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Metal accumulation and biochemical composition of selected macrophytes in an urban lake (Dal Lake), Kashmir, India

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Abstract
The present study was carried to estimate and investigate metal accumulation potential of *Ceratophyllum demersum* and *Hydrilla verticillata* in an urban lake (Dal Lake), Kashmir, India. Analyses of water and macrophyte samples were done on a monthly basis from March to August. The results showed that *Hydrilla verticillata* accumulated maximum concentration of Na, K, Mg, Cu, Zn and Cr (mg/g) compared to *Ceratophyllum demersum* at all selected sites. Data from this study may provide information on the use of *Hydrilla verticillata* as a hyper- accumulators to bio-accumulate or bio-stabilize heavy metals and metal ions from the polluted water bodies as its phytoremediation capacity increased from March to June and there after showed decreased values at all the selected sites. In *Hydrilla verticillata*, the general order of concentrations was K>Na>Mg>Cr>Cu>Zn. Biochemical parameters *viz*. chlorophyll (a, b), total chlorophyll, carotenoids and free sugar content were found to be highest during the month of June and lowest in March in both the selected plants. The present study highlights the fact that both *Hydrilla verticillata* and *Ceratophyllum demersum* absorb nutrients from water which proved them as potential phytoremediants for removing the excess concentration of nutrients and heavy metals from the Dal Lake.

Keywords: phytoremediation, bioabsorption, chromium, *Ceratophyllum demersum*, *Hydrilla verticillata*, heavy metals

Introduction

Heavy metal contamination is a major environmental concern due to their toxicity and bio-accumulative presence in the food chain (Vhahangwele and Khathutshelo 2018) [39]. These toxic pollutants are being discarded in our aquatic system, soil environment and into the atmosphere due to rapid urbanization and anthropogenic activities such as agriculture practices and metal industries, inappropriate waste disposal methods and synthetic fertilizers (Briffa *et al*. 2020) [8]. Heavy metals are difficult to degrade and can therefore accumulate in the food chain (Shue *et al*. 2014; Al-Hammad *et al*. 2016) [37, 2]. However, metals such as Cu, Fe, Zn, and Mn are important micro-nutrients for growth and development of animals and plants, while metals such as Cd, Cr, Hg and Pb have no biological activities and are extremely toxic even at low concentrations (Nicolau *et al*. 2006; Kar *et al*. 2008) [30, 23]. Experts and scientific committee are resorting to phytoremediation activities to evaluate damage produced in the environment (Marques *et al*. 2019; Weber *et al*. 2020) [27, 41]. The utilization of green plants to improve water quality is a green technology that is an efficient, environmentally sustainable, and cost-effective approach (Patel and Sahoo 2020) [32]. Aquatic macrophytes grow naturally in water bodies which are polluted by nutrient load from urban and agricultural activities. Aquatic macrophytes such as *Azolla pinnata. Pistia stratiotes*, *Hydrilla verticillata*, *Nymphaea alba*, *Eichhornia crassipes*, *Salvinia natans* and *Potamogeton crispus* have a strong potential to clean polluted water by active and passive absorption (Fawzy *et al*. 2012; Kamal *et al*. 2004; Newete *et al*. 2016; Bai *et al*. 2018; Cai *et al*. 2018) [17, 22, 29, 5, 9]. They are natural bio-filters that mitigate pollutants transported by water and have an advantage over other methods due to its cost-effectiveness and environment-friendly (Ahmood and Al-Jawasim 2019; Eid *et al*. 2019) [1, 16].

Dal Lake (urban lake) situated in the heart of Srinagar city, the capital of Jammu and Kashmir, is under immense anthropogenic pressure (Bhat and Dar 2015) [6]. The lake gets enriched by the huge quantity of domestic sewage and chemical fertilizers used in the agricultural fields and floating gardens present within the lake (Rashid *et al*. 2017) [33]; under such conditions a huge amount of toxic heavy metals may get accumulated into the water body. Later being accumulated and transferred to the food chain by the process of bio-magnification (Rather *et al*. 2019) [35]. Despite the implementation of various restoration and conservation plans prepared by national and international agencies and enforced by state authorities, the ecosystem of Dal Lake continues to deteriorate at an alarming rate (Murataza *et al*. 2010) [28].

