Applied Water Science (2022) 12:163
https://doi.org/10.1007/s13201-022-01681-9

ORIGINAL ARTICLE

In-situ anatomical and elemental response of aquatic macrophytes against nutrient enrichment in freshwater tropical lakes

Divya Dubey[1] · **Saroj Kumar**[1] · **Venkatesh Dutta**[1]

Received: 15 September 2021 / Accepted: 19 April 2022 / Published online: 20 May 2022

Abstract

Nutrient enrichment in lakes due to municipal wastewater discharge and agricultural run-off leads to excessive growth of algae and aquatic macrophytes leading to their altered trophic states. This paper presents the effect of wastewater-induced nutrient enrichment on the anatomical changes and elemental profiling in three common aquatic macrophytes of freshwater lakes in India's Central Gangetic Plain. It is observed that with increase in trophic state, biomineral depositions are seen in the leaf anatomy of aquatic macrophytes. Elemental variations in free-floating (*Eichhornia crassipes*), submerged (*Hydrilla verticillata*) and emergent (*Typha latifolia*) macrophytes collected from three different lakes with different catchment characteristics and trophic state using EDS (Energy-Dispersive X-Ray Spectroscopy) spectra show that with increasing trophic state, elemental constituent in the aquatic macrophytes also increases. The rhizome of *Eichhornia crassipes* showed the formation of calcium oxalate crystals in SEM (Scanning Electron Microscope) images and EDS analysis. Among macrophytes, floating and submerged macrophytes show a greater number of elemental constituents as compared to the emergent macrophytes. The findings of this study show that the anatomical and elemental responses of macrophytes are dependent both on the water quality and trophic state of the lakes. In-situ responses of macrophytes are based on their tolerance level against the pollution load and environmental changes. This study has important implications for understanding the response mechanism of macrophytes with changing water quality and increasing trophic state, which may help in proper management of freshwater ecosystem.

Keywords Freshwater lake · Nutrient enrichment · Trophic state index · Anatomical changes · Calcium oxalate crystal · Elemental variation

Introduction

Nutrient enrichment in lakes refers to the increase in nutrients, especially nitrogen and phosphorous due to anthropogenic activities that are responsible for the excessive growth of algae and aquatic macrophytes, thereby increasing the primary productivity of the lakes (Hampton et al. 2018). It disturbs the whole ecological balance of the lacustrine ecosystem by changing their trophic states (Elliott and Whitfield 2011; Qin et al. 2013; Dubey and Dutta 2020).

Aquatic macrophytes are important components that contribute to the primary productivity, stabilization, storage and cycling of nutrients within the lakes. Decline in submerged vegetation and increase in abundance of free-floating macrophytes are the most significant changes along the ecological gradient of the lakes (Jeppesen et al. 2012; Poikane et al. 2018; Dos Santos et al. 2020). Macrophytes are sensitive to eutrophication, water level fluctuations, acidification, shoreline modifications, recreation activities, navigation and biological invasion (Poikane et al. 2018). The growth and life cycle of free-floating macrophytes are mainly affected by the lake water chemistry, as they are in direct contact with lake water. Rooted submerged macrophytes are affected by both water and sediment chemistry as their bodies are inside the lake water and their roots derive nutrients from

---

✉ Venkatesh Dutta
dvenks@gmail.com

Divya Dubey
divyadubey.sai21@gmail.com

Saroj Kumar
sarojbhargava73@gmail.com

[1] Department of Environmental Science, School of Earth and Environmental Sciences (SEES), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India

the benthic region sediments (Krems et al. 2013; Reitsema et al. 2018). Submerged macrophytes show better dermal absorption characteristics because of the structure of their leaves having underdeveloped lamina and skin, supporting the fast exchange of matter with the environment (Maleva and Nekrasova 2004). The emergent macrophytes are least affected in comparison to free-floating and submerged macrophytes by the water chemistry of lakes as they derive their major nutrients from the benthic sediments (Short et al. 2016).

Some aquatic macrophytes that inhabit polluted or nutrient-rich waterbodies absorb pollutants to maintain homeostasis in their internal cellular organization (Marecik et al. 2006). They develop effective defense mechanisms for maintaining homeostasis either by avoiding the uptake of metal ions from entering into them or by stopping or immobilizing the metal ions in the cell membrane and by the production and activation of various organic acids, amino acids, antioxidant particles and enzymes in their body (Mishra et al. 2006). Many aquatic macrophytes are known to have phytoremediation potential. They are tolerant to a wide range of pollutants including nitrogen, phosphorus and heavy metals as they have the ability to accumulate these pollutants into their cells, thus resulting in a high level of pollution removal (Ghosh and Singh 2005). Macrophytes are used in biomonitoring of the lacustrine ecosystems, due to their ability to tolerate and accumulate large amounts of pollutants—sometimes without damaging themselves and sometimes with anatomical and elemental changes. Biomonitoring studies of the lake ecosystem are mainly done using floating and submerged macrophytes as indicators (Kelly and Witton 1998: Ali et al. 1999; Krems et al. 2013).

Studies of macrophytic responses to eutrophication exposure on anatomical and elemental changes have been done mostly (Zhu et al. 2018) in the laboratory or controlled experimental conditions with fewer field investigations (Zhu 2012). Changes in anatomical structure along with elemental variations are key strategies against the stressed aquatic environment (Zhu et al. 2018). Consequently, the objective of this study was to investigate the change in anatomy as well as elemental constituents in common aquatic macrophytes from three different freshwater lakes having different trophic states. Anatomical and elemental traits were monitored using SEM and EDS. The changes on adaxial surface of leaves of *Eichhornia crassipes* and *Hydrilla verticillata* were examined using SEM, including stomata structure, number and surface morphology. Further, elemental changes in *Eichhornia crassipes*, *Hydrilla verticillata* and *Typha latifolia* were evaluated for three different lakes with different water quality, trophic state and catchment characteristics using EDS. This allowed to study how elemental constituents in the macrophytes increased with increase in trophic state. This is the first in-situ study reporting the anatomical and elemental responses of aquatic macrophytes against wastewater-induced nutrient enrichment in the freshwater tropical lakes of the Central Gangetic Plains. This study will be helpful in understanding the macrophytic response which shall be helpful in proper management of freshwater ecosystem.

Methodology

Study area

Three freshwater lakes have been selected with different catchment characteristics and pollution stress under the same climatic conditions in the Central Gangetic Plain of Uttar Pradesh, India. Lake 1 (Samaspur lake or L1) from the rural catchment of the Salon village in Raebareli district, Lake 2 (Kathauta lake or L2) and Lake 3 (Haibatmau lake or L3) from urban catchments of Lucknow district of North India were selected (Sampling sites images are provided in Fig. 1 of supplementary data). Water samples and macrophytes samples of selected free-floating, submerged and emergent macrophytes were collected from each of the selected lakes. A brief description of these three lakes is provided in Table 1.

Collection of water sample and water quality analysis

Physico-chemical parameters of the lake water were analyzed using standard sampling procedures (APHA 2017). Sampling was conducted in the month of October 2018. Fourteen water quality parameters including temperature, pH, secchi depth (SD), total dissolved solid (TDS), total suspended solids (TSS), electrical conductivity (EC), dissolve oxygen (DO), biochemical oxygen demand (BOD), alkalinity, hardness, nitrate, nitrite, total phosphorous (TP) and Chlorophyll a were analyzed for each of the three lakes (Lumb et al. 2011) (as given in Table 1 of supplementary data). Temp, pH, SD, TDS and DO were monitored onsite, whereas rests of the parameters were analyzed in the university laboratory. Water samples were collected from six sites of the selected lakes in triplets and the means along with standard deviation (SD) are provided in Table 2. A flowchart depicting study design and methodology is provided in Fig. 1.

Assessment of trophic state of selected lakes

The most commonly used classical method for the classification of lakes in different trophic states related to biomass is based on the productivity of lakes and is known as trophic state index (TSI). It is defined as the total weight of the

