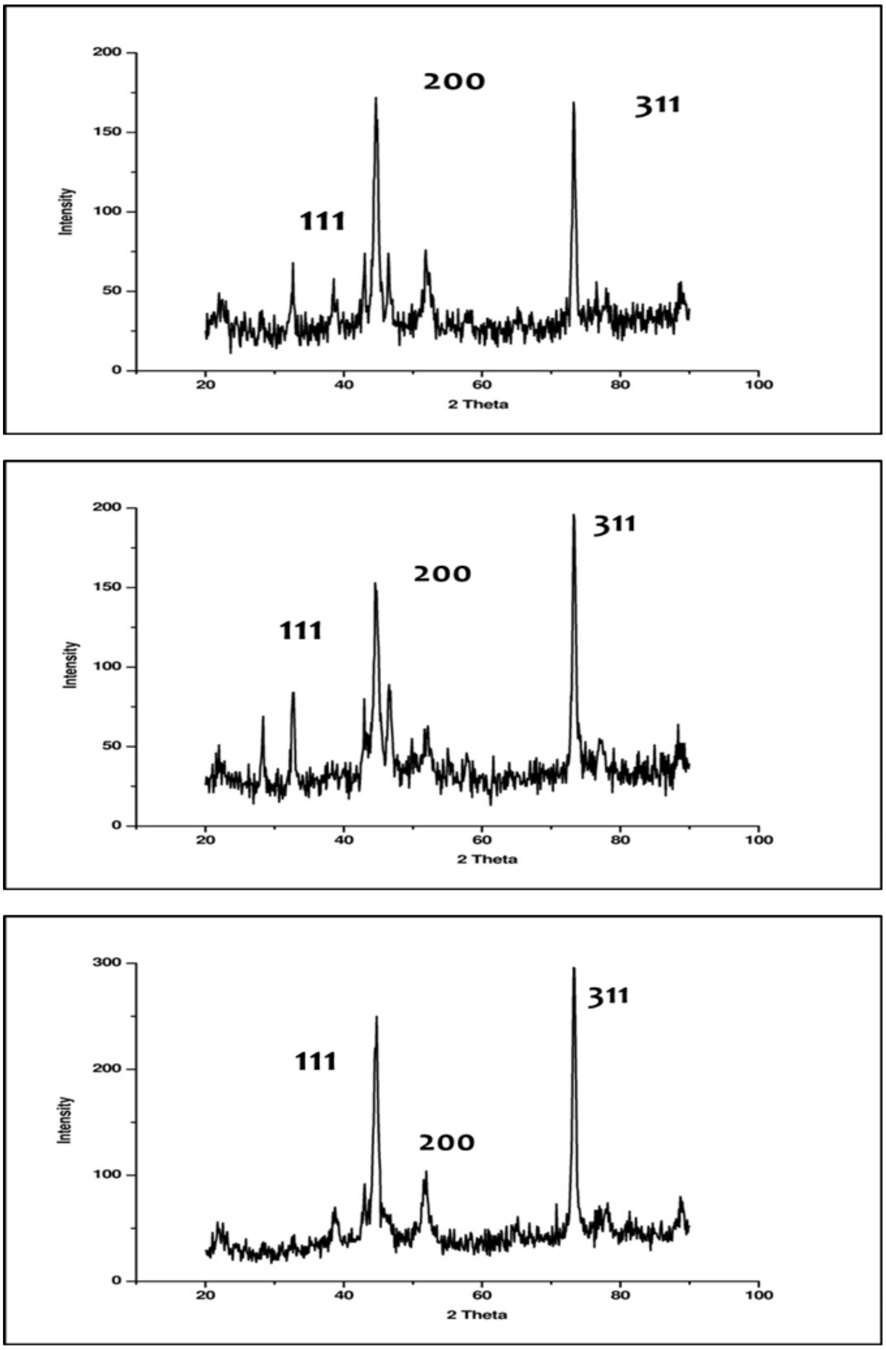


Fig. 40 X-ray diffraction pattern of Ag nanoparticles synthesised from aqueous extracts of, (**a**) *H. coronarium*, (**b**) *S. polyrhiza*, and (**c**) *T. natans*

Transmission electron microscopy, **Fig. 41(a-c)** has been employed to characterize the size, shape and the morphology of the silver nanoparticles formed by the reduction of Ag ions into Ag°. It revealed that the nanoparticles were mostly spherical and oval in shape with size ranging from 9.56 to 56.12 nm, respectively. The SAED pattern of silver nanoparticles shows its polycrystalline nature.

Photocatalytic activity of AgNPs

The photocatalytic activity of the biosynthesized nanoparticles was investigated choosing the photodegradation of four azo dyes viz. methylene blue, amido black, methyl orange and brilliant blue green as model systems. The characteristics absorption peaks at 665 nm for methylene blue, 620 nm for amido black, 465 nm for methyl orange and 610 nm for brilliant blue green was used for monitoring the catalytic degradation process. **Fig. 42(a-d)** represents the absorption spectra of the azo dyes degradation in the five different solution mixtures. It can be observed that the blank experiments performed without any of the nanoparticles as the catalysts show no significant change in dye absorbance after 120 min indicating that the dyes are not reduced effectively. The main absorption peaks ate 665, 620, 465 and 610 nm decreased gradually with the extension of exposure time in reaction mixture five with dye, nanoparticles and $NaBH_4$, indicating the photocatalytic degradation of methylene blue, amido black, methyl orange and brilliant blue green. The reason can be attributed to the large surface area of the nanoparticle increasing the reactivity and degradation process. In addition, the layer of reducing agent viz. $NaBH_4$ on the surface of the silver nanoparticles can also promote the effective adsorption between silver nanoparticles and the dyes.

