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Impact of pollution load of water and sediment of Hokersar wetland on nutrient concentration and biochemical parameters of *Trapa natans* L.: An economically important plant species of Kashmir, Jammu and Kashmir, India

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Abstract

Hokersar wetland is very important for the survival of migratory birds, but during the last four decades, the topography of the wetland has drastically changed. The wetland has reduced from 1875.04 ha in 1969 to 1300 ha in 2008 due to encroachments, sediment load carried by Doodah Ganga River and extension of willow plantations. It has shrunk and depleted due to the human settlements, domestic/sewage pollution, pollution due to the addition of fertilizers, siltation due to floods, conversion into agricultural land, encroachments and unwanted growth of aquatic weeds, *etc*. Keeping these facts in view, present study titled "Impact of polluted water and sediment of Hokersar wetland on nutrient concentration and biochemical parameters of *Trapa natans* L.: An economically important plant species of Kashmir, Jammu and Kashmir, India". In *T. natans* highest mean NPK content (4.07 × 10^4 mg kg^{-1}, 0.35 × 10^4 mg kg^{-1} and 4.47 × 10^4 mg kg^{-1}) was recorded during summer season and lowest (2.85 ×10^4 mg kg^{-1}, 0.18 ×10^4 mg kg^{-1} and 2.41 ×10^4 mg kg^{-1}) was recorded during autumn season. With respect to sites, highest mean NPK content was recorded at Inlet site (4.17 × 10^4 mg kg^{-1}, 0.30 × 10^4 mg kg^{-1}, 3.99 × 10^4 mg kg^{-1}) and least at Outlet site (control site). Similar was the case with heavy metal concentration, *i.e.*, higher concentration was recorded during summer season and lowest was recorded during autumn season. With respect to sites, highest mean heavy metal content was recorded at Inlet site and least at Outlet site (control site). The high metal concentration beyond permissible limits may damage the normal metabolism of plant. Chlorophyll 'a' varied from 1.95 to 2.14 mg/kg during all three seasons. The chlorophyll 'b' content also varied from 0.253 to 0.310 mg/kg and total chlorophyll content ranged from 2.18 to 2.46 mg/kg in three different seasons. The maximum chlorophyll 'a', 'b', and total chlorophyll contents were recorded near *Trapa* abundance site and the minimum values were recorded at Inlet. The concentration of carotenoid varied significantly from 1.10 to 1.29 mg/kg during the entire study period. The significant increase in the above studied parameters may be attributed to high nutrient availability in the wetland because of high pollution load.

1. Introduction

Hokersar has been listed under National Wetlands Conservation Programme. Hokersar wetland is differentiated into three varied zones, marshy and exposed area extending from North-to-North West, Central deeper area, South Eastern side covering most of the silted area. The North-Eastern zone comprises of diverse and dense macrophytic set up and maximum numbers of macrophyte species. The central deep area is largely a free expanse of water except at certain places where two predominant species of macrophyes *Trapa natans* and *Phragmites australis* occupy a large area. The marshy zone of the wetland and the open waters provide an efficient habitat to the migratory birds. Study by Khan *et al*. (2004) on the macrophyte community in relation to environmental stresses of Hokersar wetland reserves probably is the only long-term study carried out so far. The study indicates a shift in macrophyte community structure as evidenced by complete disappearance of *Nelumbo nucifera* and near disappearance of *Eurayle ferox* and *Acorus calamus.*

Hokersar wetland is very important for the survival of migratory birds, but during the last four decades, the topography of the wetland has drastically changed. The wetland has reduced from 1875.04 ha in 1969 to 1300 ha in 2008 due to encroachments by farmers, sediment load carried by Doodh Ganga River and extension of willow plantations. These changes in composition and structure of wetland have affected its normal functioning and deteriorated its water and sediment quality (Romshoo and Rashid, 2012).

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The wetland inhibits a number of major macrophytes like *Phragmites communis* (common reed), *Typha latifolia* (broad leaf cattail), *Nelumbium nucifera* (Indian lotus), *Trapa natans* L. (water chestnut), *Ceratophyllum demersum* (horn wort), *etc*., and minor macrophytes like *Salvinia natans* (floating fern), *Azolla pinnata* (water velvet), *Wollfia polyrhiza* Schield (duck weed), *Lemna gibba* L. (swollen duck weed), *etc*. (Afshan *et al*., 2014).

T. natans is among economically important aquatic plants. It grows as a rooted floating plant and is commonly called as Singhara. It belongs to family Lythraceae in the order Myrtales and genus Trapa. Its floating leaves are arranged in a rosette. The upper leaves are usually 2-4 cm long and the lower leaves reach up to 15 cm. The petioles of the floating leaves are 0.6-1.8 m long. The flowers are white in color and consist of petals and 4 green sepals. The fruits are four horned nut like structure about 3 cm long that develops under water. Fruits ripen in about a month and can remain viable for about 12 years. Each seed can give rise to 10-15 rosettes and each rosette may produce up to 20 seeds (Bailey, 1949). In India, water chestnut is distributed all over the country and is locally introduced in to paddy fields as alternative crop (Babu and Dwivedi, 2012). Keeping all these facts in view, the present study entitled "Impact of polluted water and sediment of Hokersar wetland on chemical and biochemical parameters of *T. natans*: An economically important plant species of Kashmir, Jammu and Kashmir, India" was under taken.

2. Materials and Methods

2.1 Study area

The present research work was conducted from July 2017 to June 2018 at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J & K). The macrophyte (*Trapa natans* L.) samples were collected from four different sampling sites, *viz*., Inlet (polluted site), Outlet (Control site), Centre and *Trapa* abundance site of Hokersar wetland during spring, summer and autumn seasons of the year. The samples were oven dried at 80^{o}C to constant weight after washing with clean tap water. The dried samples were crushed and passed through 2 mm sieve and stored at room temperature for analysis.

