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Microalgae Distribution and Diversity in the Narmada River Basin around Chutka, Madhya Pradesh, India

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ABSTRACT

Keywords

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The nutritional needs and ecological niche in food webs make microalgae as unique indicators for providing practical information of ecosystem condition. The present study aims to evaluate microalgae distribution and diversity in relation to physicochemical parameters of Narmada River basin around Chutka, a proposed place for nuclear power plant installation in Madhya Pradesh, India. Microalgae number was highest in pre-monsoon followed by the summer season and reduced in monsoon as well as in winter season. The seasonal alteration in water parameters markedly influenced microalgae abundance and diversity. Fifty-four genera belonged to five significant classes in microalgae were recorded in the Narmada River at different sampling sites under study. The successive pattern of microalgae was Chlorophyceae>Cyanophyceae>Bacillariophyceae> Euglenophyceae. Shannon diversity indices varied seasonally, and the values indicated the ecosystem was moderately polluted during monsoon and winter, while highly polluted in the summer and post-monsoon. The present study reveals continuous deterioration in the habitat parameters and microalgae diversity due to various anthropogenic activities in the area of study.

Introduction

Microalgae are mostly primary producers in aquatic food webs and play crucial roles in global geochemical cycles (Graham *et al.*, 2009). They play an essential role in the global carbon cycle and contribute around 50% of the approximately 11–117 Pg C assimilated through photosynthesis into organic matter annually (Behrenfeld *et al.*, 2001; Falkowski and Raven, 2007). Microalgae also have an essential role in global nitrogen cycles (Fowler *et al.*, 2013). Algae can assimilate dissolved carbon dioxide and bicarbonate from water for photosynthesis (Beardall and Raven, 2016). In fisheries and aquaculture, microalgae serve as essential food for the larvae and juveniles of fish, shellfish, and mollusks (Muller-Feuga, 2013). The nutritional needs and ecological niche in food webs make microalgae as unique indicators for providing practical information of ecosystem condition. Algae provide useful early warning signals of deteriorating of an ecosystem and the possible causes. Microalgae are critical components of the dam biota form the base of the pyramid of productivity. As the individual alga or its assemblage has different physiological requirements, it shows diverse responses to physicochemical parameters like temperature, pH, alkalinity, dissolved oxygen, nitrogen and phosphate contents, etc. Favourable environment induces excessive growth and accumulation of microalgae as blooms lead to the destruction of any water body resulting in dire consequences. Most research has focused on the so-called Harmful Algae Blooms (HABs), especially those who produce toxins that affect human health. Microcystin is a kind of microalgae that produces toxins which if not adequately treated and used as drinking water, may cause serious health hazards (Jochimsen *et al.*, 1998).

Biodiversity and conservation of freshwater ecosystems have been gaining the attention of researchers and policymakers for regional assessments recently since along with their terrestrial counterparts. Aquatic ecosystems have been increasingly placed under pressures to provide renewable resources. Besides, several factors such as afforestation, agriculture practices, urban, industrial development, river regulation, power generation, exotic species, dumping of solid wastes, dredging, overfishing invite threats on biodiversity in terms of conservation status. Globally the creation of reservoirs by the construction of medium to large-scale dams affects the plank tonic and macro-invertebrate populations due to their complex spatial structure and notable seasonal fluctuations in water levels (Henry *et al.*, 1998).

The Narmada River originates near Amarkantak at about 1050 m above MSL in the Maikaley highlands, flows westward through Madhya Pradesh, and Gujarat before merging with the Gulf of Cambay on the West coast. The entire Narmada basin is developed under a comprehensive river valley project programme through a series of dams. Since independence, rapid urbanization, agricultural and industrial development has taken place in all parts of the Narmada basin. Various anthropogenic activities across the basin not only deteriorated the sanctity of the River but affected abiotic and biotic parameters of the ecosystem. Recent reports demonstrated an alarming decline in the diversity of planktons in the river Narmada (Sharma *et al.*, 2013). As the Narmada is a rain-fed system and the annual run-off is dependent on the scale of water flow in the catchment areas. It is essential to maintain a suitable flow regime for managing desired and optimum habitat conditions in the dam affected river stretches of the River (Bhowmick *et al.*, 2017). Among thirty large dams, Bargi dam was constructed in Madhya Pradesh along the upper zone of the river. The upper Narmada zone of the river flows over black granitic rocks. Obstructing the river course with dams has caused alterations in basin conditions. Moreover, a large number of hills and hillocks are present in the upper valley project areas, resulting in an uneven depth profile all along the captive river basin (Bhaumik *et al.*, 2017). Recently NPCIL and DAE, Govt of India have proposed the construction of a nuclear power plant at Chuka village of Mandla district of Madhya Pradesh. Chutka situated on the right banks of River Narmada near Bargi Dam reservoir (Rani Avanti BaiLodhiSagar Dam).