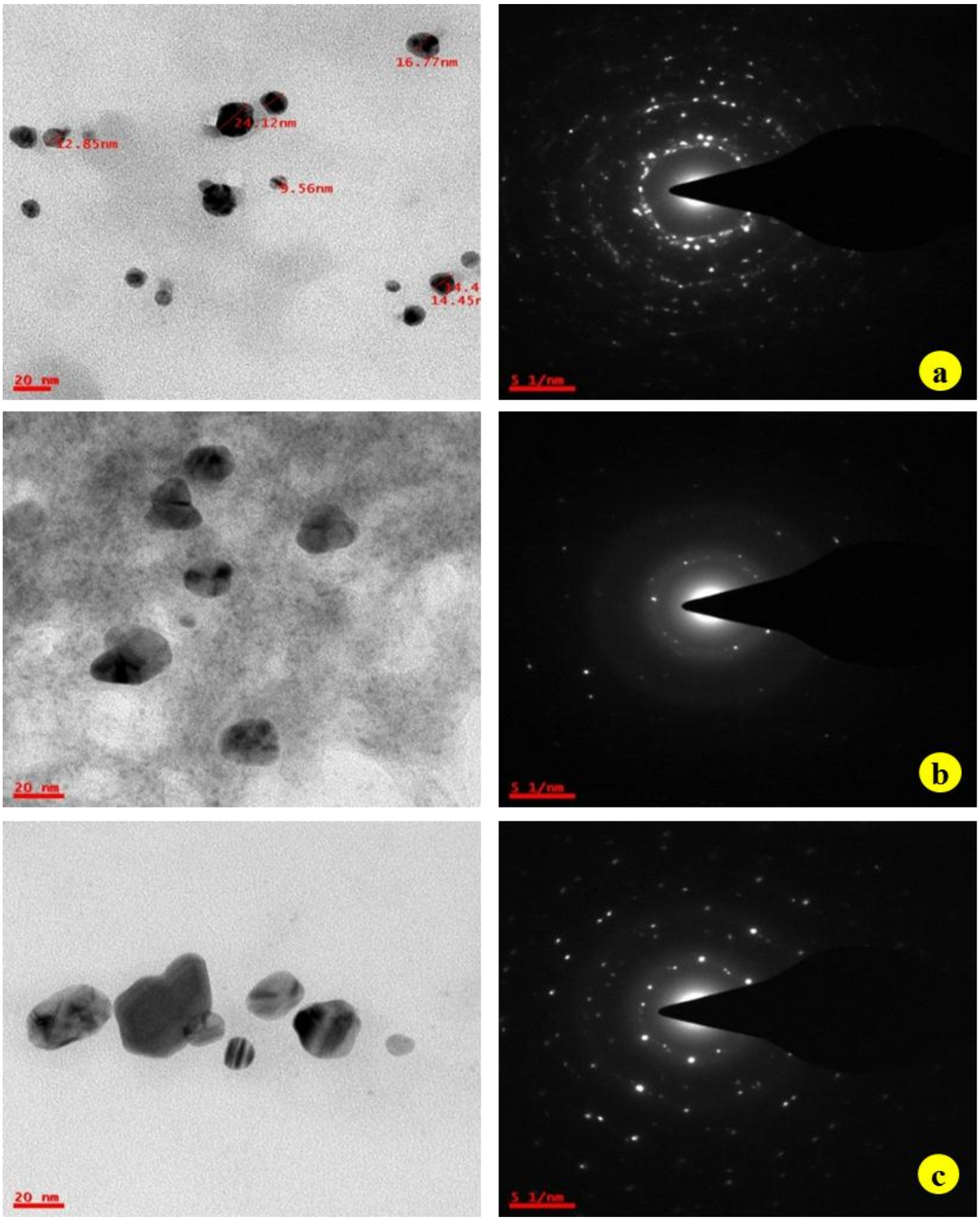


Fig. 41 (Left) High resolution TEM Ag nanoparticles and (Right) SAED pattern for, (**a**) *H. coronarium*, (**b**) *S. polyrhiza*, and (**c**) *T. natans.*

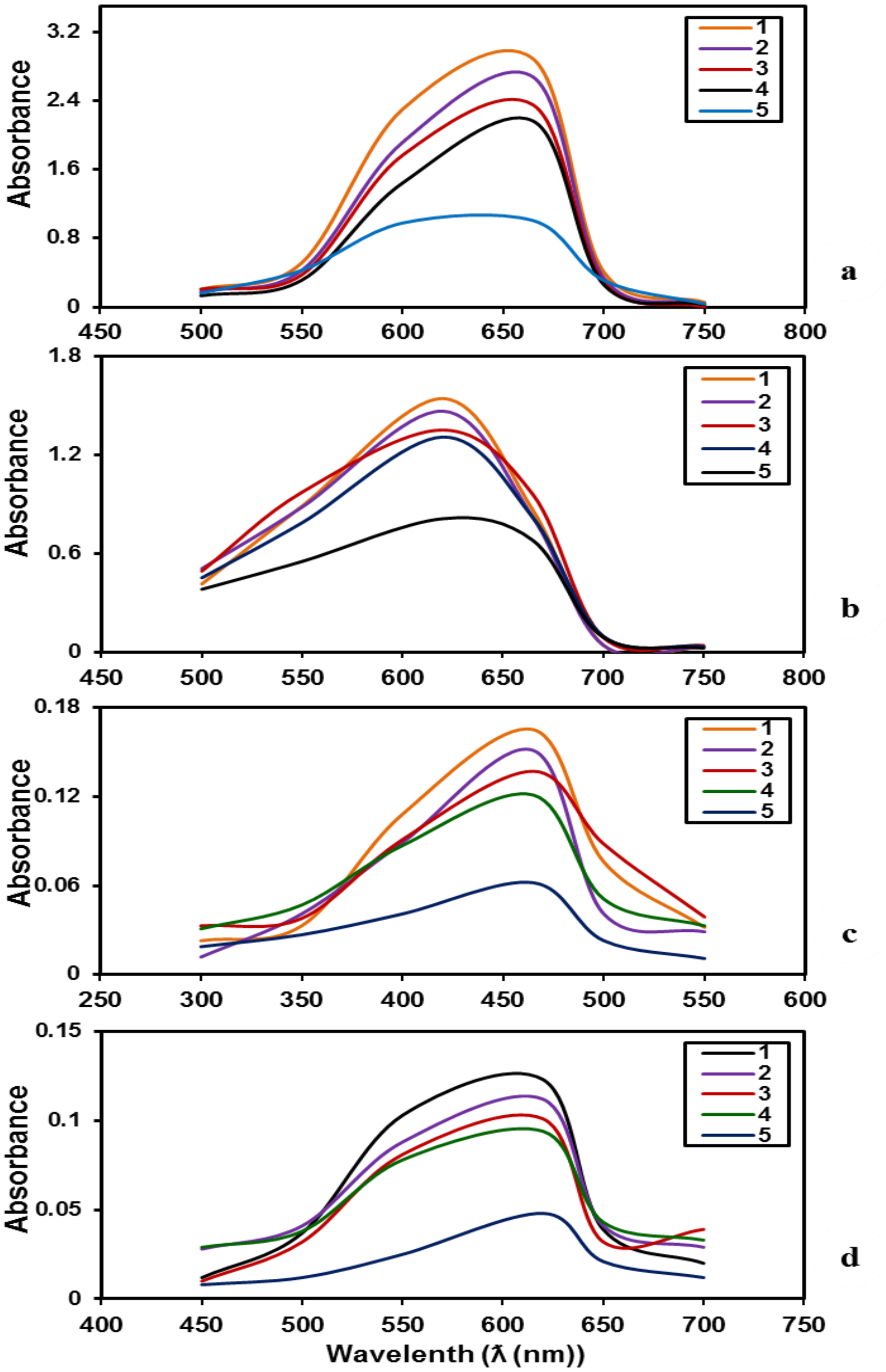


Fig. 42 Photodegradation of (**a**) Methylene blue, (**b**) amido black, (**c**) methyl orange, and (**d**) brilliant blue green by *H. coronarium* extracts .Notes: 1. Dye + water; 2. Dye + Extract + water; 3. Dye + $NaBH_4$ + water; 4. Dye + AgNPs + water; 5. Dye + AgNPs + $NaBH_4$ + water.

