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Wetland Plants' Chemical Ecology for Iron of A Ramsar Site in An Indo-Burma Hotspot: *In-Situ* Bioaccumulation and Phytoremediation Implications

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ABSTRACT

Biogeochemistry of iron in wetlands is inextricably linked with chemical ecology of aquatic biota impacting the ecology of wetland plants and human health. Therefore, its bio-accumulation in plants is of extreme eco-technological relevance in quest of potential phytoremediation tools. To this end, the Fe concentrations in water and four invasive alien macrophytes (*Eichhornia crassipes*, *Lemna minor, Pistia stratiotes* and *Salvinia cucullata*) of Loktak lake (a Ramsar Site) were measured. Further, the outcome of the present research can assess the efficiency of these plants in Fe-phytoremediation. Concomitantly, to get an explicit Fe-macrophyte's chemical ecology scenario of Loktak lake, physico-chemical parameters as well as biodiversity attributes were also investigated. Results revealed that among the four plant species, *Pistia stratiotes* accumulated the highest amount of Fe concentration and thus act as the best bio-accumulator of Fe. Further, the extent of Fe bio-accumulation was as *Pistia stratiotes>Lemna minor>Eichhornia crassipes>Salvinia cucullata*. The study revealed the importance of the selected invasive wetland plants as the potential bio-agents of Fe accumulation.

INTRODUCTION

Global wetland systems offer immense ecosystem services to humanity with their unique ecological/environmental features and biodiversity of floating and/or submerged macrophytes (Prasad et al. 2002, Bassi et al. 2014**,** Rai et al. 2018). However, recent decades witnessed a paradigm shift in wetland's health in view of the abrupt increase in environmental contaminants. In this respect, it has been well known that natural wetlands demonstrate a majestic chemical ecology in remediating the metallic contaminants. Thus, elucidating the complex chemical and ecological interactions existing in the wetland systems can pave the way for phytoremediation, hence ecological restoration (Valderrama et al. 2013, Evangelou et al. 2013, Singh & Rai 2016, Al-Baldawi et al. 2017, Rai & Kim 2019, Rai et al. 2020).

Several macrophytes of Indian Ramsar wetlands, e.g. *Phragmites australis* (of Hokersar wetlands in Kashmir Himalaya) have been identified for their phytoremediation potential of heavy metals (Chatterjee et al. 2011, Ahmad et al. 2014, Khatun 2016)**.** Moreover, in this context elucidation of mechanisms leading to integrated restoration/eco-management of Kolleru lake (a Ramsar wetland in Andhra Pradesh, India) abridged the chemistry with ecological restoration (Sharma & Sujatha 2016). Thus, the heavy metal pollution of global wetland systems and their phytoremediation attained considerable research attention (Feng et al. 2017, Rai 2018; Rai 2019, Rai et al. 2019).

The excess use of agrochemicals (including fertilizer and plant nutrients) contaminate soil, water and food crops/vegetables with heavy metals (Rai 2008, Alhashemi et al. 2011, Fatima et al. 2014, Rai et al. 2020). Also, the bioavailability of heavy metals is remarkably influenced by the geochemical factors in global wetlands (Fairbrother 2007). Moreover, the sediments of the wetland effectively sequester hydrophobic chemical pollutants which are readily available from various pollutant discharges (Rai et al. 2018).

Natural wetlands are the important sink for environmental pollutants in view of their complex physico-chemical and biogeochemical mechanisms operating inside wetlands (Jiao et al. 2014, Xin et al. 2014, Zhang et al. 2016). It has been well known that aquatic/wetland plants absorb emerging contaminants and nutrients from the water/sediments of natural wetlands in view of being interfaces with them (Xue et al. 2010, Rai et al. 2019). For a low level of environmental contamination, the aquatic plants (mostly invasive aliens) also act as ecological indicators (Pratas et al. 2012, Borisova et al. 2014, Rai & Singh 2020). Therefore, in environmental biotechnology, the screening of macrophytes for phytoremediation of metallic contaminants has also received tremendous attention (Sood et al. 2012, Borisova et al. 2014, Rai et al. 2019).