2.2 Methodology

2.2.1 Chemical parameters of *T. natans*

Total nitrogen content in the *T. natans* was estimated by micro-Kjeldahl method (Jackson, 1973). For total phosphorus, known weight of plant sample was digested in di-acid (mixture of HNO_3: $HClO_4$) in the ratio of 2:1 as per procedure described by Jackson (1973). Total potassium in plant samples was determined by the method of Toth *et al*. (1948). For finding out mineral nutrients or heavy metals, the plant samples were digested in a di-acid mixture consisting of HNO_3 and $HClO_4$. To the known amount of plant material (1g), 5 ml of concentrated HNO_3 was added and kept overnight. Next day, 12 ml of di-acid mixture (HNO_3 : $HClO_4$, 3:1) was added and digested on hot plate. The remaining digested material was diluted to 25 ml with distilled water and was then analyzed for the presence of heavy metals (Mn, Fe, Cu and Zn) by using atomic absorption spectrophotometer.

Photosynthetic pigment content, *i.e.*, the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were determined according to the method given by Hiscox and Isrealstam (1979), using dimethyl sulphoxide (DMSO). 100 g of fresh leaves were homogenized in 10 ml of DMSO and after keeping it in oven for half an hour at 40-50°C. The absorption of solution was noted at wave lengths (λ) of 480, 510, 645, 663 nm on UV spectrophotometer.

3. Results

3.1 Nutrient and mineral content analysis in *T. natans*

3.1.1 Nitrogen

In *T. natans*, the maximum mean nitrogen content (3.831 × 10^4 ± 0.005 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (3.321 × 10^4 ± 0.015 mg/kg) and least (2.491 × 10^4 ± 0.017 mg/kg) at Outlet in spring season. The maximum mean nitrogen content (4.971 × 10^4 ± 0.001 mg/kg) was observed at Inlet followed by *Trapa* abundance site (3.881 × 10^4 ± 0.001 mg/kg) and minimum (3.68 1×10^4 ± 0.001 mg/kg) at Outlet site in summer season. In autumn, the highest mean nitrogen content (3.72 ± 0.0011 × 10^4 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (2.73 ± 0.002 1×10^4 mg/kg) and least (2.33 1 × 10^4 ± 0.001 mg/kg) at Outlet. The site-wise highest mean nitrogen content (4.171 × 10^4 mg/kg) was recorded at Inlet and lowest (2.831 × 10^4 mg/kg) at Outlet (Figure 2). However, with respect to seasons, highest mean nitrogen content (4.071 × 10^4 mg/kg) was recorded during summer followed by spring (3.081 × 10^4 mg/kg) and least (2.81 × 10^4 mg/kg) during autumn season (Figure 1). Nitrogen content of sites and seasons was statistically significant from each other (Table 1).

3.1.2 Phosphorus

In *T. natans*, the maximum mean phosphorus content (0.271 × 10^4 ± 0.001 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (0.231 × 10^4 ± 0.009 mg/kg) and least (0.19 1 × 10^4 ± 0.002 mg/kg) at Outlet during spring season. Maximum mean phosphorus content (0.421 × 10^4 ± 0.008 mg/kg) was observed at Inlet followed by *Trapa* abundance site (0.371 × 10^4 ± 0.003 mg/kg) and minimum (0.281 × 10^4 ± 0.005 mg/kg) at Outlet in summer season. In autumn, the highest mean phosphorus content (0.221 × 10^4 ± 0.013 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (0.241 × 10^4 ± 0.013 mg/kg) and least (0.12 1 × 10^4 ± 0.015 mg/kg) at Outlet. However, with respect to seasons, highest mean phosphorus content (0.351 × 10^4 mg/kg) was recorded during summer followed by spring (0.221 × 10^4 mg/kg) and least (0.191 × 10^4 mg/kg) during autumn season (Figures 1, 2). Phosphorus recorded at all sites and during all seasons was seen statistically significant from each other and the interaction between sites × seasons was also found significant (Table 1).

3.1.3 Potassium

The maximum mean potassium content (3.561 × 10^4 ± 0.005 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (3.28 1 × 10^4 ± 0.008 mg/kg) and least (2.071 × 10^4 ± 0.017 mg/kg) at Outlet during spring season. Maximum mean potassium content (5.181 × 10^4 ± 0.001 mg/kg) was observed at Inlet followed by *Trapa* abundance site (4.481 × 10^4 ± 0.009 mg/kg) and minimum (3.891 × 10^4 ± 0.001 mg/kg) at Outlet during summer. In autumn, the highest mean potassium content (3.221 × 10^4 ± 0.003 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (2.451 × 10^4 ± 0.010 mg/kg) and least (1.851 × 10^4 ± 0.003 mg/kg) at Outlet. The site-wise highest mean potassium content in *T. natans* (3.991 × 10^4 mg/kg) was recorded at Inlet and lowest (2.601 × 10^4 mg/kg) at Outlet. However season-wise highest mean of potassium content (4.471 × 10^4 mg/kg) was recorded during summer followed by spring (2.881 × 10^4 mg/kg) and least (2.411 × 10^4 mg/kg) in autumn season (Figures 1, 2). All sites and seasons were statistically significant from each other with respect to potassium content and their interaction was also found significant (Table 1).