Keeping in view, the ecological significance of water bodies like Dal Lake and accumulation of nutrients in the system due to anthropogenic pressure, the present research work was aimed to evaluate the metal ion concentrations and biochemical composition in two aquatic plants *viz*; *Ceratophyllum demersum* and *Hydrilla verticillata.*

Materials and methods

Description of study area

The Dal Lake, a fluviatile fed urban lake, is important to Kashmir's tourism, economy and recreation. Located at an altitude of 1584m (ASL) between 34º5-34º6´ N latitude and 74º8´-74º 9´ E longitudes at the foot of the Zabarwan range (Rather 2012) [34]. It's a shallow lake with an average depth of 2.50 m that varies seasonally depending on rainfall and stream flow. The lake's current land use pattern and land cover include open water (10.5 km^2), aquatic vegetation (8.64 km^2), floating gardens (2.89 km^2), and settlements (2.02 km^2) (Rashid *et al*. 2017) [33]. Human settlement in the lake, floating gardens, and the construction of floating residences (house-boats) got accelerated at alarming pace during last five decades (Amin *et al*. 2014) [3] Three sites in Dal Lake *viz;* Nishat, Telibal and Hazratbal were selected for carrying out the present investigation (Fig. 1).

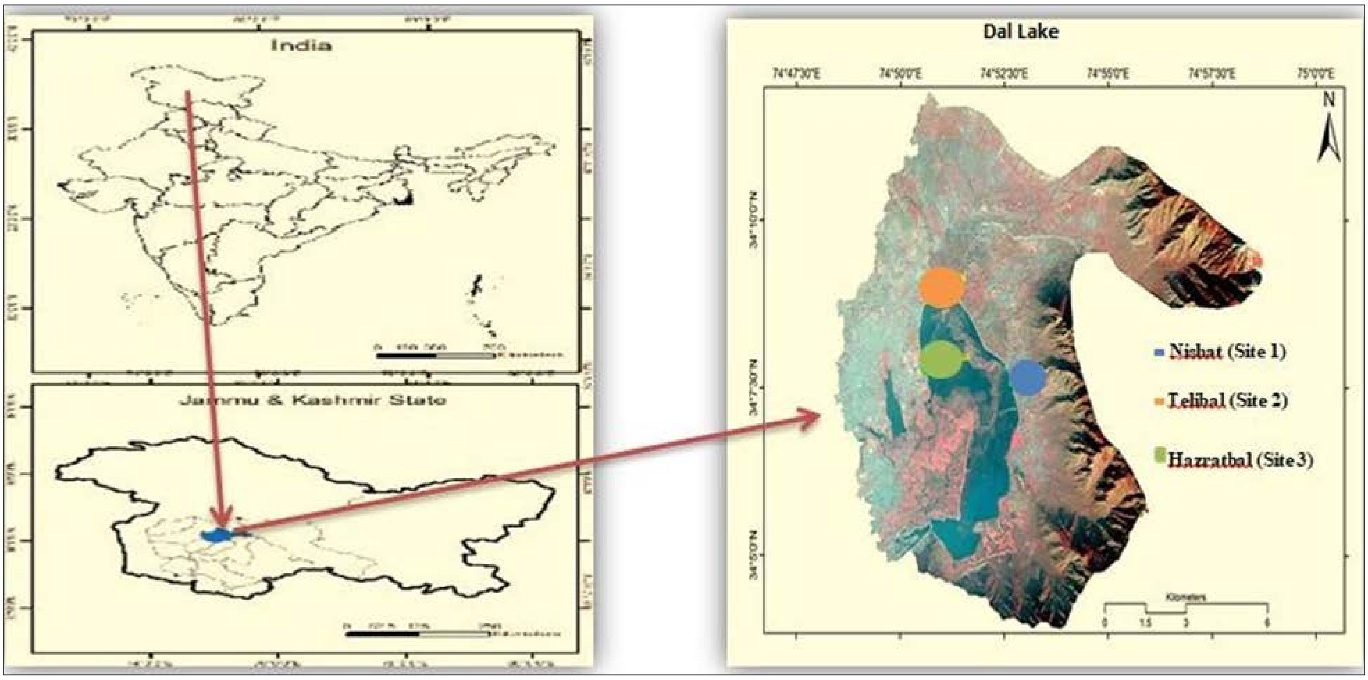


Fig 1: Geographic location of the study area

Description of sampling sites

1. Nishat (Site 1): This is a heavily populated area on the east side of the lake, close to the world-famous Nishat Garden. The site is occupied with heavy macrophytic vegetation due to heavy nutrient inputs from nearby restaurants and shops.
2. Telibal (Site 2): This is on the north side of the lake, and it is fed by the Telibal Stream. This area reflects the dynamic land use pattern, with agricultural fields, orchids, pastures and hills.
3. Hazratbal (Site 3): This densely populated area is located on the lake's western shore. The site is covered with dense macrophytic vegetation due to strong nutrient inputs from nearby houses and sewage treatment plants.