Phyto-technological investigations in global biodiversity hotspots especially from North East Indian sites are rarely investigated for pollution ecology of Ramsar wetland plants.

The sewage is eventually drained into the Ramsar wetland (Loktak lake) may also result in eutrophication as well as contamination of the lake with heavy metals of extreme human health concerns (Meitei et al. 2016, Rai 2018a). In Indian prospect, 26 Ramsar wetlands of ecological importance are listed (out of the current total of 37 wetlands under the Ramsar convention), facing the environmental perturbations in one form or other (Tombi & Shyamananda 1994; Ramsar secretariat 2013, Bassi et al. 2014, Rai 2018). The scientific investigations of Ramsar sites tend to provide their current global status in order to fill up the knowledge gap pertaining to their metals biogeochemistry and aquatic plants ecology.

Iron (Fe) plays a crucial role in various bio-systems (plants as well as animals) due to its involvement in biochemical pathways, metabolic machinery, respiratory functions (being an integral component of haemoglobin) and in DNA synthesis (Ghaly et al. 2008, Rai et al. 2018a). Nevertheless, Fe is also toxic in higher concentration ranges to living entities of the wetland ecosystem and propensity to cause human health risks in case of metals transfer through food chain. In the present study, considering the all the stress caused by the Fe (as other metallic contaminants were negligible), the authors aim to analyse and determine the amount of Fe accumulation in aquatic bodies as well as selected aquatic plants such as *Eichhornia crassipes*, *Lemna minor, Pistia stratiotes* and *Salvinia cucullata* of Loktak lake to know the efficiency of their bio-accumulation/phytoremediation. Physicochemical parameters not only affect the concentrations of metallic contaminants but also creating unique relationships among and within the biotic and abiotic components of the ecosystem (APHA 2005, Rai 2010). Henceforth, chemical/physicochemical parameters and ecological attributes (phytosociology/biodiversity) were also studied to have an integrated chemical as well as the ecological approach. It is worth mentioning that these selected wetland plants are invasive aliens, perturbing the aquatic biodiversity. Nevertheless, their controlled utilization as contaminant's hyperaccumulators can assist their sustainable management, concomitantly, decontaminating the organic/inorganic pollutants from the environment (Rai & Kim 2019).