There is plenty and continuous supply of fresh water for the smooth functioning of a power plant. The present study has been carried out from 2012 to 2015 for assessing microalgae distribution and diversity with relation to physicochemical parameters of Bargi dam around Chutka on the Narmada River basin.

Materials and Methods

Study area

Stretch of 39.5km along the Narmada River around Bargi dam was surveyed using boat. Seven locations (Table.1 and Fig.1) were selected for the samples collection on the basis of approachability and availability of water throughout the year.

Sampling sites were selected in a way, that it covered maximum habitats including shallow with rapid flow, deep with slow flowing water and lentic water (reservoir).

The sampling stations such as, Patha and Kikramal were located upstream to Chutka, whereas four stations such as, Tatighat, Poudimal, Bargi, and Tewar were situated at the downstream to Chutka (Fig. 1).

Analysis of Physicochemical parameters of water

Selected physicochemical parameters were analyzed in different seasons such as summer (16, March to 15, June), monsoon (16, June to 15, September), post-monsoon (16 September to 15, December) and winter (16 December to 15, March) during 2012 to 2015.

Surface water temperature was measured *in situ* using a mercury thermometer. pHwas measured using a portable instrument (HANNA meter model 210). Dissolved oxygen (DO), nitrate-nitrogen, nitrite-Nitrogen (NO3-N; NO2-N) and phosphate-phosphorus (PO4-P) were analyzed as per standard guidelines and procedures (APHA, 2012).

Collection and analysis of microalgae

Microalgae samples were collected from seven locations along the course of the River, as mentioned in Fig 1. Plankton net with a mouth aperture of 200 mm and mesh size of 25 µm was used to collect the microalgae. The samples filtered following the procedures described by Steedman (1976). A known volume (60 L) of water was collected from different spots of the location on the boat using the net.

All samples preserved in five percent formalin and few drops of Lugol's iodine solution was added and kept in a cold room in the dark for further analysis (Eaton *et al.*, 2005). For qualitative analysis of the microalgae, random sub-samples placed on a slide for observation using an optical microscope.

For quantitative analysis, Sedgwick-Rafter counting cell (50 x 20 x 1 mm) was used for counting the number of cells per liter. Microalgae were identified by consulting texts (Perry, 2010; Ruggiero *et al.*, 2015).

Statistical Analysis

Analysis Of Variance (ANOVA) and correlation for the water parameters and microalgae abundance was done using SPSS. Primer 5 (version 5.2.9), and Biodiversity Pro (version 2) were used to determine the diversity of the microalgae among sampling stations.

Results and Discussion

Physical and chemical characteristics of water

The mean surface water temperature ranged between 21.5±3.26°C and 24.0±3.48 °C. The maximum and minimum temperature was recorded at Tewar and Chutka respectively and did not vary (P >0.05) further in the upstream locations (Fig. 2A). The seasonal temperature showed significant wide variation (P <0.05) between 16.7°C and 31.8 °C in the winter and summer season (Fig. 2B). Temporal change in ambient temperature, though not very apparently, has been erratic and higher in the years of drought and low river discharge (Bhaumik *et al.*, 2017).

The highest and lowest mean DO values ranged between 6.3 ±0.37 mg/L and 8.05 ± 0.29 mg/L at Paudimal and Tewar, respectively. The DO values at other locations did not vary significantly and remained between 6.56 ± 0.22 mg/L and 6.99±0.86 mg/L (Fig. 3A). Analysis of variance showed that there were no statistically significant variations in dissolved oxygen (DO) values among different seasons except monsoon, which were significantly lower (p<0.05) (Fig. 3B). The dissolved oxygen at the five sampling stations along the Narmada River fluctuated between 3.1 and 6.5 mg/l and low concentration of dissolved oxygen (DO) was reported in summer (Saini *et al.*, 2015). Bhaumick *et al.*, (2017) reported the level of dissolved oxygen fluctuated over a wide range (4.4–9.1 ppm) in the middle and lower zones of the Narmada.