Collection and preparation of samples

With the aid of a ruttner sampler, water samples were obtained from three different locations of the lake from March to August at a depth of 10 to 15 cm. Samples were then stored in polypropylene bottles. However, plant samples *viz; Ceratophyllum demersum and Hydrilla verticillate* were manually collected randomly once in each month (March- August). Before being put in plastic bags, the plant samples were thoroughly washed with tap water and then rinsed with distilled water. After that, all of the samples were meticulously labelled and packed (USEPA 1991) [38]. The samples were then taken to the lab and immediately acidified with ultra-pure HNO_3 and processed at 4ºC before analysis. All equipment used for sample collection, storage and analysis were pre-cleaned by using 10% HNO3 and were rinsed with distilled water. The botanical and common names of the macrophytes collected during the study are presented in Table 1.

Table 1: The botanical and common names of the macrophytes collected during the study

Botanical name	Common name
Ceratophyllum demersum	Hornwort
Hydrilla verticillata	Hydrilla, Water-thyme

Digestion of samples and analyses of metal ion

The digestion of the water samples was carried out by mixing 100 mL of the filtered water with 5 mL concentrated sulphuric acid (H2SO4) and 5 mL concentrated nitric acid (HNO3). The volume of the mixture was then reduced to around 15 to 20 mL by heating it. The digested sample was allowed to cool at room temperature and then filtered through Whatman filter paper No. 0.45 µm. The final volume was raised to 100 mL with double distilled water and metal analysis was performed immediately. While as, plant samples were digested in a di-acidic solution consisting of 9 mL HNO_3 and 3 mL $HClO_4$. 10 mL di-acidic mixture was applied to 0.5g ground plant material, and the contents of the flask were swirled together. In a digestion chamber, the flasks were heated on a hot plate until white fumes appeared. The digestion was completed when the liquid became colorless. The digested sample was dilute to 50 mL with deionized water and filtered through Whatman filter paper No. 0.45 m and metal analysis was carried immediately using atomic absorption spectrophotometer (Lindsay and Norvell 1978) [25].

Analysis of Na^+ and K^+

Prepare stock solutions of Na and K using 2.542g NaCl and 1.9064g KCL respectively dried at 14^0C and dilute to 1000ml by adding distilled water. Dilute 10ml of this solution in 100ml of distilled water. This solution contains 100mg of Na/K per L. The intermediate solution is further diluted by taking 10ml of it and diluting it to 100ml using distilled water. This solution is used to prepare a calibration curve of Na and K ranging from 0.1-10mg/l of Na/K. The determination of Na and K in digested plant samples were carried out directly with the help of Flame Photometer (Jackson 1973) [21].

Biochemical analysis

For estimation of chlorophyll and carotenoids, 250mg fresh plant samples were extracted in 5ml 80% acetone. The leftover pellet was re-extracted with 80% acetone, and the final volume was made up to 25ml. Pooled supernatant was read at 665, 649, 510 and 480 nm in Bausch and Lomb spectronic 2000 spectrophotometer.

The chlorophyll content was calculated by using the given formula (Arnon 1949):

Chlorophyll a (mg/g) = 11.63 A665 - 2.39A649

Chlorophyll b (mg/g) = 20.11A649 – 5.18 A665

Total chlorophyll (mg/g) = 6.45 A665 + 17.72 A649

Carotenoids (mg/g) = 7.6A480 – 1.49A510 (Duxbury and Yentsch 1956) [15].

For estimation of free sugar content, a fresh weed sample of 250mg was crushed in water. To 2ml of homogenate 80% alcohol was added, and later the mixture was evaporated in a test tube till dryness. To the dried tube 2ml H_2O and 0.1 ml of 80% phenol was added. Then it was treated with 5ml of strong sulphuric acid (H_2SO_4) and kept at room temperature for 30 minutes till the brown complex was formed. The brown coloured complex formed was read at 420 nm using glucose as a standard in a spectrophotometer (Dubois *et al*. 1956) [14].

The data was subjected to ANOVA using MINITAB® Statistical Software for Windows ver. 14 (Cochran and Cox 1963) [11]. Data was compared for the heavy metal and biochemical concentrations in water and weed samples from the selected study sites using analysis of variance to test for differences amongst sites and sampling occasions ($p \leq 0.05$).