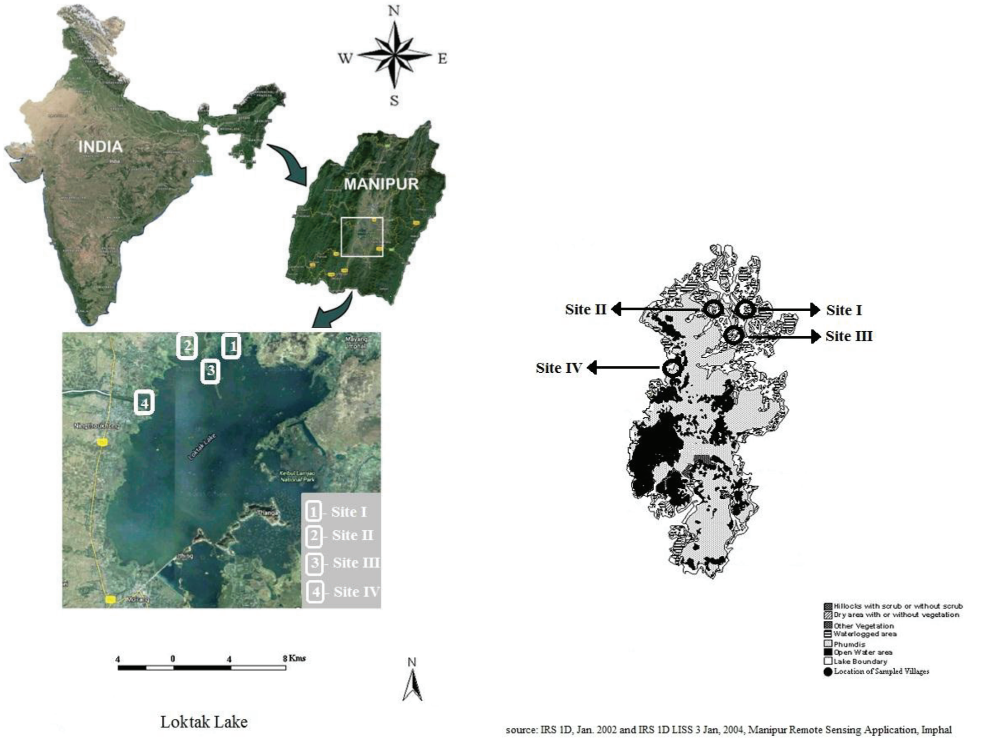
MATERIALS AND METHODS

Study Area

Loktak lake (Ramsar site; Latitude of 24º25'-24 º42' N and Longitude of 93º46'-93º55'E) lies in the middle of the state Manipur situated in the north-eastern part of India.

Sampling Sites

A total of four sampling sites were selected for analysis which is explicitly described in Fig. 1. The basis of the site selection was attributed to the source of pollution and other anthropo-



source: IRS 1D, Jan. 2002 and IRS 1D LISS 3 Jan, 2004, Manipur Remote Sensing Application, Imphal

Fig. 1: Study area and location of study in Manipur, North East India (an Indo-Burma hotspot region).

genic disturbances in the vicinity of this Ramsar lake. The Sites are as follows. Site I (Loktak Nambul vicinity); Site II (Loktak Nambol vicinity, Nambol river carries waste of the Bishenpur municipal area); Site III (Loktak Yangoi vicinity, located at the confluence of Yangoi river); Site IV (Loktak proper, near National Hydro Power Corporation Limited of Loktak lake).

Water samples in triplicate (in 2L polythene bottles) were collected in the morning between 6:30 to 9:30 a.m. in rainy, winter and summer seasons from August 2013 to July 2015, and immediately brought to the laboratory for analysis. In addition to physicochemical parameters, we confined our quest on Fe as it was the only metal recorded above the permissible limit in natural water and wetland plants (Singh & Rai 2016). Other hazardous heavy metals like Hg, Cr, Pb, Hg, As (a metalloid) and Zn were recorded in negligible/trace concentrations in water and wetland plants. Henceforth, this fact prompted us to carry out a detailed chemical and biological study in relation to screening the different wetland plants for their possible role in bio-accumulation and phytoremediation of iron (Fe).

Phytosociological Analysis

Vegetation analysis was carried out by following the standard methods as outlined in Misra (1968), Kershaw (1973) and Mueller-Dombois & Ellenberg (1974). Harvest methods were adopted for phytosociological analysis on the macrophytes and quadrats (1m × 1m) were used. Macrophytic diversity has been calculated using the following indices.

Iron Analysis of Water and Wetland Plants

The water samples were filtered through 45 µm syringe filter and metals were determined by Microwave Induced Plasma Atomic Emission Spectrophotometer (MP-AES: Agilent-4100) available in Central Instrumentation Laboratory (CIL), Mizoram University, India. The fresh macrophytes samples were weighed and kept in the oven for drying and temperature was maintained at 802°C for 24 hours. The dried plant samples were again weighed and crushed it into powder. The powdered plant samples were then digested using the di-acid method as mentioned elsewhere (APHA 2005).

RESULTS AND DISCUSSION

Iron Accumulation in Water

The Fe concentrations of water at different sites during different seasons were measured and presented in Table 1. As mentioned before, the chemical, as well as physical (physico-chemical/water quality) parameters, play a vital role in bio-availability of heavy metals in water and wetland plants (Rai 2010), therefore, it was duly monitored in conjunction with ecological studies. Fig. 2(a-f) explicitly describe the seasonal variations in different water quality parameters recorded during the study period.