A circum-neutral pH was observed during the study period, with the highest average value of 7.5±0.15 and the lowest of 7.15±0.15 at Tatighat and Paudimal, respectively (Fig. 3A). The seasonal temperature showed significant variation (P<0.05) between post-monsoon (7.54±0.05) and the rest of the seasons (7.24-7.31) (Fig. 3B). The pH of Narmada water showed alkaline condition during the period and ranged between 6.6 and 9.5 at the five different sites (Saini *et al.*, 2015).

The average alkalinity values ranged between 113.2± 0.51 mg/L and 115.87±0.48 mg/L at different locations with a maximum value at Patha and minimum at Zero Tanky (Fig. 4A). The seasonal alkalinity values were in the range of 114.15±0.39 mg/L to 115.25±0.61 mg/L, and there was no significant variation in the values among different seasons (Fig.4B).

The hardness values ranged between 76.55± 1.57 mg/L and 113.12±1.89 mg/L and showed significant fluctuations (p<0.05) among different sampling sites (Fig. 4A). The highest and lowest values were recorded at Zero Tanky and Kikra, respectively. The hardness values in different seasons did not vary significantly (p>0.05) (Fig. 4B). Alkalinity was highest in monsoon and low in winters, but no regular trend was observed with mean values of 136.66± 55.84 in Narmada River at Dograwada Ghat (Sharma *et al.*, 2015). Total hardness varied from 79 – 196 mg/l and the highest peak observed in May (196 mg/l) and lowest in July (79 mg/l) in Dograwada Ghat (Sharma *et al.*, 2015).

The highest NO3-N value recorded was 0.52 ±0.025 mg/L and the lowest of 0.15 ±0.03 mg/L at Tatighat and Tewar, respectively. The mean values were not significantly different (p>0.05) among most of the sampling sites, except Tewar and Patha (Fig. 5A).

The values in different season ranged between 0.38 ±0.05 and 0.47 ±0.05 mg/L and did not show a significant difference among the seasons (Fig. 5B). The NO2-N values showed similar levels and locational changes as recorded for NO3-N. The highest content of NO2-N was 0.56 ±0.03 mg/L at Tatighat, while the lowest was noted at Tewer (0.2

±0.01 mg/L, Fig. 5A). Results from ANOVA showed that the observed seasonal variations were not significant among different seasons (P>0.05; Fig. 5B). The nitrate nitrogen on an average annual basis recorded in the range of 0.13 mg/L to 0.30 mg/L with a mean value of 0.215mg/l during winter and post-monsoon seasons in the Narmada at Sethianghat (Bano *et al.,* 2016). PO4-P showed the highest mean concentration of 0.82 ±0.01 mg/L at Patha, whereas the lowest value was recorded at Tewar (0.28 ±0.01 mg/L; Fig. 5A). The PO4-P value of 0.49 ±0.04 mg/L was recorded in monsoon, while the highest of 0.64±0.06 mg/L was recorded in post-monsoon (Fig. 5B).

The PO4-P values were not significantly different among winter, summer, and monsoon (Fig. 5B). Very high NO3-N and PO4-P ranged from 11.1-26.5 mg/l, and 1.04-3.58 mg/l respectively were reported from the five different sites along the Narmada River (Saini *et al.,* 2015). Mean phosphate concentration of river Narmada was recorded in the range of 0.01 to 0.04 mg/L during winter and summer seasons at Sethiaghat (Bano *et al.*, 2016).

Composition of phytoplankton Communities

The microalgae community in Narmada River around the Bargi dam consisted of Bacillariophyceae, Chlorophyceae, Cyano-phyceae, Chrysophyceae, and Dinophyceae (Fig. 6). Total fifty-four genera identified during the sampling period, out of those 16 genera belonged to Bacillariophyceae, while Chlorophyceae and Cyanophyceae represented by 21and 10 genera respectively, whereas the Chrysophyceae and Euglenophyceae were represented by 2 genera each, while 3 genera belonged to Dinophyceae (Table 2). Out of all the genera, thirty one were present in all samplings and considered for statistical analysis. The previous study reported 13 genera of Chlorophyceae, 5 genera of Bacillariophyceae, 8 genera Cyanophyceae, and 1 genera of Euglenophyceae at the Dograwadaghat of River Narmada (Sharma *et al.*, 2011).