Fig. 43(a-c) shows the reduction of methylene blue by the synthesised nanoparticles in light and dark medium. *Hedychium coronarium* extract mediated nanoparticles synthesised showed the photodegradation efficiency of 67.39% in light compared to 10.10% in dark. Similarly, *S. polyrhiza* showed 80.0% reduction efficiency compared to 10.37% in dark, while *T. natans* got 89. % reduction in light compared to 8.10 % in dark. **Fig. 44(a-c)** showed 64.0% reduction of amido black in light (*H. coronarium* nanoparticles) compared to 4.36% in dark. Likewise, 64.0% in light and 6.0% in dark (*S. polyrhiza*) and 62.0% reduction efficiency compared to 8.5% in dark for *T. natans* nanoparticles. **Fig. 45(a-c)** showed 62.42% photodegradation efficiency of methyl orange in light in comparison with 9.87% in dark for *H. coronarium* nanoparticles. Similarly, 63.30% degradation in light and 8.48% in dark for *S. polyrhiza* while 55.12 and 5.61% reduction for *T. natans*, respectively. **Fig. 46(a-c)** showed a photodegradation of 60.97% of brilliant blue green in the presence of the reducing agent and nanoparticles for *H. coronarium* in light compared to 10.01% in dark medium. Likewise, 66.67 and 8.01% reduction for *S. polyrhiza* and 63.57 and 7.14% degradation efficiency were observed in *T. natans* nanoparticles mixture at 120 min.

The photodegradation properties of Ag nanoparticles in the presence of visible light may be due to the excitation of SPR, which is the oscillation of charge density that can propagate at the interface between the metal and dielectric medium (Garcia, 2011). From literature, it reveals that the photocatalytic activity strongly depends on the crystallographic structure, morphology and size of the particles (Vidhu and Philip, 2014). The nanoparticles possess a large surface area that readily acts as a substrate for the electron transfer reaction from the donor to the acceptor. Both of the reactants

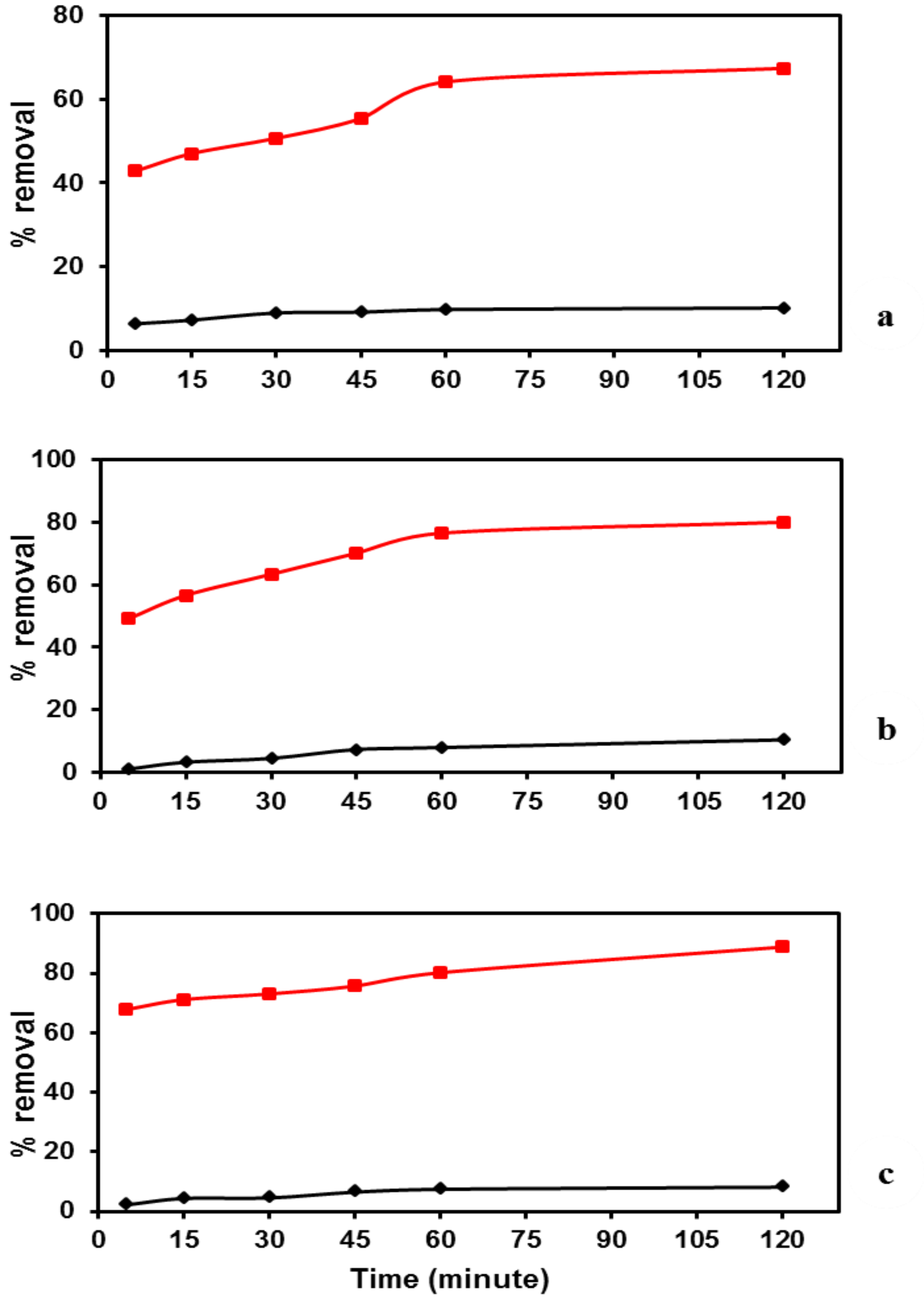


Fig. 43 Photodegradation of Methylene blue by silver nanoparticles synthesised from (**a**) *H. coronarium*, (**b**) *S. polyrhiza* and (**c**) *T. natans* extracts (Red; Light and Black; Dark).