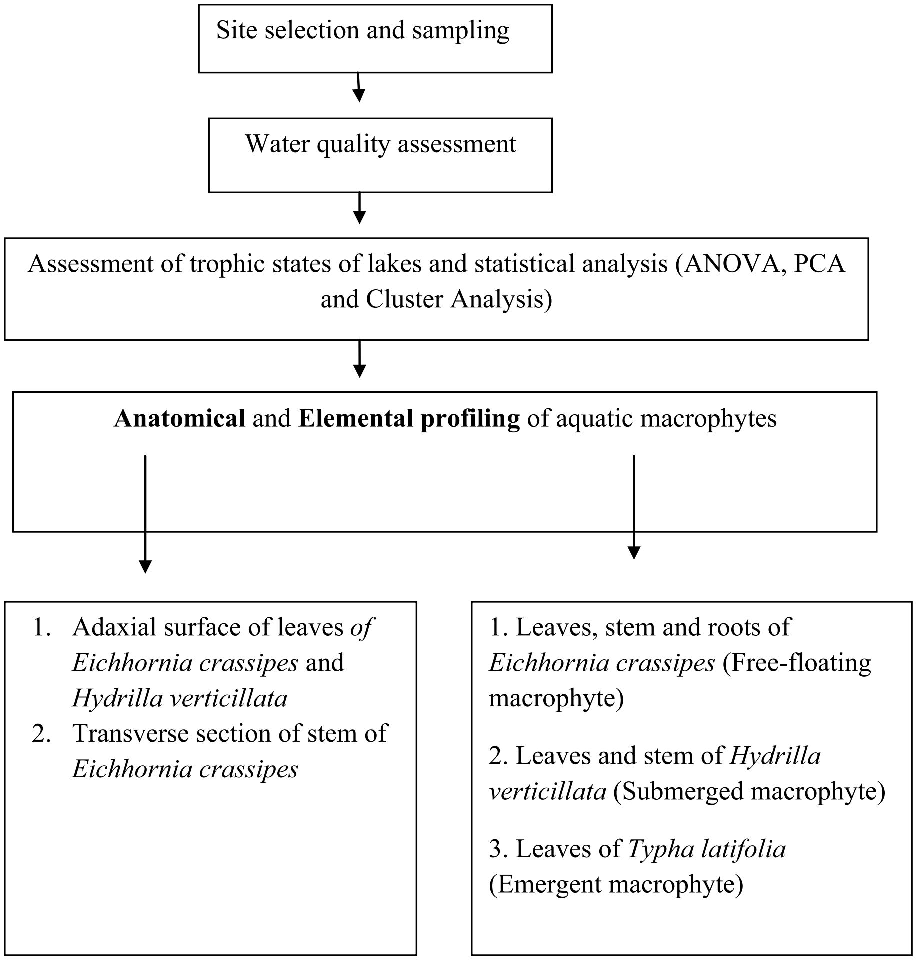


Fig. 1 Flowchart depicting study design and methodology

biomass in a lake at a precise time and location (Carlson 1977). TSI is a valuable tool in identifying trophic state and productivity of the lacustrine ecosystem (Hillsborough 2008). It also indicates the overall health of the lacustrine ecosystem in terms of its nutrient status and the biomass it supports. This method uses lakes transparency (Secchi depth), total phosphorus and chlorophyll *a* for calculating their trophic state. The Transparency of the lake was measured using Secchi disk of diameter 20 cm and values are expressed in meters. Reading was taken at the maximum depth at which disk could be seen when lowered into the lake. Total phosphorous was analyzed by the method described in (APHA 2017) and chlorophyll *a* was estimated by the Acetone method using a spectrophotometer (Marker 1972). Chlorophyll was extracted in 80% acetone and the absorbance at 660 and 620 nm was recorded. The amount of chlorophyll was calculated using the absorption co-efficient. TSI values lie between 0 and 100, which categorizes lakes in different trophic states (Carlson and Simpson 1996).

The formulae used for calculating the Carlson trophic state index (TSI) for chlorophyll *a*, Secchi depth and total phosphorous (Carlson 1977) are mentioned below:

1. $\text{TSI (Chl-}a\text{)} = 9.81 \ln \text{Chlorophyll-}a\ (\mu\text{g/L}) + 30.6$
2. $\text{TSI (SD)} = 60 - 14.41 \ln \text{Secchi depth (Meters)}$
3. $\text{TSI (TP)} = 14.42 \ln \text{Total phosphorous } (\mu\text{g/l}) + 4.15$

Where Chl-*a* is (Chlorophyll a), SD is Secchi depth and TP is total phosphorous.

The overall Carlson Trophic State of the lakes was calculated using the modified Carlson and Simpson (1996) formula:

$$\text{TSI (Overall)} = [\text{TSI (SD)} + \text{TSI (TP)} + \text{TSI (Chl} - a)]/3$$

Collection of samples of macrophytes

Areas measuring 5 m × 5 m were selected in the selected three lakes from which free-floating *Eichhornia crassipes*

Table 1 Brief descriptions of the three selected freshwater lakes

Lakes	Latitude and longitude	Morphometry	Major characteristics
L1 (Samaspur Lake)	Latitude 25° 57′–26° 01′ N and longitude 81° 21′–81° 25′ E	This lake has an area of 780 hectares. Perimeter is 18.05 km and elevation is 357.8 feet above mean sea level (amsl)	Six lakes, namely Mamuni, Gorwa, Hasanpur, Nakganj, Rohinia, and Bisaiya choti together constitute Samaspur lakes. State government established them as Bird Sanctuary in the year 1987. In 2020, this wetland has been included under ramsar site by the UNESCO
L2 (Kathauta Lake)	26.86° N latitude and 81.03° E longitude	Fragmented into two segments, large segment has an area of 59.09 acres and perimeter of 2.79 km while small segment has an area of 34.33 acres and perimeter of 1.14 km, elevation is 372 feet amsl	Larger segment of the lake is used for supplying water to the neighboring colonies. Sharda canal from Sharda river feeds water into this segment. Smaller segment of the lake is in natural condition which supports various species of flora and fauna
L3 (Haibatmau Lake)	26.75° N latitude and 80.94° E longitude	This lake has an area of 13.14 acres, perimeter of 2.04 km and elevation of 400.2 feet amsl	A major portion of the littoral area is encroached by urban settlements and badly impacted by various anthropogenic activities including discharge of municipal wastewater. Lake surface is majorly covered with *Eichhornia crassipes*

and submerged *Hydrilla verticillata* were collected for morpho-anatomical and elemental profiling of the plant. Emergent macrophytes were collected from the littoral area of the lakes. After collection of the macrophytes, they were washed with distilled water to remove the attached debris and unwanted particles attached with leaves, stem and roots for further microscopy and SEM and EDS analysis.

SEM of macrophytes

For SEM analysis, macrophyte samples of 2–4 mm were washed in 0.1 M phosphate buffer three times each for 15 min at 4 °C to remove the unreactive fixative. The plant material was then dehydrated with 30, 50, 70, 90, 95 and 100% solutions of acetone. Each dehydration was carried out for 30 min at 4 °C (Pathan 2008). Plant samples were mounted onto the aluminum stubs and carbon tape. The samples were then coated with palladium using a sputter-coater to make them conductive. The specimens were observed using a scanning electron microscope (Model JSM-6490LV, Manufacturer Jeol Japan).

EDS of macrophytes

Eichhornia crassipes, *Hydrilla verticillata* and *Typha latifolia* were collected from the selected areas of 5 m×5 m. Leaves, stems and roots of *Eichhornia crassipes*, leaves and stems of *Hydrilla verticillata* and leaves of *Typha latifolia* were washed with distilled water and samples were sun-dried for 15 days. Fine powders of the dried samples of the various vegetative parts of the macrophytes of different lakes were used for elemental analysis using EDS (OXFORD INCA X- act model 51-ADD0013 having resolution at 5.9 keV).

Statistical analysis

The means and standard deviations of the water quality parameters were calculated by using Microsoft Office Excel®. ANOVA and correlation analysis were performed using SPSS 20 software. Principal Component Analysis (PCA) and Cluster Analysis (CA) were performed using PAST 3.0 software (Hammer et al. 2001). CA employed Euclidean distance and non-weighted clustering using arithmetic mean (UPGMA) for various water quality parameters.

Results and discussion

Water quality of lakes and pollution stress

Water quality of the selected lakes was analyzed for various physico-chemical parameter such as Temp, pH, Secchi depth, TSS (Total Suspended Solids), EC (Electrical

Table 2 Water quality and trophic state of lakes during post-monsoon sampling

S. No	Parameters	L1	L2	L3
1	Temp (°C)	25.67 ± 0.58^{a}	30.33 ± 0.58^{b}	29.33 ± 0.58^{b}
2	pH	8.33 ± 0.15^{b}	7.2 ± 0.20^{a}	7.07 ± 0.12^{a}
3	Secchi depth (m)	0.67 ± 0.06^{c}	0.53 ± 0.01^{b}	0.38 ± 0.03^{a}
4	TDS(ppm)	176 ± 4.58^{a}	165 ± 5.03^{a}	306 ± 5.29^{b}
5	TSS (mg/l)	322.33 ± 2.52^{a}	625.67 ± 4.04^{b}	664 ± 5.29^{c}
6	EC (µScm-^{1})	277 ± 2.65^{b}	257.67 ± 2.52^{a}	485.67 ± 8.14^{c}
7	DO (mg/l)	5.37 ± 0.15^{b}	3.87 ± 0.12^{a}	3.67 ± 0.29^{a}
8	BOD (mg/l)	14.58 ± 0.52^{a}	41.33 ± 1.15^{b}	43.33 ± 2.89^{b}
9	Alkalinity (mg/l)	32.08 ± 2.60^{a}	122.67 ± 2.52^{b}	211.33 ± 3.21^{c}
10	Hardness (mg/l)	182 ± 2.65^{a}	194.67 ± 5.03^{b}	257.33 ± 2.52^{c}
11	Nitrate (mg/l)	3.53 ± 0.06^{a}	4.53 ± 0.42^{b}	9.75 ± 0.25^{c}
12	Nitrite (mg/l)	0.93 ± 0.07^{a}	2.29 ± 0.21^{b}	4.97 ± 0.02^{c}
13	Total Phosphorous (mg/l)	1.58 ± 0.04^{a}	7.35 ± 0.44^{c}	4.35 ± 0.56^{b}
14	Chlorophyll a (µg/l)	0.06 ± 0.01^{a}	2.67 ± 0.02^{c}	0.94 ± 0.06^{b}
	TSI calculation	26.51(Oligotrophic)	47.57(Mesotrophic)	43.09 (Mesotrophic)

Different alphabetical letters as superscripts specify significant differences between water quality parameter of the selected three lakes at $p < 0.05$

Conductivity), DO (Dissolved Oxygen), BOD (Biological Oxygen Demand), Alkalinity, Hardness, Nitrate, Nitrite, Chlorophyll a. The mean of water quality parameters of six sampling sites of Lake 1 (L1)], Lake 2 (L2) and Lake 3 (L3) is provided in Table 2. During the sampling period, Lake 2 was fragmented into two segments for cultivation of *Trapa natans* and fishing. In the fishing segment, the lake was found to have mesotrophic water quality and high algal growth. Lake 1 selected for the study is under the protection of the Uttar Pradesh Forest Department and within this lake point and non-point sources of pollution were regulated and monitored. Lake 1 was found to be oligotrophic, with a TSI value of 26.51, whereas Lake 2 and 3 were found to be in mesotrophic condition during the time of sampling, having TSI values of 47.57 and 43.09, respectively. The results of water quality analysis and trophic states along with ANOVA analysis of all the three lakes are provided in Table 2. The water quality parameters showed significant differences among the selected three lakes.