Table.1 Geographical locations and physiography of sampling sites

Serial no.	Sampling Points	Latitude (°E)	Longitude (°N)	Physiography
S1	Tewar	22°97’.071	79°87’.919	Shallow &rapids
S2	Zero tanky	22°92’79	79°90’235	Reservoir
S3	Poudimul	22°84’774	80°01’284	Deep/ slow flowing
S4	Tatighat	22°76’93	80°08’368	Deep/ slow flowing
S5	Chutaka	22°78’213	80°09’301	Reservoir
S6	Patha	22°84’774	80°01’284	Reservoir
S7	Kikara mal	22°77’552	80°18’989	Deep slow flowing

Table.2 Microalgae genera recorded in the Narmada River at seven locations.

Cyanophyceae (10)	Dinophyceae (3)	Chlorophyceae (21)	Bacillariophyceae (16)	Chrysophyceae (2)	Euglenophyceae
Anabanea sp.	*Peridinium sp.*	*Gonium sp.*	*Fragilaria sp.*	*Synura sp.*	*Phacus sp.*
Anacystis sp.	*Urosolenia sp.*	*Mougeotia sp.*	*Pinnularia sp.*	*Dinobryon sp.*	*Euglena sp.*
Chlorococcus sp.	*Ceratium sp.*	*Microspora sp.*	*Tabellaria sp.*		
Merismopedia sp.		*Cosmarium sp.*	*Gomphonema sp.*		
Microcystis sp.		*Tetraspore sp.*	*Nodularia sp.*		
Nostoc sp.		*Chlamydomonas sp.*	*Amphora sp.*		
Oscilatoria sp.		*Euastridium sp.*	*Netzschia sp.*		
Phormidium sp.		*Eudorina sp.*	*Lyngbya sp.*		
Spirulina sp.		*Cosmarium sp.*	*Synendra sp.*		
Cyclotella sp.		*Lepocinclis sp.*	*Thalassionema sp.*		
		Actinastrum sp.	*Gyrosigma sp.*		
		Crucigenta sp.	*Diatom asp.*		
		Cladophoroa sp.	*Gonicloris sp.*		
		Ulothrix sp.	*Cymbella sp.*		
		Pediastrum sp.	*Pseudonetzchia sp.*		
		Closterium sp.	*Aulacoseira sp.*		
		Scenendesmus sp.			
		Staurastrum sp.			
		Oedogonium sp.			
		Volvox sp.			
		Chlorella sp.			

Fig.1.Portal map of sampling locations along the Narmada River around Chutka

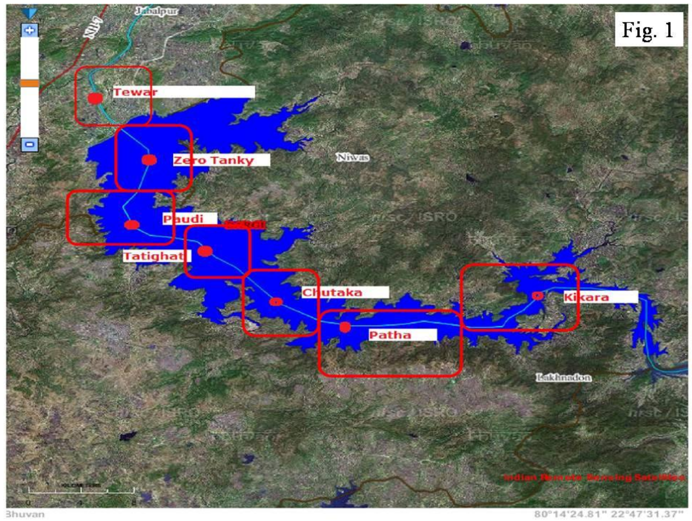


Fig.2.Locational and seasonal variation of water temperature in Narmada. Values are mean±SEM. Different letters depict statistically significant (p<0.05)