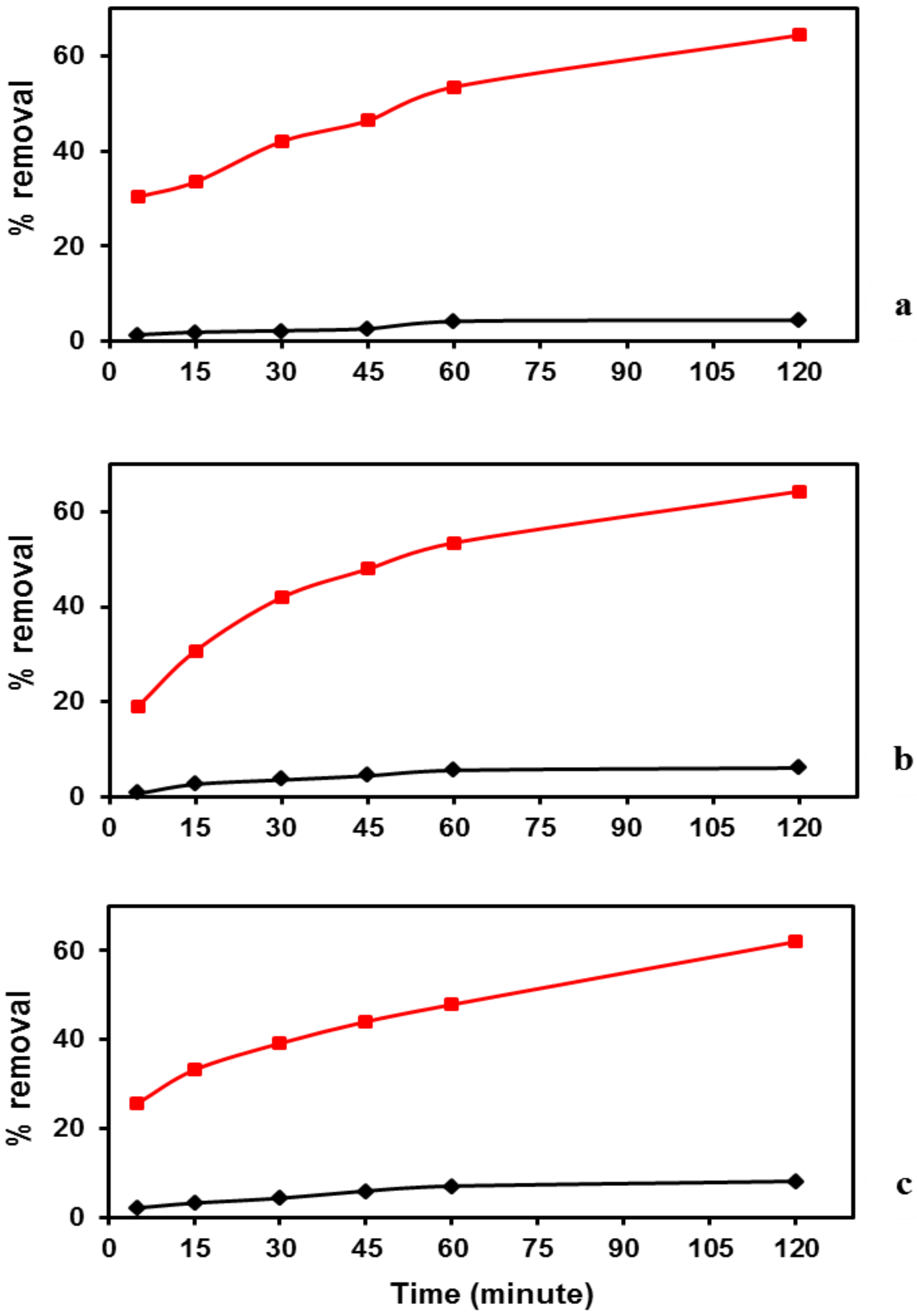


Fig. 44 Photodegradation of Amido black by silver nanoparticles synthesised from (**a**) *H. coronarium*, (**b**) *S. polyrhiza* and (**c**) *T. natans* extracts (Red; Light and Black; Dark).

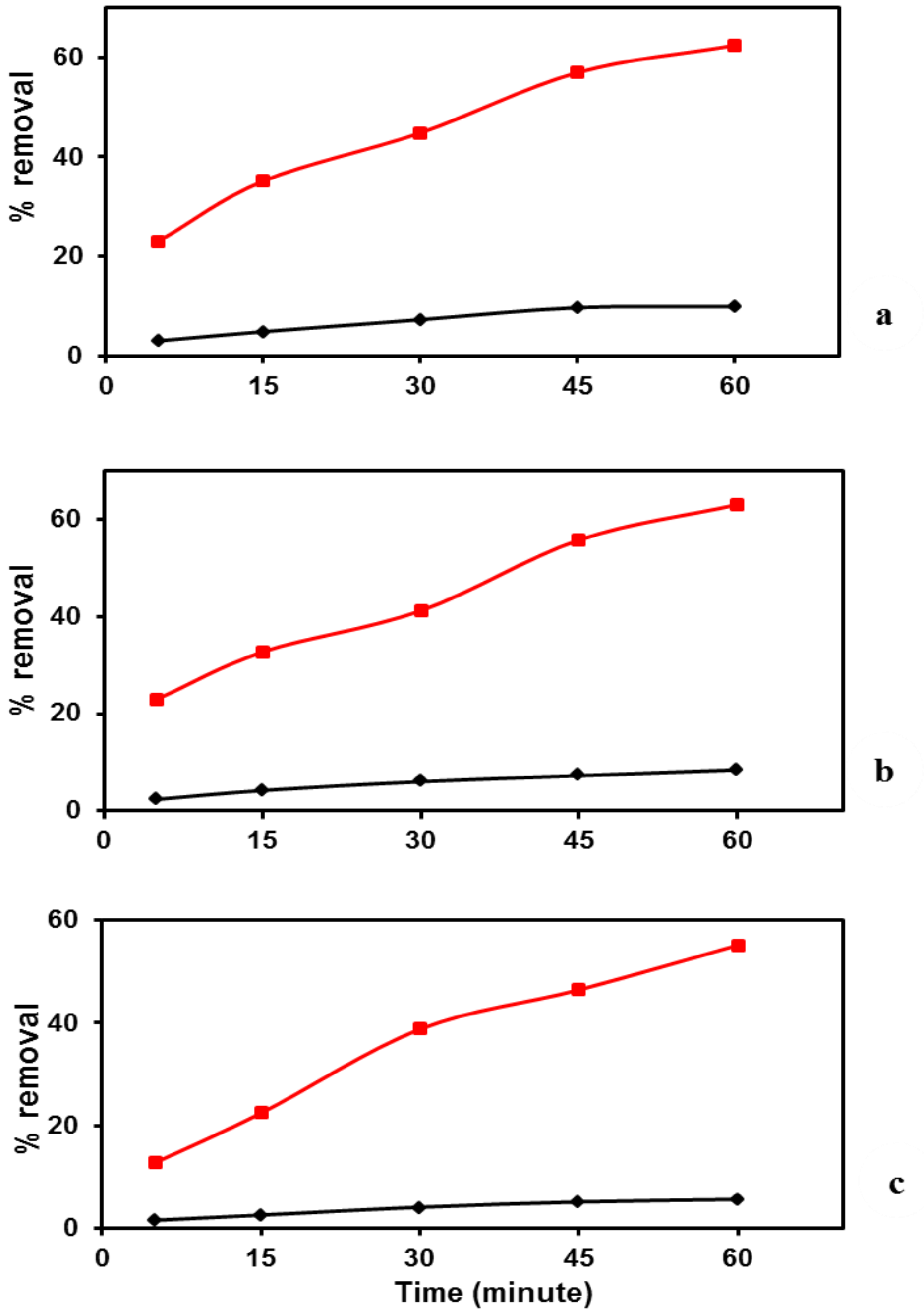


Fig. 45 Photodegradation of Methyl orange by silver nanoparticles synthesised from (**a**) *H. coronarium*, (**b**) *S. polyrhiza* and (**c**) *T. natans* extracts (Red; Light and Black; Dark).