Pertaining to the metal concentrations (analysed in the year 2013-15), the highest value of Fe concentration was measured as 0.17 $mg.L^{-1}$ at Site IV during winter season of 2014 followed by 0.15 $mg.L^{-1}$ at Site II and Site IV during the winter season and 0.13 $mg.L^{-1}$ at Site II during the winter season of the same year 2014. The lowest value of 0.01 $mg.L^{-1}$ was observed at Site I during the rainy season and summer season of 2014, as demonstrated clearly in Fig. 3. Seasonal variations revealed that Fe accumulation in lake water is higher during the winter season. However, comparatively low values were measured during the rainy season (attributed to dilution) and summer season for all the sampling periods.

Fe Accumulation in Plants

The four plant species samples were collected in triplicate from all the four sites in winter season. The trends of Fe concentrations are shown in Figs. 4-7. Results revealed that among the plants, the highest Fe concentration was measured 28.29 $mg.kg^{-1}$ in *Pistia stratiotes* at Site II followed by 13.01 $mg.kg^{-1}$ and 12.68 $mg.kg^{-1}$ in *Pistia stratiotes* at Site I and Site III, 12.74 $mg.kg^{-1}$ and 12.52 $mg.kg^{-1}$ in *Lemna minor* at Site I and Site II. The lowest value of 1.68 $mg.kg^{-1}$ was measured in *Salvinia cucullata* at Site I (Table 2). The highest value 28.29 $mg.kg^{-1}$ is higher than the permissible limit set by World Health Organisation (WHO), i.e. 20 $mg.kg^{-1}$. *Salvinia cucullata* has the lowest Fe as compared to the other plant species, i.e. *Eichhornia crassipes*, *Lemna minor* and *Pistia stratiotes*.

Table 1: Fe concentrations ($mg.L^{-1}$) of water from different study sites (average of three replicates).

Study Sites	2013-2014			2014-2015		
	Rainy	Winter	Summer	Rainy	Winter	Summer
Site I	0.02	0.07	0.03	0.01	0.08	0.01
Site II	0.08	0.13	0.1	0.07	0.15	0.09
Site III	0.04	0.1	0.06	0.06	0.12	0.03
Site IV	0.08	0.17	0.06	0.05	0.15	0.07