Principal component analysis (PCA) and cluster analysis (CA)

The result of analysis of the 14 physicochemical parameters was also subjected to multivariate exploratory principal component analysis (PCA). PCA showed that 99.99% of data variation is explained by two components. Component 1 accounts for 80.61% variation and Component 2 accounts for 19.38% variation. According to the rotated matrix component, Component 1 has main variables as Total Suspended Solids (TSS), BOD, Alkalinity, Hardness. According to the loading value of PCA, Component 2 was heavily influenced by TDS (Total Dissolved Solids) and Electrical Conductivity (EC) according to the loading value of PCA (Fig. 1 Supplementary file Table 1). The data show that the main vectors that influence component 1 are directly related to the degraded water quality of Lake 2 and Lake 3, which are in mesotrophic condition. High Electrical Conductivity (EC), Total dissolved solids (TDS), Hardness, Alkalinity, Total suspended solids (TSS) indicate the major effects of anthropogenic activities on Lake 2 and Lake 3 as shown in (Fig. 2). This finding confirms the utility of PCA in the context of environmental parameters, specifically related to water quality of the lakes.

In addition to PCA, Cluster analysis using Euclidean distance and non-weighted clustering using arithmetic mean (UPGMA) was conducted using PAST 3.0® software analyzing 14 physico-chemical parameters after the elimination of variables with commonality values lower than 0.6. The phenogram generated is shown in Fig. 3. The phenogram shows two distinct groups separating oligotrophic Lake 1 and mesotrophic lakes 2 and 3 as seen in Fig. 2.

Anatomical analysis of leaves of floating and submerged macrophytes using scanning electron microscope (SEM)

SEM analysis of adaxial surface of leaves of *Eichhornia crassipes* and *Hydrilla verticillata* from the selected lakes was conducted to analyze stomatal structure and distribution on the leaf structures collected. In mesotrophic lake (L3), submerged macrophytes *Hydrilla verticillata* were not found, due to overgrowth of free-floating macrophytes that affected the pelagic submerged floral diversity. Changes in

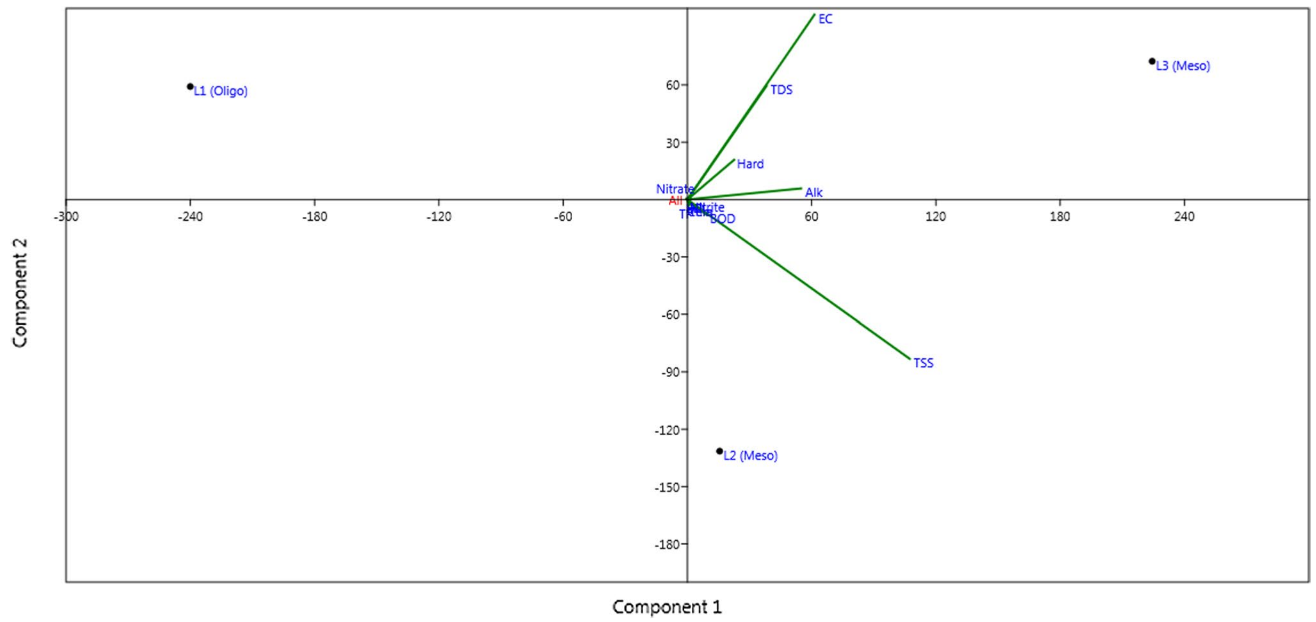


Fig. 2 Principal Component Analysis (PCA) of water from the selected lakes (i.e., Lake 1 = L1 (Oligo), Lake 2 = L2 (Meso) and Lake 3 = L3(Meso))

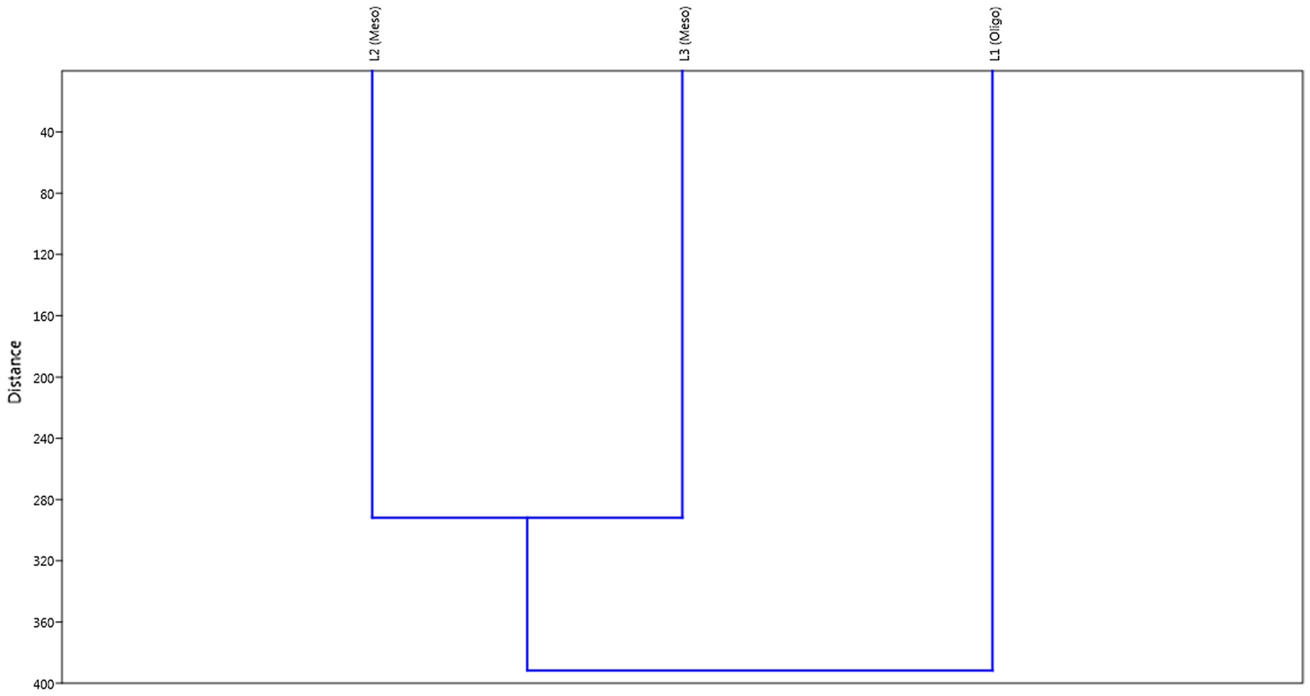


Fig. 3 Cluster Analysis of water quality parameters from the selected lakes (i.e., Lake 1 = L1 (Oligo), Lake 2 = L2 (Meso) and Lake 3 = L3 (Meso))

macrophytic communities have been reported elsewhere due to anthropogenic factors, leading to overgrowth of exotic invasive macrophytes and extinction of submerged macrophytes (Poikane et al. 2018).