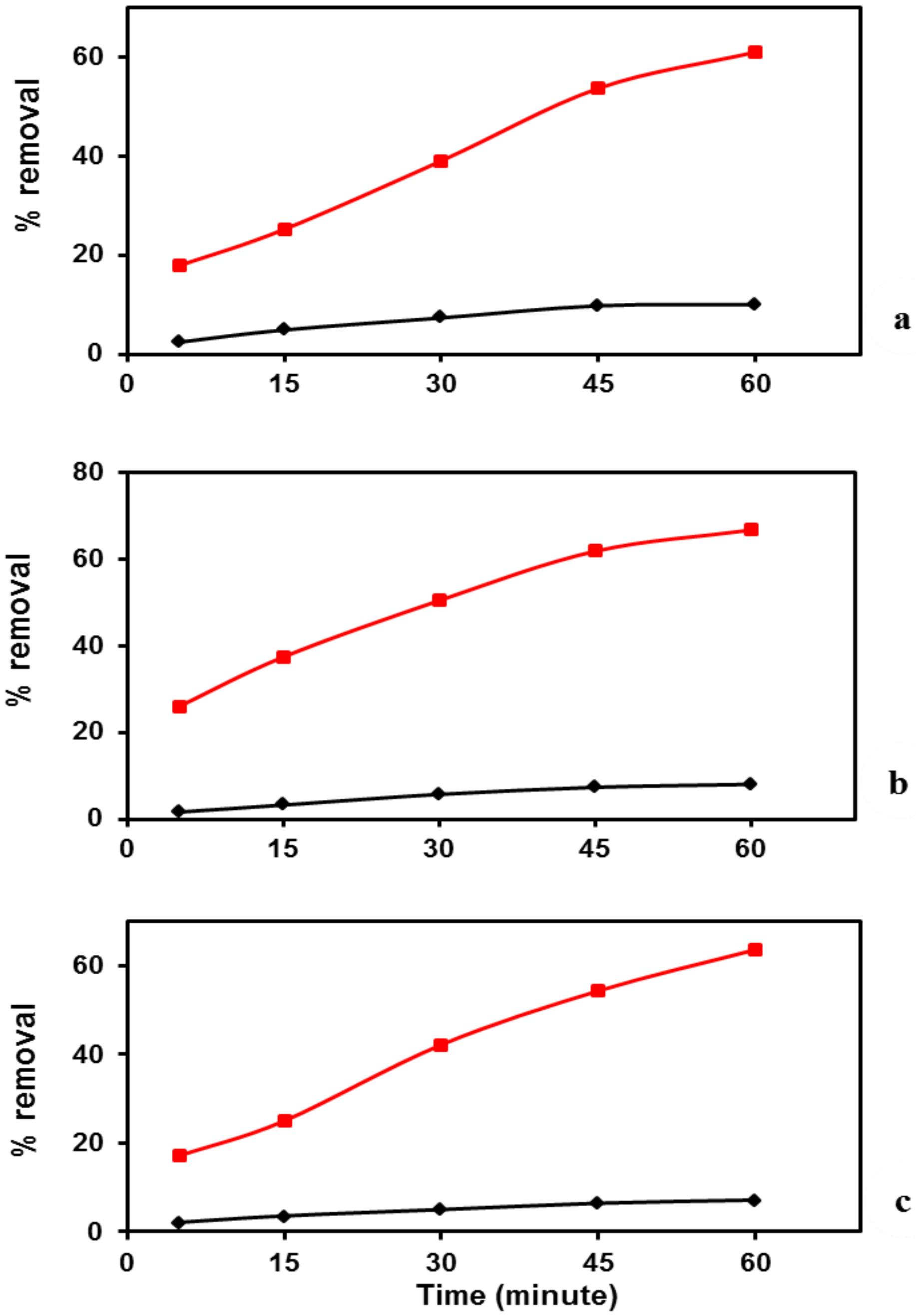


Fig. 46 Photodegradation of Brilliant blue green by silver nanoparticles synthesised from (**a**) *H. coronarium*, (**b**) *S. polyrhiza* and (**c**) *T. natans* extracts (Red; Light and Black; Dark).

are adsorbed on the metal surface during the electron transfer reaction, leading to the gain of an electron by the reactant and is reduced. Thus, the silver nanoparticles acts as an efficient catalyst thought the electron transfer process during the catalytic reactions (Ghosh et al., 2002). The results revealed that the Ag nanoparticles are good, highly efficient and stable photocatalysts under ambient temperature with the illumination of visible light for degrading various organic dyes in wastewater environment.

Conclusions

The present investigation describes for the first time, *H. coronarium*, *S. polyrhiza* and *T. natans* extract mediated phytosynthesis of Ag nanoparticles and its application in photocatalytic degradation of dyes (methylene blue, amido black, methyl orange and brilliant blue green) under normal visible light irradiation. The synthesis of the nanoparticles was affected by different parameters such as pH, contact time, extract concentrations, salt concentrations and temperature. The FTIR spectra indicate that the functional groups -OH, -CH, C=O, C-O and C-N may be responsible for the reduction and stabilizing of sliver nanoparticles. The XRD profile of the synthesised nanoparticles showed diffraction peaks at 38.55°, 44.65° and 73.25° (*H. coronarium*), 38.35°, 44.65° and 73.25° (*S. polyrhiza*) and 38.85°, 44.75° and 73.25° (*T. natans*) [assigned to the (111), (200) and (311) planes of a faced center cubic (fcc) lattice of silver]. The results of the EDX give a clear idea about the elements present in the nanoparticles and the strong signal of the Ag atoms indicates the crystalline property. The sizes of the synthesized nanoparticles are approximately 24.12 nm (*H. coronarium*), 31.12 nm (*S. polyrhiza*) and 39.82 nm (*T. natans*). The SAED pattern of AgNPs reveals its crystalline nature. The photodegradation of methylene blue, amido black, methyl orange and brilliant blue green as observed in the form decreased absorbance at λ_{max} of 665 nm, 620 nm, 465 nm and 610 nm, respectively shows the ability of the pytoextract to degrade azo dyes. Thus, the agricultural waste material produced from the *phoomdi* can be used for the consistent and quick synthesis of silver nanoparticles. Further, it also gave an additional phytotechnological application for the *phoomdi* biomass for its use in the restoration of ecosystems degraded by various organic dyes.