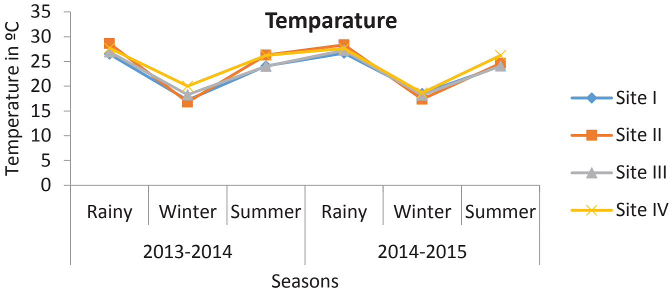


Fig. 2a

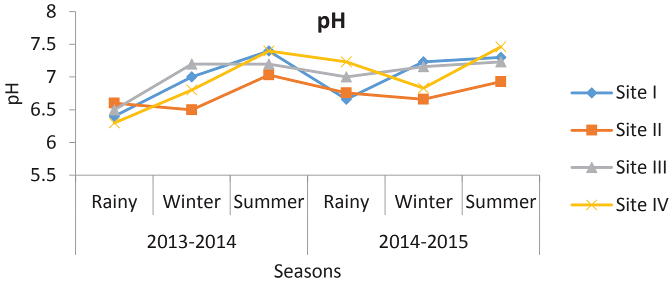


Fig. 2b

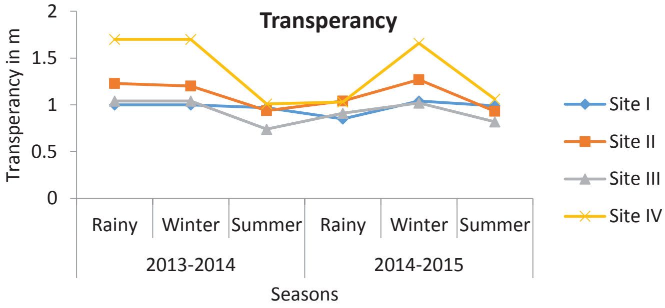
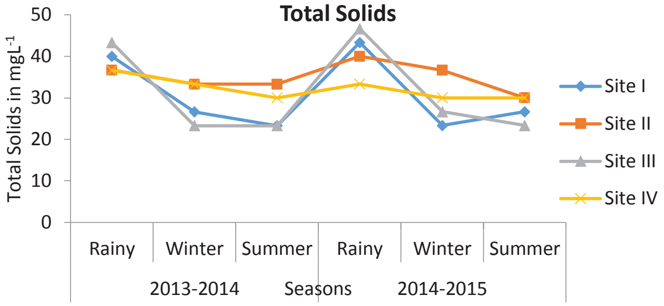


Fig.2c



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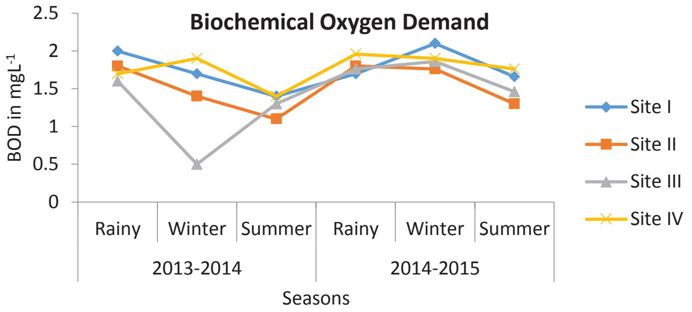

Fig. 2e

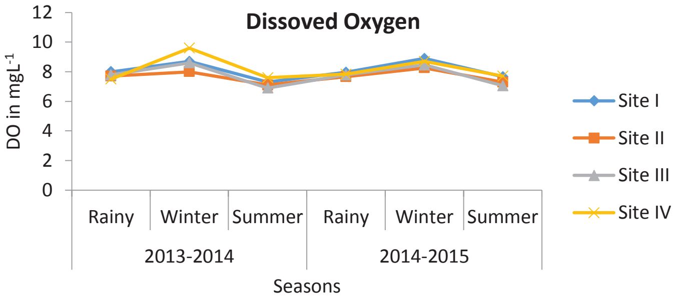

Fig. 2f

Fig. 2a-f: Seasonal variations in chemical/physicochemical/water quality parameters at all the four sites of the study area, i.e. Ramsar wetland (Loktak lake, N.E. India of biodiversity hot spot).

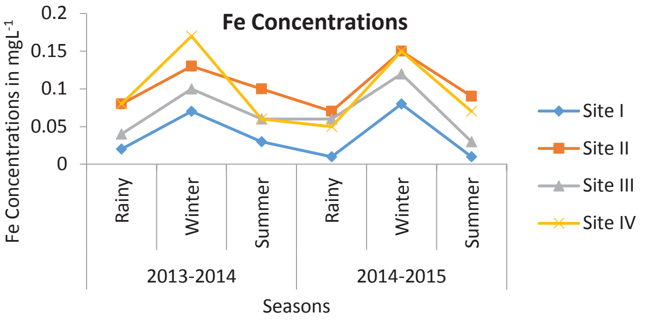

Fig. 3: Seasonal variations of Fe concentrations (in mg.L^{-1}) of water from different study sites.

Table 2: Fe concentrations (in mg.kg^{-1}) of plants from different study sites.

Name of the plants/macrophytes	Site I	Site II	Site III	Site IV
Eichhornia crassipes	0.72	9.77	9.07	0.53
Lemna minor	12.52	12.74	9.92	9.41
Pistia stratiotes	13.01	28.29	12.68	1.09
Salvinia cucullata	1.68	3.14	2.72	2.25

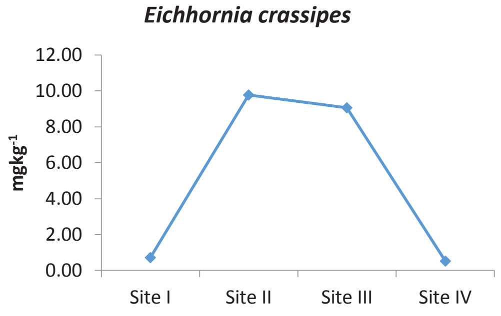

Fig. 4: Fe concentrations (in mg.kg^{-1}) in *Eichhornia crassipes* of different study sites.