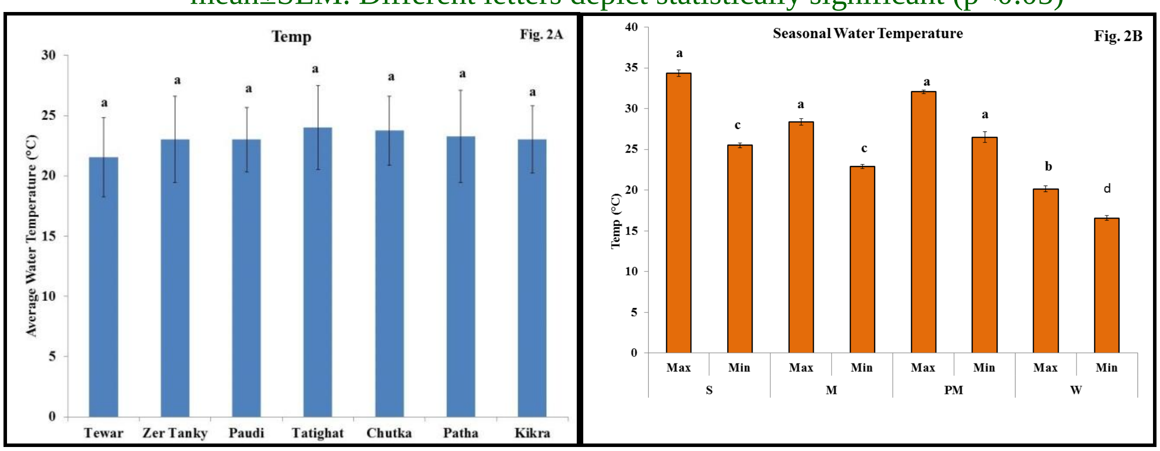


Fig.3. Spatial and temporal changes in dissolved oxygen (DO) and pH of water during study period. Values are mean±SEM. Different letters donote significant difference (p<0.05)

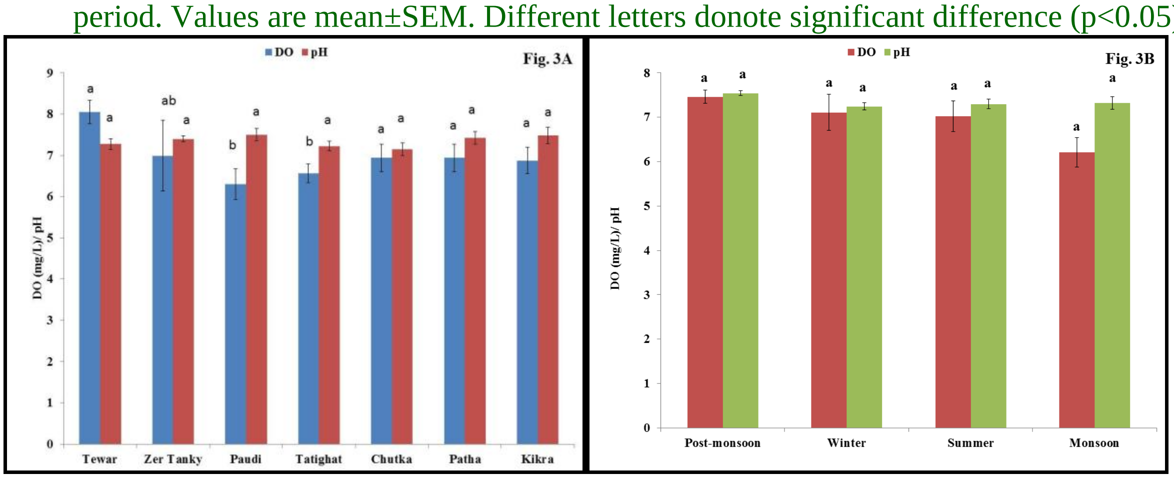


Fig. 4.Variation of alkalinity and hardness in the Narmada water at different sampling sites and seasons. Values are mean±SEM. Different letters depict significant difference ($p<0.05$)

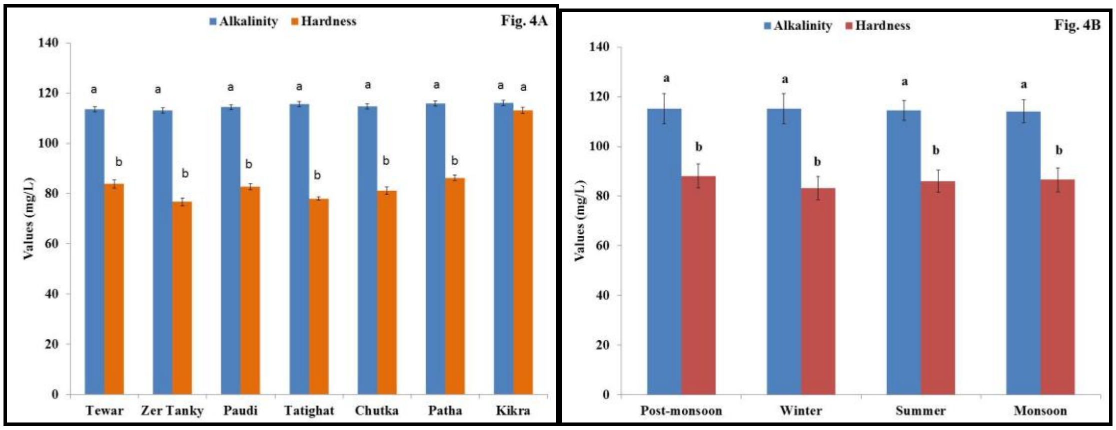


Fig.5 Variation of phosphate-phosphorus (PO4-P), nitrate-nitrogen (NO3-N), nitrite-nitrogen (NO2-N) in the Narmada water at different sampling sites and seasons. Values are mean±SEM. Different letters depict significant difference ($p<0.05$).