Table 1: Average seasonal variation in nitrogen, phosphorus and potassium contents of *T. natans* at different sites in Hokersar wetland

Parameters	Seasons	Sites				Mean	C.D ($p \leq 0.05$)
		Inlet	Centre	Trapa abundance site	Outlet		
Nitrogen (1×10^4 mg/kg)	**Spring**	3.83 ± 0.005	2.68 ± 0.015	3.32 ± 0.015	2.49 ± 0.017	**3.08**	Seasons (S):0.025
	Summer	4.97 ± 0.001	3.77 ± 0.005	3.88 ± 0.001	3.68 ± 0.001	**4.07**	Sites (S):0.029
	Autumn	3.72 ± 0.001	2.63 ± 0.001	2.73 ± 0.002	2.33 ± 0.001	**2.85**	Seasons ×
	Mean	**4.17**	**3.02**	**3.31**	**2.83**		Sites:0.050
Phosphorus (1×10^4 mg/kg)	**Spring**	0.27 ± 0.001	0.21 ± 0.004	0.23 ± 0.009	0.19 ± 0.002	**0.22**	Seasons(S):0.014
	Summer	0.42 ± 0.008	0.34 ± 0.002	0.37 ± 0.003	0.28 ± 0.005	**0.35**	Sites (S):0.016
	Autumn	0.22 ± 0.013	0.17 ± 0.013	0.24 ± 0.015	0.12 ± 0.015	**0.18**	Seasons ×
	Mean	**0.30**	**0.24**	**0.28**	**0.19**		Sites:0.027
Potassium (1×10^4 mg/kg)	**Spring**	3.56 ± 0.005	2.63 ± 0.015	3.28 ± 0.008	2.07 ± 0.017	**2.88**	Seasons(S):0.073
	Summer	5.18 ± 0.001	4.34 ± 0.029	4.48 ± 0.009	3.89 ± 0.001	**4.47**	Sites (S):0.084
	Autumn	3.22 ± 0.003	2.12 ± 0.020	2.45 ± 0.010	1.85 ± 0.003	**2.41**	Seasons ×
	Mean	**3.99**	**3.03**	**3.40**	**2.60**		Sites:0.146

C.D. = Critical difference

3.1.4 Iron

The maximum mean iron content (105.31 ± 0.014 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (89.528 ± 0.015 mg/kg) and least (76.74 ± 0.015 mg/kg) was seen at Outlet during spring season (Figures 1, 2). The maximum mean iron content (119.96 ± 0.012 mg/kg) in *T. natans* was observed at Inlet followed by *Trapa* abundance site (96.66 ± 0.065 mg/kg) and minimum (72.85 ± 0.023 mg/kg) at Outlet site during summer. In autumn, the highest mean iron content (79.58 ± 0.001 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (58.77 ± 0.001 mg/kg) and least (46.73 ± 0.007 mg/kg) at Outlet. The site-wise highest mean iron content in *T. natans* (101.61 mg/kg) was recorded at Inlet and lowest (65.44 mg/kg) at Outlet. However, with respect to seasons, highest mean iron content (95.42 mg/kg) was recorded during summer followed by spring (89.76 mg/kg) and least (60.38 mg/kg) during autumn season. All sites and seasons were statistically significant from each other with respect to iron content and interaction between sites × seasons was also found significant (Table 2).

3.1.5 Copper

The maximum mean copper content (9.81 ± 0.025 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (8.14 ± 0.043 mg/kg) and least (6.91 ± 0.059 mg/kg) at Outlet in spring season (Table 33). The highest mean copper content (16.87 ± 0.001 mg/kg) was observed at Inlet followed by *Trapa* abundance site (9.97 ± 0.045 mg/kg) and minimum (7.79 ± 0.001 mg/kg) at Outlet during summer season. In autumn, the highest mean copper content (7.89 ± 0.032 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (6.98 ± 0.029 mg/kg) and least (5.77 ± 0.041 mg/kg) was observed at Outlet. The site-wise highest mean copper content (11.55 mg/kg) was recorded at Inlet and lowest (6.82 mg/kg) at Outlet. However, with respect to seasons, highest mean copper content (10.88 mg/kg) was recorded during summer followed by spring (7.96 mg/kg) and least (6.75 mg/kg) was recorded during autumn season (Figures 3, 4). The mean copper content of sites and seasons was found statistically significant and interaction between sites × seasons was also found significant (Table 2).

3.1.6 Manganese

The maximum mean manganese content (157.93 ± 0.077 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (123.73 ± 0.020 mg/kg) and least (90.13 ± 0.015 mg/kg) at Outlet during spring season. Maximum mean manganese content (235.88 ± 0.013 mg/kg) was observed at Inlet followed by *Trapa* abundance site (167.88 ± 0.018 mg/kg) at and minimum (112.84 ± 0.019 mg/kg) was recorded at Outlet in summer season. In autumn, the highest mean manganese content (98.66 ± 0.025 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (75.74 ± 0.026 mg/kg) and least (37.850 ± 0.024 mg/kg) at Outlet. The site-wise highest mean manganese content in *T. natans* (164.15 mg/kg) was recorded at Inlet and lowest (80.27 mg/kg) at Outlet. However, with respect to seasons, highest mean manganese content (168.86 mg/kg) was recorded during summer followed by spring (122.84 mg/kg) and least (70.48 mg/kg) during autumn season (Figures 3, 4). The mean manganese of all sites and seasons was statistically significant from each other. Interaction between sites × seasons was also found significant (Table 2).