Adaxial surfaces of *Eichhornia crassipes* of Lake 1 (Samaspur Lake) show the normal anatomy with proper shaped and uniformly distributed stomata along with few deposits of biominerals on the surfaces and around the stomata, In contrast, the adaxial surfaces of the leaves of the

Lake 2 (Kathauta) and Lake 3 (Haibatmau) showed an abundant deposition of biominerals on the leaves and around the stomata. In Lake 3 (Haibatmau) non-uniform distributions of stomata were reported on the adaxial surface of *Eichhornia crassipes* leaves.

The biomineral deposition on the adaxial surface and around the stomata of the leaves of *Eichhornia crassipes* seen in SEM images of the mesotrophic lakes L2 and L3 clearly indicates that they are transported from lake water through roots and that during the process of transpiration they are deposited around the stomata of leaves. High water hardness in Lake 2 and 3 (as shown in Table 2) correlated with the biomineral deposition. This shows that with increase in trophic state and deteriorating water quality, the anatomy of the leaf surface is affected with biominerals depositions. The shape, size and number of the stomata vary among the leaves of macrophytes present within the same trophic states as seen in Lake 2 and 3.

A variety of adaptations are developed in macrophytes tolerant to nutrient-enriched conditions, resulting in altered leaf characteristics to cope up with various environmental constraints. In tolerant macrophytes, there is often deposition of different inorganic salt on the leaf surface due to inorganic salt and nutrient excretion from glandular cells during the process of transpiration (Grašič et al. 2017). Macrophytes in high nutrient and salt conditions have developed a variety of adaptations with multiple functions that enable them to cope with environmental constraints (Flowers et al. 2015). In vascular aquatic plants, the main adaptive mechanism restricts the intake of excessive nutrients in the cell cytoplasm and in various other mechanism which relies on the leaf or stem succulence (Butnik et al. 2001) which affects the water management and cation concentrations, and manages nutrient redistribution within the different plant macrophytic organs (Flowers and Colmer 2008; Munns and Tester 2008). Excess nutrients are released through the leaf tissues into the atmosphere in volatile form (Larcher 2003). The strategy of macrophytes for salt and nutrient elimination depends on the concentration of nutrients in the lake water and is also based on the species-specific potential for this particular mechanism (Katschnig et al. 2013). Some of these adaptations in aquatic macrophytes such as epidermal structures, nutrient and salt excretion can alter leaf morphology and anatomy significantly (Grašič et al. 2017).

As a result of their constant submergence in polluted water, submerged macrophytes are highly affected by the water quality of the lake as compared to free-floating macrophytes, (Jamnická et al. 2006). In SEM analysis of stomata deficient, adaxial surfaces of *Hydrilla verticillata* leaves showed the deposition of biominerals. High deposition is observed in *Hydrilla verticillata* leaves of mesotrophic lakes (L2 and L3) (Supplementary Figs. 3 and 4) as compared to *Hydrilla leaf* surface of oligotrophic lake (L1) (Supplementary material Fig. 2). The water quality analysis showed maximum total suspended solids in the mesotrophic Lake 2 and 3 as compared to oligotrophic Lake 1 as shown in Table 2 which was further verified by PCA analysis (Fig. 1). Anatomical changes in leaves of *Eichhornia crassipes* and *Hydrilla verticillata* are shown in Figs. 2, 3 and 4 of supplementary material (Figs. 4, 5).

Anatomical study of rhizome of floating macrophytes *Eichhornia crassipes*

Transverse Sections (TS) of the rhizome of *Eichhornia crassipes* of all the three selected lakes were analyzed under the research microscope and results are discussed below. The effects of nutrient enrichment on the rhizome anatomy of free-floating macrophytes are clear as they are responsible for the transport of minerals from the lake water to the upper macrophytic body of the plant.

Eichhornia crassipes from oligotrophic lake 1

TS of the *Eichhornia crassipes* of the oligotrophic Lake 1 show the presence of colorful crystals in the aerenchyma of the rhizome (Fig. 3[SA] [SB] [SC]). Some crystals were seen attached to the aerenchyma wall (Fig. 3[SD]). These depositions were unusual, as they were not reported in the two mesotrophic lakes. SEM and EDS analysis was carried out to determine the chemical composition and morphology of these crystals. The reasons for the variation in the size and color of these depositions (Fig. 3 [SA] [SB] [SC] [SD]) are not clear and have not been reported previously in the literature yet.

Eichhornia crassipes from mesotrophic lake 2

The invasive species *Eichhornia crassipes* was prominently present in the mesotrophic lake (L2). TS of the *Eichhornia* rhizome showed a normal anatomy under the research microscope (Fig. 3 [KA] [KB] [KC] [KD]), which indicates that they have developed tolerance mechanisms which resists anatomical changes (Bianchini et al. 2008). Tolerance in aquatic macrophytes is mainly determined from its transport mechanism across tonoplast and plasma membranes. The ecophysiological responses of aquatic plants help in their tolerance mechanisms (Tanwir et al. 2020). Defense mechanism activation against chelating substances and oxidative stress help in preventing biological damage (de Souza Reis et al. 2020). The ability to resist changes is associated with the development of intercellular strategies and different

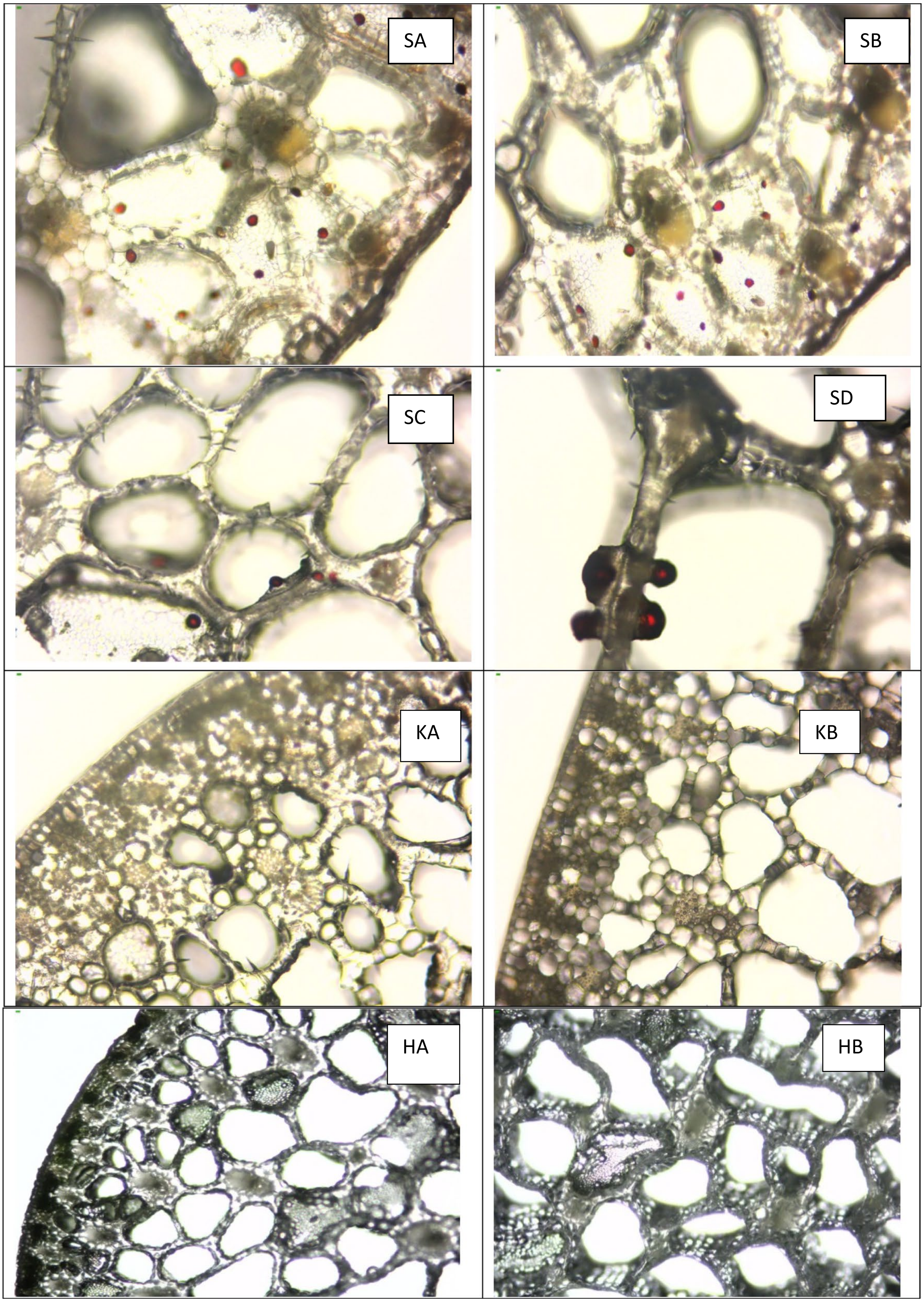


Fig. 4 [SA] [SB] [SC] [SD] Transverse section of rhizome of the *Eichhornia crassipes* of the oligotrophic lake (Lake 1) showing the colorful crystals. [KA][KB][KC][KD] TS of rhizome of the *Eichhornia crassipes* from different sites of the mesotrophic lake (Lake 2) [HA] [HB]—TS of rhizome of the *Eichhornia crassipes* of the mesotrophic lake (Lake 3) as seen under the research microscope

ecotypes dealing with the tolerance level in *Eichhornia crassipes* (de Souza et al. 2021).