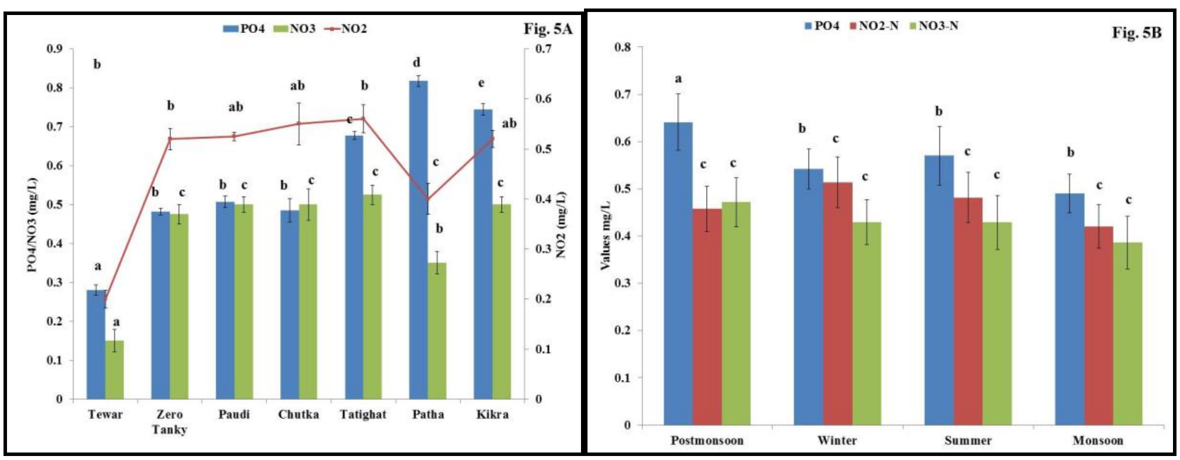


Fig.6.Seasonal variation of individual microalgae group

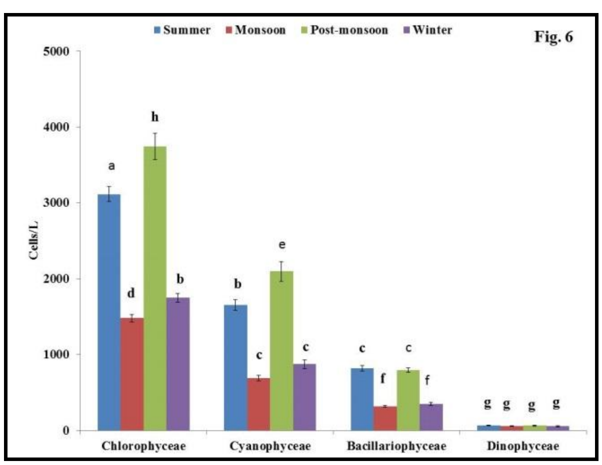


Fig.7 Composition of microalga community (7A) and seasonal variation in abundance of microalgae (7B).