Results and discussions

Heavy metal concentration in water samples

Heavy metal concentrations in water samples from various locations indicated that the order of heavy metals was Cu>Cr>Zn (Fig. 2). Copper plays an important role in human life, but excessive quantities can accumulate in the tissues of skin, liver, and kidneys and causes renal and intestinal irritations, anemia death, and Wilson's disease (Gratten *et al*. 2003) [19]. Cu (mg/L) concentration in water samples ranged from 0.019 to 0.003 during the experiment, with a mean value of 0.008±0.005 for all months (Fig. 2a). Fig. 2b shows that Zn (mg/L) concentration ranged from 0.19 to 0.005 with a mean value of 0.07±0.10. As shown in Fig. 2c, the amount of Cr (mg/L) in water samples ranged from 0.12 to 0.002, with a mean value of 0.04±0.06. Except for Cr, the concentration of Cu and Zn in water at all sites were below the acceptable level (Table 2). The heavy metal content of water samples from three locations differed significantly, with the highest levels of Cu, Zn, and Cr detected at Hazratbal (site 3) and the lowest at Telibal (site 2). At all sites studied, the highest concentration of heavy metals were found in the months of June and July, while the lowest concentration was recorded in month of March. The investigated samples were found to be moderately contaminated by heavy metals, as shown in Fig. 2. According to Wanjala *et al*. (2020) [40], heavy metal concentrations in water samples ranging from 0–1 ppm are classified as unpolluted to moderately polluted. Domestic activities such as domestic sewage, refuse dumps, houseboats, washing and bathing water may have contributed to the presence of heavy metals like Cu, Zn, and Cr in Dal Lake. Our results are in accordance with the finding of Showqi *et al*. (2018) [36] who reported that regular inputs of pollutants from natural and anthropogenic activities such as large amounts of sewage sludge, agricultural run-off from agricultural fields, and household effluents can cause an increase in heavy metals and metal ions concentrations in Dal Lake. Furthermore, higher Cr and Cu concentrations could be due to organic contamination of animal origin and domestic wastes (Ganie *et al*. 2012) [18].

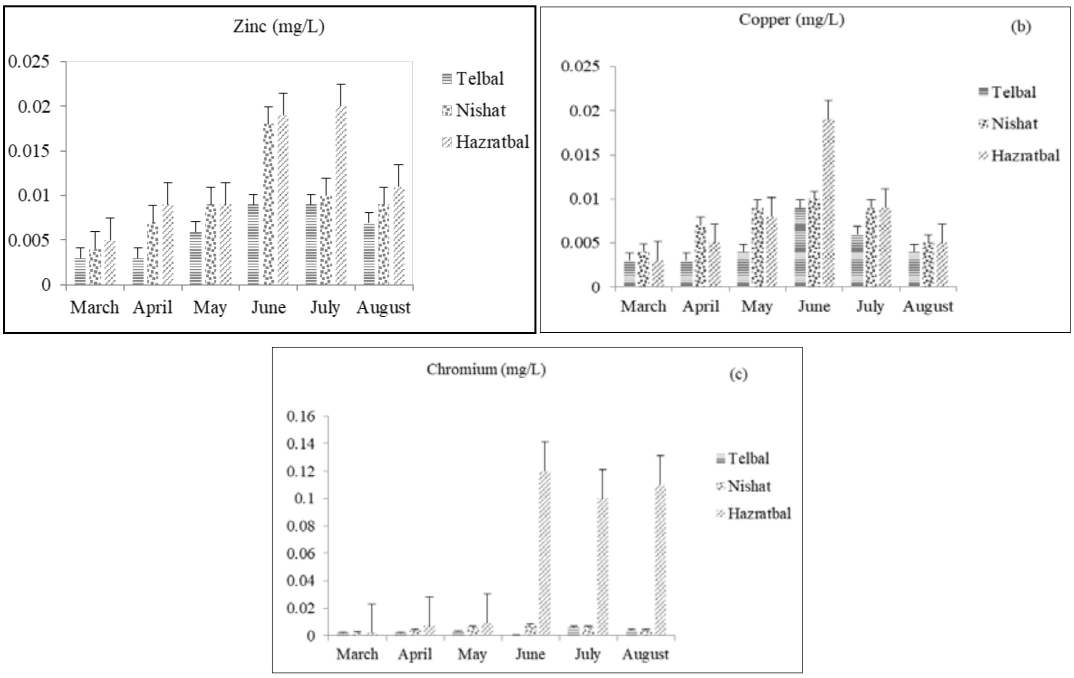


Fig 2: Monthly variation in Zn, Cu and Cr concentration in water of Dal Lake (Mean +SE)