Summary

&

Conclusions

Summary and conclusions

Loktak plays an important role in the ecological and economic security of the region. The characteristics feature of the wetland is the presence of floating vegetation, locally known as *phoomdi*. A large population living in and around the wetland depends upon the wetland resources for their sustenance. At present, more than 2000 people live on *phoomdi* with fishing as their sole profession. Several *phoom* based indigenous techniques have been developed for fishing by the local communities, particularly by *phoom* hut dwellers. Various plant species of *phoomdi* are utilized by local communities as source of food, fuel and fodder. *Phoomdi* are also utilized, in a limited manner, for paddy cultivation and for growing vegetables. *Phoomdi* play an important role in the ecological processes and functions of the wetland ecosystem. They provide biological key nutrients and govern water quality of the wetland and its nutrients regimes. The wetland, with its numerous floating islands, supports a rich biological diversity. The wetland at present faces severe pressure due to increased siltation from the degraded catchment area, rapid proliferation of *phoomdi* and decline in the water quality. Several factors like the practice of shifting cultivation, uncontrolled use of fertilizers in the agricultural lands and direct dumping of sewerage from the city are primarily responsible for the rapid deterioration of the wetland ecosystem. The accelerated growth of *phoomdi*, particularly after the construction of the Ithai barrage has reduced the carrying capacity of the wetland ecosystem and enhanced its siltation. The nutrient rich sediments eroded from the catchments and discharge of domestic waste flowing directly or indirectly into the wetland are causative factors for prolific growth of *phoomdi*. The changes in the hydrological regimes have greatly contributed to the deposition of nutrients and organic matter at the wetland bottom leading to the deterioration of water quality and loss of biodiversity. Loktak Development Authority, at present, is involved in developing strategies for conservation and management of the wetland by integrating social, economic, and ecological dimensions for sustainable development of Loktak. But, the absence of baseline data on wetland ecology and even in understanding the resource management or sustainable resource development had limited its scope. Keeping this in mind; the study was conducted to develop strategies for the utilization and management of *phoomdi* in Loktak, **Fig. 47**.

Forty seven traditionally important *phoomdi* species with different mode of usage, viz. medicine, wild edible, fodder, fuel, handicraft, fishing, and house making were registered. A total of 27 wild edible and 27 medicinal species were recorded. There were 16 *phoomdi* edible plants traded in the local bazaars for income generation. More than 19,000 tons of fish was harvested form Loktak and adjoining wetlands of Manipur during 2010-11, with 39% of the harvest from *athaphoom* fishing. It explains the importance of *phoomdi* in the socio-economic and cultural life of the communities and their dependency on the wetland.

Iron concentrations were found in high levels compared to other nutrients and metals in Loktak water. It suggests the need to treat the freshwater for domestic consumption and agricultural applications (for Fe). Sediment characteristics showed high retention of nutrient and metals. Target Health Quotient values calculated for the metals showed that the consumption of the *phoomdi* wild edibles is not free of risks. Relative high BCFs obtained depict the probable use of *phoomdi* in phytoextraction of nutrients and metals from the wetland and use in re-vegetation of waterlogged

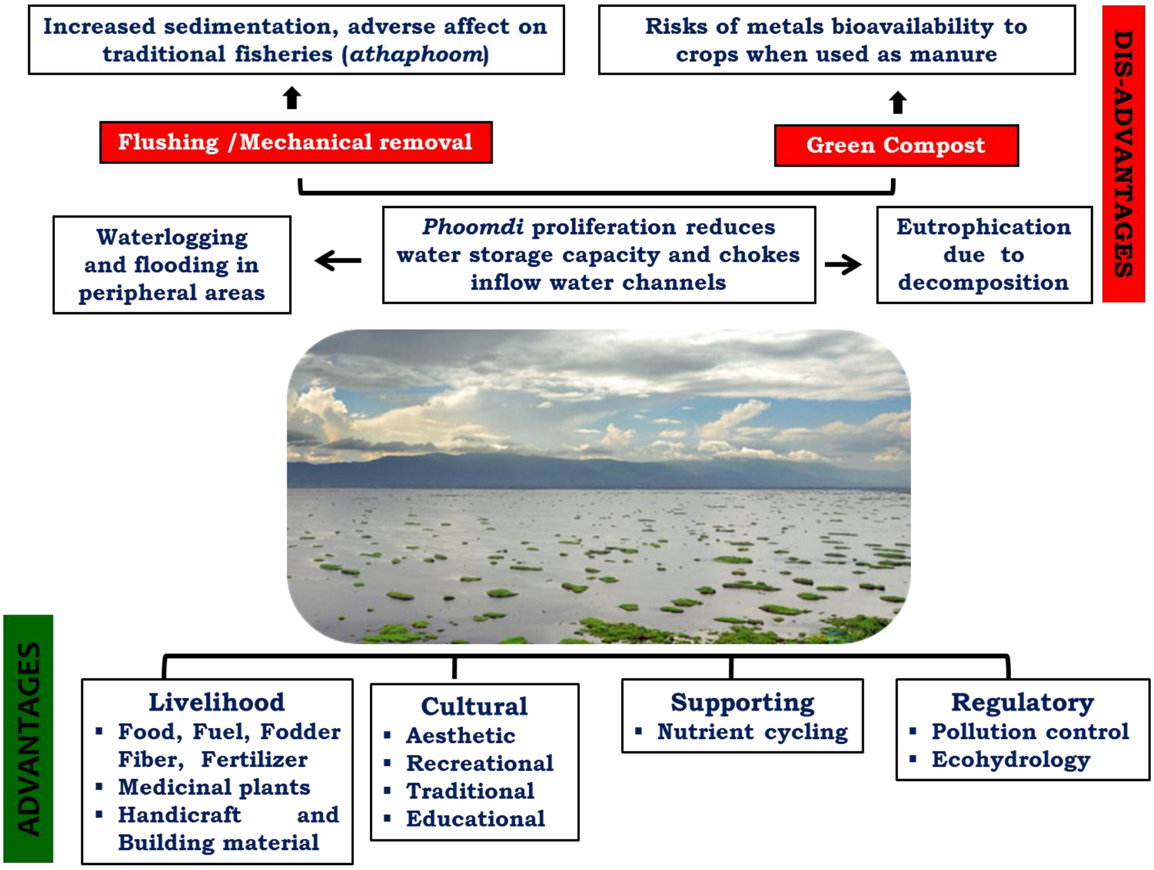


Fig. 47 Advantages and disadvantages of *phoomdi* occurrence in relation with Loktak.

contaminated sites and constructed wetlands. The results suggest the need of proper and in-depth scientific investigation by the concerned bodies (LDA and Forest Department), if they planned to remove *phoomdi* from the surface of the freshwater wetland, Loktak.