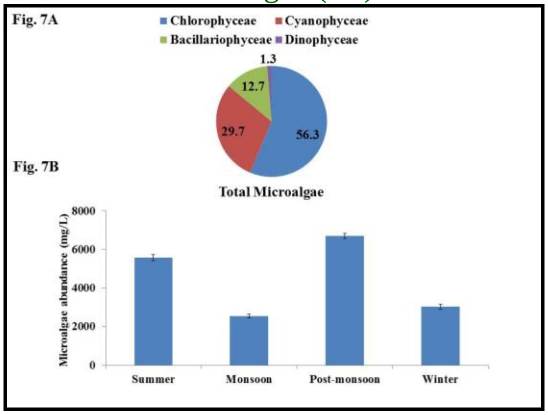


Fig.8 Microalgae diversity in different seasons

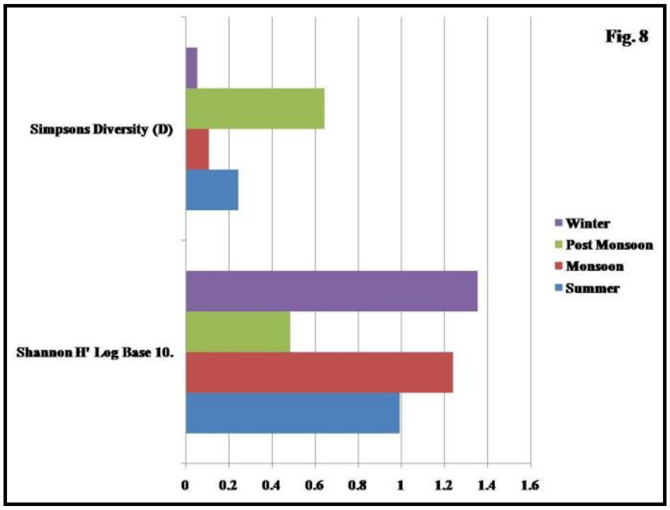
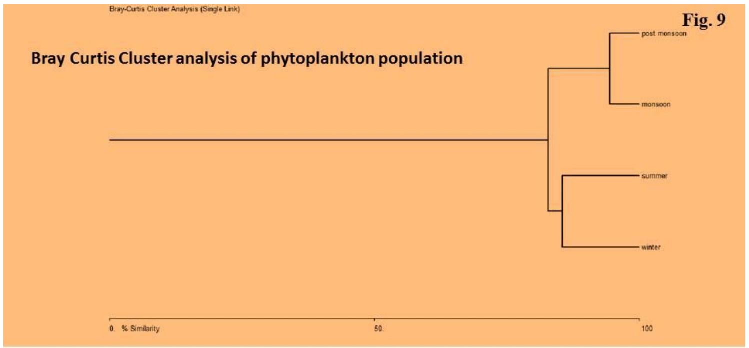


Fig.9 Similarity of microalgae community during different seasons



Similarity Matrix	Winter	Summer	Monsoon	Post-monsoon
Winter	*	85.49	68.38	71.45
Summer	*	*	80.75	82.77
Monsoon	*	*	*	90.4
Post-monsoon	*	*	*	*

A detailed study conducted by Unni (1996) on the Narmada River along 500 km stretch between Amarkantak and Sethanighat reported a total of 174 species of phytoplankton. Out of the total, 101 species in 27 genera belonged to Bacillariophyceae, while 46 species in21 genera were Chlorophyceae. Cyanophyceae, Euglenophyceae, Dinophyceae were represented by19, 4, and 3 species, respectively, and Chrysophyceae was repre-sen-ted by a single species. A recent report revealed that four families represented the phytoplankton community in the River Narmada near Jabalpur. Bacillariophyceae is the most diverse family consist of 10 species with 559 organisms (46%), while Chlorophyceae had 409 organisms (34%) belonged to 8 species. Cynophyceae consisted of 216 organisms (18%) belonged to 6 species and Euglenophyceae had 22 organism (2%) of 1 species in a six-month study during July to December 2015 (Rai *et al.,* 2016).

Seasonal and locational variations in microalgae abundance

The percentage contribution of the four major groups of microalgae varied from place to place and with the time and was related to various abiotic factors. In the present study, Chlorophyceae and Cynophyceae shared 56.3% and 29.7% respectively, while Bacillariophyceae and Dinophyceae contributed 12.7 and 1.3 % in the total population (Fig. 7A).

The similar trends of succession such as, Chlorophyceae>Cyanophyceae>Bacillariophyceae>Euglenophyceae(Fig. 6) in microalgae community were noticed in the earlier studies on the Narmada River basin time to time (Sharma *et al.,* 2011; Sharma *et al.,* 2015). However, different succession with a dominant group as Bacillariophyceae was recorded from the mixed zone of Narmada River (Saini *et al.*, 2015, Rai *et al.*, 2016). The average microalgae density showed significant differences ($p<0.05$) among the seasons. The abundance was highest in pre-monsoon season (6710 cells/L), followed by summer (5562.7 cells/L), monsoon (2537.1 cells/L) and winter 3029.4 cells/L) (Fig.7B). The total number of microalgae in different stretches of the Narmada River was recorded in the range of 317-751 cells/L in along the stretch of Lamhetaghat to Bhedaghat near Jabalpur (Saini *et al.*, 2015), 1206 cells/L at Jabalpur region (Rai *et al.*, 2016), and 1458-1505 units/L in four locations along the River basin (Sharma *et al.,* 2015).