Table 2: Permissible limits of heavy metals in water

	Zinc	Lead	Copper	Chromium	References
	5	0.05	-	0.05	WHO (2003)
Water (mg/l)	-	-	-	-	WHO (2006)
	5	0.05	0.05	0.05	BIS (2009)

Plant analysis

Metal ions concentration

The concentration of metal ions (Mg, Na, and K) in *Ceratophyllum demersum* and *Hydrilla verticillata* shows variation from month to month at all locations, with an increase in concentration during the summer months (Fig. 3). *Hydrilla verticillata* accumulated maximum Na and Mg concentrations of 2.3±0.66 and 1.8±0.29 mg/g at the Hazratbal site, respectively (Fig. 3a, b). However, the highest value of K (2.9±0.72 mg/g) was found at the Nishat site in June (Fig. 3c). During the analysis, the lowest concentration of metal ions was found in *Ceratophyllum demersum* at the Telibal site in March. *Hydrilla verticillata* had the highest concentration of metal ions (Na and Mg) and thus could be used as an alternative tool for extracting excess hardness from water, according to the findings of Lone *et al*. (2013) [26]; Oladejo *et al*. (2015) [31] who reported that aquatic plants have a high Na content and are more effective at removing excess hardness from contaminated water.

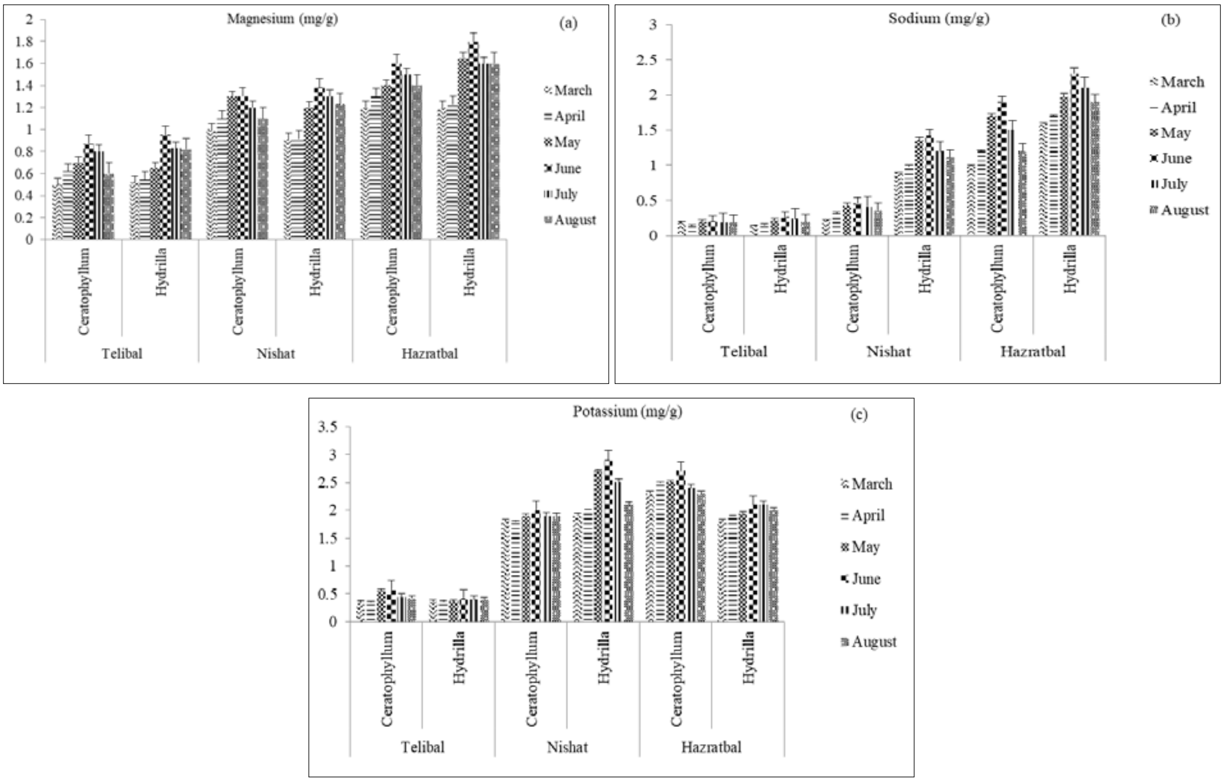


Fig 3: Monthly variation in Mg, Na and K concentration in *Ceratophyllum demersum* and *Hydrilla verticillata* of Dal Lake (Mean +SE)