A laboratory scale bioassay set up showed high accumulation and removal of Fe using *Typha latifolia* L. a *phoomdi* species and the aggravation of Fe uptake by Cu supplementation. The initial concentration of iron in water samples was reduced to 1.67 ± 0.076 mg L^{-1} from 30.00 ± 0.00 mg L^{-1} during 336 hours (14 days). Copper addition increased the uptake of iron compared to Fe alone treated *T. latifolia*. The present work showed the effectiveness of *T. latifolia* in removing Fe and Cu from wastewaters and its possible application in constructed wetlands for the phytoextraction of waters contaminated with Fe. Construction of artificial wetlands along the bank of the river Nambul and Loktak may act as biofilters and can remove high loads of nutrients and other pollutants including metals from the river water and the wetland.

Spirodela polyrhiza possessed a good Pb (II), Cd (II), Cu (II), Mn (II) and Zn (II) ions adsorption quality. The adsorption efficiency of the heavy metal ions was found to be significantly higher as compared to reported biomasses, with maximum adsorption capacities of 137 mg g^{-1} for Pb (II), 52.6 mg g^{-1} for Cu (II), 36.0 mg g^{-1} for Cd (II), 35.7 mg g^{-1} for Mn (II), and 28.5 mg g^{-1} for Zn (II), respectively. The results provided a new, low-cost and effective adsorbent, and explored a new phytotechnological application of the *phoomdi* macrophyte, *S. polyrhiza*. It paved a way towards sustainable utilization of the biomass, floating in Loktak, and led to the generation of a useful by-product in the form of an adsorbent.

Stable AgNPs were synthesised using water extract of *Hedychium coronarium* J. Koenig shoots, *Spirodela polyrhiza* (L.) Schleiden whole plant and *Trapa natans* L. fruits by the reduction of Ag^+ ions, as a measure to utilize the *phoomdi* waste produced. It is the first report of its kind for the *phoomdi* species and its application in photocatalytic degradation of dyes (methylene blue, amido black, methyl orange and brilliant blue green) under normal sunlight irradiation. Pure methylene blue, amido black, methyl orange and brilliant blue green has a λ_{max} of 665 nm, 620 nm, 465 nm and 620 nm, respectively. After addition of the dye with the nanoparticles and exposure to light, the absorbance decreased and shifted to higher wavelength. The decrease of the absorbance is indicative of the ability of the pytoextract to degrade the organic dyes. Thus, the *phoomdi* waste material produced was used for the consistent and quick synthesis of silver nanoparticles and treatment of wastewaters.

Despite of the great economic utility of the *phoomdi*, its rapid proliferation in the present years has been a cause of serious concern in the communities. The key to the management of *phoomdi* lies in the integration of traditional knowledge of the communities with the technical know-how to evolve a strategy which ensures livelihood security to the locals as well renders ecological integrity to the wetland ecosystem. Proper management and ecodevelopment of the wetland requires an adequate understanding of the structure and dynamics of the whole system in place if ad-hoc methods practiced today, **Fig. 48**. At the conceptual level, the approach should be three pronged; comprising of dynamic conservation, sustainable development and equitable access to benefits of conservation. Dynamic conservation refers to setting up various means of an effective alternative for attaining the ecosystem condition of the wetland as it existed many decades ago. Sustainable development refers to the form of development that caters the need of the present generation without compromising with the needs of the future generations from the wetland. Likewise, the third aspect concentrates on ensuring equitable access to the benefits of the development to be implemented through the participation of various institutions and the local communities.

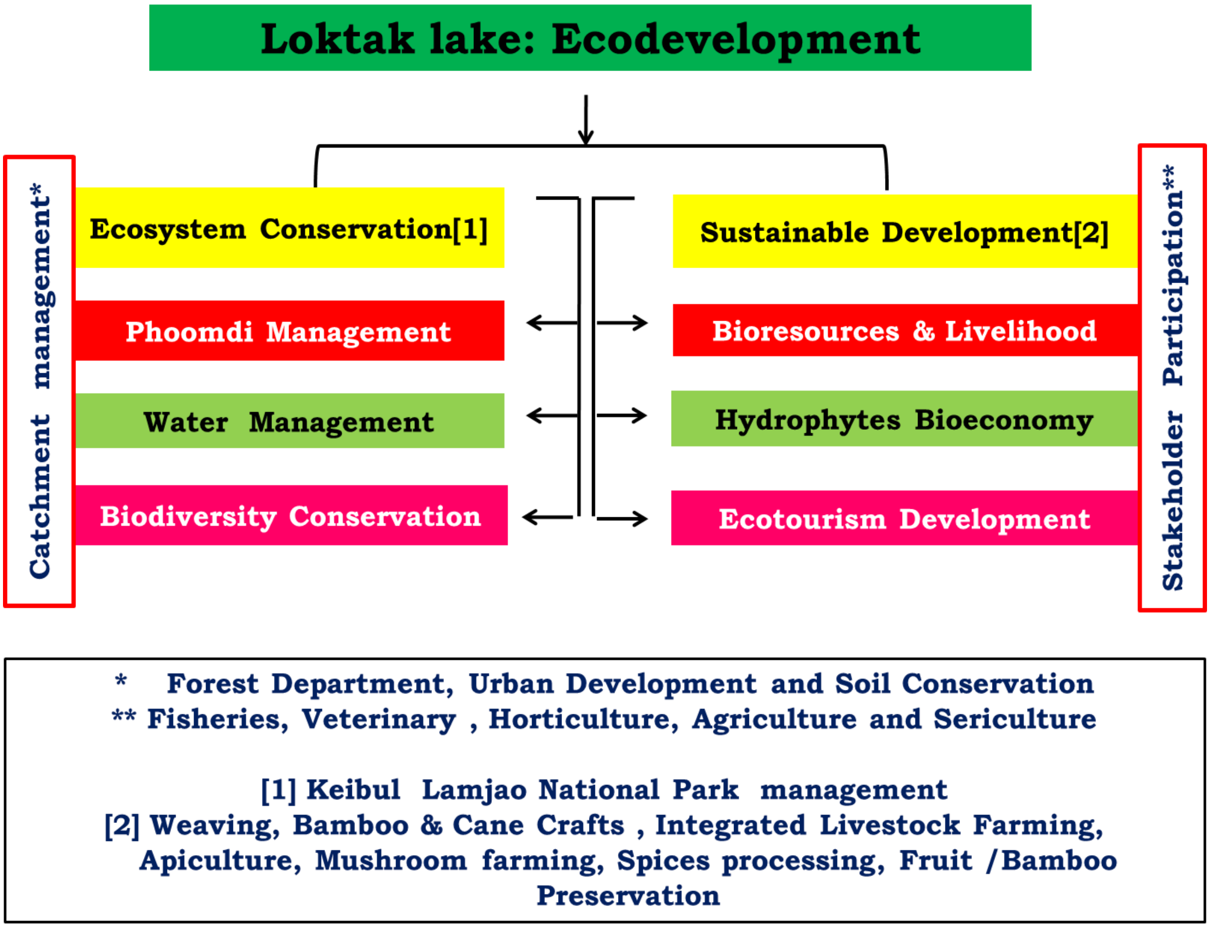


Fig. 48 Loktak and the parameters associated for Ecodevelopment. (Sustainable utilization of *phoomdi* and other wetland bioresources with equitable access of the benefits to the communities and conservation approach to preserve the habitat, Loktak).