Correlation of water parameters and microalgae

Significant correlations were calculated between microalgae density and water parameters at different locations. The overall microalgae and Dianophyceae abundances showed a highly significant and positive correlation with temperature ($r = 0.88$ and $r = 0.92$) similar to the earlier report (Sharma *et al.,* 2015). Contrary, negative correlation ($r = 0.97$) existed between nitrite-nitrogen and total phytoplankton. The Chlorophyceae, Cyanophyceae, and Bacillariophyceae density showed a significant and positive correlation with nitrate-nitrogen ($r = 0.71$-0.85) and phosphate ($r = 0.81$-0.93) as reported in Tungabhadra and Ogun River (Suresh, 2015; Odulate *et al.*, 2017). Thus, NO3 and total phosphate are nutrients for phytoplankton growth, whereas all the groups showed a negative correlation with nitrite-nitrogen. Nitrate-nitrogen showed positive correlation ($r = 0.98$) with PO4. Saravi *et al.,* (2011) suggested that the majority of phytoplankton species have large tolerance to fluctuating water quality, which varies from year to year.

Seasonal diversity in microalgae community

Overall microalgae diversity was recorded in the range of 0.487 to 1.354 during different seasons in the Narmada River around Chutka. Seasonal Shannon diversity indices represented the most diverse community in the winter season (1.354), followed by monsoon (1.24) and summer (0.994) and least varied in post-monsoon (0.487; Fig. 8). Although similar seasonal diversity pattern was reported in Sular Lake, Coimbatore, however, the diversity values were comparatively higher than the present study (Mancikam *et al.*, 2017). *Microcystis*is, one of the dominant genera, contains several species which often form massive blooms and which produce toxins (Borowitzka, 2018). The Microcystis has been expanding worldwide mainly due to eutrophication of ponds, lakes, and rivers, often together with increased temperatures, and have significant environmental and human and animal health impacts (Carmichael and Boyer, 2016). In the present study, *Microcystis* bloom developed in the post-monsoon season showed a negative correlation (r = - 0.83) with the microalgae diversity, indicates that the *Microcystis* bloom was a post-monsoon phenomenon and harmed variety of microalgae.

The evenness of microalgae community was comparatively higher in post-monsoon season (0.645), and the value decreased abruptly in summer through monsoon and reached lowest limit in winter (Fig. 8). The overall species evenness of phytoplankton was recorded in the range between 0.76 and 0.97 in Sulur lake waters (Manickam *et al.*, 2017).

Bray-Curtis similarity cluster analysis was done to evaluate similarities among the microalgae community of the different seasons. The investigation revealed that there was a significant difference among the seasons in microalgae community compositions (Fig. 9). The similarity was most notable in post-monsoon and monsoon (94.39%) compared to summer (85.49%; (Fig. 9).

Shannon-Wiener diversity index (H') is a common indicator for evaluating pollution level in an aquatic system. Wilm and Dorris (2007) suggested a relationship between species diversity and pollution status of aquatic system and classified as follows; > 3 = clean water, 1-3 = moderately-polluted,< 1 = heavily polluted. In the present study (H') value ranged between 0.487 and 1.35 (Fig. 8), suggested that the locations were moderately polluted during monsoon and winter, while highly polluted in the summer and post-monsoon. The probable reasons for pollution include high microalgae abundance in the post-monsoon and too some extent in summer. In addition, stable hydrological factors and low water level may be responsible for summer bloom, whereas agricultural and waste run-off attributed to nutrient enrichment and microalgae bloom in the post-monsoon season. Perturbation of a lotic aquatic ecosystem may not be precisely quantified based on the Shannon-Wiener species diversity of microalgae communities in the fluviatile environment since those organisms may be of allochthonous in origin despite been detected *in situ* even in highly polluted zone (Jhingran *et al.*, 1989).

The present study provides baseline information on microalgae distribution, abundance, diversity, and evenness in the River Narmada around the Chutka, a proposed area for installing a nuclear power plant shortly. Microalgae number was highest in pre-monsoon followed by the summer season and reduced in monsoon as well as in winter season. The results reveal that seasonal alteration in water parameters influenced microalgae abundance and diversity. Fifty-four genera belonged to five significant classes in microalgae were recorded in River water. The successive pattern of microalgae was as Chlorophyceae>Cyanophyceae> Bacillariophyceae>Euglenophyceae during the period of study. Shannon diversity indices varied seasonally, and the values indicated the ecosystem was moderately polluted during monsoon and winter, while highly polluted in the summer and post-monsoon. The present study reveals deterioration in the habitat parameters and microalgae diversity due to various anthropogenic activities in the area of study.

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