Eichhornia crassipes from mesotrophic lake L3

TS of *Eichhornia crassipes* collected from mesotrophic Lake (L2) shows the normal anatomical structure shown in Fig. 3 [HA] [HB].

SEM and EDS analysis of rhizome of *Eichhornia crassipes* of oligotrophic lake 1 showing crystal formation

SEM analysis confirms the presence of crystals in the *Eichhornia* rhizome and EDS spectra confirm the presence of calcium oxalate and calcium carbonate crystals. Biomineralization phenomena are commonly observed in aquatic plants (Foster 1956; Franceschi and Horner 1980; Franceschi and Horner 1980; Franceschi and Nakata 2005; Borrelli et al. 2011). Calcium oxalate and calcium carbonate crystals are commonly found within idioblast near veins (Frank 1967; Cuéllar-Cruz et al. 2020). In *Eichhornia crassipes* calcium oxalate crystals are also found in the aerenchyma tissue (Kausch and Horner 1981; Xu et al. 2011; Cai et al. 2018; Cuéllar-Cruz et al. 2020).

In this study, calcium oxalate and calcium carbonate crystal formation in macrophytes was greater in the lesser-polluted oligotrophic lake (L1) compared to the two mesotrophic lakes (Lake L2 and L3). This shows that increasing trophic state and nutrient enrichment is not responsible for the formation of calcium oxalate crystals instead, crystal formation is due to high pH and alkalinity and lake morphometry is responsible for crystal formation within lake (L1) in the aquatic macrophytes (Downing 2010, Anderson et al. 2019, Cuevas et al. 2020). Water quality of oligotrophic lake (L1) shows high pH and low alkalinity. It seems that biomineralization and calcification in aquatic plants are brought about by shifting the inorganic carbon equilibrium in the lake ecosystem. The hardness of water within the lake L1 is the lowest among all the three lakes, indicating that biomineralization in aquatic plants is closely linked to photosynthetic inorganic carbon metabolism and less to calcium fluxes and calcium metabolism (Borowitzka 1984; Gwenzi 2019; Karabourniotis et al. 2020).

Role of biomineralization through aquatic macrophytes in carbon sink

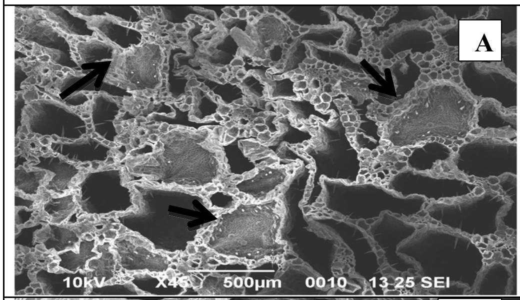
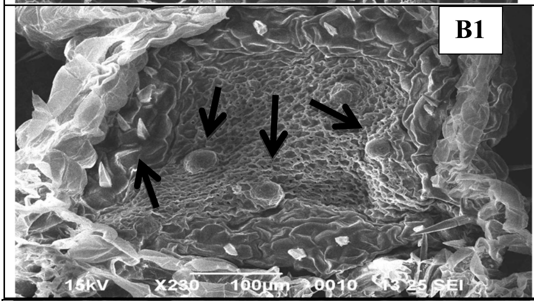
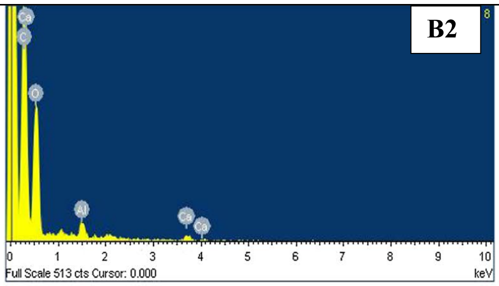
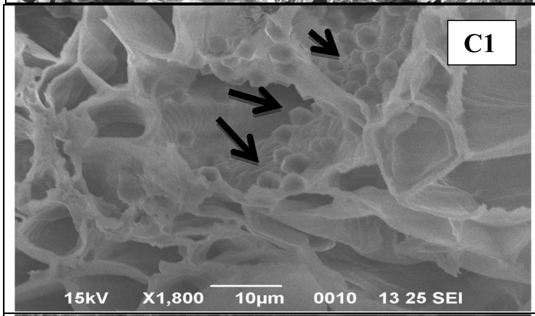
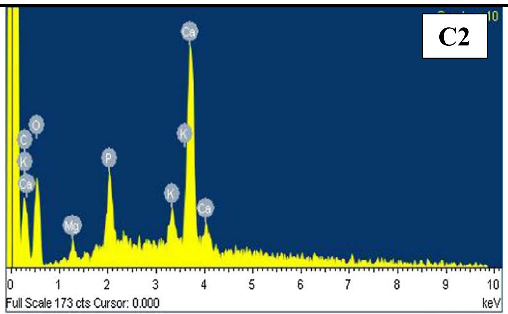
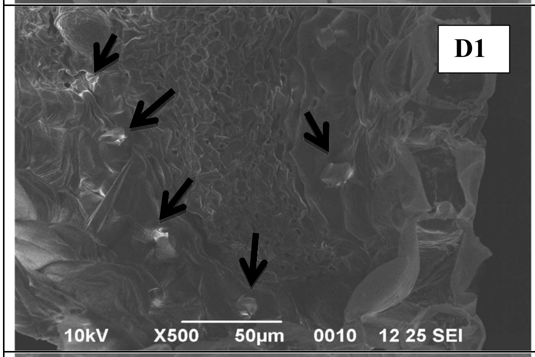
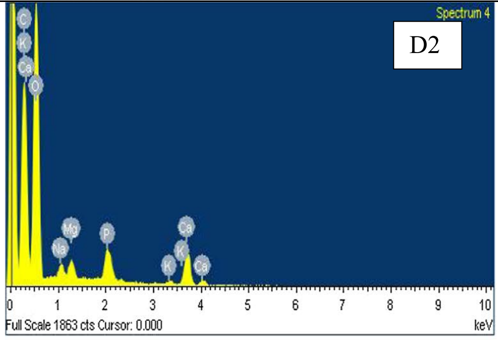
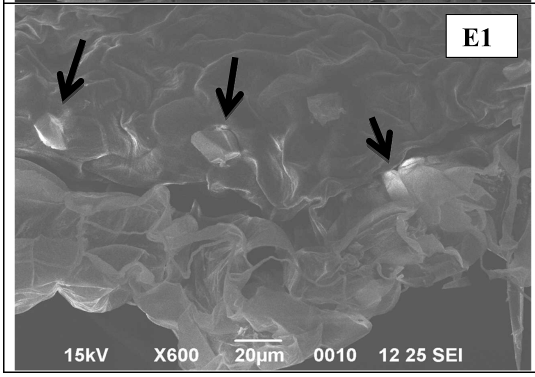
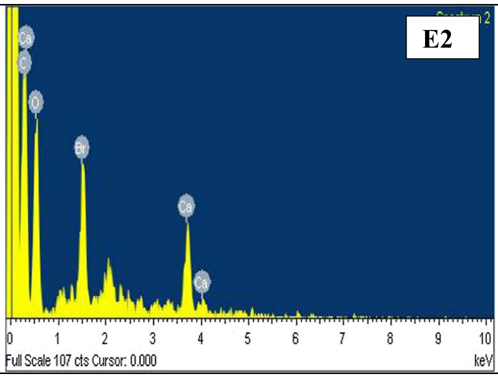
Research in lacustrine ecosystem carbon dynamics and primary productivity often overlooks calcification in aquatic macrophytes that supports photosynthesis by making protons and forming carbon dioxide. Calcification causes higher decrease in a dissolved inorganic carbon (DIC) and prevents detrimental pH increase within aquatic ecosystem (Anderson et al. 2019).

$$\mathrm{HCO_3^-} + \mathrm{H_2O} \rightarrow \mathrm{CH_2O} + \mathrm{OH^-} + \mathrm{O_2} \qquad (1)$$

$$\mathrm{Ca^{2+}} + 2\mathrm{HCO_3^-} + 2\mathrm{H_2O}^{-}\mathrm{CaCO_3}\downarrow + \mathrm{CH_2O} + \mathrm{O_2} \qquad (2)$$

The availability of CO_2 drops both by direct uptake of CO_2 directly and by increasing the pH which accompanies photosynthesis. Increasing pH in the lake ecosystem changes the balance between all species of inorganic carbon which includes carbon dioxide (CO_2), bicarbonate ($HCO_3{}^{-}$) and carbonate ($CO_3{}^{2-)}$.Changes in form occur as CO_2 is transformed to $HCO_3{}^{-}$ and eventually to $CO_3{}^{2-}$ as seen in Eqs. 1 and 2. This conversion challenges the ability of aquatic macrophytes to acquire CO_2 for photosynthesis and uses carbon dioxide and bicarbonate required for their growth. Many aquatic macrophytes overcome this challenge by the process of calcification; that is by the formation of the $CaCO_3$ crystals that accompany CO_2 release, which help in meeting the inorganic carbon demands for photosynthesis in alkaline water (McConnaughey and Whelan 1997).