Evaluation of heavy metals

Since aquatic plants are the first trophic stage of the food chain in an aquatic environment, they usually lead to the accumulation of heavy metals, which has far-reaching implications for the biota and indirectly for humans (Xing *et al*. 2018). Fig. 4 shows the heavy metal concentrations in *Ceratophyllum demersum* and *Hydrilla verticillata* across all study sites were found to be substantially different.

During the experiment, *Hydrilla verticillata* accumulated the highest Cu and Zn concentrations of 0.20±0.04 and 0.19±0.02 mg/g at Hazratbal site, respectively, while the highest Cr concentration of 0.27±0.07 mg/g was observed at Nishat site in June. At Telibal, the lowest concentration of heavy metals was found in *Ceratophyllum demersum* in the month of March.

The heavy metal concentrations in *Hydrilla verticillata* were noticeably higher than *Ceratophyllum demersum* at all three sites studied, so this result agrees with the finding of Bai *et al*. (2018) [5]. Furthermore, the high accumulation of heavy metals by two macrophytes in months with higher temperatures may be due to greater biomass production of *Ceratophyllum demersum and Hydrilla verticillata* in Dal Lake (Kumar *et al*. 2008). Dixit *et al*. (2011) also reported that submerged weeds (*Ceratophyllum demersum, Hydrilla verticillata*, etc) accumulate heavy metals (Cu, Zn, and Cr) in their biomass, which helps to purify waste water.