3.1.7 Zinc

The maximum mean zinc content (53.87 ± 0.015 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (43.67 ± 0.011 mg/kg) and least (31.73 ± 0.015 mg/kg) at Outlet during spring season (Figures 3, 4). The maximum mean zinc content (83.55 ± 0.002 mg/kg) in *T. natans* was observed at Inlet followed by *Trapa* abundance site (67.94 ± 0.008 mg/kg) and minimum (43.88 ± 0.001 mg/kg) at Outlet during summer season. In autumn, the highest mean zinc content (39.93 ± 0.021 mg/kg) was recorded at Inlet followed by *Trapa* abundance site (33.82 ± 0.039 mg/kg) and least (26.61 ± 0.017 mg/kg) was observed at Outlet. The site-wise highest mean zinc content in *T. natans* (59.11 mg/kg) was recorded at Inlet and lowest (34.07 mg/kg) at Outlet. However, season-wise highest mean zinc content (65.76 mg/kg) was recorded during summer followed by spring (43.03 mg/kg) and least (33.31 mg/kg) in autumn season. All sites and seasons were statistically significant from each other with respect to zinc content. The interaction between sites × seasons was also found significant (Table 2).

Table 2: **Average seasonal variation in iron, copper, manganese and zinc (mg/kg) contents of *T. natans* at different sites in Hokersar wetland**

Parameters	Seasons	Sites				Mean	C.D ($p \leq 0.05$)
		Inlet	Centre	Trapa abundance site	Outlet		
Iron	**Spring**	105.31 ± 0.014	87.46 ± 0.038	89.52 ± 0.015	76.74 ± 0.015	**89.76**	Seasons(S):0.192
	Summer	119.96 ± 0.012	92.21 ± 0.041	96.66 ± 0.065	72.85 ± 0.023	**95.42**	Sites (S):0.221
	Autumn	79.58 ± 0.001	56.47 ± 0.011	58.77 ± 0.0001	46.73 ± 0.0007	**60.38**	Seasons ×
	Mean	**101.61**	**78.71**	**79.98**	**65.44**		Sites:0.384
Copper	**Spring**	9.81 ± 0.025	7.01 ± 0.049	8.14 ± 0.043	6.91 ± 0.059	**7.96**	Seasons(S):0.336
	Summer	16.87 ± 0.001	8.89 ± 0.050	9.97 ± 0.045	7.79 ± 0.001	**10.88**	Sites (S):0.338
	Autumn	7.89 ± 0.032	6.37 ± 0.029	6.98 ± 0.021	5.77 ± 0.041	**6.75**	Seasons ×
	Mean	**11.55**	**7.42**	**8.36**	**6.82**		Sites:0.672
Manganese	**Spring**	157.93 ± 0.077	119.57 ± 0.002	123.73 ± 0.020	90.13 ± 0.015	**122.84**	Seasons(S):0.324
	Summer	235.88 ± 0.013	158.84 ± 0.023	167.88 ± 0.018	112.84 ± 0.019	**168.86**	Sites (S):0.375
	Autumn	9 8.66 ± 0.025	69.68 ± 0.028	75.74 ± 0.026	37.850 ± 0.024	**70.48**	Seasons ×
	Mean	**164.15**	**116.03**	**122.45**	**80.27**		Sites:0.649
Zinc	**Spring**	53.87 ± 0.015	42.86 ± 0.034	43.67 ± 0.011	31.73 ± 0.015	**43.03**	Seasons(S):0.250
	Summer	83.55 ± 0.002	67.66 ± 0.009	67.94 ± 0.008	43.88 ± 0.001	**65.76**	Sites (S):0.288
	Autumn	39.93 ± 0.021	32.89 ± 0.014	33.82 ± 0.039	26.61 ± 0.017	**33.31**	Seasons ×
	Mean	**59.11**	**47.81**	**48.48**	**34.07**		Sites:0.499

C.D. = Critical difference

3.2 Analysis of biochemical parameters in *T. natans*

3.2.1 Chlorophyll 'a'

The maximum mean chlorophyll 'a' content (2.30 ± 0.004 mg/kg) was recorded at *Trapa* abundance site followed by Centre (2.10 ± 0.003 mg/kg) and least (1.90 ± 0.009 mg/kg) at Inlet during spring season. The maximum mean chlorophyll 'a' content (2.45 ± 0.05 mg/kg) in *T. natans* was observed at *Trapa* abundance site followed by Centre (2.30 ± 0.004 mg/kg) and minimum (1.85 ± 0.010 mg/kg) at Inlet site during summer season. In autumn, the highest mean chlorophyll 'a' content (2.10 ± 0.011 mg/kg) was recorded at *Trapa* abundance site followed by Centre (2.05 ± 0.003 mg/kg) and least (1.75 ± 0.002 mg/kg) at Inlet. The site-wise highest mean chlorophyll 'a' content in *T. natans* (2.28 mg/kg) was recorded at *Trapa* abundance site and lowest (1.88 mg/kg) at Inlet. However, with respect to seasons, highest mean chlorophyll 'a' content (2.14 mg/kg) was recorded during summer followed by spring (2.08 mg/kg) and least (1.95 mg/kg) in autumn season (Figures 5, 6). All sites and seasons were statistically significant with respect to chlorophyll 'a' content and interaction between sites × seasons was also found significant (Table 3).