The coupling of photosynthesis and calcification seen in Eq. 2 is pH neutral. As a result photosynthesis can proceed without increase in pH (Sand-Jensen 1983, van den Berg et al. 2002., Sand-Jensen et al. 2018). The importance of calcification for sustaining photosynthesis has been documented in many freshwater macrophytes (Sand-Jensen et al. 2018), where it accompanies carbonate dissolution and carbon precipitation (Andersen et al. 2016). Calcification can occur spontaneously in the water column when regular photosynthesis (Eq. 1) drives pH upwards, converts bicarbonate (HCO3^{-}) to Carbonate (CO_3^{2-}) and induces formation of small calcite crystals when carbonate solubility is exceeded (Kalff 2002). It accompanies HCO_3^{-}utilization by aquatic macrophytes (Eq. 2) that precipitate carbonate in alkaline bands along cell surfaces by active calcium extrusion (McConnaughey 1991; Nõges et al. 2016). Shallow lakes which are rich in submerged macrophytes may accomplish these ideal conditions because they stratify into distinct layers on a daily basis. Stratification separates the upper photic epilimnion from the lower aphotic hypolimnion. The epilimnion is characterized by intensive photosynthesis, calcification and

SEM images of *Eihchornia crassipes* rhizome	**EDS result of the adjacent images which confirm calcium oxalate crystal in the *Eichhornia crassipes***
	TS of *Eichhornia crassipes* of Samaspur Lake (L1) at 45-500µm showing scattered calcium oxalate and calcium carbonate crystals in the arenchyma of the rhizome
	
	
	
	

◂**Fig. 5** Scanning electron microscope (SEM) and Energy-dispersive X-ray spectroscopy (EDS) of rhizome of *Eichhornia crassipes* showing calcium oxalate and calcium carbonate crystals due to biomineralization **A** SEM of the transverse section of the rhizome of the *Eichhornia crassipes* of oligotrophic Lake 1 at 450–500 µm magnification **B1** SEM images showing calcium oxalate crystal at 280–100 µm magnification **B2** EDS spectrum of the B1 sample confirming calcium oxalate crystals **C1** SEM images showing calcium oxalate crystal *at* 1,800–10 µm magnification **C2** EDS spectrum of the sample C1 **D1** SEM images showing shining calcium oxalate crystal in the rhizome at 500–50 µm magnification **D2** EDS spectra of sample D1 **E1** SEM images showing calcium oxalate at 20 µm magnification **E2** EDS spectra of the E1 sample [Note- Arrow in the images shows the deposition of calcium carbonate crystals]

dissolve inorganic carbon, whereas the aphotic hypolimnion is characterized by intensive respiration and high CO_2 concentration conducive to carbonate dissolution and dissolve inorganic carbon regeneration (Andersen et al. 2017). In a shallow water column, nocturnal surface cooling is sufficient to break the stratification by convection and allow DIC from bottom waters to mix with surface waters and support photosynthesis (Martinsen et al. 2019).

Calcification plays an important role in photosynthesis and carbon dynamics of aquatic ecosystems. Photosynthetic experiments with macrophytes confirmed that calcification rates were low at pH 7.5, while similar rates of calcification and carbon assimilation were attained when external pH approached 9.5 as carbon dioxide is saturated (Sand-Jensen et al. 2018, Anderson et al. 2019). Calcification plays the essential role in producing protons and making carbon dioxide available for assimilation from bicarbonate without further pH rise. This process is important because photosynthesis within macrophytes is markedly reduced as external pH approaches 9.5 (Christensen et al. 2013) and it usually stops entirely close to pH 10 (Sand-Jensen et al. 2018). Carbon is commonly the favored unit used in quantification of respiration and production as well as import, export and storage of organic matter in lake ecosystems (Wetzel 2001). Calcification can contribute to carbon dioxide oversaturation in lake waters and release to the atmosphere (Marcé et al. 2015). Biomineralization that causes formation of calcium oxalate and calcium carbonate crystals in plants represents a considerable carbon sink in the ecosystem with long residence time at both the ecosystem and global level (Anderson et al. 2019). This raises the possibility of exploring the role of calcium carbonate crystals in global carbon cycle and determining its effect on the climate change process (Tooulakau et al. 2016). Despite the role of carbon in biogeochemical cycling of carbon, calcium and silicon and their roles in sequestering atmospheric carbon. To date, less attention has been given in the literature to the impact of pollution and changing trophic state on biomineralization (He et al. 2014; Gwenzi 2019). Calcium carbonate and oxalate crystals play a major role in the physiological and structure functions of the macrophytes (Franceschi and Nakata 2005). Calcium oxalate and calcium carbonate crystals serve as a natural sink of calcium ions and carbon, thereby preventing calcium and carbonate accumulation in aerenchyma of the free-floating aquatic macrophyte *Eichhornia crassipes* (Prychid and Rudall 1999; Franceschi and Nakata 2005; Karabourniotis et al. 2020).

Calcium oxalate and calcium carbonate crystal formation in aquatic macrophytes has for some time been an important topic of research among plant scientists, even though the factors responsible for their formation and their functional role is still poorly understood (Nakata 2012; He et al. 2014). The main functions of calcium oxalate crystals reported in the literature are the regulation, balance and sequestering of calcium from the aquatic environment, ion balance, light collection and reflection, plant protection against herbivores and detoxification of heavy metals and oxalates (Libert and Franceschi 1987; Tooulakou et al. 2016).

The proposed pathways for oxalate production in aquatic macrophytes include photorespiratory glycolate/glyoxylate oxidation, hydrolysis of oxaloacetate and cleavage of ascorbate (Nakata 2003). Uneven distribution of calcium oxalate or calcium carbonate crystals has been reported in aquatic macrophytes (Jones and Ford 1972; Rahman et al. 2006). The current study confirms the presence of an unusual type of crystal idioblasts are found connected with aerenchyma. These crystals may protrude into the air spaces (Fran- ceschi and Horner 1980). Many studies consider that oxalate is the end product of the plant metabolism and that excess concentration may be toxic to the plants (Franceschi and Loewus 1995; Cuéllar-Cruz et al. 2020). Thus crystal and crystal forming idioblasts are a means of isolating this product in the macrophytic body and that is why they have been classified as the excretory idioblast in removing excess calcium (Foster 1956; Cai et al. 2018). Calcium oxalate crystallization may also help in removing excess oxalic acid, which is not toxic to plants, and may cause osmotic problems (Raven and Smith 1974). Aquatic macrophytes deposit their calcium carbonate crystal extracellularly as well as intracellularly in free-floating *Eichhornia crassipes*. As a result, the calcium oxalate concentration tends to increase as the plant matures (Torell et al. 2005). There is, however, evidences of calcium oxalate and calcium carbonate resorption in times of calcium depletion (Sunell and Healey 1979; Karabourniotis et al. 2020).

Energy-dispersive X-ray spectroscopy (EDS) of the submerged, floating and emergent macrophytes

Measurements of the roots of the macrophyte *Eichhornia crassipes* show the presence of more elements, due to their direct contact with lake water as compared to stem and leaves. Furthermore, leaves, stem and root of *Eichhornia* *crassipes* within oligotrophic lake 1 show fewer elements as compared to the macrophytes of mesotrophic lake 2 and lake 3. In submerged macrophyte *Hydrilla verticillata* leaves and stems within mesotrophic lakes (lake 2 and 3) and show greater number of elements in comparison to the macrophytes of the oligotrophic lake 1 (Fig. 5 of Supplementary material).

How does increasing pollution load and trophic state affect the elemental composition in the macrophytes?

The concentration of elements accumulated or present in macrophytic bodies is directly related to the concentration of that element present in the water and sediments of the lacustrine ecosystem (Guilizzoni 1991; Huang et al. 2017). Quantitative assessment of lacustrine pollution shows the relevant correlation between concentration of the elements in the lake water to the concentration of the elements present in the aquatic macrophytes (Guilizzoni 1991; Krems et al. 2013). Ion exchange is the main process by which absorption of metals takes place via surface of aquatic macrophytes (Schneider et al. 2001). Change in water chemistry due to increasing pollution load and trophic state, will affect the elemental composition in the free-floating, submerged and emergent macrophytes in the lake ecosystem. Quantitative assessment of lacustrine pollution indicates a correlation between the concentration of the elements into the lake water and the concentration of the elements present in the aquatic macrophytes (Krems et al. 2013).

Elemental bioabsorption processes in the macrophytic cells occur through surface absorption as well as through intracellular and extracellular accumulation (Veglio and Beolchini 1997, Moe et al. 2019, Dalla Vecchia et al. 2020). Bioabsorption of elements in macrophytes is defined as a reversible and fast process that is based on physico-chemical binding of metal cations through ion exchange, adsorption, microprecipitation, complexing and chelation.