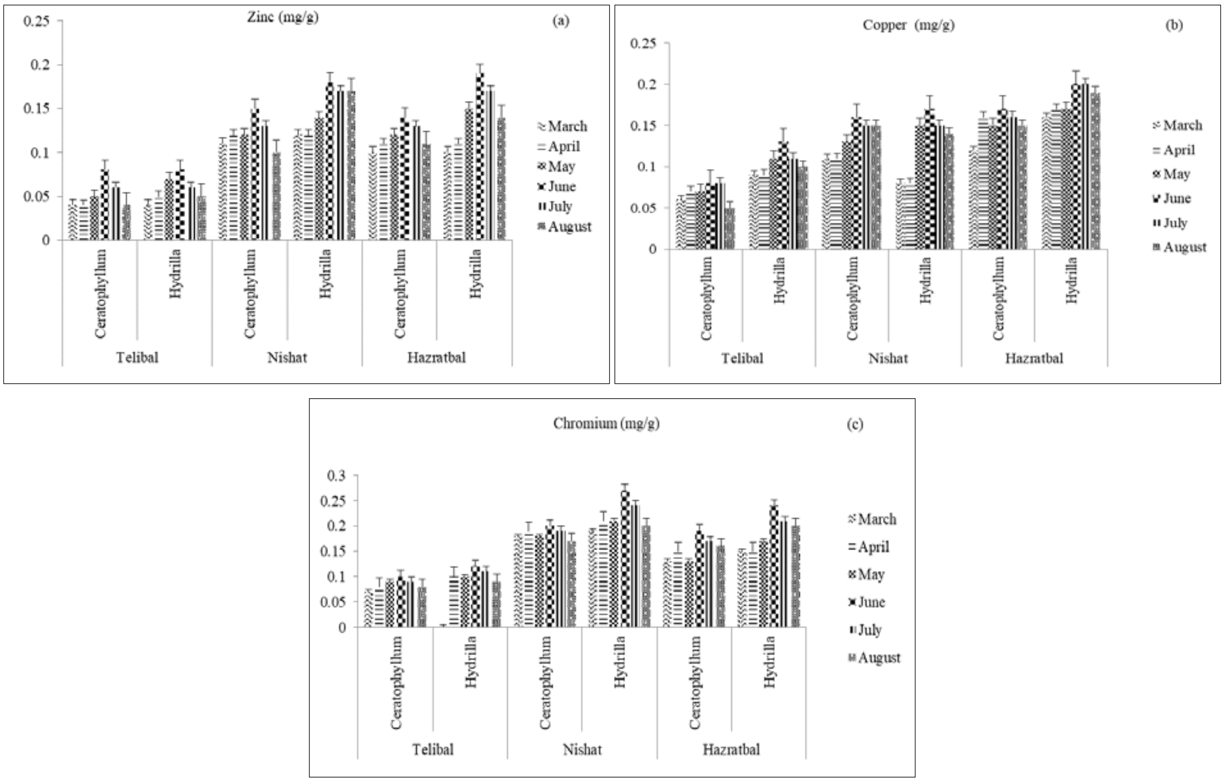


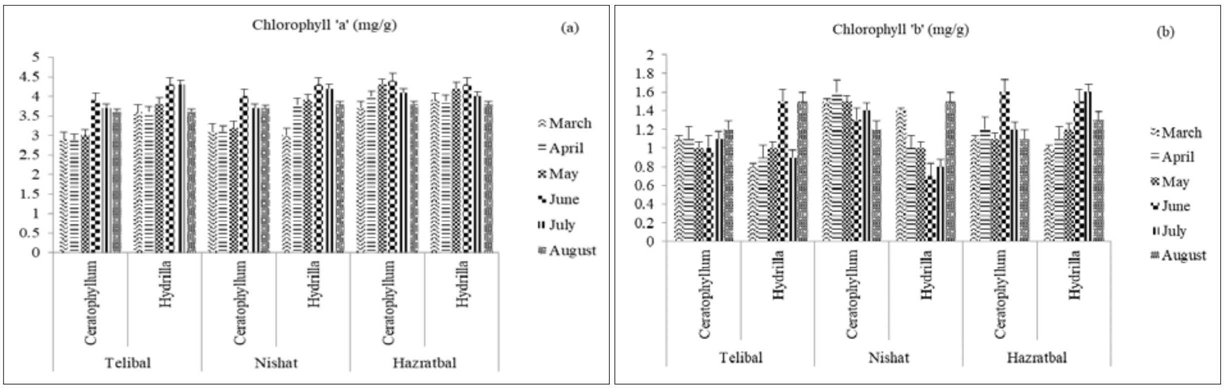
Fig 4: Monthly variation in Zn, Cu and Cr concentration in *Ceratophyllum demersum* and *Hydrilla verticillata* of Dal Lake (Mean +SE)

Biochemical analysis

The results indicated that photosynthetic pigments in *Ceratophyllum demersum* and *Hydrilla verticillata* show an increasing trend from March to June and there after it decreased at all three locations (Fig. 5). In the month of June at Hazratbal site (Fig. 5a), the maximum content of chlorophyll 'a' (4.4±0.52 mg/g) was reported in *Ceratophyllum demersum* followed by *Hydrilla verticillata* (4.3±0.52 mg/g), whereas the minimum content of chlorophyll 'a' (2.9±0.79 mg/g) was recorded in *Ceratophyllum demersum* at Telibal site in the month of March. In the experiment, highest chlorophyll 'b' content was recorded in *Ceratophyllum demersum* (1.6±0.36 mg/g) at Nishat site in the month of April. In contrast, the lowest content of chlorophyll 'b' (0.7±0.17 mg/g) was recorded in *Hydrilla verticillata* at Nishat site in the month of June (Fig. 5b).

In terms of total chlorophyll content, the highest concentration was found in *Hydrilla verticillata* (5.80±1.22 mg/g) in June at the Hazratbal site, while the lowest concentration was found in *Ceratophyllum demersum* (4.00 ±0.79 mg/g) in March at the Telibal site (Fig. 5c). The highest carotenoid content (4.30±1.47 mg/g) was found in *Hydrilla verticillata* at Hazratbal in June, while the lowest content (3.00±0.98 mg/g) was found in *Ceratophyllum demersum* at Telibal in March (Fig. 5d).

The highest free sugar content (4.00±0.20 mg/g) was recorded in *Hydrilla verticillata* as compared to *Ceratophyllum demersum* which recorded free sugar content of 3.60±0.81 at the Hazratbal site in the month of June (Fig. 5e). Whereas, both *Ceratophyllum demersum and Hydrilla verticillata* recorded the lowest value of free sugar content of 2.90±1.05 and 3.1±0.17 mg/g at Telibal and Nishat site, respectively in the month of March. *Ceratophyllum demersum* and *Hydrilla verticillata* in Dal Lake showed major variations in chlorophyll and carotenoid content across months and sites, which are in close agreement with the findings of Dar *et al*. (2012) [12]. Chakraborty and Bhattacharya (2012) [10] also reported significant differences among aquatic plants in response to biochemical parameters *viz;* chlorophyll, carotenoids, etc. Furthermore, an increase in free sugars may be attributed to an increase in chlorophyll, which boosted sugar production as a by-product of photosynthesis (Hasibuan *et al*. 2020) [20].