3.2.2 Chlorophyll 'b'

The maximum mean chlorophyll 'b' content (0.358 ± 0.004 mg/kg) was recorded at *Trapa* abundance site followed by Centre (0.297 ± 0.009 mg/kg) and least (0.256 ± 0.008 mg/kg) at Inlet during spring season. The maximum mean chlorophyll 'b' content (0.358 ± 0.005 mg/kg) in *T. natans* was observed at *Trapa* abundance site followed by Centre (0.314 ± 0.006 mg/kg) and minimum (0.289 ± 0.004 mg/kg) at Inlet during summer. In autumn, the highest mean chlorophyll 'b' content (0.285 ± 0.013 mg/kg) was recorded at *Trapa* abundance site followed by Centre (0.268 ± 0.001 mg/kg and least (0.224 ± 0.003 mg/kg) at Inlet. The site-wise highest mean chlorophyll 'b' content (0.326 mg/kg) was recorded at *Trapa* abundance site and lowest (0.256 mg/kg) at Inlet (Figures 5, 6). However, with respect to seasons, highest mean chlorophyll 'b' content (0.310 mg/kg) was recorded during summer followed by spring (0.297 mg/kg) and least (0.253 mg/kg during autumn season. All sites and seasons were statistically significant from each other with respect to chlorophyll 'b' and the interaction between sites × seasons was also determined significant (Table 3).

3.2.3 Total chlorophyll content

The mean total chlorophyll content was recorded maximum (2.65 ± 005 mg/kg) at *Trapa* abundance site followed by Centre (2.38 ± 0.003 mg/kg) and least content (2.15 ± 0.009 mg/kg) at Inlet during spring season (Table 3). The mean highest total chlorophyll content (2.81 ± 0.006 mg/kg) in *T. natans* was observed at *Trapa* abundance site followed by Centre (2.62 ± 0.002 mg/kg) and minimum (2.14 ± 0.008 mg/kg) was recorded at Inlet during summer season. In autumn, the highest mean total chlorophyll content (2.38 ± 0.004 mg/kg) was recorded at *Trapa* abundance site followed by Centre (2.34 ± 0.001 mg/kg) and least (1.97 ± 0.007 mg/kg) at Inlet. The site-wise highest mean total chlorophyll content in *T. natans* (2.61 mg/kg) was recorded at *Trapa* abundance site and lowest (2.14 mg/kg) at Inlet. However, with respect to seasons, highest mean total chlorophyll content (2.46 mg/kg) was recorded during summer followed by spring (2.37 mg/kg) and least (2.18 mg/kg) was recorded during autumn season (Figures 5, 6). All sites and seasons were statistically significant from each other with respect to total chlorophyll content (Table 3).

Table 3: Average seasonal variation in chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content (mg/kg) contents of *T. natans* at different sites in Hokersar wetland

Parameters (mg/kg)	Seasons	Sites				Mean	C.D ($p \leq 0.05$)
		Inlet	Centre	Trapa abundance site	Outlet		
Chloro-phyll 'a'	Spring	1.90 ± 0.009	2.10 ± 0.003	2.30 ± 0.004	2.05 ± 0.006	**2.08**	Seasons(S):0.001
	Summer	1.85 ± 0.010	2.30 ± 0.004	2.45 ± 0.005	1.97 ± 0.008	**2.14**	Sites(S):0.001
	Autumn	1.75 ± 0.002	2.05 ± 0.003	2.10 ± 0.011	1.80 ± 0.007	**1.95**	Seasons ×
	Mean	**1.88**	**2.08**	**2.28**	**1.95**		Sites:0.003
Chloro-phyll 'b'	Spring	0.256 ± 0.008	0.297 ± 0.009	0.358 ± 0.004	0.276 ± 0.002	**0.297**	Seasons(S):0.001
	Summer	0.289 ± 0.004	0.314 ± 0.006	0.358 ± 0.005	0.303 ± 0.011	**0.310**	Sites (S):0.001
	Autumn	0.224 ± 0.003	0.268 ± 0.001	0.285 ± 0.013	0.238 ± 0.012	**0.253**	Seasons ×
	Mean	**0.256**	**0.286**	**0.326**	**0.279**		Sites:0.002
Total chloro-phyll	Spring	2.15 ± 0.009	2.38 ± 0.003	2.65 ± 0.005	2.30 ± 0.010	**2.37**	Seasons(S):0.001
	Summer	2.14 ± 0.008	2.62 ± 0.002	2.81 ± 0.006	2.27 ± 0.012	**2.46**	Sites (S):0.001
	Autumn	1.97 ± 0.007	2.34 ± 0.001	2.38 ± 0.004	2.04 ± 0.011	**2.18**	Seasons ×
	Mean	**2.14**	**2.38**	**2.61**	**2.23**		Sites:0.002
Carotenoid content	Spring	0.87 ± 0.001	1.26 ± 0.006	1.45 ± 0.003	1.26 ± 0.003	**1.21**	Seasons(S):N/S
	Summer	0.95 ± 0.001	1.51 ± 0.001	1.60 ± 0.048	1.11 ± 0.001	**1.29**	Sites(S):0.067
	Autumn	0.75 ± 0.083	1.16 ± 0.001	1.28 ± 0.010	1.21 ± 0.010	**1.10**	Seasons ×
	Mean	**0.856**	**1.31**	**1.44**	**1.19**		Sites:0.116

C.D. = Critical difference

3.2.4 Carotenoid

The carotenoid content was recorded maximum (1.45 ± 0.003 mg/kg) at *Trapa* abundance site followed by Center (1.26 ± 0.006 mg/kg) at and least (0.87 ± 0.001 mg/kg) was seen at inlet during spring season. The highest carotenoid content (1.60 ± 0.048 mg/kg) in *T. natans* was observed at *Trapa* abundance site followed by Center (1.51 ± 0.001 mg/kg) at and minimum (0.95 ± 0.001 mg/kg) was recorded at inlet site during summer (Table 3). In autumn, the highest carotenoid content (1.28 ± 0.010 mg/kg) was recorded at *Trapa* abundance site followed by Center (1.16 ± 0.001 mg/kg) and least (0.750 ± 0.003 mg/kg) was observed at Inlet. The site-wise highest mean carotenoid content in *T. natans* (1.44 mg/kg) was recorded at *Trapa* abundance site and lowest (0.856 mg/kg) at Inlet. However, with respect to season, highest mean carotenoid content (1.29 mg/kg) was recorded during summer followed by spring (1.211 mg/kg) and least was recorded during autumn season (1.10 mg/kg) (Figures 5, 6). The mean carotenoid content of sites and interaction between sites × seasons was statistically significant but seasons were determined statistically non-significant (Table 3).