Elemental variation in free-floating, submerged and emergent macrophytes varies with the trophic state of the lakes. Floating and submerged macrophytes contain higher elemental concentrations of cations than the emergent macrophytes. Increases in trophic state and pollution lead to accumulation of cations with macrophytes.

Conclusion

This study shows that within the Central Gangetic Plain, trophic State Index provides a comprehensive tool for water quality assessment of freshwater lakes. Furthermore, the study has shown how the increasing trophic state and deteriorating water quality affect the anatomy of macrophyte leaf surfaces through biomineralization of their surfaces. Anatomical changes in macrophytes shown in this study include effects on the leaf surface, changes in stomatal structure and depositions around it. It is clear from the study that within mesotrophic lakes such as L2 and L3, nutrient enrichment and pollution stresses affect the leaves of the macrophytes through the accumulation of biogenic materials, whereas in oligotrophic lake L1 macrophytes show normal morphology with proper stomatal structures.

The anatomies of rhizomes of free-floating *Eichhornia crassipes* in lakes L2 and L3 were found to be normal in spite of being in mesotrophic condition and polluted states of the lake. In contrast within the oligotrophic L1, the presence of calcium oxalate and carbonate crystals was noted within macrophytes. Environmental and genetic factors are the main factors responsible for biomineralization within aquatic macrophytes (Bauer et al. 2011). Water quality of oligotrophic lake (L1) shows high pH and low alkalinity. Biomineralization and calcification in *Eichhornia,* therefore, appear to be mainly brought about by achieving supersaturation of the lacustrine ecosystem, which increases carbonate ion activities by shifting the inorganic carbonate ion equilibrium. The main functions of calcium oxalate and calcium carbonate crystals in aquatic ecosystems are regulating cytoplasmic free calcium levels in the macrophytes. They also help in biogeochemical cycling of carbon, calcium, and silicon and in the sequestration of atmospheric $CO_{2,}$ alleviating both salt and temperature stresses. It has also been reported that they regulate ion balance (e.g., sodium and potassium), detoxifying oxalic acid, and reduce alkalinity generated by nitrate assimilation (He et al. 2014).

The study has also shown that the trace elemental concentration within aquatic macrophytes varies with change in trophic state from samples collected from different lakes. Macrophytes of the mesotrophic lakes contain higher trace elemental activities compared with the macrophytes within oligotrophic lake. The study shows that the trace elemental concentrations in floating macrophytes are higher than in submerged and emergent macrophytes. It seems, therefore, that the trophic state and water quality are the principal determinants of the elemental variation in aquatic macrophytes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13201-022-01681-9.

Funding The doctoral fellowship provided by the University Grants Commission (UGC) to the first author is greatly acknowledged.

Declarations

Conflict of interest The authors declare no potential conflicts of interest concerning authorship, research and publication of this paper.

Human or animal rights The research did not involve human participants or animals.

References

Ali MB, Tripathi RD, Rai UN, Pal A, Singh SP (1999) Physico-chemical characteristics and pollution level of lake Nainital (UP, India): role of macrophytes and phytoplankton in biomonitoring and phytoremediation of toxic metal ions. Chemosphere 39(12):2171–2182

Andersen MR, Sand-Jensen K, Iestyn Woolway R, Jones ID (2016) Profound daily vertical stratification and mixing in a small, shallow, wind-exposed lake with submerged macrophytes. Aquat Sci. https://doi.org/10.1007/s00027-016-0505-0

Andersen MR, Kragh T, Sand-Jensen K (2017) Extreme diel dissolved oxygen and carbon cycles in shallow vegetated lakes. Proc R Soc B Biol Sci. https://doi.org/10.1098/rspb.2017.1427

Andersen MR, Kragh T, Martinsen KT, Kristensen E, Sand-Jensen K (2019) The carbon pump supports high primary production in a shallow lake. Aquat Sci 81(2):24

APHA (2017) Standards methods for examination of water and wastewater, 23rd edn. American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC

Bauer P, Elbaum R, Weiss IM (2011) Calcium and silicon mineralization in land plants: transport, structure and function. Plant Sci 180(6):746–756. https://doi.org/10.1016/j.plantsci.2011.01.019

Bianchini I, Cunha-Santino MB, Peret AM (2008) Oxygen demand during mineralization of aquatic macrophytes from an oxbow lake. Braz J Biol 68:61–67. https://doi.org/10.1590/S1519-69842008000100009

Borowitzka MA (1984) Calcification in aquatic plants. Plant Cell Environ 7(6):457–466

Borrelli N, Honaine MF, Altamirano SM, Osterrieth M (2011) Calcium and silica biomineralizations in leaves of eleven aquatic species of the Pampean Plain, Argentina. Aquat Bot 94(1):29–36

Butnik AA, Japakova UN, Begbaeva GF (2001) Halophytes: Structure and Adaptation. In: Breckle S-W, Veste M, Wucherer W (eds) Sustainable Land Use in Deserts. Springer, Berlin, pp 147–153

Cai X, Ge IC, Xu C, Wang X, Wang S, Wang Q (2018) Expression analysis of oxalate metabolic pathway genes reveals oxalate regulation patterns in spinach. Molecules 23:1286

Carlson RE (1977) A trophic state index for lakes. Limnol Oceanogr 22(2):361–369

Christensen J, Sand-Jensen K, Staehr PA (2013) Fluctuating water levels control water chemistry and metabolism of a charophyte-dominated pond. Freshw Biol 58:1353–1365. https://doi.org/10.1111/fwb.12132

Cuéllar-Cruz M, Pérez KS, Mendoza ME, Moreno A (2020) Biocrystals in plants: A short review on biomineralization processes and the role of phototropins into the uptake of calcium. Curr Comput Aided Drug Des 10(7):591

Cuevas Madrid H, Lugo Vazquez A, Peralta Soriano L, Morlan Mejia J, Vilaclara Fatjo G, Sanchez Rodriguez MDR, Carmona Jimenez J (2020) Identification of key factors affecting the trophic state of four tropical small water bodies. Water 12(5):1454

Dalla Vecchia A, Villa P, Bolpagni R (2020) Functional traits in macrophyte studies: current trends and future research agenda. Aquat Bot 167:103290

de Souza DC, Fontaneli AC, Peron AP, Froehner S (2021) Physiological effects of exposure to copper and chromium in three floating aquatic macrophyte species. Water Air Soil Pollut 232(1):1–12

de Souza Reis IR, Alves de Oliveira J, Ventrella MC, Otoni WC, Marinato CS, Paiva de Matos L (2020) Involvement of glutathione metabolism in Eichhornia crassipes tolerance to arsenic. Plant Biol 22(2):346–350

Dos Santos NB, de Arruda EC, Neto AG, de Oliveira AF (2020) Assessing the effects of water quality on leaf morphoanatomy, ultrastructure and photosynthetic pigment content of Salvinia auriculata Aubl.(Salviniaceae). Ecotoxicol Environ Saf 190:110061. https://doi.org/10.1016/j.ecoenv.2019.110061

Downing JA (2010) Emerging global role of small lakes and ponds: little things mean a lot. Limnetica 29(1):0009–0024

Dubey D, Dutta V (2020) Nutrient Enrichment in Lake Ecosystem and Its Effects on Algae and Macrophytes. Environmental Concerns and Sustainable Development. Springer, Singapore, pp 81–126

Elliott M, Whitfield AK (2011) Challenging paradigms in estuarine ecology and management. Estuar Coast Shelf Sci 94(4):306–314

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963

Flowers TJ, Munns R, Colmer TD (2015) Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann Bot 115:419–431

Foster AS (1956) Plant idioblasts: remarkable examples of cell specialization. Protoplasma 46(1):184–193

Franceschi VR, Loewus FA (1995) Oxalate Biosynthesis and Function in Plants and Fungi. Calcium Oxalate in Biological Systems, pp 113–130

Franceschi VR, Horner HT Jr (1980b) A microscopic comparison of calcium oxalate crystal idioblasts in plant parts and callus cultures of Psychotria punctata (Rubiaceae). Z Pflanzenphysiol 97(5):449–455

Franceschi VR, Nakata PA (2005) Calcium oxalate in plants: formation and function. Annu Rev Plant Biol 56:41–71

Frank E (1967) Zur bildung des kristallidioblastenmusters bei canašalia ensiformis DC. I. Z Pflanzenphysiol 58:33–48

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of it's by products. Asian J Energy Environ 6(4):18

Grašič M, Budak V, Klančnik K, Gaberščik A (2017) Optical properties of halophyte leaves are affected by the presence of salt on the leaf surface. Biologia 72(10):1131–1139

Guilizzoni P (1991) The role of heavy metals and toxic amterials in the physiological ecology of submersed macrophytes. Aquat Bot 41(1–3):87–109

Gwenzi W (2019) Carbon Sequestration via Biomineralization: Processes, Applications and Future Directions. Sustainable Agriculture Reviews. Springer, Cham, pp 93–106

Hammer Ø, Harper DA, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1):1–9

Hampton SE, McGowan S, Ozersky T, Virdis SG, Vu TT, Spanbauer TL, Kraemer BM, Swann G, Mackay AW, Powers SM, Meyer

MF (2018) Recent ecological change in ancient lakes. Limnol Oceanogr 63(5):2277–2304