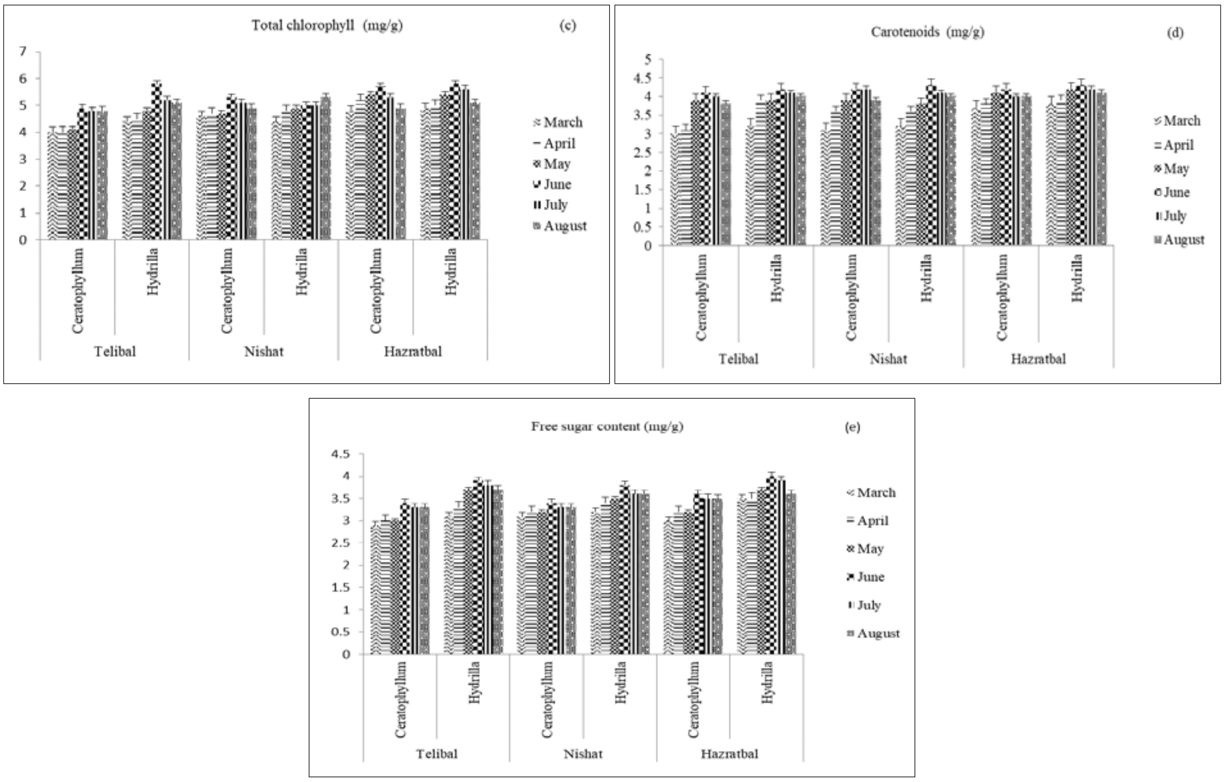


Fig 5: Biochemical composition of *Ceratophyllum demersum* and *Hydrilla verticillata* at different locations of Dal Lake (Mean +SE)

Conclusions

According to the results of this study, the spatial and monthly variations in metal concentrations in water and aquatic plants (*Ceratophyllum demersum and Hydrilla verticillata*) were found due to regular inputs of untreated human wastes from houseboats and settlements adjacent to the lake. While all metals (Zn, Cu and Cr) were present at all sampling sites, the concentration of metals at the Hazratbal site (site 3) was higher than at the Nishat and Telibal sites. Both *Ceratophyllum demersum* and *Hydrilla verticillata* extract nutrients and elements from water, indicating that they may be used as phytoremediants in order to eliminate excess nutrients and heavy metals. When compared to *Ceratophyllum demersum, Hydrilla verticillata* is better at extracting metal toxicants from contaminated water bodies. Since both species are edible and are an important part of cattle feed, the heavy metals measured will later bioaccumulate in humans via the food chain; thus, further extensive research is required to determine the heavy metal presence in other biotic components of Dal Lake.

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Data availability statement: The data that support the findings of this study can be obtained from the corresponding author upon request

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Conflict of interest

Authors have no conflict of interest

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