4. Discussion

Phosphorus (P) is an essential structural component of cell membranes and nucleic acids and necessary for maintaining the vital biological activities in plant. Phosphorus plays an important role in photosynthesis, respiration, energy storage, cell division, cell enlargement and several other processes in plants. Mean phosphorus content in *T. natans* ranged from 0.18 × 10^4 to 0.35 × 10^4 mg/kg in all three seasons. Highest mean phosphorus content in *T. natans* was recorded at Inlet site and lowest at Outlet site (Table 1). This may attributed to the availability of nutrient load at inlet site. The findings were in agreement with findings of Smal and Olszewska (2008) and Ruiz and Velasco (2010). The highest mean P content was observed during summer followed by spring and lowest in autumn. The decrease in pH due high rate of decomposition in sediments with enhanced microbial activity, could be the main reason in accumulation of more P in summer than autumn and spring. Phosphorus showed negative correlation with all mineral contents and biochemical parameters (Table 4). The present findings are in agreement with the findings reported by Eid *et al.* (2012).

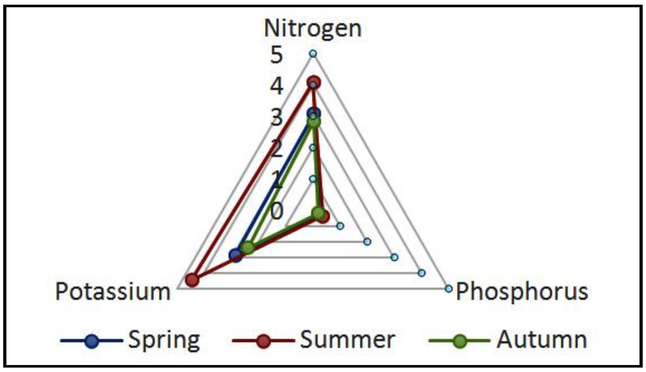

Figure 1: Seasoiial variation in N(1×10^4 mg/kg), P(1×10^4 mg/kg) andK(1×10^4 mg/kg) content of *T. natans.*

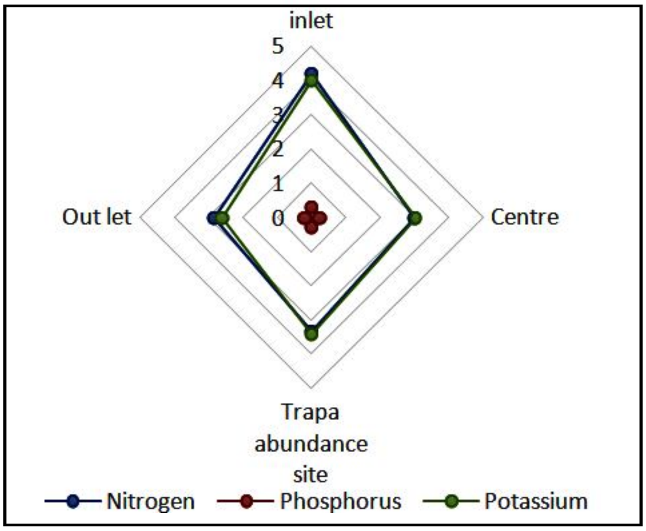

Figure 2: Variation in N(1×10^4 mg/kg), P(1×10^4 mg/kg) and K(1×10^4 mg/kg) content of *T. natans* at different sites.

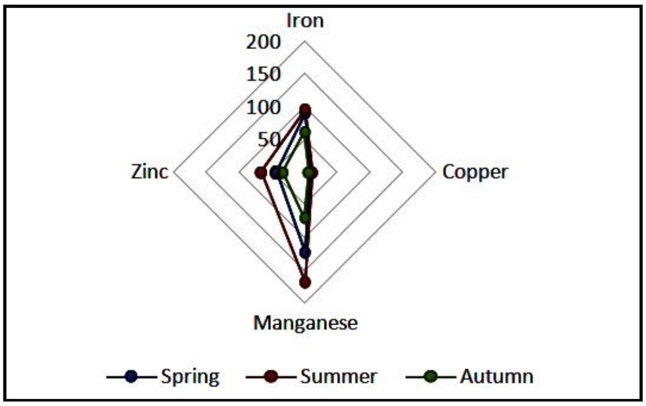

Figure 3: Seasonal variation in mineral content of *T. natans* (mg/kg).

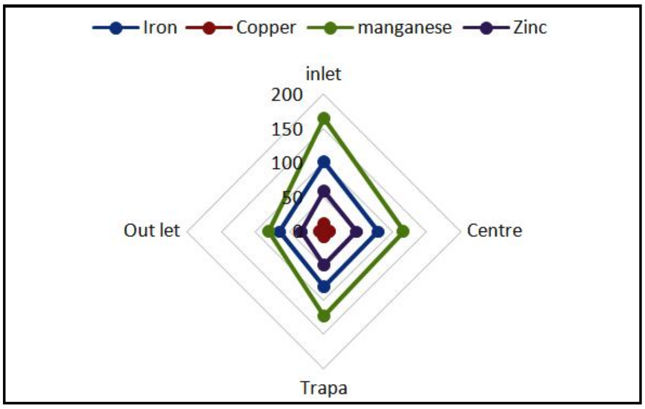

Figure 4: Variation in mineral content of *T. natans* (mg/kg) at different sites.