He H, Veneklaas EJ, Kuo J, Lambers H (2014) Physiological and ecological significance of biomineralization in plants. Trends Plant Sci 19(3):166–174

Hillsborough (2008) Trophic State Index (TSI) in Hillsborough Community Atlas, City of Tampa, Florida: Hillsborough County. URL: http://www.hillsborough.communityatlas.usf.edu/

Huang W, Liu J, Shi Q, Xing W (2017) Characteristics of the elemental stoichiometry of submerged macrophytes and their relationships with environments in Honghu Lake (China). Fundam Appl Limnol 190(4):299–308

IPCC (2014) Impacts, adaptation, and vulnerability. In: Field CB et al (eds) Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 1–44

Jamnická G, Hrivnák R, Oťaheľová H, Skoršepa M, Valachovič M (2006) Heavy metals content in aquatic plant species from some aquatic biotopes in Slovakia. Proc 36th Int Conf IAD Wien: Austrian Committee Danube Research/IAD 4:336–370

Jeppesen E, Søndergaard M, Søndergaard M, Christoffersen K (eds) (2012) *The Structuring Role of Submerged Macrophytes in Lakes*. Springer Science Business Media

Jones RJ, Ford CW (1972) Some factors affecting the oxalate content of the tropical grass setaria sphacelata. Aust J Exp Agric 12(57):400–406

Kalff J (2002) Limnology: Inland Water Ecosystems. Prentice Hall, New Jersey

Karabourniotis G, Horner HT, Bresta P, Nikolopoulos D, Liakopoulos G (2020) New insights into the functions of carbon–calcium inclusions in plants. New Phytologist 228(3):845–854

Katschnig D, Broekman R, Rozema J (2013) Salt tolerance in the halophyte Salicornia dolichostachya Moss: growth, morphology and physiology. Environ Exp Bot 92:32–42

Kausch AP, Horner HT (1981) The relationship of air space formation and calcium oxalate crystal development in young leaves of Typha angustifolia L. Typhaceae. Scan Electron Microsc

Kausch AP, Horner HT (1983) The development of mucilaginous raphide crystal idioblasts in young leaves of Typha angustifolia LTyphaceae. Am J Bot 70(5):691–705

Kelly MG, Whitton BA (1998) Biological monitoring of eutrophication in rivers. Hydrobiologia 384(1–3):55–67

Krems P, Rajfur M, Wacławek M, Kłos A (2013) The use of water plants in biomonitoring and phytoremediation of waters polluted with heavy metals. Ecol Chem Eng S 20(2):353–370

Larcher W (2003) Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups, 4th edn. Springer, Berlin, p 514

Libert B, Franceschi VR (1987) Oxalate in crop plants. J Agric Food Chem 35(6):926–938

Lumb A, Sharma TC, Bibeault JF (2011) A review of genesis and evolution of water quality index (WQI) and some future directions. Water Qual Expo Health 3(1):11–24. https://doi.org/10.1007/s12403-011-0040-0

Maleva MG, Nekrasova GF (2004) The response of hydrophytes to environmental pollution with heavy metals. Russ J Ecol 35(4):230–235

Marcé R, Obrador B, Morguí J-A, Riera JL, López P, Armengol J (2015) Carbonate weathering as a driver of CO2 supersaturation in lakes. Nat Geosci 8:107

Marecik R, Króliczak P, Cyplik P (2006) Fitoremediacja–alternatywa dla tradycyjnych metod oczyszczania œrodowiska. Biotechnologia 3(74):88–97

Marker AFH (1972) The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Freshw Biol 2(4):361–385

Martinsen KT, Andersen MR, Sand-Jensen K (2019) Water temperature dynamics and the prevalence of daytime stratification in small, shallow lakes. Hydrobiologia 826:247–262

McConnaughey T (1991) Calcification in Chara corallina: CO2 hydroxylation generates protons for bicarbonate assimilation. Limnol Oceanogr 36(4):619–628

McConnaughey T, Whelan J (1997) Calcification generates protons for nutrient and bicarbonate uptake. Earth Sci Rev 42(1–2):95–117

Seema Mishra S, Srivastava RD, Tripathi R, Kumar CS, Seth D.K. Gupta (2006) Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65(6):1027–1039

Moe TF, Hessen DO, Demars BOL (2019) Functional biogeography: stoichiometry and thresholds for interpreting nutrient limitation in aquatic plants. Sci Total

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nakata PA (2003) Advances in our understanding of calcium oxalate crystal formation and function in plants. Plant Sci 164(6):901–909

Nakata PA (2012) Plant calcium oxalate crystal formation, function, and its impact on human health. Front Biol 7(3):254–266

Nõges P, Cremona F, Laas A, Martma T, Rõõm E-I, Toming K, Viik M, Vilbaste S, Nõges T (2016) Role of a productive lake in carbon sequestration within a calcareous catchment. Sci Total Environ 550:225–230

Pathan AK, Bond J, Gaskin RE (2008) Sample preparation for scanning electron microscopy of plant surfaces—horses for courses. Micron 39(8):1049–1061. https://doi.org/10.1016/j.micron.2008.05.006

Poikane S, Portielje R, Denys L, Elferts D, Kelly M, Kolada A, van den Berg MS (2018) Macrophyte assessment in European lakes: diverse approaches but convergent views of ‘good’ecological status. Ecol Ind 94:185–197

Prins H, Snel J, Helder R, Zanstra P (1979) Photosynthetic bicarbonate utilization in the aquatic angiosperms Potamogeton and Elodea. Hydrobiol Bull 13:106–111

Prychid CJ, Rudall PJ (1999) Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. Ann Bot 84(6):725–739

Qin B, Gao G, Zhu G, Zhang Y, Song Y, Tang X, Deng J (2013) Lake eutrophication and its ecosystem response. Chin Sci Bull 58(9):961–970

Rahman MM, Niimi M, Ishii Y, Kawamura O (2006) Effects of season, variety and botanical fractions on oxalate content of napiergrass (Pennisetum purpureum Schumach). Grassland Sci 52(4):161–166

Raven JA, Smith FA (1974) Significance of hydrogen ion transport in plant cells. Can J Bot 52(5):1035–1048

Reitsema RE, Meire P, Schoelynck J (2018) The future of freshwater macrophytes in a changing world: dissolved organic carbon quantity and quality and its interactions with macrophytes. Front Plant Sci 9:629

Sand-Jensen KAJ (1983) Photosynthetic carbon sources of stream macrophytes. J Exp Bot 34(2):198–210

Sand-Jensen K, Jensen RS, Gomes M, Kristensen E, Martinsen KT, Kragh T, Baastrup-Spohr L, Borum J (2018) Photosynthesis and calcification of charophytes. Aquat Bot 149:46–51. https://doi.org/10.1016/j.aquabot.2018.05.005

Schneider IA, Rubio J, Smith RW (2001) Biosorption of metals onto plant biomass: exchange adsorption or surface precipitation. Inter J Mineral Process. 62(1–4):111–120

Short FT, Kosten S, Morgan PA, Malone S, Moore GE (2016) Impacts of climate change on submerged and emergent wetland plants. Aquat Bot 135:3–17

Sunell LA, Healey PL (1979) Distribution of calcium oxalate crystal idioblasts in corms of taro (Colocasia esculenta). Am J Bot 66(9):1029–1032

Tanwir K, Javed MT, Shahid M, Akram MS, Haider MZ, Chaudhary HJ, Lindberg S (2020) Ecophysiology and Stress Responses of Aquatic Macrophytes Under Metal/Metalloid Toxicity. Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives I. Springer, Singapore, pp 485–511

Tooulakou G, Giannopoulos A, Nikolopoulos D, Bresta P, Dotsika E, Orkoula MG, Karabourniotis G (2016) Alarm photosynthesis calcium oxalate crystals as an internal CO2 source in plants. Plant physiol 171(4):2577–2585

Torell R,Young JA, Kvasnicka B (2005) Halogeton poisoning. Cooperative Extension. University of Nevada. Fact sheet-00-20

Van den Berg MS, Coops H, Simons J, Pilon J (2002) A comparative study of the use of inorganic carbon resources by Chara aspera and Potamogeton pectinatus. Aquat Bot 72(3-4):219–233. https://doi.org/10.1016/S0304-3770(01)00202-9

Vincent R, Horner HT (1980a) Calcium oxalate crystals in plants. Bot Rev 46(4):361–427

Wetzel RG (2001) Limnology: lake and river ecosystems. Gulf Professional Publishing, Houston

Xu GX, Tan C, Wei XJ, Gao XY, Zheng HQ (2011) Development of secretory cells and crystal cells in Eichhornia crassipes ramet shoot apex. Protoplasma 248(2):257–266

Young IR, Zieger S, Babanin AV (2011) Global trends in wind speed and wave height. Science 332:451–455. https://doi.org/10.1126/science.1197219

Zhu G, Yuan C, Di G, Zhang M, Ni L, Cao T, Wu G (2018) Morphological and biomechanical response to eutrophication and hydrodynamic stresses. Sci Total Environ 622:421–435

Zhu GR (2012) Studies on the effects of eutrophication and floods on the biomechanical characteristics of aquatic macrophytes. Ph.d. Thesis. Institute of hydrobiology, The Chinese Academy of Sciences, China