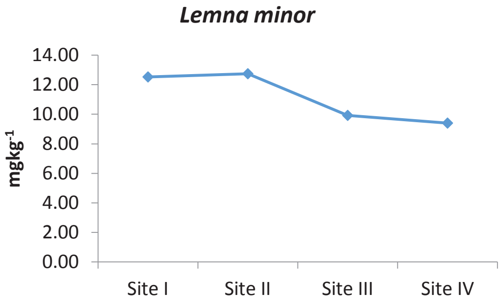

Fig. 5: Fe concentrations (in mg.kg^{-1}) in *Lemna minor* of different study sites.

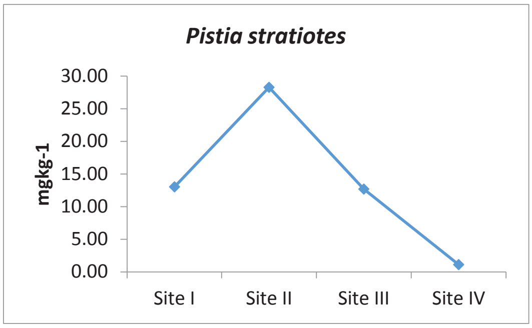

Fig. 6: F e concentrations (in mgkg^{-1}) in *Pistia stratiotes* of different study sites.

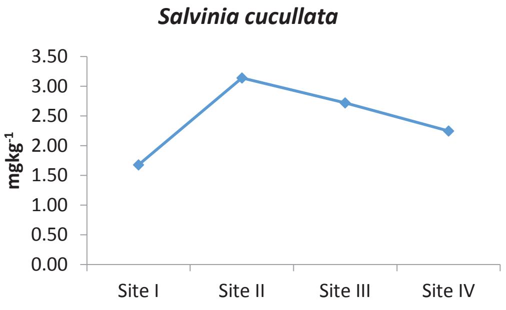

Fig.7: Fe concentrations (in mgkg^{-1}) in *Salvinia cucullata* of different study sites.

Table 3: Ecological/phytosociological attributes of wetland plants/macrophyte species in Ramsar (Loktak) lake.

Parameter	Site I	Site II	Site III	Site IV
Number of Families	8	11	9	15
Number of Genera	8	12	11	20
Number of Species	10	13	12	21
Simpson Index of Dominance	0.37	0.27	0.42	0.12
Shannon-Weiner Diversity Index	1.31	1.68	1.37	2.37

Table 4: Sorenson's Similarity Index between different sites of Ramsar (Loktak) lake.

	Site I	Site II	Site III	Site IV
Site I				
Site II	0.87			
Site III	0.73	0.72		
Site IV	0.65	0.71	0.61	

Table 5: Family-wise distribution of wetland plants/macrophyte species in Ramsar (Loktak) lake.

Sl. No.	Family	Site I	Site II	Site III	Site IV
1	Amaranthaceae	1	1	1	1
2	Apiaceae	-	-	-	1
3	Araceae	1	1	1	1
4	Asteraceae	-	1	-	-
5	Azollaceae	1	1	1	1
6	Ceratophyllaceae	1	1	-	1
7	Convolvulaceae	-	-	1	1
8	Hydrocharitaceae	1	1	-	2
9	Lemnaceae	2	2	2	2
10	Menyanthaceae	-	1	-	1
11	Nymphaeaceae	-	-	-	2
12	Poaceae	-	1	1	3
13	Polygonaceae	-	-	2	-
14	Pontederiaceae	1	1	1	1
15	Potamogetonaceae	-	-	-	-
16	Salviniaceae	2	2	2	2
17	Trapaceae	-	-	-	1

(-) Absent

From the present studies, it has been noted that *Pistia stratiotes* accumulate high amount of Fe concentration from the lake. The recorded trend of Fe bio-accumulation was as *Pistia stratiotes*>*Lemna minor*>*Eichhornia crassipes*>*Salvinia cucullata*. In this context, high concentrations of Fe in water samples might be attributed to the pollution caused by draining rivers and the domestic/urban waste from human settlements.