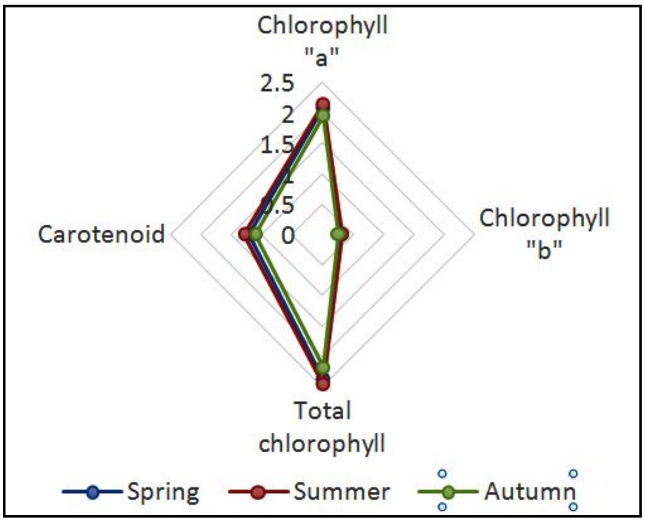

Figure 5: Seasonal variation in chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content (mg/kg) contents of *T. natans.*

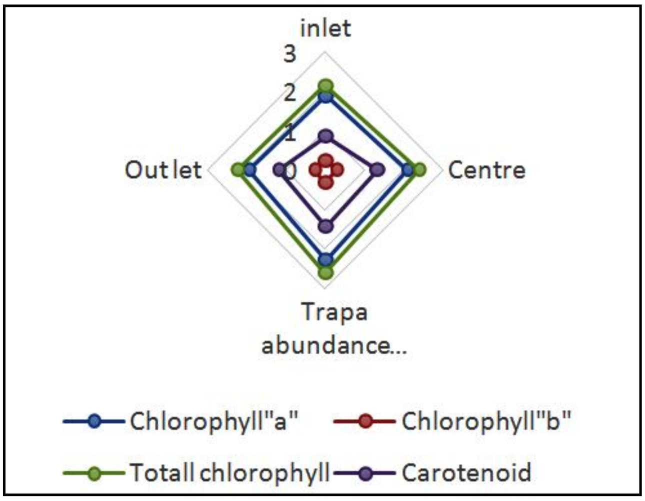

Figure 6: Variation in chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content (mg/kg) content of *T. natans* at different sites.

Potassium (K) belongs to the group of major macronutrients, responsible for plant growth and primary production. It affects the plant shape, size, colour and taste. It regulates the opening and closing of stomata and is essential for the production of adenosine triphosphate (ATP). The mean concentration of K in Hokersar wetland ranged from 2.41 × 10^4 to 4.47 ×10^4 mg/kg during all the three seasons. High accumulation of K in *T. natans* was recorded at Inlet site and lowest at Outlet (Table 1). This may due to the high nutrient load in the form of pollution influx from sediments, agricultural fields and natural activities. These findings are in concordance with the findings reported by Vardanyan and Ingole (2006) and Parvaiz *et al.* (2013). Highest content of K was recorded in summer followed by spring and lowest in autumn season (Table 1). The higher mean K content in *T. natans* during summer may be due to the availability of K in sediments by way of discharge of waste water, agricultural sewage and weathering of rocks. Since, aquatic plants absorb wide range of macro and micronutrients in the polluted environment and subsequently accumulate them in their tissues. Similar findings were reported by (Parvaiz *et al.* (2013) and Eid *et al.* (2012).

Manganese (Mn) is a plant micronutrient and is naturally occurring element found in rock, soil and water. Manganese is one of nine essential nutrients that plants require for growth. Many processes are dependent on this nutrient, including chloroplast formation, photosynthesis, nitrogen metabolism and synthesis of some enzymes. Manganese content significantly varied between 70.48 to 168.86 mg/kg in *T. natans*. Higher mean Mn content seen at Inlet site, may be because of higher accumulations of Mn in sediment due to heavy pollution load. The macrophyes have a great potential to absorb trace elements along with nutrients. The content of Mn in exceeded the natural content (52 mg/kg) as given by Brooks and Robinson (1998), but was within the toxic range (50 - 500 µg/g) given by Allen (1989) and Bonanno (2013). The excessive amount of Mn is directly related to addition sewage sludge and the injudicious use of fertilizers in the adjoining agricultural fields. Furthermore, highest mean Mn content was seen during summer season followed by spring and lowest in autumn (Table 2). The high availability of Mn during summer season may be due to the discharge of untreated water, agricultural run-off and weathering of rocks. The results are in agreement with Fitzgerald *et al.* (2003), Deng *et al.* (2004) and Klink *et al.* (2013).

Copper (Cu) is an important micronutrient for plants and is a constituent of enzymes which catalyze oxidative reactions in variety on metabolic pathways. It also activates enzymes which are involved in lignin synthesis, but at high concentration, it can be very toxic. It is an essential component in photosynthesis, plant respiration and carbohydrate metabolism. In the present study, Cu content ranged from 6.75 to 10.88 mg/kg in *T. natans* during all the three seasons. High mean concentration of Cu was reported at Inlet site and lower content at Outlet site (Figures 3, 4). This suggests the intense anthropogenic influence due to settlements near Inlet within the catchment area of the wetland. However, highest mean Cu content was seen during summer followed by spring and least in autumn (Table 2). This may be because of direct discharge or wet and dry depositions of contaminants in aquatic ecosystems. Similar findings were reported by Kumar *et al.* (2008).