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Annexure:

Curriculum vitae; List of publications; conferences abstracts and reprints

MAIBAM DHANARAJ MEITEI

Research Scholar
Department of Plant Sciences
University of Hyderabad, Hyderabad -500046
E-mail: rajjhcu@yahoo.in
Phone no: +91 8985995627.

EDUCATIONAL QUALIFICATIONS

Year	Degree	Board / Institute	Stream / Subject	Percentage / CGPA
2003	X^{th}	Board of Secondary Education Manipur	Eng. Man. Math. Sc. SSc. CSc.	72.8
2005	XII^{th}	Council of Higher Secondary Education Manipur	Eng. Man. Phys. Chem. Bio. Math	68.6
2008	BSc	Manipur University	Bot (Hons). Zoo. Chem. Eng.	81.3
2010	MSc	University of Hyderabad	Plant Biology and Biotechnology	9.03

PROJECTS

Year	Institute	Supervisor	Details
2009-10	University of Hyderabad	Prof. MNV Prasad	Iconic report on the Ecology and Environment of Loktak Lake, Manipur

ACADEMIC ACHIEVEMENTS

- Best student award from Standard Robarth English School (2003) for securing high marks in X^{th} Examination.
- First student to secure 80% in Manipuri for Standard Robarth English School in X^{th} Examination (2003).
- Highest mark in Manipuri - 82 for the state (2005), Manipur (XII^{th}).
- Gold medal in BSc for Topper in Botany honours (2008) Graduation Examination - Manipur University.
- Gold medal (2008) for securing the highest mark among all the honours papers in Manipur University.

- Manipuri Poems and Short stories published for DM College Journals (2006-2008).

- French learning course - A credit from University of Hyderabad (2009).

- Presented poster entitled "Iconic report on the Ecology and Environment of Loktak Lake, Manipur" at National Symposium on Role of Biology and Biotechnology in Conservation of Biodiversity and Sustainable Development, Gulbarga university (2009), Master project.

- Best poster award in 2nd Indian Biodiversity Congress (2012), Bengaluru for the poster; "Ecosystem services of *phoomdi* (floating islands), Loktak lake, Manipur, India".

- Post Graduate Diploma in Human Rights from Centre for Distance and Virtual Learning, University of Hyderabad (2015-).

PERSONAL INFORMATIONS

- **Father**: Maibam Deben Singh
- **Mother**: Maibam Inao Devi
- **Date of Birth**: 15-02-1988
- **Nationality**: Indian
- **State of Domicile**: Manipur
- **Qualification**: M.Sc
- **Hobbies**: Writing, gardening and philately.
- **Address**: Kyamgei Maning Leikai, M.U.Canchipur - 795003, Imphal.

List of Publications

1. **Maibam Dhanaraj Meitei**, Majeti Narasimha Vara Prasad. 2013. Lead (II) and cadmium (II) biosorption on *Spirodela polyrhiza* (L.) Schleiden biomass. **Journal of Environmental Chemical Engineering**. 1: 200-207.

2. **Maibam Dhanaraj Meitei**, Majeti Narasimha Vara Prasad. 2013. Phytotechnological applications of *phoomdi*, Loktak lake, Manipur, North-East India. **Current Science**. 105: 569-570.

3. **Maibam Dhanaraj Meitei**, Majeti Narasimha Vara Prasad. 2014. Adsorption of Cu (II), Mn (II) and Zn (II) by *Spirodela polyrhiza* (L.) Schleiden: Equilibrium, kinetic and thermodynamic studies. **Ecological Engineering**. 71: 308-317.

4. **Maibam Dhanaraj Meitei**, Majeti Narasimha Vara Prasad. 2014. *Phoomdi* - a unique plant biosystem of Loktak lake, Manipur, North-East India: Traditional and Ecological Knowledge. **Plant Biosystem**. 10.1080/11263504.2013.870250.

5. **Maibam Dhanaraj Meitei**, Abhay Kumar, M.N.V. Prasad, P. Malec, A. Waloszek, G. Maleva, K. Strzalka. 2014. Photosynthetic pigments and pigment-protein complexes of aquatic plants under heavy metal stress. In: Golovko, T.K, Gruszecki, W.I, Prasad, M.N.V, and Strzalka, K (Eds). **Photosynthetic pigments: chemical structure, biological function and ecology**. Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences, Syktyvkar, pages 319-334.

6. **Maibam Dhanaraj Meitei**, Majeti Narasimha Vara Prasad. 2015. Metal bioavailability in *phoomdi*-compost from Loktak lake (Ramsar site), Manipur, north-east India. In: J. Rinklebe, A.S. Knox, M. Paller (Eds). **Trace elements in temporary waterlogged soils and sediments**. Taylor & Francis. (accepted)

Abstracts published in seminar / conferences

1. **Maibam Dhanaraj Meitei,** Majeti Narasimha Vara Prasad. 2012. Ecosystem services of *phoomdi* (floating islands), Loktak lake, Manipur, India. In: 2nd Indian Biodiversity Congress. 9-12 December, Indian Institute of Sciences, Bengaluru, India. **Recipient of the best poster award (Poster)**

2. **Maibam Dhanaraj Meitei,** Majeti Narasimha Vara Prasad. 2013. Phytotechnological applications of *Spirodela polyrhiza* (L.) Schleiden, a *phoomdi* species of Loktak lake, Manipur, India. In. National Conference on Heavy Metals in the Environment. 28-30 November, Kottayam, India. **(Oral)**

3. **Maibam Dhanaraj Meitei,** Majeti Narasimha Vara Prasad. 2014. *Phoomdi* - a unique plant biosystem of Loktak lake, Manipur: Traditional Knowledge. In. 6th Plant Sciences Colloquium. 7th March. Hyderabad, India. **(Oral)**

4. **Maibam Dhanaraj Meitei,** Majeti Narasimha Vara Prasad. 2014. Compost production from *phoomdi* (floating islands) of Loktak lake (Ramsar site), Manipur, north-east India - *pros* and *cons*. In. IMETE Summer School program. 8-13 September. Ghent, Belgium. **(Poster)**

5. **Maibam Dhanaraj Meitei,** Majeti Narasimha Vara Prasad. 2014. Compost production from *phoomdi* (floating islands) of Loktak lake (Ramsar site), Manipur, north-east India - *pros* and *cons*. In. APAS Golden Jubilee Science Congress. 13-15 November. Hyderabad, India. **(Poster)**

6. **Maibam Dhanaraj Meitei**, Majeti Narasimha Vara Prasad. 2015. Metals in sediment, water and *phoomdi* of Loktak, India: Health risk assessment and Opportunities for phytoremediation. In. 13th International Conference On the Biogeochemistry of Trace Elements (ICOBTE). 12-16 July. Fukuoka, Japan. (**Oral**)