From the phytosociological/ecological studies of the different sites of Loktak lake, altogether a total of 24 wetland plant species belonging to 23 genera and 17 families were recorded. Of this, 10 species belonging to 8 genera and 8 families, 13 species belonging to 12 genera and 11 families, 12 species belonging to 11 genera and 9 families and 21 species belonging to 20 genera and 15 families were reported from Site I, Site II, Site III and Site IV respectively (Table 3). To this end, among 24 plants recorded, *Alternanthera philoxeroides* Griseb., *Azolla pinnata* Lam., *Eichhornia crassipes* Linn., *Lemna minor* Linn., *Pistia stratiotes* Linn.,*Salvinia cucullata* Roxb., *Salvinia natans* Hoffm., and *Spirodela polyrhiza* (Linn) Schleid. were the plants with higher density at all the sites. Biodiversity/phytosociological attributes related data of macrophyte/wetland plant species is calculated in the study (Table 4). Shannon-Weiner diversity index for macrophyte species was highest at the Site IV i.e. 2.37 and lowest in the Site I i.e. 1.31. However, a reverse trend in the results was observed in the case of the Simpson index of dominance. The Simpson index of dominance was maximum at Site III i.e. 0.42 and minimum at Site IV i.e. 0.12. Table 5 shows the present dominance and diversity of wetland plants in this Ramsar lake. As the Simpson's index of dominance values decreases, Shannon-Weiner diversity increased which is quite appropriate in ecological perspective.

Pertaining to Fe bio-accumulation/remediation, many studies have documented the Fe accumulation and ability of these wetland plants from wastewaters which are rich in nutrients (Singh & Rai 2016). Ghaly et al. (2008) studied the accumulation of Fe by different aquatic plants including broad leaved cattail, soft stem bulrush, soft rush and wool grass plants from the contaminated water.

P. stratiotes has also been extensively used for removal of heavy metals other than Fe (Rai 2018a). The removal of heavy metals by *P. stratiotes* in the laboratory was tested (Miretzky 2010). Lu et al. (2011) reported that *Pistia* is a hyper-accumulator of Cu, Fe, and Pb. Also, *Pistia stratiotes* was found to be the best phytoremediator for Cu and Pb in removing 66.5% and 70.7% of these metals (Lone et al. 2008). To this end, remarkable advances in instrumentation assisted metals analysis in wetland plants (Feng et al. 2017). However, to our best of knowledge, the present study is the first report on *Pistia stratiotes* as a better tool as an ecological indicator of Fe.

CONCLUSION

From the findings of the present study, it can be concluded that the water of the Loktak lake is contaminated with Fe to a reasonable extent and may further exacerbate in due course of time. However, the other heavy metals were well below the permissible limit in water sediments and biota. To this end, wetland plants can be a remarkable tool and this study revealed differential extent of Fe bio-accumulation among macrophytes (*Pistia stratiotes*>*Lemna minor*>*Eichhornia crassipes*>*Salvinia cucullata*). The present study can possibly be the first report on *Pistia stratiotes* as a better tool in relation to bio-accumulation of Fe. Henceforth, the wetland plants play an important role in indicating the Fe concentration of the water as well as the extent of accumulation in the plants itself. Interestingly, the studied macrophytes are actually invasive aliens, perturbing the aquatic ecology/biochemistry. Nevertheless, their controlled utilization as contaminant's hyperaccumulators can assist in their sustainable management, concomitantly, decontaminating the organic/inorganic pollutants from the total environment.

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