Zinc (Zn) is one of the eight essential micronutrients needed by plants. It is key component of many proteins and enzymes and helps the plant to produce chlorophyll. It contributes to the hormone production and internode elongation. The mean concentration of Zn content in *T. natans* ranged from 33.31 to 65.76 mg/kg during the entire study period. Maximum mean Zn content was found at Inlet site as compared to Outlet (Figures 3, 4). This may be attributed to high pollution load at Inlet due to application of high amount of fertilizers in the agricultural fields and waste water discharge from households. These findings were in agreement with Fitzgerald *et al.* (2003), Deng *et al.* (2004), Baldontini*et al.* (2009) and Klink *et al.* (2013). High mean content of Zn in *T. natans* was recorded during summer season followed by spring and lowest in autumn (Table 2). This is because of high concentration of Zn in sediments trigger the absorption by macrophyes as the rate of photosynthesis is higher during this period because of favorable environmental conditions, hence more Zn get accumulated in plants during summer season. Similar observations were reported by Bragato *et al.* (2009), Duman *et al.* (2007), Lessage *et al.* (2007) and Maddison *et al.* (2009). Zinc showed significant positive correlation with all mineral contents and with nitrogen and potassium parameters (Table 4).

Iron (Fe) is plant uptake iron in its oxidized forms Fe^{2+} and Fe^{3+} forms. It is an important constituent of proteins involved in the transfer of electrons like ferredoxin and cytochrome. It activates catalase, peroxidase and nitrogenase enzyme and is essential for synthesis of chlorophyll. Iron is required in large amounts in comparison to other micronutrients. Fe content significantly varied from 60.38 to 95.42 mg//kg during three different seasons. The highest mean Fe content in *T. natans* was reported at Inlet site and lowest at Outlet (Table 3). This could be attributed to the pollution load, caused by both natural as well as anthropogenic activities. The availability and accumulation of micronutrients by aquatic plants depends upon several factors like trace elements, content in bottom sediment, plants species present in water, climatic factors and related factors (Fitzgerald *et al*., 2003; Deng *et al*., 2004; Klink *et al*., 2013). Furthermore, with respect to seasons, highest mean Fe content *T. natans* was reported during summer followed by spring and lowest during autumn (Table 2). The high availability of Fe could be due to the discharge of domestic sewage, use of fertilizers, dumping of hospital wastes and decomposition of organic matter due to high rate of microbial activity with increased temperature in sediments readily absorbs by roots of water plants and eventually accumulates in their tissues. The present results are in concordance with results reported by Parzych *et al.* (2015). According to Allen (1989), 40 - 500 mg g^{-1} Fe concentrations are considered as toxic to plants. The results indicated higher concentration of Fe during different seasons as well as at different sites also. Heavy metals in higher concentration may damage the normal metabolism plants.

4.1 Biochemical analysis of *T. natans*

In the present study, chlorophyll ‘a’ varied from 1.95 to 2.14 mg/kg during all three seasons. The chlorophyll ‘b’ content also varied from 0.253 to 0.310 mg/kg and total chlorophyll content ranged from 2.18 to 2.46 mg/kg in three different seasons. The maximum chlorophyll ‘a’, ‘b’, and total chlorophyll contents were recorded near *Trapa* abundance site and the minimum values were recorded at Inlet. The concentration of carotenoid varied significantly from 1.10 to 1.29 mg/kg during the entire study period. The significant increase in the above mentioned parameters may be attributed to high nutrient availability at the mentioned sites, which is in agreement with Taher-u-Zaman and Kushari (2002). Furthermore, all the biochemical parameters were recorded highest during summer season followed by spring and lowest in autumn (Table 3).

Table 4: Correlation between available nutrients, mineral content and biochemical parameters of *T. natans* growing at different sites in Hokersar wetland

	N	P	K	Mn	Cu	Zn	Fe	C.a	C.b	T.C
P	.168									
K	.954**	.003								
Mn	.898**	–.118	.917**							
Cu	.889*	–.058	.831**	.914**						
Zn	.893**	–.121	.947**	.964**	.885**					
Fe	.826**	–.023	.817**	.956**	.829**	.867**				
C.a	.070	–.436	.271	.241	–.013	.291	.214			
C.b	0.279	–.047	.294	.149	.231	.278	–.021	.170		
T.C	0.105	–.452	.308	.269	.026	.320	.228	.997**	.169	
Ca	–.448	–.564	–.256	.413	–.456	–.315	–.559	.395	.215	.400

*$p<0.05$ and **$p<0.01$; where, C.a = chlorophyll "a", C.b = Chlorophyll "b", T.C = Total Chlorophyll, Ca = Carotenoids

5. Conclusion

Presence of higher concentration of NPK and heavy metal content at Inlet site in the macrophyte is indication of the fact that Inlet site is highly polluted as compared to Outlet site (control site). High heavy metal concentration in the macrophyte recorded during summer season and lowest during autumn season may be because of high degradation rate of wastes during summer season. The biochemical parameters in *T. natans, viz.*, chlorophyll 'a' (2.28 mg/kg), chlorophyll 'b' (0.326 mg/kg), total chlorophyll (2.61 mg/kg) and carotenoid (1.44 mg/kg) were seen maximum at *Trapa* abundance site and lowest {(1.88 mg/kg, 0.256 mg/kg, 2.14 mg/kg and 0.856 mg/kg)} at Inlet site. Thus, it can be said that *T. natans* have good potential to absorb nutrients and accumulate trace elements from polluted environment.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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